The Neuroscience of Dementia

Volume 2

Genetics, Neurology, Behavior, and Diet in Dementia

Edited by

Colin R. Martin

Victor R. Preedy

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Colin R. Martin—*I would like to dedicate this book to my beautiful daughter Dr. Caragh Brien, of whom I am so proud.*

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Foreword

I am gratified to write the foreword to this comprehensive book on dementia. Professors Preedy and Martin's purpose is to improve and enhance the care of individuals who have been diagnosed with dementia.

Given the scope of what dementia is—a catch-all term for a constellation pathologies that impact deleteriously and chronically on brain function—understanding the spectrum of what we consider the disease and process of dementia is inevitably a complex task. Yet, as this inspiring new book reveals, we are making significant headway.

The two professors, one from the University of Hull and the other from King's College London, leading authorities themselves in applied health research, have brought together other leading authorities from around the world who specialize in dementia clinical and applied research. Their focus is on contemporary treatment, management, and research innovation from a primarily physiological perspective, whilst implicitly emphasizing that the fundamental purpose is enhancing and improving the understanding and care of those with a diagnosis of dementia.

They have not forgotten the vital role that family, friends, and other carers play in supporting the patient with dementia and that these carers are often long-term partners who are themselves very elderly, maybe with multiple pathology themselves, or offspring, no longer in the first flush of youth. Their needs are vital. Improving the outcomes for each individual patient with dementia integrates the patient, family, friends, and other carers with health and social care practitioners in a unique partnership aimed at improving care and quality of life for both patient and those close to the patient.

The impact of the dementia diagnosis is likely to be met by fear, anxiety, and trepidation and perhaps, shame. I am old enough to remember when a diagnosis of cancer was met with the same emotions and, to some extent, stigma. We have learned so much more about cancer, the care of patients with a diagnosis of cancer, and the needs of carers. This book helps move us along the road to an increasingly evidence-based treatment of dementia, a recognition of the trauma of the diagnosis. It enables improved education and support of family, carers, and the public, and, not least, practitioners and researchers.

Surprisingly, though we know much regarding the psychosocial aspects of dementia care from an integrated perspective, the underlying biological substrates and layers of dementia are less clearly understood, particularly from an integrated perspective.

This book has special resonance for me at many levels. It balances the biological aspects of disease, evidence-based treatment, the care of patients and carers, and acknowledges the complex web that enables effective care.

My own journey through nursing and health visiting led me to research the role of the health visitor with older people. It makes me smile, even now, as I remember the lady who opened the door and I asked to see her mother. "She's dead," the lady replied. I quickly discovered the lady to whom I was speaking was 90. Hale and hearty. But there were others, very ill in their fifties and sixties. Others too, desperately caring for their partners. Feeling frightened and alone. Patients with dementia who had other illnesses not diagnosed. So much to do.

I understand well the complex web that produces integrated care, as I went on to research interprofessional, interorganizational relations, as with this book, with the sole purpose of assisting with the development of care for patients and their carers. Influencing policy and advocating are as important as the "hard" sciences, social sciences, in improving care. It is exciting. Fundamental is to be caring, this is my interest in ethics, moral behavior—why are people ill-treated?

It has been a pleasure to write the foreword for this stimulating new book. Victor Preedy and Colin Martin have produced a work of considerable value to both clinicians and researchers—often the same people. The discovery of new knowledge is always exciting, and in this instance, it can help also to prevent ill treatment.

Carolyn Roberts

Lady Roberts is Pro Chancellor at the University of Hull.

Her career has straddled clinical practice, research, consultancy, and management. She has a keen interest in clinical ethics and bioethical issues. As well as experiencing the ups and downs of life, she is ever seeking to overcome disadvantage, to be holistic—as this book does, integrating all aspects of life—plus of course, always interested in the exciting search for new knowledge, new skills, and continuous improvement.

Preface

There are many different types of dementia, and the most common of these include Alzheimer disease and Lewy body, mixed and vascular dementias. Together they account for about 90% of all dementias, though there are others. Globally there are 50 million people living with dementia. In the United States there are 5 million people with dementia costing an annual 250 billion dollars, or more. The present trajectory suggests that by 2050 the number of people in the United States with dementia will reach 16 million. Connected with this are the unpaid carers, which presently number 15 million in the United States alone.

Whilst the day-to-day impact of dementia on the individual and family unit can be anecdotal, the detailed evidence-based understanding of dementia is diffuse, appearing in different scientific domains. This is addressed in *Genetics, Neurology, Behavior, and Diet in Dementia: The Neuroscience of Dementia* which brings together different fields of dementia into a single source material. The book covers a wide range of subjects which encompasses and interlinks genetics, polymorphisms, cell signalling, microstructures, brain regions, inflammation, imaging, sleep, hypertension, atrophy, delirium self-consciousness, syndromes, cognition, violence, depression, nutritional status, micronutrients, obesity, heavy metals, modelling systems, resources and other important areas too numerous to mention here.

The book has over 50 chapters and is divided into the following subsections:

[1] Genetics, Molecular and Cellular Biology
[2] Neurology, Physiology and Imaging
[3] Behaviour and Psychopathology
[4] Diet, Nutrition and Environment
[5] Models, Modelling and Resources

There are of course always difficulties in ascribing chapters to different sections and placing them in order. Some chapters are equally at home in more than one section. However, the excellent indexing system allows material to be rapidly located.

Genetics, Neurology, Behavior, and Diet in Dementia: The Neuroscience of Dementia bridges the multiple disciplinary and intellectual divides as each chapter has:

- *Key facts*
- *Mini-dictionary of terms*
- *Summary points*

Genetics, Neurology, Behavior, and Diet in Dementia: The Neuroscience of Dementia is designed for research and teaching purposes. It is suitable for neurologists, psychologists, health scientists, public health workers, doctors, and research scientists. Those working in

the fields of genetics, molecular and cellular biology, diet and nutrition, modifiable factors and modelling systems will also find the book of interest. It is valuable as a personal reference book and also for academic libraries, as it covers the domains of neurology and health sciences. Contributions are from leading national and international experts including those from world-renowned institutions. It is suitable for undergraduates, postgraduates, lecturers, and academic professors.

The Editors

PART I

Genetics, molecular and cellular biology

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CHAPTER 1

The neuron navigator 2 gene and Alzheimer's disease

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List of abbreviations

AAO age at onset
AD Alzheimer's disease
ADHD attention-deficit/hyperactivity disorder
APOE apolipoprotein
ASD autism spectrum disorders
atRA all-trans retinoic acid
BIN1 bridging integrator 1
cDNA complementary DNA
CLU clusterin
CR1 complement receptor 1
DEGs differentially expressed genes
DTC distal tip cell
GWAS genome-wide association study
MPH methylphenidate
NAV2 neuron navigator 2
NGS next-generation sequencing
NRXN3 neurexin 3
nsSNP nonsynonymous single-nucleotide polymorphism
PICALM phosphatidylinositol binding clathrin assemble protein
UNC uncoordinated protein
UNC-53 adapter protein unc-53 [C- elegans]

Introduction

Alzheimer's disease (AD) is a quite common neurodegenerative disorder with a high degree of heritability and clinically characterized by insidious onset and progressive impairment of memory and other cognitive functions, ultimately resulting in complete dependency and death (Bettens, Sleegers, & Van Broeckhoven, 2010). This disease extracts a marked toll in terms of morbidity and economic burden and also imposes a tremendous burden on patients and the health system in general. The major risk factors of AD include increased age, lifestyle, and a positive family history of dementia, and these

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factors can be classified as modifiable risk (lifestyle) and nonmodifiable risk (age, genetics). Moreover, AD is the major cause of dementia and has a strong genetic predisposition (60%–80% of attributable risk) (Gatz et al., 2006). With advances in technology, the results of genome-wide association studies (GWASs), meta-analyses, and candidate gene studies have suggested a few potential risk variants for AD, which have been reported primarily in populations of European descent. In addition to apolipoprotein (APOE), recent next-generation sequencing (NGS), high-throughput GWASs, and meta-analyses have identified several new variants in the genes of ABCA7, CENPO, clusterin, complement receptor 1, MS4A, phosphatidylinositol binding clathrin assemble protein, RAB10, and bridging integrator 1 (Table 1.1). Previous studies suggest that the common and late onsets of AD are associated with a defect in peripheral Aβ peptide clearance, implying that the amyloid cascade hypothesis could be relevant not only in AD monogenic forms (Lambert & Amouyel, 2011). However, the complete picture of AD genetics is still not fully understood. In addition, several genes/loci that contribute to the genetic basis of AD collectively account for only a small fraction of the observed heritability of this trait.

Among AD-associated genes suggested by previous studies, we are interested in the neuron navigator 2 (NAV2) gene that encodes a member of the neuron navigator gene family and plays an important role in cellular growth and migration. In terms of NAV2's variants, function, biological pathways, and associated phenotypes, more animal than human studies have been performed.

Previous studies suggest that this gene may be involved in nervous system development (Coy et al., 2002) and associated with episodic memory scores in AD (Yan et al., 2015). Wang, Liu, Xu, Liu, & Luo (2017) reported that polymorphisms in NAV2 were associated with risk and age at onset (AAO) of AD using a family-based association study (Wang, Liu, Xu, Liu, & Luo, 2017). Previous studies also revealed that NAV2 may play a role in shaping the development of the mammalian nervous system, blood pressure regulation (McNeill, Roos, Moechars, & Clagett-Dame, 2010), development of atherosclerosis (Dong et al., 2012), and colorectal cancer (Tan et al., 2015). These findings indicate that NAV2 may be one of the mechanisms linking blood pressure, cancer, cardiovascular disease, and neurodegenerative diseases. One recent epidemiology study showed a possible inverse association between cancer and dementia in a Chinese sample (Lin, Lin, Tseng, Chen, & Hsu, 2016). Moreover, in an animal model study comparing wild-type littermates, homozygous mutant mice showed a progressive reduction in body weight with hypoactive and reduced exploratory behavior; however, those mice had no motor defects. Those mice also had impaired olfactory, auditory, and visual acuity as well as visual defects that were associated with hypoplasia of the optic nerve (Peeters et al., 2004). Some of phenotypes may be similar to AD clinical phenotypes.

We conducted a systematic review and focused on exploring present findings that assess NAV2. We first searched for articles through PubMed using "NAV2" as the key word. This resulted in 67 related articles. Next, we filtered the results by focusing on

Table 1.1 Additional evidence for top 10 genes associated with Alzheimer's disease and cognitive function.

Gene (location)	Samples size	Study method	Main findings	Potential pathophysiology	Authors, year
APOE (19q13.32)	57,979 of non-Hispanic Caucasians from 27 independent research studies	A meta-analysis using data from 12 institutions to identify how sex and APOE genotype affects the risk of developing AD	Men and women with the APOE e3/e4 genotype have similar odds of developing AD from ages of 55 −85. Women have increased risk at younger age	The APOE e4 allele represents a gain of toxic function, a loss of neuroprotective function, or both	Neu et al. (2017)
BIN1 (2q14.3)	11,832 late-onset of AD and 18,133 controls from East Asian, American, and European populations	A meta-analysis based on 22 independent studies	The first large meta-analysis shows that BIN1 SNP rs744373 is associated with susceptibility to AD under the additive model	BIN1 may modulate tau pathology and regulate endocytosis, immunity, and inflammation of the brain	Zhu, Liu, & He, (2017b)
CLU (8p21.1)	19,829 AD and 30,900 controls from 26 studies of Asians	A meta-analysis of the CLU gene associated with AD in Asian populations	rs11136000 C allele is associated with AD susceptibility	Animal studies from 10 years ago linking CLU/APOJ to amyloid deposition	Zhu, Liu, & He, (2018b)
ABCA7 (19p13.3)	4,915 brain autopsies	Discovery study: a GWAS study and an analysis of known genetic risk loci for AD	Discovered new genes with specific pathologic changes in a large brain autopsy study of AD and related dementias	1. ABCA7 deficiency exacerbates Aβ pathology; 2. it mediates phagocytic activity in macrophages; 3. it is involved in microglial Aβ clearance pathway	Beecham et al. (2014)

Continued

Table 1.1 Additional evidence for top 10 genes associated with Alzheimer's disease and cognitive function.—cont'd

Gene (location)	Samples size	Study method	Main findings	Potential pathophysiology	Authors, year
CR1 (1q32.2)	rs6656401 (2752 AD and 2313 controls) rs3818361 (2547 AD and 2338 control)	A meta-analysis	An association of both CR1 rs6656401 and CR1 rs3818361 polymorphism with LOAD susceptibility	Impacts of CR1 include amyloid-β pathology, tauopathy, immune dysfunction and glial-mediated neuroinflammation	Luo et al. (2014)
PICALM (11q14.2)	6,972 AD patients and 10,199 controls	A meta-analysis based on 16 case-control studies that evaluated the role of rs3851179 gene variants in AD patients	A meta-analysis suggests that PICALM-rs3851179 is associated with AD among Asians and Caucasians	Gene variants may impact on modulating production, transportation, and clearance of β-amyloid (Aβ) peptide	Zhu, Li, Zhang et al. (2018a)
CENPO (2p23.3)	35,298 healthy individuals of European ancestry	GWAS meta-analysis across 24 cohorts and polygenic score analyses	Novel SNP in the CENPO gene associated with cognitive performance ($P<5 \times 10^{-8}$)	Highly expressed in the basal ganglia and thalamus of the human brain	Trampush et al. (2017)
MS4A (11q12.2)	34,119 AD and 56,956 controls	19 studies were included in this GWAS meta-analysis by searching the PubMed, MEDLINE, and AlzGene databases	An association of rs610932 in Asian populations under additive [odds ratio = 0.82, $P = .03$] and dominant models (odds ratio = 0.82, $P = .006$)	MS4A may impact immune-system dysfunction, which is present as a pathogenetic force in the process of AD	Zhu, Liu, & He, (2017a)

RAB10 (2p23.3)	200 AD-resilient individuals, defined as >75 year individuals, cognitively normal, and carry at least one APOE e4 allele	1. linkage analyses; 2. whole genome sequences in linkage regions; 3. replicated SNPs from linkage peaks in an independent dataset; 4. experimentally characterized replicated SNPs	1. rs142787485 in RAB10 confers protection against AD and confirmed in an independent cohort; 2. knockdown of RAB10 resulted in decrease in Aβ42; 3. RAB10 expression is elevated in human AD brains	RAB10 may be responsible for aberrations in the vesicle trafficking observed in AD leading to neurodegeneration	Ridge et al. (2017)
NAV2 (11p15.1)	1266 AD and 1279 controls (initial study) and two replications in 791 AD and 863 AD	Candidate gene studies (initial and replication studies)	An association of NAV2 SNPs with AD followed by confirmation based on two independent cohorts	NAV2 may be responsible for aberrations in cellular growth, migration, and nervous system development	Wang et al. (2017) and unpublished data (Tables 1.1 −1.5)

AD, Alzheimer's disease; *APOE*, apolipoprotein; *BIN1*, bridging integrator 1; *CLU*, clusterin: *CR1*, complement receptor 1; *GWAS*, genome-wide association study; *PICALM*, phosphatidylinositol binding clathrin assemble protein; *SNP*, single-nucleotide polymorphism.

English language articles and human subjects. This narrowed down the number of research articles to 27. There was further inquiry in PubMed through several combinations of searches relating to NAV2, AD, age-related disorders, or neurodegenerative disorders, which resulted in three studies remaining. We decided to review all 27 studies on NAV2 with the filters of human research and in the English language.

Because there is limited study of NAV2 in human age-related phenotypes, in this review we also have added our recent confirmation study of the genetic association of NAV2 with AD (unpublished).

Research findings of neuron navigator 2 based on animal studies

Previous reports of animals and cell models demonstrated that human NAV2 has a homolog of the *Caenorhabditis elegans* adapter protein (UNC-53) gene (Merrill, Plum, Kaiser, & Clagett-Dame, 2002; Muley et al., 2008). UNC-53 plays an important role in longitudinal migration of a number of cell types, such as neurons (Stringham, Pujol, Vandekerckhove, & Bogaert, 2002). In 2002, Merrill et al. conducted subtractive complementary DNA (cDNA) library screening for an all-trans retinoic acid (atRA)-treated neuroblastoma cell line and identified NAV2 (Merrill et al., 2002). The UNC-53 mutations in *C. elegans* result in both behavioral and anatomical abnormalities. Those phenotypes were also observed in patients with AD. The results of immunocytochemistry and electron microscopy suggested neuroanatomical defects in the most longitudinal nervous tracts, and UNC-53 is also required for normal mechanosensory neuron elongation (Hekimi & Kershaw, 1993). In this study, using the NAV2/U−53H2 hypomorphic mutant mouse, the authors demonstrated that mammalian NAV2 plays an important role in the attainment of normal cerebellar size and migration. The authors also observed that NAV2 facilitates cytoskeletal rearrangement and neurite outgrowth and concluded that NAV2 plays a role in the development and proper formation of the cerebellum. Based on a statistically oriented asymmetric localization model, another study reports that uncoordinated protein (UNC)-5 acts via the UNC-53 (NAV2) cytoplasmic protein to regulate UNC-40 asymmetric localization in response to UNC-6 and EGL-20 extracellular cues (Limerick et al., 2018). UNC-53/NAV2 is considered an adaptor protein and can make the protein to bind to actin and complex with other proteins to regulate migrations of different cell types along the AP axes of *C. elegans* (Pandey, 2014; Stringham et al., 2002). A recent *C. elegans* study identified UNC -53/NAV2 as a novel component of signaling pathways that may regulate distal tip cell (DTC) migrations along the AP and dorsoventral axes. UNC-53/NAV2 negatively regulates and functions downstream of ced-10/Rac pathway genes ced-10/Rac and Mig-2/RhoG, which are required for proper DTC migration (Pandey, Yadav, Sharma, Khurana, & Pandey, 2018). A study also suggests that an inducible knockdown NAV2 in SH-SY5Y cells can eliminate atRA-stimulated neurite outgrowth (neuronal process) (Muley et al., 2008).

To understand the involvement of epigenetic changes in somatic stem cell aging, recent findings using mouse hematopoietic stem cells showed a global loss of DNA methylation of ~5% with age; however, in certain CpG islands, DNA methylation increased. These age-specific differentially methylated regions include NAV2 variants located at intronic regions and containing multiple regulatory regions, and they may be a potential enhancer region. One age-related hypomethylated NAV2 variant is located at the exonic region associated with H3K4me1. The authors compared those differentially methylated regions with the recent data (Beerman et al., 2013) and found that NAV2 is hypermethylated with age in both reports. Moreover, in one of the review papers, the authors provided unpublished data indicating that expression of NAV2 in *C. elegans* leads to near complete rescue of the truncated posterior lateral microtubule neurons, which again suggests that human NAV2 is an ortholog of *C. elegans* UNC-53. The author concluded that NAV2 may play an important role in retinoid-mediated neurite outgrowth in mammals (Clagett-Dame, McNeill, & Muley, 2006).

In summary, the findings based on genetics, genomics, and epigenetic studies in animal and cell models suggest that NAV2 might be involved in AD pathophysiology; however, more studies are needed to confirm the findings.

Research findings of neuron navigator 2 based on human studies

Increasing numbers of AD-associated variants have been suggested; over 155 GWASs on many different AD-related traits are reported based on date in the National Human Genome Research Institute European Bioinformatics Institute catalog. Among these suggested genes, we are interested in NAV2, which is a member of the neuron navigator family that also includes NAV1 and NAV3 (Maes, Barcelo, & Buesa, 2002). Two major transcripts were expressed at variable levels in all adult brain subregions examined (http://omim.org/entry/607026). In addition, a family of neuron navigator proteins was found to comprise NAV1, NAV2, and NAV3 (Maes et al., 2002). The NAV2 open reading frame encodes for a 261 kDa protein with a number of conserved domains (Muley et al., 2008). Multiple transcript variants encoding different isoforms have been found for this gene. The NAV2 gene is located at chromosome 11p15.1 (Maes et al., 2002), is highly expressed in the brain, and contains 738 amino acids (Nagase, Kikuno, Ishikawa, Hirosawa, & Ohara, 2000). In 2002, Coy et al. cloned partial cDNA encoding NAV2 that is a novel differentially spliced gene predominantly expressed in the nervous system (Coy et al., 2002).

In addition to structural and functional studies, NAV2 is associated with a number of human traits, uterine sarcoma (Davidson et al., 2017), prostate cancer (Sun, Jia, Hou, & Liu, 2016), and colorectal cancer (Cancer Genome Atlas, 2012; Tan et al., 2015). In recent findings of methylphenidate (MPH)-regulated gene expression in lymphoblastoid cells from patients with attention deficit/hyperactivity disorder (ADHD), the authors

concluded that MPH treatment affects specific NAV2 expression in patients with ADHD (Schwarz et al., 2015), which may suggest that ADHD and AD share a similar genetic basis as seen for the cholinergic receptor nicotinic alpha 7 gene (Sinkus et al., 2015). A study also showed shared configural and affective face processing abnormalities between ADHD and AD (Feuerriegel, Churches, Hofmann, & Keage, 2015).

Neuron navigator 2 is associated with Alzheimer's disease as well as age-related and neurodevelopmental-related phenotypes

The various associations of NAV2 with AD and age-related and neurodevelopmental-related phenotypes are summarized in Table 1.2.

Neuron navigator 2 variants are associated with carotid plaque, which is a pathophysiologic change for Alzheimer's disease

A growing body of evidence suggests associations of atherosclerotic and carotid plaques with AD and multiple other neurodegenerative diseases (Bennett, Grant, & Aldred, 2009; de la Torre, 2008; Luoma, 2011; Weller, Massey, Kuo, & Roher, 2000). A study demonstrates that NAV2 variants (rs2702663, intronic; rs1442710, coding synonymous) are associated with carotid plaques in Dominican families followed by a replication study in an independent subcohort, a more generalized population (Dong et al., 2012).

Knockdown neuron navigator 2 gene results in a reduction in all-trans retinoic acid–mediated neurite outgrowth, which is a pathophysiologic change for Alzheimer's disease

To understand NAV2 protein interaction partners, a recent study showed that knockdown NAV2 in human neuroblastoma cells results in a reduction in atRA-mediated neurite outgrowth (Marzinke, Mavencamp, Duratinsky, & Clagett-Dame, 2013). Promotion of neurite outgrowth might be one type of treatment for patients with AD because several clinical trials have proposed improved neurite outgrowth via targeting of multiple key pathways of the AD pathogenesis to halt disease progression. A wealth of evidence suggests that neurite dystrophy and significant loss of synaptic connectivity of neurons in the brains from AD or neurodegenerative disorders result in cognitive decline (Chong, Ai, & Lee, 2017; Liu, Li, Holscher, & Li, 2015).

Differentially expressed neuron navigator 2 was identified in the hippocampal brain region from patients with Alzheimer's disease and from normal aging-related groups

To investigate hippocampal transcriptional changes in patients with AD and normal aging-related groups, a recent systematic review and meta-analysis identified 1291 differentially expressed genes (DEGs) shared among natural aging groups and AD patients using the Gene Expression Omnibus database of one healthy aging-related and three

AD-related data sets from the hippocampal region. The authors investigated DEGs followed by further analyzing gene ontology terms, pathways, and functions of these genomic regions, which showed DEGs. In addition to their top finding of the aging-related neurexin 3 (NRXN3) gene, the NAV2 gene was listed as one of the top 50 DEGs and associated with normal aging and AD (Zheng et al., 2018) (Table 1.2). Their results suggest that the low expression of aging-related NRXN3 and NAV2 may increase AD risk via an unidentified mechanism, which requires further clarification.

Neuron navigator 2 de novo mutations have been discovered in autism spectrum disorders

Importantly, de novo single-nucleotide variants (missense and silent mutations) in the NAV2 gene were observed in two unrelated probands with autism spectrum disorders (ASDs) in a recent study using whole-exome sequencing of 928 individuals, including 200 phenotypically discordant sibling pairs, published in *Nature* in 2012 (Sanders et al., 2012). In this study, many recurrent, rare de novo mutations were identified, including NAV2 mutations with sufficient statistical power despite a high degree of locus heterogeneity and the contribution of intermediate genetic risks. These results again support previous findings of sharing clinical phenotypes (such as motor abnormalities) (Peralta & Cuesta, 2017), genetic basis (Caputo, Geier, Ouyang, Kreitner, & Stephan, 2012 and our recent findings), autophagy (Sragovich, Merenlender-Wagner, & Gozes, 2017), and molecular hybrids (Matias, Silvestre, Falcao, & Alves, 2017) between ASD and AD as well as other neuropsychiatric disorders and neurodevelopmental disorders.

Neuron navigator 2 genetic variants showed strong associations with Alzheimer's disease in a family study followed by a confirmation study in two independent cohorts

Based on the aforementioned cellular, functional, and indirect genetic findings of NAV2 in association with nervous system development, episodic memory scores, and AD in both animal and human studies, we conducted a genetic association study of NAV2 using a family design (Wang et al., 2017) in human subjects. There was no previous report on the association of the NAV2 gene with the risk or AAO of AD at the time or our study. We hypothesized that NAV2 gene polymorphisms might play a role in AD. Based on the genotype data from the National Institute on Aging - Late Onset Alzheimer's Disease Family Study: Genome-Wide Association Study for Susceptibility Loci (Study Accession: phs000168.v1.p1), we conducted a genetic association analysis of 317 NAV2 single-nucleotide polymorphisms (SNPs) with the risk and AAO of AD with a family-based sample using the FBAT-Wilcoxon statistic. Single SNP analysis showed that 20 SNPs were significantly associated with the risk of AD (the top SNP was rs7112354 with $P = 8.46 \times 10^{-4}$), and 11 SNPs were associated with AAO (e.g., SNP rs1354269 with $P = 2.87 \times 10^{-3}$). More important, SNPs rs17614100 and rs12364788 were

associated with both AD risk ($P = 1.7 \times 10^{-2}$ and 2.71×10^{-2}) and AAO ($P = 1.85 \times 10^{-3}$ and 6.06×10^{-3}). Haplotype analyses further supported the results of single SNP analysis. Moreover, NAV2 gene expression was detected across 10 human brain regions and was significantly correlated with APOE expression in 4 of 10 brain regions. This is the first study providing evidence of a number of NAV2 variants influencing the risk and AAO of AD (Wang et al., 2017).

Next, we believed a confirmation study was needed for our findings. Therefore, we conducted a case–control study by focusing on the NAV2 gene in two independent cohorts (Tables 1.3–1.6. unpublished data).

A total of 791 patients with AD and 782 controls with genotype and phenotype information in a Canadian sample (Table 1.3) were selected from the Multi-Site Collaborative Study for Genotype-Phenotype Associations in Alzheimer's disease and Neuroimaging component of Genotype-Phenotype Associations in Alzheimer's disease (Study Accession: phs000219.v1.p1). The details of these subjects have been described elsewhere (Filippini et al., 2009; Li et al., 2008). Genotyping was conducted using the Affymetrix technique. There were 230 SNPs within the NAV2 gene.

A total of 863 patients with sporadic AD and 1435 unrelated controls were selected from the Columbia University Study of Caribbean Hispanics with Familial and Sporadic Late Onset Alzheimer's disease (dbGaP Study Accession: phs000496.v1.p1). The details of these subjects have been described elsewhere (Cheng et al., 2011; Reitz et al., 2010). Genotyping was conducted using the Illumina technique. There were 438 SNPs within the NAV2 gene.

In the two cohorts we examined, a total of 19 SNPs were found to be associated with AD (the top two SNPs were rs2568127 and rs2729863 with P values of 4.59×10^{-4} and 5.31×10^{-4}, respectively) in the Caucasian sample, and 44 SNPs were associated with AD (the top two SNPs were rs2568127 and rs3802799 with P values of 6.61×10^{-5} and 1.63×10^{-4}; the top two SNPs were associated not only with AD but also with AAO, with P values of 4.30×10^{-2} and 1.36×10^{-2}) in the Hispanic cohort. NAV2 haplotypes from two cohorts were also significantly associated with increased risk for AD, which further supports the findings from single SNP analysis.

Next, we checked to see whether any AD-associated SNPs were shared among the three cohorts (family data and Caucasian and Hispanic samples), although two different platforms were used for these cohorts—the Illumina technique for family data and Hispanic samples and the Affymetrix technique for the Caucasian sample. Only four SNPs were shared among the three cohorts, again suggesting genetic and phenotype heterogeneity of AD (Table 1.6).

To examine whether those AD-associated SNPs were located at regulatory- and species-conserved regions, we used NIH SNP Function Prediction (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). We found 12 AD-associated SNPs (Table 1.5) located at the species-conserved and gene-regulatory regions, respectively, which suggests the regions containing biological function (Hardison, 2000). Having established

Table 1.2 Studies of the neuron navigator 2 gene in association with Alzheimer's disease as well as age-related and neurodevelopmental disorders.

Studies	Method	Main findings	Author, year
A systematic review and meta-analysis of low expression of aging-related genes	GEO database of one healthy aging-related and three AD-related datasets of the hippocampal region	Differentially expressed genes of NRXN3, NAV2, and other genes were associated with AD and aging-related groups	Zheng et al. (2018)
Family-based association analysis of NAV2 gene with the risk and age at onset of Alzheimer's disease (AD)	Genotyped 317 single-nucleotide polymorphisms (SNPs) in a family-based sample (1266 AD cases and 1279 unaffected family members)	A number of variants in the NAV2 gene showed association with AD and age at onset of AD	Wang et al. (2017)
Study of linkage regions reveals multiple candidate genes for carotid plaque in dominicans	Genotyped 3712 SNPs under the four linkage regions	An association ($P<.0005$) between two SNPs in the NAV2 gene and carotid plaque, the findings were confirmed in a case control study (384 dominicans)	Dong et al. (2012)
A confirmation study of NAV2 associated with Alzheimer's disease	Examine 230 SNP in the *NAV2* with AD in a Caucasian sample and 438 SNPs with AD in a Hispanic sample.	In the Caucasian sample, a total of 19 SNPs were associated with AD. In the Hispanic sample, 44 SNPs were associated with AD. In addition, two SNPs were associated with age at onset	Wang and xu, unpublished data

Continued

Table 1.2 Studies of the neuron navigator 2 gene in association with Alzheimer's disease as well as age-related and neurodevelopmental disorders.—cont'd

Studies	Method	Main findings	Author, year
De novo mutations revealed by whole-exome sequencing are strongly associated with autism	Whole exome sequencing of 928 individuals, including 200 phenotypically discordant sibling pairs with autism	Many recurrent rare de novo mutations were identified, including NAV2 mutations (missense and silent mutations) with sufficient statistical power	Sanders et al. (2012)

AD, Alzheimer's disease; *de novo*, new mutation, and new variant; *GEO database*, gene expression omnibus database.

Table 1.3 Descriptive characteristics of Alzheimer's disease and controls in our confirmation study.

Variable	Caucasian sample		Hispanic sample	
	AD	Controls	AD	Controls
Sample size (n)	791	782	863	1435
Mean AAO (years ± SD[a])	72.3 ± 8.5	—	75.3 ± 9.2	—
Range of age at onset (years)	40—97	—	44—100	—
Mean age at entry (years ± SD)	77.6 ± 8.6	73.4 ± 7.9	78.9 ± 8.7	70.1 ± 8.5
Range of age at entry (years)	43—100	48—94	48—100	35—100

AAO, age at onset; *AD*, Alzheimer's disease.
[a]*SD* refers to the standard deviation of the mean.

strong associations of AD-associated SNPs with AAO and the risk of AD, we next tested whether the genotypes of these SNPs are associated with levels of gene expression in brain tissue or the central nervous system based on data from Genotype-Tissue Expression (https://gtexportal.org/home/). We hypothesized that the effects of SNP genotypes on these AD risks may reflect genotype-based differences in the levels of gene expression in postmortem samples from 100 to 120 normal individuals from the genotype-tissue expression data set. Among the AD-associated SNPs we tested, none showed an association with allelic expression. Our NAV2 genetic findings of the three cohorts again highlight the importance of common genetic variation as a risk factor for one brain disorder, AD.

Our results indicated NAV2 variants might be a marker for AD risk, though replication in large samples is required, and the exact functional roles of NAV2 in AD pathophysiology need to be explored.

Future outlook and conclusions

Based on the findings at cellular and molecular levels in both animal and human studies, we hypothesized that AD-associated variants in the NAV2 gene facilitate neurite

Table 1.4 Single marker analysis of risk of Alzheimer's disease in the Caucasian cohort in our confirmation study.

SNP	Position[a]	MA[b]	MAF[c]	HWE[d]	OR-AD[e]	p-AD[f]	β-AAO[g]	p-AAO[h]
rs2568127	19,947,274	A	0.21	0.703	0.71(0.58, 0.86)	4.59E-04	0.26(−0.20, 0.72)	0.272
rs2729863	19,373,878	G	0.09	0.144	1.54(1.21, 1.96)	5.31E-04	−0.43(−0.93, 0.07)	0.0919
rs2252099	19,950,510	G	0.17	0.095	0.74(0.60, 0.92)	5.77E-03	0.30(−0.20, 0.80)	0.241
rs1822276	19,953,874	A	0.23	0.501	0.78(0.65, 0.93)	7.12E-03	−0.05(−0.47, 0.37)	0.823
rs2585784	19,833,505	A	0.16	0.281	1.29(1.07, 1.56)	8.41E-03	0.36(−0.05, 0.77)	0.0846
rs10833240	20,062,960	G	0.23	0.094	0.80(0.67, 0.95)	0.0121	0.24(−0.18, 0.65)	0.259
rs10766556	19,402,051	G	0.31	0.045	1.21(1.03, 1.42)	0.0173	−0.17(−0.52, 0.17)	0.325
rs2625301*	19,861,136	G	0.19	0.353	1.24(1.04, 1.48)	0.0185	0.37(−0.01, 0.75)	0.0587
rs4757808	19,396,372	T	0.32	0.040	1.21(1.03, 1.42)	0.0189	−0.16(−0.50, 0.19)	0.378
rs10833102	19,374,810	A	0.36	0.669	0.83(0.72, 0.97)	0.0199	0.02(−0.33, 0.37)	0.913
rs2255677*	20,071,925	A	0.44	0.912	0.85(0.73, 0.98)	0.0259	0.11(−0.22, 0.43)	0.524
rs4757809	19,416,660	C	0.20	0.120	1.23(1.03, 1.48)	0.0259	−0.01(−0.41, 0.39)	0.972
rs10766557	19,404,123	C	0.32	0.035	1.19(1.02, 1.40)	0.0295	−0.17(−0.52, 0.17)	0.325
rs2585753*	19,852,332	G	0.20	0.411	1.21(1.02, 1.44)	0.0338	0.36(−0.02, 0.74)	0.0619
rs10766570	19,535,631	G	0.17	0.015	1.22(1.01, 1.46)	0.0353	−0.10(−0.50, 0.30)	0.611
rs2255674	20,071,853	C	0.40	0.621	0.86(0.74, 0.99)	0.0409	0.01(−0.33, 0.34)	0.994
rs2625312	19,860,333	A	0.20	0.235	1.20(1.01, 1.43)	0.0434	0.41(0.03, 0.79)	0.0339
rs2625332	19,962,912	T	0.12	0.856	0.79(0.63, 0.99)	0.0438	0.01(−0.53, 0.56)	0.960
rs10741813	20,042,952	T	0.20	0.545	1.20(1.01, 1.44)	0.0451	−0.24(−0.64, 0.16)	0.238

* *SNP* with * indicates the SNP locates at the potential regulatory region. *AD*, Alzheimer's disease.
[a]Physical position (bp).
[b]Minor allele.
[c]Minor allele frequency.
[d]*P*-value for Hardy-Weinberg equilibrium test.
[e]Odds ratio for AD.
[f]*P*-value for AD.
[g]Regression coefficient for AAO.
[h]P-value for AAO.

Table 1.5 Single marker analysis of risk of Alzheimer's disease in the Hispanic cohort.

SNP	Position[a]	Function	MA[b]	MAF[c]	HWE[d]	OR-AD[e]	p-AD[f]	β-AAO[g]	p-AAO[h]
rs4757857	19,922,410		A	0.36	0.016	0.77(0.68, 0.90)	6.61E-05	−0.39(−0.78, −0.01)	0.043
rs3802799	20,065,673	nsSNP/regulatory/ conserve	A	0.16	0.124	0.71(0.59, 0.80)	1.63E-04	−0.69(−1.23, −0.14)	0.0136
rs7935182	19,916,098		A	0.34	0.269	1.26(1.11, 1.40)	3.51E-04	0.42(0.05, 0.78)	0.025
rs2028608	19,928,344		G	0.47	0.348	1.24(1.10, 1.40)	4.55E-04	0.55(0.20, 0.90)	2.10E-03
rs11025134	19,450,627		A	0.25	0.057	0.79(0.68, 0.90)	1.32E-03	−0.29(−0.72, 0.14)	0.181
rs2028609	19,928,395		G	0.39	1.00	0.83(0.73, 0.90)	3.21E-03	−0.39(−0.76, −0.03)	0.0341
rs11025365	20,078,496	Regulatory	A	0.31	0.029	0.82(0.71, 0.90)	3.29E-03	−0.39(−0.81, 0.02)	0.0601
rs10766603	19,933,123		A	0.28	0.115	0.82(0.71, 0.90)	3.88E-03	−0.49(−0.89, −0.10)	0.0148
rs11828964	19,580,698		A	0.13	0.828	1.27(1.07, 1.50)	5.68E-03	0.15(−0.34, 0.63)	0.555
rs11025130	19,440,246		G	0.15	1.00	0.78(0.65, 0.90)	7.33E-03	−0.45(−0.98, 0.08)	0.0973
rs16936975	19,579,262		A	0.14	0.295	1.26(1.06, 1.50)	7.35E-03	0.27(−0.20, 0.75)	0.259
rs2654009	19,377,224	Regulatory	A	0.05	0.181	0.64(0.46, 0.90)	7.38E-03	0.40(−0.63, 1.43)	0.444
rs7113364	19,580,018		C	0.14	0.251	1.26(1.06, 1.50)	7.42E-03	0.29(−0.19, 0.77)	0.233
rs11828892	19,580,327		A	0.13	0.341	1.26(1.06, 1.50)	7.50E-03	0.13(−0.17, 0.79)	0.204
rs11025356	20,037,120		A	0.08	0.869	1.33(1.08, 1.60)	8.28E-03	0.05(−0.55, 0.65)	0.870
rs16937045	19,623,652		G	0.09	0.876	1.30(1.06, 1.60)	0.0109	0.35(−0.21, 0.91)	0.223
rs4757880	20,049,859	Conserve	A	0.47	0.656	1.17(1.04, 1.30)	0.0110	0.21(−0.14, 0.56)	0.247
rs1978947	20,086,764		C	0.26	0.157	0.83(0.72, 1.00)	0.0112	−0.22(−0.63, 0.20)	0.302
rs10734289	20,098,670		C	0.33	0.655	0.84(0.74, 1.00)	0.0115	−0.02(−0.41, 0.37)	0.911
rs1559664	19,749,929		A	0.13	0.001	1.24(1.05, 1.50)	0.0118	0.11(−0.37, 0.59)	0.658
rs12785600	20,087,212		G	0.44	0.726	0.85(0.76, 1.00)	0.0126	0.21(−0.15, 0.57)	0.255
rs2707106	19,923,487		G	0.34	0.581	1.18(1.03, 1.30)	0.0135	−0.10(−0.48, 0.28)	0.607
rs2584840	20,023,619		C	0.15	0.030	0.80(0.67, 1.00)	0.0135	0.11(−0.42, 0.63)	0.684
rs1364792	19,748,992		A	0.27	0.001	1.18(1.03, 1.30)	0.0153	0.10(−0.27, 0.48)	0.591
rs11025224	19,748,584	Regulatory	G	0.28	0.001	1.17(1.03, 1.30)	0.0161	0.13(−0.24, 0.51)	0.488
rs11025105	19,373,169		A	0.08	0.075	0.75(0.59, 1.00)	0.0192	−0.31(−1.02, 0.40)	0.387
rs2702671	19,423,358		A	0.35	0.357	1.16(1.02, 1.30)	0.0207	0.03(−0.34, 0.40)	0.878
rs2006636	20,050,205		G	0.18	0.081	1.19(1.03, 1.40)	0.0212	0.06(−0.35, 0.48)	0.776

rs11025359	20,054,591		A	0.15	0.845	1.21(1.03, 1.40)	0.0224	0.17(−0.28, 0.63)	0.456
rs12276043	20,055,663		C	0.48	0.844	1.15(1.02, 1.30)	0.0234	0.25(−0.11, 0.60)	0.174
rs10766606	19,960,636	Regulatory/ conserve	A	0.33	0.403	0.86(0.76, 1.00)	0.0251	−0.29(−0.67, 0.08)	0.127
rs920671	20,050,052		A	0.24	0.116	1.17(1.02, 1.30)	0.0265	0.10(−0.28, 0.49)	0.602
rs12286036	19,740,656	TFBS	A	0.09	0.137	1.25(1.02, 1.50)	0.0296	0.40(−0.17, 0.96)	0.170
rs11604874	19,995,837		A	0.16	0.312	1.20(1.02, 1.40)	0.0304	−0.05(−0.50, 0.39)	0.819
rs11025120	19,425,234		A	0.05	0.803	0.72(0.53, 1.00)	0.0315	−0.21(−1.15, 0.72)	0.654
rs2625295	19,998,919		A	0.20	0.487	1.18(1.01, 1.40)	0.0326	0.04(−0.39, 0.47)	0.866
rs10833229	20,063,760	Conserve	A	0.44	0.484	1.14(1.01, 1.30)	0.0368	0.52(0.15, 0.88)	6.05E-03
rs10833211	19,996,984		G	0.48	0.001	1.13(1.01, 1.30)	0.0371	0.20(−0.14, 0.55)	0.249
rs12282066	19,738,228	TFBS	A	0.08	0.229	1.26(1.01, 1.60)	0.0389	0.23(−0.39, 0.86)	0.465
rs10833106	19,422,628	Conserve	G	0.17	0.223	1.18(1.01, 1.40)	0.0394	0.01(−0.47, 0.47)	0.998
rs744853	19,669,526		A	0.17	0.725	1.18(1.01, 1.40)	0.0414	0.14(−0.31, 0.58)	0.554
rs7125647	20,067,064	Regulatory/ conserve	G	0.41	0.335	1.13(1.00, 1.30)	0.0458	0.19(−0.16, 0.54)	0.277
rs11025239	19,774,304		G	0.10	0.491	1.22(1.00, 1.50)	0.0477	−0.07(−0.63, 0.50)	0.816
rs7101488	20,048,928	Regulatory/ conserve	A	0.10	0.267	1.22(1.00, 1.50)	0.0496	0.23(−0.33, 0.80)	0.423

* *SNP* with * indicates the SNP locates at the potential regulatory region. *AD*, Alzheimer's disease; *nsSNP*, nonsynonymous single-nucleotide polymorphism (nsSNP) in a gene affects its protein product; TFBS for transcription factor binding site.
[a]Physical position (bp).
[b]Minor allele.
[c]Minor allele frequency.
[d]*P*-value for Hardy-Weinberg equilibrium test.
[e]Odds ratio for AD.
[f]*P*-value for AD.
[g]Regression coefficient for AAO.
[h]*P*-value for AAO.

Table 1.6 Common Alzheimer's disease–associated single-nucleotide polymorphisms shared among a family study, a Caucasian cohort, and a Hispanic cohort.

SNP	Position[a]	MA[b]	MAF[c]	*P* values		
				A family study (Wang et al. 2017)	AD from the Hispanic samples (current unpublished data)	AAO from the Hispanic samples (current unpublished data)
rs2028608	19,928,344	G	0.47	1.05E-02	4.55E-04	2.10E-03
rs2625295	19,922,410	A	0.36	1.48E-02	0.0326	0.866
rs10833106	19,422,628	G	0.17	3.78E-02	0.0394	NA
rs11025365	20,078,496	A	0.17	4.98E-02	3.29E-03	0.0601

AD, Alzheimer's disease; *AAO*, age at onset.
[a]Physical position (bp).
[b]Minor allele.
[c]Minor allele frequency.

outgrowth, which could be a potential mechanism involved in AD pathogenesis. However, future testing of this hypothesis is needed, such as knockdown of the AD-associated variants in an AD-like animal model to better understand the pathophysiology of AD. Moreover, fine mapping of the NAV2 gene with denser markers (e.g., SNPs) in a large sample and using a high-throughput genotyping technique is needed to confirm our findings of AD-associated variants. In addition, gene sequencing for NAV2 should be targeted in patients with AD because this method has proven useful in rapidly replicating findings based on the signals of GWASs and/or whole-genome sequencing; it has also proven to be a reliable confirmation for clinical suspicion (Nicastro & D'Antiga, 2018). Further functional, genomic, and epigenomic studies of these AD-associated variants in the NAV2 gene, such as AD postmortem brain samples, would help us better understand the pathogenesis of AD. Furthermore, complex diseases such as AD result from the interplay of many genetic and environmental factors (age, lifestyle), gene–environment interactions (e.g., NAV2 and lifestyle choice), and epigenomic modifications (Fig. 1.1). To create a comprehensive catalog of common and rare variants in individuals with AD, it is useful to combine the results of GWASs, gene–gene and gene–environment interactions, gene expression, and epigenetics given the recent rapid technological advancements in NGS.

In this new genomic era, a number of major AD research projects based on large-scale NGS, GWAS, and omics (e.g., genomics, epigenomics, transcriptomics, proteomics and metabolomics) studies are ongoing, and new novel findings or discoveries could be expected soon (Sancesario & Bernardini, 2018).

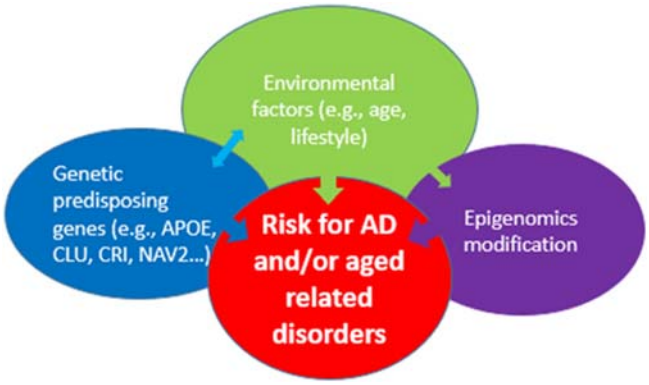

Figure 1.1 Interactions of the environment, genetics, and epigenetic modification in Alzheimer's disease. *AD*, Alzheimer's disease.

Key facts of neuron navigator 2 gene and Alzheimer's disease

- AD is a polygenetic or multifactorial disease with genetic and nongenetic factors (e.g., lifestyle, epigenomic modification) and their interactions.
- ADHD is a brain disorder marked by an ongoing pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development.
- Many genes and/or genetic variants have been suggested, but these disease-associated variants account for a small proportion of disease risk.
- NAV2 encodes a member of the neuron navigator and plays a role in cellular growth and migration. Future NAV2 genetic markers might be useful for early diagnosis, prognosis, or new drug intervention for AD.
- GWAS is an observational study of a genome-wide set of genetic variants in different individuals to see whether any variant is associated with a trait or disease.
- A SNP is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population (e.g., $> 1\%$). This type of variation may be associated with increased or decreased risk for certain diseases.
- Nonsynonymous SNPs are the most common DNA sequence variations associated with diseases in humans, and thus determining the clinical significance of each nonsynonymous SNP is of great importance.
- Genotype-Tissue Expression is an established data resource and tissue bank for studying the relationships between genetic variation and gene expression in multiple human tissues. Genotype-Tissue Expression resources are valuable tools for exploring the genetic basis of complex human diseases.

Summary points

- This chapter focuses on the NAV2 gene in association with AD.
- NAV2 encodes a member of the neuron navigator gene family and plays a role in cellular growth and migration.
- Human NAV2 is a homolog of the *C. elegans* UNC-53 gene.
- UNC-53 mutations in *C. elegans* result in both behavioral and anatomical abnormalities. Those phenotypes were also observed in patients with AD.
- NAV2 is involved in nervous system development and associated with episodic memory scores in Alzheimer's disease.
- In humans, we and others have demonstrated that NAV2 genetic variants are associated with carotid plaque.
- Differentially expressed NAV2 was identified in the hippocampal brain region from patients with AD and normal aging-related groups.
- NAV2 genetic variants showed strong associations with AD in a family study followed by a confirmation study in two independent cohorts.

Disclosure

All authors have reported no financial interests or potential conflicts of interest.

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CHAPTER 2

Interlinking polymorphisms, estrogens, and Alzheimer disease

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List of abbreviations

AD Alzheimer disease
BBB Blood–brain barrier
ERs Estrogen receptors
ERT Estrogen replacement therapy
HRE Hormone response elements
LD Linkage disequilibrium
SERM Selective estrogen receptor modulator
SHBG Sex hormone–binding globulin
SNPs Single nucleotide polymorphisms
WHIMS Women's Health Initiative Memory Study

Mini-dictionary of terms

Aβ deposition Aβ is 36–43 amino acids peptides derived from the amyloid precursor protein. The Aβ peptides can further aggregate and deposit to form amyloid plaque, a pathological hallmark of Alzheimer disease (AD) brain.

Blood–brain barrier The blood–brain barrier is a semipermeable membrane barrier composed of endothelial cells in the central nervous system. The function of the barrier is to pass small molecules (e.g., water, O_2/CO_2, glucose, amino acids, and lipid-soluble molecules) and restrict large and hydrophilic molecules (e.g., bacteria and lipid-insoluble molecules) into the cerebrospinal fluid.

Estrogen receptors Estrogen receptors are nuclear receptor proteins that can be activated by estrogen. There are two types of estrogen receptors, ERα and Erβ, with different binding affinity to E1, E2, and E3.

Neurosteroids Neurosteroids refers to steroids synthesized in either the central or peripheral nervous system, differing them from steroids produced by gonads and adrenals. This concept was first introduced by Baulieu and his colleagues in the 1980s.

Sex hormone–binding globulin Sex hormone–binding globulin is a glycoprotein carrying both androgen and estrogen in the serum.

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Introduction

Alzheimer disease (AD), a central neurodegenerative disorder presenting with cognitive, psychological, behavioral, language, and functional impairments, is the most prevalent type of dementia in elderly people. Epidemiological studies have reported a difference in the prevalence of AD between men and women, and women are more prone to AD than men (Hy & Keller, 2000; Wang et al., 2000). Women with AD have a poorer performance in some cognitive tasks than men with AD (Henderson & Buckwalter, 1994). Consistently, transgenic AD mice models have displayed more Aβ deposition and senile plaque loading in the female brain compared to the male brain (Hirata-Fukae et al., 2008; Wang, Tanila, Puolivali, Kadish, & van Groen, 2003). It is hypothesized that the underlying causes for AD vulnerability among women are multifactorial. In addition to the differences in education level as well as life expectancy, the effects of sex steroid hormones have been suggested to contribute to the AD susceptibility in the different genders.

Sex hormones, including estrogen, androgen, and progestogen, belong to the steroid hormone family. Gonads (ovaries and testes) and adrenals are organs producing peripheral steroid hormones. Sex steroid hormones are mostly bound to the sex hormone—binding globulin (SHGB) in serum. Only the nonbound free forms of estrogen hormones are physiologically active and bind to hormone receptors located in the cells' nucleus of target tissues through classical estrogen signaling. After forming the hormone-receptor complex by combination between sex hormones and the receptors, the complex triggers hormone response elements (HREs), a specific DNA sequence in the nucleus, and activates the downstream genes' transcription.

Sex hormones are lipophilic, and cross the blood—brain barrier (BBB) to enter the brain (Kancheva et al., 2011). Additionally, the brain also synthesizes neurosteroids (Baulieu & Robel, 1990). The presence of P450 enzymes, which are required for neurosteroids biosynthesis and metabolism, have been identified at both mRNA and protein levels in various regions of the brain (Melcangi & Panzica, 2009; Mellon & Griffin, 2002). For example, aromatases, which are necessary for converting the testosterone/androstenedione to estrogen, have been detected in neurons and astrocytes, especially in areas of amygdala, temporal cortex, hippocampus, and thalamus (Biegon et al., 2010; Roselli, Liu, & Hurn, 2009; Wang, Wahlstrom, & Backstrom, 1997). Currently, accumulating evidence suggests the neuroprotective effects of sex steroid hormones in memory and cognition, stress and emotion, and brain injuries, etc., besides their primary function in inducing body sex characteristics and metabolism (Zheng, 2009).

Estrogen, cognition, and AD

There are three types of estrogen: estrone (E1), 17β-estradiol (E2), and estriol (E3). Although E2 is the most potent and prevalent form of estrogen in women before

menopause, E1 is the most prevalent form of estrogen in women after menopause. The natural changes of estrogen type before menopause, during menopausal transition, and the postmenopausal period affect the brain cognitive function and the neuropathological abnormalities associated with AD.

Clinically, protective effects of estrogen on cognition differ with timing of estrogen replacement therapy. Verbal memory can be maintained and protected in women undergoing total abdominal hysterectomy by supplemental estrogen (Phillips & Sherwin, 1992; Sherwin, 1988). In a Longitudinal Study of Aging conducted in Baltimore, Maryland, supplement estrogen has shown its beneficial effects on improving visual memory as well as visual perception in postmenopausal women (Resnick, Metter, & Zonderman, 1997). Furthermore, the neuroprotective effects of estrogen on improving some cognitive domains, such as verbal memory, vigilance, and reasoning by supplement estrogen, have been suggested by a meta-analysis (LeBlanc, Janowsky, Chan, & Nelson, 2001). Conversely, women undergoing estrogen withdrawal due to unilateral or bilateral oophorectomy prior to their natural onset of menopause without supplemental estrogen have shown an increased risk of cognitive deterioration and dementia development in later life (Rocca et al., 2007).

In peripheral circulation, female AD patients have shown significant lower estrogen levels compared to controls (Manly et al., 2000). In central nervous system, significant reduced estrogen levels have been detected in cerebrospinal fluid (Schonknecht et al., 2001) and postmortem brains of AD women (Rosario, Chang, Head, Stanczyk, & Pike, 2011; Yue et al., 2005), respectively. Consistently, investigations on aromatase expression in the brain specific region related with memory, for example hippocampus, have suggested downregulated aromatase mRNA expression levels, which reflect estrogen deficits in the specific region of AD brain (Butler et al., 2010; Ishunina, Fischer, & Swaab, 2007). Moreover, the reduced estrogen levels were correlated with increased Aβ levels in the cerebrospinal fluid of AD patients (Schonknecht et al., 2001).

In vivo, early onset and increased Aβ deposition in the brain have been shown using an estrogen-deficient transgenic mouse model (Yue et al., 2005). In vitro, E2 has been found to reduce Aβ production/aggregation (Amtul, Wang, Westaway, & Rozmahel, 2010; Thakur & Mani, 2005), and to even prevent Aβ-induced cell death in a murine cholinergic cell line (Marin et al., 2003). However, the effectiveness of estrogen replacement therapy (ERT) in reducing AD risks is not confirmed by clinical trials and is still debatable. The most influential randomized, double-blind, placebo-controlled clinical trial is the Women's Health Initiative Memory Study (WHIMS) (Craig, Maki, & Murphy, 2005; Gleason et al., 2015) based on 4532 postmenopausal women aged 65 years or older, which has shown no benefit, but deterioration of cognitive function and increased risk for dementia (Shumaker et al., 2003). The participants recruited in WHIMS were much older (aged 65 years or older) compared to other studies showing a protective association based on relatively younger menopausal women (Henderson, 2006).

Therefore, the "Critical Window Hypothesis" or the "Window of Opportunity" was suggested and further investigated by different researchers. Henderson et al. found a significant protective effect of ERT in women aged 50–63 years old (Henderson et al., 2005). Moreover, in a population-based Cache County Study that followed 1768 women, Shao et al. reported that only women who had taken ERT within 5 years of menopause had significantly less AD development (Shao et al., 2012). Besides the time of intervention, the period of intervention may lead to the variations in clinical findings. It has been suggested that long-term (>10 years) (Imtiaz et al., 2017) instead of short-term (Roberts, Cha, Knopman, Petersen, & Rocca, 2006) estrogen administration would reduce AD risk in postmenopausal women. Other factors, like the difference in estrogen formulation or difference in administration method among studies, may also cause the variations in clinical outcomes. Further longitudinal clinical studies, to appraise all those confounding factors to understand the true impact of ERT on AD, are warranted.

Estrogen receptors and their genetic polymorphisms in AD

ERα and ERβ

There are two types of estrogen receptors (ERs), namely ERα and ERβ (Kuiper, Enmark, Pelto-Huikko, Nilsson, & Gustafsson, 1996). Although the binding affinity to E2 is similar for both receptors, E1 has shown preferential binding to Erα, and E3 has shown preferential binding to ERβ. Using immunofluorescence technology, ERα has been revealed to be widely distributed among hypothalamus, hippocampus, amygdala, brainstem, and forebrain (Mitra et al., 2003; Osterlund, Keller, & Hurd, 2000; Rettberg, Yao, & Brinton, 2014). However, compared to ERα′s, ERβ′s distribution is more concentrated, mainly in hippocampus, claustrum, and cerebral cortex of human brain (Osterlund, Gustafsson, Keller, & Hurd, 2000). Both ERα and ERβ have effects on hippocampal formation and therefore regulating hippocampal function, for example, working memories (spatial and episodic memories) or other hippocampal-dependent learning/memory performance (Spencer et al., 2008).

Ishunina et al. have reported increased expression of ERα in neurons of medial mammillary nucleus in postmortem AD brain, the area that also participates in memory performance. Interestingly, such an ERα increase is found to be more prominent in AD men compared to AD women (Ishunina, Kamphorst, & Swaab, 2003). However, in hippocampus, evidence based on paraffin-embedded human samples from the Netherlands Brain Bank has shown distinctly increased ERβ immunoreactivity in AD patients compared to control subjects (Savaskan, Olivieri, Meier, Ravid, & Muller-Spahn, 2001). Further study from the same research team has identified significantly decreased expression of ERα in hippocampal neurons of CA1-CA4 subfields in AD patients (Hu et al., 2003). Clinically, a significant correlation between frontal cortex ERα levels, but not ERβ levels, and the Mini-Mental State Examination scores has been revealed

using multiple linear regression analysis in postmortem AD patients (Kelly et al., 2008). Additionally, Bonomo et al. have reported an upregulation of ERs' mRNA levels in peripheral leukocytes (Bonomo et al., 2009).

Collectively, results from previous studies have proposed that the abnormalities of ERs have an impact on receptors' mediated estrogen signaling pathway and thereby in part contribute to the pathogenesis of AD, which have been further validated by subsequent genetic studies (Fig. 2.1).

ESR1 in AD

The *ESR1* located on chromosome 6q25.1 and encoding ERα has been suggested to be involved in cognitive deterioration and AD pathogenesis. A number of single nucleotide polymorphisms (SNPs) in *ESR1* have been investigated in AD. Among the investigated SNPs in *ESR1*, rs9340799 (XbaI) and rs2234693 (PvuII), which are located in the first intron of the gene, are the two most popular studied polymorphisms.

In addition to investigating the impact of *ESR1* polymorphisms on cognitive impairment by prospective studies (Ma, Tang, Leung, Fung, & Lam, 2014; Ryan et al., 2013; Yaffe et al., 2009; Yaffe, Lui, Grady, Stone, & Morin, 2002), increasing evidence has suggested their roles in AD pathogenesis. At present, there are 24 case-control studies investigating the impact of *ESR1* SNPs on AD susceptibility according to the summarization of the AlzGene database (http://www.alzgene.org/), among which 16 studies are conducted in Caucasian populations, including those in Spain, Italy, Sweden, the

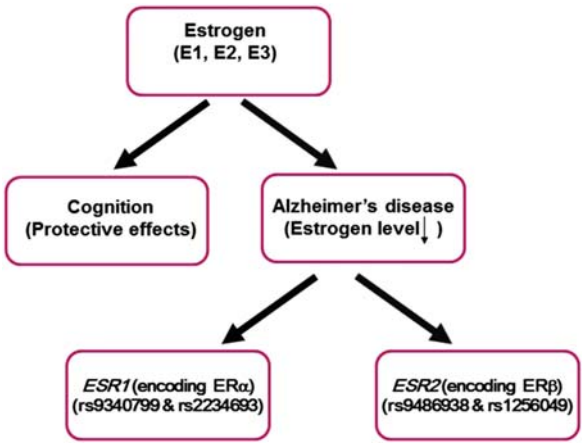

Figure 2.1 ***Estrogen, Alzheimer disease and estrogen receptor polymorphisms.*** The figure shows, in addition to identifying lower estrogen levels in Alzheimer disease patients' brain and cerebrospinal fluid, that genetic studies have revealed abnormalities in estrogen receptor genes. *E1*, Estrone; *E2*, 17β-estradiol; *E3*, Estriol; *ERα*, Estrogen receptor α; *ERβ*, Estrogen receptor β; *ESR1* = Estrogen receptor α gene; *ESR2* = Estrogen receptor β gene.

Netherlands, and Scotland. The first investigation was carried out by Brandi et al. based on an Italian population in 1999 using candidate gene approach, in which findings demonstrated significant associations between rs9340799 as well as rs2234693 and AD. Following this Italian study, significant disease associations for rs9340799 and/or rs2234693 have been further suggested by a number of other studies (Corbo, Gambina, Ruggeri, & Scacchi, 2006; Mattila et al., 2000; Monastero et al., 2006; Porrello et al., 2006). More recently, Boada et al. conducted a large-scale case-control genetic study based on 1130 AD patients and 1109 controls using the candidate gene approach in Spain, in which findings confirmed significant AD association with rs2234693 (Boada et al., 2012). In addition to the supporting evidence based on the candidate gene approach, the susceptible role of *ESR1* polymorphisms has also been indicated using a genome-wide association approach, which provides extrapolymorphism evidence of *ESR1* rs3844508 as a genetic risk factor for AD (Li et al., 2008; Myers et al., 2002). Nevertheless, results are discrepant across different populations and research settings (den Heijer et al., 2004; Lambert et al., 2001; Maruyama et al., 2000). In a more recent study conducted by Goumidi et al. based on 1007 AD patients and 647 controls recruited from Northern France, no significant association has been detected for *ESR1* SNPs (Goumidi et al., 2011).

In Asian populations, investigations have been performed in Japan and China. Both Isoe-Wada et al. (1999) and Ji, Urakami, Wada-Isoe, Adachi, and Nakashima (2000) have shown positive associations between rs9340799 as well as rs2234693 and AD in Japanese populations, although inconsistent findings have been revealed by the research team of Maruyama et al. (2000) and Usui et al. (2006), separately. In Chinese populations, findings are controversial between Northern and Southern Chinese. The first Chinese study was conducted in 2003 in Shanghai (Northern China), and findings from this study demonstrated significantly higher rs9340799 G alleles as well as rs2234693 C alleles in 30 AD patients compared to 125 control subjects (Lin et al., 2003). Ma et al. (2009) have conducted the second Chinese study in Hong Kong (Southern China) based on a relative larger sample size (233 AD patients and 245 control subjects). Contrarily, their findings have revealed disease associations for another five SNPs (rs1514348, rs2347867, rs6557171, rs9397456, and rs1801132) instead of rs9340799 and rs2234693. In a more recent Chinese study that was conducted in Hong Kong by our research team, based on 426 AD patients and 350 control subjects, no association with AD was found for rs2234693 (Chen et al., 2017), which is in accord with Ma et al.'s findings. The negative association findings for rs2234693 in Northern China are also supported by a meta-analysis, which suggests that it might be a risk factor in Caucasian populations but not in the Asian populations (Cheng, Liang, Hao, & Zhou, 2014).

Interestingly, the gender dimorphism for *ESR1* polymorphisms and AD has been shown in both Caucasian and Asian populations. Monastero et al. have found rs9340799 A allele associated with increased AD risk in women in an Italian sample

(Monastero et al., 2006). In another Italian study conducted by Porrello et al., both rs9340799 A allele and rs2234693 T allele have been found to increase disease risk by synergistically interacting with *APOEε4* only in AD women (Porrello et al., 2006). In order to conquer the inherent selection restriction of the case-control study design, a prospective multicenter longitudinal French study that recruited 6488 subjects found the CC genotype of rs2234693 associated with higher AD risk by interacting with *APOEε4* allele in women (Ryan et al., 2014). Conversely, Corbo et al. have reported rs9340799 and rs2234693 associated with AD risk in Italian men but not Italian women (Corbo et al., 2006). Moreover, a study conducted by Boada based on a Spanish population has suggested *ESR1* rs3844508 G alleles conferring decreased AD risk only in men (Boada et al., 2012). In agreement with findings in Caucasian populations that demonstrate the *ESR1* polymorphisms taking effect in a gender-specific way, in a Chinese population, Lin has found more significant AD associations for rs9340799 and rs2234693 in women in addition to total sample (Lin et al., 2003). Meanwhile, in our recent Hong Kong study, significant associations have been detected for two other *ESR1* polymorphisms, namely rs2179922 and rs932477, in AD men but not AD women (Chen, 2012) (Table 2.1).

The meta-analysis has suggested ethnic differences for distribution of *ESR1* SNPs, which may partially explain the resulting discrepancies and variability across different populations (Luckhaus & Sand, 2007). Functionally, although the exact mechanisms of *ESR1* polymorphisms still remain unknown, rs9340799 and rs2234693, being the two most popular studied, have been found to influence the plasma E2 levels (Scarabin-Carre et al., 2014; Schuit et al., 2005). It has been proposed that the rs9340799 and rs2234693 polymorphisms would affect the *ESR1* transcription activity by modifying the transcription factor binding site and therefore regulate the expression of gene, or alter the *ESR1* splicing site and therefore produce a different functional isoform (Herrington et al., 2002; Schuit et al., 2005).

Table 2.1 Genotypic association analysis for two *ESR1* SNPs (rs2179922 and rs932477) in male Chinese subjects.

dbSNP	Genotype	Cases/Controls (n, %)	Odds ratio (95%CI)	*P*-value
rs2179922	AA	18(13.8%)/14(14.1%)	2.56 (1.38−4.76)	0.0029
	AG	68(52.3%)/37(37.4%)		
	GG	44(33.8%)/48(48.5%))		
rs932477	AA	29(22.5%)/18(18.4%)	2.33 (1.25−4.35)	0.0081
	AG	60(46.5%)/36(36.7%)		
	GG	40(31.0%)/44(44.9%)		

The table shows that the genotypic distributions for both rs2179922 and rs932477 are significant differences between cases and controls in Chinese men (unpublished). *CI*, Confidence interval; *ESR1*, Estrogen receptor α gene; *OR*, Odds ratio.

ESR2 in AD

Similar to *ESR1*, the *ESR2* located on chromosome 14q22-24 and encoding ERβ has also been identified to participate in cognitive deterioration and AD pathogenesis. A number of SNPs in *ESR2* have been investigated, among which rs9486938 located in 3′ untranslated region and rs1256049, located in the fifth exon, have been widely studied in AD.

In addition to investigating the impact of *ESR2* polymorphisms on cognitive impairment by prospective studies (Ryan et al., 2013; Yaffe et al., 2009), increasing evidence has suggested their roles in AD development. It has been noticed that the *ESR2* polymorphisms take effect in AD in a gender-specific way, with more prominent effect in women. Previously, several studies have suggested the association between *ESR2* polymorphisms and disease susceptibility in Caucasian populations, including American, German, and Finnish (Janicki et al., 2014; Luckhaus et al., 2006; Pirskanen et al., 2005; Zhao et al., 2011). In one case-control study conducted by Pirskanen et al. based on 387 AD patients and 467 control subjects, TT genotype of both rs1271573 and rs1256043 in *ESR2* have been found to associate with increased AD risk only in Finnish women (Pirskanen et al., 2005). In the other case-control study, Luckhaus et al. have reported significant disease associations with rs9486938 and rs1255953 by polymorphisms' interaction based on 126 AD patients and 111 control subjects (Luckhaus et al., 2006). Apart from case-control studies, two prospective studies have also been performed in AD. In the first prospective cohort study conducted by Zhao et al., four SNPs in *ESR2* (rs4986938, rs17766755, rs4365213, and rs12435857) have been reported to associate with AD risk in women with Down syndrome after follow-up at 4 years (Zhao et al., 2011). More recently, in a prospective Washington Heights Inwood Columbia Aging Project that recruited 1686 female subjects in the United States, another four *ESR2* SNPs (rs944045, rs1256062, rs10144225, and rs22747050) have been found to associate with increased AD susceptibility in women of Caucasian ancestry (Janicki et al., 2014).

Although the aforementioned case-control as well as prospective studies do suggest associations between *ESR2* SNPs and AD susceptibility, the significant associations have been challenged by other studies reporting negative findings. In one study derived from Caucasians in the United Kingdom, Lambert et al. have reported no allelic and genotypic distribution differences for *ESR2* rs4986938 between AD patients and control subjects (Lambert et al., 2001). Two other subsequent case-control studies (Fernandez-Martinez et al., 2013; Goumidi et al., 2011) and one prospective cohort study (Ryan et al., 2014) have also failed to detect any significant AD association with *ESR2* SNPs.

In Asian populations, the investigations in China were only recently performed with some positive findings reported by our research team (Chen et al., 2017). In our Southern Chinese population, the frequency distributions for A allele of both rs4986938 and rs867443 were significantly lower in AD patients than in control subjects.

Following genotypic association, analysis showed that those two SNPs would decrease AD risk by around 50% in the total sample (Chen et al., 2017). However, these studies differed from findings in Caucasian populations showing *ESR* polymorphisms presenting with greater impact in women; in the Chinese population, the disease association for rs4986938 was marginally significant in AD men after multiple testing corrections, which may be due to the limited sample size of male subjects (Chen, 2012). Although results' discrepancies may be accounted for by different variants' linkage disequilibrium (LD) patterns in different ethnic backgrounds, findings in Chinese men were consistent with findings reported by Forsell et al. which also revealed the protective effects of *ESR2* polymorphisms in Swedish men (Forsell et al., 2001). If such protection phenomenon of the *ESR2* gene can be further replicated and validated in additional large and independent male samples, this might give us some clues regarding why men are less prone to develop AD compared to women.

The rs4986938, being the most extensively studied SNP in AD, has been predicated to be the hsa-miR-758 binding site. The microRNAs are posttranscriptional regulators that play important roles in translational repression, translational degradation, and gene silencing during synaptic development (Schratt, 2009; Shukla, Singh, & Barik, 2011). It is speculated that the function of rs4986938 would decrease translational efficiency of microRNA during posttranscription stage due to the changes of microRNA's binding site. In terms of rs1256049, it has been linked with specific cognitive domains, although the underlying mechanism of this polymorphism is still unknown. The rs1256049 is assumed to link (which we call in LD) with some other polymorphisms that play important roles in AD. While, currently those functional important polymorphisms haven't been reported which may due to resources restrictions or other reasons.

Conclusions

As reviewed and discussed by this article, estrogen exerts a wide range of neuroprotection effects on cognition and AD, which effects are partially mediated by ERs. Accordingly, the polymorphisms in *ESR1* and *ESR2* have been suggested to confer AD susceptibility in both Caucasian and Asian populations in a gender-specific manner. Although findings from clinical studies are still controversial, estrogen-based ERT is still a worthwhile target for drug development in the prevention of AD and further research to explore the mechanisms of estrogen signaling pathway is advancing. Raloxifene, a selective estrogen receptor modulator (SERM), has shown to improve cognitive function and lower the risk of AD in postmenopausal women (Yang, Yu, & Zhang, 2013). Moreover, a novel ERβ-selective phytoestrogenic formulation, known as phyto-SERM formulation, is in phase I/IIa clinical trials to evaluate its protective effect on memory problem in menopausal women (Zhao, Mao, Chen, Schneider, & Brinton, 2013). Subsequently, continuing discovery of novel polymorphisms in ERs and biological elucidation of the function of those detected polymorphisms will shed new light on translating research findings from neurosteroids studies to clinical hormone application in AD.

Key facts of the "critical window hypothesis"

- The "critical window hypothesis," also known as the "window of opportunity" or "timing hypothesis."
- The content of the hypothesis is to emphasize that the initiative time of hormone replacement therapy with regard to women's menopause onset is critical and will have an impact on clinical results of therapy.
- The hypothesis was suggested following the Women's Health Initiative Memory Study (WHIMS), whose findings were published in 2003.
- The WHIMS, being a multicenter, randomized, double-blind, placebo-controlled clinical trial, was to assess whether hormone therapy would decrease the risk of dementia in aged women.
- However, findings from WHIMS showed substantially increased dementia risk instead of decreased risk in women aged 65 years and older, who initiated hormone therapy many years after menopause.
- The subjects included in WHIMS with relatively advanced age and late administration of hormone therapy are supposed to lead the biased clinical outcomes.

Summary points

- It has been found that women are more vulnerable for developing Alzheimer disease (AD).
- The neuroprotective effects of estrogen on cognition and AD, which are partially mediated by estrogen receptors (ERs), have been suggested by both animal and human experiments.
- The effectiveness of estrogen replacement therapy (ERT) on AD continues to be debated, with negative findings from the Women's Health Initiative Memory Study but positive findings from other studies.
- Accordingly, genetic studies based on both Caucasian and Asian populations have shown that polymorphisms in the estrogen receptor α gene (*ESR1*) and estrogen receptor β gene (*ESR2*) confer AD susceptibility in a gender-specific manner.
- Therefore, it is necessary to continue exploring novel polymorphisms in ER genes, and elucidate the function of detected polymorphisms to develop new ERT in AD.

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CHAPTER 3

Linking EEGs, Alzheimer disease, and the phosphatidylinositol-binding clathrin assembly protein (PICALM) gene

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List of abbreviations

AD Alzheimer disease
aMCI amnestic mild cognitive impairment
ApoE apolipoprotein E
APP amyloid precursor protein
Aβ beta-amyloid
BBB blood–brain barrier
CME clathrin-mediated endocytosis
EEG electroencephalography
EOAD early onset AD
ERP event-related potential
GWAS genome-wide association study
LOAD late-onset AD
PRES-2 presenilin-2
PSEN-1 presenilin-1
SV synaptic vesicle

Mini-dictionary of terms

Clathrin-mediated endocytosis Is a process by which cells absorb large extracellular molecules by the invagination of the plasma membrane. Clathrin-mediated endocytosis functions include the internalization of receptors, recycling of membrane components, reformation of synaptic vesicles, and uptake of low-density lipoproteins and iron-saturated transferrin.

Electroencephalography Is a measurement of brain electrical activity, which reflects the integrated excitatory and inhibitory postsynaptic potentials of large neuron populations. The method can be used for detection of functional cerebral abnormalities such as epileptiform activity or encephalopathy.

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Reference single-nucleotide polymorphism identifier number ("rs#") Reflects that the single-nucleotide polymorphism in question has been officially registered and given an (rs) identifier by the database maintained by the US National Institutes of Health.

Single-nucleotide polymorphism Is a precise position along a chromosome where the deoxyribonucleic acid of different people may vary. Generally, two alternate alleles are found at a particular single-nucleotide polymorphism.

The P300 component of the event-related potentials Is a positive waveform. P300 is elicited using oddball paradigm, in which the subject must identify rarely occurring target items interspersed among more frequently occurring nontarget items. P300 is quantified by latency and amplitude measures. The information-processing cascade associated with attention, decision-making, and memory mechanisms is reflected in the P300 signal.

Introduction

Alzheimer' disease (AD) is a fatal neurodegenerative disease characterized by progressive impairment of cognitive functions. AD is the most common form of dementia, affecting up to 38% of people over 85 years (Alzheimer's Association, 2017).

The greatest known risk factors for AD are genetic predisposition and older age. Early onset AD (EOAD), which develops before the age of 65 years, is caused by mutations in the amyloid precursor protein (*APP)*, presenilin-1 (*PRES-1*), and presenilin-2 (*PRES-2*) genes (Goate et al., 1991; Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). Late-onset AD (LOAD) affects people older than age 65. Polymorphism of the apolipoprotein E (*ApoE*) gene has been found to be the most prevalent genetic risk factor for LOAD (Saunders et al., 1993).

Recent genome-wide association studies (GWASs) have identified approximately 20 novel genetic risk variants for AD (Harold et al., 2009; Lambert et al., 2009). Among the genes found by GWAS, the phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (*PICALM*, chrm 11q14) gene is considered to be one of the top six most prevalent genetic risk factors for AD according to data in the AlzGene database (http://www.AlzGene.org).

Unraveling the biological pathways through which genes identified in association studies influence pathogenesis of neurodegenerative diseases could potentially contribute to an earlier diagnosis and the development of personalized prevention strategies (Illarioshkin et al., 2004; Rogaev et al., 1995; Xu, Tan, & Yu et al., 2015). The role of *PICALM* in AD development remains incompletely understood.

In addition to analyzing the genetic effects of metabolic signaling pathways at the cellular level, genetic influences on AD pathogenesis can be revealed in vivo in the human brain by neurophysiological methods. Electroencephalography (EEG) and event-related potentials (ERPs) are effective tools for the analysis of specific brain alterations associated with particular genes and can help to reveal in which stage of the pathological process these alterations can occur. These neurophysiological methods

show great promise in the research of the mechanisms through which genes influence neuronal activity and cognitive functions in AD pathogenesis.

In this chapter, we will present an overview of the metabolic pathways of the *PICALM* genotype and their possible impact on the development of AD. We will then review neurophysiological alterations during normal ageing and during AD development as they are revealed by EEG and ERP. In the last section we will summarize our current knowledge regarding the association of the *PICALM* genotype with neurophysiological alterations during ageing, and we will discuss the mechanisms underlying these alterations and their role in AD pathogenesis.

Functions of PICALM and their potential influence on AD development

PICALM and AD risk

PICALM is a 112 kb gene located on chromosome 11q14. Several single-nucleotide polymorphisms (SNPs) in *PICALM,* including rs3851179, were replicated in independent studies as genetic susceptibility loci for LOAD (Harold et al., 2009; Lambert et al., 2009). The rs3851179 SNP is located in a noncoding region of *PICALM* with no known function. The *PICALM* G allele increases the risk of AD, while the A allele has a protective effect. Other SNPs in the *PICALM* gene were also found as susceptibility loci, including rs541458 and 592297, which are in linkage disequilibrium with rs3851179 (Xu et al., 2015, review). It was suggested that the association between *PICALM* and LOAD may relate to changes in the expression of PICALM or particular PICALM isoforms.

PICALM is ubiquitously expressed protein. In the brain, the presence of PICALM has been identified in neurons, astrocytes, and oligodendrocytes. PICALM is also highly expressed in the brain capillary endothelium, which forms part of the blood—brain barrier (BBB) (Parikh, Fardo, & Estus, 2014).

PICALM is an accessory adaptor protein implicated in clathrin-mediated endocytosis (CME) (Moshkanbaryans, Chan, & Graham, 2014). CME functions include the internalization of receptors, recycling of membrane components, reformation of synaptic vesicles, and uptake of low-density lipoproteins and iron-saturated transferrin (Xu et al., 2015).

The metabolic pathways of PICALM can be subdivided into two groups. The first group consists of pathways that contribute directly to the development of pathological hallmarks of AD, including Aβ deposition and the formation of neurofibrillary tangles. The second group consists of pathways that are not linked to the pathological hallmarks of AD but whose impairment may influence the development of AD through abnormalities in other important processes: synaptic function, iron metabolism, and cholesterol metabolism. *PICALM* may influence the development of AD through its role in both groups of pathways.

Role of PICALM in amyloidogenesis

A hallmark pathological feature of AD is the amyloid plaque, which consists of extracellular deposits of aggregated beta-amyloid (Aβ) peptide. Aβ is a proteolytic fragment of the APP, a transmembrane glycoprotein found primarily in neurons, which can be cleaved by several enzyme complexes (Hardy & Selkoe, 2002).

The study of neuropathological data from 4914 brain autopsies demonstrated that *PICALM* genotype is associated with the presence of Aβ-containing amyloid plaques (Beecham et al., 2014). PICALM is required for the CME of the amyloid precursor protein (APP) and for endocytic production of Aβ.

PICALM was found to influence Aβ production by modifying the trafficking of APP (Xiao et al., 2012). On the intracellular level, PICALM is involved in inhibiting the production of Aβ peptides through autophagy processes by lysosomes. PICALM promotes the transportation of APP-cleaved C-terminal fragments (CTF) from the plasma membrane into the lysosome, which allows the fusion of autophagosomes and endosomes, leading to the degradation of APP-CTF lysosomes and indirectly preventing the generation of Aβ (Xu et al., 2015, review).

Taken together, these results indicate that PICALM may function as a modulator for the uptake, trafficking, and processing of APP and the generation of Aβ.

Role of PICALM in Aβ clearance

PICALM was suggested to contribute to LOAD by changing the efficiency of Aβ clearance through the BBB into the bloodstream, the major pathway for removal of extracellular Aβ peptides in brain parenchyma (Parikh et al., 2014). PICALM is expressed in the brain capillary endothelium and is ideally situated to regulate the function of brain capillary endothelial receptors.

Inducible pluripotent stem cell-derived human endothelial cells carrying the rs3851179 protective allele had higher PICALM levels and enhanced Aβ clearance through the BBB. Reduced expression of PICALM in the brain endothelium accelerated Aβ deposition in APP transgenic mice (Zhao et al., 2015).

A change in PICALM abundance or function would affect the uptake and transcytosis of Aβ to the bloodstream.

Role of PICALM in tau clearance

Apart from Aβ plaques, another pathological hallmark of AD is intraneuronal neurofibrillary tangles, composed of hyperphosphorylated tau protein. Tau is normally a microtubule-associated protein that plays an important role in ensuring axonal transport, but in tauopathies, tau becomes hyperphosphorylated and disengages from microtubules,

with consequent misfolding and deposition into inclusions that affects neurons and glia (Hardy & Selkoe, 2002).

GWAS performed on neuropathological data from 4914 brain autopsies revealed the association of the *PICALM* genotype with the presence of neurofibrillary tangles (Beecham et al., 2014). In neurons, tau is cleared through PICALM-dependent autophagy. Immunohistochemistry revealed that PICALM was associated with neurofibrillary tangles, colocalizing with hyperphosphorylated tau in LOAD (Ando et al., 2013).

Altered PICALM expression exacerbates tau-mediated toxicity in zebrafish transgenic models. PICALM influence was demonstrated on different stages of the autophagy pathway, from autophagosome formation to autophagosome degradation (Moreau et al., 2014). Impaired autophagy could result in neurotoxicity and, consequently, might also be related to the spreading of tau pathology.

All this evidence indicates that the *PICALM* modulates tau accumulation and spreading of tau pathology in the brain.

PICALM and synaptic functions

PICALM is involved in synaptic function, contributing to the recycling of synaptic vesicle (SV) proteins (Xu et al., 2015). In the presynaptic terminal, neurotransmitter release begins with the fusion of SV to the presynaptic plasma membrane. SV fusion is mediated by vesicle-associated membrane proteins (VAMP), the most abundant of which is VAMP2. The retrieval of VAMP2 and other SV proteins is accomplished by CME, in which PICALM is a key component.

The expression level of PICALM can affect the amount of VAMP2 at the plasma membrane by regulating endocytosis (Harel, Wu, Mattson, Morris, & Yao, 2008). PICALM can also modulate synaptic functions by influencing the cell surface level of the glutamate receptor subunit GluR2 (Harel, Mattson, & Yao, 2011).

Therefore, *PICALM* may influence synaptic function by facilitating neurotransmitter delivery. This process may be compromised by pathological factors such as Aβ.

PICALM and cholesterol metabolism

PICALM influences metabolism of cellular cholesterol by modulating its internalization and transportation (Xu et al., 2015, review). The data suggest that cholesterol metabolism may be abnormal when PICALM activity is perturbed.

PICALM and iron homeostasis in AD

PICALM plays an important role in iron homeostasis; PICALM functions as a modulator of transferrin-receptor internalization (Xu et al., 2015, review). Mounting evidence has

indicated a robust association between iron homeostasis and AD. It was suggested that intracellular iron accumulation results in increased oxidative damage, which would contribute to AD.

The results of the abovementioned studies of metabolic pathways show that the *PICALM* genotype can contribute to the accumulation of Aβ and hyperphosphorylated tau proteins in the brain, which are known key molecular players in AD neurodegenerative processes affecting the neocortex and hippocampus (Hardy & Selkoe, 2002). In addition, PICALM can modulate synaptic function and neuronal membrane properties, which will influence brain neurophysiology in the preclinical stage of AD.

All these data suggest that the *PICALM* genotype can cause brain function alterations that may be revealed by neurophysiological methods in humans, even in the preclinical stage of AD. In the next sections, we will discuss the results of the studies on the association of *PICALM* polymorphism with changes in neurophysiological characteristics during human ageing.

EEG - method for studying the effect of genetic influence on brain function in normal ageing and Alzheimer's disease

EEG is a powerful, noninvasive and cost-effective method for studying brain function. EEG reflects the integrated excitatory and inhibitory postsynaptic potentials (EPSP and IPSP, respectively) of large neuron populations, allowing the observation of changes in brain functional activity during both normal and pathological ageing (Babiloni et al., 2014; Prichep et al., 2006; Van Straaten, Scheltens, Gouw, & Stam, 2014). Although EEG has lower spatial resolution compared to MRI, it has very good temporal resolution on the order of milliseconds. Changes in the amplitude of activity within specific frequency bands are robustly found to be associated with variations in overall arousal level as well as with different cognitive processes. EEG is a sensitive indicator of brain circuits' excitatory/inhibitory (E/I) balance and epileptiform alterations. Because of these properties, the study of EEG may yield unique biomarkers of the presence or predisposition to a variety of cognitive disorders.

Resting EEG is the recording of ongoing spontaneous brain electrical activity while a patient is relaxing with closed or open eyes.

Slowing of resting EEG in AD is a uniform finding. The quantitative EEG (qEEG) abnormalities found in the resting EEG of AD patients include a shift of the power spectrum to lower frequencies as well as a decrease in the coherence of fast rhythms and other synchrony measures and reduced EEG complexity (Babiloni et al., 2014; Jeong et al., 2004; Ponomareva, Korovaitseva, & Rogaev, 2008; van Straaten et al., 2014).

Cortical networks hyperexcitability is an early event in AD development (Lizio et al., 2011). Epileptiform activity in the form of spikes and sharp waves was found to occur more than four times more frequently in patients with AD than in healthy controls (Vossel et al., 2016).

Patients with amnestic mild cognitive impairment (aMCI), which is in most cases a prodromal stage of AD, have EEG characteristics intermediate between those of normal subjects and AD patients (Babiloni et al., 2014). Elevated slow-wave power in the EEG reflects the degree of synaptic dysfunction during MCI and AD development (Lizio et al., 2011, review). Synaptic loss is directly related to cognitive decline in AD and MCI. Longitudinal studies have found EEG predictors of future cognitive decline in MCI patients and even in healthy elderly subjects (Prichep et al., 2006; Babiloni et al., 2014).

EEG and PICALM genotype

Resting EEG is stable throughout healthy adult life and is highly heritable. The EEG studies estimated the heritability of EEG patterns to be on the order of 0.80. EEG is considered an important source of endophenotypes, heritable biomarkers that can already manifest in the preclinical stage of the disease (De Geus, 2010).

Recent studies have shown an association between EEG characteristics and AD risk variants in the *ApoE* and *CLU* genes in AD, MCI patients and even in healthy adults (Lizio et al., 2011, review; Ponomareva et al., 2008; 2013).

In our recent study, the possible effect of the *PICALM* rs3851179 genotype on resting qEEG was examined in 137 nondemented volunteers (age range 20–79 years) subdivided into cohorts of those younger than and those older than 50 years of age. The homozygous presence of the AD risk variant *PICALM GG* was associated with an increase in beta1 and beta2 relative power in the older cohort (Fig. 3.1) (Ponomareva et al., 2017). Such elevated beta relative power in resting EEG, as was consistently demonstrated, is an indicator of the disinhibition of cortical networks, hyperarousal and/or hyperexcitability (Cannon et al., 2014; Jin, Lipponen, Koivisto, Gurevicius, & Tanila, 2018).

Beta rhythm is one of the essential functional features of the brain. Beta oscillations are involved in regulating cognitive and motor functions (Cannon et al., 2014). Beta oscillations within corticocortical, corticohippocampal, and corticobasal ganglia-thalamocortical circuitries are dependent on the E/I balance between excitatory glutamatergic pyramidal cells and inhibitory interneurons, with GABAergic cells playing the role of pacemakers (Cannon et al., 2014).

Increased cortical beta power, associated with spike-wave discharges, was found in transgenic APP/PS1 mice (Jin et al., 2018). These alterations were mediated by the influence of Aβ pathology on thalamocortical circuitry. It has been shown that Aβ application decreases inhibitory processes by downregulating $GABA_A$ receptors (Busche et al., 2019; Palop & Mucke, 2009).In addition, as PICALM protein modulates glutamatergic neurotransmission (Harel et al., 2011), it is possible that the EEG beta rhythm alteration in the carriers of the *PICALM GG* genotype is worsened by the dysfunction in glutamatergic networks.

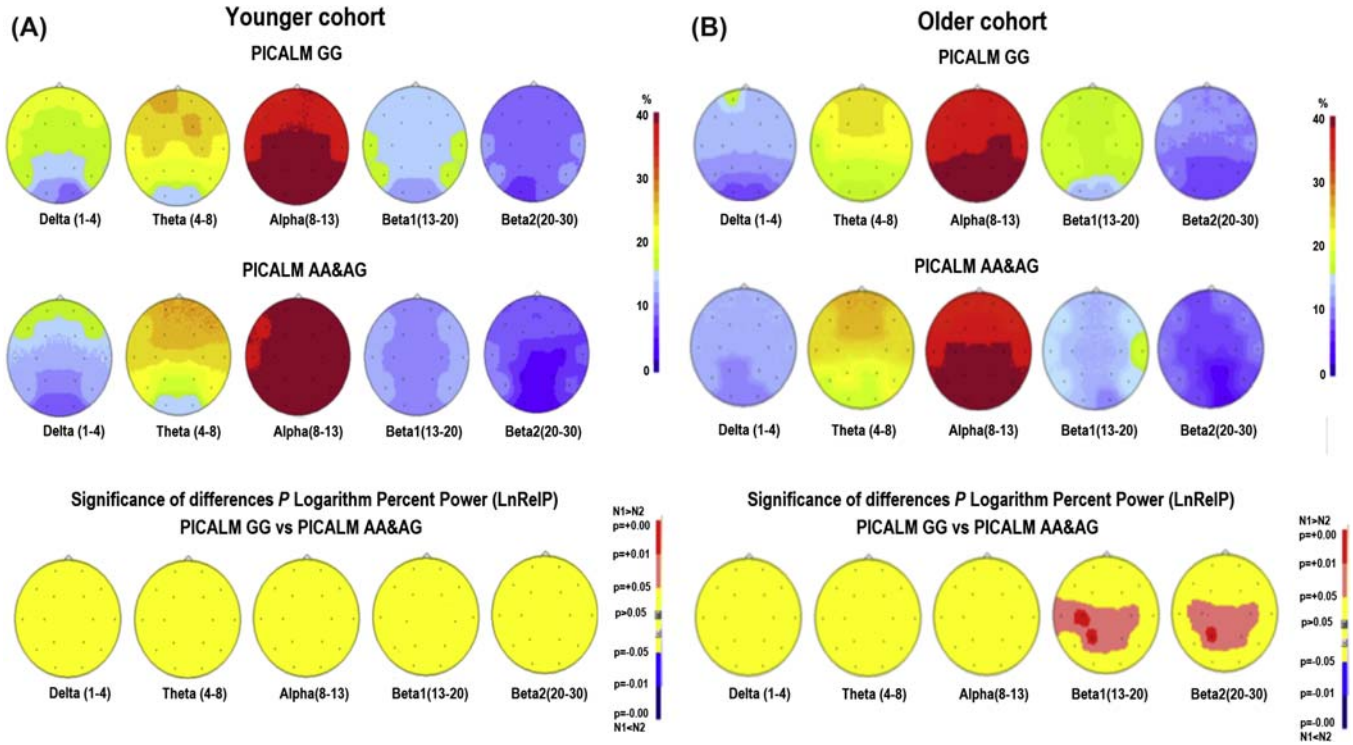

Figure 3.1 ***Average topographic maps of relative power for delta, theta, alpha, beta1, and beta2 frequency bands in the nondemented younger (A) and older (B) subjects with the PICALM GG and PICALM AA&AG genotypes.*** In the older carriers of the *PICALM GG* genotype beta1 and beta2 relative power is significantly increased compared to the older carriers of the *PICALM AA&AG* genotypes.

The association of the *PICALM GG* genotype with neurophysiological signs of hyperexcitability or disinhibition suggests that carriers of this genotype may be more susceptible to stress reactions. According to previous studies, long-standing distress in midlife increases the risk of AD (Johanson et al., 2014).

While at the preclinical stage of AD the beta power in resting EEG can increase, at the dementia stage of AD, a decrease in beta power is observed (Jin et al., 2018; Ponomareva et al., 2017). This nonlinear trajectory of beta activity is probably related to a series of pathophysiological processes that occur over the decades of AD development. Recently, in a study on transgenic mice harboring Aβ and tau, it was demonstrated that these proteins have opposing effects on the activity of neuronal circuits. Aβ alone causes hyperactivity, whereas tau alone suppresses activity and promotes silencing of many neurons (Busche et al., 2019). The authors suggested that hyperactivation is more prominent in AD patients who have relatively higher Aβ than tau levels, that is, at very early, possibly presymptomatic stages of the disease when Aβ deposits occur throughout the cortex but neurofibrillary tangles are limited to the medial temporal lobe. These experimental data corroborate the EEG feature of hyperexcitability associated with the AD risk *PICALM GG* genotype in nondemented older adults and the slowing of EEG and beta power reduction in AD patients.

Although the EEG alterations in the carriers of the *PICALM GG* genotype are not similar to those in the *ApoE ε4* allele carriers, both of these genetic risk variants for AD are associated with cerebral disinhibition and/or hyperexcitability. In the *ApoE ε4* allele carriers, neurophysiological signs of hyperexcitability were characterized by the manifestation of synchronous high-voltage delta and theta and sharp waves under hyperventilation (Ponomareva et al., 2008).

Abnormal network hyperactivity contributes to cognitive decline through changes in the expression of genes and the remodeling of neuronal circuits (Palop & Mucke, 2009). Optogenetic studies directly demonstrated that chronic activation of the hippocampal perforant pathway increases Aβ pathology in the dentate gyrus and hippocampus (Yamamoto et al., 2015).

Several studies demonstrated that low doses of the antiepileptic drug levetiracetam can improve neurophysiological and cognitive dysfunctions in mouse models of AD and in patients with aMCI (Baxter, 2012; Sanchez et al., 2012). This warrants further investigation.

Event-related potentials, P300 and PICALM

Cognitive ERPs provide a powerful tool for studying the brain's function in ageing and AD. The most commonly studied positive ERP component reflecting the neurophysiological process underlying cognitive function in normal ageing and in the development of AD is P3. It is also called P300, as it occurs with a latency between stimulus and

response of approximately 300 ms in healthy adults. The presence, magnitude, topography, and timing of the P300 reflect the speed and efficiency of cognitive processes.

The extensive ERP literature has described a gradual age-related increase of P300 latency in physiological ageing. In AD, an even greater latency increase (~2 standard deviations above the mean of normal older individuals) is commonly reported (Polich, 2007). Patients with MCI showed decreased P300 amplitude and prolonged latency compared to controls. A meta-analysis by Jiang et al. (2015) verified that P300 latency is an objective and sensitive tool for discriminating MCI from control and AD patients.

Several ERP studies have found P300 alterations in clinically healthy subjects with increased genetic risk of AD compared to the general population. Golob et al. (2009) reported delayed P300 latencies in asymptomatic persons with familial AD mutations.

In our study of the association between the *PICALM* rs3851179 polymorphism and the parameters of the P300 component of auditory ERP in 87 nondemented volunteers (age range 19–77 years), we found that, in the subjects older than 50 years of age, P300 latency was significantly increased in the carriers of *PICALM GG* compared to the carriers of *PICALM AA&AG* genotypes (Fig. 3.2) (Ponomareva et al., 2018).

The delayed P300 latency may be related to the smaller volume of the hippocampus and decreased entorhinal cortex thickness in nondemented adults that have been associated with the *PICALM* genotype (Biffi et al., 2010; Furney et al., 2011). The effect of the *PICALM* is probably related to neuronal dysfunction, subclinical neurodegeneration,

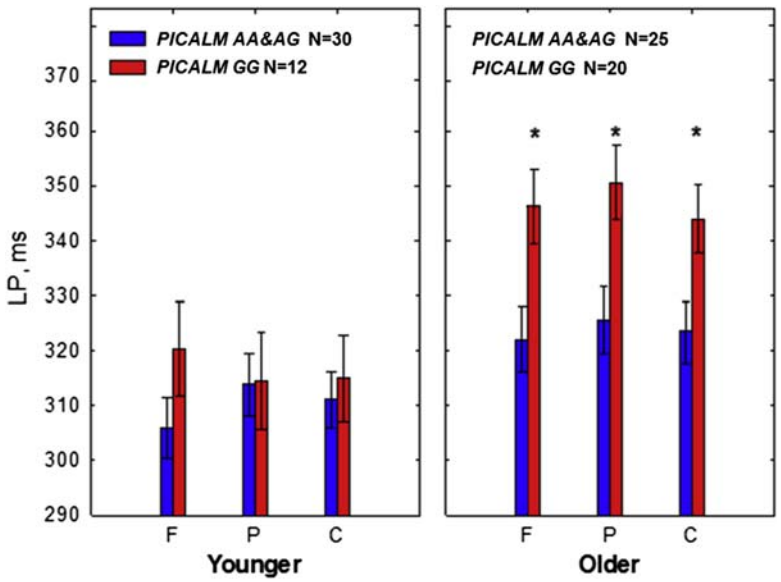

Figure 3.2 ***Latency (latent period, LP) of the P3 component of ERP in healthy subjects younger and older than 50 years of age carrying different PICALM genotypes.*** The histograms show the mean +SEM of the LP of the P3 component. F, P, C, frontal, parietal, and central brain areas, respectively, *$P < .05$ significant differences between *PICALM GG* and *PICALM AA&AG* genotype carriers.

and changes in white matter integrity. As shown by diffusion tensor imaging, the elevation of Aβ and tau pathology may influence the trajectory of white matter alterations in the preclinical stage of AD (Van Dinteren, Arns, Jongsma, & Kessels, 2014).

Preserved P300 latency in the carriers of the *A PICALM* allele may underlie the protective effect of this allele on the rate of cognitive decline and the risk of AD development. This evidence is also in line with the results of neuropsychological studies demonstrating a consistent but weak association of the *PICALM* rs3851179 *A* allele with better cognitive functioning in nondemented, elderly men (Mengel-From, Christensen, McGue, & Christiansen, 2011).

Conclusion

The *PICALM* genotype is associated with LOAD in both Caucasian and Asian populations; in particular, the *PICALM* rs3851179 A allele may serve a protective role, while the G allele serves as a strong genetic risk factor. PICALM protein expression is modestly increased with the rs3851179 AD-protective A allele. PICALM is involved in CME and has been shown to contribute to AD pathogenesis through several pathways, including Aβ production, impaired clearance of Aβ and tau proteins, and synaptic functions. Even in nondemented carriers of *PICALM* genetic AD risk variant, these pathways may lead to alterations in brain function.

The neurophysiological alterations in nondemented carriers of *PICALM GG* rs3851179 AD risk variant manifest in age-dependent beta power elevation in resting EEG. This EEG feature indicates cortical disinhibition and hyperexcitability. *PICALM GG* carriers also exhibit delayed ERP P300 latency, which reflects a pronounced decline in cognitive processing speed in the hippocampal and frontoparietal networks in ageing. This slowing of information processing and neuronal hyperexcitability may partially account for age-related cognitive decline and constitutes a neurophysiological dysfunction that may itself contribute to the pathogenesis of AD. Fig. 3.3 illustrates the role of the *PICALM* genotype in the pathophysiological alterations of the brain during ageing.

The research into various aspects of *PICALM*'s influence on AD development highlights that, when studying how candidate genes affect cognitive functions, both genetic polymorphisms and age should be considered. Cost-effective and noninvasive identification of predisposition to AD using EEG and ERP biomarkers may provide the possibility for prophylactic and early therapeutic intervention before the appearance of any clinical symptoms.

Key facts of the role of *PICALM* genotype in metabolic dysregulation and neurophysiological alterations during ageing

- *PICALM* polymorphism confers susceptibility to Alzheimer disease.
- PICALM protein is a key component in clathrin-mediated endocytosis, by which cells absorb extracellular molecules.

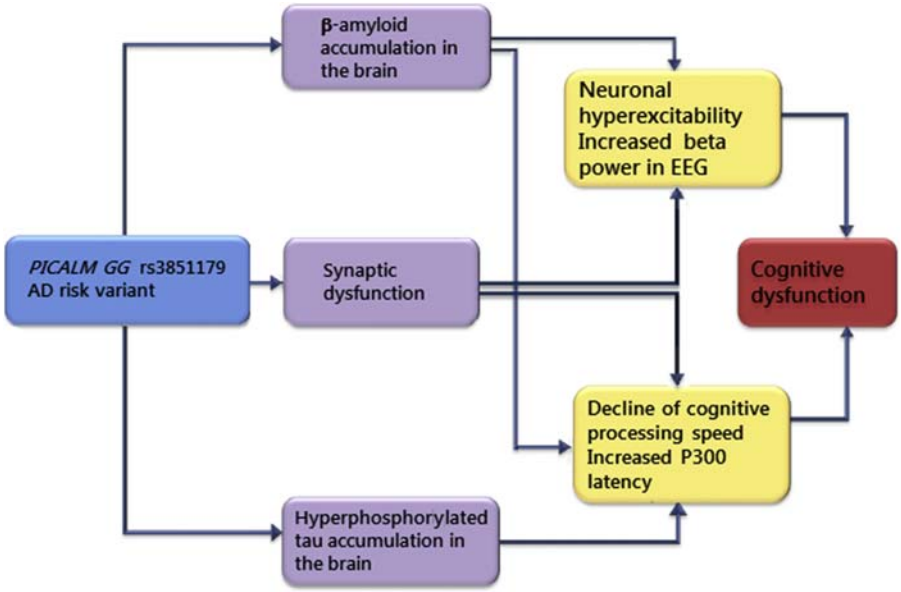

Figure 3.3 ***PICALM-driven mechanisms involved in AD pathophysiology.*** Schematic presentation shows that PICALM may contribute to the risk of AD by influencing the accumulation of beta-amyloid and tau in the brain and synaptic function. These factors can negatively affect the neurophysiology of an ageing brain and cause cognitive dysfunction.

- Clathrin-mediated endocytosis is implicated in accumulation in the brain of beta-amyloid and tau proteins, which are the main components of Alzheimer disease-related pathology.
- Beta-amyloid and tau accumulation in the brain and synaptic dysfunction can lead to neurophysiological alterations even in nondemented adults.

Summary points

- *PICALM* genotype is a highly validated genetic risk factor for Alzheimer disease.
- PICALM can contribute to the pathogenesis of Alzheimer disease through several pathways that lead to accumulation of amyloid-beta and tau proteins in the brain and synaptic dysfunction.
- The functional consequences of the metabolic pathways dysregulation related to *PICALM* genotype can be revealed using neurophysiological methods.
- The *PICALM* risk variant for Alzheimer disease is associated with an increase of beta power in resting electroencephalography in older nondemented adults, suggesting cortical disinhibition and hyperexcitability.
- In ageing, carriers of the Alzheimer disease risk variant *PICALM GG* exhibit delayed latency of the ERP component P300 compared to *PICALM GG* noncarriers. This indicates a decline in cognitive processing speed.

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CHAPTER 4

CD36 gene polymorphisms and Alzheimer's disease

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List of abbreviations

ApoE apolipoprotein E
APP amyloid-β (abeta) precursor protein
BACE1 beta-site APP-cleaving enzyme 1
CD36 cluster determinant 36
CNS central nervous system
DNA deoxyribonucleic acid
HDL high-density lipoprotein
IDE insulin-degrading enzyme
IL-18 interleukin-18
IL-1α interleukin-1α
IL-1β interleukin-1β
MAPK mitogen-activated protein kinase
MMP-9 matrix metalloproteinase 9
NADPH nicotinamide adenine dinucleotide phosphate, reduced form
NCBI National Center for Biotechnology Information
NEP neprilysin
NF-κB nuclear factor kappa B
NIH National Institutes of Health
NLM National Library of Medicine
NLRP1 NOD-like receptor family, pyrin domain containing 1
NLRP3 NOD-like receptor family, pyrin domain containing 3
NOD nucleotide binding oligomerization domain
NRF2 nuclear factor erythroid 2-related factor 2
p130Cas Cas scaffolding protein family member 1
PPARγ peroxisome proliferator-activated receptor gamma
ROS reactive oxygen species
SCARA class A scavenger receptor
SCARA-1 class A1 scavenger receptor
SCARB-1 class B1 scavenger receptor
SCARB-2 class B2 scavenger receptor

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TLR toll-like receptor
TSP-1 thrombospondin 1
VEGF vascular endothelial growth factor

Mini-dictionary of terms

Abeta Absence of the α-secretase cleavage of amyloid precursor protein (APP) leads to the internalization of APP molecules into endocytic compartments where they are subjected to cleavage by β- and γ-secretases to generate abeta (Aβ40 and Aβ42).

Alois Alzheimer Alois Alzheimer (June 14, 1864—December 19, 1915) was a German psychiatrist and neuropathologist, coworker of Emil Kraepelin, who first described the symptoms of a disease now known as Alzheimer's disease. In 1901, a 51-year-old Mrs. Auguste Deter was admitted to the hospital in Frankfurt am Main with signs of dementia. In 1903, Alzheimer left Frankfurt and moved to the Royal Psychiatric Clinic, Munich, which was headed by Emil Kraepelin. Alzheimer reported about Mrs. Deter on November 3, 1906, at the 37th Meeting of South-West German Psychiatrists in Tübingen in a lecture titled, "*Über einen eigenartigen, schweren Erkrankungsprozeß der Hirnrinde*" ("On a peculiar serious disease process of the cerebral cortex"). His report noted distinctive plaques and neurofibrillary tangles in the brain histology. The case of Mrs. Auguste Deter was published in 1907. In 1910, Kraepelin published the eighth edition of his textbook, *Psychiatrie*, where he included a report on Mrs. Auguste Deter and proposed calling this peculiar illness "Alzheimer's disease." Since then this name has been used to describe the clinical condition. In 1912, Alzheimer became Director of the Psychiatric and Neurological Clinic of the Silesian University of Friedrich Wilhelm in Breslau. He is buried in Frankfurt next to his wife.

Gene polymorphisms The human genome is stored in 23 pairs of chromosomes in the form of linear DNA with a total length of 3.1 billion nucleotides, in which 20,203 protein-coding genes are encoded. Four nucleotides—adenine, thymine, guanine, and cytosine—are sequentially combined in a chain that constitutes the structure of DNA; the sequence of the four nucleotides encodes the genetic information. Any one of these nucleotides can be exchanged for another, thus altering the genetic information. Such exchanges are referred to as "polymorphisms." When only one nucleotide is exchanged for another in a particular location, it is called a "single nucleotide polymorphism" (SNP). Combinations of SNPs together with other types of DNA polymorphisms form the basis of genetic differences between individuals. Some SNPs may produce unreadable sequences, thus potentially blocking or severely modifying expression of the encoded protein, while others merely result in a substitution of one amino acid for another producing a "full-length" protein but with a potentially altered function. SNPs can lead directly to a specific condition or, in some cases, alter the risk of ("predispose to") one or more diseases. Each SNP has its number. The "rs" prefix is added to all of the genetic polymorphisms officially recognized by the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH) of the United States Department of Health and Human Services.

Microglia Microglia are a subtype of the glial cells of the central nervous system. Microglia are essential for brain function, having multiple roles in both health and disease. Microglia act primarily as phagocytes (scavenging of cellular debris) but they are also involved in synaptic modeling, production, and secretion of trophic factors and signaling molecules. Activation of microglia can trigger inflammatory cascades potentially leading to cell and tissue damage and adversely affecting learning, memory, and other cognitive functions in the process.

Pyroptosis Pyroptosis is a form of programmed cell death. Pyroptosis is dependent on specific pyroptopic caspases (human caspase-1, caspase-4, caspase-5) to induce cell death. Activation of pyroptopic caspases results in rupture of plasma membrane, and the released cellular cytosol leads to an inflammatory process.

Taste sense It has recently been recognized that the taste receptors in the tongue and oral cavity can specifically perceive not only sweet, sour, savory (salt), bitter flavors, and umami, but also the taste of fatty acids. The CD36 receptor is one of the molecules present on the surface of tongue epithelium that contributes to the taste perception of fatty acids in food.

Introduction

Scavenger receptors are primary pathogen receptors involved in the defense reactions against a variety of pathogens. Scavenger receptors are defined as a family of molecules that share the ability to bind polyanionic ligands. They are structurally unrelated membrane receptors present on the surface of phagocytic cells such as microglia, macrophages, and dendritic cells. Currently, six classes of scavenger receptors are known; however, some scavenger receptors are yet to be categorized (Wilkinson & El Khoury, 2012). Additionally, scavenger receptors play important roles in the development of atherosclerosis and in the mechanisms of tumor growth and metastasis; yet their role in the pathogenesis of neurodegenerative disorders remains virtually unknown.

CD36 is a class B2 scavenger receptor (SCARB-2). It is an 88-kDa membrane glycoprotein present on surfaces of many types of cells. CD36 receptor is expressed in microvascular epithelium, phagocytes, dendritic cells, microglial cells, astrocytes, retinal pigment epithelium, conjunctiva, cornea, hepatocytes, adipocytes, cardiac and skeletal myocytes, etc. (Abumrad & Goldberg, 2016). Various CD36 splice variants have particular functions and are involved in mechanisms of vascular growth, internalization of pathogens (bacteria, fungi), internalization of abeta protein, gustatory perception of fatty acids (Sayed et al., 2015; Šerý et al., 2017), central mechanisms of olfactory processing (Glezer, Bittencourt, & Rivest, 2009) and probably many more.

CD36 receptor has two transmembrane domains, short intracytoplasmic domains of 5–7 and 11–13 amino acids and a large extracellular domain with six conserved cysteines linked in three disulfide bridges (Fig. 4.1). The extracellular domain has many ligand-binding sites. The 93–120 amino acid region forms a binding site for thrombospondin-1. The 155–183 amino acid region is binding oxidized phospholipids, oxidized low-density lipoproteins, etc. The amino acids 146–164 or 145–171 regions are associated with CD36 receptor binding to erythrocytes infected with *Plasmodium falciparum*. The CD36 receptor can interact with the various coreceptors, thereby extending its function. CD36 receptor coreceptors include, for example, some toll-like receptors, integrins, CD9, CD47, CD81, and others (Garcia-Bonilla, Park, & Iadecola, 2014).

Pathogenesis of Alzheimer's disease

The following four hypotheses are among the most commonly discussed as potential explanations of the pathogenesis of Alzheimer's disease: amyloid-β-deposition in brain

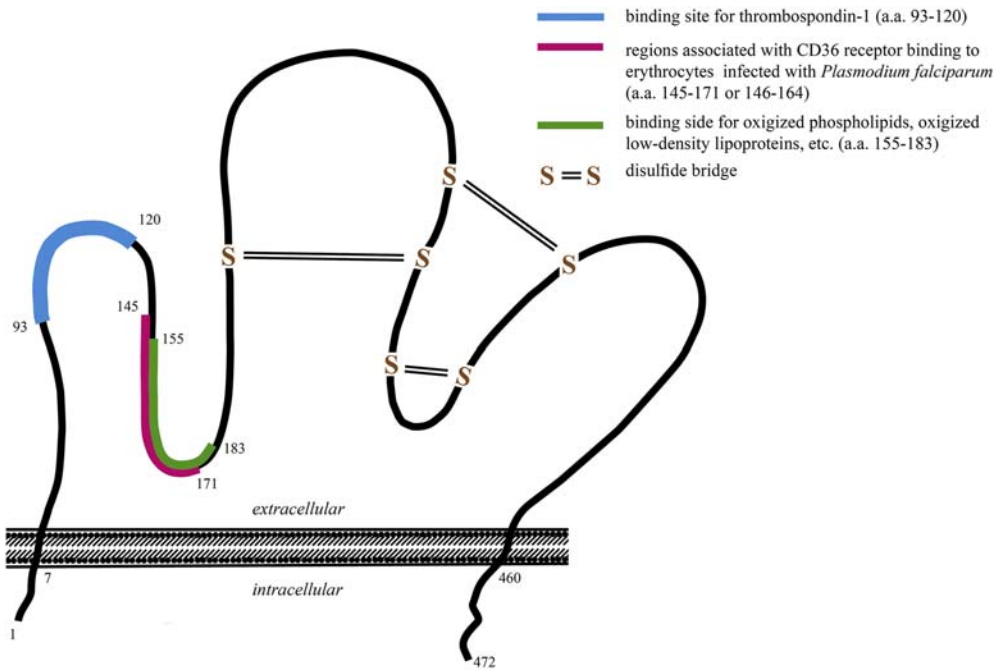

Figure 4.1 ***CD36 receptor structure and the position of binding sites.*** *a.a.,* amino acid.

("amyloid hypothesis"), disturbances of cholesterol metabolism, inflammation, and oxidative stress in the central nervous system (CNS) (Fig. 4.2). Interestingly, CD36 receptor is known to be involved in all of the above processes (Fig. 4.3). The present review focuses on the four hypotheses while examining potential roles of CD36 receptor in each one of them and, in turn, in the pathogenesis of Alzheimer's disease.

Amyloid hypothesis and CD36 receptors

In 1906, Alzheimer's disease was first described as "presenile dementia" by German psychiatrist Alois Alzheimer. Alzheimer analyzed brain of his patient Auguste Deter post mortem and noted "*miliary foci*" in the cortex that are now recognized as senile plaques (amyloid beta or *abeta*). More than 110 years after describing of Alzheimer's disease, two major pathological processes—amyloid beta extracellular deposition and hyperphosphorylated tau protein intracellular accumulation— observed by Alois Alzheimer, remain the hallmarks of AD pathology as well as potential causative factors in the AD-associated neurodegeneration (Šerý, Povová, Míšek, Pešák, & Janout, 2013).

In the CNS, microglia are major phagocytic cells. CD36 receptors are expressed on the surface of microglia and they can bind abeta proteins. It has been shown that astrocytes can also phagocyte abeta proteins, and that this phagocytosis is dependent

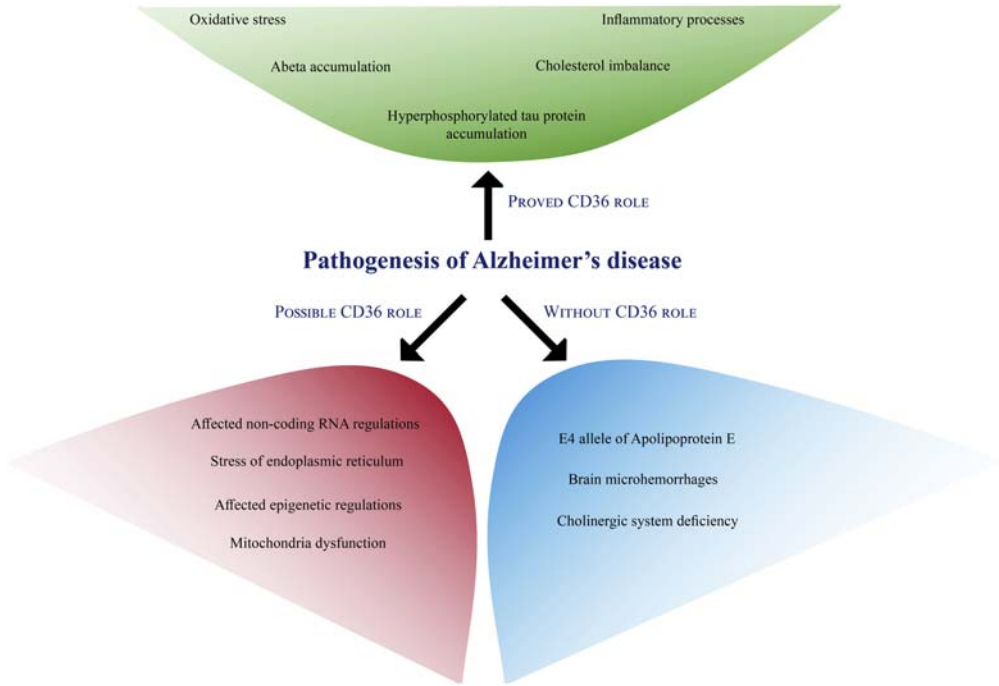

Figure 4.2 ***Major pathologies leading to Alzheimer's disease and its eventual relationship with CD36 receptor.*** Oxidative stress (CD36 receptor is involved in ROS production), abeta accumulation (CD36 expressed on the surface of microglia binds to abeta proteins), hyperphosphorylated tau protein accumulation (CD36 receptor stimulates IL-1β production that induces tau protein phosphorylation), inflammatory processes (CD36 receptor is an activator of inflammasome formation), cholesterol imbalance (CD36 affects blood cholesterol levels), affected noncoding RNA regulations (changes in some microRNAs levels have been associated with Alzheimer's disease), mitochondria dysfunction (CD36 receptor is present at mitochondrial membrane), affected epigenetic regulations (dysregulation of epigenetic modifications related to cytosine), stress of endoplasmic reticulum (protein misfolding leading to neurodegeneration), E4 allele of apolipoprotein E (ApoE4 allele is involved in the regulation of cholesterol levels, and it is also a potent transcription factor regulating expression of various genes involved in inflammation and cell death), brain microhemorrhages (brain microbleeding has been associated with Alzheimer's disease), and cholinergic system deficiency (deficiency in cholinergic system was described in Alzheimer's disease, for a review see Hálová et al. (2018)).

on CD36 receptor (Jones, Minogue, Connor, & Lynch, 2013). CD36 receptor itself does not internalize abeta, but it influences the phagocytosis by other scavenger receptors, e.g., SCARA-1, SCARB-1 (Yang et al., 2011). Ursolic acid is an inhibitor of abeta interaction with CD36 receptor and it could be considered as a potential therapeutic agent in Alzheimer's disease (Wilkinson, Boyd, Glicksman, Moore, & El Khoury, 2011).

Microglia exhibit an age-dependent decrease in the expression levels of CD36 while the presence of abeta in the brain, which is likely to increase with age even in healthy nervous tissue, actually stimulates CD36 expression (Ricciarelli et al., 2004). The abeta

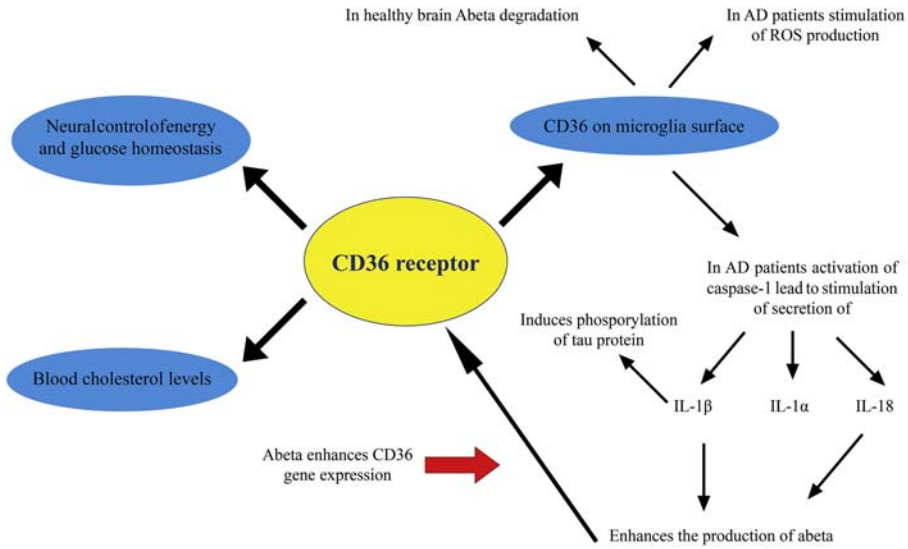

Figure 4.3 ***Overview of the role of CD36 receptor in the pathogenesis of Alzheimer's disease.*** IL-18 (interleukin-18), IL-1α (interleukin-1α), IL-1β (interleukin-1β), ROS (reactive oxygen species).

accumulation in the early stages of Alzheimer's disease would, therefore, increase the expression of CD36 receptor, activating microglia/macrophage in the process and, in turn, facilitating the elimination of abeta deposits. The upregulation of CD36 can thus be viewed as an early protective mechanism against extracellular accumulation of abeta proteins.

The question remains, however, as to the fate of the abeta proteins that are phagocytosed by microglia. Microglia express abeta-degrading enzymes including insulin-degrading enzyme, neprilysin, and matrix metalloproteinase 9, yet inhibitors of these enzymes failed to have any effect on abeta degradation. It has been reported that more than 40% of abeta internalized by SCARA was degraded by cysteine proteases including cathepsin B (Yang et al., 2011).

Binding abeta protein to the CD36 receptor activates a signaling cascade that promotes migration, adhesion, and phagocytosis of microglia. In this cascade, the scaffolding protein p130Cas is rapidly tyrosine-phosphorylated via kinase Fyn eventually colocalizing with CD36 in the cytoplasmic membrane (Stuart et al., 2007).

CNS oxidative stress, inflammatory processes and CD36

The stimulation of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) oxidase-dependent reactive oxygen species (ROS) production, is another mechanism by which microglial CD36 receptor in the brain may act in the pathogenesis of Alzheimer's disease (Coraci et al., 2002; Park et al., 2011). It turns out that at a low

concentration of the abeta protein in the brain, the CD36 receptor has a rather protective role (that is, the abeta protein is degraded by microglia). At later stages of Alzheimer's disease, the accumulation of abeta protein in brain tissue is the most characteristic feature of the pathology. At higher concentrations of abeta protein in brain tissues, as abeta protein further increases CD36 expression, the role of the "protective" receptor is reversed; it now stimulates the production of ROS, thus effectively promoting oxidative damage and actually contributing to the process of neurodegeneration. Furthermore, as the presence of CD36 receptor seems essential for the abeta-induced oxidative stress in the cerebral vasculature (Park et al., 2011), the damage may extend beyond the brain parenchyma, potentially compromising the cerebral blood supply or even the blood–brain barrier.

CD36 receptor, along with toll-like receptor (TLR) heterodimer of TLR4–TLR6, recognize endogenous ligands such as oxLDL and fibrillary abeta and lead to an inflammatory response. CD36 receptor is an activator of inflammasome formed by the cytoplasmic sensor NLRP3 (NOD-like receptor family, pyrin domain containing 3) (Sheedy et al., 2013). Abeta-stimulated NLRP3-inflammasome complex regulates activation of caspase-1. After NLRP3, it recruits apoptosis-associated speck-like protein, which assembles into helical fibrils and in turn recruits procaspase-1, causing its activation to form the mature caspase-1 that cleaves prointerleukin-1β and prointerleukin-18 and stimulate secretion of interleukin-1β (IL-1β), interleukin-1α (IL-1α), and interleukin-18 (IL-18). High expression of caspase-1 was found in the brains of patients with Alzheimer's disease. Inflammasomes cleave precursors of IL-18 and IL-1β to produce their active forms. An increased NLRP1-mediated caspase-1-dependent "pyroptosis" in cultured cortical neurons in response to abeta has been observed in transgenic mice model of Alzheimer's disease (Tan et al., 2014). IL-1β has been associated with various disorders of the CNS that are linked to inflammatory processes (e.g., Alzheimer's disease, Parkinson's disease, multiple sclerosis). IL-1β and IL-18 belong to the group of proinflammatory cytokines that are produced by microglia and astrocytes. IL-1β secreted from astrocytes enhances the production of amyloid-β precursor protein (APP) and abeta from neurons, induces phosphorylation of tau protein, and mediates the formation of neurofibrillary tangles via the MAPK-p38 pathway (Frost & Li, 2017; Sheng et al., 2001). Patients with mild Alzheimer's disease have significantly increased IL-18 levels, but no significant changes have been reported in the levels of IL-18 in patients with severe Alzheimer's disease (Malaguarnera, Motta, Di Rosa, Anzaldi, & Malaguarnera, 2006). IL-18 stimulates increases in APP, beta-site APP-cleaving enzyme 1, N-terminal fragment of presenilin-1 and presenilin enhancer-2 (components of gamma-secretase complex). IL-18 facilitates abeta production via the amyloidogenic pathway.

The expression of CD36 in brain is influenced by a number of factors. The CD36 gene promoter region contains nuclear factor kappa B and peroxisome proliferator-activated receptor γ (PPARγ) response elements. Activation of PPARγ response elements increases

CD36 expression and abeta phagocytosis (Yamanaka et al., 2012). Abeta itself has been shown to upregulate the expression of CD36 (Ricciarelli et al., 2004).

Expression of CD36 on the surface of microglia is regulated by nuclear factor erythroid 2-related factor 2 (NRF2). NRF2 is known to be a part of the antioxidative stress defense mechanisms. Higher levels of NRF2 in the nucleus increase CD36 expression and increase the scavenger effects of CD36 receptor. It has been shown that the expression of NRF2 in brains of Alzheimer's disease patients is significantly lower (Ramsey et al., 2007).

Disturbances of cholesterol metabolism and CD36

An important role of cholesterol in the pathogenesis of Alzheimer's disease has been demonstrated via the action of apolipoprotein E (ApoE), which is a cholesterol transport protein. The ApoE genotype, with known links to the dynamics of cholesterol transport, has been clearly implicated as a significant risk factor for Alzheimer's disease. The ApoE4 allele increases the risk of Alzheimer's disease and lowers the age of onset of cognitive impairment in a dose-dependent manner: the presence of one E4 allele is associated with a 2–3-fold increase in the risk, while two copies of E4 allele are associated with a 5–10-fold increase in the risk of developing AD. ApoE and its genotypes regulate cholesterol levels in the plasma. Elevated cholesterol levels in the brain have also been shown to increase the level of abeta in brain tissue (Chen, Hui, & Geiger, 2014). CD36 affects blood cholesterol levels in a similar way as ApoE (Chien, Hsu, Liu, Lin, & Chen, 2012; Elbers et al., 2012).

Indeed, CD36 genotypes were associated with elevated levels of cholesterol in blood (Yanai, Chiba, Fujiwara et al., 2000a, Yanai, Chiba, Morimoto et al., 2000b). Presence of high-cholesterol levels in blood negatively affects the blood–brain barrier, which becomes more permeable not only to cholesterol but also to toxic substances and pathogens (Freeman & Granholm, 2012).

Taste sensation and CD36 receptor

CD36 receptor is present on the apical surface of circumvallate and foliate papillae in the human tongue and also in the olfactory epithelium (Sundaresan et al., 2012; Xavier & Glezer, 2018). The CD36 receptors in these locations play an important role in the mediation of gustatory and olfactory perception of fatty acids. This is a recently discovered sensory modality; variations (polymorphisms) in the CD36 receptor gene may be responsible for differences in individual perception of fatty acids in food. CD36 receptor gene polymorphisms could, therefore, influence individual preferences for fatty foods, which, in turn, has an impact on fat intake and obesity (Daoudi et al., 2015; Karmous et al., 2018; Mrizak et al., 2015; Plesník, Šerý, Khan, Bielik, & Khan, 2018; Sayed et al., 2015). The CD36 receptor is also present in the proximal small

intestine (enterocytes), where it affects release of secretin and cholecystokinin. Whether or how the CD36 receptor is integrated in the general function (including possible internal food sensing) of the enteric nervous system or in the function of "gut–brain" axis (Furness, 2012) is yet to be determined. There is good evidence, though, that CD36 protein is involved in fatty acid sensing by the brain. As such, CD36 plays an important role in the neural control of energy and glucose homeostasis, including feeding behavior and insulin secretion (Magnan, Levin, & Luquet, 2015).

CD36 gene polymorphisms

CD36 gene (Fig. 4.4) is located on chromosome 7q21.11 and covers 72 kilobases, including 19 known exons. According to the National Center for Biotechnology Information (NCBI) database, there are more than 10,000 DNA polymorphisms in the CD36 gene. Existence of alternatively spliced transcript variants encoding different isoforms of CD36 protein has been well documented in the literature.

DNA polymorphisms and gene mutations of CD36 gene and their relationships to diseases have been studied for the past 25 years. The first mutation in the CD36 gene was found in a Japanese study in patients with type II platelet glycoprotein IV deficiency (Kashiwagi et al., 1993). Later, Yanai, Chiba, Fujiwara et al. (2000a), Yanai, Chiba, Morimoto et al., (2000b) reported that Pro90Ser mutation and insertion of A at nucleotide 1159 were the major causes of type I and II CD36 deficiencies. The relationship between CD36 gene polymorphism, HDL-cholesterol, apolipoprotein A1, and body mass index was reported by Hong et al. (2002). In 1989, Ockenhouse, Tandon, Magowan, Jamieson, and Chulay (1989) described CD36 receptor as a binding site on malaria-infected erythrocytes. Aitman et al. (2000) described mutations of CD36 receptor related to malaria susceptibility in African populations. Ma et al. (2004) described the relationship between haplotypes composed of five CD36 gene polymorphisms and increased fasting levels of free fatty acids, triglycerides, and coronary artery disease. Kuriki et al. (2005) investigated the association between CD36 polymorphism and meat consumption

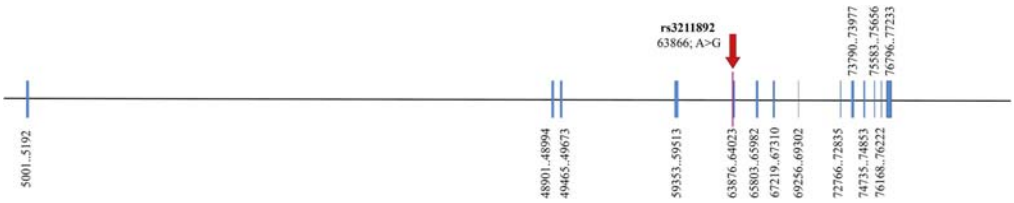

Figure 4.4 ***Schematic illustration of the CD36 gene.*** Exons according to transcript variant 2 are displayed as blue stripes (NCBI Reference Sequence NG_008192.1). The position of rs3211892 polymorphism, which is known to be significantly associated with the risk of Alzheimer's disease (Šerý et al., 2017), is marked by the *red arrow*.

preference. It was the first study that revealed the relationship between CD36 receptor and taste preference. Other studies have found the association between the CD36 gene polymorphisms and insulin resistance, metabolic syndrome, type 2 diabetes mellitus, cardiovascular risk, acute myocardial infarction, obesity, plasma vitamin E concentration, left ventricular mass, as well as intraocular pressure elevation following intravitreal application of anti-VEGF agents (Furuhashi, Ura, Nakata, & Shimamoto, 2003; Hall et al., 2011; Lecompte et al., 2011; Lopez-Carmona et al., 2017; Love-Gregory et al., 2008; Matušková et al., 2018; Rać et al., 2012). The team of Nada Abumrad were the first to describe the relationship between CD36 gene polymorphism and oral sensitivity to fat (Pepino, Love-Gregory, Klein, & Abumrad, 2012). Since then, several teams led by the team of Naim Khan have investigated the taste of fatty acids and polymorphisms of the CD36 gene (Daoudi et al., 2015; Karmous et al., 2018; Mrizak et al., 2015; Plesník et al., 2018; Sayed et al., 2015). An association between Alzheimer disease and rs3211892 polymorphism of CD36 gene was originally described by our team (Šerý et al., 2017).

The research outlined above strongly suggests the role of the CD36 receptor in the pathogenesis of Alzheimer's disease. From this, the influence of different polymorphisms or mutations of the CD36 receptor gene in the pathogenesis of Alzheimer's disease can be deduced. It is necessary to consider not only polymorphisms in exons but also polymorphisms in regulatory regions of the CD36 gene. Therefore, sequencing of the entire CD36 gene in Alzheimer's disease patients is important, as it will lead to the identification of all the polymorphisms that could alter the risk of Alzheimer's disease.

Conclusions and future directions

The scavenger receptor CD36 is involved in mechanisms of vascular growth, internalization of pathogens (bacteria, fungi), internalization of abeta protein, and gustatory perception of fatty acids. Disturbances of CD36-related processes could also contribute to the development of Alzheimer's disease. At high abeta levels, the CD36 stimulates ROS production via NADPH oxidase activation and secretion of IL-1β, IL-1α, and IL-18. Therefore, inflammasome-mediated pyroptosis could be important in the pathogenesis of Alzheimer's disease.

From the above, it should be clear that polymorphisms or mutations of the CD36 receptor genes should be further investigated and evaluated with respect to the risk of Alzheimer's disease. Optimally, one should consider not only the polymorphisms in exons but also the polymorphisms in regulatory regions of the CD36 gene, which may have an impact on the CD36 expression (and abundance) in specific cells, organs, and/or brain regions. We propose that sequencing of the entire CD36 gene in Alzheimer's disease patients should be carried out, aiming specifically at identification of all associations between CD36 gene polymorphisms and the risk of Alzheimer's disease.

Key facts of CD36 receptor in brain and Alzheimer disease

- CD36 receptors are expressed on the surface of microglia and they can bind abeta proteins;
- Astrocytes are able to phagocyte abeta proteins, and this phagocytosis is driven by CD36 receptors;
- CD36 receptor itself does not internalize abeta into microglia but it influences the phagocytosis by other scavenger receptors;
- Promotion of neurodegeneration in the brain: via stimulation of reactive oxygen species (ROS) production;
- Stimulation of production of inflammatory cytokines: this could lead to progression of Alzheimer's disease

Summary points

- This chapter focuses on the role of CD36 receptor in the pathogenesis of Alzheimer's disease
- CD36 is class B scavenger receptor
- CD36 receptor itself does not internalize abeta into microglia but it influences the phagocytosis by other scavenger receptors
- At high abeta levels, the CD36 stimulates ROS production via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and secretion of IL-1β, IL-1α, and IL-18
- Inflammasome mediated pyroptosis could be important in the pathogenesis of Alzheimer's disease
- CD36 receptor affects blood cholesterol levels
- Different polymorphisms or mutations of the CD36 receptor gene are involved in the pathogenesis of Alzheimer's disease
- It is necessary to consider not only the polymorphisms in exons but also polymorphisms in regulatory regions of the CD36 gene to assess their role in the development of Alzheimer's disease.

Acknowledgments

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CHAPTER 5

Genetic contributions to sporadic frontotemporal dementia

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List of abbreviations

AD Alzheimer's disease
ALS amyotrophic lateral sclerosis
bvFTD behavioral variant frontotemporal dementia
CBD corticobasal degeneration
CBS corticobasal syndrome
FET FUS/EWS/TAF15
FTD frontotemporal dementia
FTLD frontotemporal lobar degeneration
GWAS genome-wide association study
HLA human leukocyte antigen
MND motor neuron disease
nfvPPA nonfluent variant of primary progressive aphasia
PPA primary progressive aphasia
PSP progressive supranuclear palsy
PSP-S progressive supranuclear palsy syndrome
SNP single-nucleotide polymorphism
svPPA semantic variant of primary progressive aphasia
UPS ubiquitin-proteasome system

Mini-dictionary of terms

Autosomal dominant a form of genetic inheritance in which an individual inheriting a single copy of a pathogenic variant will develop the disease
Compound heterozygous when both copies of a given gene each harbor a distinct variant
Familial disease that occurs in the context of a family history of neurodegenerative or psychiatric disease and/or dementia syndrome (depending on the study)
Frontotemporal dementia a clinical diagnosis for a heterogeneous group of disorders presenting with behavioral and/or language abnormalities
Frontotemporal lobar degeneration a neuropathological diagnosis of frontal and temporal lobe atrophy due to underlying FTLD pathology (e.g., FTLD-tau, FTLD-TDP, FTLD-FET, FTLD-UPS)
FTD-disease spectrum a range of diseases including FTD clinical diagnoses (bvFTD, svPPA, nfvPPA) as well as FTD-MND, CBS, and PSP-S, which share similar underlying neuropathology
Genome-wide association studies studies that detect common genetic variants associated with disease by comparing the variant frequencies between disease cases and controls

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Missing heritability the portion of disease cases for which a genetic cause is expected (i.e., due to family history) but not known

Neuropathology examination upon autopsy of the degeneration of brain structures and/or accumulation of pathological proteins in various cell types

Penetrance the degree to which a genetic variant exerts its effect (e.g., "100% penetrant" means every individual carrying that variant will develop the disease)

Single-nucleotide polymorphism a genetic difference between individuals or chromosomes at a single nucleotide

Sporadic disease that occurs in the absence of known family history of neurodegenerative or psychiatric disease and/or dementia syndrome (depending on the study)

Variant a genetic difference at a particular locus/position between individuals (which may be pathogenic, benign, or of uncertain significance)

Accurate diagnosis of neurodegenerative disease is currently achieved only through postmortem brain autopsy. Thus, we rely on clinical presentation and syndromic clinicopathological relationships to deduce the most likely underlying disease etiology. Frontotemporal lobar degeneration (FTLD) encompasses a group of neuropathological diagnoses that predominantly affect the frontal and temporal lobes of the brain. Individuals with FTLD pathology usually present with clinical syndromes within the frontotemporal dementia (FTD) spectrum. FTD is an umbrella term for a complex, clinically heterogeneous group of diagnoses characterized by changes in behavior, movement, and/or language and cognitive function resulting from neurodegeneration of the frontal and temporal lobes of the cerebral cortex. In autosomal-dominant genetic forms of FTLD, there are clear links to specific neuropathology that provide insight into disease etiology. However, in nonfamilial ("sporadic") forms of disease, we rely on our ability to characterize clinical and biomarker features to deduce the most likely underlying disease pathology, which may inform prognosis and could dictate therapeutic treatment in the future.

In this chapter, we will provide an overview of the field's current knowledge of genetic contributions to clinical FTD syndromes, particularly in sporadic disease, and how these relate to FTLD pathobiology.

Heterogeneous clinical presentations and underlying neuropathology complicate the identification of genetic causes of disease

In genetic studies of familial neurodegenerative disease, pathogenic variants are typically linked to particular protein pathology, which in turn is associated with a relatively predictable clinical outcome. For example, *APP*, *PSEN1*, or *PSEN2* variants lead to alterations in amyloid processing and cause Alzheimer's disease (AD), which is characterized by amyloid-β as well as tau pathology. In the case of FTD spectrum disorders, however, the relationship between gene, neuropathology, and clinical presentation is

much less clear. While genes that contribute to FTD risk are associated with specific FTLD pathologies, they are often implicated in a variety of clinical presentations, likely due to the accumulation of pathology in specific brain regions responsible for the functions lost in a given clinical FTD presentation. Furthermore, most of these individual clinical presentations can be caused by a variety of underlying genetic factors (Fig. 5.1). For example, disease-associated *MAPT* variants give rise to tau pathology but can present clinically as behavioral-variant FTD (bvFTD), corticobasal syndrome (CBS), progressive supranuclear palsy syndrome (PSP-S), primary progressive aphasia (PPA), FTD-motor neuron disease (MND), or AD-like presentations. bvFTD, in turn, can be caused by tau, TDP-43, FUS/EWS/TAF15 (FET; sometimes referred to simply as FUS), or ubiquitin-proteasome system (UPS) pathology, and each of these pathologies may arise from a multitude of genetic etiologies. Complicating matters further, cases with several distinct underlying neuropathologies are not rare, and patients with so-called frontal variants of other neurodegenerative diseases may meet clinical criteria for FTD diagnosis

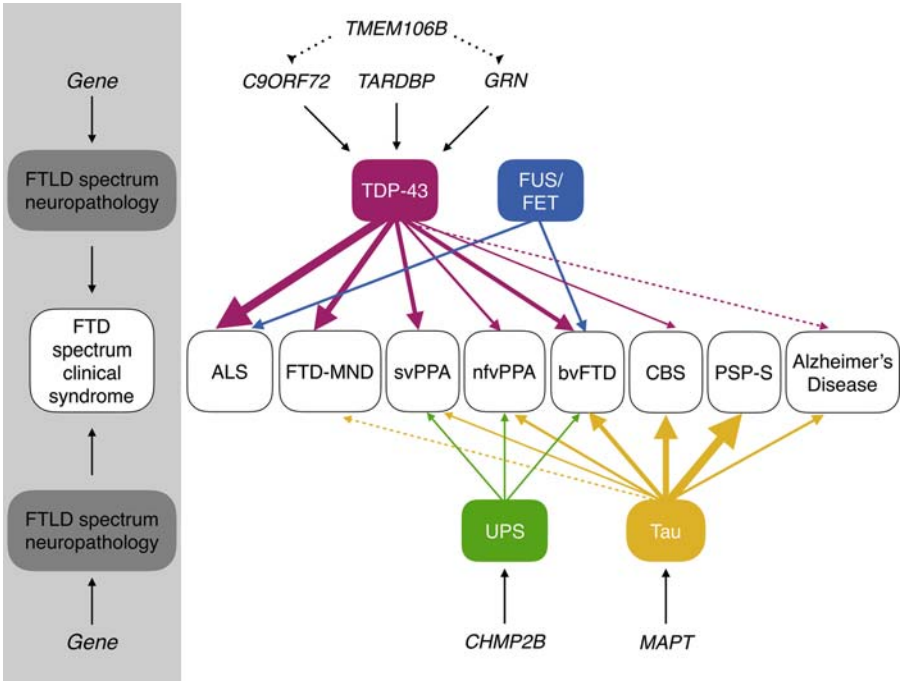

Figure 5.1 ***Genetic and neuropathological contributors to clinical presentation of frontotemporal dementia (FTD)-spectrum diseases.*** The variety of clinical presentations of FTD-spectrum diseases (*white boxes*) can be due to several underlying neuropathologies (*colored boxes*), which in turn can be caused by pathogenic variants in a number of genes (*italics*). Size of *arrow* indicates approximate frequency of pathology in given clinical presentation.

but have distinct underlying protein pathology; a frontal presentation of AD, for example, may account for up to 17% of clinical FTD diagnoses (Mackenzie & Neumann, 2016). The convoluted relationship between clinical presentation, underlying neuropathology, and genetic variation complicates studies that attempt to identify genetic contributions to this group of diseases. Simply put, the phenotypic focus of a genetic study will define its results, interpretation, and contribution to the field's understanding of the corresponding biology. For example, studying a clinical diagnosis (such as bvFTD) will help elucidate the biology that leads to selective vulnerability of the brain networks resulting in that particular clinical syndrome (Fig. 5.2A). Conversely, studying a pathological diagnosis (such as FTLD-tau) will provide insight into the biology of the underlying disease pathology (e.g., protein and/or type and/or location of inclusion), which may manifest as one or more clinical syndromes depending on where the pathology is located in the brain (Fig. 5.2B). In this framework, the cohort being studied will affect our understanding of genetic contributions to disease. This is an important point to keep in mind when interpreting results of genetic studies and how they relate to other studies in the field.

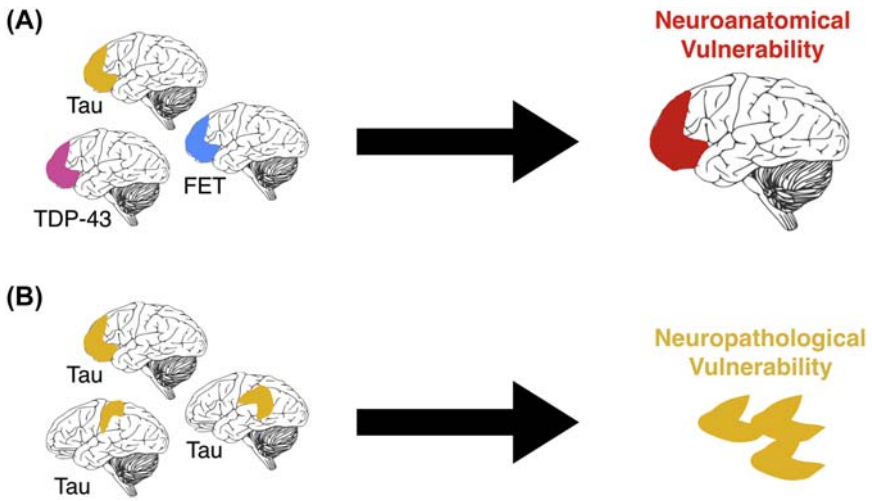

Figure 5.2 ***Study design dictates relevance and interpretation of genetic findings to underlying disease biology.*** (A) In studies of a clinical syndrome, the patient cohort may reflect a variety of underlying neuropathologies. Genetic associations with the clinical syndrome may therefore identify variation that contributes to vulnerability of the affected neuroanatomical regions and their associated functions. (B) In studies of broad classes of protein pathology, the disease cohort may reflect a variety of clinical syndromes that all result from common protein pathology occurring in distinct brain regions. Genetic associations with protein pathology therefore indicate variation that contributes to aggregation of that particular protein.

Clinical presentation defines syndromic diagnosis of frontotemporal dementia subtype

The FTD spectrum consists of three FTD subtypes—bvFTD, semantic variant of PPA (svPPA), and nonfluent/agrammatic variant of PPA (nfvPPA)—characterized by clinical presentation as well as several other disorders with similar symptomatology and shared neuropathology (Fig. 5.3). Separate from clinical presentation, FTLD is subdivided based on the type of neuropathology present upon autopsy, of which there are four main subtypes: tau, TDP-43 (TAR DNA-binding protein 43), FET, or UPS (Mackenzie & Neumann, 2016). The majority of FTLD cases have either tau (45%) or TDP-43 (45%) inclusions, while a smaller proportion are characterized by FET (9%) or UPS (1%) (Ling, Polymenidou, & Cleveland, 2013). While several different neuropathologies may be implicated in a given clinical presentation, the brain regions affected drive clinical presentation of each FTD subtype as described below.

bvFTD, historically called Pick's disease, accounts for approximately half of all FTD cases. bvFTD is characterized by changes in behavior such as disinhibition, apathy, loss of empathy, stereotyped or compulsive behaviors, dietary changes, and/or deficits in executive function with comparative sparing of the patient's memory and visuospatial

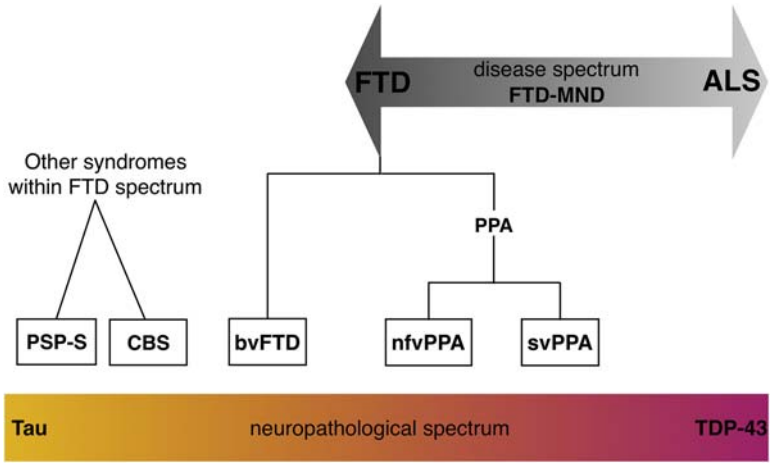

Figure 5.3 ***Clinical diagnoses within the frontotemporal dementia (FTD) disease spectrum.*** FTD exists on a disease spectrum with motor neuron disease (MND), with pure FTD and pure amyotrophic lateral sclerosis (ALS) representing the ends of the spectrum. FTD can be subdivided into the behavioral variant form (bvFTD) when presenting with behavioral symptoms, or primary progressive aphasia (PPA) when presenting with language symptoms. PPA can be further subdivided into a nonfluent variant (nfvPPA) or semantic variant (svPPA) based on the type of language dysfunction. Progressive supranuclear palsy syndrome (PSP-S) and corticobasal syndrome (CBS) are considered part of the FTD-disease spectrum and present with additional motor symptoms. FTD-spectrum diseases usually have underlying TDP-43 or tau pathology as shown by the neuropathological spectrum bar.

skills (Woollacott & Rohrer, 2016). bvFTD is associated with atrophy of the frontal lobes, insula, anterior cingulate, and anterior temporal lobes and is roughly symmetrical across hemispheres (Sieben et al., 2012). A study of 117 autopsied cases meeting "possible" to "definite" criteria for bvFTD found that approximately 30% had underlying FTLD-tau pathology, while ~50% had FTLD-TDP pathology and less than 7% had FTLD-FET pathology; the remainder of cases were explained by other pathologies, such as AD (Perry et al., 2017). Notably, FET pathology is only associated with bvFTD (with or without MND) and no other clinical presentations of FTD (Sieben et al., 2012).

FTD patients who present with initial problems in language rather than behavior are diagnosed with PPA, which can be further divided into two subcategories. svPPA is characterized by fluent speech with poor single-word comprehension and is sometimes referred to as semantic dementia. svPPA is associated with atrophy of the anterior inferior temporal lobe (usually with one side more predominant), and TDP-43 is usually the underlying neuropathology, though tau pathology can also result in svPPA (Sieben et al., 2012). nfvPPA is typified by slow, effortful speech with early sparing of word comprehension and is sometimes called progressive nonfluent aphasia (Gorno-Tempini et al., 2011). nfvPPA is associated with atrophy of the anterior perisylvian cortex, usually in the patient's dominant hemisphere, and is most often associated with underlying tau pathology (Sieben et al., 2012). A third type of PPA, logopenic variant PPA, is not generally considered part of the FTD spectrum, as it usually results from underlying AD pathology (amyloid-β and tau) (Woollacott & Rohrer, 2016).

Two other diagnoses-CBS and PSP-S-fall into the broader category of FTD spectrum disorders and are often associated with underlying neuropathological features found in other forms of FTD. These syndromes, the majority of which are due to underlying tau pathology including corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), are characterized by movement dysfunction in addition to changes in language and/or behavior.

Beyond the clinical heterogeneity within the FTD diagnoses just described, FTD itself is considered to exist on a disease spectrum with amyotrophic lateral sclerosis (ALS), a type of MND. Historically, FTD and ALS were thought to be separate diseases, but the relatively recent discovery of shared genetic risk factors and the high prevalence of motor neuron involvement in FTD patients (and behavioral features in ALS) have made it clear that these diseases are linked in terms of both underlying neuropathology and clinical presentation.

Defining familial and sporadic disease

For reasons of practicality, most studies focus on clinically diagnosed FTD cases for which neuropathological status is most often unknown. These clinical cases are generally divided into two categories based on the family history of the presenting patient: "familial" cases in which there is a history of FTD or related neurodegenerative disorders in the close relatives of the patient, or "sporadic" cases in which there is no known family

history. The three primary FTD clinical subtypes vary in their likelihood of being inherited: bvFTD has the highest heritability, followed by nfvPPA, with svPPA patients having the lowest incidence of family history (Deleon & Miller, 2018). As expected, familial cases have a much higher frequency of pathogenic variants in known FTLD-causing genes, such as *MAPT, GRN*, and *C9ORF72*. Approximately 25%–50% of familial cases (and 10%–30% of overall FTD cases) can be characterized by an autosomal-dominant mode of inheritance (Blauwendraat et al., 2018; Goldman et al., 2005; Rascovsky et al., 2011; Rohrer et al., 2009; Rosso et al., 2003; Seelaar et al., 2008); other familial cases have a family history with a less clear pattern of disease segregation, often complicated by variability of clinical presentations even within individual families (Pottier, Ravenscroft, Sanchez-Contreras, & Rademakers, 2016). Despite the higher frequency of known disease-causing genes in familial cases, there remains a large degree of "missing heritability" in familial FTD. A recent unbiased genetic analysis of 121 consecutive FTD subjects in Germany found that only 34% of familial cases carried a pathogenic variant in a known FTLD-associated gene (Blauwendraat et al., 2018). In the same study, 11% of sporadic cases (in which zero first- or second-degree relatives were affected by any neurodegenerative disease) carried pathogenic or likely pathogenic variants; other studies have estimated this percentage at ~6% (Rademakers, Neumann, & MacKenzie, 2012). It is important to keep in mind that the percentages referenced throughout this chapter are the distillation of several individual reports indicating prevalence of pathogenic variants identified in a select case series often from a single center; ranges, rather than specific values, are thus presented in order to provide the reader with the best estimate available based on current literature.

Despite their widespread usage, the terms "familial" and "sporadic" are inherently problematic and contribute to variability between studies. For example, sporadic disease may arise in the context of lack of knowledge of family history. Sporadic disease may also reflect disease that is in fact familial but that presents with incomplete penetrance, which would mask the effect of disease-associated genes in some gene-carrier relatives. Moreover, the definition of familial can range by study from a first-degree relative with confirmed FTLD to a general family history (including second- and third-degree relatives) with any neurodegenerative and/or psychiatric disease (particularly for studies of bvFTD). Despite the intrinsic flaws with this terminology, many studies use familial and sporadic designations, and we focus on sporadic disease in this chapter.

Genetics of sporadic disease: involvement of established familial frontotemporal lobar degeneration genes

We will first review the most common genetic contributors to familial FTLD, as pathogenic variants in these genes have also been identified in individuals with sporadic disease (Fig. 5.4). As in other neurodegenerative diseases such as AD, a minority (~25%–45%) of FTD cases have a positive family history. Nevertheless, identification

of genes explaining familial forms of FTLD has greatly informed research within the field, particularly within the context of therapeutic development.

Pathogenic variation within the *MAPT* gene, which encodes for microtubule-associated protein tau, was discovered in families with chromosome 17-linked FTD in 1998 (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). Mutations in *MAPT* result in aggregation and/or hyperphosphorylation of tau or an imbalance of the three-repeat and four-repeat splice variants of tau (Deleon & Miller, 2018). While tau inclusions are present in 45% of FTLD cases, *MAPT* variants are associated with only a low frequency of sporadic FTD, with estimates ranging from 0% to 4% (Table 5.1), suggesting that variation in genes beyond *MAPT* can promote tau pathology. Pathogenic variants in *MAPT* are most commonly associated with bvFTD, though they have also been found in patients with nfvPPA, CBS, PSP-S, and a single case of svPPA (Deleon & Miller, 2018). Pathogenic variation within *GRN* (encoding progranulin protein), which is also on chromosome 17, was discovered in 2006 (Baker et al., 2006; Cruts et al., 2006). All *GRN* pathogenic variants are thought to result in haploinsufficiency of the progranulin protein. *GRN* pathogenic variants are implicated in ~1%–4% of sporadic cases (Table 5.1), most of which present with bvFTD, though *GRN* variant carriers can also present with nfvPPA, CBS, and FTD-MND (Deleon & Miller, 2018). Five years after the discovery of shared TDP-43 pathology in FTLD and ALS

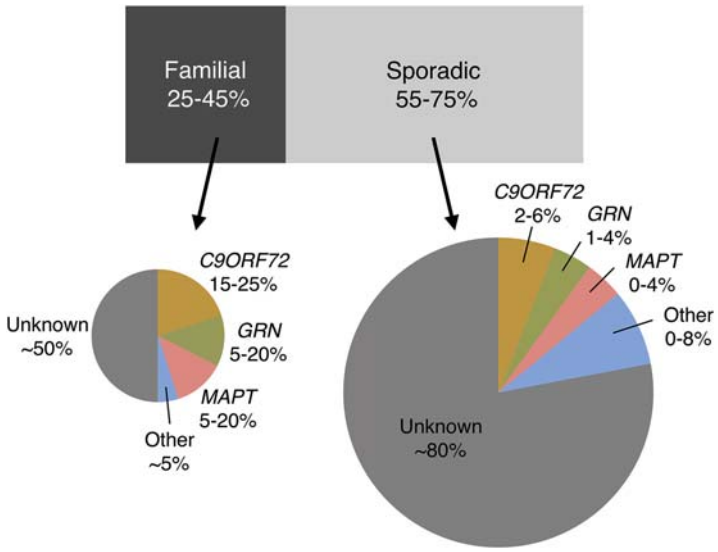

Figure 5.4 ***Genetic contributions to familial and sporadic frontotemporal dementia (FTD).*** Approximately 25%–45% of FTD cases are familial while the remaining cases (55%–75%) are sporadic. Familial FTD cases are most commonly caused by pathogenic variation in *C9ORF72*, *GRN*, or *MAPT*, though some cases are due to other known genes and some do not have a known genetic cause. Most sporadic FTD cases do not have a known underlying genetic variant. However, *C9ORF72*, *GRN*, *MAPT*, and other genes have been implicated in sporadic cases.

Table 5.1 Selected studies assessing frequencies of pathogenic variants in sporadic frontotemporal dementia (FTD).

Gene		Frequency in sporadic FTD
Reference	**Cohort description**	
MAPT		**~0–4%**
Houlden et al. (1999), Rizzu et al. (1999), Poorkaj et al. (2001), Binetti et al. (2003), Pickering-Brown et al. (2008), Blauwendraat et al. (2018)	380 sporadic FTD cases (across 6 studies)	0%
Che et al. (2017)	82 sporadic Chinese FTD cases	1.2% (1/82)
DeJesus-Hernandez et al. (2011)	203 sporadic FTD cases	1.5% (3/203)
Tang et al. (2016)	45 sporadic Chinese FTD cases	2.2% (1/45)
Stanford et al. (2004)	25 sporadic FTD or FTD-MND cases	4% (1/25)
GRN		**~1–4%**
Gass et al. (2006)	234 sporadic FTD cases[a]	0.85% (2/234)
Tang et al. (2016)	45 sporadic Chinese FTD cases	2.2% (1/45)
Che et al. (2017)	82 sporadic Chinese FTD cases	2.4% (2/82)
DeJesus-Hernandez et al. (2011)	203 sporadic FTD cases[b]	3.0% (6/203)
Le Ber et al. (2007)	158 sporadic FTD cases	3.2% (5/158)
Blauwendraat et al. (2018)	73 sporadic German FTD cases	4.1% (3/73)
C9ORF72		**~2–6%**
Simón-Sánchez et al. (2012)	224 sporadic Dutch FTD cases	2.2% (5/224)
Blauwendraat et al. (2018)	73 sporadic German FTD cases	2.7% (2/73)
DeJesus-Hernandez et al. (2011)	203 sporadic FTD cases[c]	3.0% (6/203)
Mahoney et al. (2012)	163 sporadic FTD-spectrum cases from UCL	3.7% (6/163)
Majounie et al. (2012)	981 sporadic white European FTD cases	6.0% (59/981)
TARDBP		**~0–1%**
Blauwendraat et al. (2018)	73 sporadic German FTD cases	0%
Borroni et al. (2010)	172 sporadic FTD cases	1.2% (2/172)
Benajiba et al. (2009)	78 sporadic French FTD-MND cases	1.3% (1/78)
UBQLN2		**~1–3%**
Blauwendraat et al. (2018)	73 sporadic German FTD cases	1.4% (1/73)
Synofzik et al. (2012)	36 sporadic German FTD cases	2.8% (1/36)
VCP		**Rare-3%**
Bersano et al. (2009)	1 Italian case with sporadic IBM-PDB-FTD	Rare
Shi et al. (2016)	38 Chinese FTD cases[d]	2.6% (1/38)
CHCHD10		**~1%**
Dols-Icardo et al. (2015)	342 sporadic FTD cases	0.3% (1/342)
Blauwendraat et al. (2018)	73 sporadic German FTD cases	1.4% (1/73)

Continued

Table 5.1 Selected studies assessing frequencies of pathogenic variants in sporadic frontotemporal dementia (FTD).—cont'd

Gene		Frequency in sporadic FTD
Reference	**Cohort description**	
CHMP2B		**Rare**
Skibinski et al. (2005)	311 sporadic European FTD cases	0.3% (1/311)
TBK1		**Rare**
Gijselinck et al. (2015)	350 sporadic Belgian FTD cases	0.3% (1/350)

Description of selected studies assessing frequencies of pathogenic variants in *MAPT*, *GRN*, *C9ORF72*, *TARDBP*, *UBQLN2*, *VCP*, *CHCHD10*, *CHMP2B*, and *TBK1* in cohorts including cases of sporadic FTD. Studies focused on a particular patient group (e.g., "German") are mentioned in the table.
[a]*GRN* variants in 7/234 = 3% if including "not documented" family history.
[b]Also includes 53 sporadic FTLD-TDP cases of which *GRN* is responsible for 15% (8/53).
[c]Also includes 53 sporadic FTLD-TDP cases of which *C9ORF72* is responsible for 15% (8/53).
[d]26% of entire cohort (including AD cases) was familial but unclear what percentage of FTD cases were familial versus sporadic.

(Neumann et al., 2006), a shared genetic cause of both diseases was identified—an expanded GGGGCC repeat in *C9ORF72* (DeJesus-Hernandez et al., 2011; Renton et al., 2011). *C9ORF72* encodes a protein that putatively acts as a Rab GTP-GDP exchange factor. Unaffected individuals typically harbor 2–23 hexanucleotide repeats within the first intron of *C9ORF72*, but expansions in repeat lengths above the pathogenic threshold of ~30–35 (the threshold varies by study) segregate with FTD/ALS, and patients can have up to 700–1600 repeats (DeJesus-Hernandez et al., 2011). This repeat has been hypothesized to contribute to FTD/ALS pathogenicity through a variety of mechanisms including haploinsufficiency and toxic gains of function (reviewed in Ling, Polymenidou, & Cleveland, 2013). Expanded *C9ORF72* repeats are the most common single-gene explanation for both familial and sporadic cases, accounting for 2%–6% of sporadic FTD cases (Table 5.1), and carriers typically present with bvFTD, FTD-MND, or ALS, though some variant carriers have been diagnosed with nfvPPA or CBS as well as rare cases of svPPA (Deleon & Miller, 2018).

Together, *MAPT*, *GRN*, and *C9ORF72* are estimated to account for the vast majority of familial FTLD cases with a known genetic cause, though some familial cases do not have pathogenic variants in any known FTD-associated genes. While pathogenic variants in these genes are most common in the context of autosomal-dominant familial FTD, they also occur in families without a clear autosomal-dominant mode of inheritance and in sporadic FTD, as will be described in the next section (Fig. 5.4). A proportion of familial cases can be explained by pathogenic variants in other genes, including *TARDBP, UBQLN2*, *SQSTM1*, *VCP*, *CHCHD10, CHMP2B, TIA1,* and *TBK1.* However, many familial cases still do not have a known genetic cause, indicating that there are unidentified genetic factors contributing to familial disease.

Genetics of sporadic disease: involvement of rare familial disease genes

Several genes beyond *MAPT*, *GRN*, and *C9ORF72* have been implicated in sporadic FTD, though generally less frequently than the variants previously described (Table 5.1). While more often associated with ALS, pathogenic variants within *TARDBP* have also been implicated in familial and sporadic FTD with or without motor symptoms. *TARDBP* encodes the TDP-43 protein, the primary pathological hallmark of ALS, that is also present in ~45% of FTLD cases. Suspected pathogenic variants in *TARDBP* have been found in ~1% of sporadic cases in several European cohorts (Benajiba et al., 2009; Borroni et al., 2010). Initially identified in family studies of ALS patients with dementia, *UBQLN2* (which encodes ubiquitin-like protein ubiquilin-2, a component of the UPS) has been found in sporadic (but not familial) cases of FTD, accounting for ~1%–3% of sporadic cases (Blauwendraat et al., 2018; Synofzik et al., 2012). *VCP*, also involved in the ubiquitin-proteasome pathway, is implicated in autosomal-dominant inheritance of inclusion body myopathy with Paget disease of the bone and FTD but has also been found in rare cases of sporadic FTD (Bersano et al., 2009), including in non-European populations (Shi et al., 2016). Another gene involved in ubiquitination, *CCNF*, has also been implicated in sporadic FTD cases (Williams et al., 2016). *CHCHD10*, which encodes a mitochondrial protein and has been implicated in familial FTD and ALS, has been shown to account for approximately 1% of sporadic cases in several studies (Blauwendraat et al., 2018; Dols-Icardo et al., 2015; Zhang et al., 2015). Pathogenic variants within *CHMP2B*, encoding a protein involved in endosomal sorting, were initially identified in a Danish family with autosomal-dominant FTD but have also been found in a very small proportion (0.3%) of sporadic cases (Skibinski et al., 2005). Similarly, pathogenic *TBK1* variants were first identified in Belgian families with FTD and/or ALS and subsequently identified in rare (0.3%) sporadic cases of FTD as well (Gijselinck et al., 2015). *TBK1* encodes for a kinase involved in inflammation and autophagy that phosphorylates several downstream targets, including optineurin; variants in *OPTN* have also been linked to sporadic FTD (Pottier et al., 2015). Pathogenic variants in other genes, such as *SQSTM1* (Rubino et al., 2012), have been linked to FTD in cohorts that included a large proportion of sporadic cases, but without a detailed reporting of the presence/absence of family history in variant carriers, it remains unclear whether these variants are responsible for sporadic cases of disease. Recent studies have identified homozygous or compound heterozygous (a different pathogenic variant on each chromosome) variants in *TREM2* as associated with bvFTD, including in seemingly sporadic cases (Peplonska et al., 2018), and a recent meta-analysis found significant associations between two single-nucleotide polymorphisms (SNPs) in *TREM2* and FTD risk (Su et al., 2018). Rare variation in *TREM2*, which encodes a

receptor on myeloid cells including microglia, has been studied extensively for its contribution to AD risk, and homozygous variation in *TREM2* can cause Nasu Hakola disease, a disease including early-onset FTD symptoms. The identification of *TREM2* variants associated with FTD risk supports the role of immune dysfunction in FTD disease pathogenesis and highlights how some genes contribute to multiple neurodegenerative diseases (genetic pleiotropy).

Common variant risk factors: genome-wide association studies

Despite the complexity of performing genetic studies in a heterogeneous disorder encompassing varied clinical presentations and resulting from diverse neuropathologies, careful phenotyping of clinical subtypes or focusing on neuropathologically diagnosed cases can increase researchers' ability to identify novel genetic contributions to FTD. The first common variation linked to FTD disease spectrum risk was the H1 haplotype of *MAPT*, which was initially linked to PSP in 1999 (Baker et al., 1999) and has been further implicated in FTLD-tau cases (Pottier et al., 2016). More recently, two unbiased genome-wide association studies (GWASs) of FTD have led to the discovery of novel, common genetic variants (which occur at 5% or greater frequency in the population) that contribute to modest disease risk (reviewed in Ferrari, Manzoni, & Momeni, 2018). The first GWAS of FTD was published in 2010 and focused on pathologically diagnosed TDP-43 cases and *GRN* pathogenic variant carriers (since *GRN* haploinsufficiency results in TDP-43 pathology) (Van Deerlin et al., 2010). This study identified three SNPs with genome-wide significance all within chromosome 7p21, which includes the gene *TMEM106B*, and identified *TMEM106B* as a potential disease modifier of *GRN* pathogenic variant carriers (Table 5.2). The second study, published in 2014 and consisting of over 3500 clinically diagnosed FTD cases, conducted separate GWASs of three FTD subtypes (bvFTD, svPPA, and nfvPPA) as well as FTD-MND and combined all subtypes together via meta-analysis (Ferrari et al., 2014). This study identified several SNPs associated with FTD risk, including the human leukocyte antigen (*HLA*) region and a locus near *RAB38/CTSC,* implicating the immune system and endolysosomal system, respectively, as potential pathways linked to FTD risk (Table 5.2).

Common variant risk factors: pleiotropy

To complement unbiased GWASs, new statistical methods are being utilized to leverage the concept of genetic pleiotropy, or shared genetic risk between distinct diseases/traits. In such studies, the fact that diseases or traits share common underlying biology—and may therefore also share genetic contributions—is leveraged to increase statistical power to identify novel risk loci. One study interrogated the overlap between sporadic FTD,

Table 5.2 Most significant single-nucleotide polymorphisms (SNPs) identified in genome-wide association studies (GWASs) of frontotemporal dementia (FTD).

GWAS of FTLD cases with TDP-43 pathology (Van Deerlin et al., 2010) Discovery: 515 FTLD-TDP pathology cases Replication: 89 FTLD-TDP pathology cases			
SNP	**OR (95% CI)**	***P*-value**	**Notes**
rs1990622 rs6966915 rs1020004	0.61 (0.52–0.71) 0.61 (0.53–0.71) 0.60 (0.51–0.70)	1.08×10^{-11} 1.63×10^{-11} 5.00×10^{-11}	Block of linkage disequilibrium in 7p21 containing ***TMEM106B***; rs1990622 retained significance in replication cohort
GWAS of clinical FTD cases (Ferrari et al., 2014) Discovery: 2154 clinical FTD cases Replication: 1372 clinical FTD cases			
SNP	**OR (95% CI)**	***P*-value**	**Notes**
rs9268856 rs9268877 rs1980493	0.81 (0.76–0.86) 1.20 (1.11–1.30) 0.78 (0.69–0.86)	5.51×10^{-9} 1.05×10^{-8} 1.57×10^{-8}	Discovery + replication cohorts for all FTD clinical subtypes combined. All SNPs within 6p21.3 region that contains ***HLA*** (human leukocyte antigen) region.
rs302668 (proxy) rs16913634 (proxy)	0.81 (0.71–0.92) 1.25 (1.14–1.37)	2.44×10^{-7} 8.15×10^{-4}	Discovery + replication cohorts for bvFTD cases alone. SNPs within region on chromosome 11 containing ***RAB38/CTSC*** genes.

The most significant SNP findings from the 2010 GWAS of FTLD cases with TDP-43 pathology (Van Deerlin et al., 2010) and the 2014 GWAS of clinical FTD cases (Ferrari et al., 2014).

AD, and Parkinson's disease and found shared genetic overlap within the *HLA*, *MAPT,* and *APOE* regions (Ferrari et al., 2017), highlighting three established risk factors as points of convergence between FTD and other neurodegenerative diseases. To specifically probe the genetic signals implicating immune dysregulation in FTD, a follow-up pleiotropy analysis assessed genetic overlap between FTD spectrum disorders (FTD, CBD, PSP, and ALS) and immune-mediated diseases (including Crohn's disease, ulcerative colitis, rheumatoid arthritis, type 1 diabetes, celiac disease, and psoriasis) (Broce et al., 2018). FTD shared a very high genetic enrichment with many of the immune-mediated disorders, while CBD, PSP, and ALS showed comparatively low levels of genetic enrichment with immune-mediated diseases. The genetic enrichment between

FTD and immune disorders was primarily driven by SNPs in the *HLA* region, and postmortem analysis of gene expression levels in the brains of FTD cases versus controls revealed significantly different expression levels of *HLA* genes. While the *HLA* region is clearly associated with risk for FTD (and other neurodegenerative disorders), future fine-mapping studies will be required to clarify the particular alleles of this complex genomic region driving the association with FTD risk.

Summary

While our current understanding of the genetic landscape of sporadic FTD spectrum diseases is complicated by the variety of clinical presentations, underlying neuropathologies, and inherent limitations of "familial" and "sporadic" designations of disease, it is clearly evident that there are genetic contributions to FTD. Many of the genes contributing to sporadic FTD are also implicated in familial FTD and link the pathobiology of FTD to ALS and risk for other neurodegenerative diseases. The genetic factors identified thus far as contributors to FTD risk converge on several key proteins and biological processes, suggesting possible targets for future therapeutic development. Chief among these are the genes and proteins that have been directly linked to FTLD: tau, progranulin, TDP-43, and *C9ORF72* pathogenic expansions, which are currently being studied at the basic level and/or in clinical trials (Tsai & Boxer, 2016). Additional genetic variation implicates the immune system as a potential mediator of disease risk. Further exploration of the functional consequences of these disease-associated variants will illuminate underlying mechanisms of disease with the goal of identifying specific targets for therapeutic intervention.

Key facts of frontotemporal dementia

- Frontotemporal dementia (FTD) is the second most common form of presenile (<65 years old) dementia after early-onset Alzheimer's disease.
- FTD affects 4–22/100,000 people and is a particularly devastating form of neurodegenerative disease given the early age of onset (45–65 years) and the common presence of behavioral changes.
- A number of genes contribute to FTD risk, ranging from variants that show autosomal-dominant inheritance with 100% penetrance to common risk factors that confer only modest increases in disease risk.
- Approximately 25%–45% of FTD cases are familial while 55%–75% of cases are considered sporadic (no known family history of neurodegenerative disease).
- No treatments exist for this group of diseases, and a substantial proportion of FTD heritability remains unexplained.

Summary points

- Frontotemporal dementia can occur in the context of a family history of neurodegenerative disease (familial) or in the absence of clear family history (sporadic).
- Genes implicated in familial FTD (such as *MAPT*, *GRN*, and *C9ORF72*) can also result in sporadic disease.
- Known disease genes are implicated in a high percentage of familial disease and account for a small portion (6%–11%) of sporadic cases.
- Genes implicated in FTD highlight the involvement of protein clearance pathways including the proteasomal and endolysosomal systems as well as the immune system.
- Pleiotropy analysis has identified many shared genetic risk factors between FTD and other neurodegenerative diseases as well as with several immune-mediated diseases.

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CHAPTER 6

Clinical response to cholinesterase inhibitors in dementia: the role of *CYP2D6* and *APOE* genetic polymorphisms

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List of abbreviations

AD Alzheimer disease
APOE apolipoprotein E
APP amyloid precursor protein
Aβ amyloid-beta
ChEI cholinesterase inhibitor
CYP2D6 cytochrome p450 2D6
CYP3A4 cytochrome p450 3A4
DPC donepezil plasmatic concentration
EMs extensive metabolizers
IMs intermediate metabolizers
MMSE Mini-Mental State Examination
NMDA N-metil D aspartate
NSAI nonsteroidal antiinflammatory drugs
PMs poor metabolizers
UMs ultra-rapid metabolizers

Mini-dictionary of terms

Amyloid-beta (Aβ) Peptides with 36–43 amino acids that constitute the main component of amyloid plaques observed in the brain of patients with Alzheimer disease.
Apolipoprotein E (APOE) Lipoprotein particle found in the bloodstream. Its main function is the transport of lipids in the plasma, mainly cholesterol, essential for central nervous system (CNS) metabolism.
***CYP2D6* gene** Located on chromosome 22q13.1. It presents nine exons that encode for a 497-amino acid enzyme, primarily expressed in the liver, but also in the CNS.
Cytochrome P450s Enzyme superfamily with a large number of members, including phase I metabolizing enzymes, which catalyze various types of oxidation-based reactions.
Mini-Mental State Examination (MMSE) Widely used global cognitive screening test, which includes 30 brief questions.
Single nucleotide polymorphisms (SNPs) Variation in a single nucleotide that occurs at one position in the genome with a frequency greater than 1% of the population.

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Dementia: concepts and risk factors

The core clinical criteria to define dementia are the presence of cognitive or behavioral symptoms that interfere with the ability to function at work or at usual activities, represent a decline from previous levels of functioning, and are not explained by *delirium* or major psychiatric disorder (McKhann et al., 2011).

Cognitive impairment is detected through clinical history obtained from the patient and a close informant and by objective cognitive assessment or, whenever necessary, through formal neuropsychological testing.

Dementia has a great impact on patients and caregivers, including depression in caregivers (Amieva et al., 2012). Alzheimer disease (AD) is the most common cause of dementia worldwide (Kalaria et al., 2008; Lopes & Bottino, 2002; Nitrini et al., 2004).

The major risk factors related to dementia are advanced age (Wu et al., 2012), low education (Cacabelos, 2007), female sex (Aguera-Ortiz, Frank-Garcia, Gil, & Moreno, 2010; Carter, Resnick, Mallampalli, & Kalbarczyk, 2012; Kalaria et al., 2008; Sales et al., 2011), late-life depression (Weisenbach, Boore, & Kales, 2012), and being a carrier of the *APOE* ε4 allele (Liu, Kanekiyo, Xu, & Bu, 2013).

APOE

Lipoproteins are particles found in the bloodstream. The main function of the lipoproteins is the transport of lipids in the plasma, mainly cholesterol. APOE is one of the most abundant lipoproteins found in the brain, synthetized by astrocytes and, to a much lesser extent, by microglia. APOE plays an important role in axonal growth, synaptic formation and remodeling, essential events for memory, learning, and neuronal repair. It also regulates neuronal inflammation, aggregation and depuration of beta amyloid (Aβ) found in the AD brain (Huynh, Davis, Ulrich, & Holtzman, 2017).

The *APOE* gene is polymorphic and located on chromosome 19q13.12. It can originate isoforms from three allelic forms: ε2, ε3, and ε4. There are three APOE isoforms (E2, E3, E4), which are formed from the expression of different alleles. APOE E4 is less efficient than E3 and E2 isoforms in cholesterol transport and neuronal regeneration (Ojopi, Bertoncini, & Neto, 2004).

One major mechanism by which APOE affects AD pathology is through its influence on the accumulation of amyloid plaques in the brain and cerebral vasculature. APOE E3 binds to Aβ more efficiently than APOE E4. Hence, E3 allele eliminates Aβ more efficiently than E4 (Huynh et al., 2017).

According to Liu et al. (2013), the frequency of AD and mean age at clinical onset are 91% and 68 years in ε4 homozygotes, 47% and 76 years in ε4 heterozygotes, and 20% and 84 years in ε4 noncarriers.

Pharmacological treatment

Smith and Swash (1978) published one of the first works showing that the cholinergic deficit in the brain is a main cause for the memory loss in AD, as the hippocampus is affected by this cholinergic deficiency. The low level of this neurotransmitter may be responsible for behavioral symptoms, such as apathy, depression, anxiety, hallucination, sleepiness, and cognitive problems, such as episodic memory and visuospatial deficits.

Some drugs act through the inhibition of acetylcholinesterase, an enzyme that degrades acetylcholine, therefore increasing the neurotransmitter level at the synaptic cleft. Consequently, these drugs (cholinesterase inhibitors; ChEIs) are indicated for treatment of AD: donepezil and galantamine, which inhibit; and rivastigmine, which inhibits acetylcholinesterase and butyrylcholinesterase (Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016). ChEIs are effective in mild, moderate, and severe stages of AD dementia (Birks & Harvey, 2018). ChEIs are also indicated for treatment of dementia with Lewy bodies and dementia due to Parkinson's disease (O'Brien et al., 2017).

Donepezil remains up to 70 h in the bloodstream, while the other ChEIs remain for a shorter period of time in the plasma. Donepezil reaches peak plasmatic concentration around 3 to 4 h, and the therapeutic doses vary from 5 to 10 mg daily. The drug is metabolized by the liver, through cytochrome p450 enzymes CYP3A4 and CYP2D6. The latter is also the main enzyme involved in the metabolism of antipsychotics and antidepressants. The ideal therapeutic level of donepezil ranges from 30 to 75 ng/mL. About 50% of acetylcholinesterase inhibition is obtained if donepezil concentration reaches 15.6 ng/mL, and is considered optimal if plasmatic level is higher than 50 ng/mL. An adequate plasma level of this drug is important for treatment efficacy. However, this level is not reached by all patients, because of interaction with other medications. Moreover, antipsychotics and antidepressants could inhibit CYP2D6 (Koeber et al., 2012).

Rivastigmine metabolism occurs in the synaptic cleft, and the therapeutic doses ranges from 6 to 12 mg daily. This drug exerts inhibition of both acetylcholinesterase and butyrylcholinesterase (Winblad et al., 2007).

The therapeutic dose of galantamine ranges from 16 to 24 mg daily. The drug also inhibits acetylcholinesterase and acts as an allosteric agonist of nicotinic receptors of acetylcholine. The liver metabolizes the drug and elimination is made by the kidneys (Huang & Fu, 2010).

The ChEIs do not change the natural course of AD, but may attenuate the symptoms of the disease and improve daily functioning. According to Massoud, Desmarais, and Gauthier (2011), these drugs may be effective for 2–5 years.

Memantine was the last drug to be approved for treatment of dementia due to AD. The drug binds to the N-metil D aspartate receptors of glutamate, leading to inhibition of glutamate excitatory action. The therapeutic dose is 20 mg daily, being indicated for moderate and severe AD dementia.

The concept of good, neutral, and bad clinical responders

Good clinical responders may be defined as individuals who score at least two additional points in the Mini-Mental State Examination (MMSE) after 1 year of treatment compared to baseline MMSE. Neutral responders may be defined as those who remain with the same baseline score after 1 year, or they lose or gain one point in the MMSE. Finally, bad responders are those who lost two or more points in the MMSE in comparison to baseline.

Raschetti et al. (2005), in a naturalistic study, followed 5462 patients with mild and moderate AD dementia after the first ChEI prescription for an average period of 10.5 months. By the end of 9 months of follow-up, 2853 patients (52.2%) concluded the study. Overall, the patients presented a small improvement in the MMSE, namely, they scored on average 0.5 points above the mean baseline score. However, only 17.3% of the sample were considered good responders (i.e., those who scored two additional points compared to baseline) at 9 months. There were no differences between users of donepezil, galantamine, or rivastigmine.

Medication response

Clinical trials have shown that cognitive improvement occurs with 9 to 12 months of treatment, and between 3 and 6 months for global clinical improvement. At 6 months, there is a better functional result (Cortes et al., 2008).

There is a consensus that ChEIs have a positive effect on cognitive performance and behavioral symptoms, although improvement is modest. Considering that AD is a degenerative disease, the drug effect is time-limited and, as mentioned before, its benefits range from 2 to 5 years (Birks & Harvey, 2018; Massoud et al., 2011).

Predictive factors of response

Raschetti et al. (2005) studied the predictive factors of response to ChEI, such as age, sex, MMSE score at baseline, comorbidities, concomitant use of other drugs with action on the central nervous system (e.g., antipsychotics, antidepressants), type of ChEI, doses of ChEI, and response at 3 months of treatment. They found that good clinical response at 3 months of treatment and fewer comorbidities were the two predictive factors for better drug response at 9 months.

Wattmo et al. (2011), in another naturalistic study, also investigated predictive factors of response to treatment with ChEI. They analyzed age, sex, schooling, functioning, number of medicines, *APOE* ε4 allele carrier status, living alone, and taking nonsteroidal antiinflammatory drugs (NSAIDs). The authors concluded that carriers of *APOE* ε4, NSAID use, male sex, older age, poor schooling, and high doses of ChEI were all significantly associated with better treatment response.

CYP2D6 phenotype and genetic variants

The cytochrome P450s (CYPs) is an enzyme superfamily with a large number of members, including phase I metabolizing enzymes, which catalyze various types of oxidation-based reaction, as hydroxylation, N-, O- and S-dealkylation, sulfoxidation, epoxidation, deamination, desulfuration, dehalogenation, peroxidation, and N-oxide reduction of a large number of endogenous and exogenous substances (e.g., medications). The families of CYPs, CYP1s, CYP2s, and CYP3s are responsible for oxidative metabolism of more than 90% of drugs (He, Hoskins, & McLeod, 2011; Nebert, Wikvall, & Miller, 2013; Newsome, Nelson, Corran, Kelly, & Kelly, 2013).

The *CYP2D6* gene is located on chromosome 22q13.1. It presents nine exons that encode for a 497-amino acid enzyme, which is expressed not only in the liver but also in the central nervous system (Gervasini, Carrillo, & Benitez et al., 2004; Kimura, Umeno, Skoda, Meyer, & Gonzalez, 1989) (Fig. 6.1). This gene produces five alternatively spliced transcripts, encoding four different proteins with 497, 494, 446, and 180 amino acids, which are associated with a large interindividual variation in the enzyme activity. At least 160 drugs are metabolized by CYP2D6, including those acting on the central nervous system (Zhou, & Zhou, 2009). Although CYP2D6 represents only 2% of all enzymes, it is responsible for the metabolism of the most antidepressants, antipsychotics, and ChEIs (Cacabelos, Llovo, Fraile, & Fernandez-Novoa, 2007).

In the brain, CYP2D6 protein is found in cortical pyramidal cells, pyramidal cells of the hippocampus, and Purkinje cells of the cerebellum, suggesting an endogenous function of CYP2D6 in the metabolism of neurotransmitters (Wang, Li, Dong, & Yue, 2014).

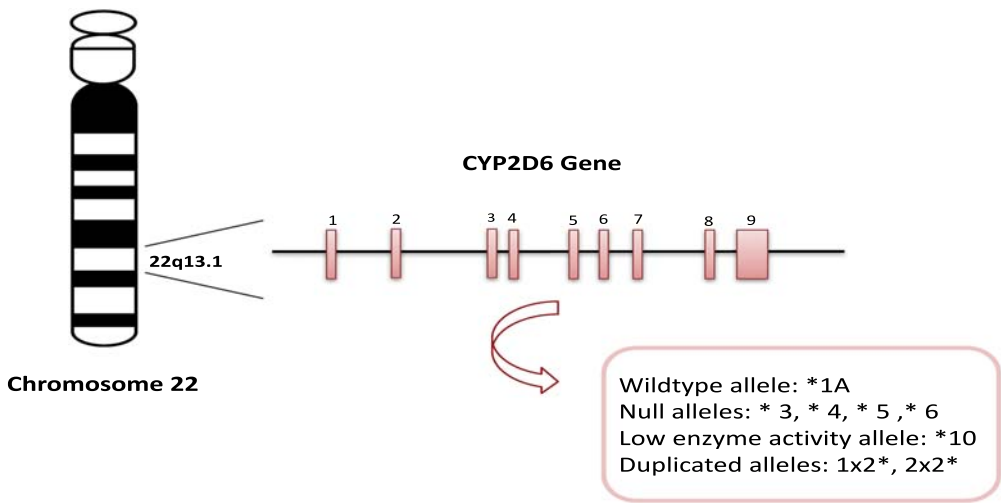

Figure 6.1 Location and structure of *CYP2D6* gene and its polymorphisms.

Physiological factors, such as ontogeny, sex, pregnancy, and fasting, as well as renal and liver diseases, diabetes mellitus, and inflammatory status, can alter CYP2D6 activity. However, the most important factors that determine this variability are the genetic characteristics. In fact, approximately 120 *CYP2D6* genetic variants have been described, which result from point mutations, deletions, insertions, rearrangements, and deletion or duplication/multiplication of the entire gene. Functionally, they are synonymous alleles, but some alleles can increase or decrease enzyme activity.

These alleles are associated to four phenotypes related to the drug metabolized by CYP2D6: extensive metabolizers (EMs), intermediate metabolizers (IMs), poor metabolizers (PMs), and ultra-rapid metabolizers (UMs). The distribution of these alleles and phenotypes is different in each ethnic group (He et al., 2015). The PMs present high drug serum levels—in this case, the individual carriers two null alleles; in UMs, the metabolism occurs very rapidly, also without achieving the expected effect—in this condition, there are several copies of the gene; EM patients present expected range of the drug metabolism and are associated with the presence of two alleles with normal function. Therefore, in IMs, a null allele (nonfunctional) and another allele with decreased function are usually found (He et al., 2011). According to Zanger, Raimundo, and Eichelbaum (2004), the drug oxidation capacity in the IM phenotype is reduced and may be similar to that presented by PMs.

Although most individuals are EMs, 5%−10% of Caucasians, 6.7% of Africans, and 1%−4% of other ethnic groups have decreased CYP2D6 activity (PMs) and are at risk for toxicity if they use the usual dose of medication. Asians display the lowest frequency (0.9%) of PMs. From 1% to 7% of Caucasians and up to 20% of individuals from Eastern Europe have the highest enzyme activity (UMs) and may not reach the plasma therapeutic concentration under the same treatment (Cacabelos & Martinez-Bouza, 2011).

The allele *1A refers to the wild type. The null alleles of the *CYP2D6* gene do not encode a functional protein. The alleles *3, *4, *5, and *6, which are the most frequent in the Caucasian population, encode a protein with no residual activity, being responsible for approximately 97% of the PM phenotype in this population (Noetzl et al., 2014) (Table 6.1). The *3 and *6 alleles present deletions at specific sites of the gene, leading to interruption of the reading phase and resulting in a nonfunctional protein (Noetzl et al., 2014). The *CYP2D6* * 3 allele contains the deletion of an adenine (A) at position 2549 in exon 5, and the *CYP2D6* * 6 allele contains a thymine deletion (T) at position 1707 of exon 3. In the *CYP2D6* *4 allele, the most common null allele among Caucasians with a frequency of 20%−25% (Zhou, 2009), there is a change from guanine (G) to adenine (A) at position 1846 of exon 4, leading to a splicing defect. The *CYP2D6* allele *5 shows complete deletion of the gene. The *CYP2D6* *10 allele, widely found among Asians, leads to a significant decrease in the enzymatic activity of CYP2D6, since it corresponds to the replacement of a cytosine by thymine at position 100 of the gene. Moreover, the single nucleotide polymorphism (SNP) −1584C > G (rs1080985) in the

Table 6.1 *CYP2D6* alleles and their effect on enzymatic activity.

CYP2D6					
Allele	*3	*4	*5	*6	*10
Genotype EM	wt/wt	wt/wt	wt/wt	wt/wt	CC
IM	wt/*3	wt/*4	wt/*5	wt/*6/*	CT
PM	*3/*3	*4/*4	*5/*5	6*6	TT
Effects in the gene	Deletion	Deletion	Deletion	Deletion	Substitution
Enzymatic activity	Null	Null	Null	Null	Diminished

Polymorphisms of *CYP2D6*; most of them are deletions. The results of drug level in the plasma are represented by four types of categories of metabolizers: extensive, intermediate, poor, and ultra-rapid (which are not found in this work).

CYP2D6 gene promoter has been associated with the enzyme activity, since the −1584G allele is related to higher gene expression when compared to the −1584C allele (He et al., 2015). Duplication or multiple gene copies are frequently found in populations from Saudi Arabia (21%) and Ethiopia (29%), but in other populations the frequency reaches approximately 10% (Matimba, Del-Favero, Van Broeckhoven, & Masimirembwa, 2009).

It is important to highlight that *APOE* gene variants can interfere in CYP2D6 activity probably via regulation of hepatic lipid metabolism, whereas it influences triglycerides levels, liver steatosis, transaminase activity, and/or cytochrome P450-related enzymes. Cacabelos (2009) showed that the presence of the *APOE* ε4/ε4 genotype was able to convert EMs into PMs. Furthermore, higher frequency of *APOE* ε4/ε4 was observed among CYP2D6 PMs and UMs (Cacabelos, 2009; Cacabelos & Martinez-Bouza, 2011).

Table 6.2 shows the variety of distribution of alleles in different populations (Brazil, Europe, and China). Miranda et al. (2017) conducted their work in Brazil, a country with high miscegenation of the population. Cacabelos (2011) evaluated predominantly Caucasians. Regarding this study data, the sum does not complete 100%, because the author investigated other polymorphisms that are not in the table. Zhong et al. (2013) conducted their study in China, where the presence of *CYP2D6* *10 is the most frequent and do influence the clinical response.

CYP2D6 genetic variants as Alzheimer disease risk factor

Some studies investigated the association between *CYP2D6* genetic variants and phenotypes with AD, whereas the presence of null alleles, especially the most frequent *CYP2D6* *4, may lead to a lower decrease of choline acetyltransferase in the brain. It may also prolong the time of exposure to neurotoxins, thus leading to environmental toxin-induced damage (Chen et al., 1995; Lu et al., 2014). However, this association remains unclear.

Table 6.2 Genotypic and allelic frequencies in patients with Alzheimer disease.

Genotypes alleles n (%)	Miranda et al., 2015	Cacabelos, 2009	Zhong et al., 2013
CYP2D6*3	wt/wt 35 (89.8) *3/wt 2 (5.1) *3/*3 2 (5.1)	wt/wt 358 (55.6) *3/wt 14 (2.2) *3*3 0 (0)	–
CYP2D6*4	wt/wt 36 (78.2) *4/wt10 (21.8) *4/*4 0 (0)	wt/wt 358 (55.6) *4/wt 153 (23.8) *4/*4 24 (3.8)	–
CYP2D6*5	wt/wt 44 (92.5) *5/wt 4 (7.5) ***5/***5 0 (0)	wt/wt 358 (55.6) *5/wt 19 (2.9) *5/*5 2 (0.3)	–
CYP2D6*6	wt/wt 96 (100) *6/wt 0 (0) ***6/***6 0 (0)	wt/wt 358 (55.6) *6/wt 13 (2.0) *6/*6 0 (0)	
CYP2D6*10	CC 26 (61.7) CT 14 (33.3) TT 2 (5.0)	–	CC 27 (27.5) CT 25 (25.5) TT 46 (47.0)

Data from different populations. Variety of distribution of alleles in populations from Brazil, Europe, and China.

In order to evaluate the relationship between CYP2D6 phenotypes and *CYP2D6* *4 polymorphism and the increased incidence of AD, Lu et al. (2014) conducted a meta-analysis including 11 studies involving 643 AD cases and 1375 controls for *CYP2D6* *4 polymorphism, and four studies including 411 AD cases and 603 controls for CYP2D6 phenotypes. The meta-analysis suggested that the *CYP2D6* *4A/G polymorphism was significantly associated with an increased risk of AD in the allelic model of A versus G, AA versus GG; and recessive genetic model AA versus AG + GG. For the CYP2D6 phenotypes, the analysis revealed that they were not associated with AD risk in all compared models. In conclusion, the study showed that the *CYP2D6* *4 allele might be associated with increased AD risk, but it did not demonstrate the association between CYP2D6 phenotypes and AD occurrence.

CYP2D6 and Alzheimer disease treatment response

A variable therapeutic response to donepezil has been observed in AD patients, from 40% to 58% of treated individuals with improvement in cognition, and approximately 6%–13% of patients exhibiting side effects with donepezil use. Moreover, it is suggested that genetic variants may account for 20%–95% variation in drug response. Consequently, the investigation of genetic polymorphisms associated with an effective response to ChEI treatment could present high benefit for AD patients (Cacabelos, 2007, 2008; Xiao, Bin, Weiwei, Beisha, & Shen, 2016). Although few studies evaluated the effect of *CYP2D6* genetic polymorphism on the response to donepezil, the results are not completely clear.

Table 6.3 Association of donepezil plasmatic concentration with *CYP2D6* wild-type wt/wt and the polymorphism wt/*5 at 3, 6, and 12 months. Univariate analysis.

		wt/wt	wt/*5	p	Test
Donepezil plasmatic concentration (ng/mL)	3 months (n = 16; n = 3)	28.32(8.6–107.9)	30.5(10.9–43.2)	0.737	Mann–Whitney
	6 months (n = 15; n = 3)	49.0 ± 19.9	36.9 ± 4.7	0.316	T student
	12 months (n = 18; n = 3)	51.70(0.2–127.6)	46.28(40.7–87.9)	0.688	Mann–Whitney

n, Number of patients. The information of two patients at 3 months and three patients at 6 months were lost. There is no statistical difference between the groups. In other words, the donepezil concentration does not vary between the wild-type group and in the group in which a deletion occurs.

Miranda at al. (2017) found that donepezil plasma concentrations between the groups (wild type wt/wt and the polymorphism wt/*5) did not attain statistical difference throughout the treatment period in terms of worse response (Table 6.3). Xiao et al. (2016) published some data consistent with a slight improvement in favor of decreased/nonfunctional groups at 3, 6, and 12 months.

CYP2D6 genetic variants may cause differences in the pharmacokinetics of donepezil. After oral administration, more than 90% of a dose undergoes first-pass metabolism by the hepatic CYP2D6, resulting in the production of several metabolites, almost all of them inactive products. Some studies have shown that carriers of *CYP2D6* alleles who present increased function (UMs) may present higher enzymatic activity, lower donepezil concentration in the plasma, and no effect with a standard dose of donepezil. Contrary, *CYP2D6* alleles that decrease the enzyme function and activity (PMs) are associated with higher donepezil concentration and increased risk of adverse effects with standard dose of the drug (Noetzli et al., 2014; Tiseo, Perdomo, & Friedhoff, 1998; Xiao et al., 2016).

In their naturalistic study, Miranda et al. (2017) observed that donepezil was the most prescribed ChEI (52.6%). There was a predominance of patients with *APOE* ε3ε3 genotype. No association was found between *APOE* genotypes and drug response. Regarding *CYP2D6* polymorphisms, the wild-type allele (wt/wt) was the most frequent, being small or absent the frequency of deletions *3, *4, *5, and *6 in all studied groups (Tables 6.1 and 6.2). Table 6.4 shows the plasmatic concentrations of donepezil and the relationships with doses and polymorphism of *APOE* and *CYP2D6*.

Comparison of plasma levels of donepezil (doses of 10 mg) over the treatment period revealed that the concentrations at 12 months were higher than at 3 and 6 months. When taking into account donepezil plasma concentrations in relation to clinical response, a significant difference emerged between good and neutral responders, suggesting that better response occurs with highest plasma drug levels (Miranda et al., 2017).

Table 6.4 Plasmatic concentrations of donepezil (10 mg) in wild alleles and *APOE* and *CYP2D6* polymorphisms over 12 months (42 patients).

	3 months	6 months	12 months	p	p-value
DPZ (ng/mL)	29.7(8.0−107.9)	49.4(6.0−91.8)	56.9(0.2−127.6)	<0.001[1]	3−6 m 0.003 3−12 m 0.000 6−12 m 0.003
APOEε3/ε3 (ng/mL)	28.1(10.9−88.7)	41.6(16.8−91.8)	55.5(0.2−100.3)	0.001[1]	3−6 m 0.107 3−12 m 0.053 6−12 m 0.07
APOE- ε3/ε4 or ε4/ε4 ng/mL	33.5(8.0−107.9)	41.7(36.9−46.5)	51.5(36.4−127.6)	0.001[1]	3−6 m 0.006 3−12 m 0.003 6−12 m 0.056
CYP2D6 3 wt/wt	30.2(8.0−107.9)	46.7(6.0−91.8)	51.7(0.2−106.8)	<0.001[1]	3−6 m 0.021 3−12 m 0.005 6−12 m 0.013
CYP2D6 3 wt/*3 or *3/*3	29.56(19.9−37.5)	66.35(49.4−69.9)	123.75(48.9−127.6)	0.097[1]	
CYP2D6 4 wt/wt	36.14 ± 20.89	48.61 ± 17.11	63.79 ± 31.76	<0.001[2]	3−6 m 0.004 3−12 m 0.000 6−12 m 0.002
CYP2D6 4 wt/*4	59.94 ± 32.26	54.08 ± 28.39	54.65 ± 30.23	0.779[2]	
CYP 2D6 5 wt/wt	33.06(18.0−107.9)	52.08(22.5−91.8)	54.42(0.2−127.6)	0.004[1]	3−6 m 0.028 3−12 m 0.048 6−12 m 0.075
CYP 2D6 5 wt/*5	30.50(10.9−43.2)	37.94(31.8−41.0)	46.28(40.7−87.9)	0.097[1]	
CYP 2D6 6 wt/wt	30.17(8.0−107.9)	49.44(6.0−91.8)	54.42(0.2−127.6)	<0.001[1]	3−6 m 0.006 3−12 m 0.001 6−12 m 0.004
CYP 2D6 10 CC	30.03(8.0−107.9)	47.67(6.0−73.9)	60.45(0.2−122.7)	0,001[1]	3−6 m 0.100 3−12 m 0.003 6−12 m 0.008
CYP 2D6 10 CT/TT	35.41(18.0−88.7)	47.46(17.9−91.8)	48.68(19.5−124.8)	0.197[1]	
DPC good responders[3]	37.44 ± 16.31	45.51 ± 14.31	68.36 ± 24.76	0.021[2]	3−6 m 0.070 3−12 m 0.008 6−12 m 0.015
DPC neutral responders[4]	28.26(10.9−87.0)	55.2(6.0−73.9)	60.46(2.4−127.6)	0.012[1]	3−6 m 0.022 3−12 m 0.016 6−12 m 0.084
CPD bad reponders[5]	42.5 ± 30.93	46.55 ± 22.29	50.42 ± 21.00	0.463[2]	

[1]Friedman test;
[2]ANOVA repeated measures;
[3,4,5]are explained in the Key Facts of Metabolized Drugs. *DPZ*, donepezil; *DPC*, donepezil plasmatic concentration. Comparing the plasma levels of donepezil (with doses of 10 mg), the concentrations at 12 months are higher than at 6 months and 3 months.

Xiao et al. (2016), in a meta-analysis, also concluded that AD individuals with normal function of *CYP2D6* alleles may have a better response to donepezil treatment. Nevertheless, patients bearing both the *APOE* ε4 and rs 1080985-G alleles show worse response.

In conclusion, there are different clinical responses to drug treatment in dementia due to AD. It depends on several factors. There is no clarity regarding the influence of *APOE* polymorphisms in relation to ChEI treatment, and it seems that individuals carrying the wild-type *CYP2D6* allele may have a better response (at least to donepezil).

Key facts of metabolized drugs

- Donepezil remains up to 70 h in the bloodstream, while the other cholinesterase inhibitors (ChEI) remain for a shorter period of time in the plasma.
- CYP2D6 protein is found within cortical pyramidal cells, pyramidal cells of the hippocampus, and Purkinje cells of the cerebellum, suggesting an endogenous function of CYP2D6 in the metabolism of neurotransmitters.
- Good clinical responders may be defined as individuals who score at least two extra points in MMSE after 1 year treatment with ChEI comparing to baseline MMSE scores.
- Neutral responders may be defined as subjects who maintain the same MMSE score obtained at baseline after 1 year of treatment with ChEI, or, alternatively, they lost or gained one point.
- Bad clinical responders may be defined as individuals who lost two points in the MMSE after 1 year of treatment with ChEI in comparison to baseline.

Summary points

This chapter focuses on dementia and *CYP2D6* polymorphisms:

- Dementia is characterized by cognitive impairment and behavioral symptoms that significantly interfere with daily activities. It must represent a decline from previous levels of functioning, not explained by *delirium* or a major psychiatric disorder.
- Alzheimer disease (AD) is the most common etiology of dementia.
- The main risk factors for dementia are age, low education, female sex, late-onset depression, and carrier of *APOE* ε4 allele.
- Early onset AD may be caused by mutations in the amyloid precursor protein (APP) or in genes encoding presenilin 1 or presenilin 2.
- APOE promotes the homeostasis of cholesterol and regulates neurons inflammation, aggregation, and depuration of complex Aβ.
- In 1976, two independent studies associated AD with acetylcholine deficiency.
- Cholinesterase inhibitors (ChEIs) constitute a drug class indicated for treatment of AD symptoms. Donepezil, galantamine, and rivastigmine are the ChEIs currently used.

- The clinical response concept used in this chapter is based on the MMSE score after 1 year of treatment, namely, good, neutral, or bad.
- ChEI may increase cognitive performance and alleviate behavioral symptoms in AD.
- *CYP2D6* genetic variants have been described, resulting from point mutations, deletions, insertions, rearrangements, and deletion or duplication/multiplication of the entire gene.

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CHAPTER 7

A1 and A2 purinergic receptor expression in dementia

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List of abbreviations

A1R A1 purinergic receptor
A2R A2 purinergic receptor
AD Alzheimer's disease
D2R Dopamine receptor2

Mini-dictionary of terms

Aβ Amyloid peptide A peptide that induces Aβ plaques by the amyloidogenic pathway.
A1 purinergic receptor A receptor activated by adenosine and attached to a Gi protein, inducing the inhibition of adenylate cyclase as well as a decrease in cAMP.
A2 purinergic receptor A receptor activated by adenosine and coupled to a Gs protein, inducing the release of cAMP by adenylate cyclase activation.
Huntington disease Is a genetic malady characterized by motor and cognitive impairments.
Neurodegenerative disease A disorder associated with destroyed neurons.
Parkinson's disease Is a neurodegenerative disease characterized mainly by motor deficiency.
Schizophrenia disease Is a disease with severe brain changes associated with abnormal behavior and hallucinations.
Tau protein Protein type that stabilizes and promotes the assembly of tubulin to microtubules.

Introduction

Dementia is a term that describes a wide variety of cognitive symptoms that portray a patient's diminished daily performance (Puzzo, Gulisano, Arancio, & Palmeri, 2015). By definition, dementia is an acquired and persistent loss of intellectual functions that affects at least three of the following spheres: memory, language, visuospatial perception, personality, and knowledge (abstraction, calculation, execution functions, reasoning, etc.). That is, dementia is a syndrome characterized by the presence of a persistent cognitive impairment that interferes with the capabilities of an individual to carry out their personal, professional, and/or social activities. Such cognitive impairment is independent of the presence of changes in the level of consciousness (i.e., it does not occur as a result of an acute state of confusion or delirium) and it is caused by a disease that affects the central nervous system (Ritchie et al., 2017).

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It is estimated that 46.8 million people suffer from dementia worldwide, and this number is expected to double every 20 years, reaching 74.7 million by 2030 and 131.5 million by 2050, according to the World Alzheimer Report (2015). Alzheimer's disease (AD) is the main cause of dementia in older adults; it is a progressive and irreversible neurodegenerative ailment characterized by cognitive and memory impairment. There are two pathological hallmarks of AD, the extracellular accumulation of Aβ-peptides in Aβ-plaques and the formation of neurofibrillary tangles from hyperphosphorylated tau protein.

The first of these two pathological hallmarks refers to the generation and accumulation of Aβ-peptide as neuritic or diffuse plaques, or oligomeric forms, proposed by the amyloidogenic pathway hypothesis. This hypothesis suggests that Aβ-peptides are generated by the proteolytic cleavage of amyloid precursor protein by β-secretase and γ-secretase (Mendiola-Precoma, Berumen, Padilla, & Garcia-Alcocer, 2016). The second pathological hallmark, the intracellular accumulation of tau protein, is due to a hyperphosphorylation of tau protein, a microtubule-associated protein. Tau protein is one of these protein types that stabilizes and promotes the assembly of tubulin to microtubules. Tau protein is expressed in the neurons as a cytoskeletal protein; it is primarily located in the axons, but also accumulates in the soma and the dendrites of the neurons. There are six tau-protein isoforms expressed within the adult brain in various combinations; these isoforms are the result of mRNA alternative splicing. According to Hasegawa (2016), based on the hyperphosphorylated tau protein isoform and the combination of these six tau-protein isoforms, the pathological characteristics of a specific type of dementia are manifested.

Purinergic receptors

Purine actions have been studied since 1929; among the first researchers were Drury and Szent-Gorgyi who studied heart and blood vessels. In 1970, Burnstock studied noradrenergic and noncholinergic substances with a function within the gastrointestinal tract, yet the concept of purinergic receptors was proposed later (Burnstock, 2006).

Purinergic receptors are activated by nucleotides as well as nucleosides, and they have been classified into two groups. The first is the P1 type, which is activated by adenosine and is classified into four receptors: A1, A2A, A2B, and A3 (Stockwell, Jakova, & Cayabyab, 2017); amongst these receptors the A1 and the A3 are attached to a Gi protein, inducing the inhibition of adenylate cyclase as well as a decrease in cAMP. The A2 receptors are coupled to a Gs protein, inducing the release of cAMP by adenylate cyclase function; the possibility that the P1 receptor subtype may couple to other G proteins has also been reported (Woods, Ajit, Camdem, Erb, & Weisman, 2016); their function has been associated with neuromodulator effects within the brain.

The A1 receptor (A1R) is the most important receptor in the P1 group due to its high affinity as well as its wide distribution throughout the brain; this function has been associated with its inhibitory effects by presynaptic neurotransmitter release, as well as by

different second messenger pathways, related to kinase and phosphatase activation, which affect the function of intracellular transporters and receptors such as NMDA and AMPA receptors (Stockwell et al., 2017).

On the other hand, the P2 receptors are activated mainly by ATP. In 1985 those receptors were divided by Burnstock into two groups, the P2X and P2Y receptors; P2X receptor activation induces cation channel function and P2Y receptors are metabotropic. In the P2X family seven subtypes have been reported (P2X1-7), all of which are distributed in neurons and glial cells in the central nervous system. The P2X receptors have been studied in pathologies such as neurodegenerative diseases; nonetheless, further studies are necessary.

The final group of purinergic receptors are the P2Y receptors. In this group, eight different subtypes have been proposed (Woods et al., 2016), coupled to G proteins Gq, Gs, and Gi; these receptors are activated by ATP as well as ADP amongst other molecules such as UDP-glucose. P2Y receptor distribution has been reported in neurons, glial astrocytes, oligodendrocytes, and microglia.

A1 and A2 purinergic receptors in neuropathologies

Neurodegeneration has been associated with ageing and with various pathologies such as AD, Huntington's disease, Parkinson's disease, and stroke amongst others. The A1R is proposed to be neuroprotective due to its inhibitory effect, while the A2AR is associated with neurodegeneration due to its excitatory effect (Fig. 7.1).

The A1R and A2R were studied in ageing by the modulation of long-term potentiation in rat brain slices treated with A1 and A2R antagonists. The results indicated increased magnitude of long-term potentiation in young rats, but not in older ones. At the other extreme, with these same treatments an increase in A2A mRNA expression in the hippocampus has been demonstrated (Costenia et al., 2011).

The changes in the brain observed in ischemic stroke induced an increase in extracellular concentration of adenosine along with purinergic receptor activation, mainly of A1R due to its high affinity and distribution. Initially, A1R activation induces neuroprotection, but during its prolonged activation it exhibits neurodegeneration associated with A1R internalization, AMPA receptors endocytosis and increased A2R (Stockwell et al., 2017).

Huntington's disease is a genetic malady characterized by motor and cognitive impairments. In this neuropathology, the neuroprotective effect of A1R agonists has been reported. In 2002, Blum et al. studied in a Huntington's disease animal model the effect of adenosine amine congener (ADAC), a specific A1R agonist that decreased the brain lesion and hind limb dystonia and increased striatal regeneration; thus, the authors proposed further purinergic receptor studies in other HD models (Blum et al., 2002). The A1R was also studied in R6/2 mice, an animal model of HD with cyclopentyladenosine, an A1R agonist that induces a decrease in synaptic transmission in corticostriatal slices (Ferrante et al., 2014). Furthermore, the A2R also was studied in a rat model of

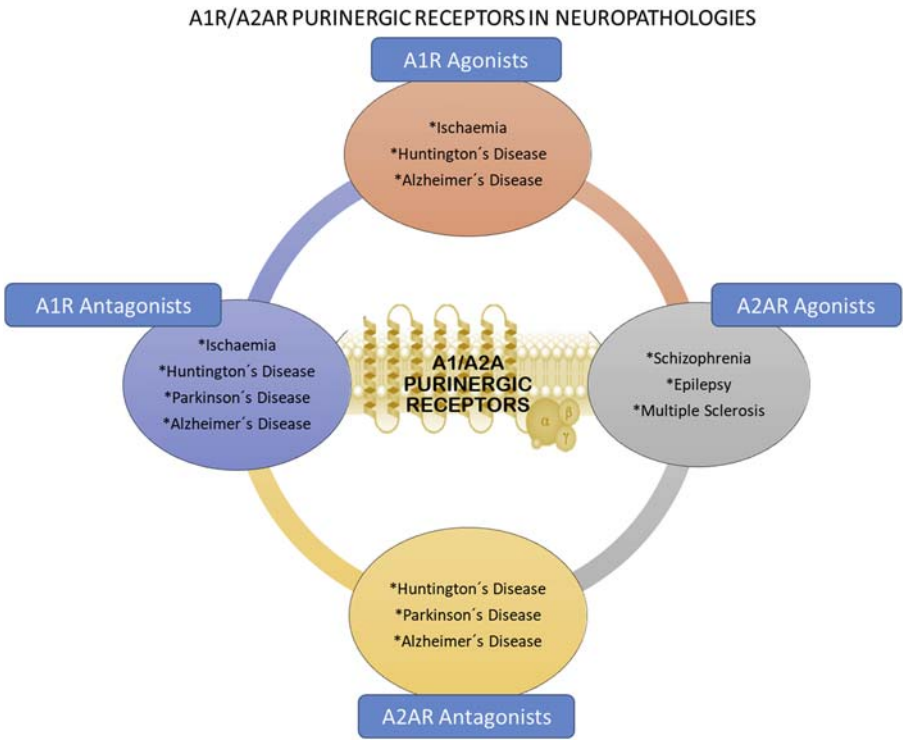

Figure 7.1 Use of A1R/A2AR agonists or antagonists in some neurodegenerative disorders.

HD treated with the A2AR antagonist [5-amino-7 (2 phenyethyl)-2-(2-furyl)-pyrazolo (4.3-e)-1,2,4 triazolo-(1,5-c)pyrimidine] and the results indicate a neuroprotective effect at low doses (Popoli et al., 2002).

As for Parkinson's disease, the importance of treatment without dopaminergic drugs has been proposed; thus the A2AR antagonist was considered as a potential drug due to its adrenergic modulation. That modulation could also be induced by caffeine, a nonselective purinergic receptor antagonist (Gołembiowska, Wardas, Noworyta-Sokolowska, Kaminiska, & Górsoka, 2013). Istradefylline, an A2AR antagonist approved in Japan, along with the use of other A2A purinergic receptor antagonists as treatment in Parkinson's disease was studied (Chen, 2014; Pinna, 2014). The study on the use of docking proposed different A2A purinergic receptor antagonists; thus, those were tested in a cerebral hypoxic-ischemic rat model with improved results (Tian, Bibi, Dale, & Imaray, 2017).

The positive participation of A2R ligands in schizophrenia was proposed in 1995 by Erfurth and Schmauss. Additionally, A1R and A2R have been studied in the brains of postmortem patients with schizophrenia, and the results did not indicate changes in A1R, but the patients showed reduced transcriptional and translational levels of A2AR (Villar-Menéndez et al., 2014). The use of molecules acting at both A1R and A2R

receptors has been proposed for schizophrenia treatment (Rial, Lara, & Cunha, 2014). Briefly, in a study of the importance of astrocytes in psychiatric disorders it was found that glutamate release inhibition is associated with D2R, and D2R is inhibited by A2AR in astrocytes that induce glutamate release; all of these results warrant considering the D2R-A2AR heterodimer as a possibility in glutamate release control (Cervetto et al., 2017).

A1R in Alzheimer's disease

A1Rs are widely distributed in the brain of most animals, with a high density in the neocortex and the hippocampus; thus, this receptor has been linked to many neurodegenerative diseases such as AD and Parkinson's disease amongst others. Because this receptor is able to modulate dopaminergic, cholinergic, and glutamatergic signaling, it has been implicated in various cognitive and memory functions, processes that are damaged in AD.

There is great interest in comparing studies that focus on changes in the expression of adenosine receptors by normal ageing or due to some type of neurodegeneration. For example, there are studies showing decreased A1Rs in the hippocampus of AD-patients' brains (Ulas, Brunner, Nguyen, & Cotman, 1993) and in AD-model rats induced by a high-fat diet (Mendiola-Precoma et al., 2017). There is also a report of generally decreased A1R expression, or a limited reduction of radioligand A1R binding in specific brain areas, or even without changes (Stone, Ceruti, & Abbrachio, 2009). This decrease in the A1R expression was confirmed to be in the hippocampal area by Fukumitsu et al. (2008) using a positron emission tomography (PET) study in patients with AD using the specific A1R-antagonist dipropylcyclopentylxanthine (DPCPX) as a radioligand. In contrast, A1R and the A2AR expression in prefrontal cortex in AD was increased (Albasanz, Perez, Barrachina, Ferrer, & Martín, 2008). Additionally, Prasanthi et al. (2010) demonstrated that AD were downregulate in the hippocampus of cholesterol-fed rabbits and changes in the expression of A2AR did not occur.

A1R mRNA expression in AD has been related to A1R protein expression. Our work group previously reported that A1R protein expression and A1R mRNA levels in the hippocampus were decreased in a model of AD (Mendiola-Precoma et al., 2017). Similarly, another study demonstrated that A1R gene expression was reduced in APP23 transgenic mice pretreated with 3-nitropropionic acid (3-NP) (von Arnim, Verstege, Etrich, & Riepe, 2006). However, Albasanz et al. (2008) reported that the relative A1R mRNA levels in the human frontal cortex were not changed in early or advanced stages of AD, and Angulo et al. (2003) reported a similar lack of effect, finding no alterations in the A1R gene in AD brain; they did, however, show that there is a downregulation of receptor protein expression. *In vitro* studies have shown that administration of (R)-N6-(1-methyl-2-phenylethyl)-adenosine (R-PIA) agonist after 48 h increases mRNA expression levels in primary cultures of cortical neurons (Ruiz, León, Albasanz, & Martín, 2011).

Because A1Rs can inhibit the release of neurotransmitter into the extracellular space, mainly glutamate, it has been thought that the use of agonists of this subtype of adenosine receptor can prevent excitotoxic damage generated by an increase in glutamate activity on its pre- and postsynaptic receptors. However, there is a discrepancy between whether it is the agonists or the antagonists of adenosine receptors, mainly A1R and A2AR, that produce beneficial effects in neurological diseases such as Alzheimer's and Parkinson's.

Several studies have explored the effects of A1R agonists in AD. Subcutaneous administration of an infusion with the A1R agonist adenosine amine congener produced a neuroprotective effect by reducing brain damage and preventing striatal degeneration induced by the mitochondrial toxin 3-NP (Blum et al., 2002). Other studies have demonstrated neuroprotective effects through A1R activation such as anticonvulsive properties in epilepsy as well as antiinflammatory attributes in multiple sclerosis (Fredholm, Chen, Cunha, Svenningsson, & Vaugeois, 2005). In an *in vitro* study on human SH-SY5Y neuroblastoma cells, it was shown that A1R activation by a selective agonist, (R)-N6-(1-methyl-2-phenylethyl)-adenosine or R-PIA, enhanced the production of the nonamyloidogenic fragment sAPPα, which is generated by the enzyme α-secretase, and therefore showed a neuroprotective effect; nevertheless, the opposite effect was shown using the selective A1R antagonist DPCPX (Woods et al., 2016).

Notably, another study reported a protective effect produced by the specific A1R agonist N6-cyclohexyl adenosine (CHA), where a selective protection was observed in damaged dopaminergic neurons with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Stone et al., 2009). It has been reported in various studies that several A1R agonists, including adenosine, CHA, and N6-cyclopentyladenosine (CPA) were administered in neuronal and glial cultures, exhibiting a protective effect (Mendonca, Sebastiäo, & Ribeiro, 2000). A large number of studies have shown that adenosine analogues tend to repair learning and memory functions; nevertheless, most reports of the use of A1R agonists have shown the opposite effect (Stone et al., 2009), which is why A1R antagonists have also been evaluated.

One of the first studies in which specific A1R antagonists were used was carried out by von Lubitz, Paul, Ji, Carter, & Jacobson (1994), who evaluated the effect of chronic administration of DPCPX on cerebral ischemia; however, they found that it increased neuronal damage, and that consequently there was a neurodegenerative effect. The same drug was evaluated in another study, in which it was administered intraperitoneally and chronically for 2 months in old transgenic APPswe/PS1dE9 mice; the memory of these animals did not improve and in nontransgenic mice long-term memory was impaired (Vollert, Forkup, Bond, & Eriksen, 2013).

Although it has been observed that A1 receptor agonists produce improved cognitive functions, no memory development or learning effects have been found in A1R knockout animals, the opposite of the findings in A2AR knockout animals (Düster, Prickaerts, & Blokland, 2014); therefore, there is a hypothesis that this receptor is

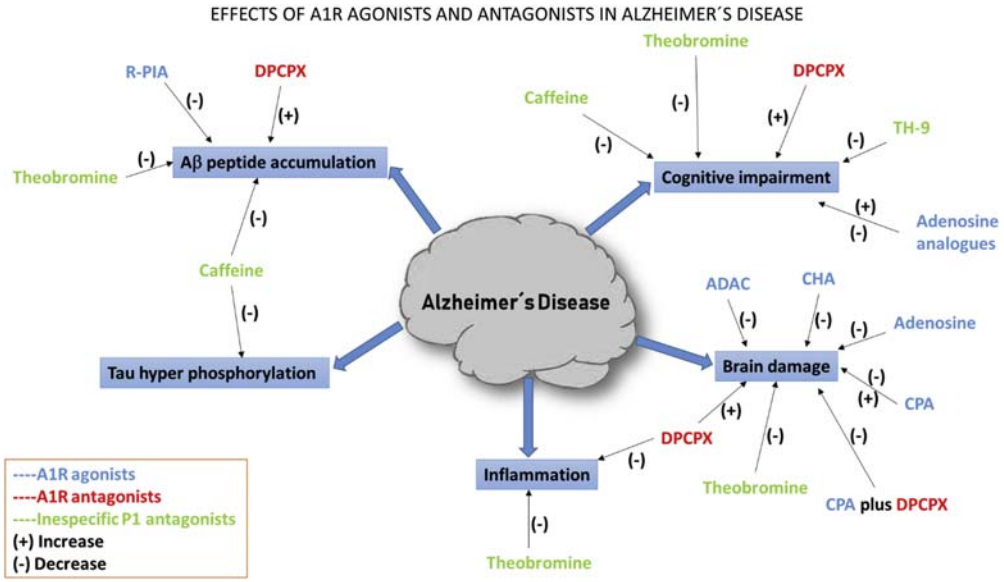

Figure 7.2 Different effects of A1R agonists and antagonists on the pathological characteristics of Alzheimer's disease. Most of the reported A1R agonists (blue) have neuroprotective effects, while the opposite effect on A1 receptor antagonists has been observed. However, the inespecific P1 receptors antagonists (green) show beneficial effects in the pathology of Alzheimer's disease.

indispensable for cognitive and memory functions. Consequently, the therapeutic interest of the A1R agonists has been limited to use as new neuroprotective drug, due to its cardiovascular effects and poor blood–brain barrier permeability, in addition to the short window of opportunity or the short bioavailability time; therefore, if A1R agonists are administered chronically, instead of having a protective effect, they would produce the opposite effect (Cunha, 2005). In recent reports, A1R activation by the administration of the agonist CPA induces neuronal death *in vivo*, and the administration of both the CPA-agonist and DPCPX-antagonist prevented such deterioration (Stockwell et al., 2017). Moreover, investigations of the therapeutic potential in AD of both agonists and antagonists of A1R and A2AR adenosine receptors have been limited to *in vitro* studies (Woods et al., 2016) (Fig. 7.2).

Surprisingly, when various A1R antagonists were used in *in vitro* studies, an a long term potentiation (LTP) enhancement was observed; however, when this effect was evaluated *in vivo* in A1R knockout mice, there was a lack of alteration in LTP (Gimenez-Llort et al., 2005). A large body of work reports that the use of A1R antagonists in neuropathologies has a neuroprotective effect and that agonists have a neurodegenerative effect (Stone et al., 2009). It has also been reported in several studies that the use of nonspecific P1 receptor antagonists, such as methylxanthines and their derivatives, has shown better neuroprotective effects than the specific agonists or antagonists of those

receptors. Therefore, the protective effect may be due to the antagonism of both receptors simultaneously. Monitoring the administration of caffeine or 1,3,7-trimethylxanthine in a rabbit model with similar characteristics to AD showed a reduction in Aβ-peptide and tau-protein levels, as well as decreased oxidative stress markers and A1R-expression restoration (Prasanthi et al., 2010). In fact, the large number of studies with caffeine in AD-transgenic models shows that caffeine improves cognitive functions, prevents neurodegeneration, and restores synaptic plasticity (Oñatibia-Astibia, Franco, & Martínez-Pinilla, 2017). Interestingly, theobromine generated a neuroprotective effect in a model of AD induced by a high-fat diet by reducing the Aβ-peptide load and restoring the A1R expression (Mendiola-Precoma et al., 2017). Another study showed that the compound TH-9 (a theophylline derivative) induced a long-term increase in excitatory postsynaptic potential in the CA1 region of the hippocampus, a mechanism involved in LTP and long term depression (LTD) (Nashawi et al., 2012).

A recent pilot study indicated the importance of structural modifications in two of the rings of 2-benzylidene-1-tetralone for the gain or loss of affinity for adenosine A1/A2A receptors. In this study, most of the compounds obtained showed selectivity for A1Rs; however, *in vivo* studies are needed to evaluate the effects on neurodegenerative diseases (Legoabe, Van der Walt, & Terre'Blanche, 2018).

As for purinergic receptor expression in senescence reported in animal models such as mice (SAM), one mouse (SAMP8) was considered an AD model, due to its abnormal amyloid precursor protein production. In this model, the A1 receptor did not increase compared to the wild type, which may be associated with the neuroprotective A1 receptor effect, absent in SAMP8 mice (Castillo et al., 2009). In conclusion, the purinergic receptors are important to be a target for the neurodegenerative disease's treatment. It is important to increase agonist and antagonist research in order to precisely determine the best neuroprotective molecules, and *in vivo* studies are needed as well.

Key facts of P1 purinergic receptors

- P1 Purinergic receptors are involved in neuropathologies.
- The A1Rs are widely distributed in brain.
- A1 receptor antagonists induce neuroprotection.
- Purinergic receptors are important as possible targets for neurodegenerative diseases.
- A2 receptor antagonists are proposed in Parkinson's disease treatment.

Summary points

- It is estimated that 46.8 million people suffer from dementia worldwide, and this number is expected to double every 20 years.
- The A1 receptor function has been associated with its inhibitory effects, which affect the function of receptors like NMDA and AMPA receptors.

- The A1R activation induces neuroprotection, but during its prolonged activation elicits neurodegeneration.
- The proposed A2A purinergic receptor antagonists by in silico studies were tested in a cerebral hypoxic-ischemic rat model with improved responses.
- A decrease in A1R expression has been documented in hippocampus of AD patients.
- A1R protein and mRNA expression in the hippocampus were decreased in an AD animal model.
- Methylxanthines, antagonists of P1 receptors, have better neuroprotective effects than the specific agonist or antagonist of these receptors, probably due to the antagonism of both A1 and A2 receptors.

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CHAPTER 8

Molecular aspects of metallothioneins in dementias

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List of abbreviations

AU arbitrary units
AD Alzheimer's disease
ADAM a disintegrin and metalloproteinase
AICD AβPP intracellular domain
ApoE apolipoprotein E
APPMT1+2KO amyloid precursor protein positive/metallothionein-1/2 deficient
APPMT3KO amyloid precursor protein positive/metallothionein-3 deficient
APPS soluble amyloid precursor protein
APPTgMT amyloid precursor protein positive/transgenic metallothionein-1 positive
APPWT amyloid precursor protein positive/metallothionein wild type
Aβ amyloid-β
AβPP amyloid-β precursor protein
cDNA complementary deoxyribonucleic acid
CNS central nervous system
CTF C-terminal fragment
DNA deoxyribonucleic acid
EOFAD early-onset familial Alzheimer's disease
Gfap glial fibrillary acidic protein
GIF growth-inhibitory factor
hAβPP human amyloid-β precursor protein
HC histochemistry
Iba-1 ionized calcium-binding adapter molecule 1
IHC immunohistochemistry
LOAD late-onset Alzheimer's disease
MPAC metal protein attenuating compound
mRNA messenger ribonucleic acid
MT1+2/3KO metallothionein-1+2/3 deficient
MT metallothionein

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NFT neurofibrillary tangle
PHF paired helical filament
Psen1/2 presenilin-1/2
TACE tumor necrosis factor-α converting enzyme
TgMT transgenic metallothionein-1 positive
WT wild type

Mini-dictionary of terms

Alzheimer's disease The most commonly diagnosed form of dementia, first described by Alois Alzheimer in 1906. It is characterized by the progressive loss of cognitive abilities as well as by the presence of amyloid deposits and neurofibrillary tangles.

Amyloid cascade The most accepted hypothesis of AD postulates that the disease is related to the production (and aggregation) of $A\beta_{40-42}$ from the proteolysis of the transmembrane protein AβPP.

Astrocyte The major and most numerous cells of the CNS that represent the main element of the brain homeostatic system.

Microglia Immune cells in the CNS that represent the first line of defense of the brain innate immune system.

Neuroinflammation Inflammatory response due to a variety of injuries in the CNS, such as infection, brain traumatic injury, neurodegenerative brain diseases (including AD), and aging.

Oxidative stress Imbalance between the production of reactive oxygen species and the capability of the organism to readily detoxify the reactive intermediates is called oxidative stress.

Tg2576 transgenic mice The most widely used and characterized AD-like mouse model. They express the 695-amino-acid isoform of human AβPP containing the so-called Swedish mutation (K670N/M671L). Aβ starts to accumulate in the brain at 6–7 months of age, and plaques appear between 9 and 12 months.

Zinc(7)-MT MT coordinates seven bivalent atoms of Zn through thiolate bonds.

Over 35 million people are affected by dementia, an emerging worldwide problem that represents high economic costs for our society (Heppner, Ransohoff, & Becher, 2015; Hurd, Martorell, Delavande, Mullen, & Langa, 2013). There are many causes of dementia (Table 8.1). Alzheimer's disease (AD) is the most commonly diagnosed dementia. AD is characterized by the presence of extracellular deposits of the amyloid-β (Aβ) peptide (senile/amyloid plaques) and intracellular deposits of hyperphosphorylated tau protein (neurofibrillary tangles), together with clear signs of neuroinflammation, oxidative stress, and eventually neuronal death in brain areas such as the hippocampus and the cortex (Bertram, Lill, & Tanzi, 2010; Heneka et al., 2015; Ittner & Götz, 2011; Lopez-Gonzalez et al., 2015). Clinically, it is defined by a progressive loss of cognitive functions, memory, and language. These conditions place a devastating burden on both patients and their families (Hurd et al., 2013).

Most cases of AD (~95%) are late onset and sporadic (LOAD), although early-onset inherited familial (EOFAD) cases also exist (~5%). EOFAD presents Mendelian inheritance of highly penetrant mutations in three genes: *Aβpp*, *Psen1*, and *Psen2*. The first encodes the Aβ precursor protein (AβPP), a transmembrane protein from which Aβ is cleaved. The other two genes encode Presenilin-1 and 2 proteins, respectively;

Table 8.1 Reversible and irreversible dementias.

Irreversible	Main cause	Illness
Degenerative	Neurodegeneration in hippocampus and cerebral cortex	Alzheimer's disease
	Progressive neuronal loss involving the frontal or temporal lobes and spindle neurons	Pick's disease (frontotemporal dementia)
	Defective huntingtin protein leads to lesions in the basal ganglia	Huntington's disease
	Dopaminergic neurodegeneration in substantia nigra	Parkinson's disease
	α-Synuclein deposits	Lewy body disease
	Neurodegeneration produced by iron accumulation in the brain	Hallervorden–Spatz disease
	Mitochondrial alterations	MELAS syndrome
Prion diseases	Spongiform encephalopathy	Creutzfeldt–Jakob disease
	Spongiform encephalopathy	Gerstmann Straussler–Scheinker syndrome
Neoplastic	Primary or metastatic brain tumors	Dementia associated with brain tumors
Reversible	**Main cause**	**Illness**
Vascular	CNS vasculitis	CNS vasculitis
Copper related	Altered copper metabolism	Wilson's disease
Endocrine alterations	Hypothyroidism	Hypothyroidism
	Adrenal insufficiency	Addison's disease
	Excess of glucocorticoids	Cushing syndrome
Metabolic alterations	Renal, hepatic, or respiratory insufficiency	Encephalopathies
	Myelin alteration	Leukodystrophies
	Alterations in heme group metabolism	Porphyria
	Diseases of lipid deposits	Gaucher's disease
Vitamin deficiencies	Cobalamine (vitamin B12)	Pernicious anemia
	Thiamin (vitamin B1)	Beriberi
	Niacin (vitamin B3)	Pellagra
Toxic agents	Alcoholism	Alcohol-related dementia
	Heavy metals (mercury, etc.)	Toxic encephalopathy
Infectious	Viruses such as HIV, etc.	AIDS, etc.

Human dementias may be irreversible and reversible, with many different putative etiologies.

these proteins form part of the catalytic center of γ-secretase, involved in the proteolytic cleavage of AβPP in the amyloidogenic pathway (Fig. 8.1). As a result of these mutations, $A\beta_{40-42}$ is overproduced and accumulates in the brain of these patients. In LOAD, there is no clear-cut genetic evidence of the disease, and it seems more related to the presence

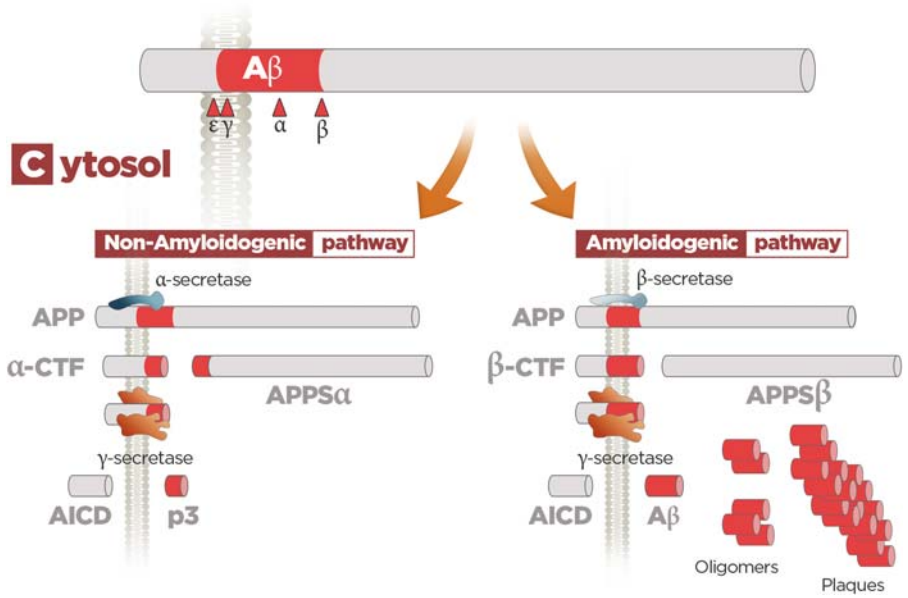

Figure 8.1 ***Amyloid-β precursor protein (AβPP) sequential proteolytic processing.*** AβPP, a type I transmembrane protein, is proteolytically processed by three proteases: α-, β-, and γ-secretases. In the amyloidogenic pathway (on the right), AβPP is consecutively cleaved by β- and γ-secretases, generating a 37- to 43-amino-acid amyloid-β peptide (Aβ) of ~4 kDa that may aggregate and form plaques. In the nonamyloidogenic pathway (on the left), AβPP is cleaved by α- and γ-secretases and produces an ~3-kDa peptide that is pathologically irrelevant. *AICD*, AβPP intracellular domain; *APP*, amyloid precursor protein; *APPS*, soluble amyloid precursor protein; *CTF*, C-terminal fragment.

of risk alleles (i.e., ε4 allele of the apolipoprotein E gene, ApoE) and aging in combination with environmental factors, such as obesity, type 2 diabetes, lack of physical activity, etc. (Bertram et al., 2010; Bertram & Tanzi, 2004; Kaminsky, Marlatt, Smith, & Kosenko, 2010).

The most accepted hypothesis for AD is the amyloid cascade hypothesis. It postulates that an altered proteolysis of AβPP leads to an overproduction of $A\beta_{40-42}$. $A\beta_{42}$ oligomerizes and deposits into extracellular plaques, promoting neuronal loss, vascular damage, and dementia (Hardy & Higgins, 1992; Heppner et al., 2015). Consequently, most transgenic mouse models are based on *Aβpp* mutations. One of the most used AD animal models is the Tg2576 mouse. This transgenic mouse model expresses the 695-amino-acid isoform of human AβPP containing the so-called Swedish mutation (K670N/M671L). Aβ starts to accumulate in the brain at 6–7 months of age, and plaques appear between 9 and 12 months (Karen Hsiao et al., 1996). This model presents cognitive impairments, such as impaired learning and spatial memory in the Morris water maze (Karen Hsiao et al., 1996), increased locomotor activity and exploration, and reduced anxiety (Manso et al., 2016; Manso, Carrasco, Comes, Adlard, et al., 2012).

In addition to amyloid plaques, Tg2576 animals also present neuroinflammation (Frautschy et al., 1998). The latter plays a key role in AD. Clinically, signs of neuroinflammation are detected in AD brains, such as activated microglia and astrocytes, increased proinflammatory cytokine levels in brain and serum, and a downregulation of antiinflammatory molecules (Comes et al., 2017; Whittington, Planel, & Terrando, 2017). Although the CNS was classically considered an immunoprivileged site, it has been shown that peripheral inflammation can contribute to chronic neuroinflammation and neurodegeneration. This inflammatory environment produces an excessive generation of free radicals and subsequent increased levels of several lipid, protein, and DNA oxidative stress markers, together with increased levels of antioxidant enzymes (Guglielmotto, Giliberto, Tamagno, & Tabaton, 2010). Oxidative stress and Aβ are closely related to each other, since Aβ induces oxidative stress in vitro and in vivo and at the same time oxidative stress increases the production, oligomerization, and deposition of Aβ (Dong et al., 2003; Guglielmotto et al., 2010).

Some facts evidence that the hypothesized amyloid cascade is not the only procedure taking part in this disease, as Aβ is insufficient to explain the accumulation of the peptide in specific brain regions in AD patients. The metal hypothesis of AD postulates that a modified form of soluble Aβ interacts with specific metals (Cu and Zn) to promote AD pathology (Bush & Tanzi, 2008). In addition, transition metals are an important source of oxidative stress and, together with Zn, have been found to be altered in AD brains (Dong et al., 2003). A number of therapeutic approaches dealing with amyloid, metal accumulation, neuroinflammation, tau aggregation, and oxidative stress have been proposed (Fig. 8.2).

Metallothioneins (MTs) constitute a nonenzymatic superfamily of low-molecular-weight heavy metal–binding proteins. Mammalian MTs are subdivided into four isoforms, MT1 to MT4, which belong to the first family of the 15 that exist. The highly conserved structure of MTs suggests that they may have an important biological function but, as of this writing, it remains unclear. MTs have similar roles in the CNS and in the peripheral tissues related to metal homeostasis, detoxification and storage of heavy metals, and antioxidant and immunomodulatory roles. In addition, MT levels are altered in certain diseases, including AD (Adlard, West, & Vickers, 1998) and multiple sclerosis (Espejo & Martínez-Cáceres, 2005; Penkowa et al., 2003), suggesting an important role for MTs in brain disorders.

The *Mt1+2* genes are widely expressed, and very early reports readily demonstrated that this was also the case in the brain (Chen & Ganther, 1975; Durnam & Palmiter, 1981). The general consensus is that MT1+2 are present throughout the brain and spinal cord, and that the main cell type expressing these MT isoforms is the astrocyte, with lower levels of expression in other cells (Chung, Hidalgo, & West, 2008; Hidalgo, Aschner, Zatta, & Vašák, 2001). The expression of the *Mt3* gene occurs predominantly within the CNS (Kobayashi et al., 1993; Palmiter, Findley, Whitmore, & Durnam, 1992),

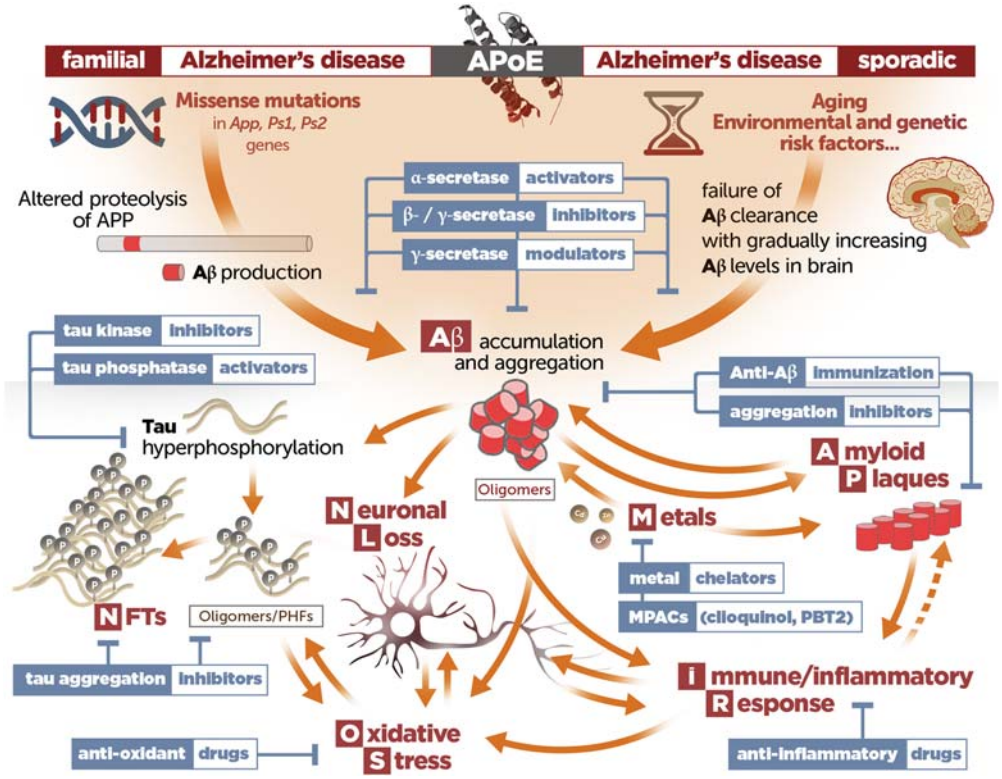

Figure 8.2 ***General overview of therapeutic approaches for Alzheimer's disease (AD) treatment.*** Multifactorial diseases, such as AD, allow for different therapeutic strategies targeting different hallmarks of the disease: amyloid-β (Aβ) aggregation (oligomeric species and plaques), hyperphosphorylation and aggregation of tau (tangles), immune/inflammatory response, oxidative stress, neuronal loss, and heavy metal imbalance. *Blue boxes* indicate the current therapies. *APoE*, apolipoprotein E gene; *APP*, amyloid precursor protein; *MPACs*, metal protein attenuating compounds; *NFTs*, neurofibrillary tangles; *PHFs*, paired helical filaments.

and although the cellular localization of MT3 is still debated, astrocytes and neurons are likely sources (Chung et al., 2008; Hidalgo et al., 2001).

MT3, the so-called brain-specific MT isoform, was initially named growth-inhibitory factor because it displayed an inhibitory role on neuronal growth in the presence of human AD brain extracts, and thus was suggested to be involved in the etiology of AD (Uchida, Takio, Titani, Ihara, & Tomonaga, 1991). Although it is still unclear whether MT3 is downregulated in AD (Manso, Carrasco, Comes, Meloni, et al., 2012), the discovery of this MT isoform increased the interest in research into the role of MT1+2 in AD and in other brain disorders. The mRNA and protein levels of MT1+2 are reported to be increased in AD brains (Duguid, Bohmont, Liu, & Tourtellotte, 1989; Hidalgo et al., 2006; Zambenedetti, Giordano, & Zatta, 1998), even at preclinical AD stages previous to

inflammation (Adlard et al., 1998). Mouse models of AD, such as Tg2576 mice, exhibit a prominent upregulation of MT1+2 in the proximity of the amyloid plaques, compatible with the inflammatory milieu (gliosis, oxidative stress, metal accumulation), whereas MT3 expression is similar in areas with and without plaques (Carrasco et al., 2006; Hidalgo et al., 2006). This suggests that MT3 would be less relevant regarding neuroinflammation in comparison with MT1+2 isoforms.

In vitro studies revealed that Zn_7MT2A decreases Aβ neurotoxicity of cultured cortical neurons, probably as a result of a metal swap among Zn_7MT2A and Cu(II)-Aβ, preventing the toxicity from Cu-mediated aggregation of $A\beta_{40}$ and $A\beta_{42}$ (Chung et al., 2010). Such protective mechanism had also been observed previously for Zn_7MT3 (Meloni et al., 2008). MT1+2 inhibit ADAM17/TACE, one of the α-secretases of the nonamyloidogenic pathway, favoring the amyloidogenic pathway and increasing the levels of Aβ peptides (Siddiq et al., 2015). Interestingly, the MT3 isoform has been reported to increase the activity of another α-secretase, ADAM10, in the mouse neuroblastoma Neuro2A Swedish APP cells (Park, Kim, Jin, Song, & Jeong, 2014), suggestive of MT isoform-specific roles.

To investigate the interaction between MTs and AD pathology, we have generated Tg2576 mice lacking either MT1+2 (Masters, Kelly, Quaife, Brinster, & Palmiter, 1994) or MT3 (Erickson, Hollopeter, Thomas, Froelick, & Palmiter, 1997) or overexpressing MT1 (Palmiter, Sandgren, Koeller, & Brinster, 1993) (Fig. 8.3). This type of longitudinal study (up to ~60 weeks of age) provides a large amount of information on the different aspects of the disease at different ages and the putative role of MTs in the disease progression.

We confirmed the premature mortality and reduced life span of transgenic mice expressing the mutated human (h) AβPP reported in previous works (Carlson et al., 1997; Hsiao et al., 1995; Moechars, Lorent, & Van Leuven, 1999). In general, all APP^{+}

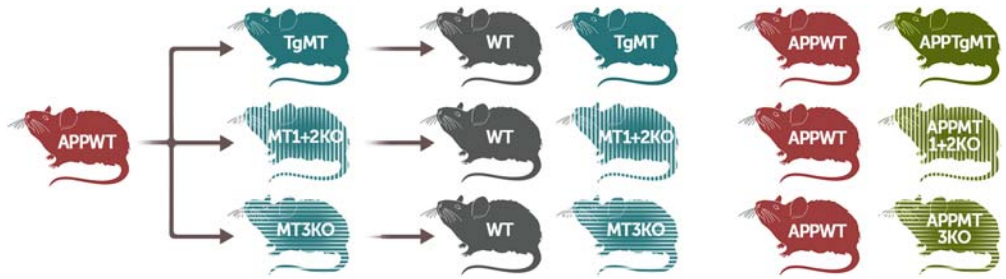

Figure 8.3 ***Cross-breeding strategy for mice.*** Hemizygous Tg2576 (amyloid precursor protein wild type [*APPWT*]) mice were appropriately crossed with mice lacking either metallothionein-1 + -2 (*MT1+2KO*) or metallothionein-3 (*MT3KO*) or overexpressing MT1 (*TgMT*), to obtain the four genotypes of interest. For instance, by crossing APPWT ($hAPP^{+/-}$/$TgMT^{-/-}$) mice with TgMT ($TgMT^{+/-}$) mice, we generated WT ($hAPP^{-/-}$/$TgMT^{-/-}$), TgMT ($hAPP^{-/-}$/$TgMT^{+/-}$), APPWT ($hAPP^{+/-}$/$TgMT^{-/-}$), and APPTgMT ($APP^{+/-}$/$TgMT^{+/-}$).

mice had a dramatically reduced survival at ~60 weeks of age, males having a higher mortality rate than females regardless of the genetic background (129S6 for the knockout mice, C57BL/6J for the TgMT line), indicating a role for sex hormones (Choi et al., 2008; Coschigano, Clemmons, Bellush, & Kopchick, 2000; Couzin-Frankel, 2011). The effects of the different MT isoforms on survival were somewhat different, and varied from early (perinatal/weaning) to later ages and in a sex-dependent manner. Thus, at weaning, MT1+2 deficiency rescued to some extent the early mortality caused by hAPP expression, and more so in female mice, suggesting a detrimental role for MT1+2; the opposite, however, occurred in APP^{-} mice (Manso, Carrasco, Comes, Adlard, et al., 2012). The overexpression of MT1, indeed, had a prosurvival effect in both APP^{+} and APP^{-} (to a lower extent) mice at weaning (Manso et al., 2016). Genetic background (Carlson et al., 1997), number of *MT1+2* alleles of the wild-type mice, and putative functional differences between MT1 and MT2 isoforms may contribute to these apparent discrepancies. In adults, MT1+2 are rather detrimental according to these two experiments (Manso et al., 2016; Manso, Carrasco, Comes, Adlard, et al., 2012). MT3 deficiency did not influence survival at early ages, but in adults it rescued partially the hAPP-induced mortality in females only, very much like that observed for MT1+2 deficiency. Thus, these three MT isoforms seem to behave as detrimental factors regarding survival in the presence of hAPP expression, particularly in female mice (Manso et al., 2016; Manso, Carrasco, Comes, Meloni, et al., 2012). This is notoriously in contrast with the general view of these proteins as protective factors.

The formation of amyloid plaques is one of the most featured and studied hallmarks of the pathology of AD. We have been evaluating the amyloid plaque burden (by immunohistochemistry [IHC] or histochemistry at 14 months of age) and cascade (by western blot at different ages) in our experiments with Tg2576 mice, especially in the most affected areas, the hippocampus and the cortex. Figs. 8.4 and 8.5 show amyloid plaques stained with the 4G8 antibody and Congo red, respectively. This is the type of plaque seen throughout the cortex and the hippocampus, regardless of the genetic background. A small but significant and consistent effect of MT1+2 was observed in the hippocampus, with MT1+2 deficiency decreasing the plaques (Manso, Carrasco, Comes, Adlard, et al., 2012) and MT1 overexpression increasing them (Manso et al., 2016); therefore, we conclude that MT1+2 promote the formation of amyloid plaques, at least in the hippocampus, in the long term and especially in female mice. Interestingly, the results for MT3 led to the same conclusion (Manso, Carrasco, Comes, Meloni, et al., 2012).

It may be important to identify different plaque types, as they may represent different stages of the pathology and might have different effects on the neuropathological events of the disease, including inflammation, metal contents, and neuronal loss. Roughly, we can differentiate between an early stage of senile plaque formation, named diffuse or preamyloid plaques, and dense core neuritic plaques, a later stage of plaque formation (see Fig. 8.4, left). The former show few evidences of neuronal damage and low levels

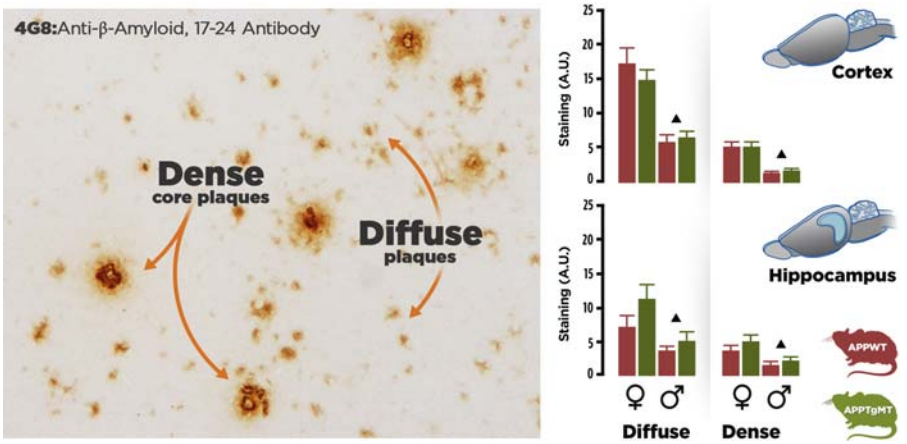

Figure 8.4 ***Effect of methallothionein-1 overexpression on dense and diffuse amyloid plaques.*** (Left) Representative 4G8 immunostaining of diffuse (an early stage of senile plaques) and dense core (a mature stage) amyloid-β plaques in the mouse brain. (Right) Unpublished quantification of these stainings (*A.U.*, arbitrary units) in the cortex and the hippocampus showed that females had significantly more amyloid-β plaques (dense and diffuse) than males in both areas and that diffuse plaques were more abundant than dense core plaques. Results are the mean ± SEM (n = 9–11); ▲$P \leq .05$ versus females.

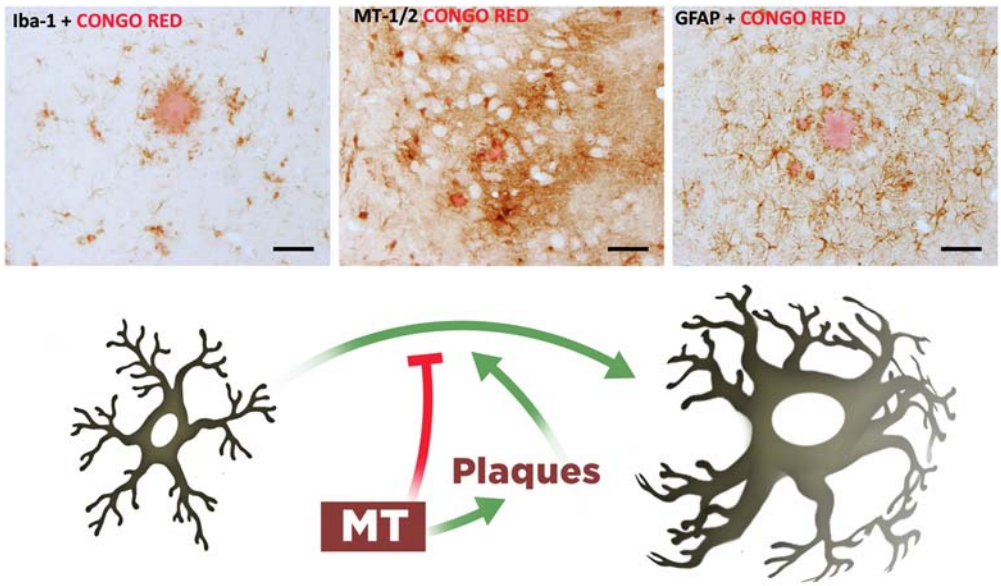

Figure 8.5 ***Amyloid plaques causes gliosis and metallothionein (MT) 1+2 upregulation.*** (Top) Dense plaques were stained with Congo red and simultaneously with antibodies specific for microglia (*Iba-1*), astrocytes (*GFAP*), and MT1+2 in Tg2576 mice; all these immunostains were increased in the vicinity of Congo red core plaques. (Bottom) Outline of the interaction between amyloid plaque formation, inflammation, and MTs. MT1+2 tend to inhibit microgliosis directly, and this is seen in young mice, whereas by their promotion of amyloid plaques, which stimulate microgliosis, MT1+2 indirectly activate microgliosis in old mice.

of associated activated microglia and astrocytes, whereas the latter are surrounded by many dystrophic neurites and high levels of associated activated microglia and astroglia (Ikeda, Yanagisawa, Allsop, & Glenner, 1990; Mrak, 2009). Interestingly, the analysis of the different plaque types in the APPTgMT model made for this study revealed that female mice have significantly more plaques (diffuse and dense) than males and that the diffuse plaques were more abundant (approximately three- to fourfold in the cortex and approximately twofold in the hippocampus) than dense core plaques (Fig. 8.4, right). Therefore, in this type of study it is of the utmost importance to differentiate between the two sexes and, if possible, to study the different regions of the brain independently. The effects of MT1 overexpression measured this way were in accordance with those observed with total plaque load (Manso et al., 2016), in principle ruling out specific effects of MTs on the transition from diffuse to dense plaques. Also, MT1+2 IHC (Fig. 8.5) did not reveal any particular trends other than high immunostaining in those cells surrounding dense plaques.

The amyloid cascade was analyzed by western blot at early (∼5 months) and advanced (∼14 months) ages. Our results suggest so far that in female mice the amyloid processing products tend to be favored by MT1+2 at early ages and decreased at advanced ages (Manso et al., 2016; Manso, Carrasco, Comes, Adlard, et al., 2012), with more inconsistent results for males. MT3 deficiency showed only minor effects, but chronic injection of MT3 altered the amyloid cascade in a complex manner (Manso, Carrasco, Comes, Meloni, et al., 2012). Perhaps this apparent decreased amyloid cascade is linked to the increased formation of plaques, which could represent a protective mechanism against oligomeric Aβ species, which have been described to play a central role in neurotoxicity and correlate better than amyloid plaque burden with disease severity (Braak et al., 1999).

Inflammation is intimately related to AD pathogenesis as a general phenomenon and particularly to amyloid plaques, in which activated microglia and astroglia are present (Glass, Saijo, Winner, Marchetto, & Gage, 2010), in the Tg2576 mouse model (see Fig. 8.5). MT1+2 are considered important proteins in the physiological events elicited in the brain to cope with tissue injury and neuroinflammation (Chung & West, 2004; Molinero et al., 2003) and, accordingly, MT1+2 IHC was increased in the vicinity of the plaques (Comes et al., 2017). In the Tg2576 model, however, minor effects of MT1+2 deficiency (Manso, Carrasco, Comes, Adlard, et al., 2012) or MT1 overexpression on gliosis (Comes et al., 2017) were observed. Yet, the results still point out that in young animals MT1+2 have a significant inhibitory effect on glial cells, whereas the opposite seems to happen in old animals. Probably the latter is the consequence of the promotion of plaques caused by MT1+2, which will induce astrogliosis and microgliosis despite the probable direct inhibitory effects of MTs. This is summarized in Fig. 8.5 at the bottom.

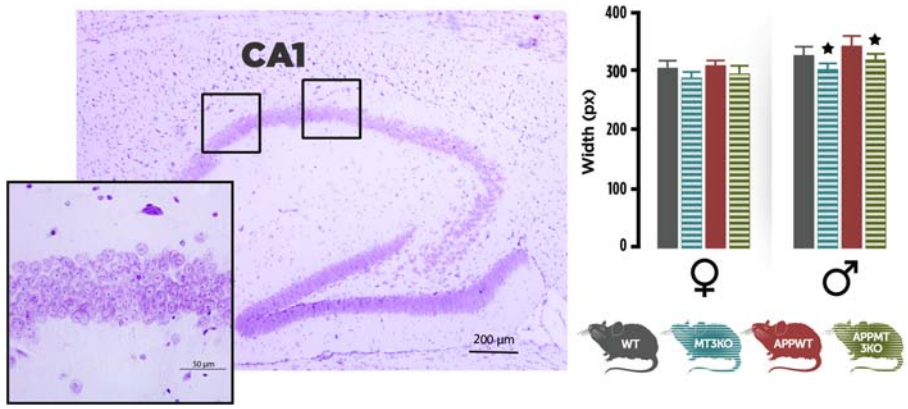

Figure 8.6 ***Effect of metallothionein-3 (MT3) on hippocampal CA1 neurons.*** (Left) Representative Nissl body staining of neurons of the hippocampus (original magnification 4×) and a higher magnification of the CA1 pyramidal layer (original magnification 20×). Three measures of the width of the pyramidal layer in two different areas of the CA1 (indicated in the *black-outlined squares*) were analyzed. (Right) Unpublished results indicate that MT3 deficiency significantly decreased the width of the CA1 layer in males, and the same trend was observed in females. Results are the mean ± SEM (n = 9–11); ★$P \leq .05$ versus wild type (*WT*) and APPWT.

Oxidative stress, inflammation, and amyloid plaque formation are involved in AD neuronal loss in zones prone to oxidation insults, like the hippocampus. As reported in previous works, we studied neuronal loss in the cornu ammonis 1 (CA1) hippocampal area where a significant thinning of the pyramidal layer had been described in APP^+ mice carrying the Swedish mutation (Calhoun et al., 1998); a selective neuronal loss in CA1 has also been observed in AD patients (West, Coleman, Flood, & Troncoso, 1994). We confirmed such a phenotype in the APPTgMT cross, but MT1 overexpression did not affect significantly the neuronal survival (Comes et al., 2017). For this study we analyzed the same hippocampal CA1 area of the APPMT3KO mice (Fig. 8.6). Surprisingly, the layer was not decreased in the APP^+ mice, but, interestingly, the deficiency of MT3 decreased it significantly in male mice, and the same trend was observed in females. Previous studies have suggested that MT3 may be especially relevant for neurons (Erickson et al., 1997; Lee, Kim, Palmiter, & Koh, 2003).

Key facts of neuroinflammation

- Neuroinflammation is the inflammatory response of the nervous system against any harmful stimuli.
- Persistence of neuroinflammation (chronic inflammation) may result in deleterious effects on the CNS.
- The main inflammatory cells of the CNS are the resident microglia and astrocytes.

- Activated microglia are the main source of reactive oxygen species and reactive nitrogen species among other neurotoxic factors that may contribute to the chronic oxidative stress present in most neurodegenerative diseases such as AD.
- Neuroinflammation has a key role in the development and progression of AD. Microglia and astrocytes are closely associated with amyloid plaque deposits and are thought to contribute to their formation and/or clearance.
- Antiinflammatory drugs are a therapeutic approach to AD treatment.

Key facts of metallothioneins

- MTs constitute a nonenzymatic superfamily of low-molecular-weight heavy metal–binding proteins with a highly conserved structure, suggesting an important biological function.
- The MT1+2 isoforms are expressed in most tissues, whereas MT3 is mostly expressed in the CNS.
- MT1+2 and MT3 are synthesized in the CNS mainly, but not exclusively, by astrocytes.
- MT1+2 are considered stress proteins, responsive to a wide range of inflammatory and prooxidant factors and to psychological stress, and are upregulated in neurodegenerative diseases such as AD. They seem to be tissue-protective proteins by means of their potent antioxidant and antiinflammatory effects.
- MT3, in contrast, responds poorly to tissue injury conditions and rather tends to be downregulated in AD. It has been proposed that such downregulation could contribute to AD.

Summary points

- This chapter focuses on the heavy metal–binding proteins, MTs, which have been shown to be altered by AD, the most prevalent form of dementia.
- The most accepted hypothesis for AD is the amyloid cascade hypothesis, which postulates that the aggregation into plaques of $A\beta_{40-42}$, originating from a particular proteolysis of AβPP, is critical for the disease.
- The transgenic mouse Tg2576 is one of the most studied animal models of AD. As in the human disease, MT1+2 tend to be upregulated in the vicinity of the amyloid plaques, whereas the results for MT3 are less clear-cut.
- The Tg2576 mice have premature mortality and reduced life span.
- At weaning, MT1+2 (but not MT3) deficiency rescues to some extent the early mortality in female Tg2576 mice.
- In adults, all three MT isoform deficiencies rescue the decreased life span up to ~60 weeks of age in female Tg2576 mice.

- Overexpression of MT1 has a general prosurvival role at weaning and rather the opposite in adult male mice.
- MTs favor the formation of Aβ plaques in susceptible areas of the brain, such as the hippocampus and the cortex, especially in females.
- MT1+2 have an inhibitory effect in microglia and astrocytes in young animals, while MT3 does not seem to have a relevant role in glial cells. In contrast, MT1+2 tend to promote gliosis in old animals, likely because of their effects on amyloid plaques.
- A significant thinning in the CA1 pyramidal layer of the hippocampus of Tg2576 mice is observed in some but not all of the crosses carried out. Mice lacking MT3 isoform showed a tendency to decrease this layer.

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CHAPTER 9

Implication of microRNAs in Alzheimer's disease pathogenesis

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List of abbreviations

AD Alzheimer's disease
APP amyloid precursor protein
Aβ amyloid-β
BACE1 β-secretase 1
CDK cyclin-dependent kinase
GSK glycogen synthase kinase
MAPT microtubule-associated protein tau
miRNA, miR microRNA
NFT neurofibrillary tangle
PSEN1 presenilin 1
SNP single-nucleotide polymorphism

Mini-dictionary of terms

Amyloid-β Aβ peptides are short (36–43 amino acid residues) peptides associated with AD pathogenesis, derived from the stepwise processing of APP by secretases.
Endocytosis The term endocytosis is a general term describing the process of engulfing substances and particles from the extracellular space by the cell.
Microtubule-associated protein tau The tau protein promotes microtubule assembly and stability. Its hyperphosphorylation and accumulation in the form of NFTs are well-established hallmarks of AD.
MicroRNA An miRNA is a short noncoding RNA molecule (19–22 nucleotides) that regulates gene expression.
Phagocytosis Phagocytosis is a type of endocytosis. It is a multistep process in which cells engulf other cells or large solid particles.

Introduction

Genetics, epigenetics, and pathology of Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized neuropathologically by the accumulation of neurofibrillary tangles (NFTs) containing hyperphosphorylated microtubule-associated protein tau (MAPT), as well as the appearance of amyloid

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oligomers and plaques in the brain (Jack et al., 2018). Pathogenic AD-causing mutations have been identified in such genes as presenilin 1 and 2 (*PSEN1* and *PSEN2*) and amyloid precursor protein (*APP*) (Lane, Hardy, & Schott, 2018). In addition, apolipoprotein E4 (*APOE4*), triggering receptor expressed in myeloid/microglial cells 2 (*TREM2*), clusterin (*CLU*), sortilin-related receptor 1 (*SORL1*), phosphatidylinositol binding clathrin assembly protein (*PICALM*), and Myc-box-dependent interacting protein 1 (*BIN1*) have been identified in genome-wide association studies (GWASs) as genes modulating AD risk (Guerreiro, Gustafson, & Hardy, 2012). The physiological functions of the proteins encoded by the AD-implicated genes, early synaptic dysfunctions, and progressive accumulation of NFTs indicate that aberrant APP processing, perturbed lipid metabolism, impaired endocytosis, phagocytosis, and compromised synaptic physiology, as well as alterations in MAPT, could all contribute to AD pathogenesis.

The expression of AD-implicated genes might be regulated by short (19—22 nucleotides long) noncoding RNAs, known as microRNAs (miRNAs), generated in a multistep process presented in Fig. 9.1. Accordingly, several studies have demonstrated alterations in the levels of a number of miRNAs in the brains of AD patients compared with healthy controls (Lau et al., 2013; Salta & De Strooper, 2017). In addition, functional annotations of AD-associated single-nucleotide polymorphisms (SNPs) revealed six lead and two proxy SNPs that are likely to affect the mRNA recognition and targeting by miRNAs (Han, Huang, Gao, & Huang, 2017). In this chapter we review potential functions of miRNAs in the development of AD (Fig. 9.2).

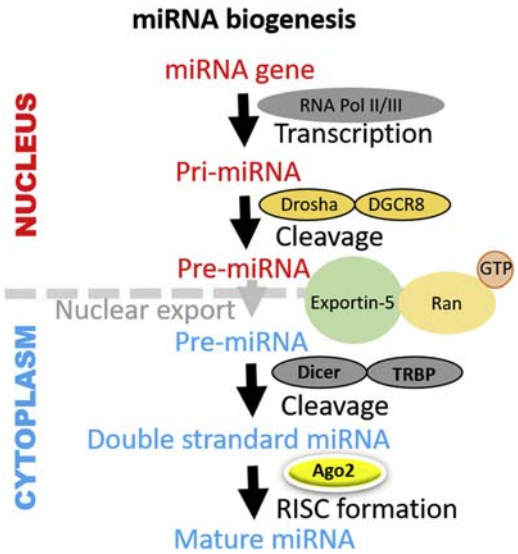

Figure 9.1 ***MicroRNA (*miRNA*) biogenesis pathway.*** The scheme illustrates the steps of miRNA biogenesis, starting from transcription and cleavage, taking place in the nucleus, through nuclear export and final processing in the cytoplasm. *RNA Pol II/III*, RNA polymerase II/III; *RISC*, RNA-induced silencing complex.

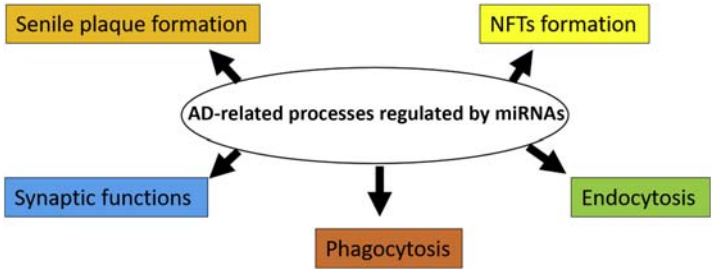

Figure 9.2 ***MicroRNA* (miRNA) *implication in Alzheimer's disease (AD).*** The scheme summarizes the potential physiological and Alzheimer's disease–related pathological processes that might be regulated by miRNAs. *NFTs*, neurofibrillary tangles.

MicroRNAs regulating amyloid precursor protein expression and processing

APP is a transmembrane protein that exists in several isoforms resulting from alternative splicing. It plays an important role in multiple physiological processes, including synaptogenesis and synaptic plasticity. APP expression is regulated at genomic, transcriptional, and translational levels, and miRNAs, including miR-106a and -520c (Patel et al., 2008); miR-17, -20a, and -106a/b (Hébert et al., 2009); miR-147, -655, -323-3p, and -153 (Delay, Calon, Mathews, & Hébert, 2011); and miR-16 and -101, are directly involved in this regulation (Table 9.1). For instance, miR-16 and -101 suppress APP production, its cleavage, and accumulation of fibrillar amyloid-β (Aβ) (Liu et al., 2012; Long & Lahiri, 2011; Vilardo, Barbato, Ciotti, Cogoni, & Ruberti, 2010). Moreover, AD-associated SNPs potentially modulating miR-147 and -20a binding sites have been discovered in the *APP* 3′UTR (Delay et al., 2011; Mallick & Ghosh, 2011).

Interestingly, miRNAs may not only regulate APP levels, but also influence the *APP* mRNA splicing (Smith, Hashimi, Girard, Delay, & Hébert, 2011). Ectopic expression of

Table 9.1 Summary of microRNAs involved in direct amyloid precursor protein regulation.

Name	Target gene	References
miR-16	APP	Liu et al. (2012), Zhang, Chen, Wang, and Lin, (2015)
miR -17, -20a, -106a/b	APP	Hébert et al. (2009)
miR-101	APP	Barbato et al. (2014), Vilardo et al. (2010)
miR- 106a, -520c	APP	Patel et al. (2008)
miR-124	APP	Smith et al. (2011)
miR-147, -20a, -655, -323-3p, -153	APP	Delay et al. (2011)

The miRNAs that regulate protein levels of APP are listed. *APP*, amyloid precursor protein.

neuron-specific miR-124 in vitro has been demonstrated to result in skipping of *APP* exons 7 and 8. Interestingly, this miRNA is downregulated in AD brain (Lukiw, 2007).

miRNAs may also indirectly influence Aβ production from APP, by modulating levels and activity of secretases, essential for Aβ generation (Gleichmann, Chow, & Mattson, 2011). While the cleavage of APP by α-secretase prevents Aβ deposition, cleavage of APP by β-secretase (BACE1), and then by γ-secretase complex, releases Aβ peptides. The majority of the described miRNA-mediated indirect regulation of APP processing refers to BACE1, while only a few known miRNAs regulate other secretases (miR-107, -144, -27a-3p, -24, -186, -455, -34a, -125b, and -146a) (Table 9.2).

Wang and colleagues showed that levels of miR-107 decrease early in AD, with a parallel increase in BACE1 levels. Later it was demonstrated that miR-107, -339-5p, -186, and -195 recognize sites in the 3′UTR of *BACE1* mRNA and reduce BACE1 expression (Kim, Yoon, Chung, Brown, & Belmonte, 2016; Long, Ray, & Lahiri, 2014; Nelson & Wang, 2010; Wang et al., 2008; Zhu et al., 2012). Moreover, miR-29a/b-1 cluster levels are significantly lower in the anterior temporal cortex in sporadic AD. This corresponds to increased BACE1 protein levels. However, the *BACE1* mRNA amount remains unchanged (Hébert et al., 2008). Finally, overexpression of miR-188-3p in an AD mouse model has been demonstrated to significantly decrease BACE1 and Aβ levels, reduce neuroinflammation, and improve long-term synaptic plasticity, spatial learning, and memory (Zhang, Hu, Teng, Tang, & Chen, 2014).

Importantly, there is a reciprocal cross talk between miRNAs and Aβ. Several studies suggest the existence of a regulatory feedback loop. For instance, they point toward a transient Aβ effect on miR-106b expression (Wang et al., 2010) and demonstrate that miR-106b regulates *APP* mRNA levels (Hébert et al., 2009). Moreover, global analysis of miRNA profiles in Aβ42-treated primary hippocampal neurons has demonstrated rapid and strong changes in the miRNA network upon Aβ42 application. Nine miRNAs (miR-9, -20b, -21, -30c, -148b, -181c, -361, and -409-3p and Let-7i), six of which present lower levels in human AD brain (miR-9, -20b, -30c, -148b, and -181c and Let-7i) (Cogswell et al., 2008; Hébert et al., 2008), are downregulated upon Aβ42 treatment (Schonrock et al., 2010). Interestingly, miR-9 is one of the most highly expressed miRNAs in the developing and adult vertebrate brain (Coolen, Katz, & Bally-Cuif, 2013).

MicroRNAs regulating microtubule-associated protein tau expression and its posttranslational modifications

MAPT promotes microtubule assembly and stability. It can undergo several posttranslational modifications, including phosphorylation, and its hyperphosphorylation and accumulation in the form of NFTs are commonly observed in AD neurons (Ballatore, Lee, & Trojanowski, 2007; Iqbal, Liu, & Gong, 2016).

Table 9.2 Summary of microRNAs involved in indirect amyloid precursor protein regulation.

Name	Target protein	References
miR-9	BACE1	Hébert et al. (2008)
miR-29a/b-1	BACE1	Hébert et al. (2008), O'Connor et al. (2008)
miR-29c	BACE1	Yang et al. (2015), Zong et al. (2011)
miR-107	BACE1	Faghihi et al. (2008), Nelson and Wang (2010), Wang et al. (2008)
miR-124	BACE1	Fang et al. (2012), Smith et al. (2011)
miR-186	BACE1	Kim et al. (2016)
miR-188-3p	BACE1	Zhang et al. (2014)
miR-195	BACE1	Ai et al. (2013), Sun et al. (2015), Zhu et al. (2012)
miR-298 and -328	BACE1	Boissonneault, Plante, Rivest, and Provost (2009)
miR-339-5p	BACE1	Long et al. (2014)
miR-107	ADAM10	Augustin et al. (2012)
miR-144	ADAM10	Cheng et al. (2013)
miR-27a-3p	PSEN1	Sala Frigerio et al. (2013)
miRs-24, miR-186, miR-455	NCSTN	Delay et al. (2014)
miRNA-34a	γ-Secretase	Dickson, Kruse, Montagna, Finsen, and Wolfe (2013), Jian et al. (2017)
miR-125b, miR-146a	TSPAN12	Lukiw, Surjyadipta, Dua, and Alexandrov (2012), Xu, Sharma, and Hemler (2009)

The miRNAs that regulate levels of proteins important for APP processing are listed. *ADAM10*, a disintegrin and metalloproteinase domain-containing protein 10 (α-secretase activity); *APP*, amyloid precursor protein; *BACE1*, β-secretase 1; *NCSTN*, nicastrin; *PSEN1*, presenilin 1; *TSPAN12*, tetraspanin 12.

Several in vitro and in vivo studies have demonstrated that miRNAs may either abrogate or exacerbate tau toxicity by regulating tau phosphorylation (Reddy et al., 2017) (Fig. 9.3). Overexpression of miR-98, downregulating insulin-like growth factor 1 (IGF-1), leads to increased phosphorylation of tau by glycogen synthase kinase (GSK) (Hu et al., 2013). Similarly, overexpression of miR-26b in rat primary postmitotic neurons induces tau hyperphosphorylation. This enhanced phosphorylation is caused by the miR-26b-dependent decrease in retinoblastoma-associated protein (Rb1) levels and consequent activation of cyclin-dependent kinase 5 (Cdk5) (Absalon, Kochanek, Raghavan, & Krichevsky, 2013). Tau hyperphosphorylation along with upregulation of p35, Cdk5, and p44/42-MAPK signaling is also caused by miR-125b overexpression in rat primary neurons (Banzhaf-Strathmann et al., 2014). These are the consequences of the downregulation of dual-specificity phosphatase 6 (DUSP6), protein phosphatase 1 catalytic subunit α (PPP1CA), and antiapoptotic factor B cell lymphoma-W (Bcl-W). The importance of miR-125b in the modulation of tau phosphorylation has been further strengthened by the observation that it downregulates forkhead box Q1 (FOXQ1), and

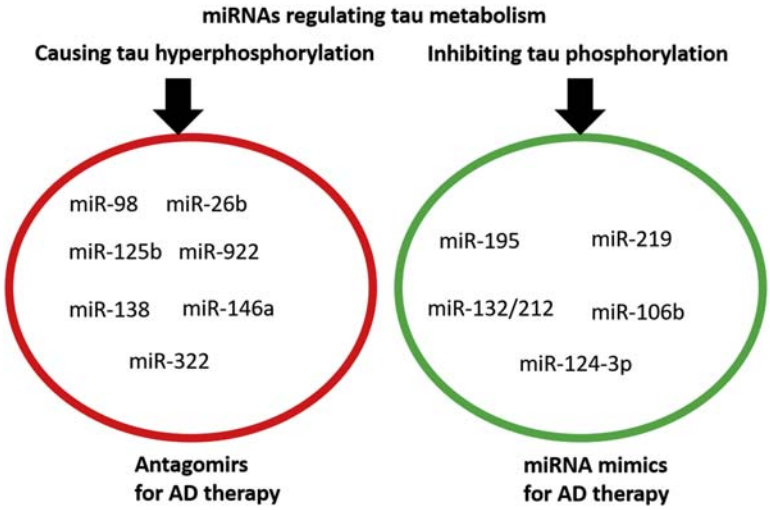

Figure 9.3 ***MicroRNAs regulating tau metabolism.*** The miRNAs influencing tau posttranslational modifications are summarized. Upregulation of miRNAs listed in the *circle* on the left leads to tau hyperphosphorylation, whereas upregulation of miRNAs listed in the *circle* on the right inhibits tau phosphorylation. *AD*, Alzheimer's disease.

consequently activates CDK5 and p35/25, leading to tau hyperphosphorylation (Ma, Liu, & Meng, 2017). Another example of the miRNA-dependent modulation of tau physiology is miR-922-mediated downregulation of ubiquitin carboxy-terminal hydrolase L1 (UCHL1), also resulting in tau hyperphosphorylation (Zhao et al., 2014). Furthermore, overexpression of miR-138 has been demonstrated to reduce retinoic acid receptor α (RARA) levels and leads to GSK3β-mediated tau hyperphosphorylation in the HEK293/tau cell line (Wang et al., 2015), and overexpression of miR-146a has been shown to cause tau hyperphosphorylation in SH-SY5Y cells (Wang et al., 2016). The last is a downstream effect of the reduced expression of rho-associated, coiled-coil-containing protein kinase 1 (ROCK1) and decreased phosphorylation of phosphatase and tensin homolog (PTEN). In addition, miR-322 enhances tau phosphorylation by negatively controlling brain-derived neurotrophic factor/neurotrophin-3 growth factors receptor (BDNF-TrkB) activation (Zhang et al., 2018). All these data provide comprehensive evidence that the miRNA network can indirectly enhance tau toxicity. Therefore, antagomirs might be worth investigating for AD therapy.

On the other hand, miRNAs may provide neuroprotection (Reddy et al., 2017). Overexpression of miR-195 in two-vessel-occlusion rats prevents tau hyperphosphorylation via downregulation of *Cdk5r1* expression and decrease in Cdk5/p35 activity (Sun et al., 2015). Moreover, another study has demonstrated that downregulation of presumably protective miR-219, directly targeting the *MAPT* gene, increases total

tau levels in a *Drosophila* model and leads to its hyperphosphorylation (Santa-Maria et al., 2015). Similarly, miR-132/212 knockout mice display increased levels of both total and phosphorylated tau, as a consequence of direct *MAPT* targeting by miR-132 (Smith et al., 2015). Consistently, loss of miR-132 enhances tau phosphorylation and increases amyloid plaque load in an AD mouse model. This is due to the direct modulation of inositol 1,4,5 trisphosphate 3-kinase B (ITPKB) levels and consequent enhanced activity of extracellular signal-regulated kinase 1/2 (ERK1/2) and BACE1 (Salta, Sierksma, Vanden Eynden, & De Strooper, 2016). In addition, miR-132 loss has been shown to directly increase the expression of nitric oxide synthase 1 (NOS1). This leads to S-nitrosylation of CDK5, dynamin-related protein 1 (DRP1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and tau hyperphosphorylation (Wang et al., 2017). Consistent with the observation of the detrimental effects of a reduction in miR-132 levels, injection with miR-132 mimic improves long-term memory in a mouse model (Salta et al., 2016). Another miRNA protective against tau toxicity is miR-106b. Its overexpression inhibits tau phosphorylation at tyrosine 18 by direct downregulation of Fyn kinase (Liu, Zhao, & Lu, 2016). Moreover, overexpression of miR-124-3p in N2a/APPSwe cells inhibits tau phosphorylation and suppresses apoptosis by downregulating caveolin-1 (Kang et al., 2017).

MicroRNAs regulating synaptic functions, endocytosis, and phagocytosis

Given that several miRNAs are enriched in the synaptic compartments, it can be expected that multiple miRNAs, expressed differentially in AD brain, might regulate the expression of genes important for synaptic functions (Table 9.3). In line with that, miR-132, which shows reduction in AD, has been reported to target GTPase-activating protein p250GAP; glutamate receptors GluA1, GluN2A, and GluN2B; methyl CpG-binding protein 2 (MeCP2); and forkhead box P2 (FOXP2). Therefore, it might regulate neuronal morphogenesis, plasticity, and synapse formation (reviewed in Bicker, Lackinger, Weiß, & Schratt, 2014).

Similarly, miR-125b and -128, enriched at the synapse, present altered expression in hippocampi and frontal gyri in human AD and APPSwe mouse brain (Cogswell et al., 2008; Lukiw, 2007). The first one, miR-125b, modulates spine morphogenesis, and its overexpression in neurons results in the formation of longer and thinner dendritic spines (Edbauer et al., 2010). In addition, miR-125b regulates synapsin-2 (Syn2) and 15-lipoxygenase (Lox15) levels. Therefore, its upregulation, detected in AD brains, might lead to decreased amounts of Syn2 and Lox15, which are important for synaptic vesicle cycling and neuroprotectin D1 (Npd1) synthesis, respectively (Lukiw, 2012; Yao et al., 2003). miR-128b has also been shown to be important for memory formation. Its expression appears to increase upon extinction training and its lentiviral-mediated knockdown impairs memory for fear extinction. This action might be exerted via

Table 9.3 Summary of microRNAs modulating synaptic functions, endocytosis, and phagocytosis.

Name	Target proteins/process	References
miR-132	p250GAP, GluA1, GluN2A, GluN2B, MeCp2, FOXP2	Bicker et al. (2014), Lau et al. (2013), Salta et al. (2016)
miR -125b	Syn2, Lox15, spine morphogenesis	Edbauer et al. (2010)
miR-128b	Creb1, Ppp1cc, Reln, Sp1, memory formation	Lin et al. (2011)
miR-124	Creb1, Ptpn1, spatial learning, dendritic spine morphology	Rajasethupathy et al. (2009), Wang et al. (2018)
miR-34a	Syt1, Grm7, TREM2, dendritic outgrowth, spine morphology	Agostini et al. (2011), Alexandrov, Zhao, Jones, Bhattacharjee, and Lukiw (2013), Morgado et al. (2015), Zhang et al. (2016), Zhao et al. (2013)
miR-154, -27b, -155, -200b, -128	Immune-related processes, endocytosis, phagocytosis	Guedes et al. (2016)

MicroRNAs that regulate levels of proteins important for the function of the synapse, endocytosis, and phagocytosis are listed. *Creb1*, cAMP response binding protein 1; *FOXP2*, forkhead box P2; *GluA1*, glutamate receptor A1; *GluN2A*, glutamate receptor N2A; *GluN2B*, glutamate receptor N2B; *Grm7*, metabolic glutamate receptor 7; *Lox15*, 15-lipoxygenase; *MeCp2*, methyl-CpG-binding protein 2; 21*p250GAP*, GTPase-activating protein; *Ppp1cc*, protein phosphatase 1cγ; *Ptpn1*, tyrosine-protein phosphatase nonreceptor type 1; *Reln*, reelin; *Sp1*, *trans*-acting transcription factor; *Syn2*, synapsin 2; *Syt1*, synaptotagmin 1; *TREM2*, triggering receptor expressed in myeloid/microglial cells 2.

targeting of several synaptic, plasticity-related genes, including these encoding cAMP response binding protein 1 (Creb1), protein phosphatase 1cγ (Ppp1cc), reelin (Reln), and *trans*-acting transcription factor (Sp1) (Lin et al., 2011).

In addition, miRNA profiling of APPSwe mouse brains has determined another central nervous system—specific miR-124 to be upregulated in the disease and demonstrated that miR-124, by targeting tyrosine-protein phosphatase nonreceptor type 1 (Ptpn1), regulates spatial learning and dendritic spine morphology. Injections of AAV1/2 particles containing the antagomir anta-miR-124 into the brains of APPSwe mice rescues memory deficits, long-term potentiation inhibition, and dendritic spine loss and increases the relative amount of mushroom spines (Wang et al., 2018). Moreover, decreased levels of miR-124 in sensory neurons lead to enhancement of long-term facilitation, at least partially due to the regulation of Creb1 expression (Rajasethupathy et al., 2009).

Yet another miRNA that might regulate synaptic function and is upregulated in AD brain, especially in the regions affected by AD pathology, as well as in the hippocampi of

3×Tg-AD (PS1M146V, APPSwe, TauP301L) mice, is miR-34a (Clement, Hill, Dua, Culicchia, & Lukiw, 2016; Sarkar et al., 2016; Zhang et al., 2016). Bioinformatics analyses have demonstrated that this miRNA might regulate the expression of synaptic plasticity protein-encoding genes, such as vesicle-associated membrane protein 2 (*VAMP2*), synaptotagmin 1 (*SYT1*), hyperpolarization-activated cyclic nucleotide-sensitive channels (*HCN*), *N*-methyl-D-aspartate receptor 2A (*NR2A, GRIN2*), and glutamate receptors (*GLUR1, GRIA1*) (Sarkar et al., 2016). Overexpression of pre-miR-34a or miR-34a in mouse neural stem cells (NSC) or cortical neurons, respectively, decreases Syt1 levels, impairs functional maturation of NSCs (Morgado et al., 2015), and negatively affects dendritic outgrowth (Agostini et al., 2011). The importance of miR-34a for synaptic signaling has been further evidenced by the observation that upregulated expression of miR-34a in 3×Tg-AD mice corresponds to the downregulation of potential miR-34a target gene *Grm7*, encoding metabotropic glutamate receptor 7, and leads to anxiety-like behavior (Zhang et al., 2016).

In addition to the regulation of synaptic functions, miR-34a has also been implicated in the modulation of phagocytosis. It has been suggested that higher levels of miR-34a in AD brains might result in reduced levels of TREM2—a receptor involved in the regulation of several processes, such as phagocytosis, cell proliferation, and survival, as well as the production of inflammatory cytokines (Ulrich & Holtzman, 2016). Diminished TREM2 levels would probably impair phagocytosis and lead to the accumulation of toxic Aβ species (Mazaheri et al., 2017; Zhao et al., 2013). Importantly, multiple GWASs have identified *TREM2* variants as strong AD risk factors (Guerreiro et al., 2013).

Compromised phagocytosis and/or endocytosis might also be due to the altered expression of other immune-related miRNAs, such as miR-154, -27b, -155, -200b, and -128, the levels of which have been reported to be altered in bone marrow-derived macrophages from AD patients compared with mildly cognitively impaired and/or control individuals (Guedes et al., 2016). This hypothesis is supported by bioinformatics predictions and experimental evidence. It has been predicted that these miRNAs might regulate the levels of AD-susceptibility genes, including complement receptor type 1 (*CR1*), *BIN1*, *PICALM*, *SORL1*, membrane-spanning 4-domains subfamily A member 4A (*MS4A4A*), myeloid cell surface antigen CD33 (*CD33*), CD2-associated protein (*CD2AP*), and *CLU* (Van Cauwenberghe, Van Broeckhoven, & Sleegers, 2016) At least some of the protein products of these genes might be involved in the endocytosis and phagocytosis processes. In addition, experimental data suggest that miR-128 is associated with decreased expression of lysosomal enzymes in monocytes and lymphocytes from AD patients and that miR-200b, by targeting β-1,4-mannosyl-glycoprotein 4-β-*N*-acetylglucosaminyltransferase (MGAT3), may impair Aβ clearance (Fiala et al., 2007; Tiribuzi et al., 2014).

Altogether these data provide compelling evidence of the importance of miRNAs in the regulation of the physiological functions of multiple cell types in the brain and highlight a critical role for miRNAs in brain physiology and in the pathogenesis of AD.

Key facts of microRNAs

- miRNAs are epigenetic fine-tuners of gene expression.
- Each miRNA can target several mRNAs and also each mRNA can be targeted by several miRNAs.
- According to the miRBase registry, there are currently 2693 mature miRNAs identified in humans.
- miRNAs are carried by vehicles such as high-density lipoprotein, exosomes, argonaute protein complex, microvesicles, and apoptotic bodies that facilitate miRNA transport and confer their stability.
- miRNA profiles are altered in several disorders, including AD.

Summary points

- This chapter focuses on the role of the miRNA network in AD pathogenesis.
- miRNAs might regulate Aβ production and amyloid pathology directly (by targeting the amyloid precursor protein [*APP*] gene) or indirectly (by targeting genes encoding α- and β-secretases and members of the γ-secretase complex).
- miRNAs might influence the formation of NFTs by regulating the expression and phosphorylation status of MAPT.
- miRNAs regulate synaptic functions, endocytosis, and phagocytosis in health and disease.
- Selected miRNA mimics and/or inhibitors (antagomirs) might be considered as novel AD therapeutics.

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CHAPTER 10

Role of cellular oxidative stress in dementia

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List of abbreviations

AD Alzheimer's disease
ADP Adenosine diphosphate
APP Amyloid precursor protein
ATP Adenosine triphosphate
CAG Cytosine–adenine–guanine
CCH Chronic cerebral hypoperfusion
CSF Cerebrospinal fluid
FENIB Familiar encephalopathy with neuroserpin inclusion bodies
FTD Frontotemporal dementia
FUS Fused in sarcoma
GPI Glycosylphosphatidylinositol
HD Huntington's disease
HNE 4-Hydroxynonenal
Htt Huntingtin protein
KO Knockout
mHtt Mutant huntingtin protein
mPTP Mitochondrial permeability transition pore
mtDNA Mitochondrial DNA
NADH Nicotinamide adenine dinucleotide
NADPH Nicotinamide adenine dinucleotide phosphate
OS Oxidative stress
PD Parkinson's disease
PrP Prion protein
RNS Reactive nitrogen species
ROS Reactive oxygen species
SOD Superoxide dismutase
TDP-43 TAR DNA-binding protein
VD Vascular dementia

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Mini-dictionary of terms

Antioxidant defense Cellular protection mechanism against oxidative stress consisting of antioxidant molecules and enzymes

Glutathione A tripeptide (glutamate, cysteine, glycine) acting as nonenzymatic cellular reducing agent, cycling between reduced (GSH) and oxidized (GSSG) forms

Glutathione peroxidase Enzyme that catalyzes the reduction of hydrogen peroxide to water oxidizing GSH to GSSG

Glutathione reductase Enzyme that reduces GSSG to GSH

Hydrogen peroxide (H_2O_2) A type of ROS and important player in redox homeostasis that is converted to water by antioxidant proteins

4-Hydroxynonenal (HNE) A secondary product of polyunsaturated fatty acid oxidation

Mitochondrial electron transport chain Proteins and nonprotein electron carriers of the inner mitochondrial membrane that pass electrons from one to the next and finally to molecular oxygen, using the energy derived from electron transfer to transport protons to the intermembrane space

Oxidative stress Cellular stress that occurs when ROS production exceeds the cell's capacity to eliminate them

Reactive oxygen species (ROS) Molecules produced by normal aerobic metabolism that play important regulatory roles in redox biology; in excess, they react with cellular components to impair their function

Superoxide dismutase (SOD) Antioxidant enzyme reducing superoxide to hydrogen peroxide; different forms include the cytoplasmic Cu/ZnSOD, the mitochondrial MnSOD, and the extracellular Cu/ZnSOD.

Introduction

This chapter summarizes abundant evidence found in patients and animal and cell culture models used to study the role of cellular oxidative stress (OS) in neurodegenerative conditions presenting with dementia. Normal cellular metabolism produces reactive oxygen species (ROS) that play a role as signaling molecules, but alterations of the redox balance due to increased ROS production or decreased antioxidant activities lead to OS that is damaging to the cell. OS is frequently associated with mitochondrial dysfunction and leads to toxic oxidation of lipids, proteins, and DNA, all found in patients as markers of disease. Neurons are particularly sensitive to OS due to their elevated energy needs and abundance of structural lipids, although not all neurons are equally responsive to OS. Often, particularly vulnerable neuronal types are affected in each pathology as described in the paragraphs that follow. The key role of OS in the pathological events underlying dementia and neurodegeneration encourages further investigation of its molecular mechanisms and possible therapeutic interventions.

Oxidative stress in Alzheimer's disease

Alzheimer's disease (AD) is the most common dementia and neurodegenerative disorder, affecting more than 35 million people worldwide. It is characterized by neuronal loss and two protein aggregates: senile plaques containing beta amyloid peptide (Aβ) and

neurofibrillary tangles. The Aβ peptide forms by cleavage of amyloid precursor protein (APP) by beta secretase 1 and the gamma secretase complex. Less than 1% of AD cases are hereditary and result from mutations in APP or presenilin 1 or 2, which affect APP processing leading to larger, more aggregative Aβ peptides. The main risk factors for sporadic AD are aging and carrying the ApoE4 haplotype (Querfurth & LaFerla, 2010).

Assessing the impact of OS in AD is difficult due to its complex etiopathology. ROS imbalance is a main feature in aging, and basal oxidative imbalance underlies AD pathology (Camandola & Mattson, 2017). Patients can undergo many years of cognitive impairment before developing AD and sometimes present oxidative alterations in cerebrospinal fluid (CSF) or plasma before Aβ pathology, with markers such as isoprostanoids increased in several body fluids (Praticò et al., 2002). Increased protein peroxidation and decreased antioxidants have been found in brains of cognitively impaired patients (Butterfield et al., 2006). Increased levels have been found of (1) 4-hydroxynonal (HNE) in temporal structures (Sayre et al., 1997); (2) malondialdehyde in the cortex (Marcus et al., 1998); (3) 2-propenal in temporal structures and the cerebellum (Williams, Lynn, Markesbery, & Lovell, 2006); (4) and F2-isoprostanes and F4-neuroprostanes in the cortex and various body fluids (Praticò, MY Lee, Trojanowski, Rokach, & Fitzgerald, 1998). Increased protein carbonyl levels were detected in the cortex (Aksenov, Aksenova, Butterfield, Geddes, & Markesbery, 2001), and higher 3-nitrotyrosines were shown in several brain regions and CSF (Tohgi et al., 1999). Moreover, several aspects of mitochondrial dysfunction have been detected in Alzheimer brains, suggesting less efficient ATP production that could increase ROS levels and oxidative imbalance (Cadonic, Sabbir, & Albensi, 2016). Interestingly, lipid peroxidation and protein oxidation products have been found regardless of the proximity of Aβ deposition (Sayre et al., 1997). Oxidative damage is also reflected by increased DNA damage in the hippocampus and cortex (Anderson, Su, & Cotman, 1996). Finally, Alzheimer's patients show decreased plasma levels of antioxidants such as albumin or vitamin E (Foy, Passmore, Vahidassr, Young, & Lawson, 1999) and decreased activity of antioxidant enzymes such as superoxide dismutase (SOD) or glutathione peroxidase in the brain (Omar et al., 1999).

In vitro assays have shown that redox metal ions such as copper, iron, or zinc bind to Aβ and catalyze ROS production (Cheignon et al., 2018), and Aβ can induce OS in cell culture models including primary neurons (Ansari, Abdul, Joshi, Opii, & Butterfield, 2009). Animal and cellular models involving mutated APP (Lott, Head, Doran, & Busciglio, 2006) or APP combined with presenilin mutations (Yang, Sun, Lashuel, & Zhang, 2012) feature Aβ toxicity that could induce oxidative imbalance. Mitochondrial dysfunction has been shown to play a role in familial AD with presenilin 1 (Sepulveda-Falla et al., 2014) or presenilin 2 (Zampese et al., 2011) mutations in the absence of Aβ toxicity, supporting the idea that OS in familial AD could result from the juxtaposition of different mechanisms (Fig. 10.1).

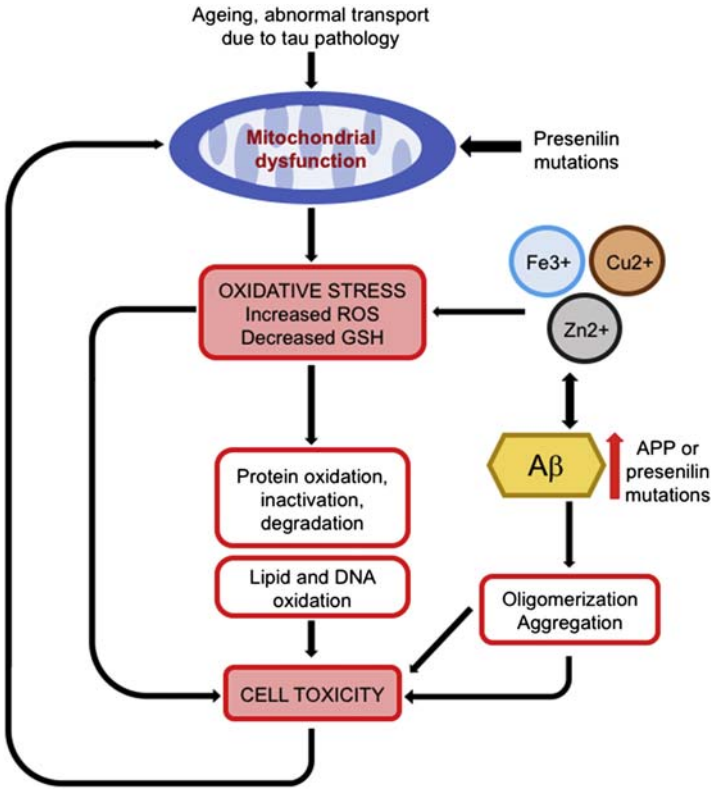

Figure 10.1 Oxidative stress in Alzheimer's disease. The diagram shows the main oxidative stress-related pathways in Alzheimer's disease (unpublished).

Oxidative stress in Parkinson's disease

Parkinson's disease (PD) is a widespread neurodegenerative condition affecting 1%–2% of people over 65 and characterized by motor symptoms (rigidity, bradykinesia, and resting tremor) (Maiti, Manna, & Dunbar, 2017), with dementia in at least 75% of patients who survive more than 10 years (Aarsland & Kurz, 2010). Most PD is sporadic and less than 5% familial and is caused by mutations in susceptible genes (including α-synuclein, Parkin, and PINK1). The histological hallmarks are eosinophilic inclusion bodies (Lewy bodies) and threadlike structures (neurofibrillary tangles) due to α-synuclein aggregation, and the main pathological event is the degeneration of dopaminergic neurons of the substantia nigra (Maiti et al., 2017). The molecular mechanisms underlying PD are complex, with OS playing an important role. Early studies found increased OS in dopaminergic neurons due to oxidative metabolism of dopamine and increased levels of oxidized glutathione (Spina & Cohen, 1989) as of Fe^{3+} and ferritin in thesubstantia nigra (Jellinger, Paulus, Grundke-Iqbal, Riederer, & Youdim, 1990).

This promotes the formation of toxic free radicals and the oxidation of biological molecules, as seen with peroxidized lipids found in the substantia nigra of PD brains (Dexter et al., 1994).

Dopaminergic neurons are exposed to several sources of OS including environmental toxins and genetic mutations that promote α-synuclein aggregation or alter mitochondrial function. They are particularly sensitive to oxidation due to the abundance of dopamine, which can give rise to quinones and oxidant metabolites such as hydrogen peroxide through autooxidation and monoamine oxidase activity. This enhances OS causing macromolecule oxidation (DNA, proteins, lipids), increased protein aggregation, and modification of proteins by quinones, altering mitochondrial function and proteostasis and causing neuronal death (Fig. 10.2) (Hastings, 2009; Lotharius & Brundin, 2002). Experimental evidence shows that hydrogen peroxide induces α-synuclein aggregation in vitro (Hashimoto et al., 1999); NADPH oxidase activity increases α-synuclein expression and aggregation in animal and in vitro models of PD (Cristóvão et al., 2012),

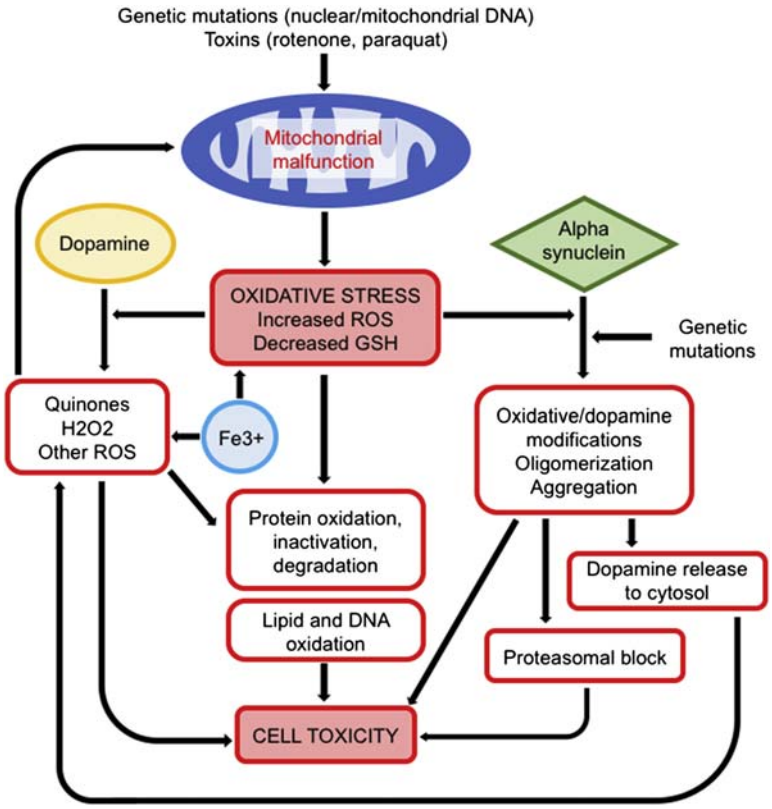

Figure 10.2 Oxidative stress in Parkinson's disease. Schematic representation of the main pathways related to oxidative stress in Parkinson's disease (unpublished).

and quinone metabolites cause irreversible inhibition of the proteasome (Zhou & Lim, 2009). Decreased proteasomal function promotes accumulation and aggregation of α-synuclein, as seen in postmortem brains and experimental models of PD (Maiti et al., 2017). Posttranslational modifications of α-synuclein due to OS affect the protein's function, interaction with membranes, and degradation and aggregation, altering mitochondrial function and cellular proteostasis in PD (Schildknecht et al., 2013).

OS is a cause and consequence of mitochondrial dysfunction in PD (Subramaniam & Chesselet, 2013). Complex I and NADH cytochrome C reductase activities are lower in mitochondria isolated from the substantia nigra of PD brains (Schapira et al., 1990), and increasing levels of free radicals and oxidation of complex I decrease the activity of the respiratory chain (Keeney, Xie, Capaldi, & Bennett, 2006). Genetic defects in mitochondrial DNA of PD patients lead to decreased complex I activity, increased ROS production, and higher susceptibility to mitochondrial toxins (Swerdlow et al., 1996). Mutations altering mitochondrial dynamics caused mitochondrial malfunction and increased ROS production in sporadic PD (Santos et al., 2015). Oligomeric and dopamine-modified α-synuclein strongly bind to the mitochondrial transporter of the outer membrane 20, reducing translocation of mitochondrial proteins and mitochondrial respiration and increasing ROS production (Di Maio et al., 2016). Finally, several toxins cause mitochondrial toxicity and OS leading to neurodegeneration in the substantia nigra similar to that found in PD (Subramaniam & Chesselet, 2013), thus supporting an environmental origin for some sporadic cases of PD.

Oxidative stress in Huntington's disease

Huntington's disease (HD) is a neurodegenerative pathology characterized by degeneration and loss of muscle and brain cells with motor, psychiatric, and cognitive alterations including dementia (Ross et al., 2014). HD is a genetic disorder caused by autosomal-dominant mutations leading to repetition of the CAG trinucleotide (glutamine) in exon 1 of the huntingtin (Htt) gene (The Huntington's Disease Collaborative Research Group, 1993). Wild-type Htt protein shuttles between nucleus and cytoplasm with functions under investigation, while mutant Htt (mHtt) presents an expanded N-terminal polyglutamine (polyQ) sequence causing loss of function and toxic aggregation, hallmarks of HD (Wheeler et al., 2000). The toxic phenotype is associated with dysregulation of several pathways including OS and failure in energy metabolism (Fig. 10.3) (Kumar & Ratan, 2016). In HD patients, increased levels of OS markers are accompanied by a decrease in antioxidant molecules (Tunez et al., 2011); plasma DNA oxidation increased with disease progression (Long, Matson, Juhl, Leavitt, & Paulsen, 2012); and higher protein nitration and lipid and fatty acid peroxidation were found in postmortem brains (Chen et al., 2007). Oxidative DNA damage could derive from either increased oxidation or impaired repair due to nuclear localization of mHtt. ROS could also arise

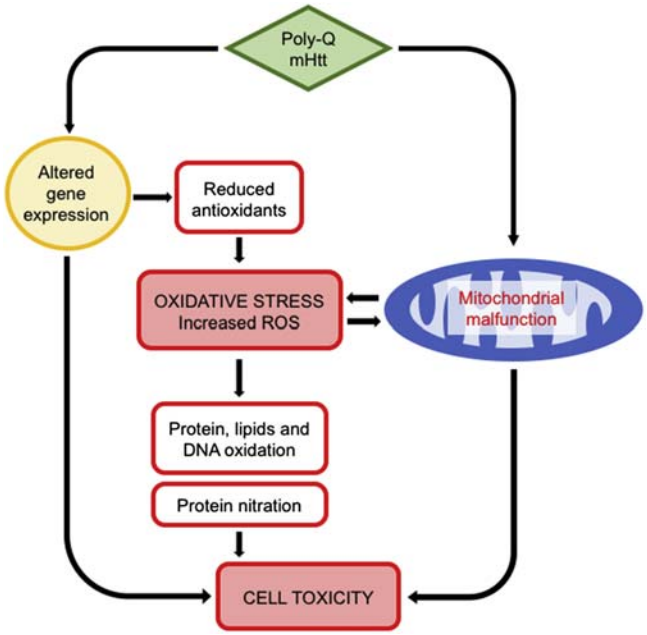

Figure 10.3 Oxidative stress in Huntington's disease. Schematic representation of the pathways involved in oxidative stress and mitochondrial dysfunction in Huntington's disease (unpublished).

from a reduced antioxidant capacity due to low levels of HACE1, a component of the cellular response to increased oxidant load, as found in the striatum of HD patients (Rotblat et al., 2014).

It is not clear whether OS is a cause or consequence of neuronal degeneration in HD. Deficiencies in neuronal metabolism have been reported in HD patients (Tabrizi et al., 1999). ROS levels are increased in cells with compromised mitochondrial function, suggesting a strong link between mitochondrial abnormalities and ROS-mediated HD pathogenesis (Reddy, Mao, & Manczak, 2009). Brains and mouse models of HD showed mitochondrial DNA (mtDNA) damage preceding the loss of striatal neurons (Acevedo-Torres et al., 2009). Excessive fission and fragmented mitochondria were reported in lymphoblasts from HD patients and in murine striatal primary cultures in correlation with increased expression of fission proteins, and this was suggested to contribute to neuronal damage (Costa et al., 2010). It has been shown that direct association of mHtt with the mitochondrial membrane disrupts mitochondrial dynamics as well as axonal transport and function, leading to mitochondrial fragmentation (Cherubini & Ginés, 2017). OS and calcium perturbations affect the opening of the mPTP (mitochondrial permeability transition pore) in the inner membrane, a key event in mitochondrial dysfunction. Mutant Htt may directly interact with the outer membrane and activate the mPTP and cause mitochondrial dysfunction (Quintanilla, Tapia, & Pérez, 2017).

Alternatively, mHtt interaction with transcriptional factors may lead to mitochondrial malfunction. In particular, HD patients present lower expression of PGC-1α, a regulator of genes involved in mitochondrial biogenesis and antioxidant defense (Johri, Chandra, & Beal, 2013).

Oxidative stress in spongiform encephalopathies

Prion diseases or transmissible spongiform encephalopathies are neurodegenerative disorders characterized by spongiform degeneration, astrogliosis, and neuronal loss. According to the "protein-only" hypothesis, the beta-sheet-rich scrapie form of the prion protein (PrP^{Sc}) generated by conformational change of its alpha-helical-rich cellular counterpart (PrP^{C}) is the infectious pathogen that produces insoluble aggregates and induces further misfolding and aggregation of PrP^{C} (Prusiner, 1982). Loss of normal PrP^{C} protein may also contribute to neurotoxicity.

One of the functions postulated for PrP^{C} is redox control conferring resistance to OS (Fig. 10.4). In fact, PC12 rat pheochromocytoma cells resistant to copper toxicity or OS have high levels of PrP^{C} expression (Brown, Schmidt, & Kretzschmar, 1997), whereas

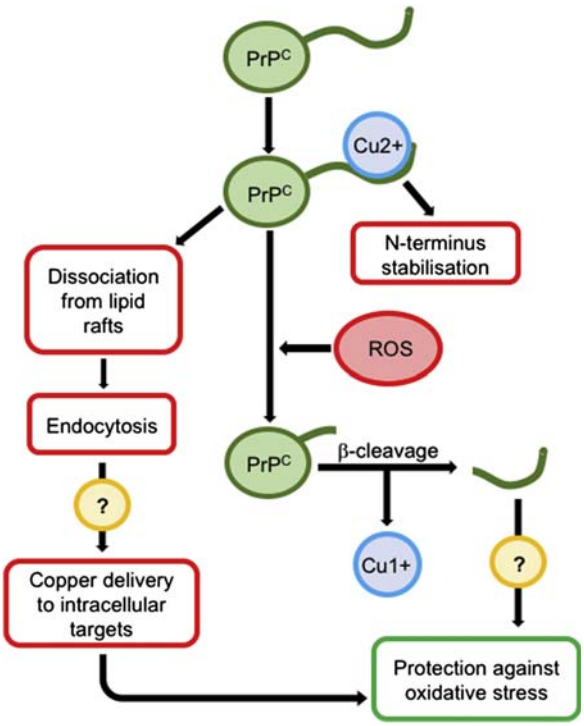

Figure 10.4 Oxidative stress and prion proteins. Involvement of prion proteins in copper homeostasis and redox control (unpublished).

primary neurons from PrP-deficient mice (PrP KO) (White et al., 1999) and prion-infected neurons (Milhavet et al., 2000) are more vulnerable to oxidative damage. Many efforts have been made to understand the underlying molecular mechanisms. The observation that recombinant PrP^C binds copper (Brown, Qin, et al., 1997) has suggested a role in brain copper metabolism. Although controversial results about copper levels in PrP KO and PrP-overexpressing mice have been obtained (Steele, Lindquist, & Aguzzi, 2007), the relationship between copper and PrP^C function has been investigated in detail. Copper binding stabilizes the N-terminus of PrP^C (Miura, Hori-i, & Takeuchi, 1996), and insertional mutations in the PrP gene leading to expansion of the octarepeat domain, the region binding four Cu^{2+} ions, is associated with familial prion disease (Stevens et al., 2009). Furthermore, PrP^C is cleaved (beta-cleavage) within or close to the octarepeat region in a copper-dependent fashion after exposure to ROS (McMahon et al., 2001). During beta-cleavage, Cu^{2+} is reduced to Cu^{1+}, suggesting a role for PrP^C as a "sacrificial quencher" of free radicals (Nadal et al., 2007). The N-terminal fragment produced by beta-cleavage protects against OS in SH-SY5Y neuroblastoma cells (Dupiereux et al., 2008), whereas inability to perform beta-cleavage renders cells more vulnerable to oxidative damage (Watt et al., 2005). Another consequence of copper binding is PrP^C dissociation from lipid rafts and endocytosis (Taylor & Hooper, 2006). A role for PrP^C in copper supply to intracellular antioxidant enzymes has been proposed, but although PrP^C expression seems to correlate with copper-loading of SOD (Brown, 1999), others have excluded the possibility that PrP^C regulates copper delivery at physiological concentrations (Rachidi et al., 2003). Investigations about dysregulation of SOD activity in mice deficient in or overexpressing PrP^C led to controversial results —while SOD activity was decreased in brain and muscle of PrP KO, others found no difference in Cu/Zn SOD activity between PrP KO or PrP-overexpressing mice compared with control (Steele et al., 2007). This is further complicated by the possibility that PrP^C itself could function as SOD, as demonstrated for recombinant PrP^C and PrP^C isolated from brain (Brown, Wong, et al., 1999), but this was excluded after in vivo experiments (Steele et al., 2007).

Oxidative stress in other types of dementia

Vascular dementia (VD) is considered the second cause of cognitive impairment after AD and involves decline in memory and cognitive functions due to chronic cerebral hypoperfusion (CCH). Increasing evidence shows that OS is critical in VD pathogenesis in both human and animal models (Liu & Zhang, 2012). Studies in animal models of VD show dysfunctions in mitochondrial activity and morphology, such as mitochondrial aggregation in hippocampal neurons of CCH rats (Jian, Yi-Fang, Qi, Xiao-Song, & Gui-Yun, 2013) and decreased activity of enzymes of the respiratory complex (I, II, IV) with disruption of mitochondrial respiration in the brains of CCH rats (Du et al., 2013). NADPH oxidase 1, a superoxide generation system and important producer of ROS,

plays an important role in neuronal death and cognitive dysfunction in VD (Choi et al., 2014). In clinical studies, a reduction of antioxidant levels in the plasma of VD patients has been described for α-tocopherol and vitamin E (Ryglewicz et al., 2002), while plasma lipid peroxidation and DNA oxidation in the cerebrospinal fluid were increased (Butterfield & Lauderback, 2002), supporting a systemic alteration of the redox state.

Frontotemporal dementia (FTD) is the third most common dementia over 65 and the second most common under 65 years of age and includes a heterogeneous group of syndromes marked by neurodegeneration of the frontal and anterior temporal lobes with deficits in executive functions, language, and behavior (Rascovsky et al., 2011). Several studies have reported OS and increased lipoxidative damage including the presence of altered levels of HNE in patient brains in correspondence with marked astrocytosis (Martínez et al., 2008). Recent findings using neurons derived from induced pluripotent stem cells obtained from FTD patients have described increased vulnerability to the mitochondrial toxin rotenone with widespread neurodegeneration that could be reverted by antioxidant molecules (Ehrlich et al., 2015) as well as inhibition of complex I, hyperpolarization of the mitochondrial membrane, increased ROS production, OS, and neuronal cell death (Esteras, Rohrer, Hardy, Wray, & Abramov, 2017).

Familiar encephalopathy with neuroserpin inclusion bodies (FENIB) is a rare neurodegenerative dementia caused by aberrant polymerization of neuroserpin within the endoplasmic reticulum of neurons, forming inclusion bodies most abundant in the cerebral cortex (Miranda & Lomas, 2006). A recent study has reported upregulation of antioxidant enzymes and increased sensitivity to oxidant insults in response to mutant neuroserpin accumulation in neural progenitor cells differentiated to neurons, supporting a role for OS in FENIB (Guadagno et al., 2017).

Key facts of oxidative stress

- Reactive oxygen species (ROS) are short lived.
- Oxidative modifications of DNA, proteins, and lipids are good biomarkers of oxidative stress damage.
- The antioxidant response of neurons diminishes with aging.
- Mitochondrial dysfunction can be both a cause and a consequence of oxidative stress and is a key event in cell death.
- Metal ions including iron, copper, and zinc ions play important roles in redox metabolism.

Summary points

- Protein aggregation underlies neuronal toxicity and is a general feature in neurodegenerative disorders.

- Oxidative stress plays an important role in most types of neurodegeneration and dementia.
- Oxidative stress is a key mechanism in the etiopathology of Alzheimer's disease and could be one of the initiating events before clinical onset.
- Dopaminergic neurons are particularly sensitive to oxidative stress and mitochondrial dysfunction due to dopamine metabolism and α-synuclein aggregation in Parkinson's disease.
- Mutant huntingtin protein elicits oxidative stress through mitochondrial dysfunction and gene expression alteration in Huntington's disease.
- Copper is a redox-active trace element essential for many cellular enzymes. Its ability to participate in free radical reactions renders it potentially toxic, so its homeostasis is finely regulated through mechanisms that partially involve the prion protein.

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CHAPTER 11

Toward an integrative understanding of the neuroinflammatory molecular milieu in Alzheimer disease neurodegeneration

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List of abbreviations

CCL C-C motif chemokine ligand
CXCL C-X-C motif chemokine ligand
DAMPs Damage-associated molecular patterns
IFN Interferon
IL Interleukin
NFκB Nuclear factor κB
PAMPs Pathogen-associated molecular patterns
PRRs Pattern recognition receptors
TGF Transforming growth factor
TLRs Toll-like receptors
TNF Tumor necrosis factor

Mini-dictionary of terms

Inflammageing Concept describing the increased levels of proinflammatory mediators and immune cells activation related to the ageing process and close related to the activity of the inflammasome.
Inflammasome Subcellular structure where proinflammatory mediators are produced.
Inflammatory milieu Concept referring to the interplay between inflammatory signaling and other cellular and molecular events affecting the functionality of a specific organ.
Microglial activation Process by which microglia change their morphology and biochemistry to manage immune challenges.
Neuroinflammation Defined as the particularities of the inflammatory response within the central nervous system, particularly, the brain.

Alzheimer disease overview

According to the World Health Organization, the world population is ageing dramatically, and the number of people over 60 will increase to an estimated 22% of the world population in 2050. Ageing is related to increased morbidity because neurodegenerative

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disorders, particularly dementia, are a relevant issue that must be addressed. Indeed, up to 20% of people over 60 are affected by some type of dementia (Alzheimer's Disease International, 2016). Although the basis of dementia involves several molecular events closely related to subjacent pathology, the main feature of this process is the loss of cognitive functioning characterized by the loss of neuronal connections and neuronal death. In this regard, among the different pathologies leading to dementia, Alzheimer disease (AD) is the most common, representing up to 70% of dementia cases worldwide (Alzheimer's Disease International, 2016). However, even though AD is closely related to ageing, it should not be considered part of a healthy ageing process. For this reason and considering the enormous human and financial costs related to AD, worldwide efforts have been committed to AD research.

Although genetic conditions, such as variations in the amyloid precursor protein (APP), presnilin-1 and presenilin-2 genes, have been related to early onset familial AD, sporadic or late-onset AD is the most common presentation (Bird, 2015).

Clinically, AD progresses from a subclinical condition, characterized by slight symptomatology, such as mood disturbances to fully compromised cognitive performance (Selkoe, 2011; Selkoe & Hardy, 2016). Histopathologically, AD displays the following pathognomonic lesions within the brain parenchyma, particularly the hippocampus: senile plaques, which are extraneuronal depositions of aggregated amyloid-β (Aβ) peptide, a proteolytic product of APP; and the intraneuronal formation of neurofibrillary tangles (NFTs), constituted by hyperphosphorylated forms of tau protein (Selkoe & Hardy, 2016). Ultimately, both lesions will induce neurite dystrophy and neuronal death, causing the collapse of the neuronal network. Notably, AD also presents with additional features. Severe neuroinflammation, represented by glial reactivity and increased levels of proinflammatory mediators, vascular compromise, mitochondrial dysfunction, calcium dyshomeostasis, and oxidative stress, are among the most relevant alterations observed during AD (Heneka, Carson et al., 2015; Heneka, Golenbock, & Latz, 2015; Selkoe, 2011; Selkoe & Hardy, 2016).

Alzheimer disease etiology

The wide spectrum of pathological features observed in AD has prompted researchers to develop different explanations regarding the genesis of AD. Since the initial observation of cholinergic system failure, giving rise to the cholinergic hypothesis, alternative and complementary hypotheses have been proposed (Carvajal & Inestrosa, 2011; Heneka, Carson et al., 2015; Heneka, Golenbock, & Latz, 2015; Selkoe & Hardy, 2016). These hypotheses are not mutually exclusive, and two of them, the amyloid and tau hypotheses, have been consolidated over the years. Although whether amyloid or tau is more determinant remains controversial, different groups have focused their attention on the amyloid hypothesis as the core of AD pathophysiology (Selkoe & Hardy, 2016).

Alzheimer disease pathophysiology and the amyloid hypothesis

According to this theory, AD results from increased levels of Aβ because of an unbalanced production/clearance rate (Singh et al., 2013; Yan & Vassar, 2014), leading to additional pathological manifestations (Selkoe & Hardy, 2016; Zolezzi, Bastías-Candia, Santos, & Inestrosa, 2014). Moreover, tau pathology (NFTs) can be explained by the detrimental effects of Aβ and its interaction with critical molecular pathways within neurons, such as the Wnt signaling pathway (Selkoe & Hardy, 2016). Aβ is a 37 to 43 amino acid peptide derived from the proteolytic processing of APP, a type I transmembrane glycoprotein. APP has three differentiated domains, including an extracellular domain (ECD), transmembrane domain, and intracellular domain (ICD). These domains are ubiquitously expressed by different cell types, but their physiological roles are not well understood. However, at the neuronal level, APP is a relevant player in synapse formation and maintenance and is also necessary for the development of short-term and long-term potentiation (STP and LTP). Moreover, APP knockout models exhibit significant neurological impairments, further confirming the relevant role of this protein in the development of the central nervous system (CNS) (Zheng & Koo, 2006; Zolezzi et al., 2014).

Traditionally, APP processing has been considered to occur through two well-defined pathways. On the one hand, nonamyloidogenic processing of APP is carried out by the sequential cleavage of alpha (α) and gamma (γ) secretases, which will release soluble APPα (sAPPα) and p3 fragments. On the other hand, the amyloidogenic processing of APP begins with the activity of beta (β) secretase (BACE1) followed by γ secretase, leading to the release of sAPPβ and neurotoxic Aβ peptide (Yan & Vassar, 2014; Zolezzi et al., 2014). In this regard, under AD conditions, the amyloidogenic processing of APP is enhanced, causing an increased production rate of Aβ. As the limiting enzyme for the amyloidogenic pathway, the modulation of BACE1 expression and activity has emerged as one of the main goals to develop new therapeutic alternatives for AD.

Importantly, in recent years, a new pathway has been identified for APP processing. The η-secretase pathway comprises the initial cleavage of APP by η-secretase, which occurs at the ECD outside of the Aβ sequence, followed by the cleavage of α- or β-secretases. This mechanism will lead to the release of several peptides, including sAPPη, Aη-α, and Aη-β, whose functions remain largely unknown (Willem et al., 2015). The implications of such a novel mechanism in AD or other pathologies are being researched and should not be omitted when describing the particularities of APP processing in AD or other APP-related pathology (Fig. 11.1).

As mentioned previously, in addition to the increased production rate of Aβ, a defective clearance mechanism substantially contributes to the development of the disease. In this regard, Aβ is removed from the brain through microglial phagocytosis and eliminated in the blood stream and cerebrospinal fluid at the blood—brain barrier (BBB)

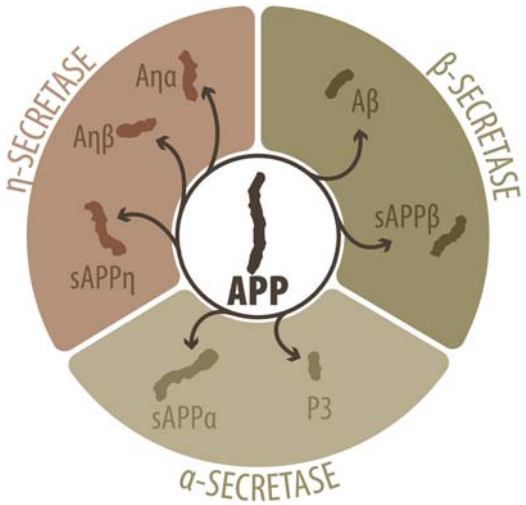

Figure 11.1 ***Main pathways associated with APP, amyloid precursor protein processing.*** The α-secretase nonamyloidogenic pathway involves the activity of α-secretase followed by γ-secretase, causing the release of soluble APPα (sAPPα) and p3 fragments. Alternatively, the β-secretase amyloidogenic pathway involves the initial cleavage of APP by β-secretase followed by γ-secretase. This pathway leads to the release of sAPPβ and Aβ peptide. Recently, a new APP processing machinery was identified. In this mechanism, η-secretase can induce an initial cut of APP, releasing sAPPη and Aη peptide. Relevantly, Aη peptide can be further cleaved by α- or β-secretase, leading to the formation of Aηα or Aηβ. The relevance of the η-secretase pathway in the context of neuropathology has not yet been elucidated.

and choroid plexus (CP), respectively. For excretion, Aβ must be bound to apolipoprotein E (ApoE), which is synthesized by astrocytes. Importantly, once in the blood stream, Aβ is finally eliminated in the liver and, to a lesser extent, by the kidneys (Zlokovic, 2011; Zolezzi & Inestrosa, 2013).

Evidently, any condition that can modify these critical nodes, overrunning the homeostatic system capacity, will induce the accumulation of Aβ and trigger the pathological processes leading to AD (Sweeney, Sagare, & Zlokovic, 2018; Zlokovic, 2011; Zolezzi & Inestrosa, 2013). In this regard, even though our knowledge regarding the molecular basis of AD has increased significantly during the last decades, we still do not know the molecular events that trigger the initial increase in Aβ production and its further aggregation.

The inflammatory milieu: the core of Aβ pathology in Alzheimer disease?

AD exhibits several cellular and subcellular alterations. Although Aβ has been considered the basis of these alterations, the slow progression of the disease and poor correlation between the initial stages of pathology and Aβ levels suggest that additional molecular

mechanisms might drive the onset of early molecular events prior to the rise of Aβ levels, setting up a detrimental scenario to facilitate Aβ-mediated neurodegeneration. During the last few decades, inflammation has emerged as a central element in the pathophysiology of AD (Ardura-Fabregat et al., 2017) (Fig. 11.2). Moreover, the close relationship established between ageing and AD, as well as between ageing and a general inflammatory status of biological systems, seems to suggest the critical role of the inflammatory process in the genesis and progression of AD.

Inflammatory response in the central nervous system

Living organisms must quickly interact with a highly demanding environment, especially, harmful challenges that can compromise system physiology. In this regard, the immune system, through its innate and adaptive subsystems, can respond to different insults, inducing a coordinated cellular and molecular cascade of events, including inflammation, leading to the detection and elimination of the damaging exogenous/endogenous insult, constraining the damage. Relevantly, pathogens, such as bacteria and viruses, express

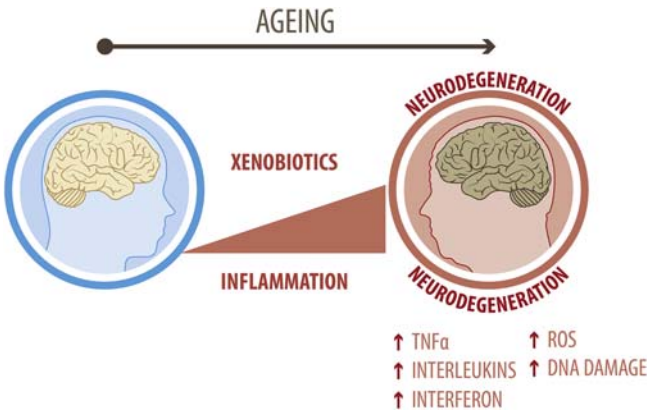

Figure 11.2 ***Setting up the scenario for neurodegeneration: the central role of the ageing-inflammatory axis.*** Ageing is a complex event that occurs naturally and develops over years. This process has been linked to the impaired functionality of different homeostatic systems, such as redox balance and immune response. Compromised immunocompetence and increased levels of proinflammatory mediators are a common feature of the ageing process. Relevantly, these age-related events also occur within the brain, changing its antiinflammatory properties to promote a proinflammatory microenvironment. Moreover, during ageing, biological systems are exposed to different xenobiotics, which tend to challenge the homeostatic capacity of the system. At certain points, when this mechanism fails, which occurs with age-related immunocompetence, xenobiotics will interact with different biological molecules, altering their function. These elements create a perfect environment for the insurgence of neurodegenerative pathologies, such as AD, in which additional stressful stimuli (Aβ) override the homeostatic system, triggering the neurodegenerative process.

different pathogen-associated molecular patterns (PAMPs), which are characteristic molecules that can activate the immune response. Alternatively, sterile processes cause the release of several cell damage-associated molecular patterns (DAMPs), such as DNA or ATP, which also induce immune activation and development of the inflammatory process. In general, whether because of a pathogen, xenobiotic and/or damaged-cell end-products, immunocompetent cells will be activated, leading to the release of several inflammatory mediators, including tumor necrosis factor 1α (TNF-1α), interleukins (IL-1, IL-8, and IL-10), and interferon γ (INF-γ) (Ardura-Fabregat et al., 2017; Heneka, Carson et al., 2015; Heneka, Golenbock, & Latz, 2015).

Because of the differential nature of harmful molecules, immunocompetent cells must express several types of immune-related receptors, termed pattern recognition receptors (PRRs). In this context, toll-like receptors (TLRs) constitute a key element of the innate immune response, including sterile pathological processes, such as AD (Hanke & Kielian, 2011).

The CNS is a highly specialized structure, and neurons require specific microenvironmental conditions to carry out its functions and to ensure that the neuronal network is properly functioning. Although the CNS is partially isolated, preventing both external and internal elements from altering brain homeostasis, some external insults, such as pathogens or environmental pollutants, and/or endogenous conditions such as autoimmune diseases, sterile pathological processes, and ageing, will eventually reach the brain parenchyma and induce neuronal damage that will require an efficient response to control and prevent the spread of damage. In this sense, because of the specialized function carried out by neurons, the immune and inflammatory response within the CNS must be tightly controlled to prevent the detrimental effects of an exacerbated process. The partial isolation of the CNS, achieved through the BBB consolidation, limits the cellular component of the immune system to the microglia, and the enrichment of the brain parenchyma with antiinflammatory mediators, such as TGF-β and IL-10, can be considered strategies to modulate and control the inflammatory response within the CNS (Malipiero et al., 2006; Strle et al., 2001). However, beyond these limitations, the CNS can develop a full immune response (Atmaca et al., 2014; Hanke & Kielian, 2011; Landreth & Reed-Geaghan, 2009).

TLR-mediated neuroinflammatory response in Aβ pathology

To date, 11–13 TLR subtypes have been described, depending on the species. Relevantly, although TLRs 1, 2, 4, 5, and 6 are expressed at the cell membrane, TLRs 3, 7, 8, and 9 are found inside cells, mainly associated with endosomes, and commonly sense viral components, such as RNA and DNA, as well as nonmethylated CpG-enriched DNA (Atmaca et al., 2014; Hanke & Kielian, 2011; Ransohoff & Brown, 2012; Takeuchi & Akira, 2010).

TLR signaling has been described extensively; however, in general terms, the classical TLR-mediated response begins with the recruitment of myeloid differentiation factor 88 (MyD88), leading to the activation and subsequent recruitment of several proteins, causing the release of NF-kB. A secondary mechanism can be triggered through TLRs 3 and 4, which will also release IFN-β along with NF-kB. Importantly, regardless of whether the classical or secondary mechanism is utilized, the final outcome of the cascade triggered by TLR activation will be the production and release of several inflammatory mediators, including interleukins and C-C and C-X-C motif ligands (Atmaca et al., 2014; Hanke & Kielian, 2011; Landreth & Reed-Geaghan, 2009; Ransohoff & Brown, 2012; Takeuchi & Akira, 2010; Zolezzi & Inestrosa, 2017).

In this context, Aβ interacts directly with TLR2 and TLR4, inducing the expression of IL-1, IL-6, IL-12, TNF-α, cyclooxygenase 2, and inducible nitric oxide synthase (Reed-Geaghan, Savage, Hise, & Landreth, 2009). Moreover, Aβ can induce the release of DAMPS, mainly because of the disruption of several neuronal processes and the alteration of different subcellular components, such as mitochondria (Selkoe, 2011; Selkoe & Hardy, 2016; Sweeney et al., 2018). In this regard, microglia establish a close interaction with neurons and remain in a "resting" state in the absence of a challenging event (Cameron & Landreth, 2010; Wake, Moorhouse, Miyamoto, & Nabekura, 2013). However, microglia are "activated" when a harmful signal, such as the loss of neuronal contact, Aβ or DAMPs, is detected by TLRs (Zolezzi & Inestrosa, 2017). Additionally, microglia express triggering receptor expressed on myeloid cells 2 (TREM2), which, beyond being involved in the phagocytosis of Aβ (Crehan, Hardy, & Pocock, 2013; Griciuc et al., 2013; Wang et al., 2015), acts as a true Aβ receptor, further modulating the microglial-driven inflammatory response (Zhao et al., 2018).

Moreover, astrocytes, oligodendrocytes, and neurons express several TLR members. In other words, Aβ affects the surrounding microglia and induces an immune/inflammatory response in neighboring neurons, astrocytes, and oligodendrocytes. This issue is the most relevant considering the perpetuation of the inflammatory cycle within the brain.

Ageing and the proinflammatory scenario

As previously mentioned, several works have noted the relevance of the ageing process and the presentation of neurodegenerative disorders. In this regard, the alteration of a wide range of cellular and molecular events has been defined as part of ageing and as key elements of the degenerative process. Accordingly, during ageing, the general status changes from an antiinflammatory to a proinflammatory condition is a process that involves increased levels of circulating inflammatory cytokines as well as the competence of the cellular components involved in the immune response. Interestingly, the CNS, including the brain, exhibits the same age-related proinflammatory deviation, further suggesting that this phenomenon can also be part of the genesis of neurodegenerative disorders (Cao & Zheng, 2018; Schwalm et al., 2014; Wang et al., 2018; Wyss-Coray, 2016).

In this sense, inflammageing defines the progressive shift from homeostasis to the inflammatory status as an age-dependent variable and is linked to the deregulated function of inflammasomes, which are intracellular structures responsible for the release of proinflammatory mediators, causing a systemic rise in the levels of these molecules, such as IL-18 (Cao & Zheng, 2018). Complementarily, increased plasmatic levels of proinflammatory mediators have a detrimental effect on the CNS microvasculature, affecting the sealing capacity of the BBB. Indeed, microbleeding, which can constitute a common feature of systemic inflammatory conditions, allows the extravasation of blood components within the brain, including the same proinflammatory signals and immune cells, which further contribute to the modification of the CNS/brain microenvironment (Cao & Zheng, 2018; Newcombe et al., 2018).

Moreover, cell senescence affects both peripheral immune cells and CNS cells. In this regard, although astrocytes are the only cells within the brain that have demonstrated senescence markers, microglia exhibit several morphological and biochemical changes linked to ageing. Overexpression of "activation markers," such as cluster of differentiation 11c and 14, increased production of inflammatory cytokines, such as TNF-α, IL-1, and IL-6, and reactive oxygen species (ROS) demonstrate the determinant role of the ageing process within CNS immunocompetence (Cameron & Landreth, 2010).

Moreover, together with genetic heterogeneity, epigenetic changes introduce another source of individual variation in the ageing process (Ashapkin, Kutueva, & Vanyushin, 2017; Masser et al., 2017). Indeed, the epigenetic profile differs between healthy and diseased individuals and between patients, making it possible to observe distinct methylation patterns of some specific genes, such as ankyrin1 (ANK1), in the case of AD patients (Lunnon et al., 2014). Our knowledge about the epigenetic modulation of immune cells is quite limited. However, regarding neurodegenerative disorders, the crossroads between epigenetics, ageing, and immunocompetence suggest that this issue should be thoroughly studied.

Ageing and the proinflammatory scenario: additional clues from menopause

Another aspect that should be considered and that seems to strengthen the critical role of the inflammatory status at the establishment of neurodegenerative pathology is the female-dependent increased risk of AD at the postmenopause stage (Fig. 11.3A).

Accordingly, several groups have demonstrated the beneficial effects of estradiol in the context of the pathological alterations observed in AD (Albert et al., 2017; Engler-Chiurazzi, Brown, Povroznik, & Simpkins, 2017; Zárate, Stevnsner, & Gredilla, 2017). Additionally, different experimental approaches have demonstrated that neuroinflammation, mitochondrial dysfunction, and Aβ levels regulation can be modulated through estradiol administration (Zárate et al., 2017; Albert et al., 2017; Engler-Chiurazzi, et al., 2017; Zhao, Mao, Woody, & Brinton, 2016) (Fig. 11.3B).

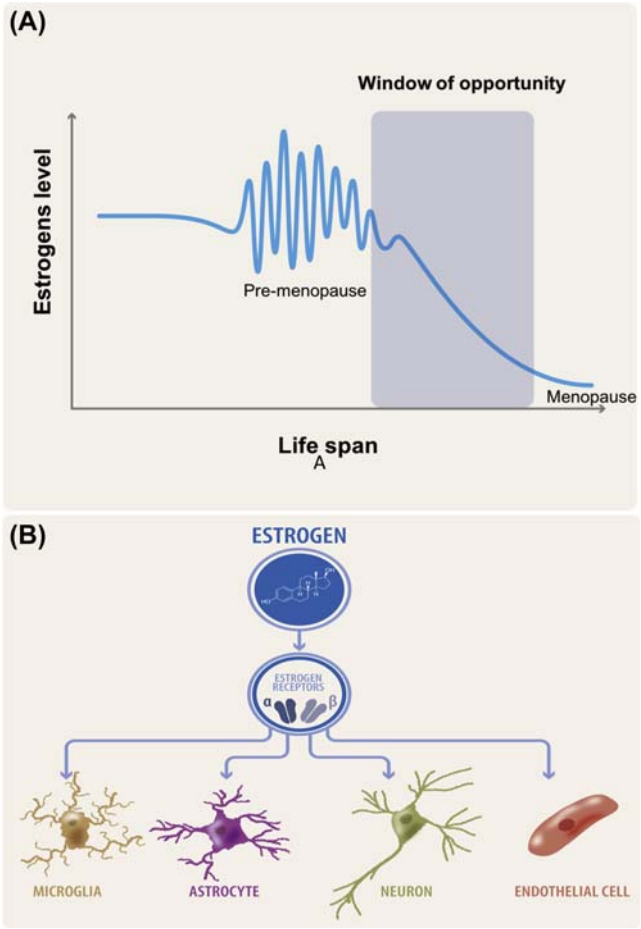

Figure 11.3 ***Estrogens, menopause and inflammation.*** An additional clue regarding the relevance of inflammation to the establishment of neurodegenerative disorders, especially AD, is related to the loss of activity of estrogens after menopause. (A) Several research groups have shown that gender represents a serious risk factor when menopause is considered and have suggested that it is possible to control this risk if hormone replacement therapy is initiated in a timely manner ("window of opportunity"). Relevantly, estrogens have demonstrated significant immunomodulatory and antiinflammatory effects in living organisms. (B) Estrogens can reduce the levels of proinflammatory mediators and modulate the physiology of different cellular components involved in the inflammatory response even in the brain. Microglia, astrocytes, neurons, endothelial cells, and mitochondria can be modulated by estrogens, protecting them from an uncontrolled or chronic inflammatory status.

Relevantly, decreased estradiol levels can be related to a systemic proinflammatory status, with increased levels of interleukins (IL-1 and IL-6) and tumor necrosis factor α (TNF-α), among other cytokines (Au et al., 2016; Mitra, Ghosh, Sinha, Chakrabarti, & Bhattacharyya, 2015; Straub, 2007; Villa, Vegeto, Poletti, & Maggi, 2016).

Similarly, activation of the nuclear factor κB (NFκB) pathway is also part of the mechanisms triggered by decreased estradiol levels, further supporting the maintenance of this proinflammatory status (Mitra et al., 2015; Straub, 2007; Sun, Yang, Zang, & Wu, 2010). Moreover, reduced estradiol levels increase the vulnerability to systemic immune-dependent conditions leading to brain proinflammatory changes in the presence of a peripheral insult (Brown, Mulcahey, Filipek, & Wise, 2010). Relevantly, this condition can be reversed when estradiol levels are restored (Brown et al., 2010).

Importantly, during an immune response within the brain, microglia express both ERα and ERβ, suggesting that estradiol can modulate the NFκB pathway within these cells, preventing the transcription of proinflammatory genes and limiting the production and release of cytokines (Caruso et al., 2013; Ishihara, Itoh, Ishida, & Yamazaki, 2015; Tripanichkul, Sripanichkulchai, & Finkelstein, 2006). Similarly, estradiol can also reduce phagocytosis and ROS production by microglia, suggesting that estradiol can exert an inhibitory effect on microglia, preventing the M1 phenotype, causing the modulation of the inflammatory response (Habib et al., 2014). Moreover, estradiol can prevent the increase in glial fibrillary acidic protein levels, a characteristic of activated astrocytes and an additional indicator of proinflammatory conditions within the brain (Fargo et al., 2017; Rozovsky et al., 2002; Sarfi, Elahdadi-Salmani, Goudarzi, Lashkar-Boluki, & Abrari, 2017).

Regarding mitochondrial dysfunction, estradiol has been linked to improved mitochondrial function through direct stimulation of mitochondrial biogenesis and mitochondrial dynamics (Simpkins, Yang, Sarkar, & Pearce, 2008). Mitochondria express both ER receptors, allowing estradiol to modulate mtDNA expression, leading to improved mitochondrial functionality, with increased energy and antioxidant capacity production together with reduced ROS release (Chen, Eshete, Alworth, & Yager, 2004; Hara et al., 2014; Hsieh et al., 2006). Similarly, estradiol can prevent Aβ-induced mitochondrial fission, further supporting the beneficial role of estradiol in the context of mitochondrial dysfunction (Sarkar, Jun, & Simpkins, 2015). Estradiol also acts at the cellular and mitochondrial levels as a modulator of Ca^{++} levels. Under physiological conditions, estradiol favors cellular calcium influx, but under pathological stimulus, it can prevent calcium overload in both neurons and mitochondria, protecting their functions. Together, these functions demonstrate that estrogens are key players in the homeostatic ROS/inflammatory balance and that disruption, which occurs after menopause, significantly increases the risk of developing AD (Fig. 11.3B).

Concluding remarks

Although our knowledge regarding the molecular mechanisms of AD has significantly increased during recent decades, the triggering events allowing for AD establishment have been very difficult to define. In this regard, considering the different hypotheses

proposed to explain the genesis of this pathology, AD, as well as other neurodegenerative disorders, potentially requires a combination of conditions to begin. Indeed, the amyloid plaques, neurofibrillary tangles, neuronal loss, and synaptic alterations in cognitive healthy subjects strongly suggest that a "detrimental scenario" sets everything in motion and allows Aβ to trigger the degenerative process. In this context, the inflammatory process emerges as a driving force of the homeostatic balance and potentially as one of the most relevant players to deviate healthy ageing to pathology.

Key facts on neurodegeneration

- The brain is a full immunocompetent organ.
- The brain microenvironment is tightly controlled and exhibits antiinflammatory features with low levels of proinflammatory mediators.
- Microglia are the only immune cells within the CNS responsible for immune surveillance.
- Microglia, astrocytes, neurons, and oligodendrocytes express toll-like receptors.
- Increased levels of inflammatory mediators at the systemic level can alter the central nervous system microenvironment, causing the "activation" of astrocytes and microglia and affecting neuronal health.
- Whether systemic or local, inflammation can induce increased levels of Aβ within the CNS.

Summary points

- This chapter focuses on neuroinflammation occurring during Alzheimer disease.
- The chronic inflammatory response is detrimental for brain functions, suggesting a relevant role in Alzheimer disease etiology and progression.
- In this regard, neuroinflammation increases as part of the ageing process before the establishment of neurodegenerative disorders.
- Moreover, systemic inflammatory conditions affect the inflammatory status of the brain, changing its microenvironment from antiinflammatory to proinflammatory.
- Inflammageing together with systemic or chronic conditions constantly challenging the immune/inflammatory response within the brain will compromise its homeostatic capacity, "setting up" the perfect conditions for amyloid-β neurodegeneration.

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CHAPTER 12

Wnt signaling and dementia

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List of abbreviations

AD Alzheimer's disease
ATR1 angiotensin II type 1 receptor
Aβ amyloid-β
BBB blood–brain barrier
calcineurin CaCn
CaMKII calcium/calmodulin kinase II
CK-1 α casein kinase 1α
CNS central nervous system
CRD cysteine-rich domain
DKK1 dickkopf-related protein 1
DVL disheveled
FTD frontotemporal dementia
FTLD frontotemporal lobar degeneration
FZDs frizzled proteins
GSK3β glycogen synthase kinase 3 β
IP3 inositol 1,4,5-triphosphate
JNK c-Jun N-terminal kinase
LRP low-density lipoprotein receptor-related protein
LTP long-term potentiation
mTORC1 mTOR complex 1
NFAT nuclear factor of activated T cells
PCP planar cell polarity
PGRN progranulin protein
PKC protein kinase C
PORCN protein-serine O-palmitoleoyltransferase porcupine
PPARγ peroxisome proliferator-activated receptor gamma
Rac1 Ras-related C3 botulinum toxin substrate 1
RhoA Ras homolog gene family member A
ROCK rho-associated protein kinase
sFRPs soluble frizzled-related proteins
TCF/LEF T-cell factor/lymphoid enhancer-binding factor
TGF-β transforming growth factor beta
TNF tumor necrosis factor
TNK tankyrase
VD vascular dementia
VEGF vascular endothelial cell growth factor

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Wnt wingless
Wnt/mTOR Wnt mammalian target of rapamycin
Wnt/STOP Wnt stabilization of proteins

Mini-dictionary of terms

β-catenin destruction complex Multiprotein complex formed by axin, GSK3β, CK1, and adenomatous polyposis coli
Frizzled receptors Family of seven transmembrane receptors containing a CRD domain
Signaling pathway Process by which linked chemical reactions occur within the cell in response to the attachment of an extracellular molecule to a membrane receptor
Targeting a signaling pathway Administration of pharmacological compounds to modify the selected pathway at different levels with the ultimate purpose of treating a disease
Wnt ligands A family of different secreted signaling lipoglycoproteins

Introduction

Wingless (Wnt) signaling is a highly conserved pathway across the species, playing a crucial role in embryogenesis. Wnt signaling in adult organisms modulates tissue regeneration and homeostasis in several organs, including the central nervous system (CNS) where it has an important function in the maintenance of neuronal homeostasis (Oliva, Montecinos-Oliva, & Inestrosa, 2018).

So far, 19 different Wnt ligands have been identified in humans. They are secreted lipoglycoproteins that bind to a family of seven transmembrane receptors, the frizzled proteins (FZDs). In addition, lipoprotein receptor-related proteins 5 and 6 (LRPs 5 and 6) act as coreceptors in the complex. Moreover, some Wnt ligands bind to the tyrosine kinase receptor families Ror and Ryk (Niehrs, 2012). Wnt signaling can be regulated by endogenous secreted proteins, mainly secreted frizzled-related proteins and proteins of the Dickkopf (DKK) family. Secreted frizzled-related proteins (sFRPs) act as an antagonist of Wnt signaling because of its ability to compete with membrane-bound receptors for ligands (Surana et al., 2014). DKK-related protein-1 (DKK1) binds LRP5/6 and disrupts the interaction of Wnt ligands with FZD receptors. Extracellular Wnt can trigger two intracellular cascades, the canonical and the noncanonical pathways, represented schematically in Figs. 12.1 and 12.2).

Canonical Wnt pathways

Canonical Wnt signaling pathways are further classified into two major types depending on the involvement of β-catenin (Fig. 12.1). The canonical Wnt/β-catenin pathway hinges on regulated proteolysis of cytoplasmic β-catenin (Fig. 12.1A). In this pathway, the binding of Wnt ligands to FZD receptor-LRP5/6 complex leads to disassembly of the "β-catenin destruction complex," which in turn induces an increase in cytoplasmic β-catenin levels and its translocation to the nucleus where it activates the transcription of

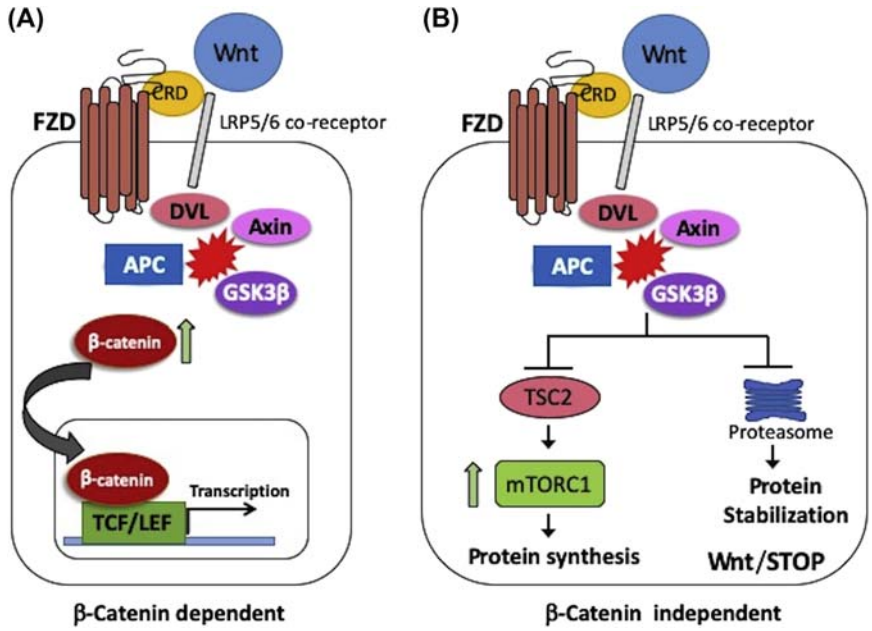

Figure 12.1 ***Schematic representation of the canonical Wnt signaling pathways.*** (A) β-catenin-dependent Wnt pathway. The binding of a Wnt ligand to the corresponding FZD/LRP5/6 receptor results in the disassembly of the "β-catenin destruction complex" and inhibition of β-catenin degradation. β-catenin then enters the nucleus and activates the expression of Wnt target genes. (B) Canonical Wnt ligands can activate intracellular signaling in a β-catenin-independent manner. In this case, the binding of the Wnt ligand to the receptor recruits GSK3β and prevents either the phosphorylation of TSC2, leading to the activation of mTORC1 (Wnt/mTOR pathway), or the phosphorylation and ubiquitination of proteins other than β-catenin, avoiding their degradation in the proteasome (Wnt/STOP).

Wnt target genes (https://web.stanford.edu/group/nusselab/cgi-bin/wnt/target_gene). Moreover, the Wnt canonical pathway can act in a β-catenin independent way (Fig. 12.1B), inducing the inhibition of tuberous sclerosis protein 2 (TSC2), which will lead to activation of the mTORC1 pathway and an increase in protein synthesis (Wnt/mTOR). In addition, the activation of the β-catenin-independent Wnt pathway can interfere with the degradation of different proteins in the proteasome causing STabilization Of Proteins (Wnt/STOP) (See Grainger & Willert, 2018 for a review).

Noncanonical Wnt pathways

The term noncanonical Wnt pathways refers to cascades activated by Wnt ligands that do not involve GSK-3β or β-catenin. They are subdivided into two, Wnt/Ca^{2+} and Wnt/planar cell polarity (PCP) (Fig. 12.2). In the Wnt/Ca^{2+} signaling pathway, ligands bind to

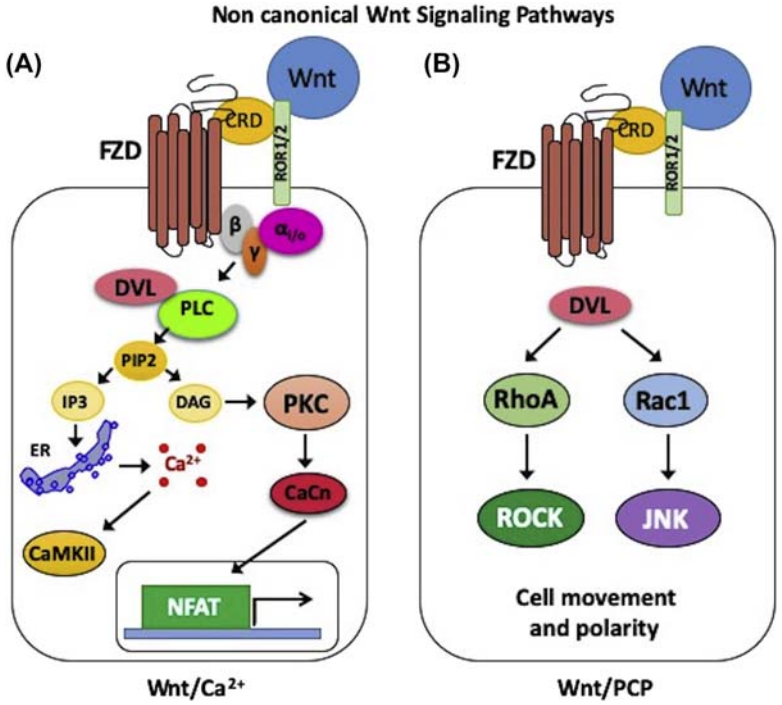

Figure 12.2 ***Schematic representation of the noncanonical Wnt signaling pathways.*** Noncanonical Wnt signaling pathways are independent of the activity of GSK3β and require either Ror1/2 or Ryk as coreceptor. (A) In Wnt/Ca^{2+} signaling, the binding of the Wnt ligand to the receptor leads to the activation of PLC, which will cleave PIP_2 into 1,2 diacylglycerol and IP_3, inducing the release of Ca^{2+} from the endoplasmic reticulum (ER) to the cytoplasm and the activation of calmodulin kinase II (CaMKII), protein kinase C (PKC), and calcineurin (CaCn). CaMKII and PKC activate the NF-κβ and CREB transcription factors, while CaCn activates the transcriptional activity of NFAT. (B) Activation of the Wnt/PCP signaling pathway leads to the stimulation of DVL and activation of two small GTPases, RhoA and Rac1, which in turn enhance ROCK and c-Jun N-terminal kinase (JNK) activity, respectively.

the FZD receptor coupled with heterotrimeric G-proteins, leading to an increase in the concentration of intracellular signaling molecules such as inositol 1,4,5-triphosphate (IP3), 1,2 diacylglycerol, and Ca^{2+}, which induces the activation of calmodulin kinase II (CaMKII) and protein kinase C (PKC) (Fig. 12.2A). These kinases regulate the activity of nuclear transcription factors such as NF-κB and CREB. Similarly, calcium ions mobilized by IP3 from endoplasmic reticulum can activate calcineurin (CaCn), which activates the protein nuclear factor associated with T cells (NFAT) via dephosphorylation, which then activates the expression of several genes (De, 2011). The Wnt/PCP pathway (Fig. 12.2B) emerged from genetic studies in which mutation in Wnt signaling components resulted in alterations in the orientation of epithelial structures. In this pathway, the binding of Wnt to the FZD receptor is followed by

the activation of two small GTPases, Rho and Rac, which in turn stimulate Rho-associated protein kinase (ROCK) and c-Jun N-terminal kinase, respectively (Fig. 12.2B). Signaling from these kinases is integrated for cytoskeletal changes for cell polarity and motility (Seifert & Mlodzik, 2007).

The role of Wnt in the central nervous system

Different Wnt molecules are implicated in the development of the CNS, playing a role in the establishment of neural polarity and synaptic connections (Ciani & Salinas, 2005). During early and late postnatal development, Wnt signaling appears to be involved in arborization and dendritic spinal formation of excitatory neurons (Ramírez, Ramos-Fernández, Henríquez, Lorenzo, & Inestrosa, 2016) and in the consolidation and recall of spatial memory (Tabatadze, McGonigal, Neve, & Routtenberg, 2014). In the adult brain, Wnt proteins are involved in neurogenesis (Inestrosa & Arenas, 2010). Interestingly, the expression of Wnt ligands is particularly high in the areas of the brain where the neurons are continuously renewed, such as cerebral cortex, olfactory bulb, hippocampus, neocortex, and thalamus (Oliva, Vargas, & Inestrosa, 2013).

In the CNS, the canonical Wnt/β-catenin pathway regulates the expression of a group of genes that encode for proteins involved in neuronal excitability, including voltage-gated ion channels, neurotransmitter receptors, synaptic vesicle proteins, and synaptic structural proteins (Wisniewska, 2013). The functioning of the β-catenin-independent canonical pathways in postmitotic neurons is less obvious. Nevertheless, there is evidence for the involvement of the PI3K/Akt/mTOR cascade in the translation of synaptic proteins in spines and dendrites (Lee, Huang, & Hsu, 2011). The activation of Wnt/STOP in the CNS could play a role in situations where the protein content must be preserved, such as in neuronal injury (Oliva et al., 2018).

Within the CNS, the activation of noncanonical Wnt/Ca^{2+} has been found to be associated with the modulation of synaptic proteins (Farías et al., 2009) and with increases in the density and formation of new spine dendrites (Varela-Nallar, Alfaro, Serrano, Parodi, & Inestrosa, 2010). It is also implicated in the modulation of long-term potentiation (LTP) in hippocampal tissue (Chen, Park, & Tang, 2006) and seems to modulate mitochondrial morphology and dynamics in rat hippocampal neurons (Godoy et al., 2014). On the other hand, noncanonical Wnt/PCP signaling is involved in converting signals into morphogenic programs and has essential functions in dendritic patterning, axonal tract development, and neuronal migration (Tissir & Goffinet, 2013).

Given the important functions of Wnt signaling pathways in the adult brain, it is not surprising that dysfunction of Wnt activity has been associated with neurodegenerative or neuropsychiatric diseases. In this review, we will preferentially focus on the involvement of Wnt cascades in dementia.

The role of Wnt signaling in dementia

The term dementia is used to describe a group of symptoms associated with a decline of cognitive functions and a reduction in the ability to perform daily life activities. Nowadays, it is estimated that dementia affects about 46 million people worldwide, with an important economic and social impact. Compelling data have indicated the existence of alterations in major cellular pathways, including Wnt cascades, in dementia (Esteras, Alquézar, de la Encarnación, & Martín-Requero, 2016; Foulquier et al., 2018; Rosen et al., 2011). A brief list of affected pathways is given in Table 12.1.

Wnt signaling and Alzheimer's disease

Alzheimer's disease (AD) is characterized by progressive loss of cognitive functions and pathologically by the presence of extracellular amyloid-β (Aβ) deposits and intracellular aggregates of hyperphosphorylated forms of protein tau, named neurofibrillary tangles (Castellani, Rolston, & Smith, 2010).

The molecular mechanisms involved in AD etiology are not fully understood. Overproduction of Aβ, hyperphosphorylation of tau, oxidative damage, synapsis loss, neuroinflammation, and reactivation of the cell cycle in postmitotic neurons have been proposed as contributors to AD pathogenesis.

Table 12.1 Signaling pathways altered in dementia.

Pathway	AD	FTD	VD
Ca^{2+}/CaM/Akt/pRB	↑		
Ca^{2+}/CaM/ERKl/2	↓		
Ca^{2+}/CaM/CaMKII	↑		↑
ERK1/2/CDK6/pRb		↑	
Insulin/IGF-l/Akt/GSK3b	↑		
Notch	↑	↑	↑
mTOR	↑	↑	↑
VEGF			↑
Wnt/β-catenin	↓	↑	↓
Wnt/Ca^{2+}	↓	↑	
PPARγ	↑		
TNFα/NF-κB		↑	
TGF-β/ATRl			↑
Mitochondrial dysfunction	+	+	+
Impaired proteostasis	+	+	
Lysosome dysfunction	+	+	

Arrows denote up- or downregulation. + indicates impaired regulation. *AD*, Alzheimer's disease; *ATR1*, angiotensin II type 1 receptor; *FTD*, frontotemporal dementia; *VD*, vascular dementia. For other abbreviated terms, please see the Abbreviations list at the start of this chapter.

Pioneering work from Inestrosa's group unveiled a strong relationship between impaired Wnt signaling activity and Aβ-induced neuronal damage (Inestrosa, Alvarez, Godoy, Reyes, & De Ferrari, 2000). This idea was further supported by independent studies showing alterations in Wnt signaling components in AD (De Ferrari et al., 2007; Ghanevati & Miller, 2005). Moreover, Wnt signaling pathways have been implicated in blood–brain barrier (BBB) formation in Aβ-induced neuroinflammation and toxicity. They seem also to play a key role in the regulation of adult neurogenesis (Lie et al., 2005) and in the formation, stabilization, and recycling of synapses in adult brains (Ciani & Salinas, 2005). Therefore, altered regulation of any or all of these processes could contribute to disease pathogenesis.

Different pieces of evidence have suggested that downregulation of canonical Wnt signaling is associated with AD onset and progression (Ferrari et al., 2014). First, it was reported that Aβ increases the expression of the secreted glycoprotein DKK1, a known Wnt antagonist (Killick et al., 2014). Elevated levels of DKK1 were found in postmortem AD brains of transgenic mice near the amyloid plaques as well as colocalizing with neurofibrillary tangles and dystrophic neurites (Caricasole et al., 2004). Second, a novel functional LRP6 gene alternative splice variant in AD was found (Ferrari et al., 2007). Third, reduction of Wnt signaling correlates with increased expression and activity of GSK3β kinase, tau phosphorylation, and neurodegeneration in human and mice models of AD (Avila et al., 2012; Killick et al., 2014). In addition, GSK3β appears to favor APP amyloidogenic cleavage (Inestrosa & Varela-Nallar, 2014) and thus the production of Aβ. GSK3β has been identified as an important regulator of inflammation, promoting the production of several proinflammatory cytokines such as IL-6, IL-1β, and tumor necrosis factor-α (TNFα) as well as decreasing the levels of antiinflammatory cytokine IL-10 (Koistinaho, Malm, & Goldsteins, 2011). And finally, it is known that presenilin proteins, which are associated with early-onset AD, are negative regulators of canonical Wnt signaling (Nishimura et al., 1999). β-catenin levels decreased in the brains of AD patients carrying presenilin-1-inherited mutations (Zhang et al., 1998). Conversely, the activation of Wnt cascades was found to recover LTP and memory impairment in (APP/Sw/PS1ΔE9) mice (Puzzo et al., 2017) and protected hippocampal neurons from Aβ-induced toxicity in vitro (Alvarez et al., 2004).

Regarding the involvement of noncanonical Wnt signaling in AD, it was reported that activation of the Wnt/Ca^{2+} pathway inactivates GSK3β, favoring β-catenin accumulation and subsequent transcription of neuroprotective genes. Moreover, increased Wnt/Ca^{2+}-dependent PKC activation promotes α-secretase activity that causes nonamyloidogenic deposition of amyloid precursor protein in the extracellular region (Skovronsky et al., 2000). A recent report carried out in hippocampal neurons, had shown that Wnt3a-mediated signaling inhibits mitochondrial membrane potential dissipation and decreases cytochrome c release, thus increasing neuronal viability (Arrázola, Ramos-Fernández, Cisternas, Ordenes, & Inestrosa, 2017). Another work

suggested the involvement of noncanonical Wnt signaling in preventing unscheduled cell cycle entry in postmitotic neurons, which in turn promote neuron survival (Zhou et al., 2017). These observations illuminate that aberrant downregulation of Wnt5a signaling is a crucial pathological step that contributes to AD-related neurodegeneration (Zhou et al., 2017).

Wnt signaling and frontotemporal dementia

Frontotemporal dementia (FTD) is a clinically, genetically, and pathologically complex neurodegenerative disorder characterized by the atrophy of frontal and temporal lobes of the brain. The clinical symptoms associated with FTD are diverse, being the most common alterations in behavior and language. Pathologically, FTD is characterized by the presence of protein aggregates in the cytosol of affected neurons. In most cases, these aggregates contain either tau or TDP-43 proteins (FTLD-tau and FTLD-TDP respectively). Around 50% of FTD cases present a positive familiar history of the disease. Mutations in *GRN, MAPT*, and *C9ORF72* genes are those most frequently associated with FTD (Takada, 2015).

In contrast to AD, overactivation of Wnt signaling cascades has been reported on both FTLD-tau and FTLD-TDP. Increased β-catenin signaling has been proposed to be an early feature of FTLD-tau in drosophila and mice models (Jackson et al., 2002; Wiedau-Pazos et al., 2009). These finding appear paradoxical, as activation of the Wnt cascade would result in downregulation of GSK3β, one of the kinases involved in tau phosphorylation. However, these authors demonstrated that the effects of canonical Wnt activation and the role of GSK3β in tau phosphorylation are independent of each other. Increased levels of β-catenin appear prior to the onset of tau hyperphosphorylation. They hypothesized that tau hyperphosphorylation is a product of physical interaction between GSK-3β and mutant tau, as they observed that these proteins colocalized in affected neurons. The early increase in β-catenin levels in FLTD-tau could explain the presence of mitotic markers in affected neurons in patients carrying *MAPT* mutations (Husseman, Nochlin, & Vincent, 2000). It has been suggested that tau pathology and neurodegeneration may be linked via abnormal, incomplete cell-cycle reentry (Andorfer et al., 2005). It is believed that aberrant cell cycle activation in neurons provides a possible explanation for the generally late onset of neurodegenerative diseases (Herrup & Yang, 2007).

Loss-of-function mutations in *GRN* cause most FTLD-TDP cases; however, the molecular mechanism by which progranulin (PGRN) protein deficit causes neurodegeneration is not well known. In 2011, the work of Geschwind's group (Rosen et al., 2011; Wexler et al., 2011) unveiled an important role for Wnt cascades in FTLD-PGRN. By performing functional genomic analysis in cultured PGRN-deficient neural progenitor cells, they found robust changes in the expression of genes in the cell cycle, apoptosis,

and ubiquitination. Further work on *GRN* KO mice indicated that PGRN deficiency upregulates the expression of the Wnt receptor FZD2 associated with unpaired apoptosis. These authors validated the in vitro results using expression data from the postmortem FTD brain. They concluded that PGRN deficiency selectively compromises neuronal survival and engages the canonical Wnt signaling pathway. Aberrant activation of Wnt signaling has also been found in induced pluripotent stem cells generated from FTLD patients carrying a *GRN* (IVS1+5G > C) mutation (Raitano et al., 2015).

Work in our laboratory demonstrated enhancement of both the Wnt/β-catenin and Wnt/Ca^{2+} signaling pathways in a cell model of FTLD-TDP: human neuroblastoma SH-SY5Y *GRN* knockdown cells (de la Encarnación, Alquézar, & Martín-Requero, 2016). We detected increased expression levels of Wnt1 and Wnt5a together with elevated nuclear content of β-catenin and increased levels of the active form of the NFAT1 transcription factor, indicating activation of canonical and noncanonical Wnt signaling, respectively. These changes were accompanied by cell cycle activation and reduced cell viability.

Moreover, we reported cell cycle disturbances and dysregulation of cell survival/death mechanisms associated with enhanced Wnt/Ca^{2+} signaling in immortalized lymphocytes from FTLD-TDP patients harboring a *GRN* loss-of-function mutation (Alquézar et al., 2014b). It was shown that PGRN deficiency led to increased Wnt5a expression levels by interfering with the TNF-α/NF-κβ pathway (Alquézar et al., 2016).

Finally, an issue that deserves investigation is the possible link between overactivation of Wnt signaling and impaired TDP-43 homeostasis in neurons and peripheral cells from FTLD-TDP patients. As opposed to canonical Wnt activation, increased Wnt/Ca^{2+} signaling might exacerbate TDP-43 phosphorylation secondary to the increased activity of GSK3β, one of the kinases involved in TDP-43 phosphorylation (Kim et al., 2009).

The role of Wnt signaling in vascular dementia

Vascular dementia (VD) is considered a neurodegenerative disorder that results from global or localized vascular alterations, with cerebral infarcts being the most common pathology contributing to cognitive deficit. The heterogeneity of VD makes it difficult to elucidate the molecular mechanisms involved, although apoptosis, autophagy, oxidative stress, and neuroinflammation have been associated with the disease (Kalaria, 2018).

The potential role of Wnt signaling cascades in VD has not been deeply investigated; however, Wnt/β-catenin signaling has been referred to as the major player for cerebrovascular development and BBB formation (Foulquier et al., 2018). On the other hand, inhibition of Wnt signaling is associated with neuronal damage in rat and mouse models of focal ischemia (Cappuccio et al., 2005). It was shown that antagonists of DKK1 or GSK3β inhibitors had neuroprotective effects (Mastroiacovo et al., 2009). Recently, it was suggested that Wnt/β-catenin signaling might have multimodal effects in protecting

brain cells from stroke by activating the expression of HIF-a and VEGF, a regulator of cellular adaptation to hypoxia and a regulator of angiogenesis, respectively (Wu et al., 2015). Environmental enrichment appears to improve spatial learning and memory in a murine model of VD by activating the Wnt/β-catenin signaling pathway (Jin et al., 2017). These authors found elevated levels of Wnt3a and β-catenin in rats with arterial occlusion housed in an enriched environment (motor, sensory, and cognitive stimulation).

Targeting the Wnt signaling pathways

Since altered signaling through Wnt cascades, rather than mutation in their components, has been associated with human diseases (Ferrari & Moon, 2006), efforts have been focused on developing small molecules that can activate or inhibit Wnt signaling and offer leads for novel treatments.

Most of the discovered Wnt modulators interfere with protein–protein interactions including FZD proteins, DVL, or β-catenin. The main sites of pharmacological intervention in Wnt signaling are production and secretion of Wnt proteins, FZD receptors, Wnt scavengers, kinase inhibitors (GSK3-β, CK1, tankyrase, and CaMKII), and modulators of β-catenin-dependent and independent transcription.

Figs. 12.2 and 12.3 summarize some pharmacological interventions. An exhaustive recompilation can be found elsewhere (Foulquier et al., 2018).

Following the synthesis of Wnt proteins, they undergo glycosylation and palmitoylation, processes that are indispensable for the secretion of active ligands (Kurayoshi, Yamamoto, Izumi, & Kikuchi, 2007) and that are regulated by the protein PORCN (Komekado, Yamamoto, Chiba, & Kikuchi, 2007). Therefore, a number of inhibitors targeting this protein have been developed. Blocking the interaction of Wnt ligands with their FZD receptors is another therapeutic strategy. At present, the options for intervention at the level of FZD receptors are very limited; some groups have used antibodies against several FZD receptors, and some of these have proven effective in a number of human cancers (Gurney et al., 2012). sFRPs are a group of molecules with the potential to both inhibit and activate the Wnt signaling pathway (Xavier et al., 2014).

Targeting the interaction of Wnt ligands/FZD receptors with the coreceptor LRP5/6 is another major point for pharmacological modulation of the canonical Wnt pathway as well as the blockade of DKK1, the natural inhibitor of this branch (Yaccoby et al., 2007).

Intracellular intervention of Wnt signaling is directed to DVL and axin proteins, and the regulator tankyrase (TNK), as well as the kinases GSK3β and CK1. The FDA-approved anthelminthic drug pyrvinium is a potent inhibitor of canonical Wnt signaling through binding and activation of CK1. In contrast, most of the known Wnt signaling activators are inhibitors of the GSK3β. Numerous small molecule inhibitors of GSK3β

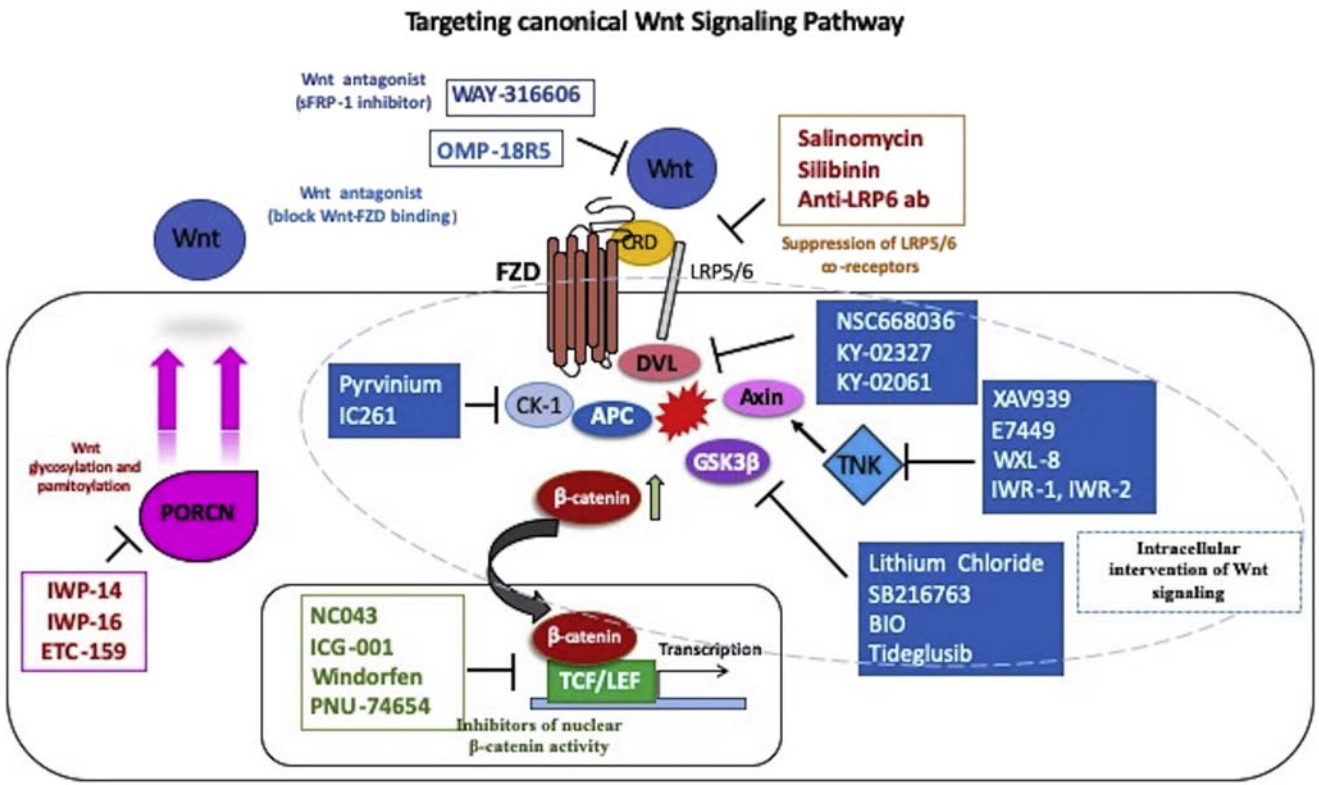

Figure 12.3 ***Diagram showing the pharmacological interventions in the canonical Wnt pathway.*** The strategies for pharmacological intervention in Wnt signaling include targeting the production and secretion of Wnt proteins, the activity of FZD receptors, the use of Wnt antagonists, inhibitors of GSK3β, CK1, and TNK kinases, and modulators of β-catenin-dependent transcription. In boxes, there is a list of the drugs that interfere at different points of the Wnt cascade.

have been developed and shown to efficiently upregulate Wnt activity. Inhibitors affecting Wnt activity have been developed. A list of inhibitors of GSK3β and nuclear β-catenin activity is shown in Fig. 12.3.

Fig. 12.4 shows molecules used to target the Wnt/Ca^{2+} signaling pathway. Most control the activity of DVL, CaMKII, PKC, and CaCn. Moreover, small molecules Wnt5a-derived, Foxy-5, and Box-5 have been used as agonists or antagonists of the Wnt5a/Ca^{2+} cascade, respectively (Jenei et al., 2009). These compounds are in phase I of clinical trials for the treatment of melanoma. Considering the increased levels of Wnt5a ligand and enhanced signaling trough of the Wnt/Ca^{2+} pathway observed in FTLD-TDP (Alquézar et al., 2014a), it is suggested that Box-5 could have potential benefits in the treatment of FTLD-TDP.

It is important to highlight the difficulty of finding clinically relevant Wnt modifiers due to the complexity of the Wnt network. Further work is needed to evaluate the efficacy and safety of drug candidates.

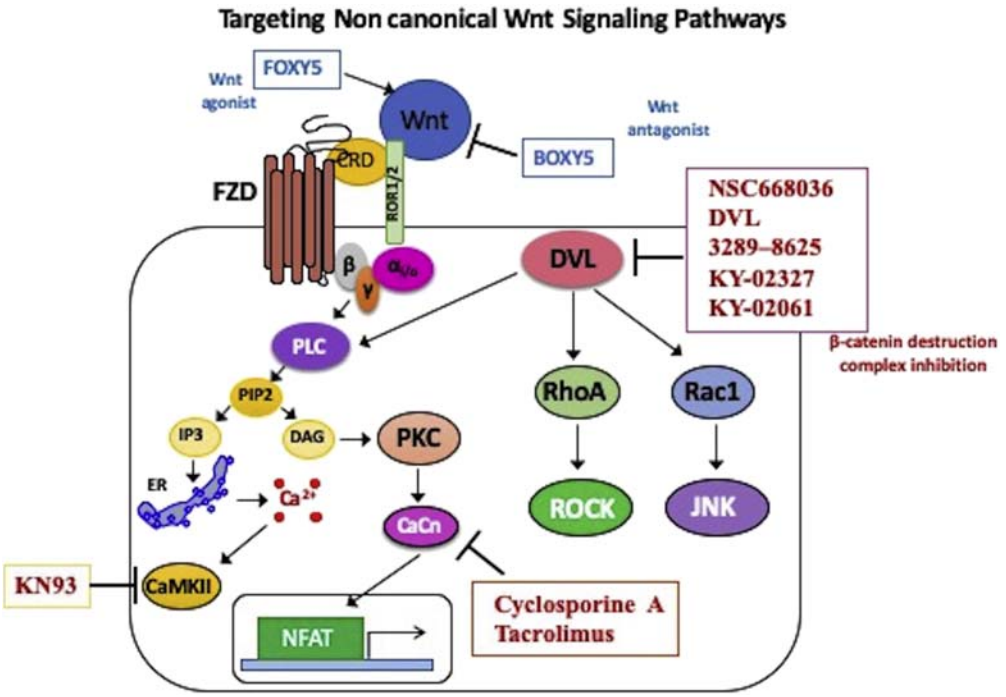

Figure 12.4 ***Pharmacological interventions in the noncanonical Wnt pathway.*** Molecules are used to target noncanonical Wnt signaling activity by blocking the activity of DVL or inhibiting the activity of kinases CaMKII and PKC and the phosphatase CaCn. Foxy-5 and Box-5 are small molecules used as agonist or antagonist of the Wnt5a ligand.

Key facts of dementia

- Dementia is caused by the selective loss of neuronal cells in specific brain regions, leading to changes in behavior, personality, and memory. With some exceptions, dementia occurs several decades after birth.
- There are different forms of dementia. Each type has a specific underlying etiology.
- AD is the most common form of dementia, followed by FTD, VD, and dementia with Lewy bodies. Rare forms of dementia include Creutzfeldt-Jakob disease and Wernicke-Korsakoff syndrome.
- Different types of dementia are associated with alterations in both canonical and noncanonical Wnt signaling pathways.
- There is no cure for dementia nowadays; however, some drugs may improve symptoms or slow the disease.

Summary points

- Impaired Wnt signaling is a common pathological feature of dementias and other neurological diseases.
- The activation of Wnt signaling pathways correlates positively or negatively with different types of dementia.
- Targeting Wnt signaling may provide long-awaited therapies for dementias.
- The difficulty of finding clinically relevant Wnt modifiers resides in the complexity of the Wnt network, affecting tissue homeostasis and repair in adults as well as in the cross-talk between Wnt and other important signaling pathways.

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CHAPTER 13

Linkage of atypical protein kinase C to Alzheimer disease

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List of abbreviations

AD Alzheimer disease
Akt protein kinase B
AMPA amino-3-hydroxy-5-methyl-4-isoxazole propionate
AMPK adenosine monophosphate—dependent protein kinase
aPKC atypical PKC
APP amyloid precursor protein
$A\beta_{1\text{-}40/42}$ amyloid-beta peptides with amino acids 1-40 and 1-42
BBB blood—brain barrier
BDNF brain-derived factor
c/nPKC conventional/novel protein kinase C
cPKC conventional PKC
DAG diacylglycerol
ERK extracellular signal-regulated receptor kinase
FoxO forkhead box-O
GSK3β glycogen synthase kinase-3-beta
Het-MλKO heterozygous muscle PKC-lambda knockout
HFD high-fat diet
HFF high-fat-fed
ICAP 1H-imidazole-4-**c**arboxamide,5-amino-1-[2,3-dihydroxy-4-[(hydroxyl)methyl]cyclo**p**entyl-[1R-(1a,2b, 3b,4a)]
ICAPP 1H-imidazole-4-**c**arboxamide,5-amino-1-[2,3-dihydroxy-4-[(phosphonoxy)methyl]cyclopentyl-[1R-(1a,2b,3b,4a)]
mTOR mammalian target of rapamycin
NGF nerve growth factor
NMDA N-methyl-D-aspartate
nPKC novel PKC
p-tau phospho-tau
PA phosphatidic acid
PDK1 protein kinase—dependent kinase-1
PDK2 protein kinase—dependent kinase-2
PGC-1α peroxisome proliferator-activated receptor-gamma coactivator-1-alpha
PI-3,4,5-$(PO_4)_3$ phosphatidylinositol-3,4,5,-trisphosphate
PI-4.5-$(PO_4)_2$ phosphatidylinositol-4,5,-bisphosphate
PI3K phosphatidylinositol 3-kinase

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PIP_3 PI-3,4,5-$(PO_4)_3$, phosphatidylinositol-3,4,5,-trisphosphate
PKC-ζ protein kinase C-zeta
PKC-λ/ι protein kinase C-lambda/iota
PKMζ protein kinase M-zeta
pY phosphotyrosine
pYXXM, phosphotyrosine - any amino acid − any amino acid − methionine
SH2 Src Homology 2
Shc Src homology 2 domain-containing protein
T2DM type 2 diabetes mellitus
TB/HetλKO total body heterozygous PKC-lambda knockout
Tg transgenic
TGN trans-Golgi network
TrK tyrosine kinase

Introduction

Prior to our current interest in Alzheimer disease (AD), we focused on studies of insulin signaling in muscle, fat, and liver, and aberrations in insulin-resistant states of obesity and type 2 diabetes mellitus (T2DM). Fortunately, we routinely saved brains for future analysis, and expected to find, as per current dogma, that insulin signaling was impaired. However, we surprisingly found in brains of three mouse and a monkey model of obesity/T2DM that activities of the two major insulin signaling factors, Akt and atypical protein kinase C (aPKC), were elevated in the resting/"basal" state to levels comparable to those provoked by maximal insulin stimulation in normal mice (Sajan et al., 2016). Similarly the phosphorylation of Akt substrates, FoxO1, FoxO3a, and FoxO4, and glycogen synthase kinase-3β (GSK3β) and mammalian target of rapamycin (mTOR) were maximally activated. We also found that hyperinsulinemia in these insulin-resistant obese/T2D models was responsible for activation of brain Akt and aPKC, as correction of hyperinsulinemia reversed resting increases in Akt and aPKC activities, and restored the ability of insulin to maximally activate Akt and aPKC (1). Subsequently, we found that the excessive activation of brain aPKC is responsible for activation of β-secretase, and increases in $A\beta_{1\text{-}40/42}$ and phospho-tau provoked by chronic hyperinsulinemia in insulin-resistant mice, and by insulin and noninsulin agents that activate aPKC in normal mice and in isolated hippocampal slices and cultured neurons (Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018) (Figs. 13.1 and 13.2). The importance of these findings escalates when it is realized that humans with nondiabetic AD have comparable elevations in Akt and aPKC activities, presumably reflecting activation by a noninsulin agonist(s) (Talbot et al., 2012).

Relationship of obesity, metabolic syndrome, and T2DM to AD

Insulin-resistant forms of obesity and other metabolic syndrome features are present in 40% of adults over age 40 and commonly progress to T2DM, which afflicts 25% of

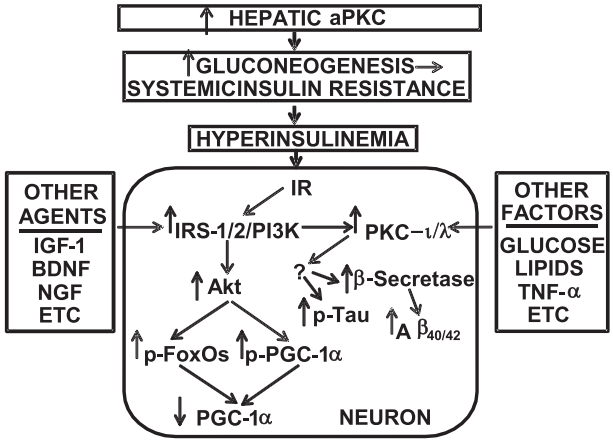

Figure 13.1 Activation of Akt and aPKC, PKC-i/λ, and alterations in downstream processes, in brain by liver-dependent hyperinsulinemia in various insulin-resistant conditions.

people over age 65. AD afflicts 10%–15% people over the age of 65 and 45% of people over the age of 85; AD prevalence in T2DM, and T2DM prevalence in AD, are generally thought to be increased twofold. Further, overt T2DM (fasting blood glucose, >125 mg/dL) and "fasting glucose intolerance" (fasting blood glucose, 110–125 mg/dL, a prediabetic insulin-resistant state) were present in 80% of AD patients seen at the Mayo Clinic (Janson et al., 2004). Accordingly, it is widely speculated that obesity and T2DM predispose the brain to develop late-onset AD.

As pre-T2D and/or T2D generally precede AD, it is speculated that insulin resistance increases AD susceptibility. And, as systemic insulin resistance is virtually ubiquitous in pre-T2DM and T2DM, it was assumed that the brain is insulin resistant

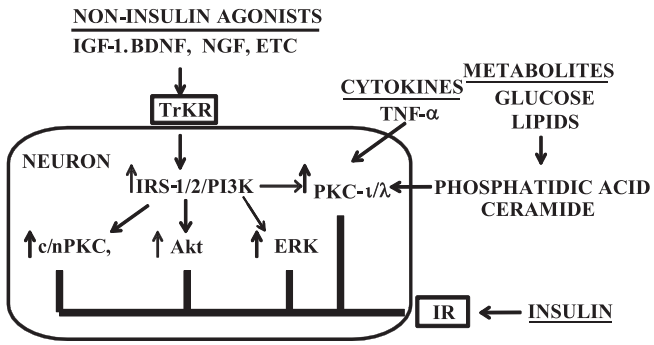

Figure 13.2 Activation of Akt and aPKC by noninsulin agonists that activate IRS1/2-PI3K via tyrosine kinase receptors (TrKRs), and activation of aPKC by agents that act directly on aPKC. Note that there are several feedback mechanisms that can lead to downregulation of the activity of insulin receptor (IR).

and deficient insulin action abets AD development. These assumptions seemed reasonable, as insulin increases cell survival and, in brain, insulin increases glucose uptake in endothelial, connective tissue and glial cells, but not in neurons. These assumptions also seemed reasonable as certain tissues are markedly insulin resistant in obesity and T2DM, e.g., in muscle, this leads to diminished glucose uptake and energy metabolism. Indeed, it was proposed that late-onset AD is "type 3 T2D." and this prompted the use of nasal insulin to treat AD. However, recent findings lead us to question these assumptions.

General aspects of insulin signaling

The insulin receptor (IR) has two outwardly facing α-subunits and two transmembranous β-subunits that have intrinsic tyrosine kinase activity. Upon interaction of insulin with α-subunits, the β-subunit is activated and phosphorylates tyrosine residues in adjacent β-subunits and intracellular proteins, Shc and insulin receptor substrates (IRSs)-1 and 2 to produce pYXXM motifs in IRS-1 and IRS-2 that interact with SH2 sites in the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and thereby activate the p110 catalytic subunit of PI3K. Shc then activates the grb2/mSOS/ras/raf/MEK1/ERK pathway, which is important for cellular differentiation and proliferation. Most importantly, PI3K acts upon PI-4,5-$(PO_4)_2$ in plasma and other membranes to produce the acidic phospholipid PI-3,4,5-$(PO_4)_3$ (PIP_3), which interacts with basic arginine and lysine residues in PH domains of protein kinase dependent kinase-1 (PDK1) and Akt, and basic arginine residues of the aPKC pseudosubstrate (Ivey, Sajan, & Farese et al., 2014). These interactions lead to phosphorylation of activation loop sites of Akt and aPKC by protein kinase−dependent kinase-1 (PDK1), and subsequent phosphorylation of the serine-473 residue in the downstream turn areas of Akt by PDK2, and phosphorylation of downstream threonine-555/560 residues of PKC-λ/ι and PKC-ζ by PDK2 and/or auto/transphosphorylation.

It may be noted that (a) Akt and aPKC are activated by many growth factors that activate tyrosine kinase (TrK) receptors, IRSs and PI3K; (b) aPKCs-ι/λ/ζ are also activated directly by ceramide and acidic phosphatidic acid (PA); (c) Akt and aPKC mediate most metabolic effects of insulin; (d) insulin increases diacylglycerol (DAG) production and also activates DAG-dependent PKCs, including, conventional PKCs (cPKCs) (α,β1,β2) and novel PKCs (nPKCs) (δ,ε,θ); and (e) DAG is increased and c/nPKCs are activated by many agonists that hydrolyze PI-4.5-$(PO_4)_2$ by phospholipase C, and by glucose and fatty acids that provide substrate for de novo synthesis of PA and DAG (Farese, 2001).

Insulin signaling in brains of humans afflicted by nondiabetic AD

Before discussing diabetes-associated AD, we will first discuss AD thought to be unassociated with T2DM. In support of the hypothesis that insulin action in brain is

deficient in AD, levels of insulin itself and certain insulin signaling factors have been found to be diminished in brains of AD humans. However, these alterations may also reflect nonspecific pathology in AD brain. And, in a very well-controlled study of postmortem brains of humans thought to have nondiabetic AD, Talbot et al. (2012) found that the *activity* of the IR in hippocampal and other brain areas is diminished, such that the IR response to 1 nM insulin was moderately reduced by 30%–35%, but reduced by only 15%–20% at 10 nM insulin (and presumably less at higher insulin levels, owing to "spare receptors").

It was also found by Talbot et al. (2012) that although resting/basal IR activity (β-subunit phosphotyrosine (pY) content) was "normal" in AD brains, IRS-1 and IRS-2 activities (pY content), and their binding to the p85α subunit of PI3K were *increased*, and, most importantly, accompanied by *increased* activities of Akt, aPKC, and ERK, i.e., signaling factors activated by insulin and other growth factors that act via TrK receptors to activate PI3K and Shc. Although it was concluded that IR function is impaired in nondiabetic-AD brain, note that: (a) whereas the *relative* effect of insulin on Akt activity was markedly decreased in AD hippocampi, the resting *baseline* activity of Akt was increased 193%, i.e., maximally, and, understandably, unresponsive to insulin treatment; (b) modest decreases in IR activity can be bypassed by hyperinsulinemia in insulin-resistant states via operation of "spare" IRs; and (c) agonal stress and nonspecific activation of c/nPKCs, ERK, and/or other factors may have exerted downregulating effects on the IR in AD brain samples.

That activities of IRS-1, IRS-2, PI3K, Akt, and aPKC were elevated in brains of humans with nondiabetic AD is best explained by activation of a noninsulin agonist(s) that operates via IRS/PI3K to activate Akt and aPKC. Indeed, insulin itself negatively feeds back on IR activity, and preagonal inanition can rapidly reverse clinical insulin resistance. Regardless of the mechanism for IR downregulation, as Akt, aPKC, and ERK are strongly activated in brains of humans with nondiabetic AD (Talbot et al., 2012), and, as nasal insulin therapy for AD is now in clinical trials (Craft, 2007; Craft et al., 2017; Holscher, 2014), it may be questioned if activation of these factors by insulin has beneficial effects. It may also be questioned if insulin therapy has adverse effects mediated by aPKC activation. This may explain why AD progression may accelerate with nasal insulin therapy.

As to noninsulin agonists that may underlie PI3K/Akt/aPKC/ERK activation and negative feedback on IR activity in AD brain, N-methyl-D-glutamate (NMDA) receptors are thought to provoke neurodegenerative effects via aPKC activation in cultured human neuronal cells, and/or via uptake of neurotoxic amounts of Ca^{++} (Brennan-Minnell, Shen, & Swanson, 2013; Koponen et al., 2003) (Fig. 13.2). Interestingly, agents that block NMDA receptors are currently used to treat AD. In addition, brain-derived nerve factor and nerve growth factor activate TrK receptors, and operate via PI3K/Akt/aPKC/ERK.

Despite uncertainties of the agonist(s) that negatively feeds back, the impairment in brain IR activity may diminish salutary effects of insulin in normoinsulinemic nondiabetic states, and in hypoinsulinemic states, including, later phases of T2DM when insulin secretion diminishes owing to pancreatic islet β-cell failure, or in type 1 diabetes mellitus. Thus, in these circumstances, insulin therapy to overcome IR blockade may have beneficial effects by (a) improving cognitive/memory functions by maintaining PKMζ levels/activity, (b) increasing neuronal longevity by Akt-dependent antiapoptotic effects, and (c) improving overall glucose metabolism and energy metabolism in certain CNS cell types. This may explain why AD symptoms may improve with nasal insulin therapy.

Insulin signaling in brains of humans afflicted with AD associated with T2DM and pre-T2DM

The status of brain IR and other insulin signaling factors has not been elucidated in humans with AD *associated with diabetes or prediabetes*. Moreover, the reasoning on the importance of purported decreases in brain IR activity in pre-T2D/T2DM-associated AD does not take into account that: insulin resistance *at the IR level* in liver and adipocytes is readily overcome by hyperinsulinemia via operation of "spare IRs," meaning that maximal downstream responses are elicited by activation of only a fraction of IRs; and the severity of insulin resistance in skeletal muscle reflects impairment of *IRS-1-dependent PI3K activation*, i.e., *a postreceptor defect that impairs insulin activation of Akt and a* PKC, and thereby impairs glucose transport (Sajan, Standaert, Nimal et al., 2009; Sajan, Standaert, Rivas, et al., 2009). Indeed, in contrast to IRS-1/PI3K, muscle IRS-2/PI3K activation by insulin is well maintained in HFF mice (Sajan, Standaert, Nimal et al., 2009; Sajan, Standaert, Rivas, et al., 2009), undoubtedly via spare IRs. Finally, recall that in HFF mice, existing levels of hyperinsulinemia maximally activate the brain IR (Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018), suggesting little or no impairment of brain IR.

Insulin signaling in brains of animal models of insulin-resistant forms of obesity and T2DM

Surprisingly, we found, in whole brain and hippocampal and anterior cortical neurons of hyperinsulinemic HFF, ob/ob mice, and heterozygous muscle-specific PKC-λ knockout (Het-MλKO) mice (where impaired glucose transport in muscle initiates systemic insulin resistance that secondarily involves liver), and monkeys with long-standing diet-dependent obesity/T2DM, that insulin signaling to the IR, Akt and aPKC, and phosphorylation of Akt substrates, GSK3β, mTOR, FoxO1, FoxO3a, and FoxO4, are uniformly increased to levels seen with acute 15-min maximal insulin treatment (Sajan

et al. 2016; Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018). Moreover, hyperinsulinemia appeared to be responsible, as correction of hyperinsulinemia (elicited by improvement in hepatic abnormalities following treatment with aPKC inhibitor, aurothiomalate, that acts in liver, but *not* in brain), was attended by full return of all brain insulin signaling increases to normal basal levels, and restored ability of insulin to acutely reactivate Akt and aPKC.

Accordingly, persistent hyperactivation of brain Akt would phosphorylate/inhibit activities of all FoxO's; and, whereas FoxO1 inhibition may acutely diminish apoptotic activity (Zhang, Tang, Hadden, & Rishi, 2011), long-term deficiencies of FoxO1/3a/4 have detrimental effects on neuronal integrity and memory function (Paik et al., 2009; Renault et al., 2009; Salih et al., 2012). Additionally, Akt-dependent phosphorylation of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) would inactivate PGC-1α and diminish important transcriptional activities. More importantly, hyperactivation of brain aPKC provokes increases in β-secretase, $A\beta_{1\text{-}40/42}$, and phospho-thr-231-tau (p-tau).

In summary, our findings suggest that systemic insulin resistance that originates in liver (Sajan, Standaert, Nimal et al., 2009; Sajan, Standaert, Rivas, et al., 2009; Sajan, Acevedo-Duncan et al., 2014; Sajan, Ivey et al., 2014; Sajan, Jurzak et al., 2014; Sajan, Ivey, Farese, 2015; Sajan, Ivey, Lee, Farese, 2015; Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018), muscle (Farese, 2007; Sajan et al., 2012), or adipose tissue (Xia et al., 2015) is generally accompanied by hepatic aPKC activation and liver/aPKC-dependent aberrations that produce insulin resistance and hyperinsulinemia, which leads to activation of brain aPKC and increased production of $A\beta_{1\text{-}40/42}$ and p-tau, which produce neurotoxic β-amyloid plaques and p-tau tangles.

Central role of the liver in insulin-resistant states of obesity and T2DM

As discussed, the liver plays an essential role in both diet-initiated insulin-resistant states, e.g., in HFF and ob/ob mice and ad lib-fed monkeys, and other models, e.g., mice with impaired glucose transport in muscle that leads to a liver-dependent insulin-resistant state. In each model, hepatic aPKC activity is inordinately increased by hyperinsulinemia, ceramide, and/or PA. The ability of hyperinsulinemia to hyperactivate hepatic aPKC therein reflects that IRS-2/PI3K mediates insulin activation of *hepatic* aPKC, and IRS-2 does not downregulate; this contrasts with skeletal and cardiac muscle, where IRS-1/PI3K mediates insulin effects on aPKC, and, unlike IRS-2, IRS-1 activity downregulates (Fig. 13.3). Also note that insulin effects on phosphorylation/inhibition of hepatic FoxO1 and PGC-1α are coordinated by their recruitment, along with Akt and aPKC, to scaffolding protein WD40/ProF; and, unfortunately, aPKC hyperactivation in insulin-resistant conditions displaces Akt from WD40/ProF, FoxO1 and

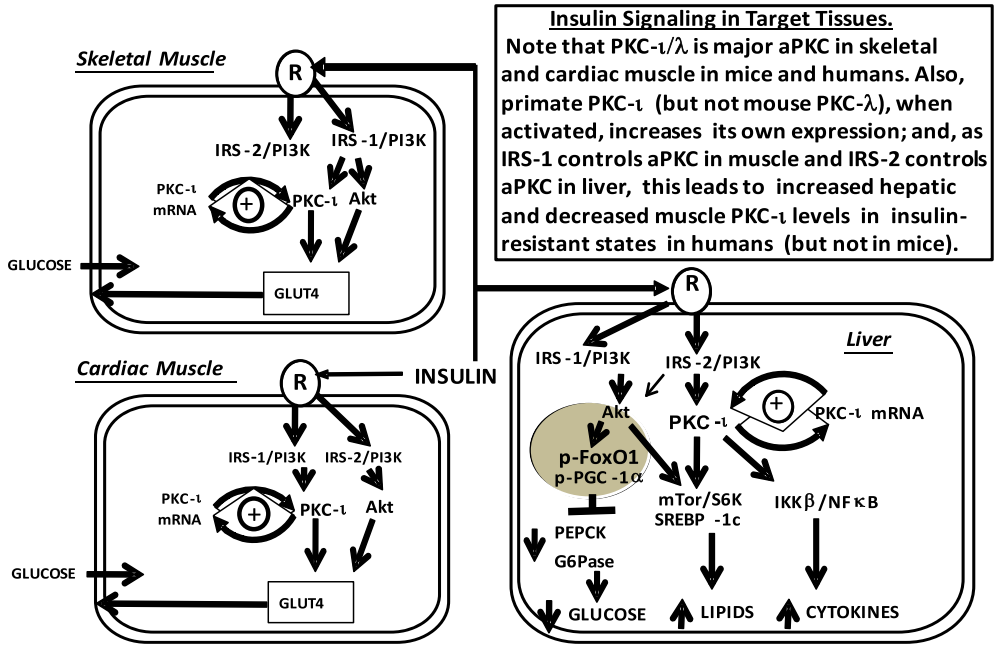

Figure 13.3 Insulin signaling in liver, skeletal muscle, and cardiac muscle. Whereas IRS-1 largely controls Akt activation in liver and skeletal muscle, IRS-2 appears to be the major factor in cardiac muscle. Whereas IRS-1 controls aPKC activation in skeletal and cardiac muscle, IRS-2 is the major factor in liver. Both Akt and aPKC are required for regulation of glucose transport in skeletal and cardiac muscle, and for increases in lipogenesis in liver. But, very importantly, whereas Akt suppresses hepatic gluconeogenic enzyme expression by phosphorylating/inhibiting FoxO1 and PGC-1a (which takes place in the WD40/ProF compartment depicted by the shaded area), aPKC impairs this effect of Akt by displacing Akt from the compartment. Accordingly, inhibition of hepatic aPKC, improves Akt action and diminishes expression of both gluconeogenic and lipogenic enzymes.

PGC-1α activities increase, and gluconeogenic enzyme expression increases (Sajan, Acevedo-Duncan et al., 2014; Sajan, Ivey et al., 2014; Sajan, Jurzak et al., 2014; Sajan, Ivey, Farese, 2015; Sajan, Ivey, Lee, Farese, 2015; Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018). These abnormalities are compounded later, as hepatic Akt activation diminishes with downregulation of hepatic IRS-1.

This dependence of systemic insulin resistance on hepatic aPKC explains how treatment with liver-*selective* inhibitors of aPKC corrects hyperinsulinemia, reduces brain aPKC activity, and improves β-secretase, $A\beta_{1-40/42}$, and p-tau, and high-fat-diet (HFD)-induced memory impairments (Sajan et al., 2016; Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018).

Supporting evidence of Akt and aPKC activation in brains of AD humans and HFF mice

Other investigators have reported similar evidence of brain Akt/aPKC activation in AD: (a) increased Akt activity and phosphorylation of Akt substrates in temporal cortical

neurons of AD humans (Griffin et al., 2005); (b) increased Akt activity in enterorhinal, hippocampal, and temporal lobe neurons in AD humans (Pei et al., 2003; Rickleet al., 2004); (c) decreased FoxO3a/6 expression in brains of HFF mice (Zemva et al., 2012); (d) increased CNS Akt activity and GSK3β phosphorylation in HFF mice (Macpherson, Baumeister, Peppler, Wright, & Little, 2015); and (e) increased Akt and aPKC activities in hippocampi of humans with nondiabetic AD (Talbot et al., 2012).

Supporting evidence that insulin increases $A\beta_{1\text{-}40/42}$

In concert with our findings indicating that insulin increases $A\beta_{1\text{-}40/42}$ production via aPKC activation in brains of insulin-resistant mice and monkeys: (a) neuronal knockout of the IR or the IGF-1 receptor *protects* against generation of $A\beta_{1\text{-}40/42}$ peptides in Tg2576 AD-transgenic mice (Stohr et al., 2013); and (b) in *Caenorhabditis elegans*, mutational inhibition of DAF-2, an IR analogue, is accompanied by reduced production and neurotoxicity of $A\beta_{1\text{-}40/42}$ (Florez-McClure, Hohsfield, Fonte, Bealor, & Link, 2007).

Knockout of mouse PKC-λ diminishes insulin-simulated increases in $A\beta_{1\text{-}40/42}$ and p-Tau

Initially, we relied largely upon the use of chemical inhibitors to show that PKC-λ/ι is required for increases in β-secretase $A\beta_{1\text{-}40/42}$ and p-thr-231-tau. As chemical agents may have nonspecific effects, we recently used a knockout approach to show a PKC-λ requirement. Although total body deletion of PKC-ι/λ alleles is embryonic lethal, mice haploinsufficient for PKC-λ (total body heterozygous PKC-λ knockout [TB/HetλKO]) are seemingly normal. But the loss of one *hepatic* aPKC allele leads to constitutive FoxO1 phosphorylation/inactivation, and constitutive downregulation of gluconeogenic enzymes (Sajan, Acevedo-Duncan et al., 2014; Sajan, Ivey et al., 2014b; Sajan, Jurzak et al., 2014). Thus, TB/Het-λKO mice are *fully* resistant to developing abnormalities in glucose and lipid metabolism *and hyperinsulinemia* when challenged with an HFD. With this metabolic protection and the failure to develop hyperinsulinemia, we found (unpublished) that TB/HetλKO mice are protected from developing resting increases in brain PKC-ι/λ, β-secretase, $A\beta_{1\text{-}40/42}$, and p-thr-231-tau, that are seen in HFF wild-type mice. Moreover, the 50% decrease in *brain* PKC-λ in TB/HetλKO mice leads to commensurately diminished effects of insulin on PKC-ι/λ, $A\beta_{1\text{-}40/42}$ and p-thr-231-tau. Furthermore, knockout of one PKC-λ allele had no effect on Akt activation, 50 kDa PKC-ζ activity, or memory function tests.

Tau phosphorylation in insulin-resistant states

Despite Akt hyperactivity and therefore phosphorylation/inhibition of GSK3β, thr-231-tau phosphorylation is increased in ob/ob mice and T2D monkeys (Sajan et al., 2016), db/db obese/T2D mice (Kim, Backus, Oh, Hayes, & Feldman, 2009), and HFF mice (Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018). Thus, increases in p-thr-231-tau and other p-tau's in hyperinsulinemic states reflect operation of factors distinct from GSK3β, e.g., aPKC, c/nPKCs, ERK, mTOR.

Use of nasal insulin for AD treatment

Nasal insulin may be beneficial in nondiabetic AD where systemic insulin resistance and hyperinsulinemia are absent, and brain IR activity is downregulated by noninsulin factors that activate the IRS/PI3K/Akt.aPKC pathway, but also by agents that directly activate aPKC, e.g., ceramides, PA and TNF-α, or by proinflammatory cytokines and oxidants that hydrolyze PI-4.5-$(PO_4)_2$ to produce inositol-trisphosphate and DAG, and thereby increase intracellular Ca^{++} and c/nPKC activity.

However, the rationale for insulin therapy for AD is more uncertain if decreases in IR activity reflect actions of noninsulin agonists that activate aPKCs, such as, NMDA receptor (Brennan-Minnell et al., 2013; Koponen et al., 2003) agonists that increase neuronal death by an aPKC-dependent mechanism, or AMPA receptor (Ren et al., 2013) agonists, or noninsulin agonists that activate tyrosine kinase receptors and thus activate aPKC and Akt via IRS-1/2 and PI3K (Miranda, Miele, Pierotti, & Van Obberghen, 2001), such as, neurotropin and brain-derived neurotrophic factor, which is also known to activate aPKC (Melemedjian et al., 2013).

The rationale for using insulin is even more questionable in situations where blood insulin levels are elevated beyond that needed to act via spare IRs and maximally activate brain Akt and aPKC. Such elevations of brain Akt and aPKC activities were seen in hyperinsulinemic monkeys with long-standing diet-dependent obesity/T2DM, and aPKC elevation was accompanied by increases in β-secretase, $Aβ_{1-40/42}$, and p-tau. On the other hand, insulin therapy may be helpful in T2D-associated AD when insulin secretion diminishes to levels that cannot overcome the IR downregulation. Fig. 13.4 summarizes expectations in various phases of pre-T2DM/T2DM.

Potential salutary effects of insulin in AD

Salutary effects of insulin in the brain, particularly in normoinsulinemic or hypoinsulinemic states, are that insulin may improve cognitive/memory functions by ensuring PKMζ activation, increase neuronal longevity by Akt-dependent antiapoptotic effects that are mediated by inhibition of FoxO1, and improve glucose and energy metabolism in certain

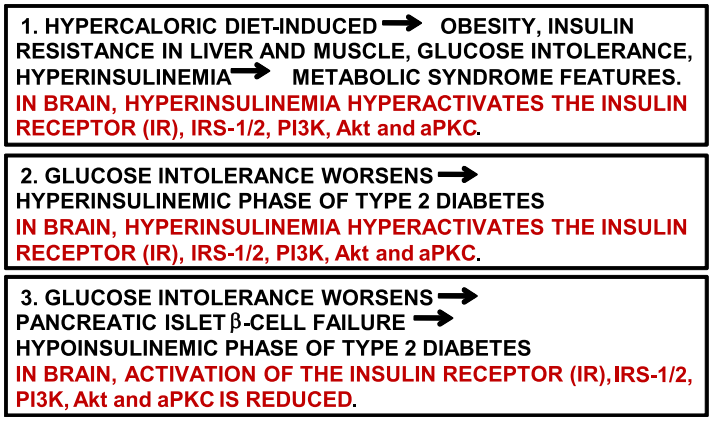

Figure 13.4 Phased development of diet-induced obesity, the metabolic syndrome, and type 2 diabetes.

brain cell types. This may explain why some AD patients improve with insulin therapy (Craft et al., 2017). On the other hand, some studies have noted declines in memory functions in human AD patients treated with nasal insulin (Holscher, 2014), or increases in AD pathology in mouse studies following nasal insulin therapy (Anderson et al., 2017; Kamei et al., 2017).

Alterations in brain insulin signaling factors in transgenic AD models

AD pathology and impairments in cognitive/memory functions in Tg 2576 mice and other Tg-AD mice are intensified by IR activation (Stohr et al., 2013) and HFD (Ho et al., 2004), and improved by IR knockout (Stohr et al., 2013) and caloric restriction (Qin et al., 2008). Although some have interpreted AD-exacerbating effects of HFD as being due to increases in *brain* insulin resistance, we believe that HFD-induced *hyperinsulinemia* is responsible.

Another confounding problem in Tg 2576 and 3XTg-AD mice is that these mice, for uncertain reason (perhaps reflecting coimpairment of hypothalamic brain centers that regulate appetite and energy expenditure), commonly develop obesity and glucose intolerance, before or after developing significant AD pathology (Macklin et al., 2017; Vandal et al., 2017). Thus, hyperinsulinemia may contribute to AD development in these transgenic mice.

aPKC inhibitors

We have used chemical inhibitors of aPKC to examine aPKC requirements during insulin-induced increases in β-secretase, $A\beta_{1\text{-}40/42}$, and p-thr-231-tau. These inhibitors were identified by high-throughput screening of the crystallographic structure of the

catalytic domain of PKC-ι. Thus, we identified a number of compounds that target a site present in the catalytic domains of aPKCs, but absent in c/nPKCs. Three agents were studied in detail: (1) 1H-**i**midazole-4-**c**arboxamide, 5-**a**mino-1-[2,3-dihydroxy-4-[(**p**hosphono-oxy)methyl]cyclo**p**entyl-[1R-(1a,2b,3b,4a)] (ICAPP), which potently (IC50, 1−10 nM) and selectively inhibits recombinant PKC-ι/λ; (2) 1H-**i**midazole-4-**c**arboxamide, 5-**a**mino-1-[2,3-dihydroxy-4-[(hydroxyl)methyl]cyclo**p**entyl-[1R-(1a,2b, 3b,4a)] (ICAP), which is converted to active phosphorylated ICAPP intracellularly by adenosine kinase (Sajan et al., 2013); and (3) 2-**a**cetyl-**c**yclo**p**entane-1,3-**d**iketone (ACPD), which comparably inhibits recombinant forms of both PKC-λ/ι and PKC-ζ (IC50s 10−30 nM) (Sajan et al., 2012, 2013; Sajan, Acevedo-Duncan et al., 2014; Sajan, Ivey et al., 2014; Sajan, Jurzak et al., 2014; Sajan, Ivey, Farese, 2015; Sajan, Ivey, Lee, Farese, 2015).

Doses of these agents that effectively inhibit hepatic aPKC do *not* inhibit muscle or adipocyte aPKC, and have no effect on Akt in any tissue. Moreover, by improving hepatic aberrations in mouse models of obesity and T2DM, these inhibitors improve/increase insulin activation of aPKC and Akt in muscles and adipose tissues of HFF, ob/ob and Het-MλKO mice (Sajan, Standaert, Nimal et al., 2009; Sajan, Standaert, Rivas, et al., 2009; Sajan et al., 2012; Sajan et al., 2013; Sajan, Acevedo-Duncan et al., 2014; Sajan, Ivey et al., 2014; Sajan, Jurzak et al., 2014; Sajan, Ivey, Farese, 2015; Sajan, Ivey, Lee, Farese, 2015) by correcting adverse liver-to-muscle cross talk and thereby improving muscle IRS-1/PI3K activation by insulin. Importantly, these inhibitors have no effects on recombinant forms of cPKCs (α,β) or nPKCs (δ,ε,θ), AMP-activated protein kinase (AMPK), and an array of 35 other kinases.

In vivo effects of aPKC inhibitors on liver versus brain aPKC

At lower doses, ICAP, ICAPP, and ACPD tissue selectively inhibit hepatic aPKC and largely or fully reverse hepatic aPKC-dependent signaling clinical abnormalities, while having little or no effect on brain aPKC. However, at higher doses, ICAP/ICAPP and ACPD (Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018) additionally inhibit brain 70 kDa PKC-λ/ι, but not 50 kDa PKMζ, the constitutively active putative memory protein. Thus, in hyperinsulinemic states of obesity and early stages of T2D, ICAP, ICAPP, and ACPD can reduce brain PKC-λ/ι activity, indirectly by inhibiting liver aPKC and directly by inhibiting brain aPKC.

In vitro effects of PKC-ι/λ activators and inhibitors

In addition to in vivo studies, we found in *human neuroblastoma-derived neuronal cells and mouse hippocampal slices* that ICAPP and dominant-negative aPKC inhibit stimulatory effects of aPKC activators, i.e., insulin, metformin, and constitutively active aPKC,

on PKC-ι/λ activity, β-secretase-1 activity, $A\beta_{1\text{-}40/42}$ production, and tau phosphorylation, without inhibiting Akt or 50 kDa PKMζ (Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018).

Selective inhibition of brain PKC-λ/ι by ICAP

That ICAP is converted to active ICAPP in brain is clear from studies in which intracranially injected ICAP inhibited 70 kDa-PKC-ι/λ, but not 50 kDa-PKMζ, the putative memory protein needed for long-term potentiation (LTP) in the hippocampus (Sacktor, 2008; Tsokas et al., 2016). We also have clear evidence that parenterally and orally administered ICAP passes the blood–brain barrier and strongly inhibits 70 kDa PKC-ι/λ in the brain.

ICAP and AICAR: similarities and differences

ICAP is identical to the commonly used AMPK activator, AICAR, except that the oxygen atom in the glycosidic ring of AICAR is replaced by a carbon atom to make a cyclopentane ring. Like ICAP, AICAR is phosphorylated intracellularly by adenosine kinase to produce active phosphorylated ZMP. However, despite structural similarities, ICAP has no effect on AMPK. On the other hand, AICAR and metformin *activate* aPKC in hepatocytes, neurons, myocytes (Chen et al., 2002; Sajan et al., 2010; Sajan, Ivey et al., 2018; Sajan, Lee et al., 2018) and brain (Wang et al., 2012) via activation of AMPK, ERK, and phospholipase D-dependent PA, and thereby increase β-secretase, $A\beta_{1\text{-}40/42}$, and p-tau.

Potential mechanisms of β-secretase activation by aPKC

The rapidity of insulin activation of β-secretase-1 and $A\beta_{1\text{-}42}$ production suggests operation of a signaling factor, such as aPKC, which rapidly phosphorylates specific substrates containing serine or threonine residues flanked by X-R(K)-R(K) residues. In this regard, note that insulin stimulates movement of β-secretase-containing vesicles from the trans-Golgi network (TGN) to the plasma membrane (Gasparin et al., 2001), and aPKC, by increasing phosphorylation of serine-498 in β-secretase-1, stimulates movement of β-secretase-containing vesicles both from the plasma membrane to endosomes (Tan & Evin, 2012), and from endosomes to the TGN (Sun & Zhang, 2017). As β-secretase activity is increased by acidity, and, as endosomes and TGN provide acidic environments, aPKC may increase β-secretase-1 activity and $A\beta_{1\text{-}42}$ production by stimulating serine-498 phosphorylation and trafficking of β-secretase-containing vesicles to endosomes and TGN, as mediated by the interaction of β-secretase-1 with Golgi-associated adaptor proteins and sortilin.

aPKC requirements for memory functions

Many findings suggest that CNS 50 kDa PKC-ζ ("PKMζ"; which, lacking a 30 kDa regulatory domain and its autoinhibitory pseudosubstrate, is constitutively active) functions in LTP and long-term memory (LTM) (Sacktor, 2008). However, although PKMζ knockout does *not* impair LTP/LTM, this may be explained by a compensatory increase in 70 kDa PKC-λ/ι in PKMζ null mice (50), as suggested by impaired LTP/LTM following selective inhibition of PKC-ι/λ by ICAP in PKMζ null mice, *but not in normal mice that rely on PKMζ* (Tsokas et al., 2016). Moreover, from the lack of effect of ICAP on *on-going* LTP and memory function in normal mice (Tsokas et al., 2016), it may be argued that 70 kDa PKC-λ/ι is not required for *on-going* LTP/LTM. Indeed, in HFF mice, ACPD fully *improved* HFD-induced memory dysfunction (novel object recognition) while inhibiting insulin-stimulated increases in PKC-ι/λ activity, and sparing 50 kDa PKMζ activity (Sajan, Ivey et al., 2018& Sajan, Lee et al., 2018). Moreover, as discussed, 50% loss of brain PKC-λ in PKC-λ haploinsufficient mice, and a commensurate decrease in insulin-stimulated PKC-λ activity did not impair memory function in both *radial arm water maze and novel object recognition tests.*(unpublished) Nevertheless, a hippocampal-specific homozygous full knockout of PKC-ι/λ suggested an enhancing but unessential role for PKC-ι/λ in an advanced learning test in which two training sessions (instead of the customary four) were used (Sheng et al., 2017).

Potential uses of inhibitors of brain PKC-ι/λ for AD treatment

We initially used aurothiomalate to inhibit hepatic aPKC and rapidly reverse aPKC-dependent hepatic aberrations and correct hyperinsulinemia-induced increases in brain Akt and aPKC in insulin-resistant obese/T2D mice. Subsequently, we used ACPD in a dose that inhibits liver and brain over a 3-month period to correct not only liver-dependent hyperinsulinemia and brain aberrations but also to block insulin-induced increases in brain aPKC, β-secretase, $A\beta_{1\text{-}42}$, and p-tau in HFF mice. Furthermore, ACPD reversed HFD-induced defects in memory, and had no adverse effects over 3 months. Similar safety was seen with 3-month ICAP usage. However, to more fully assess efficacy and safety, there is a critical need to use ICAP and ACPD over longer periods, in doses that inhibit liver alone or brain plus liver, in models of both diabetes-associated AD and nondiabetic AD.

As another therapeutic approach, it appears that nasal insulin therapy has salutary effects on cognitive/memory functions in studies of human with AD, and these improvements probably reflects Akt activation. However, some studies are less optimistic, and nasal insulin may be problematic, as insulin activation of PKC-ι/λ would activate β-secretase, and increase $A\beta_{1\text{-}40/42}$ and p-tau. This conundrum may be obviated by using aPKC inhibitors along with nasal insulin.

Bullet points

- Atypical PKC activates β-secretase and controls production of Aβ- peptides
- Phosphatidylinositol 3-kinase activators and other agents that activate atypical PKC simultaneously increase β-secretase, Aβ-peptides, and phospho-tau
- Hyperinsulinemia in insulin-resistant states activates brain atypical PKC and simultaneously increases β-secretase, Aβ-peptides, and phospho-tau
- Hyperinsulinemia is commonly present in obesity, the metabolic syndrome, and type 2 diabetes
- Brain atypical PKC activity of uncertain etiology is also reportedly elevated in humans with nondiabetic, presumably normoinsulinemic Alzheimer disease
- Haploinsufficiency of atypical PKC diminishes induced increases in β-secretase, Aβ-peptides, and phospho-tau
- Chemical inhibitors of atypical PKC that pass the blood—brain barrier diminish increases in β-secretase, Aβ-peptides, and phospho-tau

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CHAPTER 14

Linking histone deacetylases and phosphodiesterase 5 in novel treatments for Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
APP amyloid precursor protein
Aβ amyloid-β peptide
BBB blood–brain barrier
cAMP cyclic adenosine monophosphate
cGMP cyclic guanosine monophosphate
CREB cAMP response element binding
CSF cerebrospinal fluid
HDAC histone deacetylase
HDACi histone deacetylase inhibitor
LTP long-term potentiation
PDE5 phosphodiesterase
PDE5i phosphodiesterase inhibitor
PS presenilin

Mini-dictionary of terms

Histone deacetylases Family of enzymes that remove acetyl groups from lysine amino acids in histones or other proteins.

Long-term potentiation Long-lasting increase in signal transmission between two neurons produced by a specific pattern of synaptic activity. LTP shares the same molecular mechanism as memory, and it is considered one of the molecular mechanisms underlying learning and memory.

Multifactorial disease A disease caused by a combination of genetic and environmental factors in a manner that is not fully understood.

Off-target effect Side effect of a drug that is different from the expected effect derived from an interaction with its biological target.

Phosphodiesterases Family of enzymes that hydrolyze the cyclic nucleotides cAMP and cGMP, producing inactive metabolites.

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The molecular basis of dementia

Alzheimer's disease (AD) is a multifactorial disease that is difficult to treat because of the complexity of the pathological mechanisms involved in its etiopathology. It is estimated that, as of 2020, there were up to 5.5 million people in the United States who had AD, with the associated health care costs predicted to exceed $170 billion per year (Alzheimer's Association, 2017a, 2017b). The prevalence of dementia increases with age, and therefore, these numbers are projected to triple by 2050 due to the increase in life expectancy. AD is the most common form of dementia in the Western world (60%–80% of cases), but there are other causes of dementia, such as vascular dementia or stroke or that produced by vitamin deficiencies. In each condition, the brain regions affected and the molecular bases underlying the dementia differ.

One of the first brain areas affected in AD is the hippocampus, which is involved in the formation of new semantic and episodic memories and in the retrieval of old memories. In fact, the inability to learn new information and impaired spatial and episodic memory are the earliest symptoms of AD. However, other brain functions are affected as the disease progresses, making it difficult for the patients to carry out their daily activities. The principal hallmark of AD is the accumulation of abnormal insoluble proteins: extracellular deposits of the amyloid-β peptide (Aβ; senile plaques) and intracellular accumulation of the phosphorylated tau protein (tangles). While senile plaques are specific to AD, the accumulation of tau also occurs in other neurodegenerative diseases. These plaques and tangles provoke synaptic dysfunction that ultimately leads to the loss of neurons, reflected in the atrophy observed in the brains of AD patients. Astrogliosis, neuroinflammation, and vascular alterations are also characteristic hallmarks of AD, although of all these histological markers, the loss of synapses is that which best correlates with the severity of memory impairment (Terry et al., 1991).

Aging constitutes the greatest risk factor for developing sporadic AD, but despite much research, it is still unclear which factors might trigger the onset of the disease and what are the neurodegenerative mechanisms associated with disease progression. This lack of knowledge is probably one of the reasons an effective treatment to cure or stop the progression of the disease has not yet been found. Current treatments targeting cholinergic and glutamatergic neurotransmission aim to alleviate the symptoms, and they are only partially effective in the early stage of the disease. Currently, there is a huge worldwide effort being made to search for targets that will allow effective disease-modifying treatments to be developed that stop the progression of the disease.

Clinical trials of AD therapies have a high failure rate compared with other therapeutic areas, exceeding 99% (Cummings, Morstorf, & Zhong, 2014). Most clinical trials for AD have been carried out on therapies based on the amyloid hypothesis, approaches that might well be effective if administered at very early stages of the disease, but not years after disease onset, when clinical symptoms are evident. While it is clear that dementia is not a direct consequence of amyloid deposition, it is the outcome of a long process that probably starts decades before and that is referred to as the "cellular phase" of AD (De Strooper and Karran, 2016). Thus, it is obvious that a therapy that simply targets Aβ is unlikely to be

the best option to restore memory in AD patients. As such, new therapies are needed that focus on alternative mechanisms involved in disease progression. Indeed, there is an extremely urgent need to identify new compounds or therapies that can be readily translated to the clinical environment. As such, a promising drug development strategy to accelerate clinical uptake involves the repositioning of existing drugs. In fact, galantamine, an acetylcholinesterase inhibitor currently used to treat AD patients, was first approved by the US FDA for poliomyelitis (Cummings and Zhong, 2006).

Both genetic and nongenetic factors play a role in AD onset. Ninety-five to ninety-nine percent of AD cases are sporadic, and they are associated with an interaction between genetic and environmental factors that will epigenetically affect the expression of AD-related genes (Kivipelto and Mangialasche, 2014). As such, drugs that target nongenetic risk factors, like those targeting epigenetic processes, may represent new alternatives to antiamyloid therapies to prevent and treat AD. Likewise, considering the complex and multifactorial nature of AD, pharmacological interventions directed at multiple target ligands may represent the best therapeutic option. Accordingly, we have recently validated a new therapeutic approach based on the concomitant inhibition of the epigenetic enzymes histone deacetylases (HDACs) and phosphodiesterase 5 (PDE5) (Cuadrado-Tejedor et al., 2015). In this chapter, we will describe the effects of HDAC and PDE5 inhibitors on AD and the synergistic therapeutic effects obtained when both enzymes are inhibited simultaneously.

The role of histone deacetylase inhibition in Alzheimer's disease

Lifestyle and environmental factors may epigenetically modify the expression of genes involved in the pathogenesis of AD, accelerating the onset of the disease. These epigenetic changes include DNA methylation and histone modifications like methylation and acetylation, which may affect chromatin structure and gene transcription. Indeed, while histone deacetylation is associated with transcriptional repression, an increase in histone acetylation can lead to the activation of gene transcription (de Ruijter, van Gennip, Caron, Kemp, & van Kuilenburg, 2003). The enzymes catalyzing histone acetylation and deacetylation are histone acetyl transferases (HATs) and HDACs, respectively. There is considerable evidence linking the balance of histone acetylation and deacetylation with memory function, and histone acetylation was seen to increase in different areas of the rat brain following a memory training session as early as 1979 (Schmitt and Matthies, 1979). Later studies demonstrated that HAT activity was required for intact memory and that the increase in histone acetylation associated with the use of HDAC inhibitors (HDACi's) could restore memory deficits in mice with aberrant HAT activity (Alarcón et al., 2004; Barrett et al., 2011; Korzus, Rosenfeld, & Mayford, 2004; Oliveira, Wood, McDonough, & Abel, 2007; Vecsey et al., 2007; Wood, Attner, Oliveira, Brindle, & Abel, 2006). Interestingly, the group of Dr. Li-Huei Tsai at the Picower Institute for Learning and Memory (Massachusetts Institute of Technology) demonstrated that HDACi's combat neurodegenerative conditions in several disease models, even after massive neuronal loss has occurred (Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007). The mechanism of action proposed involved

activating the transcription of disease-modifying genes and equilibrating histone acetylation homeostasis involved in the neurodegenerative processes.

Weaker acetylation of the promoters of neuroplasticity genes was demonstrated in the brain of the CK-p25 AD mouse model, which may contribute to the memory loss evident in these animals (Graff et al., 2012). This epigenetic blockade of gene transcription is mediated by the overexpression of HDAC2 in the brain, a finding that was also confirmed in the hippocampal CA1 area of AD patients. Using a proteomic approach, the levels of histone acetylation were seen to be significantly lower in the temporal lobe of AD patients (Zhang, Schrag, Crofton, Trivedi, Vinters, & Kirsch, 2012) and, more recently, enhanced HDAC2 expression was coupled to a marked decrease in H3 acetylation at Lys9 in peripheral blood mononuclear cells in a twin affected with AD relative to the unaffected one (D'Addario et al., 2017). All these findings seem to confirm the involvement of epigenetic alterations in AD.

Interestingly, epigenetic changes are dynamic and can be manipulated pharmacologically. Specifically, HDAC2 can be specifically targeted to counteract the epigenetic blockade of gene transcription that contributes to neurodegeneration (Graff et al., 2012). However, selective HDACi's are not available, and almost all such inhibitors tested in mouse models are pan-HDACi's, targeting class I and II enzymes. One of the first studies to show that enhancing histone acetylation reinstates memory function in AD was carried out on the Tg2576 AD model chronically administered phenylbutyrate (PBA), a class I and IIb HDACi (Ricobaraza et al., 2009). Transgenic animals chronically treated (5 weeks) with PBA had more synapses at apical and basal dendrites of hippocampal neurons compared with untreated transgenic animals, demonstrating that PBA was capable of recovering memory in Tg2576 mice even after synaptic failure had occurred. Similar results were reported in other AD models administered pan-HDACi's such as sodium butyrate, valproate, trichostatin, or vorinostat (Govindarajan, Agis-Balboa, Walter, Sananbenesi, & Fischer, 2011; Kilgore et al., 2010; Qing et al., 2008; Zhang and Schluesener, 2013), confirming the influence of HDACs on cognitive function.

Although the specific mechanisms of action are not fully understood, it seems that HDACi's restore homeostatic histone acetylation and gene transcription, addressing several features of the disease, including the amyloid pathology, synaptic plasticity, and neuroinflammation, and producing a positive therapeutic effect in animal models (Benito et al., 2015; Ricobaraza et al., 2009; Volmar et al., 2017; Zhang and Schluesener, 2013). However, the lack of specificity of most current HDACi's is associated with widespread side effects. Furthermore, an aberrant increase in H3 acetylation can worsen the amyloid pathology by enhancing amyloid precursor protein (APP) expression and/or that of the APP-processing enzymes BACE1 and PS1 (Guo, Wu, Ren, Liu, & Li, 2011). Accordingly, while the use of HDACi's in AD may offer some promise, optimal doses and adequate administration schedules must be selected with care.

While HDAC6 is a class IIB enzyme known to be overexpressed in the brain of animal models of AD and in patients (Ding, Dolan, & Johnson, 2008), its role in AD remains controversial. Due to its distribution, HDAC6 catalyzes the deacetylation of cytosolic proteins, thereby destabilizing microtubules by reducing tubulin acetylation. Furthermore, its C-terminal domain can interact with cytoskeletal proteins like myosin II and dynein, which participate in the transport of different cargos. This dual activity implicates HDAC6 in the most important mechanisms of protein clearance in the cell (reviewed in Moreno-Gonzalo, Mayor, & Sánchez-Madrid, 2018), making this enzyme particularly interesting in diseases associated with protein aggregation, like AD. In fact, the administration of HDAC6i's ameliorates the symptoms of AD in animal models (Majid, Griffin, Criss, Jarpe, & Pautler, 2015; Zhang et al., 2014), and it was demonstrated that HDAC6 dampens the acetylation of the tau residues critical for tau aggregation in vitro. Indeed, HDAC6i's reduce the tau levels in primary neuronal cultures (Carlomagno et al., 2017).

In summary, the clinical application of HDACi's in AD represents a promising therapeutic approach, yet some issues must still be resolved, such as the potential basal toxicity of nonspecific HDACi's and the fact that their prolonged use at high doses may compromise their neuroprotective effects. Research is under way to develop specific HDAC2i's that provide more effective and safer treatment. Moreover, it may be possible that combining HDACi treatment with that of another drug will produce additive or even synergistic effects, while reducing toxic side effects, suggesting that combinatorial approaches should be pursued.

The role of phosphodiesterase 5 inhibition on Alzheimer's disease

Phosphodiesterases (PDEs) catalyze the hydrolysis of the cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) nucleotides, second messengers that regulate signal transduction in different biological systems (Beavo, 1995). The PDE families can be distinguished functionally on the basis of their cyclic nucleotide substrate specificity, and PDE5, for example, is involved in the regulation of cGMP-specific signaling. Protein kinase G (PKG) phosphorylates key enzymes or transcription factors involved in important signal transduction pathways, and it seems to be the most important effector of cGMP (Puzzo, Sapienza, Arancio, & Palmeri, 2008).

In the central nervous system (CNS), there is evidence that the cGMP/PKG pathway influences learning and memory, and that enhancing CREB (cAMP response element-binding) protein phosphorylation may have a beneficial effect on synaptic transmission (reviewed in Puzzo, Sapienza, Arancio, & Palmeri, 2008). Moreover, cGMP levels seem to be higher in the immature (development) than in the adult brain, its levels decreasing with age (Chalimoniuk and Strosznajder, 1998). Indeed, cGMP has been postulated as a key molecule in neural transmission and it is thought to enhance long-term potentiation (LTP) (Zhuo, Hu, Schultz, Kandel, & Hawkins, 1994).

Several studies have shown that administering PDE5 inhibitors (PDE5i's) induces cognitive improvement in different animal models of cognitive impairment (Prickaerts, Sik, van der Staay, de Vente, & Blokland, 2005; Reneerkens et al., 2012). Indeed, the PDE5i sildenafil appears to enhance hippocampal and/or prefrontal cortex memory function in different animal species, as witnessed in various behavioral tests, like the Morris water maze and the passive avoidance and novel object recognition tests (Baratti and Boccia, 1999; Boccia, Blake, Krawczyk, & Baratti, 2011; Erceg et al., 2005; Rutten et al., 2005; Rutten et al., 2008). Thus, it was proposed that specific PDE5i's could prevent the onset of senile dementia by increasing cGMP levels (Domek-Lopacinska and Strosznajder, 2010), consistent with the decrease in cGMP associated with aging (Chalimoniuk and Strosznajder, 1998). Regarding the use of PDE5i's in humans, repeated administration of udenafil (a PDE5i) appears to improve cognitive function in patients with erectile dysfunction (Shim et al., 2011, 2014), although other studies failed to show any positive effects on cognition after a single dose (Grass et al., 2001). However, there are as yet no clinical studies assessing whether the risk of developing dementia is altered in individuals taking a PDE5i.

Regarding the use of PDE5i's to treat AD, we reported enhanced PDE5 mRNA expression in the hippocampus of AD patients and less cGMP in the cerebrospinal fluid of AD patients than in that of healthy control individuals (Ugarte et al., 2015). These data have been confirmed elsewhere, demonstrating that reduced cGMP in AD patients is correlated with memory impairment and tau as a marker of neurodegeneration (Hesse et al., 2017). Hence, by regulating cGMP, PDE5i's could represent good candidates to counteract the memory deficits in AD patients (reviewed in Garcia-Osta, Cuadrado-Tejedor, Garcia-Barroso, Oyarzábal, & Franco, 2012). In fact, the specific PDE5i's sildenafil and tadalafil improve memory performance and/or enhance synaptic plasticity and cognitive function in different animal models of AD (Cuadrado-Tejedor et al., 2011; Garcia-Barroso et al., 2013; Hutson et al., 2011). The specific mechanism underlying the effects of PDE5i's in AD remains unclear. On one hand, it has been suggested that cognitive performance may be indirectly improved by a PDEi-mediated increase in brain blood flow and/or brain glucose consumption, although a cerebrovascular effect of PDE5 inhibition has not been shown in experiments on memory performance using PDE5i's (Rutten et al., 2009). On the other hand, there is evidence that by elevating cGMP, PDE5i's may promote the transcription of memory-related genes by activating CREB, which is strongly associated with LTP (Garcia-Osta, Cuadrado-Tejedor, Garcia-Barroso, Oyarzábal, & Franco, 2012). It has also been shown that sildenafil and tadalafil reduce tau phosphorylation in different animal models of AD, due to cGMP-dependent inactivation of GSK3β (Cuadrado-Tejedor et al., 2011; Garcia-Barroso et al., 2013), although the results with these drugs regarding amyloid pathology are somewhat controversial. A positive effect of sildenafil on soluble Aβ levels was demonstrated in APP/PS1 mice after 3 weeks of treatment (Puzzo et al., 2009), although we found no effect on amyloid burden in the Tg2576 mouse model after 5 weeks of treatment

(Cuadrado-Tejedor et al., 2011). Similarly, while sildenafil and tadalafil restore memory deficits and revert the tau pathology in aged J20 mice (with an advanced AD phenotype), neither of them decreased the plaques nor the levels of water-soluble, detergent-soluble, or guanidinium-soluble Aβ (Garcia-Barroso et al., 2013). These results indicate that cognitive enhancement by PDE5i's may occur without any gross improvement in the amyloid pathology.

In addition to the aforementioned beneficial effects, it should be noted that PDE5i's fulfill a number of criteria that are desirable in an anti-AD drug: good blood–brain barrier penetration, a long half-life, and a good safety profile. Therefore, clinical trials should be performed to determine their potential in AD patients and also whether PDE5i's can prevent age-related cognitive decline or dementia in retrospective or prospective studies.

The effects of simultaneous histone deacetylase and phosphodiesterase 5 inhibition on Alzheimer's disease

Theoretical disease models are being increasingly used in conjunction with empirical studies to predict the effects of combination therapies as opposed to those of individual drugs (Boran and Iyengar, 2010; Severyn et al., 2011; Zheng, Fridkin, & Youdim, 2014). In fact, combination therapies represent a promising strategy to treat multifactorial diseases that involve diverse pathological mechanisms, such as cancer or AD. In this sense, many researchers believe successful AD treatment will ultimately involve a "multitherapy" cocktail of medications that are directed at several targets. Accordingly, we have worked on a new approach that targets different pathways related to important pathological aspects of AD (Fig. 14.1). The combination of two or more drugs that can produce

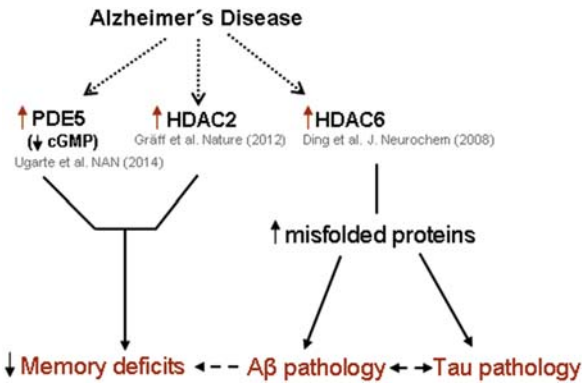

Figure 14.1 Relationship of phosphodiesterase 5 (*PDE5*), histone deacetylase 2 (*HDAC2*), and HDAC6 with Alzheimer's disease (AD). HDAC2, HDAC6, and PDE5 are overexpressed in the brain of animal models of AD and in AD patients. Furthermore, the levels of cGMP are decreased in the cerebrospinal fluid of AD patients, which can contribute, together with the increase in HDAC2, to the memory deficits in AD conditions. The accumulation of HDAC6 may alter protein clearance function in the cell, leading to amyloid and tau accumulation. *Aβ*, amyloid-β.

synergistic effects facilitates dose reduction, thereby minimizing the toxicity and off-target effects that might be associated with using larger amounts of individual drugs (Lehar et al., 2009; Zimmermann, Lehár, & Keith, 2007). Likewise, drug repositioning is a promising drug discovery strategy to identify new indications for drugs that have already been approved for their use in other conditions, which may reduce the costs and accelerate the delivery of new therapeutic agents to patients.

Based on this rationale we recently validated the efficacy of a novel therapeutic approach based on the concomitant inhibition of HDACs and PDE5 through the use of the following reference compounds: SAHA (vorinostat), an HDACi approved by the FDA for cancer treatment, and tadalafil, a PDE5i used to treat chronic diseases like chronic pulmonary hypertension and erectile dysfunction (Cuadrado-Tejedor et al., 2015).

First, we demonstrated that the combination of vorinostat and tadalafil synergistically rescued impaired LTP in hippocampal slices of APP/PS1 mice. In vivo, we demonstrated that chronic coadministration of subeffective doses of both drugs led to a synergistic effect, preventing the disrupted synaptic plasticity witnessed in Tg2576 mice. Amelioration of the memory impairment exhibited by the Tg2576 mice was accompanied by a significant reduction in the amyloid and tau pathology and a recovery of the loss in dendritic spine density. Interestingly, these effects were present even after a 4-week washout period, suggesting that targeting HDAC and PDE5 simultaneously triggers long-lasting changes in plasticity and remodeling, which may be of particular interest to counteracting the memory decline in AD. An analysis of gene expression profiles revealed that the combination therapy enriched pathways associated with synaptic plasticity, which may underlie the restoration of the AD phenotype. The restoration of LTP in APP/PS1 mice using these two drugs supports this hypothesis (see Fig. 14.2 for the different modes of action that lead vorinostat and tadalafil to reverse the pathological features associated with AD) (Cuadrado-Tejedor et al., 2015).

Interestingly, in a theoretical biological model that simulates the induction of LTP and the deficits in LTP associated with Rubinstein–Taybi syndrome, it was predicted that the combination of HDAC and PDE inhibitors would fully reverse the LTP deficits in this model (Smolen, Baxter, & Byrne, 2014). This combination displayed further synergism, obtaining a stronger effect in combination than the sum of the effects of the individual treatments alone. The results obtained using the reference compounds vorinostat and tadalafil empirically verify the beneficial synergistic effects predicted in this theoretical model.

A synergistic effect of a combination of other similar reference compounds was also demonstrated to be an antitumor therapy. Simultaneous administration of MS-275, an HDACi, and pentoxifylline, a nonselective PDEi, enhanced their individual effectiveness by slowing tumor growth and reducing toxicity in different xenograft models (Nidhyanandan et al., 2015). This provided further evidence that concomitant inhibition of HDAC and PDE may produce better clinical outcomes than single target inhibition.

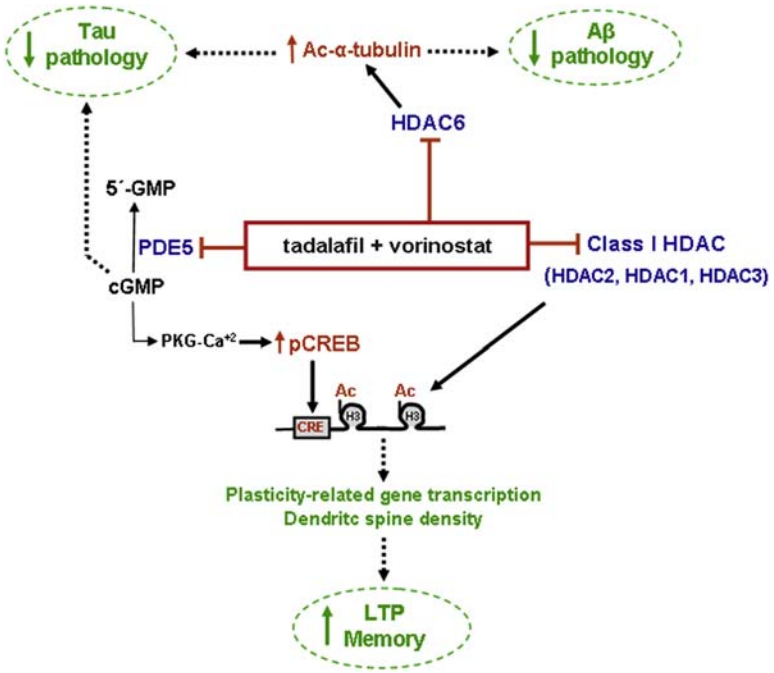

Figure 14.2 Putative mechanism of action involved in the restoration of pathological signs by the simultaneous inhibition of HDAC2/6 and PDE5. The proposed mechanism of action whereby the pathological hallmarks of Alzheimer's disease are restored following combined administration of tadalafil and vorinostat is shown. *Aβ*, amyloid-β; *Ac*, acetyl; *HDAC*, histone deacetylase; *LTP*, long-term potentiation; *pCREB*, phosphorylated cAMP response element binding protein; *PDE*, phosphodiesterase; *PKG*, protein kinase G.

Considering the positive preclinical results obtained with the combination of vorinostat and tadalafil, a novel, first-in-class, series of dual inhibitors has been designed based on the structural information and the structure—function information available for HDAC and PDE5 inhibitors (Cuadrado-Tejedor et al., 2017; Rabal et al., 2016). A lead compound, CM-414, with an optimal profile to achieve effective and safe chronic treatment, was selected from among more than 180 new compounds synthesized to be tested in an AD mouse model. CM-414 was designed with moderate class I activity (IC_{50} values of 310, 490, and 322 nM, against HDAC1, HDAC2, and HDAC3, respectively) to avoid any toxicity associated with the inhibition of HDAC class I enzymes. By contrast, it has strong activity against HDAC6 (IC_{50} 91 nM) and PDE5 (IC_{50} 60 nM). This therapeutic tool produced results similar to those achieved previously in the proof of concept with the reference compounds, suggesting that the synergistic effect of inhibiting HDAC and PDE5 means that strong inhibition of HDAC class I enzymes is not required to trigger the transcription of memory-related genes. In this sense, as HDACi's may not be the best option for chronic treatment given their adverse side effects, the use of low doses of an HDACi in combination with a PDE5i could be an appropriate alternative

to reduce therapeutic toxicity and increase efficacy. Accordingly, and considering that it can take up to 20 years to bring a new drug to the market, the reprofiling strategy using low doses of vorinostat in conjunction with tadalafil may be of special interest as a proof of concept in AD patients.

None of these drugs are yet approved for CNS disorders, yet it is important to highlight that in both cases, clinical trials for repurposing in patients with CNS disorders are ongoing: in the case of vorinostat, to determine the maximal tolerable dose in mild-AD patients (ClinicalTrials.gov identifier: NCT03056495), and in the case of tadalafil, to determine if a single dose of this drug enhances blood flow in deep brain tissue and potentially improves cognitive function in patients with cerebral small vessel disease (ClinicalTrials.gov identifier: NCT02450253).

To sum up, preclinical studies seem to indicate that concomitant inhibition of HDAC and PDE5 has a synergistic effect in terms of epigenetic responses and synaptic maintenance, as well as efficacy after a washout period. Accordingly, and considering that the optimal combined dose of both drugs may be lower, requiring a less aggressive administration schedule, a phase 1 clinical repurposing trial combining the administration of vorinostat and tadalafil in a cohort of patients with mild to moderate AD might be an option worthy of consideration.

Key facts

Key facts of Alzheimer's disease

- AD is a neurodegenerative disorder and the most common cause of dementia.
- It is estimated that in 2017 there were 5.5 million people affected by AD in the United States alone.
- It is estimated that 10% of people age 65 have AD and the percentage of affected individuals increases with age.
- AD develops as a result of the interaction of genetic and nongenetic risk factors.
- Clinical symptoms include memory loss, language difficulties, and problems with other cognitive functions that affect the patient's ability to perform his or her daily activities.
- AD is characterized by the formation of senile plaques and neurofibrillary tangles.
- There is no pharmacological treatment to slow or stop the progression of the disease.

Key facts of combination therapies

- Combination or multitarget therapy uses two or more drugs to treat a single disease.
- Combination therapy is the best option to treat multifactorial diseases.
- A combination therapy is considered successful when the efficacy of the treatment is better than that obtained with the individual drugs alone.

- When drug combinations exhibit additivity or synergy, the benefits of the therapy are enhanced.
- Combinational therapy allows the doses of the individual drugs to be lowered, minimizing toxicity.
- Together, theoretical modeling and empirical research can serve to identify optimal drug combinations for unmet medical needs.

Summary points

- This chapter focuses on the combination of HDAC and PDE5 inhibitors for the treatment of AD.
- AD is a degenerative brain disorder and the most common form of dementia.
- There is no effective treatment to stop the neuronal death that causes dementia associated with AD.
- HDAC inhibitors represent a promising therapy for neurodegenerative disorders but they are not the best option for chronic treatments because of their associated toxicity.
- PDE5 inhibitors can restore memory function in different models of AD.
- Due to the complexity of the pathological mechanisms in AD, a multitarget therapy is likely to be the best option.
- The simultaneous inhibition of HDAC and PDE5 using reference compounds had a synergistic effect in restoring AD symptoms.
- A first-in-class HDAC and PDE5 dual inhibitor, CM-414, restored synaptic function and ameliorated the histopathological hallmarks of AD in model systems.
- The synergistic therapeutic effect achieved through the inhibition of HDAC and PDE5 suggests this new approach may be a suitable alternative means to treat AD.

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CHAPTER 15

Nuclear factor erythroid 2-related factor 2 in Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
Akt protein kinase B
AMPK AMP-activated protein kinase
APP amyloid precursor protein
ARE antioxidant response element
ATG autophagy-related protein
ATP adenosine triphosphate
Aβ amyloid-beta peptide
BAG3 BCL2-associated athanogene 3
BBB blood–brain barrier
CDDO-MA CDDO-methylamide
Cul cullin
DMF dimethylformamide
Drp1 dynamin-related protein-1
FIP200 FAK-family interacting protein 200
Fis1 mitochondrial fission 1 protein
GCL glutamate cysteine ligase
GCLM glutathione cysteine ligase modulatory subunit
GFAP glial fibrillary acidic protein
GPx glutathione peroxidase
GR glutathione reductase
GSH glutathione
GSK3β glycogen synthase kinase 3 beta
GST glutathione S-transferase
HO-1 heme oxygenase 1
IBA1 ionized calcium-binding adapter molecule 1
IL interleukin
Keap1 kelch-like ECH-associated protein 1
LC3 microtubule-associated protein-1A/1B light chain 3
Maf proto-oncogene c-Maf
mETC mitochondrial electron transport chain
Mfn mitofusin
MMP mitochondrial membrane potential
mPTP mitochondrial permeability transition pore
MS multiple sclerosis

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mTOR mammalian target of rapamycin
mTORC mTOR complex NBR1 neighbor of BRCA1 gene 1 protein
NDP52 nuclear dot protein 52
NQO1 quinone recycling NAD(P)H:quinoneoxidoreductase 1
Nrf1 nuclear respiratory factor 1
Nrf2 nuclear factor erythroid 2 [NF-E2]-related factor 2
Opa1 mitochondrial dynamin-like GTPase
p62 nucleoporin p62
p70S6k ribosomal protein S6 kinase beta-1 (S6K1)
PGC-1α peroxisome proliferator-activated receptor gamma coactivator 1-α
PI3K phosphoinositide 3 phosphate kinase
PINK1 PTE induced putative kinase 1
Prx peroxiredoxin
PS1 presenilin 1
Rbx1 RING-box protein 1
RNS reactive nitrogen species
ROS reactive oxygen species
SOD superoxide dismutase
SQSTM1 sequestosome 1
Srx sulfiredoxin
tBHQ tert-butylhydroquinone
TERT telomerase reverse transcriptase
TFAM transcriptional factor A of mitochondria
TNF tumor necrosis factor
Trx thioredoxin
Txnrd Thioredoxin reductase
ULK1 Unc-51 like kinase 1
8-OHdG 8-hydroxy-2-deoxyguanosine
γ-GCS γ-glutamyl cysteine synthetase
β-TrCP Beta-transducin repeat-containing protein

Mini-dictionary of terms

Redox balance The ability of the body to maintain the balance (homeostasis) of generating harmful molecules (free radicals) and eliminating them.
Oxidative stress The accumulation of harmful molecules (free radicals) that damage the body when their production exceeds the body's ability to neutralize them.
Reactive oxygen species (ROS) Harmful free radicals containing oxygen that can damage cells and organelles.
Mitochondrial dysfunction Occurs when the energy (adenosine triphosphate, ATP)-producing ability of the mitochondria is impaired, typically resulting in ROS production.
Mitochondrial electron transport chain (mETC) A series of protein complexes within the mitochondria responsible for the production of ATP (energy) for the cell via the pumping of hydrogen ions (H+) against their electrochemical gradient.
Mitochondrial membrane potential (MMP) Charge/electrical gradient needed to produce ATP used to determine mitochondrial function.
Mitophagy The selective removal of damaged mitochondria via autophagic processes (see below).
Mitochondrial biogenesis The regenerative properties of mitochondria, allowing them to grow and divide to increase the amount of ATP (energy) produced.
Neuroinflammation Inflammation of nervous tissue including the brain.

Intracerebral injection Invasive injection made into the brain, typically for the targeted delivery of drugs/viral vectors.

Microglia Immune cells of the central nervous system that engulf foreign materials or damaged cells.

Autophagy Process that eliminates damaged intracellular organelles and unnecessary protein aggregates via incorporation into autophagosomes that eventually fuse with lysosomes for degradation.

Autophagosome Double-membrane vesicle that transports damaged organelles and proteins to the lysosome during the process of autophagy.

Introduction

Alzheimer's disease (AD) currently affects 35.6 million people and is expected to extend its devastating effect to 115 million people worldwide by 2050. Because AD develops through complicated pathogenic pathways and acquires multifaceted natures, it is untreatable by conventional single-modal treatments. Therefore, a significant amount of effort has been made to find a better therapeutic target that can affect multiple AD pathogenic pathways simultaneously. Among considered targets, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) appears to be reasonable because it plays a key role in maintaining homeostasis in redox balance, mitochondrial biogenesis, inflammation, and autophagy, specifically in the AD brain. In this chapter, we will briefly examine the role of Nrf2 in these four systems that are impaired in AD and will illuminate how Nrf2 activation attempts to repair these existing systems and restore homeostasis.

The role of nuclear factor erythroid 2-related factor 2 in redox balance

The AD brain loses endogenous antioxidant capacity (Ghosh, LeVault, Barnett, & Brewer, 2012) more quickly than normal brains during aging, accumulating a significant quantity of free reactive radicals (Kim et al., 2006). Accumulated oxidative stress damages mitochondria and allows the production of reactive oxygen species (ROS) (superoxide and hydrogen peroxide) and reactive nitrogen species (RNS) (nitric oxide, dinitrogen tetroxide, and peroxynitrite) via mitochondrial electron transport chain (Halliwell, 2001). AD patient brains contain increased levels of free reactive radicals and their derivatives (e.g., lipid peroxide) and adducts (e.g., 8-OHdG) (Butterfield et al., 2006). During AD progression, the brain loses antioxidant enzymes and activities, further contributing to oxidative stress.

Nrf2 is the main switch for the expression of a majority of endogenous antioxidant enzymes (Johnson & Johnson, 2015). It is considered a legitimate target to enhance the global antioxidant defense system. Antioxidant enzymes under the control of Nrf2 include protein enzymes involved in iron homeostasis (heme oxygenase 1 [HO-1] and ferritin), redox regulation (catalase, peroxiredoxin [Prx], sulfiredoxin [Srx], thioredoxin [Trx], and superoxide dismutase [SOD]), and glutathione synthesis (glutathione S-transferase [GST], glutathione reductase [GR], glutathione peroxidase [GPx], glutathione cysteine ligase

regulatory subunit, glutathione cysteine ligase modulatory subunit [GCLM], and glutathione synthetase, γ-glutamyl cysteine synthetase [γ-GCS]), and quinone recycling (NAD(P)H:quinoneoxidoreductase 1 [NQO1]) (de Vries et al., 2008). The functions of these antioxidant enzymes are well described in our recent review (Murphy & Park, 2017).

Several mechanisms activate Nrf2. The first is the Nrf2 repressor protein kelch-like ECH-associated protein 1 (Keap1)-mediated mechanism that sequesters Nrf2 in the cytoplasm and links Nrf2 to the ubiquitin ligase cullin-RING-box protein 1 (CuI-Rbx1) complex for proteasomal degradation (Zhang, Lo, Cross, Templeton, & Hannink, 2004). Keap1 functions as a sensor for ROS/electrophilic stress. When its cysteine residue reacts with an electrophile that is elevated during oxidative stress (Itoh, Mimura, & Yamamoto, 2010), Keap1 releases Nrf2, which in turn enters the nucleus and heterodimerizes with Maf prior to binding to the antioxidant-response element (ARE) in the promoters of the genes that encode antioxidant enzymes (Itoh et al., 1997). However, with chronic oxidative stress in the AD brain, Nrf2 becomes resistant to ROS-induced activation (Ramsey et al., 2007). The secondary mechanism that activates Nrf2 in the AD brain appears to be inhibiting glycogen synthase kinase 3 beta (GSK3β) (Rada et al., 2011). GSK3β phosphorylates Nrf2 and targets it to beta-transducin repeat-containing protein/Culin1-E3 (β-TrCP/Cul1-E3) ligase complex for proteasomal degradation (Rada et al., 2011; Zhou et al., 2014). However, phosphoinositide 3 phosphate kinase/protein kinase B (PI3K/Akt) signaling can activate Nrf2 by inhibiting GSK3β, releasing Nrf2 from proteasomal targeting (Taguchi et al., 2014) and allowing stabilized Nrf2 to translocate into the nucleus and activate gene expression. Nrf2 activation for maintenance of redox homeostasis is shown in Fig. 15.1.

In AD brains, Nrf2 is primarily located in the cytoplasm, with much less in the nucleus (Ramsey et al., 2007). Nrf2 knockout in amyloid precursor protein/presenilin 1 (APP/PS1) mice exacerbated oxidative damage (Joshi, Gan, Johnson, & Johnson, 2015). On the other hand, Nrf2 activation via 18 α-glycyrrhetinic acid and triterpenoids enhanced neuron survival against Aβ stress by increasing GCL and GSH (Ghosh, LeVault, & Brewer, 2014), and attenuated oxidative stress (Dumont et al., 2009), respectively. As such, antioxidant enzymes are expected to be decreased in AD brains; however, groups of studies have shown otherwise. In some studies, NQO1 (SantaCruz, Yazlovitskaya, Collins, Johnson, & DeCarli, 2004), GR, GPx, HO-1 (Aksenov & Markesbery, 2001; Schipper, Cisse, & Stopa, 1995), and GCLM (Tanji et al., 2013) were increased in AD brains compared with normal brains. Conversely, in other studies the expression of some Nrf2-dependent enzymes was reduced or unchanged in AD brains (Ansari & Scheff, 2010; Joshi et al., 2015). Though there is still no clear explanation about the discrepancy, it appears that Nrf2 activation is favorable for oxidative stress defense.

Activating neuronal Nrf2 should boost the production of free radical scavengers and antioxidants such as glutathione (GSH). Nrf2 activation in astrocytes also significantly contributes to the protection of neurons from oxidative stress, as astrocytes scavenge extracellular radicals and provide GSH and other antioxidants to neurons (Shih et al., 2003). To obtain this effect in vitro, a group of blood—brain barrier (BBB)-permeable

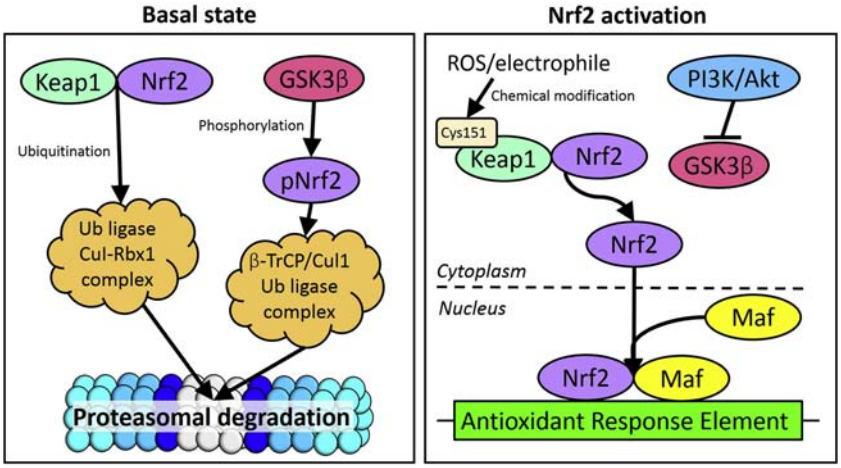

Figure 15.1 ***Nrf2 activation maintains redox balance.*** Nrf2 activation via chemical modification of repressor Keap1 allows for Nrf2 release and translocation. PI3K/Akt-mediated inhibition of GSK3β causes Nrf2 to be released from ubiquitin ligase, preventing proteasomal degradation. Unhindered Nrf2 then dimerizes with Maf and binds to the antioxidant response element, enabling redox homeostasis maintenance. *β-TrCP/Cul1*, beta-transducin repeat-containing protein/culin1; *Cul-Rbx1*, cullin-RING-box protein 1; *Cys151*, cysteine 151; *GSK3β*, glycogen synthase kinase 3 beta; *Keap1*, kelch-like ECH-associated protein 1; *Nrf2*, nuclear factor erythroid 2 [NF-E2]-related factor 2; *PI3K*, phosphoinositide 3 phosphate kinase; *Ub*, ubiquitinated.

Nrf2 activators are being developed and tested in clinical trials. For example, as a front-runner in clinical trials—phase III for multiple sclerosis (MS) treatment—dimethylformamide (DMF) reduces Nrf2 degradation by chemical modification of cysteine 151 in Keap1 (Gold et al., 2012; Linker et al., 2011). Additional Nrf2-activating compounds that show therapeutic promise include CDDO-methylamide (CDDO-MA) (Dumont et al., 2009), puerarin (Zhou et al., 2014), and gracilins A and C (Leiros et al., 2015). Furthermore, pharmacological activation of Nrf2 using tert-Butylhydroquinone (tBHQ), chitosan, kavalactone, curcumin and puerarin, and lentiviral Nrf2 overexpression could increase Nrf2-dependent antioxidant genes, GSH synthesis and long-term memory and reduce the accumulation of ROS or Aβ peptides in vitro and in vivo AD models (Fragoulis et al., 2017; Frautschy et al., 2001; Kanninen et al., 2008, 2009; Khodagholi, Eftekharzadeh, Maghsoudi, & Rezaei, 2010; Zhou et al., 2014).

The role of nuclear factor erythroid 2-related factor 2 in mitochondrial maintenance

In the AD brain, mitochondria, the major adenosine triphosphate (ATP) generator of neurons, quickly lose their function, as neurons have high rates of mitochondrial electron transport, nonregenerative postmitotic status, and weak intrinsic antioxidant defenses (Halliwell, 2006). As such, mitochondrial dysfunction is observed at very early stages in AD animal models (Hauptmann et al., 2009). Both oxidative reactive radicals and

Aβ peptides directly damage mitochondria, resulting in the uncoupling of mitochondrial electron transport chain (mETC) and the depolarization of mitochondrial membrane potential (MMP) (Galindo, Ikuta, Zhu, Casadesus, & Jordan, 2010). Damaged mitochondria stop ATP production and generate ROS (Galindo et al., 2010). In human AD brains, mitochondrial fission gene expression—dynamin related protein-1 (Drp1) and mitochondrial fission 1 protein (Fis1)—was increased while that of fusion genes (mitofusin [Mfn1, Mfn2], mitochondrial dynamin like GTPase (Opa1), and translocase of outer mitochondrial membrane 40 [Tomm40]) was decreased (Manczak, Calkins, & Reddy, 2011). Similarly, 12-month-old APP mice showed increased levels of mitochondrial fission proteins, Drp1 and Fis1, and decreased levels of fusion (Mfn1, Mfn2, and Opa1), biogenesis (peroxisome proliferator-activated receptor gamma co-activator 1-α [PGC-1α], nuclear respiratory factor 1 [Nrf1], Nrf2 and transcriptional factor A of mitochondria [TFAM]), and mitophagy (PTEN-induced putative kinase 1 [PINK1] and telomerase reverse transcriptase [TERT]) proteins compared with age-matched wild-type mice (Manczak, Kandimalla, Yin, & Reddy, 2018). This suggests the mitochondrial maintenance system is perturbed in the AD brain, and restoring mitochondrial MMP integrity is necessary to generate ATP and prevent ROS generation.

Activating Nrf2 is expected to help restore functional mitochondria in AD brain neurons because Nrf2 activation increases the expression of proteins involved in mitochondrial biogenesis and fusion (PGC-1α, Nrf1, TFAM) and decreases proteins for mitochondrial fission (Drp1) (Dinkova-Kostova & Abramov, 2015; Sabouny et al., 2017). Activation of Nrf2 also enhances mitophagy, which removes damaged mitochondria (Georgakopoulos et al., 2017). Nrf2 activation improves mitochondrial integrity by attenuating the oxidative stress-induced opening of the mitochondrial permeability transition pore (Greco, Shafer, & Fiskum, 2011). Thus, activating Nrf2 is expected to remove damaged mitochondria and provide neurons with more functional mitochondria, thus reducing mitochondrial ROS production in the AD brain. Nrf2 activation with respect to mitochondrial maintenance is shown in Fig. 15.2.

The role of nuclear factor erythroid 2-related factor 2 in antiinflammation

In the AD brain, microglia play a major role in neuroinflammation. Activated microglia (reactive microgliosis, M1) release ROS and RNS that damage and kill neurons which, in turn, further activate microglia, triggering the vicious cycle of neuroinflammation (Block, Zecca, & Hong, 2007). Activated M1 microglia also release proinflammatory mediators such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, IL-12, and IL-23. The role of Nrf2 in the antiinflammatory response is also evident in macrophages, monocytes, and astrocytes in addition to microglia (Kobayashi et al., 2016; Quinti et al., 2017). Nrf2 knockout in APP/PS1 mice increased chronic neuroinflammation, specifically increasing

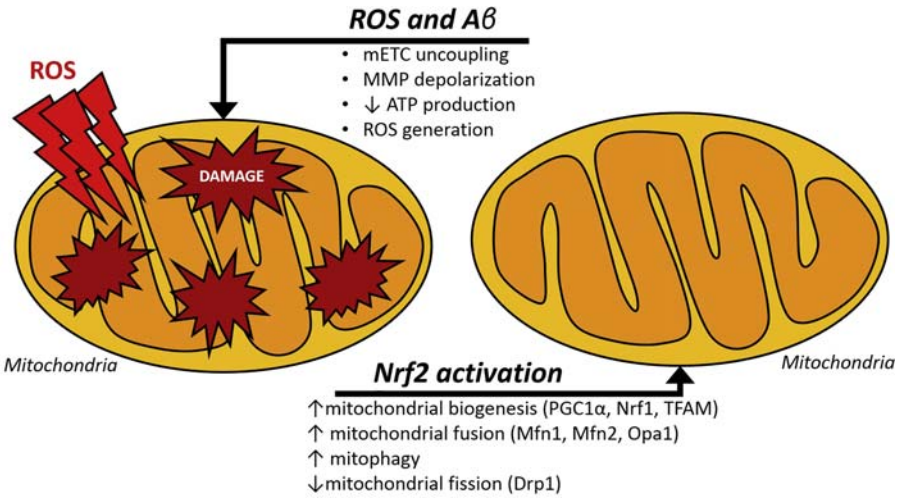

Figure 15.2 ***Nrf2 activation preserves mitochondrial function.*** In the Alzheimer's disease brain, reactive oxygen species and Aβ peptides damage mitochondria, leading to electron transport chain uncoupling, depolarization of mitochondrial membrane potential, decreased ATP production, and exacerbated ROS generation. Nrf2 activation combats mitochondrial dysfunction by increasing the expression of proteins involved in mitochondrial biogenesis, fusion, and mitophagy as well as decreasing that of fission. *ATP*, adenosine triphosphate; *Drp1*, dynamin-related protein-1; *mETC*, mitochondrial electron transport chain; *Mfn*, mitofusin; *MMP*, mitochondrial membrane potential; *Nrf1*, nuclear respiratory factor 1; *Opa1*, mitochondrial dynamin-like GTPase; *PGC*-1α, peroxisome proliferator-activated receptor gamma co-activator 1-α; *ROS*, reactive oxygen species; *TFAM*, transcriptional factor A of mitochondria.

glial fibrillary acidic protein (GFAP, astrocyte marker) and ionized calcium-binding adapter molecule 1 (IBA1, microglia marker) in the hippocampus (Joshi et al., 2015). Nrf2 is a known negative regulator of microglial activation and can inhibit neuroinflammation in the brain.

Activation of Nrf2 in the brain was shown to suppress neuroinflammation (Kim, Cha, & Surh, 2010). Specifically, activated Nrf2 protects microglia from oxidative stress and proinflammatory activation by increasing the antioxidant enzymes SOD3, GPx, HO-1, and NQO1 and reducing enzymes GCLM/C, GR, Trx, and Prx (Rojo et al., 2014). DMF exerts its antiinflammatory effect in an Nrf2-dependent manner (Linker et al., 2011). Similarly, the intracerebral injection of Nrf2 activator kavalactone methysticin into the hippocampus and cortex of APP/PS1 mice reduced microgliosis, astrogliosis, and proinflammatory cytokines with memory improvement (Fragoulis et al., 2017). Thus, activation of Nrf2 is expected to suppress neuroinflammation in AD brain. The promotion of antiinflammation by Nrf2 activation is shown in Fig. 15.3.

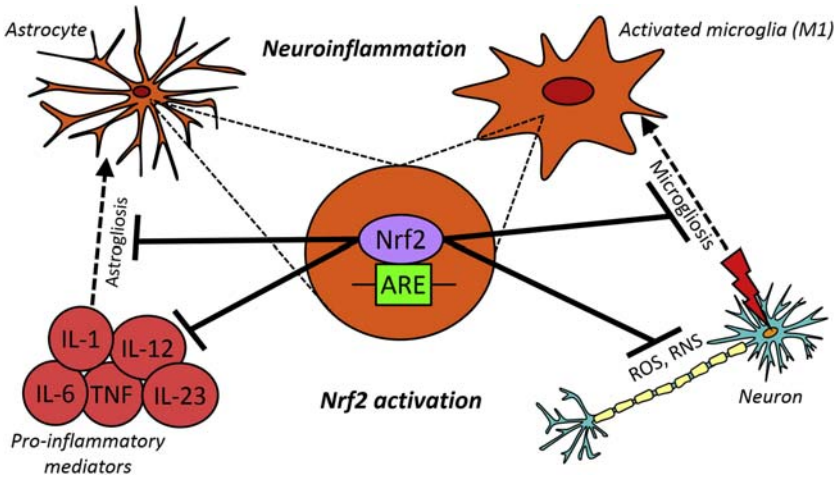

Figure 15.3 ***Nrf2 activation promotes antiinflammation.*** Neuroinflammation in the Alzheimer's disease brain is caused by activated microglia that produce radicals that damage neurons and cyclically activate microglia (microgliosis). Activated microglia also induce proinflammatory mediators that target astrocytes (astrogliosis). Nrf2 activation suppresses these neuroinflammatory actions by upregulating antioxidant enzymes and inhibiting microgliosis, astrogliosis, and proinflammatory cytokines. *ARE*, antioxidant response element; *IL*, interleukin; *RNS*, reactive nitrogen species.

The role of nuclear factor erythroid 2-related factor 2 in autophagy

Autophagy removes damaged intracellular organelles and unnecessary protein aggregates by engulfing them into multimembrane vesicles prior to delivery to lysosomes for degradation (Ogata et al., 2006). To activate autophagy, mammalian target of rapamycin complex 1 (mTORC1), a negative regulator of autophagy, is inhibited by phosphorylation by AMP-activated protein kinase (AMPK) and PI3K/Akt (Maiese, Chong, Wang, & Shang, 2012). Then, the disassembly of the Unc-51-like kinase 1-autophagy-related protein 13- FAK-family interacting protein 200 (ULK1-ATG13-FIP200) complex releases ULK1, which initiates membrane nucleation via interaction with PI3KIII, beclin-1, and autophagy-related protein 6 (ATG6) (Kim, Kundu, Viollet, & Guan, 2011). Autophagy membrane nucleation is followed by elongation and conjugation reactions by E1- and E2-like conjugating ATG enzymes (Mizushima, Noda, & Ohsumi, 1999). During elongation, p62 (sequestosome 1 [SQSTM1]) recruits polyubiquitinated proteins to the elongating autophagosomal membrane (Ichimura et al., 2008). Elongation and closure of the autophagosomal membrane is via microtubule-associated protein-1A/1B light chain 3 (LC3)-II incorporation (Weidberg, Shpilka, Shvets, & Elazar, 2010) following processing of LC3 to LC3-I and LC3-II by ATG complexes (Yu et al., 2012).

In normal brains, autophagy typically occurs fast and at a low level, so autophagosomes are scarcely found, whereas in the AD brain, distended autophagosomes accumulate along dystrophic neurites due to autophagy dysfunction (Nixon, 2007).

Moreover, hyperactivated mTOR signaling and increased lysosomal hydrolases (due to impaired autophagosomal–lysosomal clearance) were also found in the AD brain (Boland et al., 2008). Autophagy inhibition by partial deletion of beclin-1 in AD animal models further increased intracellular and extracellular Aβ load (Pickford et al., 2008). Conversely, in vivo rapamycin treatment to activate autophagy reduced intracellular Aβ peptides and cognitive defect (Caccamo, Majumder, Richardson, Strong, & Oddo, 2010) and amyloid plaque load in 3xTg-AD mice (Majumder, Richardson, Strong, & Oddo, 2011). Similarly, autophagy activation was shown to reduce Aβ peptide accumulation and alleviate memory deficits in AD mice (Yang et al., 2011). Similarly to its involvement in Aβ peptide clearance, autophagy is also considered a major mechanism to scavenge p-tau (Jo et al., 2014).

Nrf2 promotes autophagy by increasing the expression of several autophagy-mediating proteins such as p62/SQSTM1 (Jain et al., 2010). p62 exerts positive feedback on Nrf2 activation by occupying the Nrf2-binding site on Keap1, thus releasing Nrf2 (Kwon et al., 2012). Nine autophagy genes contain ARE in their promoters and are expressed in response to Nrf2 activator sulforaphane in human cells (Pajares et al., 2016). Nrf2-mediated enhancement of autophagy appears to be involved in clearance of Aβ peptide and p-tau. Nrf2 knockout reduced many autophagy-mediating proteins such as BCL2-associated athanogene 3 (BAG3), neighbor of BRCA1 gene 1 protein (NBR1), nuclear dot protein 52 (NDP52), and p62 and increased p-tau in the hippocampus of 12-month-old wild-type mice (Tang, Ji, Pallo, Rahman, & Johnson, 2018). Nrf2 knockout in APP/PS1 mice reduced autophagy-mediated proteins and thus inhibited autophagy, as evidenced with higher phospho-mTOR and phospho-ribosomal protein S6 kinase beta-1 (p70S6k) as well as accumulation of multivesicular bodies, endosomes, and lysosomes (Joshi et al., 2015). p62(−/−) mice and Nrf2 knockout mice accumulated high levels of p-tau (Jo et al., 2014; Ramesh Babu et al., 2008). In these mice, NDP52 that was reduced by Nrf2 knockout played a major role in Nrf2-dependent clearance of p-tau aggregates (Jo et al., 2014). Conversely, Nrf2 overexpression in AD animal models increased autophagy-mediated proteins and decreased Aβ peptide and p-tau (Boland et al., 2010). Thus, Nrf2-mediated autophagy enhancement appears to be significant in intracellular clearance of two major AD pathogenic factors, Aβ peptide and p-tau, in AD brains. Autophagy repair via Nrf2 activation is shown in Fig. 15.4.

Conclusion

Nrf2 activation appears to play a major role in homeostasis restoration of redox balance, mitochondrial biogenesis, inflammation, and autophagy in the AD brain. The impact of Nrf2 activation with respect to these four contributions to the AD phenotype are summarized in Fig. 15.5. For these reasons, Nrf2 is considered a reasonable therapeutic target for AD, as its activation may repair existing processes involved in AD pathology. Nrf2 activation in different physiological and experimental conditions is summarized in Table 15.1.

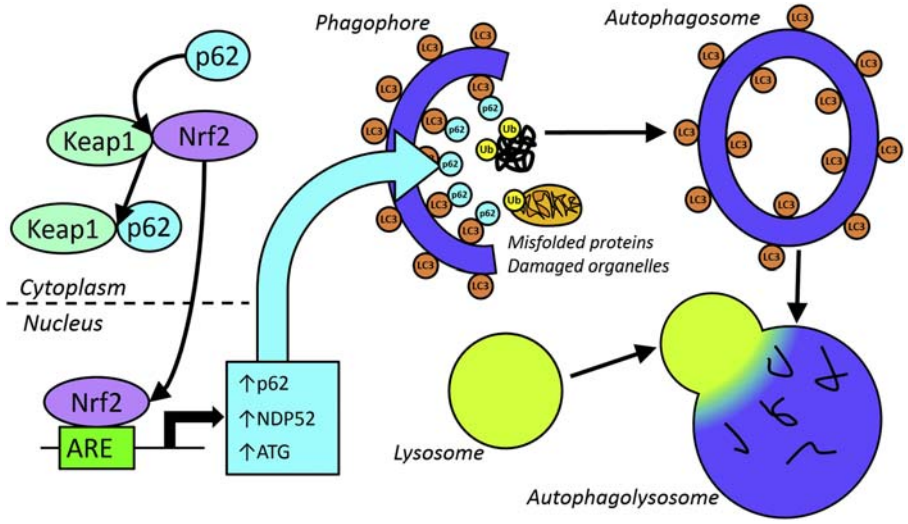

Figure 15.4 ***Nrf2 activation restores autophagy.*** Autophagy dysfunction in Alzheimer's disease can be overcome with Nrf2 activation, which increases autophagy-mediated proteins including p62. p62 binds to repressor Keap1 at the Nrf2 binding site, releasing Nrf2 and allowing for increased expression of p62 and other autophagy-mediated proteins. p62 recruits ubiquitinated proteins and mediates elongation and closure of the autophagosomal membrane. *ARE*, antioxidant response element; *ATG*, adenosine triphosphate; *LC3*, microtubule-associated protein-1A/1B light chain 3; *NDP52*, nuclear dot protein 52; *p62*, nucleoporin p62.

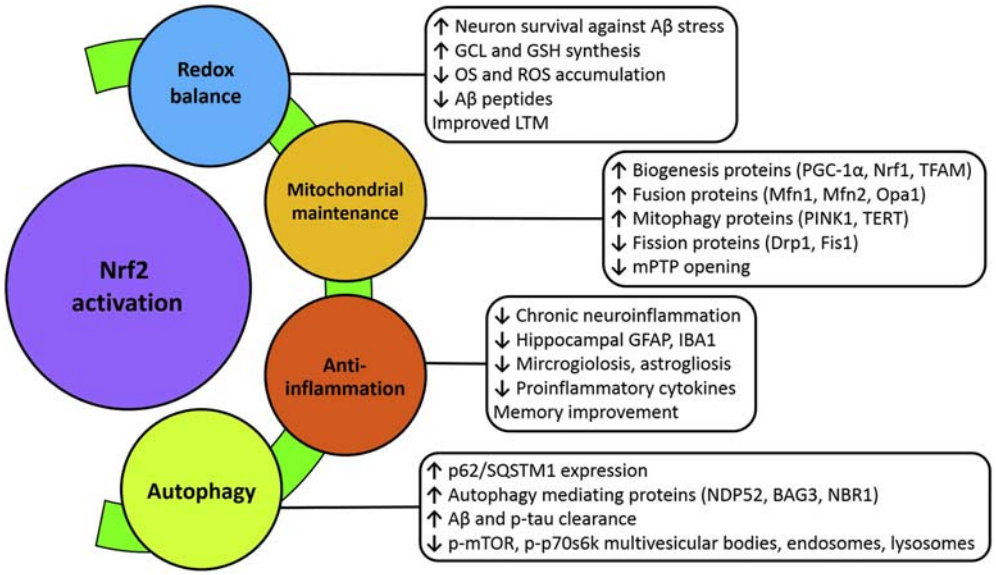

Figure 15.5 ***Summary of Nrf2 activation in Alzheimer's disease.*** Nrf2 activation may be useful for the mitigation of Alzheimer's disease progression with respect to the impaired cellular processes of redox homeostasis, mitochondrial biogenesis, neuroinflammation, and autophagy. Nrf2 activation contributes to homeostasis restoration within the brain, establishing itself as a potential efficacious therapeutic target for Alzheimer's disease. *BAG3*, BCL2-associated athanogene 3; *GCL*, glutamate cysteine ligase; *GFAP*, glial fibrillary acidic protein; *GSH*, glutathione; *IBA1*, ionized calcium-binding adapter molecule 1; *LTM*, long-term memory; *mPTP*, mitochondrial permeability transition pore; *NBR1*, neighbor of BRCA1 gene 1 protein; *OS*, oxidative stress; *PINK1*, PTEN-induced putative kinase 1; *p-mTOR*, mammalian target of rapamycin; *p-p70s6k*, ribosomal protein S6 kinase beta-1; *TERT*, telomerase reverse transcriptase.

Table 15.1 Summary of main findings of Nrf2 activation in physiological and experimental conditions.

Model Nrf2 Condition	Outcome	References
Redox homeostasis		
APP/PS1ΔE9 mice *Nrf2 KO*	↑ Oxidative damage	Joshi et al. (2015)
3xTg-AD neurons *Nrf2 activation* via 18α-glycyrrhetinic acid	↑ Neuron survival against Aβ stress ↑ GCL and GSH	Ghosh et al. (2014)
Tg19959 mice *Nrf2 activation* via CDDO-methylamide	↓ Oxidative stress ↓ Aβ levels/plaque burden ↓ Microgliosis ↑ Spatial memory retention	Dumont et al. (2009)
Various cell lines/mouse models *Nrf2 activation* via tert-Butylhydroquinone, chitosan, kavalactone, curcumin, pueratin, lentiviral Nrf2 overexpression	↑ Nrf2-dependent antioxidant genes ↑ GSH synthesis Improved long-term memory ↓ ROS accumulation ↓ Aβ peptides	Frautschy et al. (2001), Kanninen et al. (2008,2009), Khodagholi et al. (2010)
Human AD brain	↑ NQO1, GR, GPx, HO-1, GCLM	Aksenov and Markesbery (2001), SantaCruz et al. (2004), Schipper et al. (1995), Tanji et al. (2013)
Human AD brain APP/PS1ΔE9 mice	↓ Antioxidant enzymes	Ansari and Scheff (2010), Joshi et al. (2015)
Mitochondria		
Thy-1 APP mice Human AD brain APP mice (12 months old)	Mitochondrial dysfunction ↑ Fission genes (Drp1, Fis1) ↓ Fusion genes (Mfn1, Mfn2, Opa1, Tomm40) ↓ Biogenesis proteins (PGC-1α, Nrf1, Nrf2, TFAM) ↓ Mitophagy proteins (PINK1, TERT)	Hauptmann et al. (2009) Manczak, Calkins, and Reddy (2011), Manczak, Kandimalla, Yin, and Reddy (2018)
Various cell lines/mouse models *Nrf2 activation*	↑ Mitochondrial biogenesis and fusion proteins (PGC-1α, Nrf1, TFAM) ↓ Mitochondrial fission proteins (Drp1)	Dinkova-Kostova and Abramov (2015) Sabouny et al. (2017)

Continued

Table 15.1 Summary of main findings of Nrf2 activation in physiological and experimental conditions.—cont'd

Model Nrf2 Condition	Outcome	References
Mouse embryonic fibroblasts, HeLa, SH-SY5Y cells *Keap1 inhibition/Nrf2 activation*	↑ Mitophagy	Georgakopoulos et al. (2017)
Fischer 344 rats *Nrf2 activation* via sulforaphane	↓ mPTP opening	Greco, Shafer, and Fiskum (2011)
Inflammation		
APP/PS1ΔE9 mice *Nrf2 KO*	↑ Chronic inflammation ↑ GFAP, IBA1 (hippocampus)	Joshi et al. (2015)
Various cell lines/mouse models *Nrf2 activation*	↓ Neuroinflammation	Kim, Cha, and Surh (2010)
EAE mice *Nrf2 activation* via DMF	Antiinflammation effect (Nrf2-dependent)	Linker et al. (2011)
APP/PS1 mice *Nrf2 activation* via methysticin oral administration	↓ microgiosis, astrogliosis, proinflammatory cytokines Memory improvement	Fragoulis et al. (2017)
Autophagy		
AD brain	Distended autophagosomes Dystrophic neurites Autophagy dysfunction	Nixon (2007)
Human AD brain APP/PS1 mice TgCRND8 mice	Hyperactivated mTOR signaling ↑ Lysosomal hydrolases Impaired autophagosomal-lysosomal clearance	Boland et al. (2008), Yang et al. (2011)
Beclin-1(+/−) mice x APP T41 Tg mice	Autophagy inhibition ↑ Aβ (intra- and extracellular)	Pickford et al. (2008)
3xTg-AD mice Autophagy activation via in vivo rapamycin	↓ Intracellular Aβ/plaque load Improved cognition	Caccamo et al. (2010)
Various cell lines *Nrf2 activation* (p62 induced)	↑ p62 expression	Jain et al. (2010)
HEK293T and HT22 cells *Nrf2 activation* via sulforaphane	Autophagy gene expression (via ARE)	Pajares et al. (2016)

Table 15.1 Summary of main findings of Nrf2 activation in physiological and experimental conditions.—cont'd

Model Nrf2 Condition	Outcome	References
Nrf2 KO mice (12 months old) *Nrf2 KO*	↓ Autophagy-mediating proteins (BAG3, NBR1, NDP52, p62) ↑ p-tau	Tang et al. (2018)
APP/PS1ΔE9 mice *Nrf2 KO*	↑ p-mTOR, p-p70s6k, multivesicular bodies, endosomes, lysosomes ↓ Autophagy mediated proteins Autophagy inhibition	Joshi et al. (2015)
p62 (−/−) KO mice *p62 KO*	↑ p-tau, NFTs, neurodegeneration ↑ anxiety, depression ↓ working memory	Ramesh Babu et al. (2008)
Nrf2 KO mice *Nrf2 KO*	↓ NDP52 ↑ p-tau	Jo et al. (2014)
Niemann-Pick type C1, GM1 gangliosidosis, Sandhoff mice	Impaired lysosomal flux ↑ APP C-terminal fragments	Boland et al. (2010)

↑, increase; ↓, decrease; *AD*, Alzheimer's disease; *APP*, amyloid precursor protein; *ARE*, antioxidant response element;*BAG3*, BCL2 associated athanogene 3; *DMF*, dimethylformamide; *Drp1*, dynamin-related protein-1; *EAE*, experimental autoimmune encephalomyelitis; *Fis1*, mitochondrial fission 1 protein; *GCL*, glutamate cysteine ligase; *GCLM*, glutathione cysteine ligase modulatory subunit; *GFAP*, glial fibrillary acidic protein; *GPx*, glutathione peroxidase; *GR*, glutathione reductase; *GSH*, glutathione; *HO-1*, heme oxygenase 1; *IBA1*, ionized calcium-binding adapter molecule 1; *KO*, knockout; *Mfn*, mitofusin; *mPTP*, mitochondrial permeability transition pore; *mTOR*, mammalian target of rapamycin; *NBR1*, neighbor of BRCA1 gene 1 protein; *NDP52*, Nuclear dot protein 52; *NFTs*, Neurofibrillary tangles; *NQO1*, Quinone recycling (NAD(P)H:quinoneoxidoreductase 1); *Nrf1*, Nuclear respiratory factor 1; *Nrf2*, Nuclear factor erythroid 2 [NF-E2]-related factor 2; *Opa1*, mitochondrial dynamin-like GTPase; *p-*, phosphorylated; *p62*, nucleoporin p62; *p70s6k*, ribosomal protein S6 kinase beta-1; *PGC-1α*, peroxisome proliferator-activated receptor gamma coactivator 1-α; *PINK1*, PTEN-induced putative kinase 1; *PS1*, presenilin 1; *ROS*, reactive oxygen species; *TERT*, telomerase reverse transcriptase; *TFAM*, transcriptional factor A of mitochondria; *Tg*, transgenic; *Tomm40*: translocase of outer mitochondrial membrane 40. This table shows the outcomes of Nrf2 activation in specific research models and under different Nrf2 conditions (e.g., activation or knockout).

Key facts of nuclear factor erythroid 2-related factor 2

- Nrf2 is a basic leucine zipper transcription factor and has a cap "n" collar structure.
- Nrf2 contains six domains called Nrf2-ECH homology (Neh) domains that are highly conserved (*see below*).
- Neh1 allows for heterodimerization with Maf proteins; Neh2 binds to Keap1 repressor; Neh3 aids with protein stability and interacts with transcriptional machinery; Neh4 and Neh5 are transactivation domains; Neh6 may be involved with Nrf2 degradation.

- Nrf2 is the master regulator of redox homeostasis and is able to regulate many antioxidant genes via binding to the ARE in the promoter regions of target genes, initiating transcription of antioxidant proteins.
- Nrf2 is important for the upregulation of antioxidant genes in the presence of oxidative stress brought on by injury and inflammation. Specific target genes of Nrf2 include HO-1, SOD, GR, and many others.
- In basal unstressed conditions, Nrf2 is located in the cytoplasm, where it is held and proteasomally degraded. However, in response to oxidative stress, Nrf2 translocates to the nucleus to initiate transcriptional activity.
- In the AD brain, there is more Nrf2 in the cytoplasm and less in the nucleus compared with that of non-AD brains.
- Many drugs target the Nrf2 signaling pathway, specifically for diseases caused by oxidative stress.

Summary points

- This chapter focuses on Nrf2 as a therapeutic target for AD.
- Nrf2 is a transcription factor activated in the presence of oxidative stress.
- Though originally believed to just mediate redox homeostasis, recent research has highlighted a role for Nrf2 in mitochondrial maintenance, antineuroinflammation, and autophagy activation in the AD brain.
- Nrf2 activation increases the expression of endogenous antioxidant enzymes, which are lost with AD progression.
- Nrf2 activation restores functional mitochondria, which are unable to produce ATP in AD, by increasing biogenesis and fusion proteins, decreasing fission proteins, and ROS production.
- Nrf2 activation suppresses neuroinflammation due to activated microglia in AD by increasing antioxidant enzymes and decreasing microgliosis, astrogliosis, and proinflammatory cytokines.
- Activation of autophagy via Nrf2 increases autophagy-mediated proteins, which are impaired in AD.
- Nrf2 activation also reduces ROS and/or Aβ peptide accumulation and increases p-tau clearance in the AD brain.
- Nrf2 activation may mitigate AD progression with respect to the cellular processes of redox homeostasis, mitochondrial biogenesis, neuroinflammation, and autophagy.

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CHAPTER 16

Implications of alpha- and beta-secretase expression and function in Alzheimer's disease

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Mini-dictionary of terms

ADAM10 A disintegrin and metalloproteinase 10, a zinc-dependent metalloproteinase that has been shown to be the major alpha-secretase in neuronal cells

APPs-alpha Cleavage product derived from alpha-secretase activity on APP; has been reported to act as a neurotrophic and neuroprotective factor

BACE-1 Beta-site amyloid precursor protein cleaving enzyme 1, an aspartic protease that cleaves APP to yield soluble sAPP-beta and A-beta peptides

EST Expressed sequence tag; short fragments of mRNA sequences (100–800 bp) derived through single sequencing reactions performed on randomly selected cDNA library clones

SNP Single-nucleotide polymorphism, a variation in a single nucleotide occurring with a relatively high abundance within a population (>1%)

Introduction

About three decades ago, amyloid-beta (A-beta) peptides were identified as the major components of senile plaques from the brain of Alzheimer's patients (Glenner & Wong, 1984). They derive by cleavage of the amyloid precursor protein (APP) and were widely accepted as the basic prerequisite for Alzheimer's disease (AD) pathogenesis within the first years of emerging research. The thereby-deduced amyloid cascade hypothesis somewhat enforced researchers' focus on how the peptide is produced and accumulates, how its toxicity to neurons is conferred, and on therapeutic strategies strictly linked to activity of the enzymes that lead to synthesis of the toxic compound (namely beta- and gamma-secretase). In this canonical pathway, the aspartic protease beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1) initially cuts amyloid precursor protein (APP) to yield C99 and APPs-beta (Fig. 16.1). Subsequently, gamma-secretase leads to hydrolysis of the peptide-bond at variable sites within the transmembrane region. This results in fragments such as A-beta 42 and the soluble APP intracellular domain (AICD). In the nonamyloidogenic pathway, alpha-secretase cuts APP within the A-beta stretch and thereby prevents formation of the toxic peptide. This is accompanied by secretion of a soluble protein fragment—APPs-alpha—which has been linked to

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Enzyme	Name of cleavage product	Exemplary reference
alpha-secretase	APPs-alpha, CTF-alpha	(Lammich et al., 1999)
BACE-2	APPs-alpha–, CTF-alpha–like	(Fluhrer et al., 2002)
beta-secretase	APPs-beta, CTFbeta	(Vassar et al., 1999)
Caspase	C31	(Barnes et al., 1998)
Eta-secretase (MT5-MMP)	Eta-CTF-alpha, Eta-CTF-beta	(Willem et al., 2015)
gamma-secretase (zeta-, epsilon-cleavage)	A-beta (div. length), p3, AICD	(Zhao, Tan, Mao, Cui, & Xu, 2007)
IDE		(Farris et al., 2003)
Meprin-beta	APPs-beta, CTF-beta	(Bien et al., 2012)
unknown	NTE	(Welzel et al., 2014) (Portelius et al., 2010)

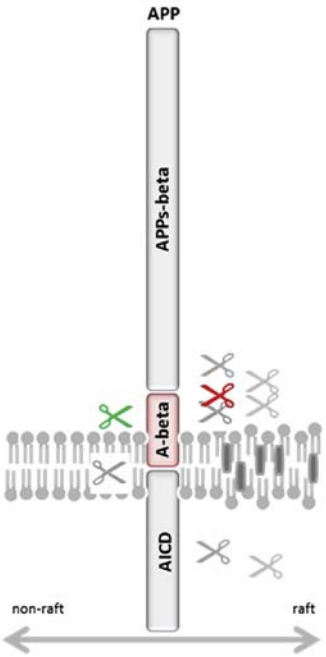

Figure 16.1 ***Proteolytic processing of the amyloid precursor protein (APP).*** APP comprises a huge ectodomain, a transmembrane domain, and a short intracellular part and is localized to both nonraft and raft regions of the cellular membrane. Especially, the region proximal to the membrane-spanning amino acids is working point for various proteases; alpha- and beta-secretase (*green and red scissor*) initiate the nonamyloidogenic and the amyloidogenic pathway, while the outcome of other APP processing events still remains elusive—e.g., N-terminally extended fragments (NTEs). Additionally, several proteolytic steps have been identified that work on single or multiple APP fragments (*gray scissors*). *AICD*, APP intracellular domain; *CTF*, C-terminal factor.

neuroprotective features (reviewed in Kogel, Deller, & Behl, 2012). The shorter C-terminal stub (C83) is also cleaved by gamma-secretase and delivers p3 peptide as well as AICD.

How myopic the strict focus on A-beta production might have been is indicated by the still-growing number of APP proteolysis products found in *in vitro* assays and biological fluids such as human cerebrospinal fluid (CSF). Selkoe and Walsh identified N-terminally extended (NTE) A-beta peptides in CHO cells, which start 40 amino acids before the beta-secretase cleavage site and end at the gamma-secretase cleavage site (Welzel et al., 2014). Moreover, more than 10 endogenous NTE species have been

observed in CSF (Portelius et al., 2010). In endosomes and lysosomes of HEK293 cells, a C-terminal fragment of APP with a mass of about 30 kDa had been detected more than 20 years before (see Haass et al., 1992) but was described just 5 years ago as a new type of APP cleavage designated as the activity of "eta-secretase," with the matrix metalloproteinase MT5-MMP potentially being responsible for this cleavage (Willem et al., 2015). Additionally, caspase 3 (Gervais et al., 1999), and a variety of other proteinases have been described to act on single or multiple APP proteolysis products, which will not be discussed here further.

However, despite all these cleavage products of APP, A-beta peptide synthesis still remains crucial in mostly all attempts to explain pathogenesis (for a very short overview on mechanisms probably involved in AD pathogenesis, see Table 16.1). An early-stage clinical trial with the experimental drug BIIB037, a naturally occurring anti-A-beta antibody, resulted in reduced A-beta in the brain accompanied by reduced cognitive decline, bringing back momentum to the old amyloid hypothesis (comment by Underwood, 2015). These and other data we will present here sufficiently legitimize research approaches regarding alpha- and beta-secretase balance, although multiple factors besides A-beta obviously contribute to AD.

Table 16.1 Potential molecular causes underlying sporadic Alzheimer's disease.

Factors associated with Alzheimer's disease	Proteins involved	Damage-causing result
Tau hyperphosphorylation	GSK3b	Destabilization of microtubules
Decreased A-beta peptide clearance	IDE, neprilysin	Accumulation of toxic A-beta peptide oligomers
Overproduction of A-beta peptides	BACE-1, gamma-secretase, ADAM10(lack of)	Accumulation of toxic A-beta peptide oligomers, reduction in neurotrophic APPs-alpha
Misbalanced immune response	e.g., TREM2	Overshooting immune reaction or decreased phagocytosis of A-beta peptide aggregates
Disturbed cholesterol metabolism	APOE, Clu, ABCA7	Accumulation of toxic A-beta peptide oligomers
Deficits in endocytosis/ autophagy	SORL1, PICALM, Beclin1	Accumulation of toxic A-beta peptide oligomers
Decreased A-beta efflux from brain	LRP1, RAGE (lack of)	Accumulation of toxic A-beta peptide oligomers within the brain

Sporadic Alzheimer's disease is a multifactorial disease with many molecular pathways thought to be causal. This table only exemplarily names proteins that directly influence the two hallmarks of the disease: neurofibrillary tangles built of Tau protein and A-beta peptide oligomers.

Balance of alpha- and beta-secretase in aging and Alzheimer's disease

The molecular identity of alpha- and beta-secretase has been resolved—the aspartic protease BACE-1 has been characterized as the main physiological beta-secretase (Vassar et al., 1999). The finding that meprin-beta is also capable of cleaving APP reminiscent of BACE-1 complicates the picture (Bien et al., 2012). Meprin-beta resembles not a pure proamyloidogenic enzyme despite its A-beta releasing function but is able to activate ADAM10 (A disintegrin and metalloproteinase 10) as has been shown by a proteomic approach (Jefferson et al., 2013).

ADAM10 together with ADAM9 and ADAM17 has been discussed as a candidate for alpha-secretase activity. ADAM9 has been shown to increase basal and stimulated APPs-alpha release (Koike et al., 1999), but in an *in vitro* cleavage assay failed to cleave an APP-derived peptide at the main alpha-secretase site (Roghani et al., 1999). Additionally, ADAM9 knockout mice showed no reduction in alpha-secretase-derived cleavage products of APP (Weskamp et al., 2002). Similar to meprin-beta, ADAM9 acts on ADAM10 (e.g., see work by Cisse et al., 2005), and thereby is able to modulate APP processing indirectly. For ADAM10 and ADAM17, data obtained in cell culture and mice suggest a role as physiological alpha-secretases: analysis of ADAM17 revealed that the enzymatic activity seems to be more correlated with physiological stimuli such as interleukin 1 (Hall & Blobel, 2012), while ADAM10 has been confirmed to act as the major constitutive alpha-secretase in neurons (Postina et al., 2004; Kuhn et al., 2010; Jorissen et al., 2010).

The emerging picture of APP-processing events therefore gets increasingly complicated, but BACE-1 and ADAM10 seem to have central roles. When their expression in murine and human tissue is compared by expressed sequence tag (EST) profile, it becomes obvious that distribution and intensities are quite different (Fig. 16.2). For example, transcripts of both proteinases are detected in muscle within human experimental data but not in mice. Moreover, the ratio of enzyme transcripts varies between species—e.g., in bone, the amount of transcript species is comparable for mouse, while in humans BACE-1 clearly dominates over ADAM10. Despite the fact that occurrence of transcripts does not report on protein amount and activity, it is important that in brain, the occurrence and ratio of transcripts are nearly indistinguishable, which supports the use of mice as models for investigating the role of both proteinases in the CNS.

Mouse models with central nervous system manipulation of A disintegrin and metalloproteinase 10/beta-site amyloid precursor protein cleaving enzyme 1 expression

Several mouse models with manipulation of both proteinases by genetic means have been established; already in 2004, ADAM10-overexpressing mice were generated as well as

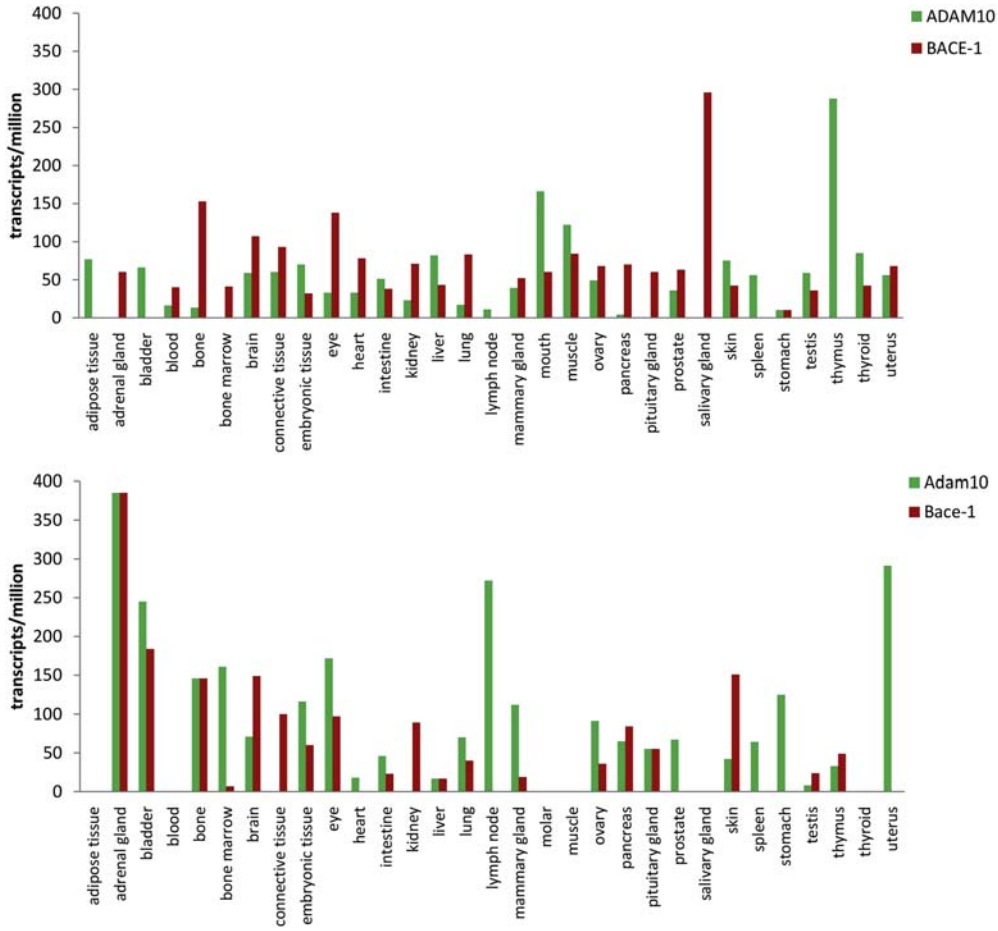

Figure 16.2 ***Comparison of secretase expression in human and murine tissue.*** Expressed sequence tag (EST) profiles are given as transcripts per million for human (upper graph) and murine (lower graph) tissue—data extracted from NCBI UniGene for ADAM10/Adam10 (Hs.578508/Mm.3037) and BACE-1/Bace-1 (Hs.504003/Mm.24044). Only tissue for which data of both species were available is included; EST data of brain are highlighted.

mice with expression of a dominant negative (dn) variant of the enzyme (Postina et al., 2004) and intensely investigated regarding APP processing and impact on the pathology of AD model mice (APPLd mice). Mice with moderate overexpression of ADAM10 (30% protein level increase above wild-type values) showed amelioration of learning deficits in crossbreeding experiments with Alzheimer model mice and displayed a shift toward nonamyloidogenic processing of APP, while dn mice showed the opposite (Postina et al., 2004). ADAM10 overexpression in Tg2576 mice reduced A-beta levels to about 30% at 3 months of age and resulted in a decrease of more than 99% at

12 months of age (Suh et al., 2013). The observed impact of ADAM10 on APP cleavage was also reflected by using wild-type mice and measuring endogenous APP-processing products (Clement et al., 2008). Expression of the transgenes was regulated by a deletion version of Thy1-promoter, restricting expression to neuronal cell populations and circumventing the embryonic lethality that had hampered investigation of knockout mice thus far (Hartmann et al., 2002). The more recent generation of ADAM10 conditional knockout mice contributed new data on ADAM10 CNS function: ADAM10 inactivation in neural progenitor cells (NPCs), NPC-derived neurons, and glial cells via a Nestin/Cre driver elongated the life span of knockout animals from E9.5 (Hartmann et al., 2002) to a perinatal time point (Jorissen et al., 2010). In these mice, a disrupted neocortex and a severe reduction of ganglionic eminence were observed, demonstrating the importance of ADAM10 for brain development. The prominent role for ADAM10 in APP processing was confirmed by a large decrease in APPs-alpha (Jorissen et al., 2010). Knockout of ADAM10 in the postnatal CNS by cross-breeding Adam10-floxed mice with CaMKII-alpha-Cre transgenic mice finally delivered adult mice with lack of the enzyme in the brain (Prox et al., 2013). Although this deficiency did not cause major morphological changes, synaptic dysfunction indicated by epileptic seizures and altered behavior, and an increase in early perinatal lethality occurred. This was accompanied by a decrease in APPs-alpha and an increase in APPs-beta, which was not observable in nontargeted brain regions. These results were reproduced by independent reestablishing of the mouse model (Zhuang, Wei, Lin, & Zhou, 2015).

In monotransgenic neuronally BACE-1-overexpressing mice, no plaques were detectable by an age of 14 months but augmented amyloidogenic APP-processing (e.g., Mohajeri, Saini, & Nitsch, 2004). Moreover, double-transgenic mice with mutated APP displayed increased A-beta levels and extracellular A-beta deposits surrounded by reactive astrocytes at 4 months of age. This indicates that BACE-1 alone is not sufficient to evoke pathogenesis in mice but that BACE-1 accelerates it. Two lines of BACE-1 knockout mice were established by exon replacement or exon deletion and both revealed no gross phenotypic deficiencies despite total loss of beta-secretase activity in primary cortical cultures and in brains (Roberds et al., 2001). Interestingly, a more recent publication reports on the knock-in of human BACE-1 in mice: the forebrain-specific expression of the human enzyme led to amyloidogenic processing of endogenous murine APP and behavioral deficits (e.g., habituation to a novel environment) at about 4 months of age (Plucinska et al., 2014). A 50% reduction in BACE-1 enzyme levels caused only a 12% decrease in A-beta levels in young AD model mice (3 months; PDAPP McConlogue et al., 2007;) but in older mice (aged 15 or 18 months) resulted in an impressive reduction in plaques, neuritic burden, and synaptic integrity. By using heterozygous BACE-1 gene knockout mice, it has been reported that this about 50% BACE-1 reduction rescued hippocampus- and cortex-dependent memory deficits of 5XFAD mice and resulted in reduced levels of C99, A-beta peptides, and plaque burden in target

regions of the brain (Devi & Ohno, 2015). In sum, these data clearly deciphered importance of BACE-1 as the physiological beta-secretase in the CNS. Inhibition of BACE-1 activity in peripheral body sites of wild-type mice or mice overexpressing APP reduced A-beta levels in the periphery (plasma) but was not sufficient to lower them in the brain (Georgievska et al., 2015). Additionally, BACE-1 heterozygous knockout mice displayed 62% reduced plasma A-beta40, whereas brain peptide level was only decreased by 11%. This suggests that BACE-1 in the periphery might not be a valuable target for AD-therapy and that it also might not be the rate limiting enzyme in the amyloidogenic processing pathway.

Evidence for a role of beta-site amyloid precursor protein cleaving enzyme 1 and A disintegrin and metalloproteinase 10 genetic variants in Alzheimer's disease

Certain mutations in APP or presenilin genes undoubtedly lead to early onset of AD. The role of gene variants of BACE-1 or ADAM10 in late-onset AD are not that evident; for the common single-nucleotide polymorphism (SNP) in exon 5 of BACE-1 (rs638405, Val262), a meta-analysis with data from four Chinese studies (248 AD patients and 224 healthy persons) showed a weak association with AD, which obtained only significance between the CC genotype and disease in the ApoE-epsilon4-positive cohort (Jo et al., 2008). This confirmed data from a study using a community-based sample of Caucasian individuals (Gold et al., 2003). BACE-1 SNP rs687740 was found to be significantly associated with AD in Down syndrome patients without any association with APOE-epsilon4 (Patel et al., 2011). By analyzing 11 different SNPs—including SNP rs687740—covering the whole BACE-1 gene, Laws and colleagues reported that no single marker or haplotype was associated with AD (Laws et al., 2011) A Swedish cohort of 269 AD patients was tested for impact of the BACE-1 gene on AD-related biomarkers. The authors reported the effect of BACE-1 gene variants on BACE-1 activity or on levels of A-beta 40, A-beta 42, APPs-alpha, and APPs-beta as well as total and phospho-tau181 (Sjolander, Zetterberg, Andreasson, Minthon, & Blennow, 2010).

When cognitively normal controls ($n = 170$) and AD patients ($n = 92$) were genotyped in regard to 19 putative regulatory-tagging SNPs within nine genes closely associated with APP processing (APP, ADAM10, BACE-1, BACE-2, PSEN1, PSEN2, PEN2, NCSTN, and APH1B), a significant association was found only after control for multiple comparisons between ADAM10 SNP rs514049 and APPs-alpha levels in an AD-specific manner (rs514049; Bekris et al., 2011). rs514049 is located 5′ to the ADAM10 core promoter region within a potential CREB/c-Jun transcription factor site at −644 bp (Prinzen, Muller, Endres, Fahrenholz, & Postina, 2005). This SNP was retested in a cohort of sporadic AD patients from the Chinese Han population and respective controls, but association with AD could not be confirmed (Zeng et al., 2015).

For SNPs at positions −279, −348, −630, and −927 within the ADAM10 promoter, no association of genotype distribution with AD was found (Prinzen et al., 2005). An elaborated study analyzing 27 SNPs covering the whole genomic sequence of human ADAM10 found no evidence for association of either SNP or haplotype with AD [50]. However, in 2012, the ADAM10 rs514049-rs653765 C-A promoter haplotype was reported to be correlated with higher postmortem brain hippocampal ADAM10 protein levels in subjects with low plaque score but not in those with high plaque score (Bekris et al., 2012).

In 2009, two rare potentially disease-associated mutations (Q170H and R181G) were identified in the ADAM10 prodomain coding sequence (Kim et al., 2009), which resulted in significantly lowered alpha-secretase activity and elevated A-beta levels in cell-culture studies. In AD model mice, introduction of both mutations in human ADAM10 attenuated alpha-secretase activity and shifted APP-processing toward the amyloidogenic pathway (Suh et al., 2013).

In sum, the emerging picture for BACE-1 and ADAM10 genetic variant contribution to AD remains rather controversial, although their role in AD-progression is undisputed.

Secretase expression in mild cognitive impairment and Alzheimer's disease

The premise of targeting beta- or alpha-secretase in regard to AD is that deregulation of expression and/or activity occurs in aging (preventive approach) or in disease (curative approach). In a former review, we collected knowledge about respective reports up to 2010 (Fig. 16.3; for exact references, see Endres & Fahrenholz, 2012). In the majority of publications, BACE-1 mRNA/protein or activity was described to be elevated in AD and also in all investigations regarding mild cognitive impairment (MCI). On the contrary, ADAM10 gene products and activity have mostly been found to be reduced in AD (11 out of 13 reports), while the sole report on ADAM10 in MCI recognized unaltered protein amounts in peripheral tissue (Gorham, Bark, Bjorkhem, Meaney, & Crisby, 2010).

Since our initial report, a limited number of publications has been published regarding secretase balance, but some of them reveal important new information; the Alzheimer's disease Neuroimaging Initiative released a manuscript describing unaltered BACE-1 activity in CSF in stable as well as progressive MCI and AD in comparison with normal controls (Perneczky & Alexopoulos, 2014). More interestingly, a report on physicochemical alterations in lipid rafts in frontal and entorhinal cortex from early AD stages might explain changes in BACE-1 activity or access to substrate: higher viscosity observed in plasma membranes obtained from diseased subjects might favor interaction of APP with the secretase and thereby amyloidogenic APP processing (Fabelo et al., 2014) even if the amount of the proteinase is not changed. This also consequently lowers substrate accessibility to cleavage by the alpha-secretase. Measurement of both

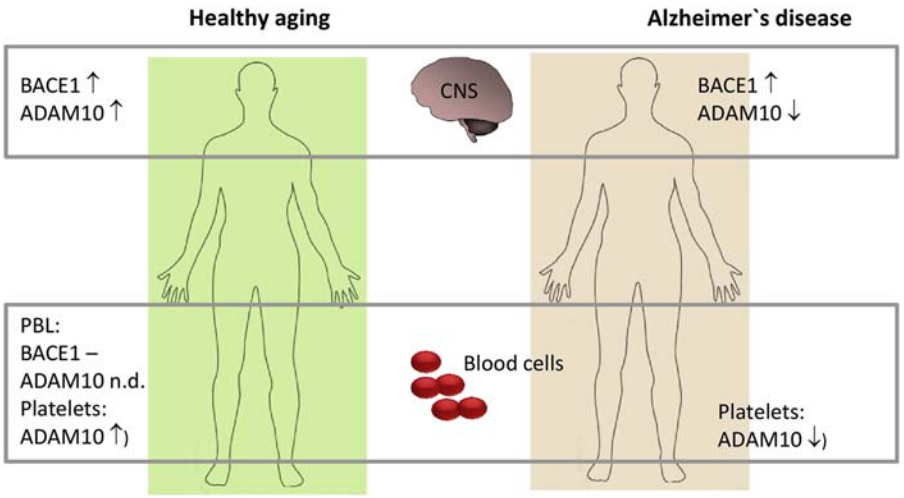

Figure 16.3 ***Secretases in the central nervous system and peripheral blood cells in cognitively healthy aging subjects and those with Alzheimer's disease (AD).*** Expression as well as enzyme activity of BACE-1 and ADAM10 in healthy and pathological aging (AD) have been analyzed. Despite some knowledge about blood cells (*PBL*, peripheral blood leukocytes; platelets), information from other peripheral tissue specimens is vastly lacking.

proteases in CNS is mostly restricted to postmortem samples based on their transmembrane characteristic; therefore, investigations using surrogate tissues from periphery occur as an attractive alternative. In 2002, Colciaghi and colleagues accounted ADAM10 from platelets to be reduced in AD (Colciaghi et al., 2002). Ensuing examination revealed that this lowered ADAM10 amount also correlates with cognitive performance in AD as demonstrated by MMSE scoring and a clock drawing test and therefore might represent a valuable biomarker (e.g., Manzine et al., 2014).

Secretase expression in normal aging

Data concerning secretase expression and/or activity in healthy aging are rather scarce but in our opinion, knowledge about this is quite important because the highest risk factor for late-onset AD is age per se. Measurement of BACE-1 activity in normal control brains (subjects ranging from 16 to 95 years; Miners, van, Kehoe, & Love, 2010) revealed that BACE-1 protein/activity and A-beta-degrading enzyme activities (IDE, neprilysin) increased with age, and BACE-1 activity closely correlated with age (Fig. 16.3). An elevation of BACE-1 was also reported for healthy, aged primates within the plaque-bearing cortex (Cai et al., 2010). Elaborating life span profiles of APP and BACE-1 delivered species-dependent results—while APP increased with age in mice, A-beta itself declined (Dosunmu et al., 2009). In monkeys, APP and BACE-1 mRNA also declined, but an

increase in both proteins as well as in A-beta was observed. Therefore, expression of one protease alone seems not valid as a predictor for balance of APP-processing, and mice probably are not the best model to investigate age-dependent processes linked to AD.

Cell culture-based approaches describe changes in both processing capacity and localization of proteins involved in APP cleavage along with aging; APP showed reduced expression in an aging human fibroblast cell line (IMR-90; Kern, Roempp, Prager, Walter, & Behl, 2006). Moreover, intracellular APP cleavage products C99, C83, and AICD declined, and a reduced secretion of soluble APP species occurred. While a progressive decrease in gamma-secretase enzymatic activity was measured, ADAM10 as well as BACE-1 protein levels remained unchanged with prolonged life span. However, BACE-1 enzymatic activity was increased in aged cells. This was confirmed by the increased protein–protein interaction of APP and BACE-1 found in the IMR-90 fibroblast cellular aging model with an endosomal accumulation of this protein complex accompanied by an increased A-beta amount (Zou, Yang, Zhang, & Dai, 2010). As human fibroblasts have been successfully reprogrammed to iPSC and further on to neurons, this might resemble a highly relevant model in future approaches to investigating APP processing in the aging neuronal cell.

Surrogates from the periphery can be obtained not only by fibroblasts but also from blood as described in the prior paragraph. An endpoint RT-PCR-analysis of samples derived from a small cohort of healthy individuals ($n = 12$; Herrera-Rivero, Soto-Cid, Hernandez, & Aranda-Abreu, 2013) aged 30–80 years revealed no changes in leukocyte BACE-1 and APP expression levels. A study that aimed at comparing brain and leukocyte APP processing reported that besides the occurrence of different APP fragments, ADAM10 was present in brain but undetected in the blood leukocyte fraction (peripheral blood leukocytes, or PBLs; Delvaux, Bentley, Stubbs, Sabbagh, & Coleman, 2013). BACE-1, on the contrary, was detected in most samples despite the age of the respective subject. This discrepancy between brain and leukocyte characteristics might hamper their use as models. Interestingly, our own study indicated ADAM10 expression in PBMCs (peripheral mononuclear blood cells (Schuck, Wolf, Fellgiebel, & Endres, 2016)) that include monocytes in contrast to mere PBLs. Because PBMCs as well as PBLs only showed a minor amount of full-length APP (Delvaux et al., 2013; Schuck et al., 2016) suitable as a substrate to the secretases, we suggest that platelets might serve as a better model to analyze age-dependent changes in APP processing. Using three groups of cognitively healthy subjects, we described the elevation of ADAM10 protein amounts as well as the catalytic function of alpha-secretase along with age (Schuck et al., 2016).

Several hints exist that might explain disturbances of APP processing during one's lifetime that might end in AD: oxidative stress as well as the accumulation of metabolic remnants such as advanced glycation end products or stress-induced insulin resistance might confer development of the disease in older age (e.g., Solas, Aisa, Tordera, Mugueta, & Ramirez, 2013). Interestingly, a *vice versa* linkage of APP secretases and

life span has been found by characterizing alpha-, beta-, and gamma-secretase as being responsible for Klotho cleavage (Bloch et al., 2009), a protein that has been designated as a longevity factor.

In sum, investigating secretase expression and function relative to aging seems difficult according to available models for CNS-centered changes in the aging process. Nevertheless, understanding which factors contribute to the persistence of balanced APP processing or how balance might be restored is crucial for therapeutic approaches and needs further investigation.

Key facts of proteases acting on the amyloid precursor protein

- Several proteases are capable of cleaving APP.
- APP can be cleaved within its ectodomain, the transmembrane proportion or even in its cytosolic domain.
- These cleavage events lead to proteolytic fragments with a potentially wide range of biological functions.
- Beta-secretase BACE-1 leads to production of neurotoxic A-beta peptides by processing APP.
- The alpha-secretase ADAM10, on the contrary, prevents A-beta formation and releases neuroprotective APPs-alpha from the precursor protein.

Summary points

- In this chapter, we summarized present knowledge on the expression/activity balance of the AD-relevant proteases BACE-1 and ADAM10.
- Genetically modified mice showed that balancing both proteases has a tremendous impact on, for example, plaque formation and learning/memory.
- Genetic association studies in humans have revealed single SNPs in both genes that may contribute to AD; however, data have sometimes not been reproducible.
- In AD, most reports indicate an increase in BACE-1 expression/activity, while ADAM10 seems to decrease.
- For the factor "aging," the most prominent risk factor for AD, data on ADAM10 and BACE-1 expression are scarce.
- Mild pharmacological inhibition of BACE-1 or increase of ADAM10 would consequently restore the physiological homeostasis of APP processing and further prevent the progression or even onset of AD.

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CHAPTER 17

Methylation analysis of DNA in Alzheimer's disease

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List of abbreviations

Aβ amyloid-beta peptide
ABCA7 ATP-binding cassette, subfamily A, member 7
AD Alzheimer's disease
ADAM17 ADAM Metallopeptidase Domain 17
ANK1 ankirin 1
APOE apolipoprotein E
APP amyloid precursor protein
BACE1 β-site APP cleaving enzyme 1 (beta secretase)
BDNF brain-derived neurotrophic factor
BIN1 bridging integrator 1
D-loop displacement-loop
DNMT DNA methyltransferase
EWAS epigenome-wide association study
HLA-DRB5 major histocompatibility complex, class II, DR beta 5
HOXA3 homeobox A3
LOAD late-onset Alzheimer's disease
LRRC8B leucine rich repeat containing 88 VRAC subunit B
MAP2 microtubule-associated protein 2
MAPT microtubule associated protein tau
MCF2L MCF.2 cell line–derived transforming sequence like
MCI mild cognitive impairment
mtDNA mitochondrial DNA
NGS next-generation sequencing
PSEN1 presenilin 1
PSEN2 presenilin 2
S100B S100 calcium-binding protein B
SLC24A4 solute carrier family 24 member 4
SORL sortilin-related receptor 1
STK32 serine/threonine kinase 32C
TET ten-eleven translocation
TMEM59 transmembrane protein 59
TREM2 triggering receptor expressed on myeloid cells 2
5-hmC 5-hydroxymethylcytosine
5-mC 5-methylcytosine

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Mini-dictionary of terms

DNA methylation The addition of a methyl group (CH_3) to cytosine forming 5-methylcytosine (5-mC), usually in a CpG dinucleotide context. DNA methylation in the promoter region is usually associated with gene silencing.

Epigenetics The term literally means above genetics and refers to molecular mechanisms regulating gene expression levels, but not involving changes of the DNA sequence. Epigenetic mechanisms include DNA methylation, histone tail modifications, nucleosome positioning, and gene silencing mediated by noncoding RNAs, and take part in several physiological processes, such as cell differentiation and development, maintenance of the cellular identity and silencing of repetitive elements in differentiated cells, genomic imprinting, X-chromosome inactivation, and learning and memory processes.

Epigenetic biomarker A measurable epigenetic signature that is an indicator of a particular biological condition or process. Particularly, the chapter describes methylation biomarkers of Alzheimer's disease.

Epigenome-wide association study (EWAS) The analysis of DNA methylation or hydroxymethylation on a genome-wide scale.

Mitoepigenetics Epigenetic modifications occurring in the mitochondrial DNA.

Introduction

It is estimated that almost 50 million people worldwide are living with dementia, and that this number will more than double by 2050, only as a consequence of the global aging of the human population (World Alzheimer Report, 2015). Alzheimer's disease (AD) is the primary form of dementia in the elderly, accounting for about 60%–80% of the cases, and the most common neurodegenerative disorder in humans (Alzheimer's Association, 2017). Only about 1% of the total AD cases are monogenic forms, and with few exceptions they are transmitted in families following an autosomal dominant inheritance pattern (Alzheimer's Association, 2017). These familial AD cases result from mutations in one of three major AD genes, namely *APP*, *PSEN1*, and *PSEN2*, coding respectively for the amyloid precursor protein, and for presenilin 1 and 2 proteins, all involved in the production of the amyloid β (Aβ) peptide, the neurotoxic peptide that accumulates in brain regions of AD patients forming the extracellular senile plaques (Reitz & Mayeux, 2014).

Most of AD is however sporadic, occurs in people of advanced age (LOAD = late-onset AD), and results from lifelong interactions among genetic, environmental, and stochastic factors, superimposed on the age-related accumulation of neuronal damage (Migliore & Coppedè, 2009). More than 20 LOAD susceptibility loci, mainly coding for proteins involved in immune response, lipid metabolism, and endolysosomal pathways, have been identified so far, but except for the apolipoprotein E (*APOE*) *ε4* allele that increases AD risk from threefold in heterozygous carriers up to twelvefold in homozygous carriers, all the other loci have a limited effect on the overall prevalence of AD either because they are rare or only slightly increase disease risk (Pimenova, Raj, & Goate, 2017). Several nongenetic factors including aging, serious head injuries, type 2 diabetes, high blood pressure, midlife obesity, hyper-homocysteinemia, stroke, smoking, depression, metal and pesticide exposure, sedentary lifestyles, and dietary habits have been suggested to

contribute to cognitive decline and dementia (Alzheimer's Association, 2017; Killin, Starr, Shiue, & Russ, 2016). In this context, epigenetic mechanisms, resulting from the interplay between the environment and the genome, could provide a mechanistic explanation that might help our understanding of AD pathogenesis (Stoccoro & Coppedè, 2018). Among epigenetic modifications, DNA methylation represents one of the most stable and studied marks, and the present chapter is a narrative review of studies investigating DNA methylation in AD samples, as well as in cell cultures and animal models of the disease. Due to either word or reference limits, and following the editorial requirements for this book, the author will not go into details of each single study or perform a systematic review of the literature but rather will provide a general overview of the state of the art in this field describing some relevant studies, and making a critical summary of the main findings.

DNA methylation and hydroxymethylation

DNA methylation consists of the addition of a methyl group to the DNA, mediated by enzymes called DNA methyltransferases (DNMTs), and represents a physiological mechanism required for genomic imprinting, X-chromosome inactivation, embryonic development, cell differentiation, and maintenance of the cellular identity, as well as for the repression of repetitive elements (Jones, 2012). Soon after fertilization both the paternal and the maternal genome are demethylated, except for imprinted regions, in order to allow embryonic stem cells to become pluripotent. After implantation, cell type–specific DNA methylation patterns are established during mammalian development by de novo DNMTs (DNMT3A and DNMT3B) and maintained in adult somatic cells by the maintenance enzyme (DNMT1) (Chen & Riggs, 2011). The best-characterized DNA methylation process is the addition of a methyl group to cytosine in CpG sites, forming 5-methylcytosine (5-mC), but in certain cells, DNA methylation occurs also at non-CpG sites (CpA, CpT, and CpC) (Jang, Shin, Lee, & Do, 2017). The general functions of CpG and non-CpG methylation include gene silencing or activation depending on the methylated regions. For example, when CpG-rich regions in the promoter of a gene are methylated, the expression of that gene is repressed because methyl-CpG-binding domain (MBD) proteins recognize and bind to the methylated DNA, and in turn recruit other epigenetic factors to enhance chromatin remodeling and transcriptional repression (Jones, 2012). However, sites of CpG and non-CpG methylation are found throughout the whole genome, including repetitive sequences, enhancers, promoters, and gene bodies. Interestingly, non-CpG methylation is restricted to specific cell types, and is prevalent in human embryonic stem cells, neurons, and glial cells (Jang et al., 2017). 5-Hydroxymethylcytosine (5-hmC) is another modification of cytosine resulting from the oxidation of 5-mC mediated by members of the ten-eleven translocation (TET) protein family. Originally, it was believed that 5-hmC was only an intermediate of an active demethylation of cytosine, but the specific

distribution of 5-hmC in mammalian brain regions and its lower affinity for MBD proteins than 5-mC has revealed that 5-hmC is another important epigenetic mark with suggested roles in neurodevelopmental and neurodegenerative disorders (Wen & Tang, 2014). Overall, it is emerging that DNA methylation at either CpG or non-CpG sites, as well as DNA hydroxymethylation, are important epigenetic players in brain development and memory formation, and that their impairment could contribute to neurodevelopmental, neurobehavioral, and neurodegenerative disorders (Coppedè, 2018; Jang et al., 2017).

Early studies of DNA methylation in cell cultures and animal models of Alzheimer's disease

Several studies were done in the early 2000s in order to investigate environmental factors potentially leading to an epigenetic dysregulation of AD-related genes in cell cultures and animal models of the disease (reviewed in Coppedè, 2010). Some of the most important findings from those investigations were the following: (1) Studies in neuronal cell cultures as well as in rodents demonstrated that deprivation of B vitamins, required for proper DNA methylation reactions, induced demethylation of the *PSEN1* gene, followed by increased production and accumulation of the Aβ peptide in animal brains (Fuso et al., 2005, 2008); (2) Early life exposure of rodents and monkeys to lead (Pb) enhanced the expression of genes associated with AD, such as *APP*, and increased the burden of oxidative DNA damage in the aged brain (Wu et al., 2008); (3) It was also shown that the Aβ peptide induced global DNA hypomethylation in murine cerebral endothelial cells (Chen et al., 2009). Collectively, those studies have revealed that early life environmental exposures can induce lifelong lasting epigenetic modifications of AD-related genes, but also that epigenetic modifications can result from the accumulation of disease-related neurotoxic peptides, such as the Aβ one. The question of whether epigenetic changes observed postmortem in AD specimens are cause or consequence of the disease is still largely debated, mostly because it is difficult, from the analysis of postmortem tissues, to discriminate between early epigenetic modifications preceding and triggering disease onset from those resulting as a consequence of the disease (Stoccoro & Coppedè, 2018). This has led to the search of peripheral epigenetic biomarkers of AD, such as epigenetic changes occurring in blood cells that could be measured in living individuals from the preclinical to the advanced disease stages (Fransquet et al., 2018).

Methylation studies in Alzheimer's disease tissues

Several approaches have been used to investigate DNA methylation and hydroxymethylation in AD specimens (Stoccoro & Coppedè, 2018). These include quantification of global DNA methylation or hydroxymethylation levels by immunohistochemistry on

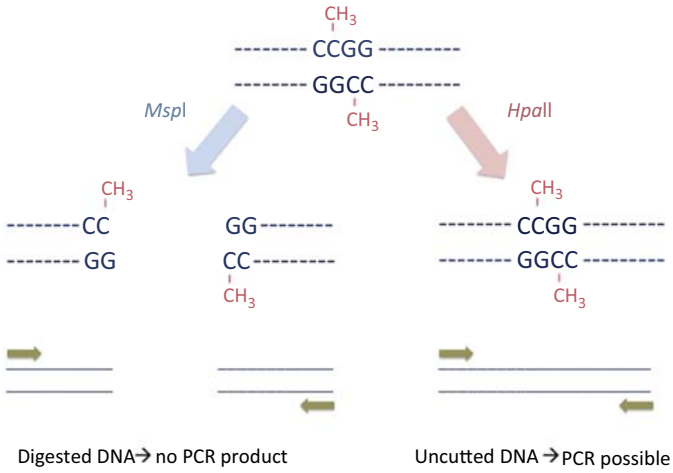

Figure 17.1 ***Detecting DNA methylation with restriction enzymes.*** *Hpa*II is a methylation-sensitive restriction enzyme that recognizes and cuts the CCGG sequence only if unmethylated. By contrast, *Msp*I is able to cut the same sequence even if methylated. Their combined use allows obtaining the methylation status of DNA regions containing the CCGG sequence in a subsequent polymerase chain reaction (PCR).

postmortem brain tissues using antibodies against 5-mC or 5-hmC (Lashley et al., 2015; Mastroeni, McKee, Grover, Rogers, & Coleman, 2009), as well as gene-specific methylation and whole-genome methylation analysis by means of approaches that basically rely on the use of restriction enzymes, on bisulfite conversion, and on affinity enrichment-based methods (Yong, Hsu, & Chen, 2016). Methylation-sensitive and methylation-dependent restriction enzymes cut respectively unmethylated or methylated DNA sequences and can be used to obtain information on their methylation status (Fig. 17.1). Treating genomic DNA with sodium bisulfite converts unmethylated cytosine into uracil, while 5-mC residues remain unchanged (Fig. 17.2). Affinity enrichment-based methods use either MBD proteins or antibodies against 5-mC to capture methylated DNA regions for subsequent investigations. After enzymatic digestion, bisulfite conversion or affinity enrichment of the genomic DNA, PCR-based methods or sequencing technologies have been used to detect the methylation levels of selected sequences (Yong et al., 2016). The advances in microarray and next-generation sequencing (NGS) technologies have allowed methylation and hydroxymethylation investigations on a genome-wide scale (Fig. 17.3), the so-called epigenome-wide studies (EWASs), as well as the possibility to perform even single-cell or single-strand methylation analysis (Yong et al., 2016). A summary of the main findings of DNA methylation studies in AD is provided (Table 17.1).

The immunohistochemical quantification of global 5-mC or 5-hmC content in postmortem AD brain regions has produced conflicting and still inconclusive results (Condliffe et al., 2014; Coppieters et al., 2014; Ellison, Abner, & Lovell, 2017; Lashley

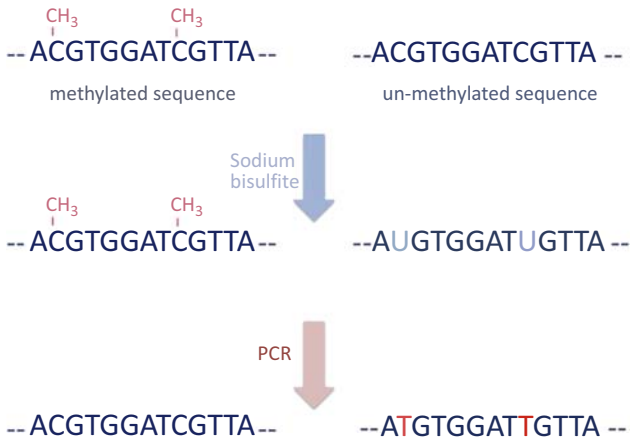

Figure 17.2 ***Bisulfite treatment for the detection of DNA methylation.*** Treating genomic DNA with sodium bisulfite converts all unmethylated cytosines (C) into uracil (U), while 5-mC residues remain unchanged. Uracil eventually converts to thymine (T) in a subsequent polymerase chain reaction (PCR), so that the methylation status of a given sequence can be easily detected.

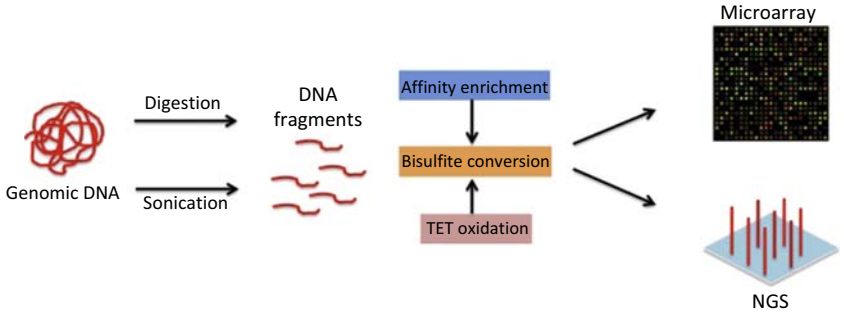

Figure 17.3 ***Whole-genome methylation analysis.*** Whole-genome methylation analyses are based on next-generation sequencing (NGS) or microarray technologies. The genomic DNA can be digested with restriction enzymes or sonicated to obtain DNA fragments, and affinity enrichment-based methods, such as proteins or antibodies that bind to methylated cytosines, are often used to capture the methylated fragments before analyzing by microarray or NGS platform. Bisulfite conversion and ten-eleven translocation (TET) oxidation of the genomic DNA, the latter allowing distinguishing between 5-mC and 5-hmC, can be used for whole-genome methylation or hydroxymethylation analyses.

et al., 2015; Mastroeni et al., 2009), likely due to the small sample size of investigated cohorts, to the different areas of the brain investigated, as well as to differences in study design that often make it difficult to compare the obtained results (Stoccoro & Coppedè, 2018). Recent studies on postmortem material have also indicated that global 5-mC or 5-hmC levels change within disease progression from preclinical dementia or mild cognitive impairment (MCI) to later dementia stages (Ellison et al., 2017), and this could be another reason for the conflicting nature of the results produced so far.

Table 17.1 Overview of DNA methylation studies in Alzheimer's disease.

Main findings	References
Certain early life environmental stressors, including deficiency of B-group vitamins or lead exposure, led to methylation changes of genes required for the Aβ peptide production, and altered the expression of proteins involved in the epigenetic machinery in neuronal cell cultures and animal models	Fuso et al. (2005), Fuso et al. (2008), Wu et al. (2008)
Global DNA methylation investigations (quantification of 5 mC and 5 hmC content, or methylation levels of repetitive elements) in brain samples as well as in peripheral blood, led to conflicting results in the comparison of AD and control tissues	Condliffe et al. (2014), Coppieters et al. (2014), Ellison et al. (2017), Lashley et al. (2015), Mastroeni et al. (2009), Ellison et al. (2017)
The methylation analysis of major AD genes, such as those involved in Aβ peptide production and in neurofibrillary tangles formation (*APP*, *PSEN1*, *PSEN2*, *BACE1*, *MAPT*), showed conflicting and inconclusive results in the comparison of AD and control blood or postmortem brain samples	Barrachina and Ferrer (2009), Brohede et al. (2010), Carboni et al. (2015), Iwata et al. (2014), Piaceri et al. (2015), Tannorella et al. (2015), West et al. (1995)
TREM2 and *BDNF* genes are among the most replicated differentially methylated genes in AD specimens, suggesting that they could represent promising epigenetic biomarkers of the disease	Celarain et al. (2016), Chang et al. (2014), Ma et al. (2015), Nagata et al. (2015), Ozaki et al. (2017), Smith et al. (2016), Rao et al. (2012), Xie et al. (2017)
EWASs in AD brain tissues have revealed several hundreds of differentially methylated and hydroxymethylated regions compared to healthy brains	Bernstein et al., 2016, Zhao et al., 2017, Gasparoni et al., 2018
Recent studies suggest a possible contribution of mitochondrial DNA methylation in AD pathogenesis	Blanch et al. (2016), Stoccoro et al. (2017)

Gene-specific methylation analysis in postmortem AD brains was initially centered on dementia-related genes, including *APP*, *PSEN1*, *PSEN2*, *BACE1*, or *MAPT*, required for the production of the Aβ peptide or involved in neurofibrillary tangle formation (Barrachina & Ferrer, 2009; Brohede, Rinde, Winblad, & Graff, 2010; Iwata et al., 2014; West, Lee, & Maroun, 1995). Those studies were conflicting, often limited to a few subjects, and overall failed to provide clear and replicated evidence that these genes could represent epigenetic biomarkers of AD; similar results were obtained when

addressing their methylation status in blood DNA of living AD patients (Carboni et al., 2015; Piaceri et al., 2015; Tannorella et al., 2015). More recent approaches that took advantage of whole-genome methylation investigation in sorted neuronal and nonneuronal cells to search for AD-associated methylation signals revealed cell-type-specific methylation changes in the *APP* gene (Gasparoni et al., 2018), suggesting that the question of whether or not major AD genes are epigenetically dysregulated in AD brains is still open.

Several other genes involved in LOAD susceptibility, inflammation, synaptic plasticity, neuronal function, and other AD-related pathways have been investigated in either blood or neuronal DNA samples (Fransquet et al., 2018). Changes in the methylation pattern of *ANK1*, *SORL1*, *ABCA7*, *BIN1*, *HLA-DRB5*, *SLC24A4*, *TREM2*, *BDNF*, *APOE*, and many other genes have been detected in DNA samples from postmortem AD brains (Celarain et al., 2016; De Jager et al., 2014; Foraker et al., 2015; Lunnon et al., 2014; Rao, Keleshian, Klein, & Rapoport, 2012; Smith et al., 2016; Yu et al., 2015). Many genes have been also investigated in over 40 case-control studies using blood DNA samples for the search of peripheral methylation biomarkers of AD (reviewed in Fransquet et al., 2018). Unfortunately, most of these studies are limited in sample size, and results are often conflicting or lack replication, so that the clinical utility of the identified biomarkers is still unclear (Fransquet et al., 2018).

Epigenetic modifications are cell-specific events, and the strong variation of cell-type proportions across brain tissue samples, coupled with the fact that the cortex cell-type composition changes upon disease progression, is likely one of the major causes of the conflicting nature of the findings observed so far when searching for AD-related methylation signals in postmortem brain samples (Gasparoni et al., 2018). Similarly, small case-control cohorts, different methodological approaches, as well as lifestyles, and nutritional, environmental, geographic, and ethnic factors, could be reasons for the differences observed when searching for peripheral epigenetic biomarkers of the disease (Stoccoro & Coppedè, 2018). For example, both *TREM2* and *BDNF* are among the most replicated methylation biomarkers of AD in both brain and peripheral tissues (Celarin et al., 2016; Chang et al., 2014; Ma et al., 2015; Nagata et al., 2015; Ozaki et al., 2017; Rao et al., 2012; Smith et al., 2016; Xie et al., 2017), and it was suggested that increased peripheral *BDNF* promoter methylation might predict the conversion from amnestic MCI to AD (Xie et al., 2017), but significant differences in average *BDNF* promoter methylation have been observed in different MCI populations, pointing to the contribution of either environmental or population-specific factors that still require clarification (Ma et al., 2015).

Several hundreds of differentially methylated or hydroxymethylated CpG sites are emerging from recent epigenome-wide methylation and hydroxymethylation studies performed in postmortem AD brain tissues (Bernstein et al., 2016; Gasparoni et al., 2018; Zhao et al., 2017). For example, a recent EWAS performed on sorted neuronal and nonneuronal nuclei from postmortem human brain tissues revealed neuronal or glia-specific associations with AD Braak stage progression at genes such as *MCF2L*, *ANK1*, *MAP2*,

LRRC8B, *STK32C*, and *S100B*, and validated previous AD-associated DNA methylation signals (Gasparoni et al., 2018). Similarly, a recent investigation of postmortem AD prefrontal cortices revealed more than 500 differentially hydroxymethylated regions associated with senile plaques and 60 differentially hydroxymethylated regions potentially involved in neurofibrillary tangle formation (Zhao et al., 2017). With respect to candidate-gene methylation investigations, often conducted in a different manner, EWASs have the advantage to use commercially available arrays or NGS platforms, so that data are often comparable among studies and meta-analyses can be performed. Indeed, a recent meta-analysis of EWASs in AD brains revealed significant methylation signals in *APP*, *ADAM17*, and *HOXA3* genes (Gasparoni et al., 2018; Smith et al., 2018).

Most of the studies addressing DNA methylation in AD focused on nuclear DNA methylation changes, and little attention was given to the mitochondrial DNA (mtDNA). Only in recent years was it suggested that epigenetic modifications of the mtDNA, the so-called "mitoepigenetics," might occur in AD and other neurodegenerative conditions, but evidence is limited to a few studies (Blanch, Mosquera, Ansoleaga, Ferrer, & Barrachina, 2016; Stoccoro, Siciliano, Migliore, & Coppedè, 2017). Particularly, aberrant DNA methylation of the mitochondrial displacement-loop region (D-loop) was observed in brain regions of individuals in preclinical stages of AD, in the substantia nigra of patients with Parkinson's disease, in AD mice, and in peripheral blood of AD patients (Blanch et al., 2016; Stoccoro et al., 2017). The D-loop region is critical for mtDNA replication and transcription, and recent studies in patients with amyotrophic lateral sclerosis revealed that an increased methylation of this region is linked to a decreased copy number of the mtDNA (Stoccoro et al., 2018). The emerging evidence of altered mtDNA methylation in AD and other neurodegenerative disorders warrants further research in the field.

Conclusions

DNA methylation changes linked to AD pathogenesis have been increasingly investigated in recent years and there is substantial evidence suggesting that hundreds of regions change their methylation status in the brain of the affected patients. The heterogeneous composition of the brain in terms of different neurons and glial cells, and the loss of neurons as a consequence of the degenerative process, are among the major sources of variability in the comparison of postmortem brain samples, likely contributing to the conflicting nature of the findings obtained so far when addressing methylation and hydroxymethylation marks. Other sources of variability include relatively small numbers of case and control brains, handling and storage conditions, and the different methodological approaches used to investigate DNA methylation (Coppedè, 2018). In addition, postmortem brain tissues from patients deceased from dementia are often from advanced-disease stages, several brain regions are atrophic as a consequence of neuronal death, and there is no way to understand if the observed methylation marks had a role in disease onset, or only resulted from amyloid

plaque burden, inflammation, oxidative stress, and several other pathways that are compromised during disease progression, posing several questions on their clinical utility (Stoccoro & Coppedè, 2018). Also the studies in peripheral blood DNA samples of living AD patients, which have been recently systematically reviewed by Fransquet et al. (2018), have not yet produced robust results, due to different panels of investigated genes among studies, different methodological approaches, and relatively low sample size of case-control cohorts (Fransquet et al., 2018). Recent genome-wide technologies have the potential to overcome these limits, cell sorting and single-cell methylation analyses are nowadays possible, arrays and platforms are commercially available, and data can be shared by different groups and used in meta-analyses to avoid the limits posed by small cohorts (Gasparoni et al., 2018). These technological improvements will allow better investigation of the epigenetic landscape in AD, and it will be possible to clarify the role of emerging epigenetic marks, such as non-CpG methylation, RNA methylation, and mtDNA methylation. Understanding the temporal and cell-type specific modifications that underlie AD pathogenesis, and determining their functional consequences, may provide novel molecular markers of the disease and potential targets for therapeutic interventions (Stoccoro & Coppedè, 2018).

Another key issue in AD epigenetics is to clarify the contribution of environmental factors to the observed DNA methylation changes, in order to set up preventative interventions. Current evidence is limited to a few factors, particularly lead exposure and B-group vitamin restriction that induced methylation changes and AD-like pathology in animal models and neuronal cell cultures (Fuso et al., 2005, 2008; Wu et al., 2008). Studies in humans are limited to a small amount of data suggesting that circulating folates and related B-group vitamins correlate with peripheral blood DNA methylation levels in both AD and matched control subjects (Grossi et al., 2016). In this regard, several studies performed in both in vitro and in vivo models of AD have shown that folic acid or other methyl donor compounds increase the DNA methylation potential and DNMT activity, modify DNA methylation, and ultimately decrease APP, PSEN1, and Aβ protein levels (Fuso et al., 2012; Li et al., 2015), so that the identification of either natural or synthetic compounds able to counteract impaired DNA methylation could represent a promising strategy to prevent or delay cognitive decline and neurodegeneration.

Key facts of DNA methylation in Alzheimer's diseases

- DNA methylation is a covalent chemical modification of the DNA and represents a physiological mechanism required for neuronal differentiation, brain development, and memory formation.
- Changes in DNA methylation have been largely documented in AD tissues at either global or single-gene level.

- DNA methylation marks are cell specific, and vary among brain regions that are composed by different cell types.
- Global DNA methylation marks change from early to late dementia stages, so that it is difficult to discriminate between early and late epigenetic changes.
- Results of different studies are often conflicting or replication is missing, so that it is still too early to translate research findings into the clinical settings.

Summary points

- The chapter focuses on the investigation of DNA methylation levels in Alzheimer's disease.
- Early life environmental stimuli led to life-lasting methylation changes of AD-related genes in brain regions of rodents and monkeys.
- Despite that the results of different studies are often conflicting, both DNA methylation and hydroxymethylation changes have been largely documented in AD tissue, and likely contribute to disease onset and progression.
- The pathological consequences of the epigenetic modifications observed in AD brains are still little understood, and the main question is if they are cause or are a consequence of the neurodegenerative process.
- The search for methylation biomarkers in peripheral blood DNA of AD patients led to encouraging results, but more robust studies are needed to confirm the preliminary findings.
- Little is still known concerning the environmental factors that contribute to AD-related epigenetic changes in humans.
- Impaired mitochondrial DNA (mtDNA) methylation could contribute to AD pathogenesis, but the available evidence is limited, and further investigation is greatly encouraged.

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CHAPTER 18

The signalosome malfunctions in age-associated neuropathologies

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List of abbreviations

AA Arachidonic acid
AD Alzheimer's disease
ALA α-linolenic acid
APP Amyloid precursor protein
BACE β-secretase
DHGA Docosahexaenoic acid
DLB Dementia with Lewy bodies
EPA Eicosapentaenoic acid
PD Parkinson's disease
PUFA Polyunsaturated fatty acid
STK Src tyrosine kinase
α-syn α-synuclein

Mini-dictionary of terms

Alpha-synuclein A small-sized protein that belongs to the synuclein family, together with beta- and gamma-synucleins. This protein is highly abundant in the cytoplasm of neurons where it appears to show a multifunctional profile. The role of alpha-synuclein in the cell has been related to the regulation of synapsis, vesicle transport, and fusion to the membrane in the synaptic terminal, and neurotransmitter release. Furthermore, this protein has the ability to self-aggregate in different configurations forming neurotoxic deposits, such as Lewy bodies and Lewy neurites. These structures are highly abundant in PD brains and other synucleopathies.

APP The amyloid precursor protein (APP) is a transmembrane protein that produces in its enzymatic processing the amyloid beta peptide. This peptide is released to the extracellular space where it can form insoluble toxic aggregates named senile plaques.

BACE1 An essential protein for the β-amyloid peptide generation. This enzyme encoded by BACE1 gene is responsible for the first step in the endoproteolytic processing of the amyloid precursor protein (APP).

Biomarker Any quantifiable indicator that allows the identification of a particular biological state or condition.

Docosahexaenoic acid The docosahexaenoic acid (DHA) is the most abundant polyunsaturated omega-3 fatty acid in phospholipids of the plasma membrane. It is particularly abundant in the brain. The docosahexaenoic acid uptake is essential for the adequate maintenance of brain function throughout a lifespan.

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Homeostasis The ability of an organism to keep its internal physiological parameters stable through autoregulation of systems in response to external incident factors.

Lipid rafts Microdomains differing from the adjacent plasma membrane. Their particular lipid and protein composition confers a higher degree of packaging, density, and viscosity. Consequently, lipid rafts are considered functional microstructures regardless of the cell membrane.

Neuronal plasticity Neurons' ability to adapt their response according to the adjacent microenvironmental inputs as well as physiological parameters. Thus, neurons can modify their neuronal network by adding or reducing connections with other neurons or create new synapses to adapt their activity to new stimuli.

Prion protein A small-sized protein with the ability to self-aggregate particularly in microenvironmental circumstances. Prions are capable of inducing the formation of aberrant molecular aggregates and toxic intracellular signaling.

Proteinopathies This term refers to a group of diseases that have the pathological misfolding and aggregation of small protein components as a response to particular microenvironmental parameters in the cell in common. These aggregates create insoluble deposits that contribute to the progression of the disease. Among the common known proteinopathies are AD, PD, synucleopathies, and prion diseases.

Src tyrosine kinases A family of kinases composed of different members that are distributed in numerous cell types. Interaction of these kinases with other proteins induces the phosphorylation of tyrosine residues. Among the members of this family that play important roles in neural cells are Src, Fyn, and Lyn. These proteins are involved in cell proliferation, differentiation, and neuroprotection.

Signalosome Signaling platforms that modulate different intracellular responses. It is a multimolecular complex formed by lipid and protein species contributing to cell adaptation to different stress conditions following the extracellular stimuli.

Synucleopathies A general term to define neurodegenerative diseases that have in common the accumulation of alpha-synuclein protein aggregates in the nervous system. These conformations are detected in different parts of the neurons, glial cells, and even nerve fibers. The most common synucleopathies are PD and dementia with Lewy bodies. People suffering from these diseases have similar symptoms, such as tremor, postural instability, and cognitive impairment.

Introduction

The brain is one of the fattiest organs in the body. Lipid content plays a crucial role in neuronal functionality and plasticity, and it is rarely used as a source of energy. The brain contains the highest amount of different phospholipid species of the whole organism. More than 5000 lipid species have been identified in the brain, distributed between gray and white matter. Lipid homeostasis in the brain is a key factor for this organ preservation, and changes in lipid content correlate with brain dysfunctioning. Characterizing neurolipidomic changes occurring in the brain during a lifespan represents a challenging and promising field whose interpretation may contribute to predict and elucidate potential anomalies associated with neuropathologies.

The neuronal membrane is a dynamic interface capable of reordering and modifying its composition to adapt to changes in the molecular microenvironment. Lipid molecules are unevenly distributed in the neuronal membrane, forming distinct microstructures that participate in neuronal activities. Currently, it is generally accepted that the plasma membrane is composed of liquid-ordered and liquid-disordered regions that exhibit different

lipid composition. It was more than a decade ago that Simons and Ikonen (1997) proposed that lipid rafts in the plasma membrane microdomains are particularly enriched in cholesterol, sphingolipids, and gangliosides that confer a liquid-ordered microstructure. In more recent times, this concept has been reviewed, and lipid rafts are presently defined as dynamic structures that can change in size and molecular composition according to specific lipid-protein and protein-protein interactions (Simons & Gerl, 2010). Furthermore, the peculiar lipid composition of lipid rafts confers particular physicochemical properties, such as higher liquid order and viscosity, as compared to other nonraft membrane regions. These properties facilitate the clustering of macromolecular complexes formed by proteins that are stabilized in lipid rafts through their interaction with different lipid constituents (Lingwood & Simons, 2010).

The signalosome concept

The multimolecular structure of lipid rafts where lipid, glycolipid, and protein compounds interact in functional complexes can be rearranged in response to extracellular signals (Collard et al. 2017). According to neuronal circumstances, these membrane microdomains participate in a plethora of cell activities that are involved in neuronal growth, maintenance, communication, and survival. Importantly, lipid rafts also play important roles in myelin formation and stability, axonal growth and neuritogenesis that are essential for functional neuronal networks (Head, Patel, Insel, 2014). Part of these actions is exerted through extracellular stimuli to activate signal transduction that ultimately modulate the final cellular response. Noticeably, the lipid raft functional complexes known as signaling platforms or signalosomes are reorganized and activated by different ligands binding to specific protein targets according to microenvironmental parameters (Hicks, Nalivaeva, Turner, 2012; Marin, 2011).

Neuronal lipid raft signalosomes contain a variety of protein classes, including transmembrane proteins, cell adhesion molecules, signal transducers such as GTPases and Src tyrosine kinases (STKs), and lipid-modified proteins (Tsui-Pierchala, Encinas, Milbrandt, & Johnson, 2002). Numerous receptors related to neuronal synapsis and differentiation are also located in lipid rafts, such as neurotransmitter receptors, hormone receptors, G protein-coupled receptors, and neurotrophin receptors (Kumari, Castillo, & Francesconi, 2013; Marin, Marrero-Alonso, Fernandez, Cury, & Diaz, 2012). This plethora of proteins interacts in lipid rafts, interacting in multimolecular complexes that develop distinct intracellular responses according to the binding of extracellular ligands that trigger signal transduction. Fig. 18.1 shows a schematic illustration of lipid raft molecular structure.

An increasing body of evidence has characterized different signalosomes that participate in a variety of brain functions, notably, neuroprotection against age-associated neuropathologies, myelination, synapsis transmission, synaptic plasticity, and cognitive and memory processes (Egawa, Pearn, Lemkuil, Patel, & Head, 2016; Marin & Diaz, 2018). A summary of the signalosomes intervening in these actions is shown in Table 18.1.

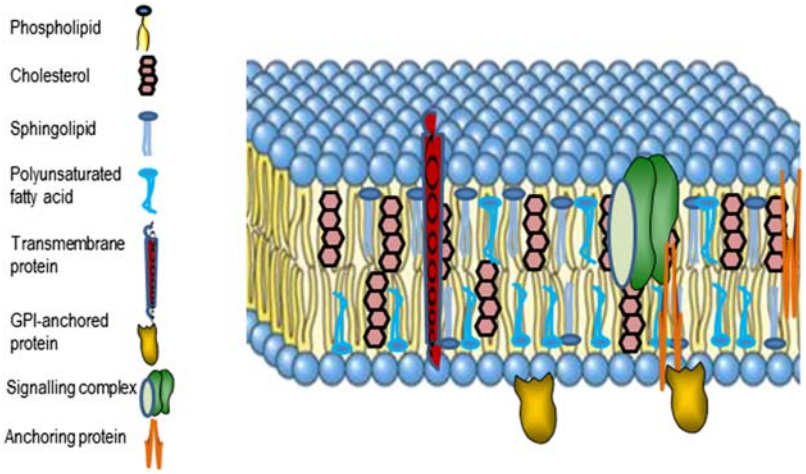

Figure 18.1 ***Schematic illustration of lipid raft microstructure.*** Lipid raft microdomains are enriched, among others, in cholesterol and sphingomyelin (in particular in the outer leaflet of the plasma membrane) and polyunsaturated fatty acids (in the inner leaflet of the plasma membrane, preferentially). This particular lipid composition allows the integration of numerous transmembrane proteins and signaling proteins clustering in signalosomes. This figure has not been previously published.

Lipid raft anomalies and neuropathology

Taking into account the variety of functions that have been related to lipid rafts, it is conceivable that alterations in the lipid composition of these membrane sites may have important consequences for neuronal stability and functionality. Recently, it has been established that a parameter promoting lipid unbalance of lipid rafts is just the consequence of brain ageing progression (Colin et al. 2016). Cerebral ageing is a complex process where multiple factors intervene, including mitochondrial impairment, metabolic alterations, a progressive neuronal detriment, loss of neuronal plasticity, increase of oxidative stress, and the accumulation of toxic protein aggregates (Bishop, Lu, & Yankner, 2010). For instance, the most relevant aged-related neuropathologies, i.e., Alzheimer disease (AD), Parkinson's disease (PD), dementia with Lewy bodies (DLB), Huntington disease, and frontotemporal dementia are generally characterized by abnormal protein oligomerization and aggregate formation that accumulates in different brain areas, contributing to the neuropathology (Cummings, 2017; Dillin & Cohen, 2011). Fig. 18.1 illustrates a potential scenario of the molecular events occurring in lipid rafts during the progression of neurodegeneration (Fig. 18.2).

Recent publications suggest a common prion-like behavior of common aged-related neurodegenerative diseases that are generally referred as proteinopathies (Espargaró, Busquets, Estelrich, & Sabaté, 2016). The prion-like concept may apply to human neurodegenerative diseases with toxic protein assemblies, including, AD, frontotemporal

Table 18.1 A summary of data related to multiprotein complexes associated with lipid rafts related to cerebral cells.

Characterized signalosomes in lipid rafts	Neuronal functioning involvement	References
Synphilin-1-alpha-synuclein in *Saccharomyces cerevisiae*	α-Synuclein aggregation; Regulation of autophagy.	Büttner et al. (2010)[a]
β-Arrestins-GRKs-Epithelial growth factor receptor	Neurotransmitter release; Membrane sensitization	Hupfeld and Olefsky (2007)[b]
G protein-coupled receptors, Adenosine A2A-Dopamine D2 receptor-Docosahexaenoic acid	Increase of receptor oligomerization; Implications for schizophrenia and Parkinson's disease.	Guixà-González et al. (2016)[c]
Stearoyl-coenzyme A desaturase 1-AKT protein kinase B-Forkhead box protein O1 (FOXO1)	Regulation of autophagy and lipogenesis.	Tan et al. (2014)[d]
Estrogen receptors-Voltage dependent anion channel-Insulin growth factor-1 receptor, Caveolin-1	Modulates rapid neuroprotective estrogen responses against Alzheimer disease	Marin et al. (2009)[e]; Canerina-Amaro et al. (2017)[f]
Src proteins family-glutamatergic receptors	Synaptic plasticity	Chen, Zhang, and Marvizón (2010)[g]
Neural protein GAP-43 bound to palmitic acid	Neuronal regeneration and synaptic plasticity	Arni, Keilbaugh, Ostermeyer, and Brown (1998)[h]
Glutamatergic receptors-glutamatergic presynaptic marker vesicular glutamate transporter 1-flotilllin	Synapsis formation; Neuroprotection	Swanwick, Shapiro, Vicini, and Wenthold (2010)[i]
Protein tyrosine kinase Pyk2-multifunctional adaptor protein Cbl-adaptor protein ArgBP2-Flotillin	Regulation of the actin cytoskeleton in neurite growth. Important in nerve regeneration after injury	Haglund, Ivankovic-Dikic, Shimokawa, Kruh, and Dikic (2004)[j]
Ret tyrosine kinase- glycosyl-phosphatidylinositol (GPI)-anchored coreceptors GFRalpha1-alpha4-Src family kinases	Neuronal signaling, differentiation, and survival	Tsui-Pierchala et al. (2002)
Secretory carrier membrane protein 2-serotonin transporter-syntaxin 1A-flotillin-1	Serotonin modulation	Müller, Wibor, and Haase (2006)[k]

Continued

Table 18.1 A summary of data related to multiprotein complexes associated with lipid rafts related to cerebral cells.—cont'd

Characterized signalosomes in lipid rafts	Neuronal functioning involvement	References
Different members of tumor necrosis factor receptor family-IkB kinases-caveolin	Sensitization of glioma cells	Tewari, Choudhury, Mehta, and Sen (2012)[l]
Numerous functional signaling clusters in lipid raft proteomes	Multiple neurodegenerative neuronal processes in a transgenic murine model of Alzheimer disease	Chadwick, Brenneman, Martin, and Maudsley (2010)[m]
Cognate prion protein (PrP^{c})-Scrapie prion protein (PrP^{sc})	Prion diseases	Taylor and Hooper (2007)[n]

Different clusters of signaling proteins associated with lipid rafts have been identified. These complexes are associated with a variety of neuronal functions by different research groups. This table is original and created for this publication.

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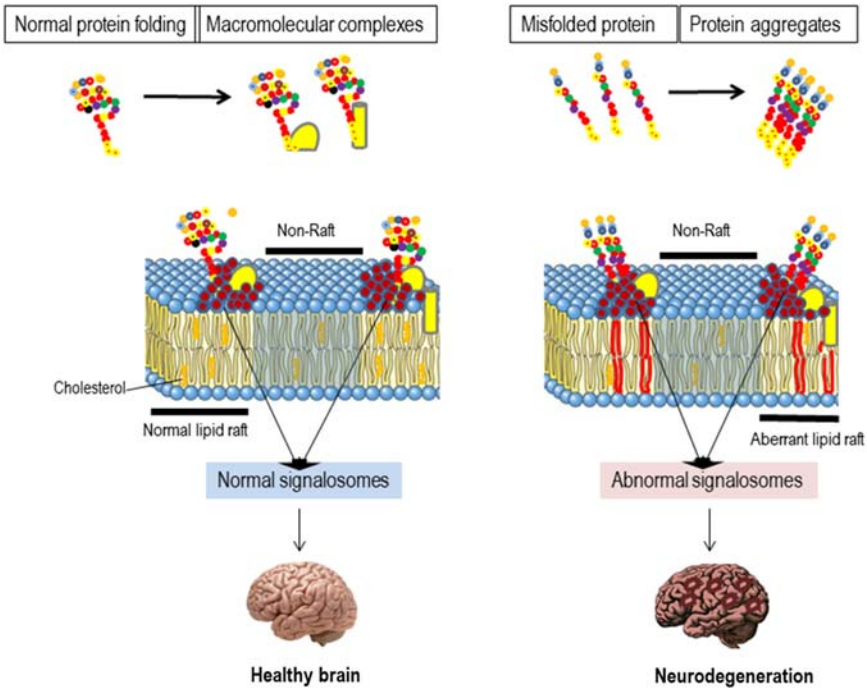

Figure 18.2 ***Schematics of the potential neuropathological lipid changes in lipid rafts.*** Molecular content and trafficking can be modified in lipid raft structures and signalosomes under pathogenic conditions. In normal physiological conditions, protein signaling complexes may contribute to normal healthy signal transduction. However, during a pathological condition, alteration in the lipid composition of lipid rafts may enhance aberrant interactions, promoting the formation of toxic aggregates and neurodegeneration. This figure has not been previously published.

dementia, PD, and DLB. Thus, oligomerization of the main protein pathological hallmarks of these diseases, the amyloid-beta peptide (Aβ) and α-synuclein (α-syn), form insoluble deposits (senile plaques and Lewy bodies, respectively) that contribute to neurodegeneration. Noticeably, self-aggregation of these proteins requires specific interrelations with membrane-integrated molecules to create the optimal biophysical and biochemical conditions for amyloid-like seeding (Ugalde, Finkeslstein, Lawson, & Hill, 2016). Once formed, these protein aggregates can propagate to other brain regions through the nervous system. For instance, in human brains, Aβ deposits appear initially in the neocortex, and later throughout the hippocampus, diencephalon, and basal ganglia (Braak & Del Tredici, 2015; Thal, Rüb, Orantes, & Braak, 2002). In the case of α-syn-enriched Lewy bodies, the first deposits appear in the olfactory bulb, and spread through the midbrain and basal forebrain to ultimately reach the neocortex (Goedert, Falcon, Clavaguera, & Tolnay, 2014).

An increasing body of evidence indicates that alterations in the lipid composition of membrane microstructures such as lipid rafts play a crucial role in the formation of aberrant protein aggregation and neurodegeneration (Marin et al. 2016). Table 18.2 summarizes the lipid changes observed in neuronal lipid rafts in AD and synucleopathies. Indeed, the detriment of cholesterol and polyunsaturated fatty acid (PUFA) content together with the increase of saturated fatty acids in lipid rafts have been correlated with an accelerating process of brain ageing and cognitive decline (Diaz, Fabelo, Ferrer,

Table 18.2 Lipid alterations in neuronal lipid rafts associated with Alzheimer disease and synucleopathies (Parkinson's disease and dementia of Lewy bodies).

Neuronal tissue	Lipid alterations in lipid rafts	Related disease	References
Frontal and entorhinal cortices, and hippocampus	Lower levels of cholesterol, sphingomyelin, and polyunsaturated fatty acids Higher levels of sterol ester and phosphatidylcholine	Early and mild stages of Alzheimer disease	Fabelo et al. (2014)[a]
Temporal cortex	Lower levels of cholesterol Higher levels of gangliosides GM1 and GM2	Early and late stages of Alzheimer disease	Molander-Melin et al. (2005)[b]
Frontal cortex	Lower levels of polyunsaturated fatty acids, monoene fatty acid 18:1n-9, gangliosides GM1 and GM2, and disproportion of saturated/unsaturated fatty acids	Late stages of Alzheimer disease	Martín et al. (2010)
Frontal cortex	Lower levels of cholesterol, sphingomyelin, oleic acid, cerebrosides, and polyunsaturated fatty acids	Late stages of Alzheimer disease and menopausal stages	Canerina-Amaro et al. (2017)[c]

Table 18.2 Lipid alterations in neuronal lipid rafts associated with Alzheimer disease and synucleopathies (Parkinson's disease and dementia of Lewy bodies).—cont'd

Neuronal tissue	Lipid alterations in lipid rafts	Related disease	References
Cortical areas	Lower levels of ganglioside GM1 Higher levels of ganglioside GM3	Parkinson's disease	Di Pasquale et al. (2010)[d]
Frontal cortex	Lower levels of cholesterol, gangliosides, plasmalogens, cerebrosides, and polyunsaturated fatty acids Higher levels of saturated fatty acids	Incidental Parkinson's disease (asymptomatic stage) Parkinson's disease	Fabelo et al. (2011)
Frontal cortex	Lower levels of cholesterol, plasmalogens, and polyunsaturated fatty acids	Dementia with Lewy bodies	Marin et al. (2017)[e]

Significant alterations in lipid species integrated in neuronal lipid rafts have been identified. These lipid-driven changes may trigger early events of age-associated neuropathologies such as Alzheimer disease and synucleopathies. This table is original and created for this chapter. It is based on factual data published in our previous publication Marin and Diaz (2018) and does not require permission.

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& Marin, 2018). It is particularly relevant that anomalies in lipid content of raft microdomains are observed from the earliest stages of these neurodegenerative diseases in different brain areas, such as the frontal cortex and hippocampus (Fabelo, Martin, Marin, Moreno, Ferrer, & Diaz, 2014; Marin et al. 2017). In the case of AD, Aβ is an amphiphilic molecule that binds to different lipid classes, which can lead to Aβ aggregation. This peptide shows high affinity for cholesterol, and changes in the membrane content of

cholesterol influence Aβ generation (Gibson, Eckert, Igbavboa, and Müller, 2003). Interaction of Aβ with lipid raft components, including gangliosides, cholesterol, and phospholipids, is accompanied by a higher accumulation of insoluble aggregates of this peptide, thereby promoting neurotoxic events in AD (Morgado & Garvey, 2015). Furthermore, in Parkinson's disease and other synucleopathies, the formation of pathological insoluble aggregates of α-synuclein (α-syn) is also associated with changes in membrane lipid turnover. This protein shows a multifunctional profile linked to synaptic activities and neurotransmitter release (Volpicelli-Daley, 2016). Although α-syn is principally a soluble, cytosolic protein, it also has the capability of binding to lipids inserted into the cellular and intracellular membranes. Following interaction with specific lipid moieties, the protein can adopt different configurations from monomers to oligomers that generate insoluble toxic fibrils (Lasuel, Overk, Oueslati, & Masliah, 2013). Thus, fibrillation of α-syn can be enhanced or inhibited depending on the phospholipid fatty acid composition, and the ratio of the protein to phospholipid (Zhu & Fink, 2003).

Lipid raft molecules as potential biomarkers of neurodegeneration

The alteration of lipid composition in raft structures together with the derangement of signalosomes are factors that may contribute to the acceleration of brain ageing as well as the progression of neurodegeneration. Taking into account these premises, it is feasible to hypothesize that identification of the early molecular events leading to lipid raft impairment may be potential tools to detect the first stages of age-associated neurodegenerative diseases. On the bases of this, investigation is now focusing on the identification of raft biomarkers detected in peripheral fluids, such as the cerebrospinal fluid and blood plasma, as a mirror of the lipid raft pathology observed in early stages of either AD or synucleopathies (Marin, Rojo, Fabelo, Fernandez, & Diaz, 2013). Fig. 18.3 summarizes the strategy to detect neuropathological alterations of protein interactions in lipid rafts that may be reflected peripherally, as potential biomarkers of the disease progression.

AD and PD dementia are presently a socioeconomic hazard and a challenge in public health, mainly due to their high incidence in the population in parallel with increased longevity, and the high cost of patient care. Therefore, the challenge is to establish accurate protocols for early detection and diagnosis of these neuropathologies. The diagnostic criteria are based on neurochemical, genetic, and clinical parameters, together with neuroimaging. However, these tools are still insufficient for accurate clinical diagnosis at the early stages of these diseases, when cardinal symptoms are still inconclusive. Among the emergent promising tools of diagnosis are biomarkers. Biomarkers were defined by the US Food and Drug Administration (FDA) in 2011 as "any characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic interventions." Thus, biomarkers for dementia may allow the detection of at-risk individuals before the

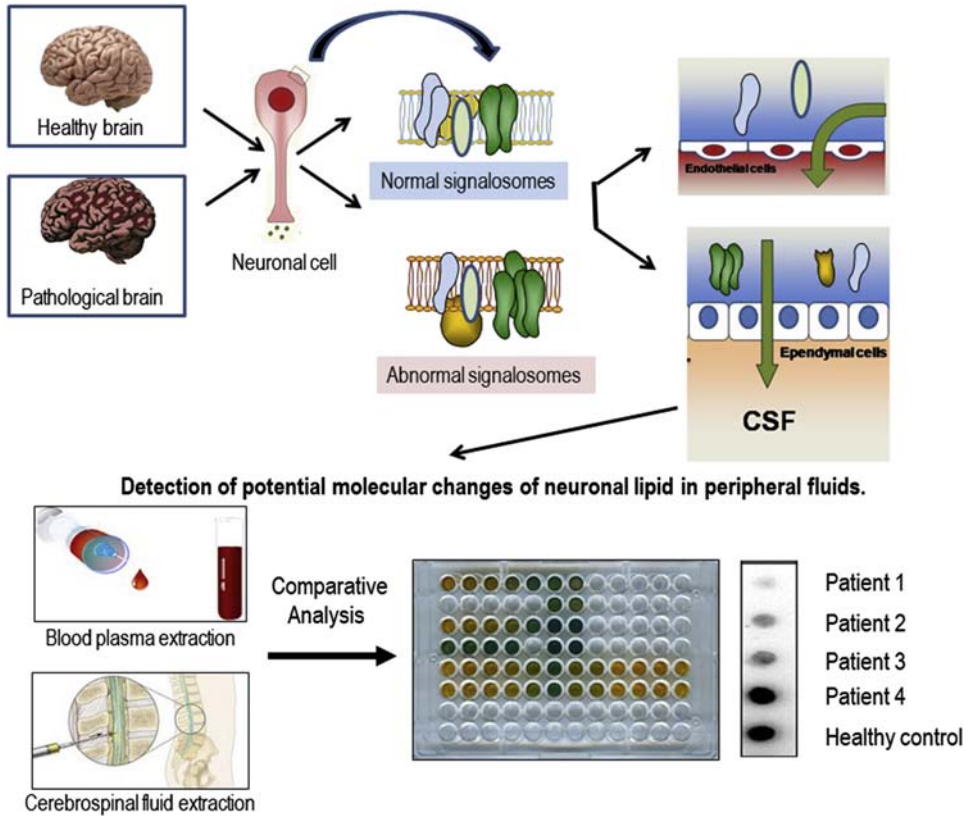

Figure 18.3 ***Potential strategy for lipid raft constituents as biomarkers for neurodegenerative diseases.*** Disruption of lipid raft signaling complexes or signalosomes during the development of neuropathological process may release specific subsets of protein and lipid species toward the brain parenchyma. These lipid raft-related molecules are poured out into the cerebrospinal fluid across the ependymal cell layer. Alternatively, these products may be released from the brain through the blood–brain barrier across the endothelial lining to the blood. Comparative quantification analyses of the raft components and by-products using multivariate approaches may provide diagnostic tools for an accurate identification of neurodegenerative diseases. This figure has not been previously published.

phenotypical features appear. Optimal biomarkers may also be able to discriminate between different neurodegenerative diseases showing similar early features, thereby aiding in establishing more efficient therapeutic interventions.

To date, there are no definitive biomarkers for AD or PD diagnosis. In the case of AD, the measurement of both Aβ fragment 1-42 and the ratio of the protein tau versus phosphorylated tau allows the detection of AD patients. However, these markers do not establish a clear distinction of mild cognitive impairment in the first stages of the disease. Related to PD, the concentration of α-syn in CSF may be a candidate biomarker for the diagnosis. However, so far the studies have demonstrated that α-syn alone is not a reliable biomarker and may require the addition of other molecular biomarkers.

Taking into account the subtle changes observed in molecular rearrangements of lipid rafts as a result of brain ageing, a potential strategy it is to target the first symptoms of neurodegeneration. Lipid raft markers may be accurate predictors of ongoing pathologies in a multiparametric analysis for preclinical diagnosis. Further identification of the altered lipid and protein factors in these microdomains will be an important hint in future biomarkers for asymptomatic stages of AD and PD.

Essential fatty acids for healthy neurons

One of the challenges in neurolipidomic studies is identifying the key lipid factors whose alteration may correlate with the process of cognitive decline. One of the most important brain fatty acids associated with learning and cognition is the docosahexaenoic acid (DHA). DHA is an n-3 PUFA highly abundant in membrane phospholipids of neural tissues (Uauy, Hoffman, Peirano, Birch, & Birch, 2001) where it plays an important role in neurogenesis, neuroplasticity, and neuroprotection throughout life (Crupi, Marino, & Cuzzocrea, 2013; Gómez-Pinilla, 2008). In the neuronal membranes, DHA is mostly found linked to phospholipids, phosphatidylethanolamine, and phosphatidylcholine, in particular (Farooqui, Horrocks, & Farooqui, 2000). It is preferentially located at the inner leaflet of membrane lipid bilayer, in contrast with the abundance of cholesterol in the outer leaflet, a fact that strongly affects membrane fluidity, lateral organization, membrane fusion, and microdomain formation (Stillwell & Wassall, 2003). The detriment of DHA in cerebral membranes and lipid rafts is a common factor of age-related neuropathologies such as AD and PD. Several studies have reported a progressive reduction in PUFA content in areas morphologically affected by AD (Prasad, Lovell, Yatin, Dhillon, & Markesbery, 1998). This reduction is particularly significant in the frontal and entorhinal cortex and hippocampus, where a 50% reduction of DHA was observed as compared to a 20% decrease in healthy elderly. In lipid rafts of cortical and hippocampal areas, the progression of AD and PD correlates with a significant disproportion in the DHA/saturated fatty acids ratio. This alteration in the lipid raft microenvironment that affects lipid lateral diffusion has important consequences in the dynamic of raft-integrated proteins that depend on the lipid-driven force.

Thus, lipid raft impairment enhances the trafficking into the raft site of the enzymes involved in amyloid-beta peptide (Aβ) biogenesis, such as the β-secretase (BACE) and the amyloid precursor protein (APP), a fact that promotes Aβ production and aggregation (Díaz et al., 2015). Moreover, changes in the asymmetric segregation of lipids between exofacial and cytofacial leaflets may provide a most favorable microenvironment for Aβ-induced neurotoxicity (Wood, Schroeder, Igbavboa, Avdulov, & Chochina, 2002). In addition to Aβ production, lipid rafts also provide a favorable environment for peptide aggregation to generate neurotoxicity. For instance, Aβ binding to gangliosides, which is abundant in lipid raft regions, enhances Aβ peptide seeding and clustering.

In addition, an interesting aspect of the amyloid in its interaction with lipids is that Aβ appears to participate in the regulation of lipid homeostasis in the plasma membrane, modulating sphingolipid and cholesterol trafficking. Therefore, Aβ peptide/membrane lipids association is bidirectional and exposed to a bidirectional regulation.

In the case of α-syn as a main contributor to synucleopathies, an interesting fact is that this protein binds to lipid membranes depending on the lipid content; α-syn binding occurs preferentially in the presence of acidic phospholipids, i.e., phosphatidylserine, in combination with DHA, monosaturated oleic fatty acid, and ganglioside GM1 (Sharon et al., 2003). Interestingly, it has been pointed out that lipid interaction of α-syn may determine its degree of oligomerization and aggregation. When bound to lipid membranes, this protein can adopt an α-helical structure, therefore providing a seeding-like template to promote further self-aggregation (Kahle, 2008). Although still a matter of controversy, it has been reported that the type of lipid bound to α-syn may determine the derived structural modification of the protein. Thus, some results have demonstrated that association with GM1 inhibits α-syn fibrillation whereas interaction with DHA may enhance a pathological oligomerization (De Franceschi et al., 2011). In contrast, other data have reported that α-syn integration in lipid membranes may prevent its aggregation and neurotoxicity (Burré, 2014). Although still unsolved issues remain, these data reflect the importance of lipid raft homeostasis in α-syn configurations and activity.

An important aspect in DHA metabolism is the fact that this fatty acid cannot be synthesized by humans, and therefore has to be incorporated in the diet. DHA is considered an essential fatty acid required in a high amount by the brain, retina, and most cell membranes. The reason for the incapability of human beings to produce DHA from its main precursor, the α-linolenic acid (ALA), is that humans do not have the enzymatic machinery of elongases and desaturases that are required for the synthesis of DHA molecular chain from ALA. For instance, in humans, only 0.2%–1% of ALA is converted to DHA, mostly in the liver. Even though neural cells require this fatty acid in high doses for their functioning, the brain does not produce DHA. Consequently, the depletion of DHA in neural phospholipids as a result of ageing or other factors cannot be mitigated by compensatory metabolic pathways even in diets enriched in ALA (Plourde & Cunnane, 2007). Taking into account the poor production in humans of DHA and other essential fatty acids of the omega-3 series, such as eicosapentaenoic acid (EPA), different nutritional interventions have been proposed in order to alleviate cognitive decline in AD, PD, and other dementia.

Supplementary PUFA to prevent cognitive decline?

Because the poor capacity of humans to produce the required high amount of the two major omega-3 PUFAs, i.e., EPA and DHA, even large amounts of the dietary ALA precursor have a negligible effect on plasma DHA concentrations. The linolenic acid is

found in vegetable oils (canola oil, walnut oil, flaxseed oil, soybean oil), nuts (walnuts) beans (red beans) and seeds (Chia seeds (*Salvia hispanica*), pumpkin seeds, flax seeds) whereas EPA and DHA are predominantly found in fish oil like tuna, herring, sardine, salmon, trout, Atlantic cod, etc. Both lipids are exclusively produced by photosynthetic microalgae of marine sources, and accumulated in marine animal consumers (Youdim, Martin, & Joseph, 2000). Another important fatty acid in the brain is the arachidonic acid (AA), an omega-6 PUFA, although this lipid is highly abundant in human nutritional habits. Indeed, the current Western diet is very rich in cereal grains, vegetable oils such as corn, sunflower, safflower, soybean, nuts (walnut, almond, peanut) and meat (veal, pork, lamb), therefore dietary deficiency of AA fatty acid is rare. Table 18.3 shows a list of food sources with these essential fatty acids and their precursor molecules.

Table 18.3 List of food sources containing unsaturated essential fatty acids.

Fatty acid common name	Molecule C:D(n-N)[a]	Food sources
Alpha-linolenic acid (omega-3)	18:3(n-9)	• fish oils • oily fish (sardine, herring, mackerel, tuna, salmon, anchovy, swordfish, cod fish) • Algae, seafood, mollusks • nuts (walnut) • seeds (pumpkin, chia, flax) • red beans
Arachidonic acid (omega-6)	20:4(n-5)	• vegetable oils (peanut, soybean, corn, walnut, sunflower, sesame, canola, palm, rapeseed) • nuts (walnut, peanut, pine nut, almond) • seeds (pumpkin, sunflower) • red meat (lamb, goat, beef, cow, deer, boar) • lean meat (veal, pork, rabbit, poultry)
Docosahexaenoic acid (omega-3)	22:6(n-3)	• fish oils (cod liver oil) • oily fish (sardine, herring, mackerel, tuna, salmon, anchovy, swordfish, cod fish) • Algae, seafood, mollusks
Eicosapentaenoic acid (omega-3)	20:5(n-3)	• fish oils (cod liver oil) • oily fish (sardine, herring, mackerel, tuna, salmon, anchovy, swordfish, cod fish) • Algae, seafood, mollusks
Linoleic acid (omega-6)	18:2(n-9)	• vegetable oils (peanut, soybean, corn, walnut, sunflower, sesame, canola, palm, rapeseed) • nuts (walnut, peanut, pine nut, almond) • seeds (pumpkin, sunflower) • red meat (lamb, goat, beef, cow, deer, boar) • lean meat (veal, pork, rabbit, poultry)

Essential polyunsaturated fatty acids must be incorporated into the diet. These fatty acids can be found enriched in different food sources. This table is original and created for this publication.

[a]"C" represents the number of carbohydrates; "D" represents the number of double bonds between carbohydrates along the molecule chain; "N" represents the position of the first double-bounded carbohydrate.

The changes of the dietary sources of PUFAs are reflected in tissue membrane composition and in the nervous system in particular. Different data have found a correlation in the loss of membrane DHA proportions and the decline in the structural and functional integrity of neuronal membranes and lipid raft microdomains. Interestingly, DHA detriment and the impairment in the balance of AA/DHA ratio appear to be enhanced in neurodegenerative disorders as AD and PD. The analysis of the lipid content in lipid rafts from cortical and hippocampal regions of AD and PD brains has shown a reduction of DHA levels and unhealthy disproportions of AA/DHA, thereby contributing to impaired signaling mechanisms underlying some of the neuropathological features (Martín et al., 2010; Fabelo et al., 2011). Cell membrane detriment of DHA is detected even before the symptoms of the disease appear, indicating that imbalance of omega-3 fatty acids may be a trigger factor of progressive cognitive impairment. For instance, alterations in brain lipid profiles of early AD subjects (with mild cognitive impairment or mild clinical dementia) were correlated with a 93% diagnostic accuracy by neuropathological findings for AD. The pathological alterations are related to neuronal dysfunction, loss of synaptic plasticity, and dementia.

Consequently, numerous nutritional initiatives have been proposed for an optimal diet of omega-6 and omega-3 lipids with the aim of delaying the onset or reducing the impact of brain functions provoked by these neurodegenerative diseases. Some evidence suggests that an adequate nutritional intake of omega-3 PUFA over a lifetime contributes to preserving cognition and prevents the onset of neurodegenerative diseases. Nevertheless, the epidemiologic assays analyzing the potential beneficial effects of DHA-enriched diets in the risk of dementia have not been conclusive. The observed divergences may be explained by numerous factors such as the frequency and doses supplied, gender, age, and molecular form administrated (Morris, 2012). The oxidation degree of the molecule is also another factor to take into account, indicating that prevention of DHA oxidation has to be optimized. Thus, even though nutritional interventions may be a potential therapeutic tool to palliate the symptoms of cognitive impairment, further studies are required to tackle optimal strategies to mitigate brain impairment during ageing.

Key facts of age-associated neuropathology

- The risk of suffering neuropathological disorders increases with age, showing a peak between 65 and 85 years, particularly in women.
- Alzheimer disease is the most common form of dementia, affecting approximately 2%–5% individuals between 65 and 69 years old, and 25%–30% individuals over 85 years of age.
- Parkinson's disease and other synucleopathies such as dementia of Lewy Bodies are the second most frequent neurodegenerative diseases, possibly accounting for up to 25% of all dementia cases.
- Common symptoms of neurodegeneration with ageing are the loss of smell, cognitive decline, memory impairment, depression, loss of communication skills, and insomnia.

Key facts of neurolipidomics in brain pathology

- The brain possesses at least 5000 types of lipid classes that are essential for its normal functioning, including neuronal communication and nerve fiber integrity.
- An important feature observed in early stages of these diseases is a loss of normal lipid load in the brain and, in particular, in neuronal cell membranes.
- The lipid distribution of neuronal membranes is irregular, forming particular regions with different structural features named lipid rafts.
- Changes in the lipid and protein composition of lipid rafts promote some neuropathological features such as aberrant protein aggregation and cell toxicity.
- Polyunsaturated fatty acids such as docosahexaenoic acid (known as omega-3 fatty acid) are very abundant in the neuronal membranes, and their detriment is related to brain disorders.
- Omega-3 lipids are not produced in the human brain and therefore must be included in the diet during the entire lifecycle.

Summary points

- This chapter discusses the importance of the neuronal membrane microdomains named lipid rafts in brain functioning.
- Lipid rafts have a distinct lipid composition providing particular physicochemical properties that allow the clustering of signaling proteins in multimolecular complexes or signalosomes.
- Signalosomes develop crucial activities in neurons in response to different stimuli thereby triggering signal transduction during cell growth, differentiation, synapsis, and neuroprotection against different insults.
- Alterations in the lipid composition of lipid rafts are at the basis of cognitive impairment in age-related neurodegenerative diseases, such as Alzheimer disease and Parkinson's disease.
- Lipid changes also affect signalosomes, inducing aberrant interactions, formation of pathological aggregates, and toxic intracellular signaling.
- A main lipid involved in lipid raft integrity is docosahexaenoic acid (DHA) which is highly abundant in the brain and the neuronal membrane.
- A DHA-deficient diet may induce the detriment of DHA in the brain, thereby promoting cognitive decline, the acceleration of brain ageing, and neuropathology.

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CHAPTER 19

FAM3C in Alzheimer's disease: a risk-related molecule and potential therapeutic target

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List of abbreviations

AD Alzheimer's disease
APP amyloid precursor protein
Aβ amyloid-β
CSF cerebrospinal fluid
CTF C-terminal fragment
EMT epithelial–mesenchymal transition
FAM3 family with sequence similarity 3
ILEI interleukin-like EMT inducer

Mini-dictionary of terms

FAM3 superfamily this superfamily was originally identified by a database search for novel proteins exhibiting predictive structural similarity to the four-helix-bundle cytokines.

FAM3C FAM3C is a ubiquitously expressed, 227-amino-acid protein that is secreted after release of the signal sequence.

γ-Secretase γ-Secretase is a macromolecular complex composed of four core components, namely, presenilins, nicastrin, anterior pharynx defective-1, and presenilin enhancer-2.

Aβ peptide Aβ contains 38–43 amino acids, and 42- and 43-amino-acid species are highly aggregation prone and pathogenic.

Presenilin Presenilins (presenilin-1 and presenilin-2 in human) are nine-pass transmembrane proteins and catalytic center-harboring components of the γ-secretase complex. Numerous missense mutations of the conserved residues are linked to familial AD.

Introduction

Although disease-modifying therapies for Alzheimer's disease (AD) are urgently required, the clinical trials conducted to date have produced unsatisfactory outcomes in terms of

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effects on clinical manifestations. Immunization against amyloid-β (Aβ), which is regarded as the causative molecule of AD, is effective for eliminating amyloid plaques but not slowing the progression of dementia, even in patients with mild AD (Holmes et al., 2008). Hence, to obtain satisfactory therapeutic effects, it is critical to detect and treat brain Aβ deposition in the very early preclinical stage.

The amyloid cascade hypothesis, which is now widely accepted, posits that Aβ accumulation in the brain triggers the subsequent molecular events leading to the development of AD. AD is heterogeneous regarding its causative factors, including genetic and environmental risks. The heritability of AD is as high as 60%–80%, and genome-wide association studies revealed that several genomic polymorphisms influence the incidence of sporadic AD. The missing heritability is explained by unidentified genomic variants or epistasis (Gatz et al., 2006). Alteration of the brain protein expression profile possibly increases the risk of AD, but the present knowledge of AD risk-associated molecules is insufficient.

Uncovering the molecular risk factors for brain Aβ accumulation can provide important clues for developing preventive pharmacological interventions. Several hundred proteins have been reported to possibly exacerbate Aβ accumulation and trigger Aβ deposition in the brain by influencing Aβ production, clearance, or aggregation (Campion, Pottier, Nicolas, Le Guennec, & Rovelet-Lecrux, 2016). Alteration of the expression or modification of these proteins could influence the epistatic risk. However, only a limited number of molecular risk factors other than genetic polymorphisms have been confirmed.

Regulation of γ-secretase cleavage and identification of family with sequence similarity 3, member C

In the brain, neuronal cells mainly produce and secrete Aβ into the extracellular milieu. γ-Secretase, an intramembrane aspartyl protease, mediates the final step of Aβ production, and it has been a major target of disease-modifying therapy for AD. However, clinical trials of γ-secretase inhibitors were discontinued due to increased cancer prevalence and worsening of dementia (Doody et al., 2013). These adverse effects could be attributable to the inhibition of Notch signaling and accumulation of C-terminal fragments (CTFs) of amyloid precursor protein (APP), which are substrates of γ-secretase (Mitani et al., 2012).

The Aβ concentration in the interstitial fluid of the brain parenchyma depends on local synaptic activity (Cirrito et al., 2005). The synapse is the major site of Aβ production in the brain, and Aβ reportedly has modulatory effects on synaptic transmission (Kamenetz et al., 2003). These findings suggest the existence of an intrinsic regulatory mechanism of Aβ production. Many proteins reportedly regulate γ-secretase activity through direct binding with the γ-secretase complex (De Strooper, Iwatsubo, & Wolfe,

2012). In addition to these proteins, a unique group of γ-secretase-interacting proteins alters Aβ production without affecting their proteolytic activity. These proteins alter the stabilization of α-secretase- and β-secretase-cleaved APP-CTFs (C83 and C99, respectively), but not full-length APP, by binding to the γ-secretase complex without modifying the proteolytic activity of γ-secretase. Among these, we have focused on family with sequence similarity 3, member C (FAM3C).

FAM3C has been identified as a γ-secretase-binding protein through tandem tag-affinity purification of the γ-secretase complex from cultured HEK293 cells (Hasegawa, Liu, Tooyama, Murayama, & Nishimura, 2014). Its binding with endogenous core components of the γ-secretase complex was confirmed by coimmunoprecipitation assays.

FAM3C reduces amyloid-β production by destabilizing APP-C99

FAM3C negatively regulates secreted Aβ peptide levels (Hasegawa et al., 2014). Neuronal SH-SY5Y and nonneuronal HEK293 culture cells express FAM3C, and short interfering RNA-mediated silencing of endogenous FAM3C increased Aβ secretion without changing the ratio of Aβ species. Conversely, FAM3C silencing had no effect on Aβ generation in a cell-free reaction mixture containing cellular microsome-derived γ-secretase complexes and recombinant APP-C99, suggesting that FAM3C binds to the γ-secretase complex but does not inhibit its proteolytic activity. FAM3C decreased both secreted Aβ and cellular APP-CTF levels. FAM3C silencing elongated the half-life of APP-CTFs but not full-length APP, suggesting that FAM3C destabilizes APP-CTFs to reduce Aβ production. FAM3C neither activated general protein degradation through lysosomal/autophagosomal pathways nor affected intracellular APP-CTF trafficking. FAM3C-mediated alteration of APP-CTF stability requires cellular expression of the γ-secretase complex, regardless of whether the complex is enzymatically active. FAM3C did not affect other γ-secretase substrates such as Notch, LRP1, and N-cadherin. These findings indicate that FAM3C binds to the γ-secretase complex and accelerates nonspecific APP-CTF degradation to reduce Aβ production (Fig. 19.1).

An earlier study illustrated that the presenilin-1/γ-secretase complex stably binds to APP-CTFs and protects them against nonspecific degradation (Pitsi & Octave, 2004). Recently, Bustos, Pulina, Bispo, et al. (2017) reported that phosphorylation of presenilin-1 at Ser^{367} destabilizes APP-CTFs by activating autophagic degradation without affecting γ-secretase activity and that transgenic mice expressing mutant presenilin-1 (Ser^{367} → Ala) exhibited dramatic increases in Aβ and APP-C99 levels *in vivo*. Similar to FAM3C, several presenilin-binding proteins were found to have positive or negative effects on the degradation efficiency of APP-CTFs. Dedicator of cytokinesis 3, a member of the guanine nucleotide exchange factor family that is also known as a modifier of cell adhesion, reportedly binds to the large cytoplasmic loop of presenilins and accelerates proteasome-mediated degradation of both full-length APP and

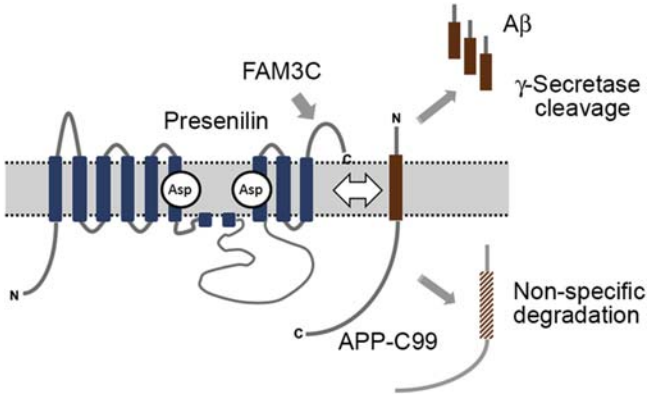

Figure 19.1 ***FAM3C binds to the γ-secretase complex and reduces cellular amyloid-β (Aβ) production.*** Scheme showing FAM3C function. FAM3C binds to the extracellular tail segment of presenilin C-terminal fragment and enhances the nonspecific degradation of APP-C99 to reduce Aβ production.

APP-CTF (Chen, Kimura, & Schubert, 2002). Adipocyte plasma membrane-associated protein, a type II glycosylated membrane protein that is specifically induced during adipocyte differentiation, stabilizes APP-CTFs but not full-length APP or other γ-secretase substrates by binding with the γ-secretase complex and APP (Mosser et al., 2015). Conversely, tetraspanin 6 binds to the γ-secretase complex and affects autophagosome—lysosomal fusion to slow APP-CTF degradation (Guix et al., 2017).

FAM3C is secreted by cells after cleavage of the N-terminal signal sequence, and it interacts with the γ-secretase complex from outside the cell. The addition of recombinant FAM3C to culture medium decreased cellular APP-C99 levels in a concentration-dependent manner. Extracellular FAM3C was internalized into the endosome system, and it bound to the γ-secretase complex (Hasegawa et al., 2014). These results indicate that FAM3C binds to the extracellular segments of a γ-secretase complex component. Using *in situ* protein cross-bridging, the direct binding partner was identified as a presenilin-1 CTF. Furthermore, alanine substitution of residues Asp^{458}, Gln^{459}, and Leu^{460} in the C-terminal tail of presenilin-1 abolished binding between FAM3C and the γ-secretase complex, and this binding is critical for reducing Aβ production. The putative FAM3C-binding site is located on the extracellular face close to the substrate-binding region of the γ-secretase complex (Fig. 19.2).

The FAM3 superfamily

FAM3A

FAM3A predominantly resides in mitochondria, and unlike other members, its secretion has not been observed. Hepatic FAM3A expression is markedly reduced in *db/db* mice

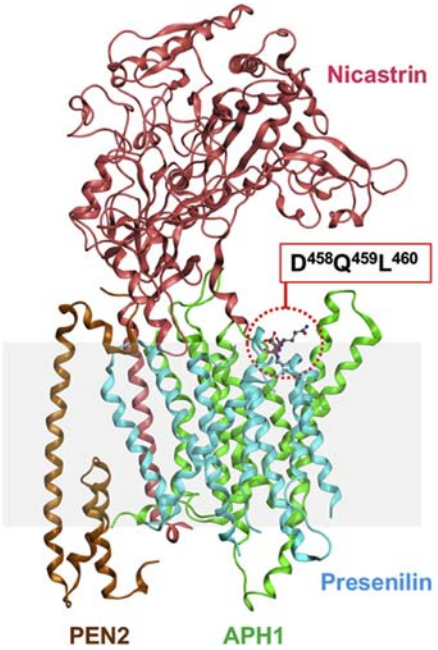

Figure 19.2 ***Putative FAM3C-binding site is located on the extracellular face of the γ-secretase complex.*** A *ribbon* diagram of the crystal structure of the γ-secretase complex (PDB ID: 5A63) was created using MOE software (Chemical Computing Group). Presenilin, nicastrin, anterior pharynx defective-1 (*APH1*), and presenilin enhancer-2 (*PEN2*) are indicated by light blue, pink, green, and orange, respectively. The putative FAM3C-binding site ($D^{458}Q^{459}L^{460}$) is located on the extracellular face under the large lobe of nicastrin.

and high-fat diet—induced diabetic mice. In prior research, FAM3A overexpression in the liver attenuated hyperglycemia, insulin resistance, and fatty liver with increased PI3K—Akt signaling and repressed gluconeogenesis and lipogenesis (Wang et al., 2014). Arterial FAM3A promotes neointima formation after injury by inducing vascular smooth muscle cell proliferation and migration by activating Akt and ERK1/2 (Jia et al., 2014). Neuronal FAM3A protects against oxidative stress and glutamate-induced neurotoxicity by suppressing cytoplasmic Ca surge (Song, Gou, & Zou, 2017).

FAM3B

The pancreatic islets of Langerhans secrete FAM3B, also referred to as pancreatic-derived factor (Cao et al., 2003). Under glucose stimulation, FAM3B is cosecreted with insulin (Burkhardt, Cook, Young, & Wolf, 2008; Wang, Guan, & Yang, 2010). Transgenic mice overexpressing FAM3B in pancreatic β-cells display fasting hyperglycemia and hepatic insulin resistance, whereas FAM3B-null mice similarly exhibit glucose intolerance (Robert-Cooperman et al., 2010, 2014). Insulin sensitivity was not impaired in

these knockout mice, but responses to glucose stimulation and hepatic insulin clearance were decreased. Hepatic expression of FAM3B receptor was predicted, and FAM3B treatment inhibited insulin signaling in hepatocarcinoma cells in a concentration-dependent manner (Yang et al., 2009). Adenovirus-mediated systemic overexpression of FAM3B decreases triglyceride levels in the liver and adipose tissue (Mo et al., 2015).

The nonsecretory splicing variant FAM3B is highly expressed in colorectal adenocarcinoma tissues and cell lines (Li et al., 2013). Stable overexpression of this variant downregulates adhesion proteins and upregulates Slug and Cdc42 to promote colon cancer cell migration, invasion, and metastasis in nude mice (Li et al., 2013). FAM3B also inhibits cell death and increases prostate tumor growth through transcriptional induction of Bcl-2 and Bcl-X_L (Maciel-Silva et al., 2018).

FAM3C

FAM3C was identified as an upregulated molecule in an expression profile of mammary gland epithelial cells undergoing epithelial–mesenchymal transition (EMT) with a metastable phenotype, and it is also referred to as interleukin-like EMT inducer (Waerner et al., 2006). FAM3C is translationally induced by tumor growth factor-β (TGF-β) signaling, and it causes EMT in epithelial cells and hepatocytes (Chaudhury et al., 2010; Lahsnig et al., 2009). Secretory epithelia express FAM3C in perinuclear vesicular structures, and its cytoplasmic expression is reportedly correlated with EMT and cancer metastasis (Waerner et al., 2006).

Genome-wide association studies have illustrated that the FAM3C locus is associated with bone mineral density (Cho et al., 2009). During osteogenic differentiation, FAM3C is expressed in the cytoplasm of bone marrow stromal cells, and its expression is induced by TGF-β1 signaling and reduced expression of Runx2, one of the major transcription factors required for osteogenic differentiation (Bendre, Buki, & Maatta, 2017). FAM3C-null bone marrow cells exhibit accelerated osteogenic differentiation and mineralization (Maatta et al., 2016).

FAM3C expression is decreased in the livers of obese diabetic mice, and its overexpression ameliorates hyperglycemia, insulin resistance, and fatty liver (Chen, Wang, Yang, Chen, Meng, Feng, et al. 2017; Chen, Wang, Yang, Chen, Meng, Geng, et al. 2017). Secreted FAM3C suppresses gluconeogenic gene expression in hepatocytes by upregulating HSF1 and activating the PI3K–Akt pathway (Chen, Wang, Yang, Chen, Meng, Geng, et al., 2017).

Other than suppressing Aβ production, the functions of neuronal FAM3C have scarcely been described. However, transcriptional profiling of mouse retinas revealed that FAM3C is highly expressed in the ganglion cell layer, and downregulation and upregulation of *Xenopus* retinal FAM3C caused photoreceptor cell dislocation and laminar disorganization, respectively (Katahira, Nakagiri, Terada, & Furukawa, 2010).

FAM3D

FAM3D, also named oncoprotein-induced transcript 1, was induced by dietary fat in the small intestine and plasma in prior research (de Wit et al., 2012). Plasma FAM3D inhibits glucagon secretion via MAPK phosphatase 1-dependent suppression of ERK1/2 signaling (Cao et al., 2017). Human neutrophils and monocytes have strong chemotaxis activity toward FAM3D. Formyl peptide receptors have been identified as candidate FAM3D receptors, and FAM3D participates in gastrointestinal homeostasis and inflammation by binding these receptors (Peng et al., 2016).

The family members share approximately 30%–55% homology at the amino acid level (Fig. 19.3). Although all FAM3 proteins participate in the homeostasis of glucose and lipid metabolism, each FAM3 member is implicated in a distinct range of physiological functions (Table 19.1). X-ray analysis of FAM3B and FAM3C crystals revealed a globular β–β–α three-layer architecture with two antiparallel β sheets and one layer of three short helices, forming a conserved water-filled cavity (Jansson et al., 2017, 2013). Prior studies emphasized that the enclosed cavities may be functionally important. In addition, these members share a large conserved surface area on one side of the molecule, suggesting their interaction with a similar class of binding partners. Recently, the same group reported the possibility that the active form of FAM3C is a domain-swapped dimer (Jansson et al., 2017).

Neuronal FAM3C expression

Earlier reports found that FAM3C is mainly expressed in the epithelial cells of multiple organs, including the lungs, liver, pancreas, spleen, kidneys, intestine, colon, and thymus

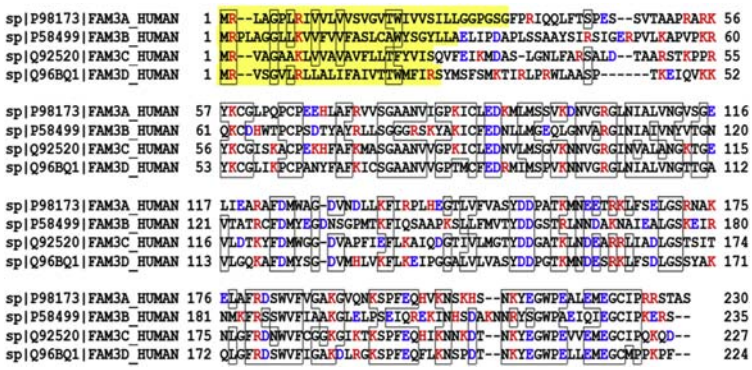

Figure 19.3 ***The FAM3 superfamily includes four members.*** An alignment of human FAM3 protein sequences is shown. ClustalW2 software was used to align the amino acid sequences of the four proteins. Conserved residues are boxed. Predicted signal sequences are highlighted by yellow. Acidic and basic amino acids are shown as blue and red characters, respectively.

Table 19.1 The FAM3 superfamily.

	FAM3A	FAM3B/ PANDER	FAM3C/ILEI	FAM3D/Oit1
Distribution	Ubiquitous	Brain, liver, pancreas	Ubiquitous	Gastrointestinal tract
Subcellular localization	Mitochondria	Perinuclear vesicles	Perinuclear vesicles, cytoplasmic	Golgi apparatus
Secretion	−	+	+	+
Functions	- Arterial neointima formation - Hepatic gluconeo-genesis and lipogenesis - Protection against oxidative and ER stress - Neuronal excitotoxicity	- Regulation of glucose and lipid metabolism - Colon cancer metastasis - Prostate tumor growth	- EMT - Tumorigenesis and metastasis - Gluconeogenesis - Osteogenic differentiation - Reduction in Aβ production - Retinal development	- Gastrointestinal homeostasis and inflammation - Glucose metabolism
Related signaling pathways	- PI3K−Akt - ERK1/2	- Insulin receptor signal - PKA −CREB - Slug and cdc42 - Bcl-2, Bcl-X_L	- TGF-β - HSF1−CaM −Akt - Runx2 - γ-Secretase complex	- Formyl peptide receptor - MAPK phosphatase 1 −ERK1/2

Data are derived from papers cited in the text. *Aβ*, amyloid-β; *EMT*, epithelial−mesenchymal transition; *ER*, endoplasmic reticulum; *ILEI*, interleukin-like EMT inducer.

(Pilipenko, Reece, Choo, & Greinwald, 2004; Zhu et al., 2002). In addition, immunostaining of mouse and human brains revealed neuronal FAM3C expression in widespread regions (Fig. 19.4) (Liu, Watanabe, Akatsu, & Nishimura, 2016). Large pyramidal neurons in layer V of the cerebral cortex display prominent immunostaining (Fig. 19.5A). In particular, piriform and entorhinal cortices express higher FAM3C levels. In the hippocampus, the granule cell layer of the dentate gyrus, the CA2, and the medial part of the CA1 are strongly positive. Lateral amygdaloid, medial habenular, and reticular thalamic nuclei exhibit stronger staining, whereas the caudate putamen and the cerebellar Purkinje cells display weaker staining.

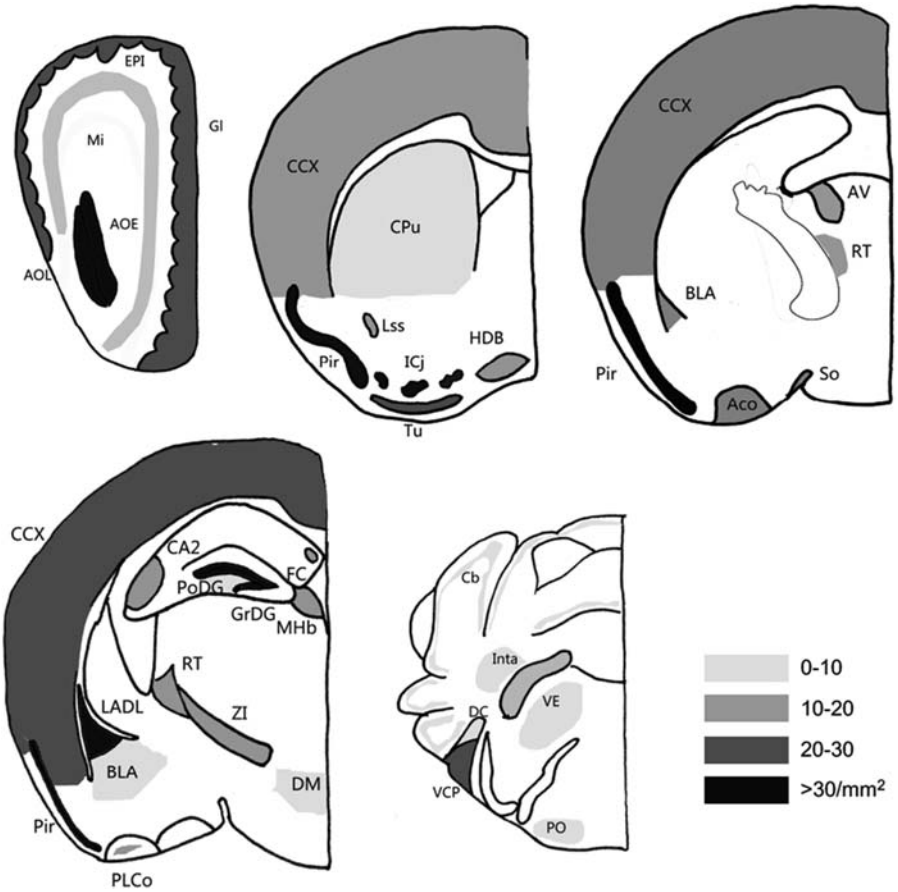

Figure 19.4 ***FAM3C is broadly distributed in the mammalian brain.*** FAM3C-positive neurons were counted in immunostained sections of mouse brain. *ACO*, anterior cortical amygdaloid nucleus; *AOE*, anterior olfactory nucleus, external part; *AOL*, anterior olfactory nucleus, lateral part; *AV*, anteroventral thalamic nucleus; *BLA*, basolateral amygdaloid nucleus, anterior part; *CA2*, field CA2 of the hippocampus; *CB*, cell bridges of the ventral striatum; *CCX*, cerebral cortex; *Cpu*, caudate putamen; *DC*, dorsal cochlear nucleus; *DM*, dorsomedial hypothalamic nucleus; *EPI*, external plexiform layer of the olfactory bulb; *FC*, fasciola cinereum; *GI*, glomerular layer of the olfactory bulb; *GrDG*, granule cell layer of the dentate gyrus; *HDB*, nucleus of the horizontal limb of the diagonal band; *ICj*, islands of Calleja; *IntA*, interposed cerebellar nucleus, anterior part; *LaDL*, lateral amygdaloid nucleus, dorsolateral part; *LSS*, lateral stripe of the striatum; *MHb*, medial habenular nucleus; *Mi*, mitral cell layer of the olfactory bulb; *Pir*, piriform cortex; *PLCo*, posterolateral cortical amygdaloid area; *PO*, posterior thalamic nuclear group; *PoDG*, polymorph layer of dentate gyrus; *Rt*, reticular nucleus; *SO*, supraoptic nucleus; *Tu*, olfactory tubercle; *VCP*, ventral cochlear nucleus, posterior part; *VE*, vestibular nucleus; *ZI*, zona incerta.

FAM3C immunoreactivity was detected in neuronal cells but not in glial or endothelial cells. Tubular and vesicular profiles were immunolabeled in the cytoplasm and proximal neurites of large pyramidal neurons in the cerebral cortex and hippocampus (Fig. 19.5A). Double-fluorescence immunocytochemistry of neuronal and nonneuronal

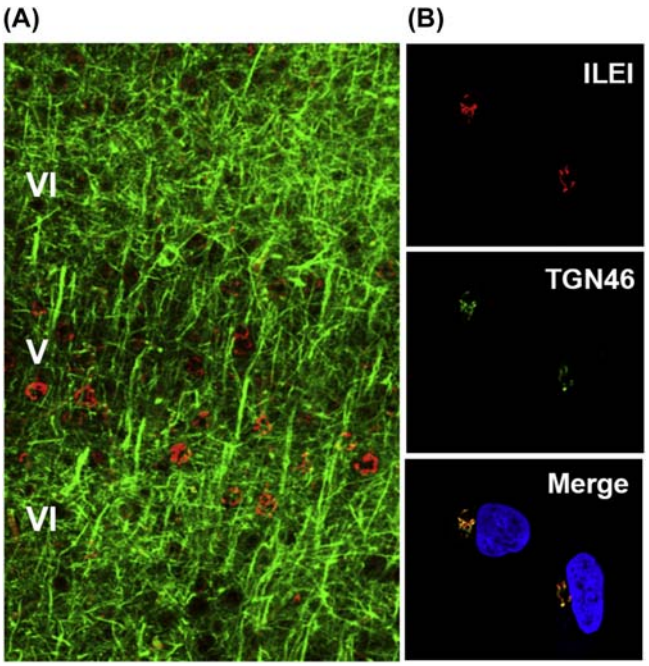

Figure 19.5 ***FAM3C resides in the* trans*-Golgi network.*** (A) FAM3C immunoreactivity is prominent in perinuclear regions of large pyramidal neurons in the mouse cerebral cortex. FAM3C and phosphorylated neurofilament protein are indicated by red and green, respectively. (B) Double immunocytochemistry of HEK293 cells indicates that FAM3C colocalizes with TGN46, a marker of the *trans*-Golgi network. Nuclei are stained with Hoechst 33342. *ILEI*, interleukin-like EMT inducer.

cells indicated that FAM3C mainly resides in the *trans*-Golgi network (Fig. 19.5B). Synaptic localization was suggested by the overlapping distribution of the presynaptic marker synaptophysin on synaptosome fractionation analysis (Liu et al., 2016). Synaptic vesicles, particularly release-ready, active-zone-docked synaptic vesicles, but not reserve pools of free synaptic vesicles, contained high levels of FAM3C. APP and the γ-secretase complex were cofractionated with FAM3C in active-zone-docked synaptic vesicles.

Reduction of brain FAM3C expression with aging and in patients with Alzheimer's disease

During mouse brain development, FAM3C expression exhibits a monophasic increase with a peak in the early postnatal period and a gradual decline thereafter with aging (Liu et al., 2016). This alteration was more prominent for the secreted form than the full-length precursor. The subcellular localization of neuronal FAM3C was consistent across all developmental stages. In a quantitative study using temporal cortex tissues from cynomolgus monkeys, which are vulnerable to age-dependent Aβ burden

(Nishimura et al., 2012), FAM3C protein and mRNA levels were significantly lower in aged brains than in young adult brains (unpublished data).

Semiquantitative immunoblotting suggested that levels of the secreted form of FAM3C were significantly reduced in AD brains compared with those in age-matched nonneurological disease controls and non-AD disease controls and inversely correlated with Aβ accumulation (Hasegawa et al., 2014; Liu et al., 2016). Immunohistochemistry also indicated that the number of FAM3C-positive neurons was decreased but that the subcellular localization of the protein was unaltered in AD brains (Liu et al., 2016). FAM3C levels are comparable between aged APP-transgenic and age-matched wild-type mice (Hasegawa et al., 2014), suggesting that the reduction in FAM3C expression is not secondary to Aβ accumulation. Instead, FAM3C suppression is a possible causative factor of age-related brain Aβ accumulation and the development of AD pathology.

Although the mechanism underlying AD-associated FAM3C suppression remains unresolved, downregulation of TGF-β signaling may decrease FAM3C levels in AD brains. Previous reports described decreases in TGF-β signaling in AD brains (Tesseur et al., 2006; Wyss-Coray et al., 2001), whereas sustained TGF-β activation enhanced the translation of FAM3C in mammary epithelial cells, lung cancer, and neuronal cells through the phosphorylation of heterogeneous nuclear ribonucleoprotein E1 (Chaudhury et al., 2010; Hasegawa et al., 2014; Song, Sheng, Zhang, Jiao, & Li, 2014).

Proteomic analysis of cerebrospinal fluid (CSF) revealed significantly decreased FAM3C levels in patients with idiopathic temporal lobe epilepsy (Xiao et al., 2009), who frequently exhibit brain Aβ accumulation (Mackenzie & Miller, 1994). This finding suggests that decreased FAM3C levels in CSF could be a biomarker for brain Aβ accumulation.

Conclusion and perspective

Age-related downregulation of FAM3C is likely a candidate epistatic causative factor of brain Aβ accumulation and the subsequent development of AD pathology. In addition, decreases in brain or CSF FAM3C levels could represent an extremely early diagnostic biomarker of the risk of Aβ accumulation.

Diagnostic screening of healthy people at high risk of AD is a prerequisite for developing preventive medicine. Pharmacological management of the molecular risks is an ideal strategy for reducing AD prevalence. Hundreds of molecules can directly or indirectly influence brain Aβ homeostasis by altering Aβ production, clearance, or aggregation. Certain populations of these molecules increase the risk of AD development. FAM3C may be a strong candidate among these molecules. The remaining risk molecules must also be identified to develop preventive medicine and treat this devastating disease.

Key facts of the molecular pathomechanism of Alzheimer's disease

- Aβ peptides are mainly produced and secreted from neurons in the brain.
- β-Secretase- and γ-secretase-mediated proteolytic processing of APP generates Aβ peptides.
- Aβ accumulation in the brain triggers the pathogenic cascade of AD.
- Accumulated Aβ is deposited in amyloid plaques, a pathological hallmark of the AD brain.

Summary points

- This chapter focuses on FAM3C, which regulates endogenous Aβ production in the brain.
- FAM3C can reduce cellular Aβ production by binding with the γ-secretase complex.
- FAM3C is expressed at higher levels in young healthy brains before declining with aging.
- FAM3C levels are negatively correlated with accumulated Aβ levels in aged brains.
- Supplementation of FAM3C activity in the brain might represent a personalized preventive intervention for Aβ accumulation and AD development.

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CHAPTER 20

Amylin and amylin receptors in Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
AM adrenomedullin
Aβ beta amyloid peptide
cAMP cyclic adenosine monophosphate
CGRPs calcitonin gene-related peptides
CRSPs calcitonin receptor-stimulating peptides
CT calcitonin
CTR calcitonin receptor
GPCR G protein-coupled receptor
hAmylin human amylin
hCT human calcitonin
IAPP islet amyloid polypeptide
LTP long-term potentiation
RAMP receptor activity-modifying protein
rAmylin rat amylin
sCT salmon calcitonin
T2DM type 2 diabetes mellitus

Mini-dictionary of terms

Amylin Also named islet amyloid polypeptide, a 37-residue peptide hormone that is cosecreted with insulin from the pancreatic β cells that plays a role in glycemic regulation and promoting satiety. Human amylin is an amyloidogenic peptide, whereas rat amylin is not.

Amyloid plaques Aggregates of misfolded proteins, which stick together to form fibrous deposits in plaque form around cells and can disrupt the healthy function of neural elements.

Amylin receptor A class B GPCR composed of heterodimers of CTR and RAMPs (RAMP1-3) and dimers of CTR, and either RAMP1, RAMP2, or RAMP3 comprise amylin receptor subtypes AMY1, AMY2, AMY3.

Calcitonin receptor A class B GPCR for peptide hormone calcitonin involved in maintenance of calcium homeostasis, bone formation, and metabolism.

Diabetes mellitus A metabolic disorder characterized by chronic hyperglycemia with defects in insulin secretion, insulin action, or both.

G protein-coupled receptors A large protein family of receptors with seven transmembrane domain structure and their cellular responses coupling with G proteins.

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Long-term potentiation Persistent increase in synaptic strength following high-frequency stimulation of synapses, one of the major cellular mechanisms underlying learning and memory.

Receptor activity-modifying proteins A class of protein that interact with and modulate the activities of several Class B G protein-coupled receptors.

β-sheet A common motif of regular secondary protein structure in which two or more β strands are connected in parallel or antiparallel by hydrogen bonds, forming a generally twisted, pleated sheet.

Introduction

Both Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are chronic, age-related protein misfolding diseases. They possess mutual risk factors and are linked at epidemiological, clinical, pathophysiological, and molecular levels (Biessels & Despa, 2018; de Matos, de Macedo, & Rauter, 2018). These conditions are characterized by the tissue deposition of protein aggregates, termed amyloid, that contain misfolded beta amyloid peptides (Aβ) in the brain, and human amylin (hAmylin) in the pancreas, respectively (Chiti & Dobson, 2017; Sala Frigerio & De Strooper, 2016; Westermark, Andersson, & Westermark, 2011). The sequence comparison of amylin and Aβ is listed in Table 20.1. During the process of the formation of amyloid fibrils, Aβ and hAmylin demonstrate similar features for sequence-specific aggregation and oligomeric intermediates that are precursors to the aggregated state. Typically, the amyloid fibrils form unbranched, polymorphic, and crossed β-sheet structures (Chiti & Dobson, 2017; Mitraki, 2010). Amyloid fibrils can be an integral part of normal cellular physiology and serve as storage reservoirs for peptide hormones within secretory granules (Maji et al., 2009). The amyloid formation is a dynamic process between protein monomers, oligomers, and fibrils. The populations of the different states and their conversion rates are governed by their thermodynamic stability. The active amyloid cross-β-sheet structures are formed with tightly interacting, stranded, repetitive intermolecular β-sheets, which enable amyloids to grow by recruitment of the same protein (Riek & Eisenberg, 2016). The characteristic architecture of amyloid state is "generic" and not encoded by specific amino acid sequences (Knowles, Vendruscolo, & Dobson, 2014). Compared with fibril forms, soluble oligomeric intermediates may serve the primary toxic species of amyloids. This oligomer toxicity is inhibited by oligomer-specific antibodies, which suggests that different types of soluble amyloid oligomers have a common structure and share a common mechanism of toxicity (Kayed et al., 2003).

Table 20.1 Sequence comparison for amylin-related peptides.

hAmylin	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	H	S	N
rAmylin	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	R	S	S
Pramlintide	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	H	S	S
hCalcitonin	C	G	N	L	S	T	C	M	L	G	T	Y	T	Q	D	F	N	K	F	H
sCalcitonin	C	S	N	L	S	T	C	V	L	G	K	L	S	Q	E	L	H	K	L	Q
$hA\beta_{1-42}$	D	A	E	F	R	H	D	S	G	Y	E	V	H	H	Q	K	L	V	F	F
$rA\beta_{1-42}$	D	A	E	F	G	H	D	S	G	F	E	V	R	H	Q	K	L	V	F	F

Amylin, a neuroendocrine hormone

Amylin, also named diabetes-associated peptide or islet amyloid polypeptide, was first isolated from amyloid-rich pancreatic extracts of type 2 diabetic patients (Zhang & Song, 2017). It is a 37-amino acid endocrinal peptide hormone cosecreted with insulin from the pancreatic β-cell (Khemtémourian, Killian, Höppener, & Engel, 2008) and belongs to the calcitonin (CT) peptide family, which comprises CT, calcitonin gene related peptides (CGRPs), adrenomedullin, and calcitonin receptor-stimulating peptides (CRSPs). These peptides all contain an N-terminal six- or seven-amino-acid cyclic structure formed by a disulfide bridge required for biological activity. hAmylin shares ~s20% sequence identity with human CT and AM and 46% with CGRPs.

The major source of amylin is pancreatic β-cell with a plasma concentration about 3–5 pM in the fasting state and 15–25 p.m. after a meal. As a regulatory hormone, amylin is involved in glucose regulation, energy metabolism, and neuronal development (Zhang & Song, 2017; Hay, Garelja, Poyner, & Walker, 2018) (Fig. 20.1). Amylin reduces energy intake by reducing satiation (the promotion of meal-ending processes). It also reduces gastric emptying, inhibits postprandial glucagon secretion, and reduces body weight and adiposity. Its satiation effect is most likely through the central nervous system mediated by amylin receptors in the area postrema, solitary tract nucleus, and lateral parabrachial nucleus. Amylin regulates insulin secretion and inhibits the action of insulin on glycogen synthesis and glucose uptake in liver and muscle cells and normalizes hexose metabolism in the liver and adipose tissue in rats with altered glucose homeostasis (Desai et al., 2014). It shows complicated autocrine action on islet cells. At low blood glucose levels, amylin (at picomolar concentration) triggers an oscillatory modulation of signal transduction and promotes β-cell proliferation. However, at high glucose levels, such as those prevailing in diabetes mellitus, amylin inhibits the very same signaling pathways (Visa et al., 2015).

Amylin receptors, multiplexed signaling, and regulation

Amylin (AMY) receptors are putative targets for amylin, and they contain heterodimerized complexes of calcitonin receptor (CTR) interacting with one-receptor activity-modifying proteins (RAMPs 1–3) that result in multiple amylin receptor subtypes designated as AMY_{1-3} (Hay, Garelja, Poyner, & Walker, 2018). CTR is a seven transmembrane domain class B G protein-coupled receptor (GPCR), while RAMP is a

N	N	L	G	P	V	L	S	P	T	N	V	G	S	N	T	Y				
N	N	L	G	P	V	L	P	P	T	N	V	G	S	N	T	Y				
N	F	P	Q	T	A	I	G	V	G	A	P									
T	Y	P	R	T	N	T	G	S	G	T	P									
T	E	D	V	G	S	N	K	G	A	I	I	G	L	M	V	G	G	V	V	I	A
A	E	D	V	G	S	N	K	G	A	I	I	G	L	M	V	G	G	V	V	I	A

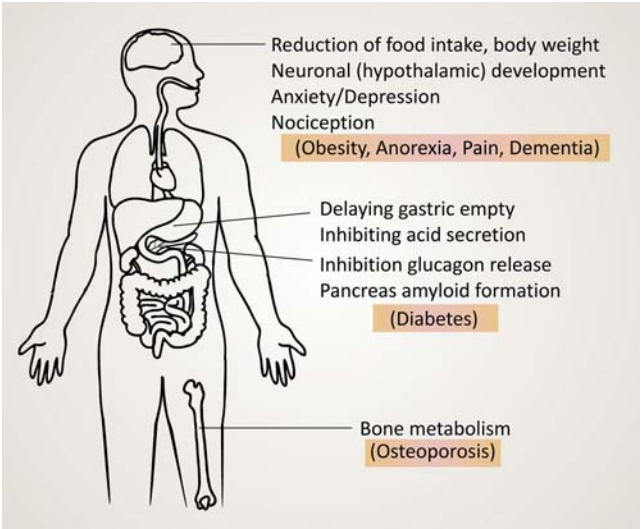

Figure 20.1 ***Amylin: Physiological functions.*** Amylin is a pancreatic β-cell hormone and activates amylin receptors producing effects in multiple organ systems, serves as a neuroendocrine hormone and involved in different pathological processes and diseases.

single-domain protein and not a receptor itself, with three currently known subtypes named RAMPS 1−3. Amylin activates amylin receptors through a variety of signal pathways, which include cyclic AMP (cAMP), intracellular calcium increases, ERK1/2, and Akt pathway activation (Fig. 20.2). Recent discovery of CTR-biased signal agonism may explain the complexity of its underlying mechanisms of action at a cellular level. CTR itself (the key component of AMY) is activated by CT and forms receptor-bound G-protein heterotrimers with different GTP affinities. Both salmon calcitonin (sCT) and human calcitonin (hCT) activate the receptor with different efficacy. The sCT shows higher affinity for the ternary complex with lower sensitivity to GTP, while hCT shows a lower affinity for the ternary complex but a higher sensitivity to GTP. This results in different attraction rates for the G-protein complex and adenylate cyclase activation time. This allosteric activation mechanism allows for divergent ligand-dependent receptor residual times for the G-protein complex and different accumulation rates for downstream second messengers (Furness et al., 2016; Gingell et al., 2016; Fu, Patel, Kimura, Soudy, & Jhamandas, 2017).

RAMPs, membrane-spanning accessory proteins, act as pharmacological switches and chaperones to alter CTR signaling and trafficking that make AMYs a far more diverse molecular structure. RAMPs dimerize with CTR to increase the accessibility of the CTR extracellular domain-binding groove and thus further enhance amylin binding. RAMPs are also multidirectional allosteric proteins that alter AMY receptor properties not only through the extracellular domain but also via the transmembrane bundle and

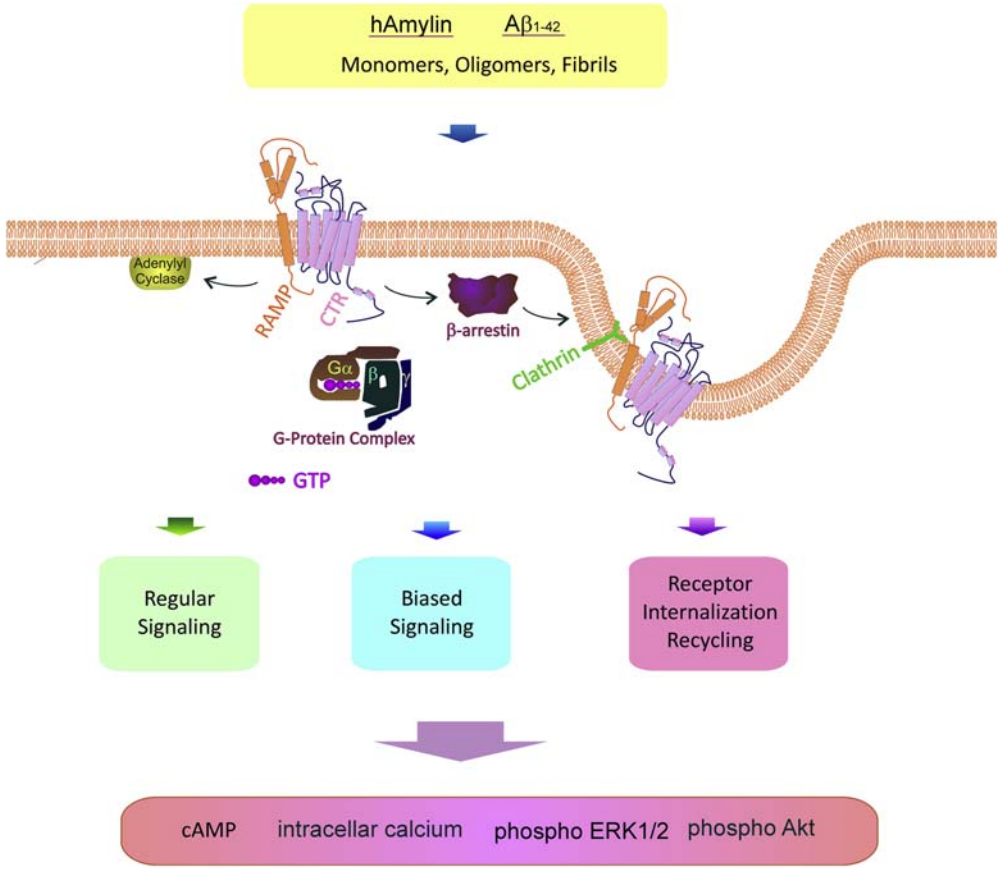

Figure 20.2 ***Amylin receptor activation mechanisms.*** Both amylin and $A\beta_{1-42}$ can allosterically activate AMY receptors and either of these peptides may be present in monomeric, oligomeric, or fibrillar forms at different ratios based on the physiological conditions. These molecular forms can act as different orthosteric or allosteric ligands and differentially activate/modulate AMY receptor subtypes. Ligand binding to the AMY receptors results in an engagement of adenylyl cyclase or β-arrestin, which trigger different downstream signaling pathways that activate ERK and/or Akt or receptor internalization and recycling.

intracellular C terminus (Gingell et al., 2016; Bower & Hay, 2016; Hay & Pioszak, 2016). This diversity of conformational states of AMY receptors makes it difficult to study the function of the receptors. Currently, it is challenging to assign specific functions to the receptor subtypes because of the lack of highly selective pharmacological tools for distinguishing the amylin receptor subtypes and receptor complexity. The AMY receptor presents multiple allosteric binding sites that can be exploited as ligand binding pockets. This permits individual ligands to preferentially bind to specific receptor conformations, which when activated trigger different downstream signaling pathways.

Internalization and recycling of AMY receptors may also relate to their signaling profiles (Hay, Garelja, Poyner, & Walker, 2018) (Fig. 20.2). The internalization rates vary based on CTR splicing variant and RAMPs. In mammalian cell lines that heterologously express the human CTR, both peptides demonstrate equipotent activity for cAMP production and β-arrestin recruitment with short-term stimulation (<2 h). However, prolonged stimulation (up to 72 h) with sCT leads to persistent cAMP accumulation, phosphorylation of ERK1/2, β-arrestin recruitment, and internalization of CTR. In contrast, hCT loses its stimulatory activity markedly earlier (Andreassen et al., 2014). The uptake or recycling of amylin into the pancreatic cells can occur through both endocytotic and nonendocytotic (translocation) mechanisms, while the predominance of a particular trafficking route is dependent on amylin concentrations and incubation times (Bhowmick, Singh, Trikha, & Jeremic, 2018). At low (≤100 nM) concentrations, internalization of AMY is an AMY receptor dependent mechanism, which can be blocked by the amylin receptor antagonist, AC187. However, cytotoxic (μM) concentrations of hAmylin enter pancreatic cells by translocation, macropinocytosis, or a clathrin-dependent pathway. For amylin oligomers, especially following longer exposure times (24 h), macropinocytosis is a major clearance mechanism. After internalization, hAmylin accumulates in the cytosol, then translocates into the nucleus and is detoxified by proteasomes. However, the excessive hAmylin accumulation in the nucleus results in increased cytotoxicity and decreased proteasome proteolytic activity. Inhibition of proteasomal proteolytic activity in turn leads to further increases in intracellular amylin accumulation and toxicity (Singh, Trikha, Sarkar, Jeremic, 2016).

Amylin in Alzheimer's disease

Common features of human amylin and beta amyloid peptide, amyloid proteins

The amino acid sequence of amylin is highly conserved across animal species but with a notable variation in the 18–29 sequence regions (Westermar, Andersson, and Westermark, 2011). The proline residues within this region are essential for inhibition of aggregation (Sala Frigerio & De Strooper, 2016). The amylin peptides with one or more proline residues within this region, such as the peptides in dog, rat, mouse, guinea pig, and cow, lack fibrillogenicity and propensity to aggregate. Although the amylin peptide in human and rat differs by only six amino acids (they share 84% sequence homology), this is sufficient to confer the property of amyloidogenicity on hAmylin as opposed to rat amylin (rAmylin) (Westermark, Andersson, & Westermark, 2011; Fortin & Benoit-Biancamano, 2015). The three proline substitutions within the segment 20–29 render rAmylin nonamyloidogenic (Zhang & Song, 2017; Westermark, Andersson, & Westermark, 2011). The NFLVH motif within the amylin segment 8–20 is also a key sequence for hAmylin to form amyloid fibrils (Fortin & Benoit-Biancamano, 2015).

Although lacking obvious sequence similarity, hAmylin and Aβ proteins are strikingly similar in their physiochemical properties including the ability to form amyloid, which

consists of long, highly ordered insoluble fibers with a characteristic crossed β-sheet pattern. These two amyloidogenic proteins also generate soluble oligomeric form intermediates, which demonstrate strong cytotoxicity through a variety of mechanisms, such as by directly disrupting plasma and organelle membranes, inducing inflammatory responses, generating reactive oxygen species, and overloading the unfolded protein response pathway (Bower & Hay, 2016; Fortin & Benoit-Biancamano, 2015).

In addition to the above similar physical properties that permit the formation of insoluble, elongated, and unbranched amyloid fibrils, there are also direct interactions between hAmylin and Aβ. Amyloids are composed of proteins that interact in a tight and orderly manner to form cross-β-sheet structures, which elongate and grow through further recruitment of the same proteins. They have the potential to self-replicate, adapt to the environment, and transmit from cell to cell, thus yielding spreading and demonstrating toxicity (Riek & Eisenberg, 2016). Interestingly, the misfolding and aggregation is not limited solely to the same protein or peptide. In Aβ -amylin cross-interaction interface, several regions can either self- or cross-interact if they show high affinity for both Aβ and amylin. These featured molecular recognition sites underlying amyloid self-assembly exist for Aβ, amylin, and most likely other amyloidogenic polypeptides (Lee, Hay, and Pioszak, 2016; Gingell et al., 2016). Amylin also inhibits self-association of Aβ into cytotoxic aggregates through direct amylin-Aβ interactions and shares common pathways underlying toxicity that lead to mitochondrial dysfunction (Wootten, Miller, Koole, Christopoulos, & Sexton, 2017; Furness et al., 2016). The cross-seeding interactions between different amyloid peptides may play an important role in progression and transmission between different amyloid diseases (Ono et al., 2014; Andreetto et al., 2010).

In an in vitro environment, Aβ and hAmylin form an Aβ-hAmylin assembly, and hybrid Aβ-hAmylin fibrils demonstrate similar morphologies to pure hAmylin fibrils (Andreetto et al., 2010; Zhang, Hu et al., 2017). Both Aβ and hAmylin can serve as a nucleus to accelerate Aβ aggregation (Andreetto et al., 2010; Zhang, Hu et al., 2017; Zhang, Yang et al., 2017). When mice overexpressing hAmylin have been crossbred with AD transgenic mice, hAmylin has been found to colocalize and aggregate within brain parenchymal deposits of amyloid plaques (Moreno-Gonzalez et al., 2017). Inoculation of pancreatic amylin aggregates into the brains of AD transgenic mice has resulted in more severe AD pathology and significantly greater memory impairment. Amylin also is found deposited in human brains in T2DM with dementia and AD patients (Jackson et al., 2013). Hyperamylinemia is commonly seen in obese and insulin-resistant type-2 diabetes patients. In these diabetic patients, amylin is not only deposited in pancreatic islets but also accumulates in the cerebrovascular system and brain and thus induces pathological changes similar to those observed in AD (Sexton, Paxinos, Kenney, Wookey, & Beaumont, 1994).

Nonamyloidogenic peptides are also capable of forming amyloid fibrils. For example, rAmylin (Table 20.1) can engage hAmylin cross-seeding to form stable complexes and coassemble into heterogeneous structures whose resultant hybrid fibrillary structures are similar to that of pure hAmylin fibrils (Andreetto et al., 2010). Similarly, pramlintide

has also been reported to form amyloid fibrils in vitro under certain experimental conditions (da Silva, Fontes, Erthal, & Lima, 2016). These amylin-based peptides thus have a tendency to cross-seed and form fibrils. Consequently, these properties of nonamyloidogenic amylin peptides raise concerns regarding their potential use for AD therapy.

Amylin receptors involved in memory and learning

AMY receptors are expressed in different CNS major cell types including neurons, glial cells (microglia, astrocytes), and endothelial cells of the brain vasculature (Fu, Patel et al., 2017). These cell types play important and somewhat distinct roles in AD pathogenesis (Fig. 20.3). AMY receptor—mediated toxicity may thus contribute to synaptic dysfunction, neuronal loss, microglial activation and inflammatory cascade, and amyloid vasculopathy affecting endothelial cells.

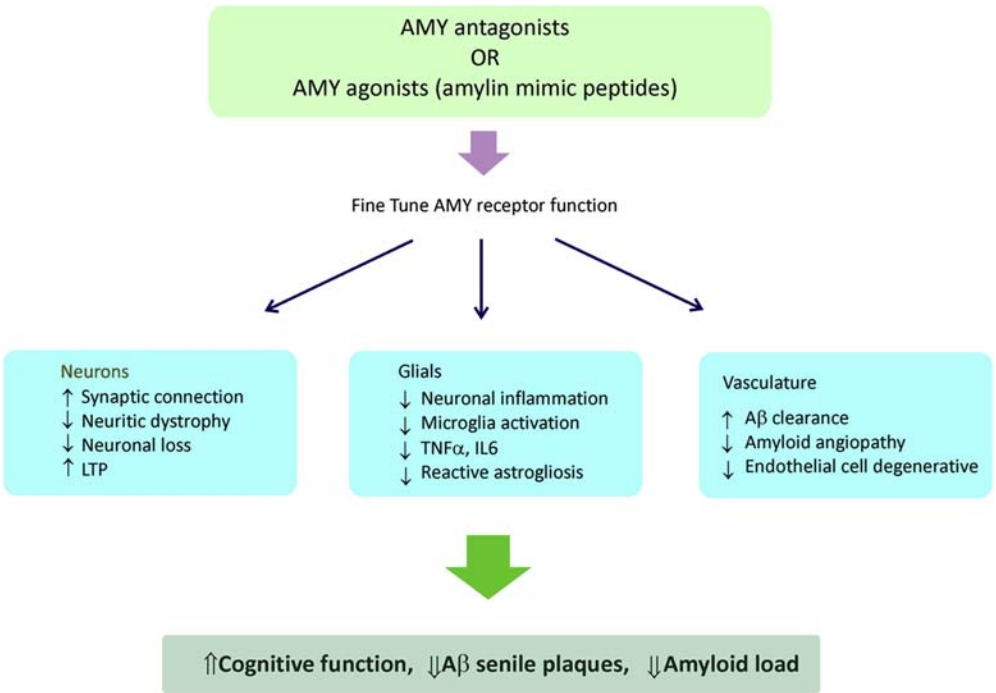

Figure 20.3 ***Targeting AMY receptors for AD therapy.*** AMY receptors distributed in widespread regions of the brain and they are expressed on different types of CNS cells that include neurons, glial cells (microglia, astrocytes), and endothelial cells of the brain vasculature. These cells constitute central players in the development of AD pathology, which includes synaptic dysfunction and neuronal loss, microglial activation and inflammation, and amyloid vasculopathy. The AMY receptors located on these cell types are a target for the deleterious effects of Aβ resulting in emergence of clinical and pathological manifestations of AD. Modulating AMY receptor receptors function with AMY antagonists or agonists (i.e., amylin mimic peptides) could attenuate these processes and improve cognitive function in AD.

Peripheral amylin majorly secreted from pancreas and the peptide can cross the blood–brain barrier (Sridhar, Lakshmi, & Nagamani, 2015; Lutz, 2012). Amylin can also originate in the brain, and the amylin-synthesizing neurons are localized in the lateral hypothalamus, arcuate nucleus, medial preoptic area, and other basal forebrain regions (Akter et al., 2016; Mukherjee, Morales-Scheihing, Butler, & Soto, 2015; Li, Kelly, Heiman, Greengard, & Friedman, 2015). Amylin receptors are also widely expressed in central nervous system areas and involved in autonomic regulation (food intake and body fluid balance) as well as memory and learning processes (Braegger, Asarian, Dahl, Lutz, & Boyle, 2014). AMY receptors express in multiple cell types including neurons, microglia, and endothelial cells that trigger different functions (Fu, Patel et al., 2017). Remarkably, the expression of AMY receptor levels in the brain of AD transgenic mice increases in parallel with Aβ in an age-dependent manner and in specific regions of the brain with a high amyloid plaque load such as the cortex, hippocampus, and basal forebrain but sparing the brainstem or cerebellum (Jhamandas et al., 2011). Although the significance of the upregulation of AMY receptors and its correlation with brain amyloid pathology in AD mice remains unknown, the relationship further highlights the close linkage between amylin and amyloid in the context of neurodegeneration.

Amylin receptors, mediated neuronal cytotoxicity

Both hAmylin and Aβ directly activate amylin receptors, and their neurotoxic effects can be blocked with specific amylin receptor antagonists (Jhamandas & MacTavish, 2004; Jhamandas et al., 2011). At higher concentrations, hAmylin not only induces pancreas β-cell cytotoxicity (Lorenzo, Razzaboni, Weir, & Yankner, 1994) but also produces cytotoxicity in neuronal cells including murine and rat neuronal cell lines and human fetal primary neuronal cell cultures (Fu et al., 2012; Jhamandas & Mactavish, 2012; May, Boggs, & Fuson, 1993; Tucker, Rydel, Wright, & Estus, 1998; Jhamandas, Harris, Cho, Fu, & MacTavish, 2003). The neuronal cytotoxicity of both hAmylin and Aβ is through the same AMY mechanism that can be blocked with amylin receptor antagonists. Their cytotoxicity may also be involved in the induction of oxidative stress genes and apoptotic-related genes. AMY receptor antagonists such as AC187 and AC253 (Table 20.2) blocked AMY receptor–mediated upregulated proapoptotic mediators and the cytotoxic effects evoked by hAmylin and Aβ in rodent and human cell culture models (Jhamandas & Mactavish, 2012, 2004). In primary cultures of human fetal neuronal cell culture, downregulation of AMY3 receptor (used CTR and RAMP3 siRNA) gene expression attenuates hAmylin- and Aβ-induced apoptotic cell death (Jhamandas et al., 2011; Jhamandas & Mactavish, 2012; Jhamandas, Harris, Cho, Fu, & MacTavish, 2003).

Both hAmylin and Aβ can act as monomers or aggregated soluble oligomers on amylin receptors. The different molecular forms may activate the subtypes (i.e., AMYs 1–3) of AMY receptors differently. As mentioned earlier, AMY receptors harbor different allosteric binding sites that permit differential activation of downstream signaling

Table 20.2 Amylin receptor antagonist sequence comparison.

sCT_{8-32}	V	L	G	K	L	S	Q	E	L	H	K	L	Q	T	Y	P	R	T	N	T	G	S	G	T	P
AC187	V	L	G	K	L	S	Q	E	L	H	K	L	Q	T	Y	P	R	T	N	T	G	S	N	T	Y
AC253		L	G	R	L	S	Q	E	L	H	R	L	Q	T	Y	P	R	T	N	T	G	S	N	T	Y
AC413	A	T	Q	R	L	A	N	F	L	V	R	L	Q	T	Y	P	R	T	N	V	G	A	N	T	Y

pathways—i.e., biased agonism (Wootten et al., 2017; Furness et al., 2016). The mechanisms for these actions need to be further elucidated. Concentration-dependent effects of hAmylin on rat hippocampal neurons appear to involve both the AMY receptor (at lower pico or nanomolar doses) and also the mechano-osmo-sensitive TRPV4 receptor (at higher micromolar doses) (Zhang, Hu et al., 2017; Zhang, Yang et al., 2017). The higher concentrations of hAmylin (high μM doses) activate TRPV4 and disturb calcium hemostasis, which could be responsible for cytotoxicity observed in these cells (Zhang, Yang et al., 2017). There is at present substantial evidence for AMY receptors to mediate hAmylin- and Aβ-evoked neuronal toxicity, but the contributions of newer receptor targets such as TRPV4 toward the actions of these peptides continue to emerge.

Amylin and amylin receptor involved in beta amyloid peptide–related neuroinflammation reaction

In a diabetic HIP rat model, which overexpresses hAmylin in the pancreas, amylin is also deposited in the brain and linked to neuroinflammation. Activated microglia/macrophages are clustered around the small cerebral blood vessels where abundant amylin infiltration is identified. Both M1 and M2 activated microglia are increased in the cortex with elevated proinflammatory cytokines TNF-α and IL-6, while the antiinflammatory cytokine IL-10 is downregulated (Srodulski et al., 2014). Amylin plaques and mixed amylin-Aβ deposits are also found in human patient brains of diabetes with AD and show increases in the proinflammatory cytokine interleukin (IL)-1β (Verma et al., 2016). AMY receptor antagonist, AC253, blocked hAmylin or Aβ induced increases in intracellular Ca^{2+}, NLRP3, Caspase1, TNFα, and IL-1β in cultured BV2 cells and primary cultures of human fetal microglia. Intraperitoneal administration of AC253 resulted in reduction in microglial activation (Iba-1 and CD68), caspase-1, TNFα, and IL-1β accompanied by a reduction in amyloid plaque burden and improvement of cognitive deficits in 5xFAD mice (Fu, Patel et al., 2017; Fu, Vukojevic et al., 2017).

Amylyin receptor involved in hippocampal long-term potentiation

Long-term potentiation (LTP) is a process involving persistent strengthening of synapses that leads to a long-lasting increase in signal transmission between neurons. It is an important process in the context of synaptic plasticity. LTP recording is widely recognized as a cellular model for the study of memory. Soluble oligomeric Aβ depresses hippocampal LTP and thus impairs glutamatergic NMDA receptor-mediated synaptic plasticity (Li et al., 2011). Both Aβ and hAmylin induced reductions in LTP at nanomolar concentrations, and the reduction can be blocked in the presence of the amylin receptor antagonist AC253 in wild-type mice (Kimura, MacTavish, Yang, Westaway, & Jhamandas, 2012). In transgenic AD mouse model (TgCRND8), LTP is chronically blunted due to elevated

ambient levels of Aβ. AC253 can reverse LTP depression and restore it to levels observed in age-matched littermate control animals.

Pramlintide, the amylin analog, has also attenuated both Aβ- and hAmylin evoked LTP depression and partially restored LTP in AD mice (Kimura, MacTavish, Yang, Westaway, & Jhamandas, 2017). This discrepancy in the ability of an amylin receptor antagonist (AC253) and pramlintide to demonstrate similar effects of LTP might be explicable by biased agonism as mentioned earlier. The pramlintide could, under some conditions and at low concentrations (nM) such as used in the LTP study, act also as a functional antagonist for amylin receptor. However, further research is warranted to confirm this notion.

Modulation of amylin receptors to improve cognitive function in Alzheimer's disease

Amyloidogenic amylin and its target receptors (AMYs) appear relevant for the pathogenesis of AD through their interactions with Aβ at the receptor level and subsequent downstream effects on amyloid plaque formation, generation of inflammatory responses, and cognitive function. Indeed, strategies aimed at either mimicking (with amylin agonists) or blocking (with amylin antagonists) amylin receptor have been reported to improve cognitive function in different transgenic AD mouse models.

Pramlintide, an FDA-approved synthetic amylin analog for the treatment of diabetes, has been used in several studies in AD transgenic mouse models (Adler et al., 2014; Zhu et al., 2015). Intraperitoneal (i.p.) injections of either pramlintide or amylin improved performance in tasks of learning and memory in AD mouse models including SAMP8, 5XFAD, and 3xTgAD mice (Adler et al., 2014; Zhu et al., 2015). These peptides reduced the amyloid burden and lowered the concentrations of Aβ in the brain, and these benefits were deemed to represent an activation of the amylin receptors. A single pramlintide injection induced a surge of Aβ in plasma in AD patients (Zhu, Stern, et al., 2017), which was attributed to an efflux of brain Aβ into the blood (Zhu et al., 2015).

In contrast to these actions of amylin mimetic peptides on cognitive function, the amylin receptor antagonists, AC253 and cyclized AC253 (cAC253), either centrally (ICV) or via i.p. administration, have demonstrated a significant improvement in spatial memory and learning in transgenic AD mice (TgCRND8 and 5xFAD) (Soudy et al., 2017). A reduction in brain amyloid plaques and neuroinflammation was observed to accompany the behavioral improvements in these mice. The beneficial cognitive effects were felt to be mediated by neuronal amylin receptors, a notion supported by strong binding of the fluorescently labeled peptide antagonist, cAC253, to amylin receptors within the hippocampus. These in vivo studies are complemented by earlier In vitro LTP experiments where AC253 and pramlintide restored the chronically depressed levels of hippocampal LTP observed in AD mice to levels comparable to those seen in age-matched wild-type control mice (Kimura, MacTavish, Yang, Westaway, & Jhamandas, 2017).

Conclusion

The diversity of amylin receptor subtypes and emerging knowledge of their participation in specific physiological and pathophysiological conditions offers a unique opportunity for their role in the development of novel AD therapeutics. The ability to allosterically modulate amylin receptors opens up a new vista for drug development beyond the use of conventional agonists or antagonists. Regardless of their perceived agonist or antagonist designation at the level of AMY receptors, the inescapable conclusion is that targeting these receptors for drug development is capable of yielding multiple synergistic and beneficial effects such as increased clearance of amyloid across the blood–brain barrier, reduction of neuroinflammation, and improved cognition in AD. As such, amylin and the amylin receptor play an important role in AD pathogenesis and represent a novel and promising strategy for the treatment of AD.

Key facts of Alzheimer's disease and amylin

- AD is a progressive neurodegenerative disease for which there is no cure.
- Accumulation of Aβ in the brain is considered an early and seminal event in AD pathogenesis.
- Amylin peptide, first isolated from pancreas of diabetic patients, shares similarities to Aβ and is regarded as a "second amyloid."
- Amylin receptor is expressed on three critical elements of AD pathology—neurons, glial cells, and vascular cells.
- Amylin receptor has been identified as a target for the expression of deleterious effects of Aβ and is considered an attractive drug target.
- Amylin receptor–based compounds have been shown to improve memory and learning in in vitro and in vivo transgenic mouse models of AD.
- Amylin receptor–based drugs represent a novel platform development of disease-modifying therapies for AD.

Summary points

This chapter focuses on amylin and amylin receptors and their role in AD pathogenesis and therapeutics.

- Both human amylin and Aβ are amyloid peptides and capable of forming amyloid plaques.
- Amylin is a neuroendocrine hormone.
- Amylin receptor is a GPCR with multiplexed signaling and regulation.
- Amylin receptors are widely distributed in the central nervous system and involved in memory and learning.

- Amylin receptors mediate Aβ cytotoxicity and neuroinflammation.
- Amylin receptors are involved in hippocampal LTP.
- Modulation of the amylin receptor function either through stimulation or blockade of the receptor improves cognitive function in AD mouse models.

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CHAPTER 21

Mammalian target of rapamycin complexes: regulation and Alzheimer's disease

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List of abbreviations

Akt protein kinase B
AMPK AMP-activated kinase
Atg autophagy-related protein 13
BDNF brain-derived neurotrophic factor
FTD frontotemporal dementia
GSK-3β glycogen synthase kinase-3β
Hif1α hypoxia-inducible factor 1-α
LTD long-term depression
LTP long-term potentiation
mTOR mammalian/mechanistic target of rapamycin
p70S6K1 p70 ribosomal S6 protein kinase 1
PI3K phosphoinositide 3-kinase
PRAS40 proline-rich Akt substrate 40 kDa
Raptor regulatory-associated protein of mTOR
Rictor rapamycin insensitive companion of mTOR
Tsc tuberous sclerosis complex
ULK1 unc-51-like kinase 1
4E-BPs 4E-binding proteins

Mini-dictionary of terms

Aging The process of change in the properties of a material occurring over a period either spontaneously or through deliberate action
Apoptosis The death of cells that occurs as a normal and controlled part of an organism's growth or development
Autophagosome A spherical structure with double-layer membranes. It is the key structure in macroautophagy, the intracellular degradation system for cytoplasmic contents (e.g., abnormal intracellular proteins, excess or damaged organelles), and also for invading microorganisms.
ER stress Under various conditions, protein folding in the ER is impaired, leading to the accumulation of misfolded proteins.
Misfolding To fold into an incorrect three-dimensional shape that is typically nonfunctional and often resistant to breakdown

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Neurofibrillary tangles Aggregates of hyperphosphorylated tau protein most commonly known as a primary marker of AD

Paired helical filament Neuropathology paired structures that are core constituents of the neurofibrillary tangles of AD and occur in Down syndrome, Hallervorden–Spatz disease, lead encephalopathy, lipofuscinosis, subacute sclerosing panencephalitis, and tuberous sclerosis

Phagophore A double membrane that encloses and isolates the cytoplasmic components during macroautophagy

Proteinopathies Protein conformational disorders, or protein misfolding diseases including Creutzfeldt–Jakob disease and other prion diseases, Alzheimer's disease, Parkinson's disease, amyloidosis, and a wide range of other disorders

Stereotactic Involving, being, utilizing, or used in a surgical technique for precisely directing the tip of a delicate instrument (such as a needle) or beam of radiation in three planes using coordinates provided by medical imaging in order to reach a specific locus in the body

STZ Streptozotocin

Introduction

Aging is the major biologic process driving neurodegeneration in Alzheimer's disease (AD) but is itself a complex accumulation of damaging changes to organ systems and their cells over time, beginning with oxidative stress and inflammation and leading to the misfolding of characteristic proteins. These processes are closely linked to mitochondrial/energy failure and insulin resistance (Butterfield, Di Domenico, & Barone, 2014; De Felice & Ferreira, 2014) and then lead to DNA damage and neuronal death. The signaling pathways involved are common to other chronic conditions such as cancer and cardiovascular disease. By controlling both protein and organelle degradation through the autophagy/lysosomal process and the synthesis of proteins via translation regulation, mammalian/mechanistic target of rapamycin (mTOR) is a master switch that integrates extracellular growth factors and the cell's nutrient status to heavily influence growth and metabolism during aging (Saxton & Sabatini, 2017).

In this chapter, we review the cellular biology and regulation of mTOR. The contribution of mTOR to AD pathogenesis is discussed, and some of the reported differences in the details of its complicated activities are summarized.

Mammalian target of rapamycin and regulation

The mammalian target of rapamycin complex 1 (mTORC1) is composed of the 289 kDa mTOR serine-threonine (S-T) kinase and its regulatory protein raptor, in addition to GβL/mLST8, the 40 kDa noncore, proline-rich Akt substrate (PRAS40), and deptor proteins. The mTORC1 signaling pathway is a crucial cellular energy and nutrient sensor as well as growth factor (insulin, IGF-1, brain-derived neurotrophic factor—BDNF) transducer. mTOR controls protein synthesis by phosphorylating downstream targets essential to the initiation step of mRNA to protein translation (eIF-4E binding protein, 4EBP1) and ribosome biogenesis (p70 ribosomal protein S6

kinase 1, p70S6K) (Swiech, Perycz, Malik, & Jaworski, 2008). Accordingly, mTORC1 has been found to support protein synthesis-dependent synaptic plasticity and dendritic spine numbers that underlie learning and long-term memory (Ebert & Greenberg, 2013; Santini, Huynh, & Klann, 2014). In skeletal muscle, knockout of either raptor or mTOR or the application of specific inhibitors results in a muscular dystrophy (Bentzinger et al., 2008; Risson et al., 2009). Thus, the inhibitor rapamycin or various genetic reductions of mTOR can block several types of memory processes such as fear conditioning and late-phase long-term potentiation (LTP) (Hoeffer & Klann, 2010; Stoica et al., 2011; Tang et al., 2002). BDNF and EGF support memory formation through mTOR activation (Ramanan et al., 2015; Slipczuk et al., 2009). mTOR activation is felt to take place at lysosomes or plasma membrane (Menon et al., 2014; Noda & Ohsumi, 1998). Inhibition of autophagy (see later in chapter) and stimulation of mitochondrial respiration are other key roles (Ramanathan & Schreiber, 2009; Schieke et al., 2006). Cell growth, division, proliferation, survival, and aging are accordingly affected.

Downregulation of mTOR occurs through the TSC 1/2 complex by inactivating Ras homology enriched in brain protein Rheb (see Fig. 21.1). Akt stimulation releases mTOR

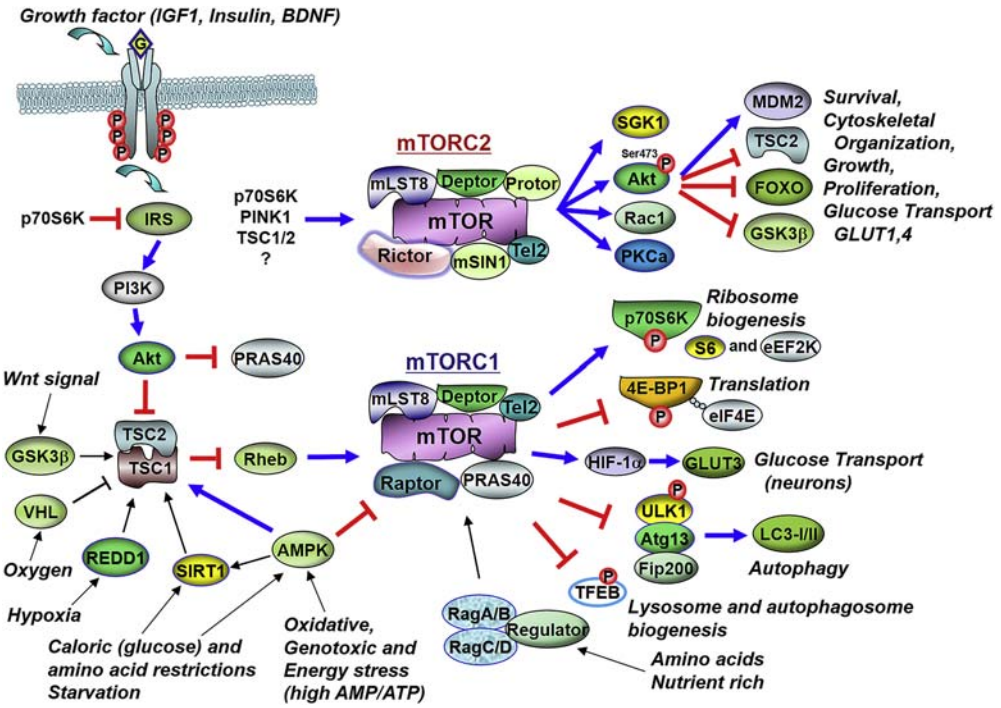

Figure 21.1 ***mTORC1 and C2 pathways.*** Growth factor/neurotrophin, energy, nutrient and oxygen tension state inputs are shown. Effects on protein synthesis, glucose transport, autophagy and cell growth/survival are indicated. PRAS and Deptor are negative C1 regulatory units. Caloric restriction/AMPK and amino acid restriction through TSC1/2 are important C1-inhibitory paths. Negative feedback onto insulin/PI3K/Akt from C1 and positive feedback loop from C2 are noted. The regulation of C2 is less clear. See abbreviations list.

from inhibition by phosphorylation and inactivation of TSC1/2 (Hers, Vincent, & Tavare, 2011; Nave, Ouwens, Withers, Alessi, & Shepherd, 1999). AMPK, an important nutrient sensor and cell energy broker, is a powerful negative regulator of mTOR. It is activated under conditions of low substrate (glucose) and low ATP (high ADP/ATP) (Steinberg & Kemp, 2009; Viollet et al., 2010). AMPK inhibits mTORC1 via TSC phosphorylation (activation) (Gwinn et al., 2008; Inoki, Zhu, & Guan, 2003) and thereby supports autophagy (Kim, Kundu, Viollet, & Guan, 2011; Malik, Urbanska, Macias, Skalecka, & Jaworski, 2013). When AMPK is inhibited, for instance in the palmitate model of insulin resistance, mTOR is stimulated (Kwon & Querfurth, 2015). Under diabetic conditions, as well as others such as ER stress and apoptosis, mTOR induction can be detrimental to cell health, which rapamycin may potentially reverse (Kwon & Querfurth, 2015; Salvado et al., 2013). Akt and AMPK can also influence mTOR directly (via PRAS40 and raptor, respectively), bypassing TSC.

One target of mTORC1, p70S6K, has an additional feedback role to downregulate insulin signaling through an inactivating phosphorylation of IRS-1 (Harrington et al., 2004). This function of stimulated mTORC1, to negatively regulate sustained PI3K/Akt activation by insulin (Harrington, Findlay, & Lamb, 2005; Tzatsos & Kandror, 2006), has central importance to the widely held notion that the AD brain is an insulin-resistant organ (Perluigi et al., 2014; Tramutola et al., 2015).

In contrast, mTORC2 is a relatively rapamycin-resistant complex comprising mTOR and Rictor in addition to GβL/mLST8, mSIN1, PRR5/protor, and deptor proteins. While not directly regulated by nutrients, mTORC2 is insulin and growth factor responsive and feeds forward to amplify the activation of the S-T kinase Akt (protein kinase B) by trophins insulin/IGF-1. It does so by acting as an Akt-S473 kinase (PDK2) (Hresko & Mueckler, 2005; Sarbassov et al., 2006). In this way, it opposes mTORC1 to fine-tune Akt action. C2 may be stimulated by activated ribosome biogenesis (Laplante & Sabatini, 2012). Other targets of mTORC2 as a metabolic regulator and cell survival promoter include the actin cytoskeleton and serum/glucocorticoid-regulated kinase 1 (Cybulski & Hall, 2009; Sparks & Guertin, 2010). mTORCs 1 and 2 are reciprocally inhibited or activated, respectively, by the TSC1/2 complex (tuberin/hamartin) (Huang, Dibble, Matsuzaki, & Manning, 2008; Inoki et al., 2003).

A second mechanism of activated mTORC1-mediated suppression of insulin action is the inhibitory T1135 phosphorylation of rictor/mTORC2 (Julien, Carriere, Moreau, & Roux, 2010), thereby dampening the insulin response at the level of Akt. Thus, by preferentially inhibiting mTORC1, short-term rapamycin treatment may activate Akt via rictor (and by p70S6K suppression), whereas long-term rapamycin disassembles mTORC2, causing insulin resistance (Lamming et al., 2012; Ye, Varamini, Lamming, Sabatini, & Baur, 2012).

Here we review the role of mTOR pathways in various degenerative conditions involving cognition, control of movement, and motor neuron function.

Alzheimer's disease

AD, the most common of neurodegenerations (60%), affects 10% of the world's population over 65. It shares cerebrovascular pathology with vascular cognitive impairment and dementia, accounting for another 20% (Trigiani & Hamel, 2017).

AD is characterized by the accumulation of protein aggregates, namely extracellular amyloid β-peptide (Aβ) and cellular tau tangles, which lead to synaptic and neuronal damage, particularly in the hippocampal and medial temporal/inferior parietal lobules regions of the brain, resulting first in memory dysfunction (Borlikova et al., 2013; Braak & Braak, 1991). Evidence points to the AD brain as affected by a unique form of insulin deficiency (Rivera et al., 2005), attenuated receptor expression (Steen et al., 2005) and resistance (Correia et al., 2011; de la Monte & Wands, 2008). Insulin resistance in the AD brain is itself a complex phenomenon, resulting from a combination of reduced ligand binding to cognate receptor (e.g., IR), IRS-1 deactivation or desensitization (de la Monte, 2017; Diehl, Mullins, & Kapogiannis, 2017), and other signal transduction impairments. β-amyloid has been experimentally shown to result in each of these. In addition, a system of protein disposal and recycling termed autophagy is altered (Nixon, 2013; Tramutola et al., 2015). Energy failure is exacerbated by accumulated mitochondrial damage.

It is important to understand what impact changes in mTOR signaling have for early AD pathogenesis, since mTOR integrating functions are essential to the regulation of brain insulin signaling, protein synthesis, toxic protein/damaged organelle handling, and mitochondrial function. mTOR's role in age extension is also relevant to neurodegeneration. In this regard, neuroprotection in various transgenic and control rodents has been achieved by: (1) downregulation of the insulin/IGF-1 signaling pathway (Douglas & Dillin, 2010; Kenyon, 2011), (2) caloric restriction/SirT1 stimulation (Ghosh, McBurney, & Robbins, 2010; Guo et al., 2011), and (3) rapamycin treatment (Harrison et al., 2009). It is to be emphasized that there is also a large body of opposing evidence in favor of upregulating or restoring insulin/IGF1 signaling-mediated neuroprotection in AD (Carro et al., 2006; Watson & Craft, 2003), creating much discussion and debate.

There are differing accounts of mTOR status in the AD brain, transgenic mice, and cell models. Several groups report dramatic upregulation of basal mTOR signaling markers (phospho-mTOR, p-4EBP1, p-eEF2K, p-p70S6K, and p-eIF4E) in the AD temporal or frontal cortex. For instance, in Western studies of AD, Down syndrome, mild cognitive impairment (MCI), and preclinical AD patients, increased ratios of p-Akt (Ser473), p-PI3K (Tyr508), p-mTOR (Ser2448), and p-p70S6K (Thr389) over their respective total protein levels were found by one group as evidence for overactivation of the PI3K/Akt/mTOR signaling axis. Correlations with decreased autophagy marker expression and increased inhibitory phosphorylation of IRS-1 were also reported (Perluigi et al., 2014; Tramutola et al., 2015). Similar abnormal activation markers have been found by at least two other groups (Griffin et al., 2005; O'Neill, 2013; Pei, Bjorkdahl, Zhang, Zhou, & Winblad, 2008).

Another group also found hyperactivation but only in severely affected AD cases (Sun et al., 2014) (see Table 21.1). The molecular cause behind the autonomous basal activation of PI3K/Akt (and mTOR downstream of that) in the early- to midstage AD brain (Griffin et al., 2005; O'Neill, Kiely, Coakley, Manning, & Long-Smith, 2012) has not been completely worked out, but it is suggested that Aβ directly inactivates PTEN and disinhibits PI3K (Bhaskar et al., 2009; Tramutola et al., 2015). On the other hand, the collateral resistance to insulin/IGF action (Talbot et al., 2012) in AD has been mechanistically linked to feedback inhibitory S616/S636 phosphorylations of IRS-1 by pS6K (O'Neill et al., 2012; Talbot et al., 2012). Aβ has been implicated in this phenomenon by directly activating mTOR in studies using transgenic models (Caccamo et al., 2011; Majumder et al., 2012) and decreases in IRS levels (Kapogiannis et al., 2015; O'Neill et al., 2012). Actual insulin resistance was convincingly demonstrated just recently in the postmortem AD brain (Talbot et al., 2012). Interestingly, the inhibited PI3K/Akt signaling response to insulin stimulation was the most impressively reduced parameter (90%) in these viable human samples, perhaps overshadowing the basal hyperactivated status of Akt and mTOR under unstimulated ex vivo conditions. As pointed out, the targeting of basal IRS-1 phosphorylation may actually involve kinases other than mTOR (Talbot et al., 2012). Additional mechanisms of proximal insulin resistance include reduced numbers and activity of insulin and IGF-1 receptors (O'Neill et al., 2012; Zhao & Townsend, 2009).

In cell models using either transgenic primary cortical neurons (PCNs) or control PCNs exposed to Aβ oligomers, abnormal phospho-activations of Akt (p-S473) and mTOR (pSer2448)/4EBP1(p-S65) pathway components were associated with aberrant cell cycle reentry (Bhaskar et al., 2009). mTOR signaling increases are also described in 3xTgAD and PDAPP transgenic mice, where inhibition of mTOR with rapamycin rescued early learning and memory deficits and activated autophagy (Caccamo et al., 2011; Spilman et al., 2010). In further experiments by the same group, intrahippocampal anti-Aβ antibody injections normalized the aberrant mTOR activation. In their model, Akt hyperactivation was deduced to drive proline-rich Akt substrate of 40 kDa (PRAS40) phosphorylation, thereby derepressing mTORC1 (Caccamo et al., 2011).

Regarding tau pathology, mTOR hyperactivation may also be responsible for hyperphosphorylation and cytoplasmic vacuolar collections (Tang et al., 2015). By acting on multiple tau kinases (e.g., p70S6K) (Pei et al., 2006) as well as by inhibiting PP2A (the major tau phosphatase) and supporting tau translation, the Akt/mTOR axis can drive tau hyperphosphorylation (Lee, Lee, & Rubinsztein, 2013; Tang et al., 2015). The situation with glycogen synthase kinase (GSK) 3β (tau kinase 1), however, remains uncertain because Akt, if stimulated by Aβ as hypothesized, would be expected to and does drive GSK inhibition (pS9) (Tramutola et al., 2015). In any case, activated mTOR marker levels were positively correlated to neurofibrillary tangle load, total and paired helical filament-tau burden, and excessive tau mRNA translation (Li, Alafuzoff, Soininen,

Table 21.1 mTOR dysregulation in Alzheimer's disease.

Model system			mTOR signaling						Akt signaling				Reference
AD or DS Brain	Transgenic mice	Cell culture or in vitro	Phospho-mTORC1 S2448,S2481	Phospho-p70S6K/4EBP1 T389,T421,S424	Autophagy markers Beclin/ LC3-ll	mTORC1 activity	Protein translation	Phospho-mTORC2	Phospho-Akt, basal S473,T508	Phospho-Akt, stim	Akt activity	Phospho-GSK3β S9	
•			↑						↑			↑	Griffin J Neurochem 2005; O'Neill Exp Gerontol 2013
•				↑/									An Am J Pathol 2003; Pei FEBS Lett 2006; Pei J Alz Dis 2008
	•	•	NC or ↑	/↑					↑				Bhaskar Mol Neurodegen 2009
	•	•	NC	↑/↑		↑							Caccamo JBC 2011,2010
•	•			↑/↑	NC								Caccamo J Neurosci 2014,2015
	•			↑/					↑			↓	Caccamo Neurobio Dis(NBD)2018
•			↑	↑/	↓				↑				Perluigi BBA 2014; NBD 2015
•			↑	↑/↑									Iyer J Neuro Exp Neurol 2014
•			↑	/↑	↓								Li FEBS J.2005
•			↑(adv. AD)	↑/				NC					Sun J Alz Dis 2014
•			↑	↑/↑	↓				↑				Tramutola J Neurochem 2015
		•		↑/↑									Norambuena Alz and Dem 2017
•			↑(↓ to Ins)						↑	↓		NC or ↑	Talbot J Clin Invest 2012
•WBC	•	•	↓	↓(biphasic)					NC				Lafay-Chebassier J Neurochem; 1994; J Neurosci Res 2006
•	•		↓	↓/↓			↓		↓		↓		Ahmad Antiox Redox Sig 2017

Continued

Table 21.1 mTOR dysregulation in Alzheimer's disease.—cont'd

Model system			mTOR signaling						Akt signaling				Reference
AD or DS Brain	Transgenic mice	Cell culture or in vitro	Phospho-mTORC1 S2448,S2481	Phospho-p70S6K/4EBP1 T389,T421,S424	Autophagy markers Beclin/ LC3-II	mTORC1 activity	Protein translation	Phospho-mTORC2	Phospho-Akt, basal S473,T508	Phospho-Akt, stim	Akt activity	Phospho-GSK3β S9	
•	•	•	↑(adv. AD)	NC or ↑	NC or ↑	↓(↓/C2)	↓	NC	↓	↓	↓		Lee HK. J Alz Dis 2017; Mol Biol Cell 2009
	•		↓	↓/↓	↓/NC								Francois J Neuroinflam 2014
	•		NC	↓/NC					↑				Damjanac Neurobiol Dis 2008
•	•	•		↓/↓					↓				Ma PLoS-1 2010; J Neurosci 2014
		•	↓							↓			Chen J Neurosci Res 2009
		•	↓		↑				↓				Xue Eur J Pharm 2014
•				↓/									Chano Brain Res 2007
	•			↓/	↑								Siman PLoS-1 2015
	•			↓/	NC				NC			↓	Avrahami J Biol Chem 2013

In a summary of the literature, the model systems employed by the various authors/laboratories referenced on the far right, are noted in the far left. "Alzheimer (AD) or Down syndrome (DS) brain" may also include other human cell types and either be post mortem fixed, frozen or ex vivo. "Transgenic mice" may also include β-amyloid- or viral transgene-injections into wild type animals. "Cell culture" may be transfected or exposed to β-amyloid and either utilize primary, immortalized lines, transfected or mouse brain tissue slices as models of amyloid/Presenilin/Tau injury. Also included therein are other in vitro assays. mTOR signal changes include: 4EBP-1 and enzymatic activity measurement as evidence for activation and direction of change (↑ indicates hyperstimulation, ↓ understimulation). Whether macroautophagy is initiated is also indicated. If data on Akt activation (pAkt, insulin-stimulated Akt, downstream phospho-GSK3β, or enzymatic activity is provided, a hyperactivation (↑) or inhibition (↓) is noted. A change qualified by context is noted by an 'or'. NC= no change. Most, but by no means all, studies favor hyperactivation of Akt and mTOR in various amyloid injury models (top half), pointing to significant differences in model employed, stage of disease severity, time course, other technical issues, etc. to account for conflicting findings. Note that very few studies report on actual enzymatic activity assay. mTORC2 is relatively understudied in AD. See abbreviations. The authors apologize for any inaccuracies and unintended omissions.

Winblad, & Pei, 2005; Oddo, 2012). In a Drosophila tauopathy, mTOR activation was also found to mediate cell cycle reentry and neurodegeneration (Khurana et al., 2006). Stereotactically injected AAV-hTauP301L into the mouse hippocampus provoked the expected pathology, which was rescued by rapamycin (Siman, Cocca, & Dong, 2015). Notably, AMPK is also a tau kinase (targeting Thr231 and Ser 396/404). Activated phospho-AMPK (Thr 172) accumulates in tangle-bearing AD neurons and other tauopathies as shown by immunohistocytology (Vingtdeux, Davies, Dickson, & Marambaud, 2011). Increased p-AMPK, and a translation target p-eEF2K, were demonstrated by Western in postmortem AD brain and double APP/PS1 transgenic mice extracts (correlated with LTP failure), indicating pathologic AMPK hyperactivation. However, mTOR status was not tested (Ma et al., 2014).

On the other hand, in a recent study of autopsy brain, levels of p-mTOR (Ser2448), p-mTOR (Ser2481), and total mTOR revealed no statistical differences across the clinical groups (AD vs. control) (Perez et al., 2015). Another study found neither total nor phospho-mTOR (or raptor) levels were significantly changed in early to moderate AD hippocampus compared with control. The same authors concluded that rictor (mTORC2) expression was unaltered in AD (Sun et al., 2014). In our work, both mTORC 1 and two levels and their respective enzymatic activities were reduced in advanced AD brain and transgenic models, autophagy markers increased, and protein synthesis inhibited (Lee et al., 2017). Nevertheless, either rapamycin treatment by further reducing mTORC1, or overexpression of C2, were found to be protective. A proteomics study of neuroblastoma cells expressing wild type or mutant mTORs and subjected to serum deprivation- induced death, also concluded that upregulation of mTORC2, but not C1, was responsible for increased cell viability. mTORC2 promoted survival by suppressing mitochondrial caspase-mediated apoptotic pathways as well as by stimulating pAkt (Tang et al., 2014). These results in AD models are consistent with mTORC2 survival-promoting functions (Goncharova et al., 2011; Jacinto et al., 2006).

Results pointing to downregulation of mTOR signaling (pS2448 and p-p70S6K) were obtained in N2A cells affected by aggregated Aβ42 in double-transgenic APP(sl)/PS1(M146L) mouse cortex and AD lymphocytes compared with controls (Lafay-Chebassier et al., 2005). Moreover, APP(swe)/PS1(deltaE9) transgenic mice display increased autophagic activity accompanied by decreased mTOR activity (Li et al., 2013). In yet another Tg model, APP(sl)/PS1(KI), mTOR itself was unchanged but downstream activation of p70S6K (pT389) was reduced rather than stimulated (Damjanac et al., 2008). Consistent with these studies but using a growth factor stimulation paradigm in rat PCNs, Aβ treatment inhibited brain-derived neurotrophic factor (BDNF)-induced Akt-mTOR signal activation (Chen, Wang, & Chen, 2009). Similar inhibition of neurotrophin-stimulated Akt/GSK3β-S9 phosphorylation was found in N2a cells exposed to oligomeric Aβ-containing fractions from 2X transgenic AD mouse brain (Jimenez et al., 2011). In the transgenic APPTg2576 model, decay of LTP was correlated with inhibited

mTOR signaling (lowered p-p70S6K and p-4EBP1), similar to results in wild type slices exposed to Aβ peptide or rapamycin (Ma et al., 2010; Morel, Couturier, Lafay-Chebassier, Paccalin, & Page, 2009). In this model, upregulation of mTOR rescued LTP (Ma et al., 2010). The role of systemic insulin resistance in modifying mTOR signaling in AD was recently probed using two rat models (T2DM: IP STZ on high fat diet and AD: hippocampal Aβ injection). In comparing the control, T2DM, and AD animal groups, the total mTOR protein and mRNA levels in hippocampus as well as the phosphorylation of tau protein were significantly increased only in the combined T2DM + AD group and not between the control and AD group (Ma, Wu, & Liu, 2013). Finally, a reduction in mTOR signaling and basal p-Akt markers as well as activity was correlated with inhibited BDNF-stimulated protein translation in synaptosomes from APP/PS1 mice and postmortem AD brain. Akt enhancement rescued protein translation (Ahmad et al., 2017). The observation that Aβ may stimulate AMPK, perhaps in a compensatory manner, may partially explain the reduction in mTOR activity observed in some studies (Cai, Yan, Li, Quazi, & Zhao, 2012; Lee et al., 2017).

As noted previously, whether in an AD transgenic model where basal mTORC1 is abnormally overactivated or in another model where downstream marker p-p70S6K was not (against a functionally hyperactive mTORC1), rapamycin had a beneficial effect on restoring memory formation and maintenance (Caccamo, Majumder, Richardson, Strong, & Oddo, 2010; Spilman et al., 2010). This underscores the duality of mTOR roles in health and disease with respect to synaptic plasticity (Bockaert & Marin, 2015).

In AD, neuronal autophagy is a major clearance mechanism for amyloid, alongside microglia, the UPS, and degrading enzymes (Das et al., 2013; Son, Jung, Shin, Byun, & Mook-Jung, 2012). In early-stage AD or animal models, autophagic vacuoles accumulate in dystrophic neurites. Rather than occurring from the basal suppression of mTOR, as found in some studies, they develop primarily because lysosomal acidification, autophagosome fusion, or clearance are reduced, resulting in net impairment of autophagic flux (Boland et al., 2008; Nixon, 2013). Recently, autophagy markers (beclin-1, LC3) were found decreased in the MCI and AD brain. These correlated negatively with amyloid load and were associated with hyperactivated PI3K/Akt/mTOR axis (Tramutola et al., 2015). Consistent with this, rapamycin, by suppressing C1, induces autophagy flux and ameliorates cognitive deficits in mice (Majumder, Richardson, Strong, & Oddo, 2011). Genetic reduction of mTOR in Tg2576 AD mice also reduced Aβ pathology, stimulated autophagy, and rescued memory deficits (Caccamo, De Pinto, Messina, Branca, & Oddo, 2014). Abnormal tau can also be cleared by mTOR- regulated autophagy (Congdon & Wu, 2012; Li, Liu, & Sun, 2017) but in turn may also impair it (Rodriguez-Martin et al., 2013). Rapamycin ameliorates tau pathology in triple-transgenic mice (Caccamo et al., 2010; Spilman et al., 2010).

Conclusion

Both the upregulation of basal mTOR activity markers and autonomous basal overactivation of the PI3K/Akt/mTOR axis are reported in several cellular and transgenic models as well as in AD brain cohorts. Aβ accumulation is at least partially responsible via mechanisms such as inactivation of PTEN (disinhibition of PI3K) and activation of mTOR. Insulin resistance and inhibited Akt activation ensue from powerful negative feedback at the level of IRS-1. Tau phosphorylation may also be driven by Akt/mTORC1 hyperactivation. However, acceptance of these details is not complete because other studies have found no change or reduced mTOR activation markers and activity in both AD samples and animal models (see Table 21.1). It is nevertheless accepted by most investigators that rapamycin treatment is neuroprotective. Thus, manipulation of mTOR is a strong treatment strategy to pursue in AD. The goal would be inhibit excessive activity or even further reduce activity to some level below normal baseline. However, in some neurodegenerative disorders or where there is clear mTOR deficiency, it may be necessary to reestablish basal levels and reactivity. These considerations will depend on the particular neurodegenerative process and proteotoxicity as well as disease stage (see part 2).

In the second chapter, the topic of mTOR inhibition as a treatment option will be discussed in the broader context of other neurodegenerations such as ALS, FTD, and PD.

Key facts

- mTOR is the main kinase subunit common to two large complexes termed C1 and C2.
- C1 and C2 have different functions determined by the presence of distinct positive and negative regulatory protein subunits.
- Stimulation of C1 by insulin and other trophic factors may, through negative feedback, lead to downregulation of insulin signaling, whereas C2 appears to amplify insulin action.
- Rapamycin (sirolimus) is a natural inhibitor of mTORC1 produced by a streptomyces bacterium isolated in soil samples from Easter Island. It binds first to a cytosolic protein termed FKBP12 before targeting mTOR.
- Rapamycin has important antiinflammatory and tumor-arresting properties, finding early uses in coronary stent patency, prevention of transplant rejection, and renal cancer.

Summary points

- mTOR plays an essential role in cellular protein synthesis, component recycling and disposal, growth, and survival in many tissue types from its position at the intersection of diverse signaling pathways.

- mTOR is a serine/threonine kinase residing in either of two enzyme complexes, mTORC1 or mTORC2.
- mTOR functions as a homeostatic nutrient sensor to regulate energy metabolism and transduce insulin action.
- It supports growth and plasticity in neurons.
- mTOR has a key function in negatively regulating autophagy.

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CHAPTER 22

Mammalian target of rapamycin complexes: protein synthesis and autophagy, Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia

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List of abbreviations

Akt protein kinase B
AMPK AMP-activated kinase
Atg autophagy-related protein 13
BDNF brain-derived neurotrophic factor
FTD frontotemporal dementia
mTOR mammalian (or mechanistic) target of rapamycin
p70S6K1 p70 ribosomal S6 protein kinase 1
raptor regulatory-associated protein of mTOR
TSC tuberous sclerosis complex
ULK1 unc-51-like kinase 1
4E-BPs 4E-binding proteins

Mini-dictionary of terms

Aging The process of change in the properties of a material occurring over a period, either spontaneously or through deliberate action
Autophagy The condition whereby the cells start to recycle damaged and garbage parts (defects) into basic elements thereby allowing the cells to remodel themselves
Elaborate (of a natural agency) produces (a substance) from its elements or simpler constituents.
Homeostasis The state of steady internal conditions maintained by living things. This dynamic state of equilibrium is the condition of optimal functioning for the organism and includes many variables, such as body temperature and fluid balance, being kept within certain preset limits.
Immunosuppression A reduction in the activation or efficacy of the immune system
Lewy bodies Abnormal aggregates of protein that develop inside nerve cells, contributing to PD
Misfolding To fold into an incorrect three-dimensional shape that is typically nonfunctional and often resistant to breakdown

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Nephrotoxicity Toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on renal function. There are various forms, and some drugs may affect renal function in more than one way

Pathophysiology The disordered physiological processes associated with disease or injury

Pneumonitis Inflammation of the walls of the alveoli in the lungs, usually caused by a virus

Proteinopathies Protein conformational disorders, or protein misfolding diseases that include such diseases as Creutzfeldt–Jakob disease and other prion diseases, AD, PD, amyloidosis, and a wide range of other disorders

Proteotoxicity The adverse effects of damaged or misfolded proteins and even organelles on the cell

Stomatitis A condition that causes painful swelling and sores inside the mouth

Tauopathy A class of diseases caused by misfolding of the tau protein

Transplantation The process of taking an organ or living tissue and implanting it in another part of the body or in another body

Introduction

In the accompanying earlier chapter, the regulation and cellular biology of mTOR were reviewed. As most studies of mTOR in neurodegeneration have been reported in the Alzheimer's disease (AD) field, that area was discussed first. Here we review mTOR's biologic action in greater detail and then review what is known of it in amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and frontotemporal dementia (FTD). Common to all neurodegenerations, aging is the major biologic process driving the accumulation of damage to organ systems and their cells, beginning with oxidative stress and inflammation leading to the misfolding of characteristic proteins and proteotoxicity. Mitochondrial/energy failure and insulin resistance (Butterfield, Di Domenico, & Barone, 2014; De Felice & Ferreira, 2014) are important factors in a process that ends with DNA damage and neuronal death. By controlling both protein and organelle degradation through the autophagy/lysosomal process and the synthesis of proteins via translation regulation, mTOR is a master switch that integrates extracellular growth factors and the cell's nutrient status to heavily influence growth and metabolism during aging (Saxton & Sabatini, 2017). Finally, the rationale and prospects for treatment of these disorders as based on mTOR are summarized.

Mammalian target of rapamycin control of protein synthesis and autophagy

mTOR's functions to control protein synthesis and autophagy largely account for its contribution to neurodegenerative diseases. In general, mTOR supports protein synthesis by regulating cap-dependent translation and transcription by phosphorylating p70 ribosomal S6 protein kinase 1 (p70S6K) and 4E binding protein 1 (4E-BP1) (Laplante & Sabatini, 2012). Rapamycin inhibits this response to the cell's energy and dietary state (Stanfel, Shamieh, Kaeberlein, & Kennedy, 2009). mTOR's supportive roles in synaptic

plasticity (Hou & Klann, 2004; Tang et al., 2002) and in hippocampal memory formation/stability (Gong, Park, Abbassi, & Tang, 2006; Parsons, Gafford, & Helmstetter, 2006) derive from these properties as occur in dendrites and their synapses (Swiech, Perycz, Malik, & Jaworski, 2008).

The most studied form of autophagy, macroautophagy, is a conserved cellular pathway for removing protein aggregates (aggrephagy) and organelles (e.g., mitophagy), whereby the contents of autophagosomes fuse with the lysosome. It is activated by cellular starvation (amino acids, glucose) as well as by protein aggregation and organelle damage (Menzies et al., 2017). mTOR kinase's phosphorylating activity inhibits the induction of autophagosome formation by blocking autophagy-related gene product 13 (Atg) and Unc-51-like kinase 1 (ULK1/2) complex formation (Jung et al., 2009; Russell et al., 2013) as well as inhibiting activation of LC3B I/II (Fujita et al., 2008; see Fig. 21.1). Autophagy itself may be the target of toxic protein oligomers and begin to fail in AD and PD (Kaushik & Cuervo, 2018; Menzies et al., 2017). Its essential role is demonstrated in mice by the neuronal deletion of Atg genes resulting in age-dependent neurodegeneration and proteostasis (Hara et al., 2006; Komatsu et al., 2006). Downregulation of mTOR signaling facilitates autophagy (Goodman, Mayhew, & Hornberger, 2011). Amino acid deprivation inhibits mTOR and stimulates autophagy, whereas nutrient-rich conditions or basal neurotrophin availability suppresses autophagy and contributes to neuronal survival (Smith et al., 2014). Inhibition of mTORC1 by rapalogs or activation of AMP-activated kinase (AMPK) facilitates autophagy and misfolded protein removal (Tian, Bustos, Flajolet, & Greengard, 2011; Wu et al., 2015) including β-amyloid (Vingtdeux et al., 2011). These interventions tend to promote Atg transcription and recruitment to the phagophore by disinhibiting ULK-1 and Vps34 complex formations (Plaza-Zabala, Sierra-Torre, & Sierra, 2017).

Parkinson's disease

PD is the second commonest neurodegeneration after AD, affecting 1%–2% of those over age 65, in which aging is also the primary risk. There is a large body of evidence that mTOR is perturbed in PD models (Xu et al., 2014). First and more so than in AD, oxidative stress (reactive oxygen species) is a major contributor to the selective degeneration of dopaminergic neurons in PD (Ciccone, Maiani, Bellusci, Diederich, & Gonfloni, 2013). Accordingly, various neurotoxins (e.g., ceramide, rotenone, H2O2, 6OH-DA) are used to model PD pathophysiology. In general, these manipulations suppress mTOR/protein kinase B (Akt) activity, and restoring mTOR or overexpression of p70S6K rescue neuronal death (Xu et al., 2014; Zhou et al., 2015). Conversely, rapamycin predictably potentiates oxidative stress (Chen et al., 2010; Choi, Kim, Ha, Kim, & Son, 2010). Nevertheless, in one MPTP mouse model, mTOR/Akt was upregulated 1 week out from a single injection, and autophagy markers

were correspondingly impaired (Giacoppo, Bramanti, & Mazzon, 2017). The nuanced role of mTOR was further elaborated using such models by showing that mTOR pathologically upregulated protein translation to toxic levels, and that rapamycin proved neuroprotective by correcting this and by restoring Akt signaling (Malagelada, Jin, Jackson-Lewis, Przedborski, & Greene, 2010). Important to note is that accumulation of the major proteinopathy comprising the Lewy bodies of PD, that of α-synuclein, may be lacking in such models.

α-Synuclein modulates synaptic activity, and point mutations in the SNCA gene cause early-onset PD (Irwin, Lee, & Trojanowski, 2013). Deficient autophagy may contribute to α-synuclein accumulation in PD, and stimulation by rapamycin may promote its clearance (Menzies, Fleming, & Rubinsztein, 2015; Scrivo, Bourdenx, Pampliega, & Cuervo, 2018). Macroautophagic removal of defective mitochondria may also be protective (Perier et al., 2005). In the PD brain, mTOR appears upregulated, and autophagosomes accumulate (Chu, Dodiya, Aebischer, Olanow, & Kordower, 2009; Dijkstra et al., 2015). In neuronal cultures and mice expressing mutant A53T α-synuclein, mTORC1 signaling is also overactivated, resulting in insulin resistance (via IRS-1, S636 phosphorylation), with effects reversed by rapamycin (Gao, Duan, Gao, Wang, & Yang, 2015; Xiong et al., 2015). In agreement, everolimus holds promise in Lewy body disease (Crews et al., 2010).

Interestingly, several familial PD-linked proteins, such as those affected by disease-causing mutations in PINK-1/parkin, LRK2, and DJ-1 genes, influence the autophagy—lysosomal pathway in response to mitochondrial damage in an mTOR-independent manner (Manzoni et al., 2016; Zhang et al., 2017). Adding to the complicated actions of mTOR in PD noted above, there is interest in stimulating autophagy and synuclein removal by means other than rapamycin and analogs—i.e., mTOR-independent autophagy. These strategies employ agents like curcumin and trehalose (Jiang et al., 2013; Redmann, Wani, Volpicelli-Daley, Darley-Usmar, & Zhang, 2017). Whereas mTOR negatively regulates transcription factor EB (TFEB) to limit autophagy, trehalose acts on the Foxo-1 transcription factor to enhance autophagy protein expressions (Castillo et al., 2013).

In cells bearing the Huntington's disease mutation that expands the CAG tract in the Htt gene, blockade of mTOR with rapamycin results in stimulated autophagy, removal of mHtt protein, and cytoprotection (Berger et al., 2006; Roscic, Baldo, Crochemore, Marcellin, & Paganetti, 2011). Nevertheless, here too there is evidence to the contrary, in a rodent model, that mTOR activation is cytoprotective (Lee et al., 2015).

Amyotrophic lateral sclerosis and frontotemporal dementia

In another expansion mutation, a hexanucleotide repeat in the C9ORF72 gene causes the most common forms of inherited ALS and FTD. The loss of its protein function promotes TDP43 accumulation in ubiquitin-containing inclusions. In a C9ORF72 knockout model, autophagic flux is increased. Additionally, mTOR activity (p-p70S6K1) is found to be reduced. Hence, C9ORF72 protein is postulated to act as a negative autophagy

regulator, perhaps in synergy with the binding of mTOR, at the lysosome membrane (Ji, Ugolino, Brady, Hamacher-Brady, & Wang, 2017; Khayati et al., 2017).

In a model incorporating another ALS-causing gene mutation, SOD-1 G93A, autophagy markers are increased in spinal motor neurons (Zhang et al., 2011). mTOR enhancement and autophagy abrogation were neuroprotective (Hsueh et al., 2016). On the other hand, mTOR-independent activation of autophagy with trehalose was neuroprotective and reduced protein aggregation. Since rapamycin actually accelerated disease progression, perhaps motor neuron viability is dependent on the stable activity of mTOR in this context (Saxena et al., 2013; Zhang et al., 2014). Progranulin (GRN) mutations resulting in haploinsufficiency also cause familial FTD, and in GRN genetic models, trehalose is also found to be neuroprotective (Holler et al., 2016).

Nevertheless, in the model context of TDP43/ubiquitin inclusions, rapamycin treatment and mTOR inhibition-autophagy activation are neuroprotective against memory loss and inclusion burden (Wang et al., 2013). Finally, in a mouse FTD tauopathy model where mTOR overactivation is associated with tau accumulation and hyperphosphorylation, rapamycin also reduced behavioral deficits and afforded neuroprotection (Caccamo et al., 2013). A phase 2 clinical trial of rapamycin in ALS is ongoing (Mandrioli et al., 2018).

Mammalian target of rapamycin–based treatment

Rapamycin (sirolimus), the prototypical mTORC1 inhibitor, is an immunosuppressant and antiproliferative FDA-approved agent for kidney transplantation, coronary stents, cardiac hypertrophy, and renal cell carcinoma (Garber, 2009). It binds FK506 and stabilizes regulatory-associated protein of mTOR (raptor-mTOR) in a kinase-inactive complex. Its protective effect in models of neurodegeneration first arose from the recognition that mTOR transduces the actions of insulin and IGF-1 via Akt in both the periphery and brain. Not only does type 2 diabetes double the risk for AD (Schrijvers et al., 2010), but the AD brain is also intrinsically insulin resistant and does not metabolize glucose where needed. Thus, much effort has been devoted to developing antidiabetic drugs for AD including metformin, glimepiride (a sulfonylurea), GLP-1, and liraglutide (a glucagon-like peptide analog) and intranasal insulin (Li, 2017; McClean, Jalewa, & Holscher, 2015). These strategies appear to enhance the Akt/mTOR signaling axis.

On the other hand, the inhibition of downstream mTOR with rapamycin proved not only to increase life-span in aging mammals (Harrison et al., 2009) as well as being proneuroregenerative (Maiese, 2014), but to also rescue cognition and restore synaptic plasticity in many animal and cell disease models. Effects also include mitigating synaptic/neuronal losses and vasculopathy and decreasing proteotoxic aggregates via autophagy/lysosome induction. Rapamycin is beneficial, perhaps regardless of mTOR basal activation status, in alleviating proteotoxicity in AD mice and other neurodegenerative models (Berger et al., 2006; Jimenez et al., 2011; see Fig. 22.1). Examples include tuberous sclerosis complex (TSC) 2 haplo-deficient mice (Ehninger et al., 2008), hippocampal

neurons under toxic stress from β-amyloid (Ramirez, Pacheco, Aguayo, & Opazo, 2014), 3X and hAPPJ20 transgenic AD mouse strains (Lin et al., 2013; Spilman et al., 2010), and transgenic tauopathy mice (Caccamo et al., 2013; Siman, Cocca, & Dong, 2015). A caveat is that once the disease becomes too advanced, rapamycin becomes ineffective (Majumder, Richardson, Strong, & Oddo, 2011).

Unfortunately, the systemic toxic profile of rapamycin, including pneumonitis, stomatitis, poor wound healing, nephrotoxicity, and immunosuppression (Li, Kim, & Blenis, 2014; Sadowski, Kotulska, & Jozwiak 2016), limits its application to foster neuroregeneration and abate neurodegeneration. Rapamycin can be toxic to mitochondrial respiration and biogenesis via peroxisome proliferator-activated receptor gamma coactivator 1 disruption (Ramanathan & Schreiber, 2009; Ye, Varamini, Lamming, Sabatini, & Baur, 2012). Long-term use can produce insulin resistance including loss of mTORC2 function, inhibition of Akt phosphorylation, IRS-2 levels, and glucose uptake (Pereira et al., 2012) and thereby exacerbate type 2 diabetes mellitus (Sarbassov et al., 2006; Yang et al., 2012).

Rapalogs such as temsirolimus, everolimus, and second-generation mTOR inhibitors represent major improvements in tolerance (Benjamin, Colombi, Moroni, & Hall, 2011; Chiarini, Evangelisti, McCubrey, & Martelli, 2015) and hold promise in aging and AD (Richardson, Galvan, Lin, & Oddo, 2015). Temsirolimus restores learning and memory in 5-month-old double-mutant AD transgenic mice, associated with autophagic clearance of

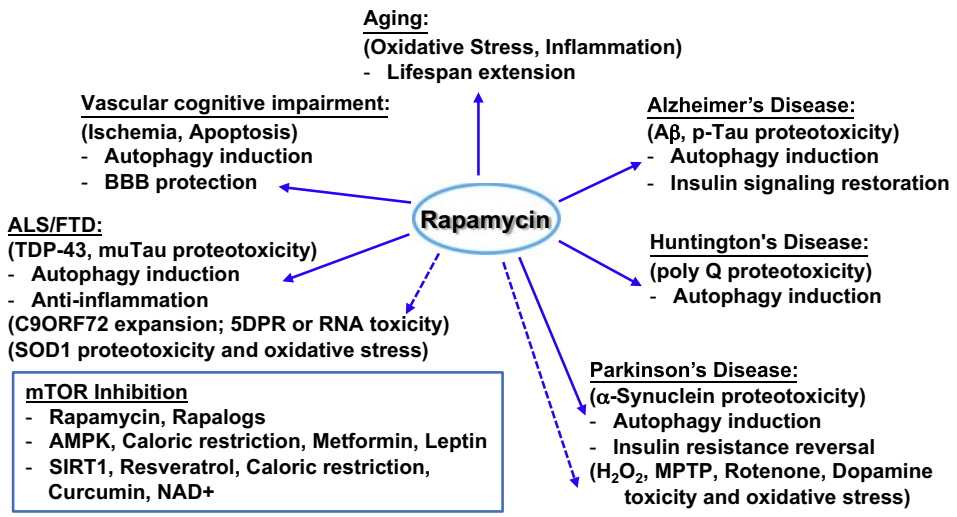

Figure 22.1 As a nutrient sensor, mTOR has important homeostatic functions to regulate energy metabolism and support neuronal growth and plasticity. However, in Alzheimer's disease (AD), mTOR alternately plays important pathogenic roles by inhibiting both insulin signaling and autophagic removal of beta amyloid and Tau aggregates. Overactive mTOR also abets the cerebrovascular dysfunction of AD. Some of the other neurodegeneration conditions, discussed herein, have similar proteotoxic mechanisms. The beneficial actions of mTOR inhibition with rapamycin are shown as arrows to the corresponding bulleted effects. Dashed arrows indicate unproven actions on these proteotoxic processes.

Aβ and antiapoptosis (Jiang, Yu, Zhu, Tan, et al., 2014a,b). Similar results in phospho-tau clearance and memory are reported in a mutant TauP301S model (Frederick et al., 2015).

Metformin, which activates AMPK (indirectly suppressing mTOR) and may also directly suppress raptor/mTOR, and stimulates autophagy like rapamycin. However, there is epidemiological evidence pointing to an increase in AD risk in those treated with metformin (Imfeld, Bodmer, Jick, & Meier, 2012). Nevertheless, one clinical trial concluded in favor of its use in MCI/AD to mitigate cognitive dysfunction (Koenig et al., 2017). Several in vitro and in vivo AD models also report conflicting results with metformin, either promoting amyloid aggregation and memory dysfunction or rescuing synaptic plasticity and preventing neuropathological changes. The agent cilostazol increases AMPK expression (SirT-1-dependent), suppresses mTOR activation, increases autophagy markers beclin-1, Atg, and LC3II, and promotes Aβ autophagic clearance of AB in N2A neurons (Park et al., 2016). Caloric restriction also stimulates AMPK to activate autophagy and prevent AD pathology in triple-transgenic mice (Salminen, Kaarniranta, Haapasalo, Soininen, & Hiltunen, 2011). Dietary curcumin and resveratrol reduce mTORC1 levels and disrupt the complex and/or can inhibit mTOR by activating AMPK, thereby inducing autophagy and rescuing cognitive impairment in 2X AD transgenic mice (Wang, Zhang, Teng, Zhang, & Li, 2014). mTOR-independent stimulation of autophagy with trehalose is another alternative (Sarkar et al., 2007).

Conclusion

Manipulation of mTOR is a strong treatment strategy to pursue in PD/Huntington's disease (HD), ALS, and AD (Fig. 22.1). The goal is to either inhibit excessive activity or restore deficient activity in order to reestablish basal levels and reactivity. This will depend on the particular neurodegenerative process and proteotoxicity as well as disease stage. Attempts to block its activity should be partial in consideration of important roles in facilitating memory formation (Ramanan et al., 2015; Yang et al., 2014) and tissue repair involving progenitor cells (Romine et al., 2015) such as following ischemia (Xie et al., 2013). Insulin/Akt axis homeostasis and mTORC1-dependent protein translation are essential and need to be maintained. mTOR also has important antiapoptosis properties. On the other hand, overactivation of mTOR carries the potential for tumorigenesis (e.g., loss of tumor suppressor TSC1/2 function in tuberous sclerosis), loss of autophagy function (Maiese, 2015), glucose intolerance via IRS-1 feedback inhibition (Harrington et al., 2004), and even learning impairment (Ehninger et al., 2008). Conversely, overinhibition of mTOR alone could also lead to feedback overactivation of Akt and unchecked tumor proliferation.

In proteotoxic processes, the overriding goal may be to stimulate autophagy and protein removal. Most studies recommend mTOR inhibition for neuroprotection—for instance, in AD (Cai & Yan, 2013). However, under other conditions, mTOR activation would appear therapeutically beneficial. These may include where ischemia/apoptosis is the overriding pathology (Yang et al., 2014; Zare Mehrjerdi et al., 2013) or where oxidative stress is high in certain PD and ALS models (Choi et al., 2010; Zhang et al., 2011).

Moreover, the timing of mTOR inhibitor treatments can affect C1 and C2 differentially (Choo, Yoon, Kim, Roux, & Blenis, 2008; Thoreen & Sabatini, 2009). Thus, it is likely that an individualized balance between mTORC1 and C2 manipulations needs to be reached for each of the proteinopathies (Lee et al., 2017; Roscic, Baldo, Crochemore, Marcellin, & Paganetti, 2011). The use of rapamycin or analogs to treat AD has promise due to its many actions to increase longevity and remove toxic proteins, but toxicity concerns persist. Yet, this leaves open other mTOR-dependent targets such as S6K1/2 (Caccamo et al., 2015; Jahrling & Laberge, 2015) and more selective brain targeting strategies (Richardson et al., 2015).

The balance between IRS inhibition (mTORC1-directed negative feedback) and AKT responsiveness to insulin (mTORC2/rapamycin-insensitive companion of mTOR-directed positive feedback) could be swung in favor of homeostatic insulin signaling.

Key facts

- AD is an insulin-resistant state affecting cerebral metabolism, resembling a form of diabetes of the brain.
- mTOR has been found to be dysregulated in the AD brain; however, there is controversy as to which direction and to what effect.
- Most studies in models agree that mTOR C1 inhibition may be beneficial in AD.
- Toxicity of rapamycin, such as infection and tissue repair, limits its potential clinical use in neurodegeneration.
- Other similar compounds or "rapalogs," exist or are in development, offering a safer strategy to promote misfolded protein removal.

Summary points

- mTOR is variably deranged in several neurodegenerative diseases.
- mTOR is a novel drug target to arrest neurodegeneration in several dementing conditions that involving misfolded protein accumulations.
- In AD, mTOR may be pathologically overactive.
- In AD, mTOR activity alternately associates with disease progression by inhibiting both insulin signaling and autophagic removal of β-amyloid and tau aggregates.
- New data suggest that the balanced actions of mTORC1 and C2 may also have implications for PD, HD, FTD, and ALS.
- Beyond rapamycin, rapalogs with lesser toxicity hold promise in arresting these age-dependent conditions.

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CHAPTER 23

Linking CD200 in brains and dementia: molecular aspects of neuroinflammation

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List of abbreviations

AD Alzheimer's disease
Aβ amyloid beta
EAE experimental allergic encephalomyelitis
IL interleukin
PD Parkinson's disease

Mini-dictionary of terms

Alzheimer's disease Most common cause of dementia in elderly, having the pathological feature of plaques and neurofibrillary tangles
Amyloid beta Putative causative factor for AD; a 4 kDa peptide that becomes aggregated and deposited as plaques in aging and AD
Experimental allergic encephalomyelitis Widely used experimental model to produce, in rodents, a demyelinating and inflammatory syndrome with similarity to multiple sclerosis; involves injection of myelin-related proteins into animals to induce autoimmune response
Lewy body disease Term for a number of diseases that have deposits of abnormal a-synuclein as the key pathological feature. The most common LBDs are PD and dementia with LBs.
Microglia Brain-resident macrophages with inflammatory and phagocytic function

Introduction

Inflammation is a feature of most diseases of man; if uncontrolled or unregulated, it can do immense damage to healthy tissue. Inflammation in the Alzheimer's disease (AD) brain has been intensely investigated for many years since the identification of significantly increased numbers of activated microglia, defined by their morphology and increased expression of the MHCII protein, associated with pathological structures (Itagaki, McGeer, Akiyama, Zhu, & Selkoe, 1989). Inflammation has been proposed as a key pathogenic entity for AD because microglia interacting with amyloid beta (Aβ)-containing plaques in diseased brains have the potential to be activated to produce damaging cytokines and other products associated with innate immune responses

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(Walker, Link, Lue, Dalsing-Hernandez, & Boyes, 2006). These earlier observations were the foundation of testing nonsteroidal antiinflammatory drugs (NSAIDs) for treating AD patients. Unfortunately, multiple clinical trials of these agents did not show effectiveness in slowing AD progression (reviewed by Miguel-Alvarez et al., 2015).

Since those initial observations, considerable refinements using modern technologies have been used to investigate the special nature of chronic inflammation in diseases such as AD or Lewy body diseases (LBDs), where the deposition of insoluble and toxic Aβ, tau, or α-synuclein proteins is a significant feature (Friedman et al., 2018). It has now become apparent from much experimental work that effective treatments for these diseases requires not just removal of these insoluble proteins, but also reduction of possible damaging inflammation.

The understanding of the involvement of inflammatory cells in AD disease processes is still incomplete, and a reevaluation of inflammation-related treatment strategies has been needed. One approach had been the use of the Aβ vaccine or an antibody strategy to stimulate Aβ plaque removal by microglia (Zotova et al., 2011), but this also has not been successful in clinical trials. Another approach includes the study of endogenous cellular mechanisms for inflammation control to determine whether they are deficient and whether they can be amplified. There has been strong evidence for a number of years that CD200 and its receptor CD200R have central roles, compared with other systems, in regulating/suppressing tissue inflammation (Hoek et al., 2000; Wright et al., 2000), and that a disruption of this system occurs in aging and AD brains (Walker, Dalsing-Hernandez, Campbell, & Lue, 2009), but this has never been translated into a therapeutic strategy (Hernangomez et al., 2014). Understanding the direct consequence of loss of CD200 function on dementia still requires further study, as this is a relatively underinvestigated area of research. We will consider the evidence that directly or indirectly suggests the significance of CD200 to AD but need to consider the involvement of fractalkine and fractalkine receptor, and CD47 and signal regulatory protein alpha (SIRP-α), significant antiinflammatory systems, in parallel with CD200 when considering future studies (Chavarria & Cardenas, 2013).

Background. CD200 is member of the immunoglobulin superfamily of cell surface proteins, which shares many features with other immune-related proteins of this group. A major feature, though, is that this molecule lacks the identifiable intracellular domains involved in cellular signaling. The protein is highly expressed in the brain, is highly glycosylated, and has a native molecular weight of approximately 43 kDa. Its molecular weight varies depending on the expressing cell type. It had been studied as OX-2 protein for a number of years prior to the identification of its function (Barclay, Clark, & McCaughan, 1986). The gene encoding CD200 is located on chromosome 3 and is adjacent to the CD200R gene that codes for its cognate receptor, CD200R1. Both proteins have similar structures, but CD200R contains an intracellular signaling domain. Activation of CD200R occurs by specific binding of certain amino acid sequences of

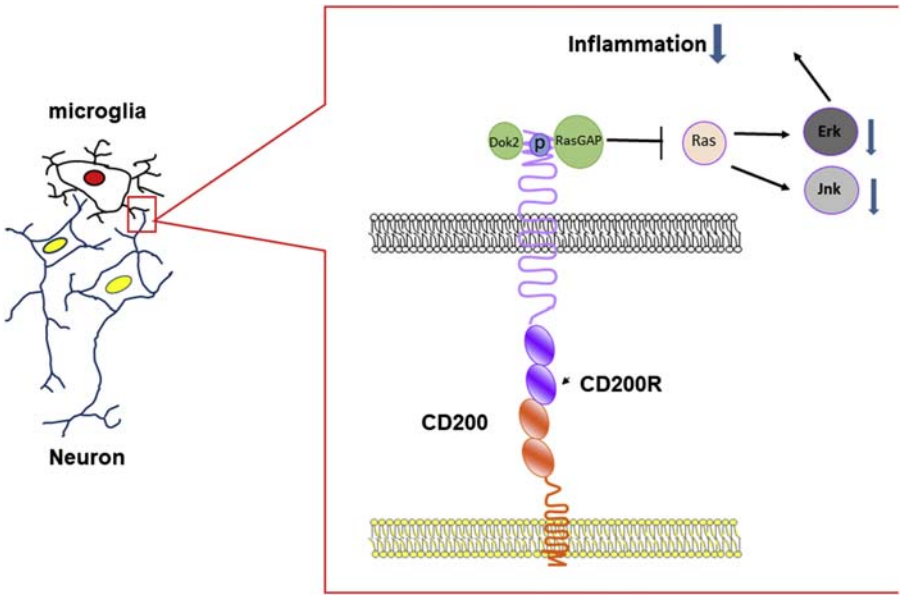

Figure 23.1 Diagram illustrating the principles behind CD200/CD200R interactions. Neuron-microglia interactions result in binding of N-terminal sequences of both proteins, leading to activation of CD200R signaling pathways.

CD200 to similar regions on CD200R (Fig. 23.1) (Chen, He, & Gorczynski, 2005; Hatherley & Barclay, 2004; Wright et al., 2000). Activation of CD200R results in phosphorylation of a cytoplasmic motif that binds DOK-1, resulting in inhibition of the Ras/MAP kinase pathways that have a central role in many macrophage activation processes (Jenmalm, Cherwinski, Bowman, Phillips, & Sedgwick, 2006). Recent data indicate that increased expression of Foxp3 was involved in CD200R signaling (Yi et al., 2016). The interaction of CD200 and CD200R1 was shown to be dependent on N-glycosylation of asparagine-44 of CD200R1 in microglia (Liu, Shen, Tang, & Gu, 2018). When this glycosylation was prevented through mutation at that site, the antiinflammatory consequences of CD200R1 activation were not observed.

CD200's role in the periphery

The possible significance of CD200 to human neurological diseases has been inferred from studies in the periphery, particularly in leukemia, tissue transplantation/rejection, and pregnancy (Clark, McCready, Harris, Malloy, & Arredondo, 2018; Gorczynski & Zhu, 2017; Yan et al., 2018). Although CD200 was first identified as antigen OX-2 as a cell surface protein expressed by neurons (Webb & Barclay, 1984), much information on its medical significance has come from its use as a biomarker for different types of

cancers, particularly leukemias but also neuroendocrine cells (Aref, Azmy, El-Bakry, Ibrahim, & Mabed, 2017; Love et al., 2017). There are relatively few studies of CD200 in human brain diseases; most studies have been carried out with experimental animal models, but the detrimental role of increased CD200 expression in innate immunity to cancer cells suggests a significant function in immune regulation in most tissues. These pathological properties assigned to CD200 in cancer are possibly beneficial in situations of neuroinflammation in neurodegenerative diseases.

It has now been shown that increased expression of CD200 by cancer cells correlates with poor prognosis for treatment and increased chance of metastasis (Stumpfova, Ratner, Desciak, Eliezri, & Owens, 2010). The mechanism involved is increased cellular expression of CD200 resulting in significant inhibition of the tumor-induced innate immune response by CD200R-positive macrophages or microglia (Liu et al., 2016). The growing literature on CD200 expression as a strong risk factor for poor prognosis to neoplasms highlights the significance of the CD200/CD200R system compared with others in inflammatory regulations. Other antiinflammatory systems exist, including fractalkine/fractalkine receptor and CD47/SIRP-α, but appear to have less significance in innate responses to CD200-positive tumor cells. Experimental studies have also shown that an intact CD200/CD200R system is essential to prevent immune-mediated rejection in pregnancy (Clark, McCready, Harris, Malloy, & Arredondo, 2018) and tissue transplantation (Yu, Chen, & Gorczynski, 2013).

CD200 in human neurodegenerative diseases. We have published several measurements of CD200 expression in human brains affected by age and neurodegenerative diseases. The first study identified significantly reduced levels of CD200 mRNA and protein in hippocampus and temporal cortex, but not the cerebellum, in AD cases (Walker et al., 2009). There were also reduced levels of CD200R mRNA in the hippocampus and temporal cortex samples from these cases. Using a custom antibody, we showed by immunohistochemistry that CD200 was primarily expressed by neurons and oligodendrocytes but could be detected in subsets of activated astrocytes. Expression in neurons was very variable, with a noticeable decrease in AD-affected areas. Although we were unable to demonstrate immunolocalization of CD200R to microglia in the brain tissue sections used in this study, the significant decrease in CD200R mRNA in the same samples indicated that CD200/CD200R interactions and antiinflammatory signaling would likely be reduced (Walker et al., 2009). Analyses showed that decreased expression of CD200 and CD200R correlated significantly with increasing severity of brain plaque and tangle loads across a spectrum of low pathology, high pathology, and AD cases. Further measurements of CD200 levels by ELISA were made in a series of human brains of progressively increasing age that demonstrated an age-associated decrease in CD200 expression (Maarouf et al., 2014, 2011). The mechanism behind this is unclear, as the regulation of CD200 expression in humans has not been determined. CD200 expression in rodent brain is upregulated by the cytokine interleukin

(IL)-4 (Lyons et al., 2007), while we and others showed that in human macrophages or microglia, CD200R was regulated by IL-4 (Koning et al., 2010; Walker et al., 2009). This cytokine did not regulate CD200R in rodent macrophage/microglia cells, and IL-4 did not seem to affect CD200 expression by cultured human neurons (unpublished observation). It is well established that inflammation increases with aging, but it is unclear whether reduction in CD200 leads to increased inflammation or whether increased inflammation results in reduced CD200. Either scenario sets up the potential of progressively increasing inflammation due to lack of effective control signaling. Using CD200 as a marker for inflammation, along with intercellular adhesion molecule (ICAM)-1, we carried out measurements of these proteins in a large series of cases with progressively increased amounts of α-synuclein Lewy body (LB) pathology. These cases included Parkinson's disease (PD), PD with dementia, AD with LBs, dementia with LBs, and control cases (Walker et al., 2017). In this study, measurements were made in the cingulate cortex and temporal cortex. Surprisingly, we found no correlation of changes in CD200 or ICAM-1 levels associated with increasing severity of LB pathology. There were however, strong correlations in these cases between the severity of AD pathology (plaque and tangle loads) with decreasing CD200 levels, and increasing ICAM-1 levels. Many of the cases used in this study had identifiable additional pathologies besides α-synuclein deposits, but cases with significant LB pathology and minimal amounts of AD pathology had levels of CD200 and ICAM-1 equivalent to pathology-free controls. This study not only defined differences between AD and LBD inflammation but also provided new data on how CD200 changes with disease and brain region (Walker et al., 2017).

The use of AD mouse models for studying many aspects of neuroinflammation has resulted in many publications about which factors are the most significant mediators of inflammation and by implication also in AD brains. There are few alternatives to transgenic mouse models for investigating AD, but differences between rodents and humans in their innate immune systems need to be appreciated. This particularly applies to the CD200/CD200R system (Franco Bocanegra, Nicoll, & Boche, 2018). Findings from these models need to be validated by appropriate measurements in human brains.

With regards to the other key antiinflammatory ligand/receptor systems of fractalkine/fractalkine receptor and CD47/SIRP-α, it has been shown that overexpression of these molecules also suppresses inflammation in model systems (Lee et al., 2010; Nash et al., 2013). Fractalkine receptor (CX3CR1) and SIRP-α are expressed by microglia, while fractalkine (CX3CL1) and CD47 are predominantly expressed by neurons. To compare their significance in AD brains versus CD200, it is now possible to access different gene-expression profiling datasets that compare global gene expression of controls and AD-affected brain tissues to study genes of interest. One recent key publication on neuroinflammation provided expression data from two analyses comparing expression of human control and AD brain tissue (Friedman et al., 2018). These data are accessible as supplemental data for the online version of this paper and from the

NCBI Gene Expression Omnibus (GEO). One dataset (GEO identifier GSE95587) used RNAseq methodology in a series of control and AD tissue samples from fusiform gyrus, while the other dataset (GSE15222) obtained data from a larger series of control and AD temporal cortex samples using an Illumina microarray platform. Both datasets showed highly significant decreased expression of CD200 mRNA in AD cases (adjusted P values of 4.5×10^{-5} and 7.8×10^{-11}, respectively), and CD200R expression was decreased in the fusiform gyrus dataset (adjusted P value of 2.4×10^{-3}), confirming our published results (Walker et al., 2009). By comparison, these datasets showed the following differences for fractalkine receptor (P values of 0.84 and 7.2×10^{-3}) and fractalkine (P values 1.4×10^{-2} and 1.1×10^{-2}), and CD47 (2.6×10^{-4} and 7.8×10^{-4}) and SIRP-α (P values of 0.11 and 0.99). Changes in these systems with disease were less consistent than for CD200/CD200R, though the lack of change in SIRP-α expression by microglia suggests this system could be maintained during disease progression.

Our work is the only published studies of CD200 and CD200R in brain tissue samples from AD and LB disease cases, but similar studies with similar findings using human brain tissue samples from multiple sclerosis (MS) cases have been carried out (Koning, Bo, Hoek, & Huitinga, 2007; Koning, Swaab, Hoek, & Huitinga, 2009). MS is a disease of white matter degeneration and a stronger immune-mediated response than with AD. Laser dissection of regions of MS plaques followed by analysis of expression of key genes, including CD200, CD200R, CD47, and SIRP-α in extracted material showed strong downregulation of CD200 and CD47 in the center and edge of active MS plaques, the areas with the strongest inflammatory activation, but not of their respective ligands CD200R and SIRP-α (Koning et al., 2007). Using a different CD200 antibody from our study, it was shown that CD200 was predominantly expressed in classes of neurons and oligodendrocytes and also subsets of activated astrocytes in MS and control tissues (Koning et al., 2009). This was similar to our findings in AD and control tissues (Walker et al., 2009).

It is important to also comment on the expression of CD200R in human tissues relative to CD200. CD200 is relatively abundant in brain tissue, with measured levels of 20–40 ng/mg protein in cingulate and temporal cortex (Walker et al., 2017), while CD200R is expressed at much lower levels than CD200 in the human brain. Expression of mRNA for CD200R can be detected in the brain but has not been detected by immunohistochemistry in microglia in human brain tissues by our group or others; however, it can be detected in infiltrating perivascular macrophages (Koning et al., 2007). Low levels of CD200R expression by microglia were confirmed in studies showing that microglia directly purified from brain had very low levels of CD200R mRNA compared with that of blood macrophages (Koning et al., 2009; Melief et al., 2012).

Deficits in CD200, CD47, and SIRP-α, but not CD200R, were shown in human samples from cases of focal cortical dysplasia type IIb and tuberous sclerosis complex at mRNA protein levels along with semiquantitative immunohistochemistry counts.

These are well-recognized causes of chronic intractable epilepsy in children (Sun et al., 2016). Although dealing with different types of neurological disease from the central topic of this chapter, this study made certain observations that should be reexamined in relation to AD-affected tissues. These authors showed an apparent link between the CD200/CD200R system and CD47/SIRP-α system, suggesting that both are required for functional protection. The cellular distribution of these proteins was similar, with CD200 and CD47 being mainly detectable in neurons. There was a significant negative correlation between CD200 and CD47 immunoreactivity scores and the number of HLA-DR-activated microglia. Using an innovative approach of tissue slice culture from biopsy samples of patients, the addition of soluble forms of CD200 or CD47 to slices resulted in significant decline in the secretion of cytokine IL-6. This study also demonstrated that expression levels of CD200 mRNA and CD47 mRNA strongly correlated with levels of IL-4 mRNA in samples from both disease groups. Diseased tissue had significantly less IL-4 mRNA than that of control tissue. This finding contradicts our findings that in humans, CD200 expression was not regulated by IL-4. Our finding suggested that only CD200R was IL-4 regulated; however, a reexamination of this in AD tissue is warranted. In our original paper, we found that IL-4 mRNA expression was generally not detectable in mRNA samples of the temporal cortex from AD and control cases (Walker et al., 2009).

CD200 in experimental models of neurodegeneration: implications for Alzheimer's disease

The link between decline in CD200 expression and aging was first established by comparison of expression in hippocampus of 3- and 24-month-old rats where significant decreases in mRNA for CD200 and the antiinflammatory cytokine IL-10 were demonstrated (Frank et al., 2006). The 24-month-old animals showed significantly increased expression of activation markers MHCII, CD86, and interferon-gamma, indicating higher levels of inflammation with aging.

CD200 gene—deficient mice have been studied to understand the consequences of complete absence of CD200 protein. In CD200-null mice, there was evidence of increased background levels of activated microglia. When these animals were subjected to experimental lesions—facial nerve transection or induction of experimental allergic encephalomyelitis (EAE)—there were enhanced levels of microglia activation compared with that of mice with normal CD200 expression (Hoek et al., 2000). The reverse was observed in a strain of mice that had genetic mutations in ubiquitin/proteasome pathway genes that led to reduced ubiquitination of CD200, which resulted in increased levels of neuronal CD200 due to its reduced degradation and removal (Chitnis et al., 2007). These CD200-overexpressing mice showed significantly reduced pathology when induced to develop EAE. This finding was replicated in vitro, neurons from

CD200-overexpressing mice were significantly protected from lipopolysaccharide (LPS)-activated microglia (Chitnis et al., 2007). In both the in vivo and in vitro models, the protective effects of CD200 neuronal overexpression were abrogated when blocking antibodies to CD200 were administered. In a study using the EAE model, a time course analysis of changes in CD200 and CD200R expression showed that decreased CD200 in the spinal cord of mice occurred before symptoms developed. In this model, an accompanying increase in CD200R expression was shown at presymptomatic stages (Valente, Serratosa, Perpina, Saura, & Sola, 2017). The results showed that levels of CD200 expression had significant negative correlation, and CD200R had significant positive correlation, with disease severity in lumbar, thoracic, and cervical spinal cord. Interestingly, this study showed a significant increase in the expression of truncated CD200 in the spinal cord. This form of CD200 lacks the domain required to bind and activate CD200R.

The consequences of overexpression of CD200 in a mouse model of AD have been investigated (Varnum, Kiyota, Ingraham, Ikezu, & Ikezu, 2015). These findings have significant implications for CD200 as an AD therapeutic target due to the disease protection outcomes demonstrated. Using viral vectors to transduce CD200 in vivo in plaque-developing mice and in vitro, several significant findings were made. Overexpression of CD200 in the hippocampus resulted in increased neurogenesis; significantly reduced expression of the classical activation marker inducible nitric oxide synthase; significantly 'increased expression of the alternative activation marker YM1; and significant reduction of levels of soluble but not aggregated Aβ. In vitro experiments confirmed that CD200-treated microglia promoted neuronal differentiation and showed enhanced phagocytosis of Aβ peptide (Varnum et al., 2015). A similar set of experiments was carried out using aged rats injected with CD200 protein in the hippocampus. These animals did not develop AD pathology but did demonstrate age-associated memory impairment and decreased long-term potentiation (LTP), an indication of memory deficit (Cox, Carney, Miller, & Lynch, 2012). These aged animals also had higher levels of microglial activation. The consequences of CD200 injection were reduced microglial activation, as demonstrated by reduced expression of MHCII and CD40, and improvement in ability to sustain LTP. Animals challenged with LPS showed greater levels of microglial activation and reduced LTP; these effects were also attenuated in the hippocampus of CD200-injected animals. One study showed that microglia that developed in the absence of CD200 had different properties from those that developed normally in the presence of CD200 protein. Microglia cultured from CD200-deficient mice, cells that do not normally express CD200, showed different responses to activation or phagocytosis of Aβ peptide compared with those of wild-type mice-derived microglia. Microglia derived from CD200-deficient mice showed minimal inflammatory responses in terms of cytokine secretion and inflammasome activation following Aβ stimulation, but demonstrated enhanced Aβ phagocytosis

and increased expression of CD68, a microglial marker for phagocytosis (Lyons et al., 2017). The mechanism for these observations is unclear in terms of how CD200 affects the maturation of microglia, but a recent study describing a method to prepare human microglia from induced pluripotent stem cells included incubation of cells with CD200 protein for 3 days as the final treatment (Abud et al., 2017).

Factors increasing the expression of CD200 in the brain

CD200 blocking has been proposed as a therapeutic target in cancer, but few studies have investigated factors that might increase its expression. Studies have suggested that CD200 is regulated by IL-4, but manipulating this cytokine might not be therapeutic due to its involvement in allergic immunity. An in vitro study using mixed cultures of rodent neurons and glia showed that both CD200 and CD200R were upregulated by the peroxisome proliferator-activated receptor gamma (PPAR-γ) ligand 15-deoxy-Delta(12, 14) -prostaglandin J2 (Dentesano et al., 2014). A number of natural prostaglandins are PPAR-γ agonists. In addition, the insulin-sensitizing thiazolidinedione class of drugs is made up of synthetic PPAR-γ agonists, while the NSAID drug ibuprofen is a weak activator. Data have suggested that endocannabinoids—for example, anandamide—can enhance CD200/CD200R interactions through binding to cannabinoid receptor CB2 (Hernangomez et al., 2012). One study demonstrated that treatment of lymphocytes with 1alpha,25-dihydroxyvitamin D3 (vitamin D) and in vivo supplementation with vitamin D resulted in increased expression of CD200 in CD4+ T cells but not B cells or epithelial cells (Dimeloe et al., 2012).

Conclusions

The role of inflammation in AD is still undefined, but there is a large amount of circumstantial human epidemiological and pathological data combined with experimental data that disturbances in microglial function with development of AD or increasing age have detrimental consequences to neuronal function. Clinical trials with relatively nonspecific NSAIDs or Aβ vaccines/antibodies have not produced significant clinical benefits. Maintaining the balance of cellular processes that can protect against inflammation might be one way of tackling this issue. As CD200 and CD200R are the key players in a critical system for controlling inflammation, further studies are warranted to investigate whether it is possible to maintain the balance as this system becomes disrupted with chronic inflammation and aging. Fig. 23.2 outlines possible consequences of insufficient activation of CD200R1 by CD200 on loss of synapses, the primary event that causes the loss of cognitive function.

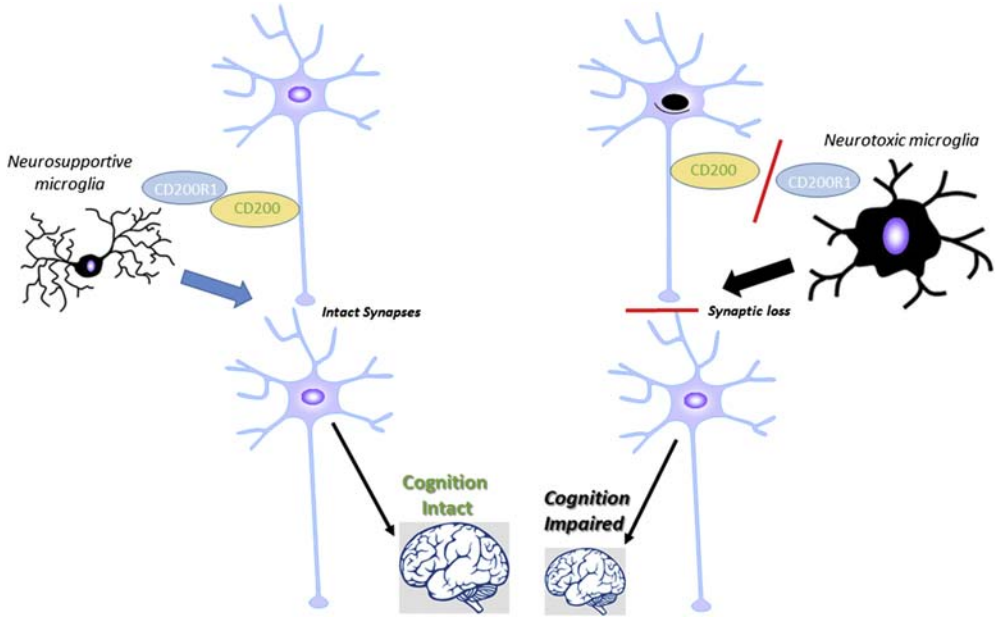

Figure 23.2 Diagram illustrating possible outcomes of insufficient interaction of CD200 and CD200R1 at the anatomical level, which can lead to microglial synaptotoxicity, a possible first stage leading to dementia.

Key facts on neuroinflammation

- Neuroinflammation is the study of inflammatory responses in the brain primarily caused by activation of microglia.
- Neuroinflammation can be a damaging response to pathology or a reparative response involving increased removal of damaged cellular materials.
- Microglia, the brain resident macrophages, respond to cellular damage by secreting cytokines and by phagocytosis.
- Neuroinflammation increases with age and is enhanced by disease pathology.
- The key feature of neuroinflammation in AD is the pronounced microglial response to amyloid plaques.
- CD200 and its receptor CD200R are one cellular control system involved in controlling neuroinflammation.
- Other systems for controlling neuroinflammation include fractalkine/fractalkine receptor, CD47, and SIRP-α.

Summary points

- This chapter focuses on the protein CD200, which is highly expressed by neurons in the brain.
- CD200 functions as a ligand for engagement with CD200 receptor, which is primarily expressed by macrophages and microglia.
- Interaction of CD200 with CD200R results in downregulation of activation signaling in monocytic cells.
- Measurements of CD200 in human brains showed that expression was decreased in AD brains.
- CD200 expression decreases with age in both human and rodent brains.
- Animal models that have reduced or null expression of CD200 show enhanced inflammation in the brain and periphery.
- Increased CD200 expression is protective from pathological damage.
- Increased CD200 expression in the brain is protective from Aβ-mediated damage
- CD200 treatment of microglia (CD200R-expressing cells) increases phagocytosis of Aβ peptide.
- CD200/CD200R interactions can be enhanced with PPAR-γ and CB2 receptor ligands.

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PART II

Neurological, physiological and imaging

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CHAPTER 24

Hippocampal atrophy associated with dementia risk factors and dementia

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List of abbreviations

AD Alzheimer's disease
Af atrial fibrillation
ApoE apolipoprotein E
Aβ amyloid beta
CSF cerebrospinal fluid
MCI mild cognitive impairment
MRI magnetic resonance imaging
PET positron emission tomography
SEM structural equation modeling
SNAP suspected non-Alzheimer pathology
VSRAD voxel-based specific regional analysis system for Alzheimer's disease
WMLs white-matter lesions

Mini-dictionary of terms

Hippocampal formation The hippocampal formation is essential for explicit memory formation. Visual, auditory, and somatic information is conveyed to the parahippocampus and perirhinal cortices, then the entorhinal cortex, the dentate gyrus, the hippocampus, the subiculum, and finally back to the entorhinal cortex.

Mediterranean diet The traditional Mediterranean diet is characterized by a high intake of olive oil, fruit, nuts, vegetables, and unrefined carbohydrates; a moderate intake of fish and poultry; a low intake of dairy products, red meat, processed meats, and sweets; and wine in moderation consumed with meals.

Structural equation modeling (SEM) The SEM is described as path diagrams, where the square boxes represent measured observations, and circles represent latent constructs. Single-headed arrows represent simple regression relationship, and double-headed arrows represent correlations. Usually, several indices of model fit are examined for SEM.

Vascular depression The vascular depression hypothesis posits that cerebrovascular disease and especially WMLs predispose, precipitate, or perpetuate some late-life depressive syndromes. Several studies have suggested that apathy (i.e., "primary loss of motivation" or "quantitative reduction of self-generated voluntary and purposeful [goal-directed] behavior") but not depression is a critical symptom of vascular depression.

Genetics, Neurology, Behavior, and Diet in Dementia
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Voxel-based specific regional analysis system for Alzheimer's disease (VSRAD) The VSRAD is free software developed by Matsuda et al. (*AJNR Am J Neuroradiol* 2012;*33*:1109−1114) based on voxel-based morphometry with statistical parametric mapping 8 (SPM8). Segmented images are spatially normalized to the customized template using Diffeomorphic Anatomical Registration Through an Exponentiated Lie algebra (DARTEL) for the VSRAD advance version.

Introduction

Dementia has historically been considered an irreversible and untreatable malady. In recent years, researchers have made substantial progress in the identification of modifiable risk factors for dementia, suggesting the possibility for primary prevention. The Lancet Commission on Dementia Prevention, Intervention, and Care presented a novel life-course model of risk for dementia by incorporating potentially reversible risk factors from different phases of the life span (Livingston et al., 2017) and found that approximately 35% of dementia is attributable to a combination of the following nine risk factors: education, hypertension, obesity, hearing loss, depression, diabetes, physical inactivity, smoking, and social isolation.

Early identification of individuals who are at risk for dementia or Alzheimer's disease (AD) is critical for the successful implementation of preventive strategies. In previous research, cognitively normal individuals with atrophy in the AD-signature cortices in areas such as the hippocampus were at an increased risk of progressing to clinical AD compared with those without atrophy (Chiang et al., 2011; Dickerson et al., 2011). In the Rotterdam Scan Study, a decline in hippocampal volume on magnetic resonance imaging (MRI) over a 10-year follow-up period was associated with an increased risk of cognitive decline (i.e., decline in delayed recall) (den Heijer et al., 2010). Recently, efficient automated analytical methods for MRI and particularly voxel-based morphometry methods have demonstrated utility in the context of neurodegenerative disease assessment (Ashburner, 2012). Figs. 24.1 and 24.2 show representative images of a subject with hippocampal atrophy and memory dysfunction. Although hippocampal atrophy is a hallmark for AD-type dementia, the hippocampus is vulnerable to a number of non-AD pathologies (Table 24.1). In this chapter, we present an overview of risk factors related to hippocampal atrophy and discuss the relevance of hippocampal atrophy to AD and vascular dementia or vascular cognitive impairment.

Dementia risk factors and the hippocampus

Diabetes

The Mayo Clinic Study of Aging identified significant associations between midlife diabetes and ischemic lesions as well as atrophy in the whole brain and hippocampus. Because an association between diabetes and mild cognitive impairment (MCI) was substantially attenuated after adjusting for brain volume indices, it was concluded that diabetes potentially affected late-life cognition through loss of whole-brain and

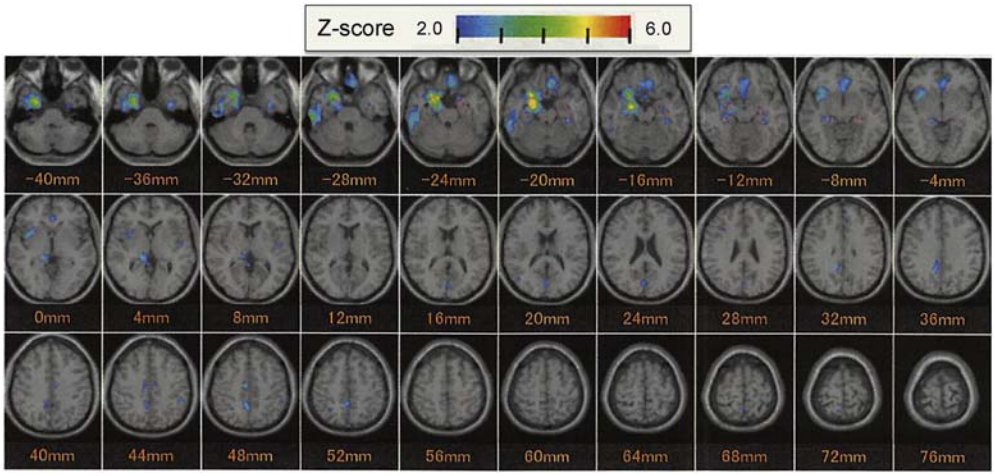

Figure 24.1 Representative images of the voxel-based specific regional analysis system for Alzheimer's disease (VSRAD) advance version. Colored areas with z-scores >2 (regions of significant atrophy) are overlaid on tomographic sections of the standardized MRI template. In this case (no.15,008, a 79-year-old woman), the severity of atrophy calculated as the averaged positive values of z-scores in the hippocampus (ZAdvance) is 2.49, and the percentage rates of the coordinates with the z-scores > 2 are 52.51% and 4.62% in the hippocampus and in the whole brain, respectively. *(Reproduced from Hashimoto, M., Araki, Y., Takashima, Y., Nogami, K., Uchino, A., Yuzuriha, T., Yao, H. (2016). Physical inactivity associated with vascular depression or apathy leads to hippocampal atrophy and memory dysfunction in community-dwelling elderly subjects: The Sefuri study*. Brain and Behavior, 7, *e00620. © 2016 The Authors. Brain and Behavior published by Wiley Periodicals, Inc.)*

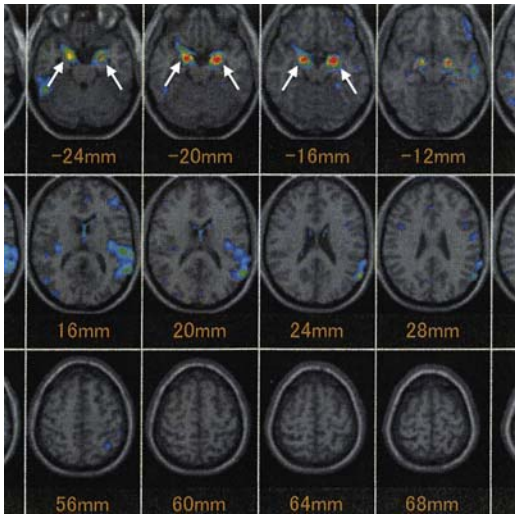

Figure 24.2 Regions of atrophy overlaid on the standardized magnetic resonance imaging (MRI) template. Medial temporal structures including hippocampus are indicated with *arrows*.

Table 24.1 Factors implicated in hippocampal atrophy.

Diabetes mellitus	Physical activity
Hypertension	Cognitive activity
Chronic kidney disease	Alzheimer disease
Alcohol	Mild cognitive impairment
Education	Vascular cognitive impairment
Depression	Small vessel disease
Hearing loss	White matter lesions
Diet	Post-stroke hippocampal atrophy
Obesity	

hippocampal volumes (Roberts et al., 2014). In other studies, higher fasting plasma glucose was associated with hippocampal atrophy or shape differences even in the normal glucose range (Cherbuin, Sachdev, & Anstey, 2012) or within the normal range and also in type 2 diabetes (Zhang et al., 2016), whereas higher plasma glucose levels at 2 h after the intake of 75 g of oral glucose were significantly associated with lower global and hippocampal volumes in an elderly Japanese population (Hirabayashi et al., 2016).

Hypertension

Untreated hypertensive subjects with a history of midlife high blood pressure had an increased risk for late-life hippocampal atrophy (Korf, White, Scheltens, & Launer, 2004; den Heijer et al., 2005). High blood pressure can lead to cerebral small-vessel pathology, which contributes to cognitive decline in patients with AD; additionally, white-matter lesion severity is also correlated with hippocampal atrophy (den Heijer et al., 2005). In patients with longstanding hypertension, the lower limits of cerebral blood flow autoregulation are shifted to higher levels, which may compromise brain to hypoperfusion in association with the blood pressure reduction. Memory decline and an increase in cerebrospinal fluid biomarkers of AD—associated with longitudinal decrease in mean blood pressure—were observed only in subjects with hypertension, yet any relationship between blood pressure and hippocampal volume was not found at baseline or longitudinally (Glodzik et al., 2014).

Chronic kidney disease

Even within the range of normal renal function (i.e., estimated glomerular filtration rate $\geqq 60$ mL/min/1.73 m^2), higher renal function has been associated with the attenuated progression of hippocampal atrophy in patients with MCI (An et al., 2017). Yet a path analysis showed that hippocampal atrophy was mediated more robustly through vascular burden and amyloid deposition than through renal function. Accordingly, the magnitude of the contribution of renal function to hippocampal atrophy is unclear.

Alcohol

Among community-dwelling adults enrolled in the Whitehall II study, higher alcohol consumption was associated with an increased likelihood of hippocampal atrophy as rated on the Scheltens visual rating score, compared with abstinence; the highest and most consistent odds were in subjects drinking in excess of 30 units a week (odds ratio 5.8, 95% confidence interval 1.8 to 18.6) (Topiwala et al., 2017). Alcohol consumption, even at moderate levels (14 to <30 units a week), was also associated with right hippocampal atrophy in this study. There was no evidence of a protective effect of light drinking on brain structure or function.

Education

In a study of healthy subjects and patients with amnestic MCI, only the amnestic MCI group showed a negative correlation between education and left hippocampal formation (parasubiculum/subiculum volumes) (Kang, Lim, Joo, Lee, & Lee, 2018).

Depression

A meta-analysis demonstrated consistent reductions in hippocampal volume in patients with depression; additionally, volume reduction in the right hippocampus was significantly correlated with the number of depressive episodes (Videbech and Ravnkilde, 2004). Addressing subtle depressive symptoms that are more relevant to the topic of this chapter, the Harvard aging Brain Study found that subthreshold symptoms of depression (i.e., dysphoric mood, apathy, and anhedonia but not anxiety) were associated with lower hippocampal volume in an analysis adjusted for multiple possible confounders. Importantly, this association was not modified by brain amyloid status (Donovan et al., 2015).

Hearing loss

Lin et al., (2014) demonstrated that individuals with hearing impairment had accelerated volume decline in whole-brain and regional volumes in the right temporal lobe including the parahippocampus. Several potential mechanisms have been proposed for this relationship between hearing impairment and cognitive status, including decreased socialization, increased depression, and excessive cognitive load on higher cortical function.

Atrial fibrillation

Atrial fibrillation (Af) may increase the risk of dementia through several potential mechanisms (e.g., cerebral hypoperfusion due to low cardiac output, increased incident stroke or silent stroke, and intermittent microembolism). Knecht et al. (2008) identified decreased hippocampal volume and poorer cognitive functions (i.e., learning and memory, and executive functions) in stroke-free community-dwelling individuals with Af compared with those without even after controlling for other vascular risk factors.

In contrast, Af was not associated with hippocampal volume, white-matter hyperintensity, amyloid deposition, or an AD-like pattern of glucose hypometabolism (Graff-Radford et al., 2016). Af with brain infarction was associated with an increased likelihood of cognitive impairment compared with Af without infarction (i.e., Af alone), suggesting that microembolism may be an important mechanism underlying the relationship between Af and dementia.

Diet and obesity

The Mediterranean diet was associated with preservation of volume in the cingulate cortex, parietal lobe, temporal lobe, and hippocampus as well as cortical thickness in the superior-frontal region in a multiethnic cohort of elderly subjects (Gu et al., 2015). This association was likely driven by high fish consumption and low meat consumption. In contrast, the Mediterranean diet was not associated with brain volume or mean cortical thickness at ages 73 and 76; rather, change in total brain volume was the only variable significantly associated with the Mediterranean diet over the 3-year period (Luciano et al., 2017).

Alternatively, an "unhealthy" Western-style diet—a dietary pattern higher in saturated fats and refined carbohydrates—was independently associated with smaller left hippocampal volumes in older adults after adjusting for covariates such as age, sex, education, depressive symptoms, physical activity, and vascular risk factors (Jacka, Cherbuin, Anstey, Sachdev, & Butterworth, 2015). Likewise, presumed lower fish consumption indicated by lower levels of omega-3 fatty acids (i.e., docosahexaenoic acid and eicosapentaenoic acid) in red blood cells was associated with smaller brain volume and hippocampal atrophy (Pottala et al., 2014; Tan et al., 2012). Yet a systematic review and meta-analysis found no evidence to support the effectiveness of omega-3 supplementation for improving cognitive function in patients with AD; nonetheless, Yassine et al. (2017) hypothesized that supplementation in APOE4 carriers might improve outcomes if the timing of the intervention preceded the onset of dementia.

Higher body tissue adiposity or obesity was correlated with the atrophy of brain regions responsible for cognitive function (e.g., the frontal lobe and hippocampus) in patients diagnosed with AD and MCI (Ho et al., 2010). Additionally, obesity was associated with hippocampal atrophy at baseline and over a follow-up period in community-dwelling healthy participants (Cherbuin et al., 2015b).

Physical and cognitive activity

In a randomized controlled study of exercise training in 120 older adults, Erickson et al. (2011) identified a 1.4% decline in hippocampal volume over 1 year in a stretching control group compared with a 2% increase in an aerobic exercise training group. Increased hippocampal volume in this study was further associated with higher serum levels of

BDNF and improved memory function. Moreover, 6 months of twice-weekly aerobic training but not resistance training preserved hippocampal volume in women 70–80 years of age with probable MCI compared with a control group (ten Brinke et al., 2015). Our path analysis based on structural equation modeling (SEM) indicated that hippocampal atrophy was associated with age, less education, and lower physical activity, and hippocampal atrophy resulted in memory dysfunction (Fig. 24.3) (Hashimoto et al., 2016). We previously showed that white-matter lesions (WMLs) were a major factor explaining apathetic behavior and that apathy had significant negative effects on physical activity in community-dwelling elderly subjects (Yao et al., 2015). Considering these two SEM analyses together, we hypothesized that vascular factors (i.e., VCI and vascular depression) facilitate hippocampal atrophy or AD as a result of physical inactivity (Fig. 24.4) (Yao, 2017).

In cognitively normal adults, brain training improves performance in the trained cognitive domain (Butler et al., 2018). This finding was largely derived from the Advanced Cognitive Training for Independent and Vital Elderly trial. Individuals with higher complex mental activity exhibited a lesser degree of hippocampal atrophy (an

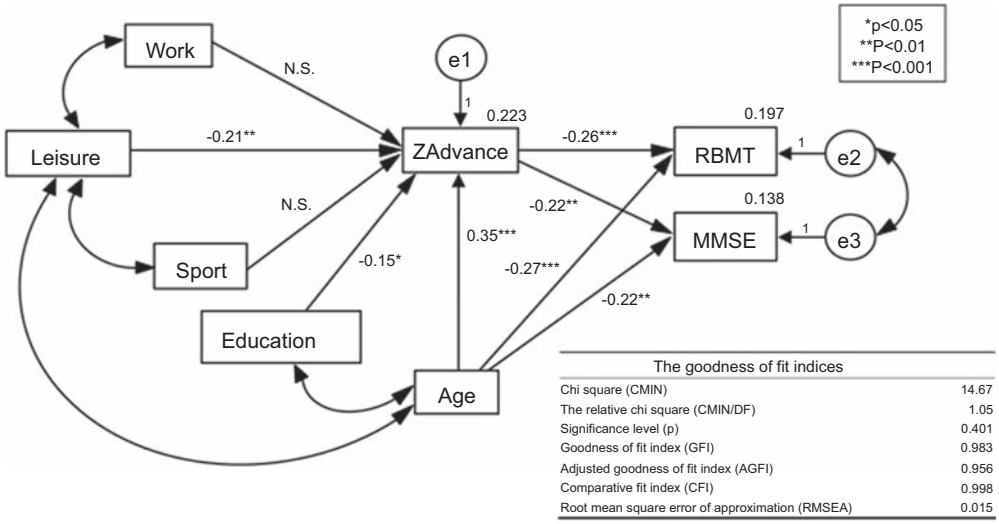

Figure 24.3 SEM of physical activity, hippocampal atrophy, and cognition. The direct paths from leisure-time physical activity, age, and education to hippocampal atrophy (ZAdvance) were significant. The direct paths from age and hippocampal atrophy to Rivermead Behavioral Memory Test (RBMT) and mini-mental state examination (MMSE) scores were also significant. Goodness-of-fit indices indicated that this model was a reasonable fit for the data. *(Reproduced from: Hashimoto, M., Araki, Y., Takashima, Y., Nogami, K., Uchino, A., Yuzuriha, T., Yao, H. (2016). Physical inactivity associated with vascular depression or apathy leads to hippocampal atrophy and memory dysfunction in community-dwelling elderly subjects: The Sefuri study.* Brain and Behavior, 7, *e00620. © 2016 The Authors. Brain and Behavior published by Wiley Periodicals, Inc.)*

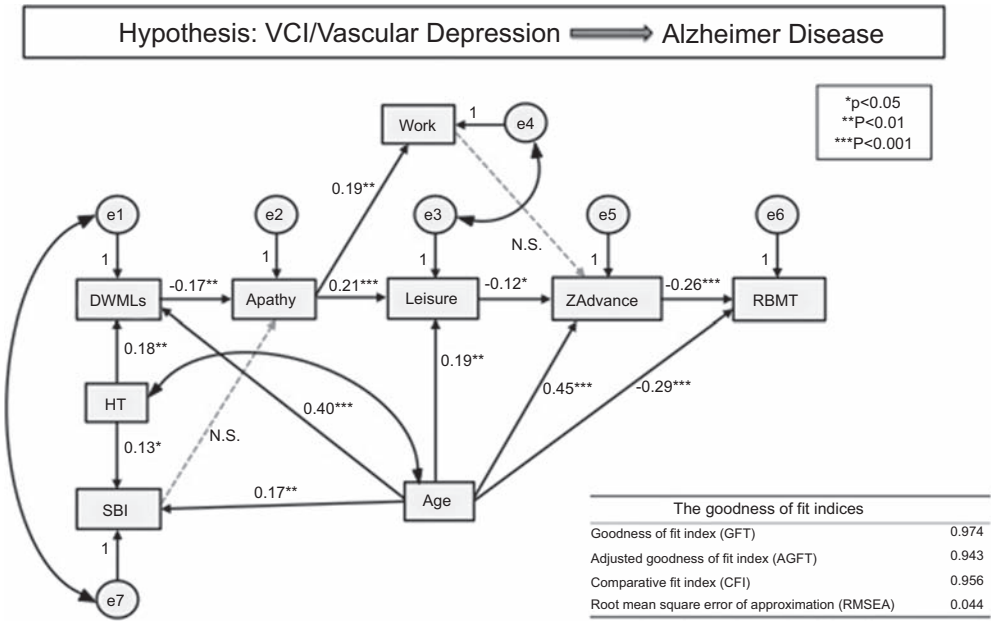

Figure 24.4 SEM of hypertension, deep white-matter lesions (WMLs), apathy, leisure-time physical activity, hippocampal atrophy (ZAdvance), and cognition (Rivermead Behavioral Memory Test [RBMT] score). The direct paths from vascular depression or apathy (i.e., loss of motivation associated with hypertension and deep WMLs) to decreased leisure-time physical activity were significant. This decreased leisure-time physical activity appeared to cause hippocampal atrophy (ZAdvance) and memory dysfunction. Therefore, we hypothesized that vascular factors (i.e., VCI and vascular depression) facilitated hippocampal atrophy or Alzheimer's disease as a result of physical inactivity. Goodness-of-fit indices indicated that this model was a reasonable fit for the data. *(Reproduced from Yao, H. (2017). Cognitive dysfunction associated with silent brain lesions in community-dwelling elderly subjects: The Sefuri study.* The Japanese Journal of Stroke, 39*, 460–464.)*

average loss of 3.6%) than those with low mental activity (an average loss of 8.3%) over a 3-year period (Valenzuela, Sachdev, Wen, Chen, & Brodaty, 2008). Similarly, Schulz et al. (2015) reported that engagement in cognitive activities pertinent to playing games was associated with higher cognitive scores and gray-matter volumes in areas vulnerable to AD pathology (i.e., the hippocampus, posterior cingulate, anterior cingulate, and middle frontal gyrus) in a cohort of at-risk, middle-aged adults. An intergenerational social health promotion program, the Baltimore Experience Corps Trial, found that while male control subjects exhibited age-related decline in hippocampal volume, men receiving a 24-month intervention showed a 1.56% increase in volume over the study period (Carlson et al., 2015).

Alzheimer-type dementia and the hippocampus

Apolipoprotein E genotype

The most important genetic risk factor for sporadic AD is the ε4 variant of the apolipoprotein E (ApoE) gene. Manning et al. (2014) demonstrated that ApoE ε4 carriers with a clinical diagnosis of AD or progressive MCI had a disproportionately greater hippocampal loss than that of noncarriers. Several previous studies have investigated whether ApoE modifies the rate of hippocampal atrophy (see review by Manning et al., 2014). Only amyloid beta (Aβ) was significantly associated with hippocampal atrophy rate in normal control subjects and patients with MCI in a model adjusted for age, gender, WMLs, and intracranial volume; the ApoE ε4 genotype was also associated with hippocampal atrophy rate in MCI (Nosheny, 2015).

Preclinical phase of Alzheimer's disease

The preclinical phase of AD is divided into three stages: stage 1, Aβ-positive; stage 2, Aβ plus neurodegeneration; and stage 3, Aβ plus neurodegeneration and subtle cognitive impairment. Aβ deposition can be measured in vivo using cerebrospinal fluid (CSF) biomarkers and amyloid positron emission imaging (PET), whereas neurodegeneration can be assessed with glucose metabolism PET and volumetric MRI. In previous studies, approximately 20% of normal elderly subjects had abnormal neurodegenerative imaging biomarkers but normal amyloid imaging (i.e., SNAP). High rates of Aβ accumulation were identified in subjects with abnormal Aβ at baseline, and the rate of accumulation was not influenced by hippocampal volume adjusted for total intracranial volume (Fletcher et al., 2016; Jack et al., 2014). Likewise, low hippocampal volumes alone did not accurately predict ongoing neurodegeneration (Chung et al., 2017; Gordon et al., 2016).

Mild cognitive impairment

Subjective cognitive failure was associated with lower hippocampal volume in subjects without objective cognitive impairment in a manner independent of WMLs (van Norden et al., 2008). In another study, subjective memory decline at follow-up, but not at baseline, was significantly associated with increased hippocampal atrophy over the 4-year study period, even after controlling for anxiety and depression symptoms (Cherbuin et al., 2015a). On this premise, the authors suggested that subjective memory decline tends to follow rather than precede hippocampal atrophy.

Older age, poorer general condition, ApoE ε4 prevalence, and lower hippocampal volume at baseline all predicted subsequent accelerated hippocampal atrophy and were proposed as markers for improving the identification of subjects at risk for progression from MCI to AD (van de Pol et al. 2007). A mediation analysis demonstrated that hippocampal volume and glucose metabolism mediated up to 25% of the effects of Aβ on episodic memory in MCI, and a combination of gray-matter volume and glucose

metabolism mediated approximately 40% of this effect (Mattsson et al., 2015). Hanseeuw, Dricot, Lhommel, Quenon, & Ivanoiu (2016) showed that while patients with MCI subjects had lower cortical metabolism than that of elderly control subjects, hippocampal volume and hippocampal metabolism were both useful for discriminating amyloid-positive versus amyloid-negative MCI.

Vascular cognitive impairment and the hippocampus

Small-vessel disease

AD and cerebrovascular disease share modifiable risk factors that lead to cognitive decline and dementia, including diabetes, hypertension, smoking, alcohol use, unhealthy diet, and physical inactivity (Liu, Wong, Law, & Mok, 2015). Although "mixed" AD pathology and vascular lesions are prevalent, and amyloid plaques frequently coexist with vascular lesions, van de Pol, Gertz, Schelten, & Wolf (2011) found that hippocampal atrophy was less severe in patients with subcortical vascular dementia than in those with AD. In patients with cognitive impairment, a path analysis showed that amyloid burden was associated with hippocampal atrophy and memory impairment, while WMLs were associated with frontal cortical thinning and executive dysfunction (Ye et al., 2015). Another path analysis demonstrated that the effects of arteriosclerosis on cognitive impairment were mediated via cortical atrophy, while two distinct pathways—one involving cortical atrophy and another involving a latent (unknown) variable—were delineated in AD pathology and cognitive impairment (Zheng, Vinters, Mack, Weiner, & Chui, 2016). Taken together, hippocampal atrophy may not be a major factor in vascular cognitive impairment.

White-matter lesions

Although most previous studies have not identified a correlation between WMLs and amyloid load or AD, several studies have shown positive relationships between WMLs and hippocampal atrophy. The RUN DMC study demonstrated that lower white-matter volume and lower hippocampal volume at baseline significantly increased the 5-year risk of dementia among nondemented participants with small-vessel disease (i.e., lacunes and/or white-matter hyperintensities on MRI) (van Uden et al., 2016). In the Ginkgo Evaluation of Memory study, amyloid retention, left and right hippocampal volume, and WML volume were independent predictors of dementia in a group of nondemented individuals ages 85 years and older (Lopez et al., 2014). WMLs were associated with disproportionately greater hippocampal atrophy in control subjects and patients with MCI relative to whole brain atrophy (Fiford et al., 2017). In contrast, Brickman et al. (2012) found that only WMLs in the parietal lobe predicted the time to incident AD, while WML volume in other areas as well as hippocampal volume did not.

Poststroke hippocampal atrophy

Medial temporal lobe atrophy on MRI was a predictor of delayed poststroke dementia in elderly patients with stroke who were not cognitively impaired at 3 months poststroke (Gemmell et al., 2012). In this study, hippocampal CA1 but not CA2 subfield neuron density was affected in poststroke and in groups of patients with AD, vascular dementia, and mixed dementia. These findings suggest that hippocampal atrophy is critical for dementia in both cerebrovascular and neurodegenerative diseases. In a comparison of hippocampal atrophy between poststroke patients and those with MCI, it was found that WMLs were important for cognitive impairment in poststroke patients but had a minor role in amyloid-positive MCI, whereas CSF biomarkers (amyloidβ1-42, total and phosphorylated microtubule-associated protein tau) were important in MCI but not in poststroke patients (Selnes et al., 2015). Moreover, Werden et al. (2017) found that the hippocampus was smaller in patients with first-time stroke compared with control subjects, and recurrent stroke was associated with further atrophy of hippocampus compared with first-time stroke. Therefore, hippocampal changes—probably caused by vascular risk factors—may precede ischemic stroke.

Conclusions

Although hippocampal atrophy is a prototypical biomarker for AD-type dementia, numerous modifiable risk and protective factors (e.g., education, diabetes, hypertension, chronic kidney disease, alcohol, hearing loss, and depression) are implicated in hippocampal pathology. Therefore, primary prevention strategies against risk factors for hippocampal atrophy may be useful in the context of AD and dementia. Furthermore, physical activity, healthy diet, and lifelong learning and cognitive training may protect against hippocampal atrophy. Cerebrovascular disease and AD share modifiable risk factors related to hippocampal atrophy. A healthy brain—emphasized by an absence of overt vascular or neurodegenerative injury—is essential for living a longer and fuller life (Gorelick et al., 2017), and hippocampal preservation is a critical characteristic in this context.

Key facts of hippocampal atrophy

- The primary function of the hippocampus is learning and memory.
- When the hippocampus is damaged bilaterally, the ability to form new memories is largely lost.
- Recent studies have examined hippocampal atrophy using voxel-based morphometry methods.
- Neurofibrillary tangles—a major histopathological hallmark of AD—tend to occur in the hippocampal formation, parahippocampal gyrus, amygdala, and the temporal association cortex.

- Hippocampal atrophy is most frequently observed in AD; however, various factors including education, risk factors for dementia, diet, and physical activity are involved in the pathophysiology of hippocampal atrophy.

Summary points

- This chapter explores the pathophysiology of hippocampal atrophy and dementia.
- Volumetry of the hippocampus—an early target of AD—is a promising prognostic approach.
- Numerous modifiable risk factors are implicated in hippocampal atrophy including education, diabetes, hypertension, alcohol, and depression.
- Physical activity, healthy diet, and lifelong learning and cognitive training may protect against hippocampal atrophy.
- Assessment of hippocampal atrophy involved in the preclinical phase of AD (e.g., subjective cognitive decline and MCI) is a plausible tool for risk evaluation.
- There is weak evidence for the direct involvement of hippocampal atrophy in the relationship between cerebrovascular disease and cognitive impairment.
- Preserved hippocampus may be a landmark of the healthy brain.

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CHAPTER 25

Inflammation and insulin resistance in Alzheimer's disease: partners in crime

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List of abbreviations

AD Alzheimer's disease
APP Amyloid precursor protein
Aβ Amyloid beta
IR Insulin receptor
IRS1 Insulin receptor substrate 1
PS1 Presenilin 1
STZ Streptozotocin
TNFα Tumor necrosis factor α

Mini-dictionary of terms

Neuroinflammation Increased activation of resident brain cells such as microglia and astrocytes together with infiltrating cells from the periphery, toward stimulation in specific brain regions. On one hand, this activation results in increased secretion of cytokines or complement system factors that may result in neuronal death. On the other hand, activation of those cells may help in clearing different pathological features such as beta amyloid and the secretion of factors that may contribute to repair.

Insulin signaling Intracellular signaling followed by the binding of insulin to the insulin receptor (IR), which prompts the internalization of extracellular glucose

Diabetes: A disease characterized by elevated glucose levels due to improper insulin signaling in the body that can be caused by a lack of insulin production (type I) or impairments in insulin signaling in target cells (type II)

Streptozotocin (STZ) A toxin that enters cells through glucose transporters that are highly expressed in pancreatic beta cells and is used to experimentally induce type I diabetes

High fat diet A nutrition regime rich in fats (up to 70%) that is used experimentally to induce obesity and type II diabetes.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by marked cognitive decline and the appearance of cerebral plaques consisting of amyloid beta (Aβ), a cleavage product of amyloid precursor protein (APP), and intracellular aggregates of phosphorylated tau called tangles (Braak & Braak, 1991). AD affects different brain cells

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beside neurons, such as astrocytes and microglia, which in turn contribute to pathological processes in AD (Farfara, Lifshitz, & Frenkel, 2008). Other prominent features of AD include a reduction in connectivity among the cholinergic neurons (Coyle, Price, & Delong, 1983) and an impairment in brain metabolism (Kahn & Suzuki, 2010), which in turn may be affected by an increase in the inflammatory brain process.

Inflammation features in Alzheimer's disease

It was reported that in AD there are significant inflammatory processes within the brain involving many cell types and cellular pathways. A long-standing marker for inflammatory processes in AD is the proinflammatory cytokine tumor necrosis factor α (TNFα)—serum levels of TNFα are elevated among AD patients compared with age-matched controls (Fillit et al., 1991), and among AD patients, TNFα levels are correlated with increased rates of cognitive decline (Holmes et al., 2009). In line with these findings, the administration of anti-TNFα antibodies was shown to mitigate behavioral deficits in a middle-aged AD mouse model, but not in elderly mice, without affecting Aβ plaque burden (Giuliani et al., 2009). Similarly, genetic knockout of TNFα in an AD mouse model was able to mitigate behavioral deficits in both middle-aged and old mice and reduce Aβ plaque burden at middle age but increased Aβ levels at an old age (Giuliani et al., 2009).

Another prominent phenotype of AD is marked gliosis—proliferation and change in the phenotype of glial cells, mainly microglia and astrocytes. Evidence from AD mice suggests that astrocyte and microglia activation precedes Aβ plaque formation, including increased expression of proinflammatory cytokines such as IL-1β and IL-6 as well as neuronal upregulation of the β-site APP cleavage enzyme (Heneka et al., 2005). With aging and the appearance of Aβ plaques, these inflammatory markers are increased, including the activation of induced nitric-oxide synthase, especially near Aβ plaques (Heneka et al., 2005).

A significant activation of the complement system can also be observed in AD. Recent postmortem analyses revealed an increase in the levels of NF-κB transcription factor and complement factor 3 (C3) in the brains of AD patients compared with ages-matched controls (Lian et al., 2015; Yasojima et al., 1999), as well as other complement system components such as C1q and C8−C9. In vitro experiments showed that C3 is upregulated in astrocytes following NF-κB activation, such as after TNFa stimulation, and that the C3-induced signaling reduces neuronal synaptic density (Lian et al., 2015). Importantly, in an AD mouse model, antagonists to C3 signaling were able to rescue cognitive performances (Lian et al., 2015). Similarly, while genetic deletion of C3 in a mouse model of APP increased the Aβ load, it also improved the cognitive performance of the mice, mitigated synaptic loss, and reduced gliosis around Aβ plaques (Shi et al., 2017). Recent findings from a mouse model also suggest that complement activation around synapses is an early event that precedes Aβ plaque deposition and that this complement activation mediates synaptic loss by microglia (Hong et al., 2016).

Microglia, which are considered the main scavenger cells of the central nervous system (Kreutzberg, 1996), play a major role in Aβ clearance and inflammatory processes. Activation of microglia by proteasome-based adjuvant treatment caused a dramatic decrease in Aβ deposition in the brain (Frenkel et al., 2005), and microglial Aβ clearance is generally considered important for preventing Aβ levels from exceeding a critical threshold (Weiner & Frenkel, 2006), through various pathways. Aβ aggregates were shown to colocalize with toll-like receptor 2 (TLR2) expression on microglia (Liu et al., 2012). TLR2 is important for microglial activation by Aβ and for its clearance by microglia, as TLR-2-deficient microglia secrete reduced levels of the proinflammatory cytokines TNFα and IL-1β following stimulation with aggregated Aβ while exhibiting increased uptake of extracellular Aβ. Interestingly, bone marrow transplantation of TLR-2-deficient myeloid cells in an AD mouse model reduced the brain levels of TNFα, caused a slight reduction in Aβ levels, and rescued neuronal synapses and behavioral deficits (Liu et al., 2012). Another prominent marker for the proper function of myeloid cells is chemokine receptor 2 (CCR2). $CCR2^{+}$ cells are recruited to Aβ plaques in the brains of an AD mouse model, but the function of CCR2 appears to affect mainly perivascular plaques rather than parenchymal plaque load. The increased Aβ deposition around blood vessels may be of specific importance, as it is linked with increased mortality among mice (Mildner et al., 2011).

Interestingly, induction of inflammation through intrahippocampal LPS (lipopolysaccharide) injection to AD mouse model animals caused marked microglial activation with opposing effects on AD-related markers—while there was a reduction in Aβ load (DiCarlo et al., 2001), the inflammatory activation increased tau phosphorylation (Lee et al., 2010), suggesting a complex interaction between inflammation and AD pathology.

A recently emerging target for research in microglia is the triggering receptor expressed on myeloid cells 2 (TREM2), which is increased in the cerebrospinal fluid (CSF) of AD patients at the mild cognitive impairment stage of disease progression but not in later stages of the disease. Likewise, levels of the soluble variant of TREM2 are positively correlated with the levels of tau and pTau but not with Aβ (Suárez-Calvet et al., 2016). In the brains of AD patients and in a mouse model, a subset of microglia cells dubbed "disease-associated microglia" whose activation is both TREM2-independent and TREM2-dependent, are located around Aβ plaques (Keren-Shaul et al., 2017). Interestingly, TREM2 expression around Aβ plaques is increased with age in AD mouse models, primarily by macrophages. Experimental deletion of TREM2 has a profound effect on the disease phenotype in mice, as it results in reduced levels of Aβ plaques; reduced astrogliosis and reduced accumulation of myeloid cells around Aβ plaques; reduces the levels of the inflammatory cytokines IL-6 and IL-1β; and reduces the levels of phosphorylated tau around Aβ plaques (Jay et al., 2015).

Inflammation-targeted treatments in Alzheimer's disease

The involvement of inflammatory processes in AD has led to extensive research aimed at modulating or blocking certain aspects of inflammation in the hope of mitigating disease progression (Heneka et al., 2015). Accumulating evidence suggests that long-term, but not short-term, consumption of nonsteroidal antiinflammatory drugs (NSAIDs) has a protective effect and reduces the risk for developing AD (Breitner et al., 2011; Etminan, Gill, & Samii, 2003; McGeer & McGeer, 2007). The consumption of NSAIDs failed, however, to slow disease progression, mitigates its symptoms among AD patients, and even showed some adverse effects among patients (Breitner et al., 2011; Pasqualetti et al., 2009; Reines et al., 2004), suggesting that mitigating symptoms through the disease course requires a different approach (Weiner & Frenkel, 2006; Wyss-Coray & Rogers, 2012). Indeed, it was suggested in recent years that modulation of immune checkpoints rather than suppression of the immune system may provide beneficial outcomes in AD. Depletion or inhibition of regulatory T cells (T-regs) in an AD mouse model was shown to reduce Aβ plaque load and rescue cognitive impairments, whereas augmentation of the immune-suppressive activity of T-regs resulted in increased gliosis, increased Aβ plaque load, and worsening of cognitive impairments (Baruch et al., 2015). Interestingly, blocking the immune checkpoint PD-1 in an animal model of AD was suggested to increase the infiltration of myeloid cells and result in a reduction of Aβ plaque load and attenuated cognitive impairment (Baruch et al., 2016).

Impairments in metabolism and insulin signaling in Alzheimer's disease

The most genetic risk factor for AD is different alleles of apolipoprotein E (APOE). A specific allele, APOE4, is linked with decreased glucose metabolism in the brain combined with increased inflammatory phenotype (Michaelson, 2014). Likewise, inflammatory features were suggested to play a role also in different metabolic diseases that affect insulin signaling (Dandona, Aljada, & Bandyopadhyay, 2004), whose complications might lead to dementia (Arvanitakis et al., 2004; Gudala et al., 2013; Profenno et al., 2010) (see Table 25.1 and Fig. 25.1). Insulin is a hormone important for the tight regulation of blood glucose levels in the periphery but also affects the other tissues such as the brain (Saltiel & Kahn, 2001) after it crosses the blood-brain barrier (Banks, Owen, & Erickson, 2012). In recent years, several population studies have shown a link between type 2 diabetes (T2D) and cognitive decline. A longitudinal study showed that prolonged disease duration of T2D leads to impairments in working memory and executive functions (Beeri et al., 2014). Moreover, meta-analyses show that T2D patients have a significantly higher risk for developing dementia compared with control subjects (Cheng et al., 2012; Gudala et al., 2013) and that T2D patients also show a 45%–65% increase in the risk for developing AD (Arvanitakis et al., 2004; Gudala et al., 2013; Profenno et al., 2010).

Table 25.1 Evidence of a potential link between insulin impairment and inflammation in Alzheimer's disease.

Disease pathology	Effects on inflammation	Metabolism and insulin signaling	Model	Animal
Accumulation of Aβ plaques and phosphorylated tau (Braak & Braak, 1991). Cognitive decline (Braak & Braak, 1991; Holmes et al., 2009).	Microgliosis and astrogliosis (Braak & Braak, 1991). Increased serum TNFα levels (Fillit et al., 1991), increased CSF TREM2 levels (Suárez-Calvet et al. 2016), and increased complement activation (Lian et al., 2015; Yasojima et al., 1999).	Type II diabetes patients have increased risk for developing AD (Arvanitakis et al., 2004; Gudala et al., 2013; Profenno et al., 2010). AD patients exhibit lower CSF insulin levels combined with higher plasma insulin levels (Craft et al., 1998). Reduced insulin signaling (Bomfim et al., 2012; Rivera et al., 2005; Steen et al., 2005), in correlation with disease progression (Rivera et al., 2005).	AD patients	Human
Improved memory performance after short periods of treatments, and reduced cognitive decline after prolonged treatments (Claxton et al., 2015; Craft et al., 2012; Reger et al., 2008). Increased plasma $A_{\beta 40}$ levels (Reger et al., 2008).			Nasal administration of insulin in humans	Human
Accumulation of Aβ plaques (Baruch et al., 2015, 2016; Jolivalt et al., 2008; Wang et al., 2010). Cognitive impairment (Baruch et al., 2015, 2016; Takeda et al., 2010; Wang et al., 2010).	Microgliosis, astrogliosis (Baruch et al., 2015, 2016; Heneka et al., 2005), increased levels of inflammatory cytokines (Takeda et al., 2010).	Reduced brain insulin levels at older ages (Li-Min et al., 2012).	APP mouse model	Mouse

Continued

Table 25.1 Evidence of a potential link between insulin impairment and inflammation in Alzheimer's disease.—cont'd

Disease pathology	Effects on inflammation	Metabolism and insulin signaling	Model	Animal
Impaired spatial learning in an APP/PS1 mice (Wang et al., 2010) Increased levels of phosphorylated tau in the hippocampus and cortex (Jolivalt et al., 2008; Murtishaw et al., 2018). Increased BACE expression, increased A_{β} monomer and plaque burden (Jolivalt et al. 2008; Wang et al. 2010).	Increased microgliosis in the hippocampus (Murtishaw et al., 2018).	Reduced plasma insulin levels (Murtishaw et al., 2018); reduced AKT and IR phosphorylation in the hippocampus (Jolivalt et al., 2008; Murtishaw et al., 2018).	Peripheral administration of STZ	Mouse
Neuronal loss and increased gliosis. Increased levels of phosphorylated tau and neuronal Aβ (Lester-coll et al., 2006).	Increased gliosis.	Reduced insulin expression in the brain; reduced IR levels in the brain, reduced insulin binding in the brain (Lester-coll et al., 2006).	Intracerebral administration of STZ in a rat model AD patients	Rat
Increased levels of tau phosphorylation (Ma et al. 2009). Increased gamma-secretase activity; increased Aβ plaque load (Ho et al. 2004).		Reduced AKT and IR phosphorylation in the cortex (Ho et al. 2004).	High-fat diet in APP mice	Mouse
Earlier onset of learning deficits. Exacerbation of cognitive impairments (Takeda et al., 2010).	Increased astrogliosis, coupled with increased gliosis (Takeda et al., 2010).	Reduced AKT phosphorylation (Takeda et al., 2010).	*db/db* APP mice	Mouse
Reduced tau phosphorylation and Aβ42 accumulation in the brain, and prevented synaptic loss in a diabetes mouse model (Li et al., 2012). Improved performance in the Morris water maze, reduced neuronal death in the hippocampus, and reduced Aβ accumulation in the hippocampus and cortex of APP mice (Ou et al., 2018).	Reduced astrogliosis and microgliosis in the hippocampus and cortex of APP mice, combined with reduced secretion of inflammatory cytokines in the cortex and hippocampus of APP mice (Ou et al., 2018).		Metformin (antidiabetic drug)	Mouse

Attenuated Aβ accumulation in the cortex and hippocampus of STZ-treated mice, and ameliorated cognitive deficits in the Morris water maze (Liu et al., 2013).			Pioglitazone (Liu et al., 2013) antidiabetic drug	Mouse
Improved performance of APP mice in the Morris water maze (Bomfim et al., 2012).		Protects neurons against Aβ-induced impairment in insulin signaling. Improves insulin signaling in APP mice (Bomfim et al., 2012).	Exenatide (antidiabetic drug) in a mouse model	Mouse
Reduced Aβ plaque load (Long-Smith et al., 2013).	Reduced astrogliosis and microgliosis (Long-Smith et al., 2013).	Improved insulin signaling (Long-Smith et al., 2013).	Liraglutide (antidiabetic drug) in a mouse model	Mouse
		Rescue of Aβ oligomers-induced insulin signaling impairments in hippocampal neurons (Bomfim et al., 2012).	Administration of TNFα-neutralizing antibodies	Mouse

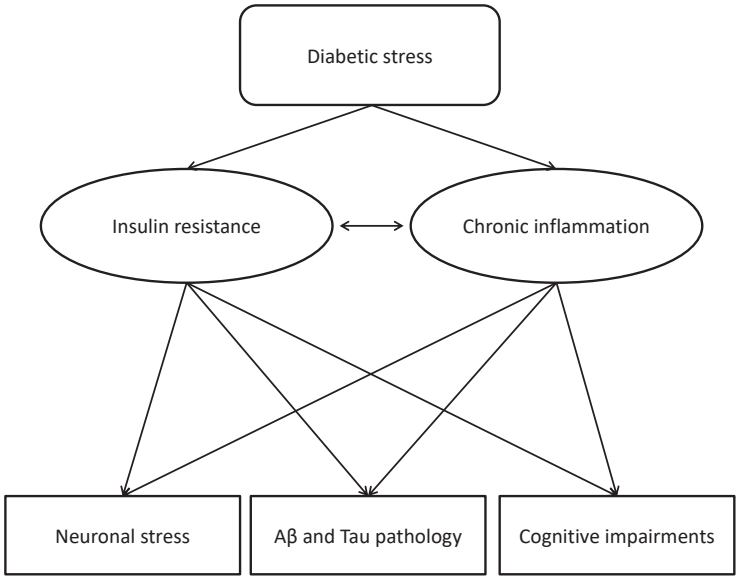

Figure 25.1 ***Summary of the proposed relationship between insulin resistance and chronic neuroinflammation in Alzheimer's disease.*** Insulin resistance and chronic inflammation can contribute independently to neurodegenerative processes and also have the potential to aggravate each other, resulting in a vicious cycle.

Interestingly, there appears to be a cross-link between the two diseases, as T2D was reported to be more prevalent among AD patients than in age-matched controls (Janson et al., 2004). AD patients with advanced dementia have lower CSF levels of insulin compared with controls, while having higher plasma-insulin levels. While control subjects have a positive correlation between CSF and plasma insulin levels, this correlation does not exist in AD patients, suggesting a disruption in the relation between brain and peripheral insulin in AD patients (Craft et al., 1998). Similar results were also observed in an AD mouse model, in which older AD mice had significantly lower insulin levels in the brain compared with WT mice (Li-Min et al., 2012). Of note, CSF insulin levels were positively correlated with better cognitive performance in AD patients as measured in the MMSE test, except for APOE4 homozygous patients (Craft et al., 1998).

Postmortem histological analysis of AD patient brains reveals reduced insulin signaling as measured by lower levels of IRS (Bomfim et al., 2012; Steen et al., 2005) and lower levels of phosphorylation on insulin receptor (IR) and AKT in the hippocampus . This is accompanied by reduced transcription of insulin and IR in the cortex, hippocampus, and hypothalamus (Steen et al., 2005). The reduction in insulin signaling also appears to correlate with disease progression, as the reduction in IR transcription and in insulin binding to the tissue is more pronounced in patients in later Braak stages of the disease (Rivera et al., 2005).

In vitro studies have produced several mechanistic explanations for impairments in insulin-signaling in AD. It appears that Aβ monomers can compete with insulin for binding to cellular membrane, interfere with insulin-induced activation of IR (Xie et al., 2002), and increase inhibitory phosphorylation of IRS1 (Bomfim et al., 2012; Ma et al., 2009). In addition, Aβ oligomers adhere to IR in neuronal spines, cause a reduction in membrane IR levels (De Felice et al., 2009; Zhao et al., 2007, 2009), and also prevent IR phosphorylation in response to insulin stimulation.

In animal models of diabetes, a causal role of diabetes in AD pathology was examined (see Table 25.1). Peripheral administration of streptozotocin (STZ), a compound with a relatively high toxicity to pancreatic β-cells, is commonly used to induce type 1 diabetes (Murtishaw et al., 2018). STZ-treated mice exhibit a reduction in plasma insulin levels, a reduction in AKT phosphorylation in the hippocampus (Murtishaw et al., 2018), and a reduction in IR phosphorylation in the brain (Jolivalt et al., 2008). Interestingly, STZ-treated mice also exhibit higher levels of phosphorylated tau in the hippocampus and cortex (Jolivalt et al., 2008; Murtishaw et al., 2018), increased Aβ levels in the brain (Jolivalt et al. 2008), and increased microgliosis in the hippocampus (Murtishaw et al., 2018). Of note, intracerebral injection of STZ to rats that does not affect pancreatic β-cells also results in neuronal loss concomitant with increased gliosis in the brain. Such STZ administration also reduces IR protein levels and insulin mRNA levels in the brain, and reduced insulin binding to the brain tissue. Finally, intracerebral STZ administration results in higher levels of phosphorylated tau and increased neuronal Aβ immunoreactivity (Lester-coll et al., 2006). These findings suggest that diabetes and impaired insulin signaling are sufficient to induce AD-related phenotypes. Of note, the effects of neuronal insulin signaling appear to have a specific relevance to tau phosphorylation—a specific deletion of insulin receptors in neurons resulting in reduced insulin-induced phosphorylation of insulin-signaling molecules such as AKT, ERK, and IRS caused a significant increase in the levels of phosphorylated tau within the brain (Schubert et al., 2004).

In line with these findings, induction of diabetes in animal models of AD appears to exacerbate disease symptoms. In an APP/PS1 animal model of AD, STZ administration reduces insulin signaling in the brain but also increases BACE expression in the brain, which results in increased APP processing, Aβ monomer levels, and Aβ plaque burden in the cortex and hippocampus. Finally, STZ administration impaired spatial learning in APP/PS1 mice (Wang et al., 2010). Similarly, the occurrence of type 2 diabetes in AD animal models in leptin receptor-deficient (*db/db*) animals exacerbates AD pathology (Takeda et al., 2010); diabetic transgenic-APP mice exhibit earlier onset of learning deficits with greater cognitive impairments prior to Aβ plaque deposition. These mice exhibit a reduction in brain AKT phosphorylation coupled with increased levels of inflammatory cytokines and astrogliosis (Takeda et al., 2010). Induction of T2D using a regime of a high-fat diet (HFD) can also exacerbate AD symptoms, as it reduces IR

phosphorylation and AKT phosphorylation in the cortex (Ho et al., 2004). HFD can also increase inhibitory phosphorylation of IRS1 and increase the phosphorylation levels of tau (Ma et al., 2009). Lastly, while HFD did not increase APP levels, it increased gamma-secretase activity in the brain, which resulted in increased levels of Aβ monomers in the hippocampus as well as an increase in Aβ plaque number (Ho et al., 2004).

Insulin signaling may also be directly involved in Aβ clearance, as short insulin stimulation can promote rapid internalization of Aβ oligomers in fibroblast cultures, and this effect is diminished in a loss-of-function mutation of IR. Moreover, insulin stimulation promotes internalization of Aβ into astrocytes, prevents oligomers-induced dendritic spine damage, and protects from surface-IR loss (Zhao et al., 2009). The relation between insulin signaling and Aβ-generating enzymes' activity appears to be bidirectional—not only does impairment in insulin-signaling increase gamma-secretase activity (Ho et al., 2004), gamma-secretase itself appears to affect insulin signaling. First it was shown that gamma-secretase cleaves an intracellular fragment of IR, as an inhibition of gamma-secretase increases intracellular levels of this intracellular domain (Kasuga et al., 2007). Moreover, a lack of gamma-secretase activity resulted in reduced IR phosphorylation following insulin stimulation (Kasuga et al., 2007), suggesting that gamma-secretase activity is important for physiological insulin signaling. Other findings suggest that a deletion of gamma-secretase results in increased IR mRNA and protein levels and increased AKT phosphorylation in response to insulin signaling, suggesting a negative modulatory role for gamma-secretase in insulin signaling (Maesako et al., 2011). In line with these findings, gain-of-function phosphorylation mutation in presinilin-1 reduced IR transcription and translation and importantly, brains of AD patients exhibit lower levels of IR in correlation with increased phosphorylation of PS1, the catalytic component of gamma-secretase (Maesako et al., 2012).

Inflammation and its effects on insulin signaling

The relation between inflammation and insulin signaling has long been studied in the context of diabetes and especially obesity. It was previously suggested that inflammatory responses can lead to impairments in insulin signaling aggravating metabolic diseases (Shoelson, Lee, & Goldfine, 2006). Furthermore, it was shown that macrophage-secreted factors, especially TNFα, can impair insulin signaling in neighboring cells as evident in reduced AKT, IR, and IRS-1 phosphorylation (Feinstein et al., 1993; Lumeng, Deyoung, & Saltiel, 2007). In addition, blockage of TNFα signaling was shown to protect cells from inflammatory-mediated impairments in insulin signaling (Uysal et al., 1997; Lumeng et al., 2007). In the context of AD, exposure of hippocampal cultures to Aβ oligomers raises TNFα levels, and usage of TNFα-neutralizing antibodies can block Aβ-induced insulin signaling impairments (Bomfim et al., 2012). Moreover, intracerebroventricular injection of TNFα that causes hypothalamic inflammation results in several diabetes-like phenotypes, such as peripheral insulin abnormalities, as measured

by increased serum insulin levels and increased basal secretion from pancreatic islets accompanied by reduced insulin signaling in the liver and muscles (Arruda et al., 2011). It was reported that the lack of TNFα signaling in a rat model of TNF-receptor knockout protects from HFD-related insulin-signaling impairments in the periphery (Arruda et al., 2011). It was previously suggested that insulin has antiinflammatory properties and that restoring insulin signaling can significantly reduce harmful inflammatory responses (Jeschke et al., 2002).

Insulin-based treatments in Alzheimer's disease

Based on the evidence linking AD pathology with impairments in insulin signaling, several therapeutic approaches to AD have been developed based on insulin signaling. Administration of the antidiabetic drugs Exenatide and Liraglutide in AD model mice reduced inhibitory phosphorylation of IRS-1 in the brain, reduced soluble Aβ levels and Aβ plaque load (Bomfim et al., 2012; Long-Smith et al., 2013), and improved cognitive performance of the mice . These animal models exhibit an increase in IR expression, especially around Aβ plaques, and this can be reduced using Liraglutide (Long-Smith et al., 2013). Likewise, the administration of the common antidiabetic drug Metformin improved AD-like symptoms observed in the db/db leptin-resistant mouse model. Metformin administration reduced tau phosphorylation, prevented loss in synaptic markers, reduced the accumulation of Aβ42 in the brain, and showed a trend toward reduction in the levels of insulin-degrading enzyme (Li et al., 2012). In an AD mouse model, Metformin improved the cognitive performance of mice, prevented neuronal death in the hippocampus, reduced Aβ accumulation in the hippocampus and cortex, and reduced gliosis and the secretion of inflammatory cytokines (Ou et al., 2018). Similarly to Metformin, administration of the pioglitazone attenuated the accumulation of Aβ42 observed in the cortex and hippocampus of STZ-treated mice and ameliorated the cognitive deficits observed in these mice (Liu et al., 2013).

Experiments in AD patients, through nasal administration of insulin or long-acting insulin analogs, have shown improvement in memory testing after short periods of treatment (Claxton et al., 2015; Craft et al., 2012; Reger et al., 2008) and reduced cognitive decline in prolonged treatments. Short-term administration of insulin also raised plasma Aβ40 monomer levels (Reger et al. 2008), possibly due to insulin's effect on the blood—brain barrier (Swaminathan et al., 2018; Vandal, Bourassa, & Calon, 2015).

Conclusions

The pathology of AD is accompanied by insulin resistance and chronic inflammation. Accumulating lines of evidence from various human and animal models suggest a unique contribution of these processes to various pathological phenotypes of AD (summarized in Table 25.1) as well as that both insulin resistance and chronic inflammation can abet their

respective "partner in crime" resulting in increased neuronal stress, Aβ and tau pathology, and cognitive impairments (Fig. 25.1). Further research targeting the link between inflammation and insulin may increase our knowledge of the progression of AD, leading to the identification of new targets for early-stage disease diagnosis and paving the way to further therapeutic approaches.

Key facts

- There is evidence of impairment in insulin signaling in AD patients.
- Meta-analyses show that T2D patients have a significantly higher risk for developing dementia compared with control subjects.
- Mouse models that overexpress Alzheimer's beta amyloid show cognitive impairment and impairments in brain insulin signaling.
- Exacerbation of diabetes risk factors in mice appears to increase deposition of brain beta amyloid.
- Antidiabetic treatments reduced Alzheimer's beta amyloid load in mouse models.

Summary points

- There is evidence that strongly links a reduction of brain insulin signaling to AD pathology. Furthermore, diabetes risk factors accelerate disease progression.
- Impairment in brain insulin signaling is correlated with an increase in neuroinflammation.
- Antiinflammatory treatments were shown to improve brain insulin signaling.
- Treatments used to treat diabetes show a potential to reduce cognitive impairment in mouse models that overexpress Alzheimer's amyloid plaques.
- Further research of the pathways through which inflammation might lead to brain insulin resistance may increase our understanding of the etiology of AD and suggest potential novel targets for both diagnosis and therapeutic interventions.

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CHAPTER 26

Brain susceptibility to hypoxia/hypoxemia and metabolic dysfunction in Alzheimer's disease: insights from animal and in vitro models

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List of abbreviations

AD Alzheimer's disease
BBB Blood–brain barrier
NVU Neurovascular unit
OGD Oxygen/glucose deprivation
OLs Oligodendrocytes
OPCs Oligodendrocyte precursor cells

Mini-dictionary of terms

Blood–brain barrier Anatomical and functional unit, composed of the highly selective brain capillary endothelial cells and the neurovascular unit.

Hypoxia/hypoxemia Total (hypoxia) or partial (hypoxemia) decrease of oxygen tension in blood and/or tissues.

Neurovascular unit Histological structure composed of basement membrane, vascular endothelial cells, pericytes, astrocytes, and microglia, regulating the blood supply to neurons.

Oxygen-glucose deprivation In vitro system modeling in vivo hypoxia/ischemia, exposing cell cultures to a time-defined glucose-deprived medium in hypoxic chamber.

Preclinical/presymptomatic phase Stage of the disease preceding clinical symptom manifestation.

Introduction

Despite the lack of success of several phase III clinical trials based on drugs designed to prevent amyloid deposition or to favor its removal and clearance (Mehta, Jackson, Paul, Shi, & Sabbagh, 2017), the amyloid hypothesis remains the most compelling working hypothesis for Alzheimer's disease (AD; Selkoe & Hardy 2016). However, two emerging pathogenic aspects pointing at neurobiological alterations occurring in the

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preclinical/presymptomatic phase of the disease, well before plaque deposition, are being integrated within the amyloid hypothesis.

The first one concerns the intracellular Aβ peptides accumulation, which occurs in neurons (Fig. 26.1), as well as other cell types (Fig. 26.2), before the appearance of plaque and cognitive symptoms. Notably, the intracellular concentration of $A\beta_{1-42}$, the most toxic Aβ variant, has been estimated to be much higher in sporadic AD patients than in control subjects in pyramidal (CA1) human neurons (Umeda et al., 2011), and we recently demonstrated a causal link between intracellular Aβ and neuron vulnerability to hypoxia and glucose deprivation (Baldassarro, Marchesini, Giardino, & Calzà, 2017).

The second one concerns the revival of the vascular hypothesis in AD. In fact, over the past 20 years the vascular hypothesis for AD emerged as a complementary mechanism to the amyloid toxicity hypothesis (De La Torre, 2010). Based on epidemiological, pathological, neuroimaging, pharmacotherapeutic, and clinical studies, the vascular hypothesis suggests that vascular "risk factors" also related to the aging process contribute to the Aβ accumulation. The cerebrovascular dysfunction causes the reduction of the cerebral blood flow, tissue hypoxia, and a blood–brain barrier (BBB) dysfunction that may lead to a defect in Aβ clearance, thus favoring Aβ accumulation (Janota, Lemere, & Brito, 2016).

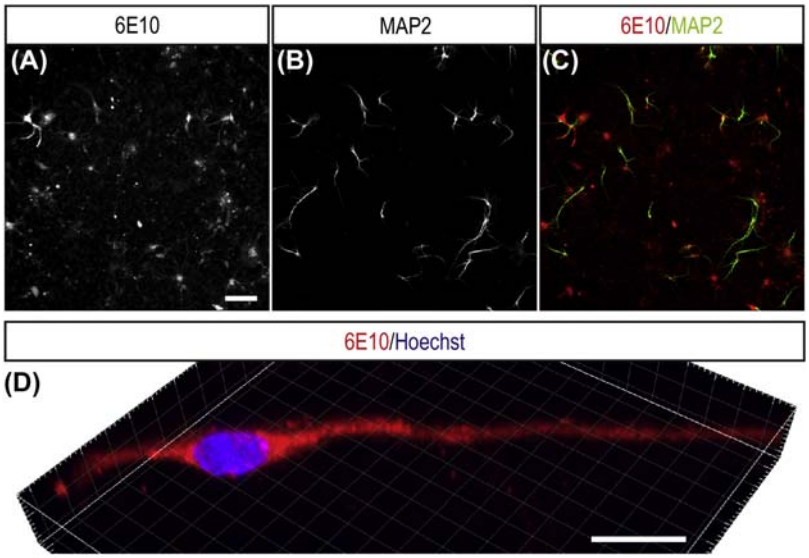

Figure 26.1 ***APP/Aβ accumulation in Tg2576-derived neurons.*** Panels show representative images of pure primary neuron cultures isolated from the cerebral cortex of newborn Tg2576 mice, after 7 days in vitro. Images were acquired by cell-based high-content screening (A–C) and z-stack acquired by confocal microscopy (D). Cultures were stained for 6E10 (A, D), an antibody marking the full human APP and Aβ fragment, showing the intracellular accumulation. Cells were also stained for a marker specific for mature neurons (MAP2s; B). Merged image of the two markers is also included in the panel (C). Scale bars in (A and G): 10 μm. Figure shows original pictures not published elsewhere. *MAP2*, microtubule-associated protein 2.

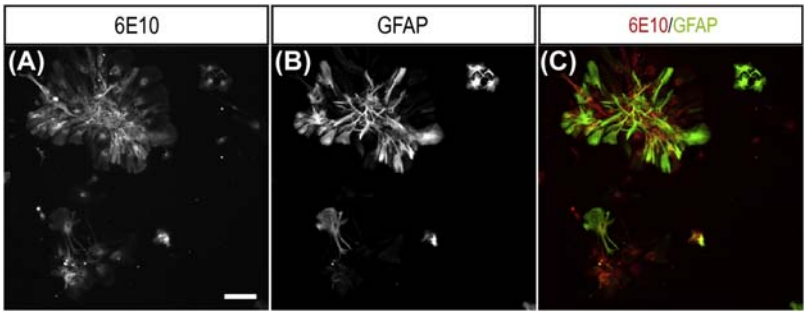

Figure 26.2 ***APP/Aβ accumulation in Tg2576-derived astrocytes.*** Panels show representative images of pure primary astrocytes isolated from the cerebral cortex of newborn Tg2576 mice, after 7 days in vitro. Images were acquired by cell-based high-content screening. Cultures were stained for 6E10 (A), an antibody marking the full human APP and Aβ fragment, showing the intracellular accumulation. Cells were also stained for a marker specific for astrocytes (GFAP; (B). Merged image of the two markers is also included in the panel (C). Scale bar in A: 10 μm. Figure shows original pictures not published elsewhere. *GFAP*, glial fibrillary acidic protein.

The anatomical and functional dysfunction of large vessels was originally regarded as the major responsible for vascular contribution to AD in the frame of "vascular dementia," including in this topic systemic diseases that may increase the risk of stroke or ischemia, such as obesity, diabetes, hypertension, and atherosclerosis (Santos et al., 2017). More recently, attention has shifted to "small-vessel disease," indicating a number of alterations of the microcirculation that finally impair the "neurovascular coupling," i.e., the physiological response that guarantees the appropriate cerebral tissue metabolic supply according to the functional activation (Iadecola, 2004).

In this chapter, we will review neurobiological aspects of the neurovascular dysfunction in AD related to the capillary net, focusing on the possible contribution of chronic hypoxemia and metabolic impairment to cell vulnerability in AD, using the translational perspective offered by AD animal models.

The neurovascular unit

The brain is a heavy energy—demanding organ compared to its mass. It represents only 2% of the total body mass in humans, but consumes about 20% of the oxygen and 25% of glucose of the whole body (Bélanger, Allaman, & Magistretti, 2011). Moreover, it lacks a metabolic reservoir, thus it is totally and continuously depending on blood for metabolic supply, also in case of mutated energy demand. This continuous connection between brain metabolic demand and blood supply is tightly regulated by a specific structure, i.e., the neurovascular unit (NVU; Iadecola, 2017). NVU is a histological structure composed of a basement membrane, vascular endothelial cells, pericytes, astrocytes, and microglia. Moreover, other cells, such as oligodendrocytes (OLs), interact with the NVU (McConnell, Kersch, Woltjer, & Neuwelt, 2017), contributing to vascular-related processes such as BBB regulation and angiogenesis (Fig. 26.3).

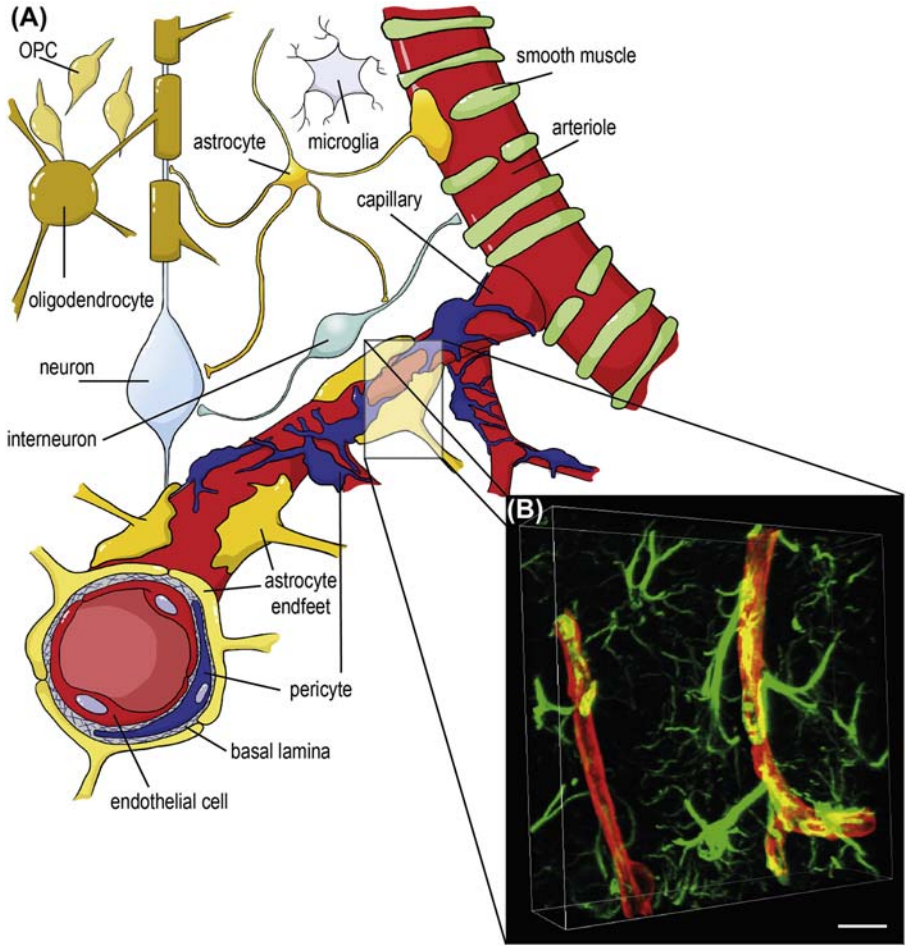

Figure 26.3 ***The neurovascular unit structure.*** Different cells residing in the central nervous system affect the neurovascular unit dynamics through direct or indirect interactions (A). Astrocyte end-feet are plastered inside the capillary basal lamina, covering 99% of the capillary surface and including pericytes. The figure shows a three-dimensional confocal image (14 μm thickness; 62× magnification), showing astrocytes (GFAP, green) covering capillaries walls (laminin, red) with their end-feet (B). Scale bar; 20 μm. Figure shows original drawing not published elsewhere. *GFAP*, glial fibrillary acidic protein; *OPC*, oligodendrocyte precursor cells.

Endothelial cells lining the brain capillaries show tight junctions (TJs) that create a physical barrier to the paracellular diffusions of ions and molecules, while specific transporters allow the selective and active movement of molecules. These TJs are stabilized by specific proteins and connected to pericytes (Liebner et al., 2018).

A physical bridge between neurons and capillaries is made by the most abundant cell type in the vertebrate central nervous system (CNS)—the astrocyte. They are multifunctional cells, regulating uptake/release of neurotransmitters, and directly supplying neurons with

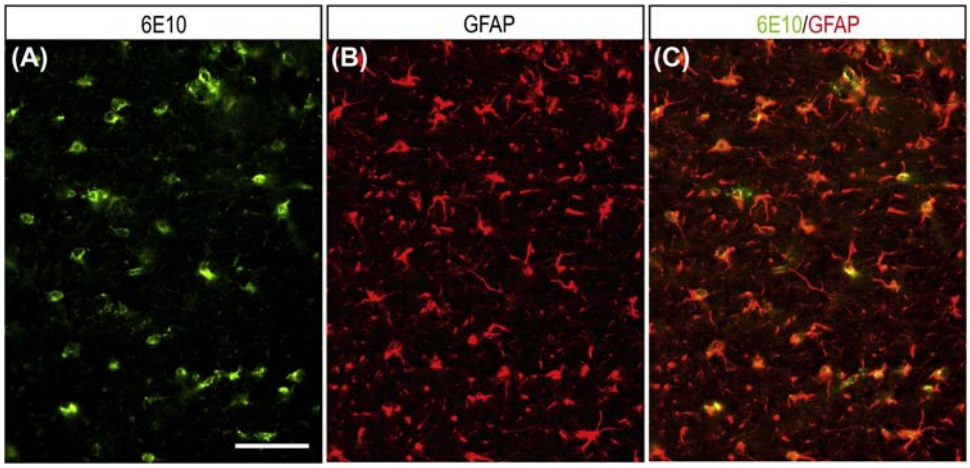

Figure 26.4 ***APP/Aβ accumulation in astrocytes of 3-month-old Tg2576 mouse.*** Panels show representative images of immunolabeling of cerebral cortex of 3-month-old Tg2576 mouse. Images were acquired by epifluorescence microscope. Slice was stained for 6E10 (A), an antibody marking the full human APP and Aβ fragment, showing the intracellular accumulation. Cells were also stained for a marker specific for astrocytes (GFAP; (B). Merged image of the two markers is also included in the panel (C). Scale bars in A: 50 μm. Figure shows original pictures not published elsewhere. *GFAP*, glial fibrillary acidic protein.

substrates for oxidative phosphorylation. In fact, the metabolic needs of neurons are totally dependent on blood oxygen supply, glucose supply supported by the astrocytic glucose transporters (mainly GLUT-1), and conversion of glycogen to lactate (González-Reyes, Nava-Mesa, Vargas-Sánchez, Ariza-Salamanca, & Mora-Muñoz, 2017). Astrocytes are physically located between neurons and endothelial cells, extending end-foot processes to the surface of brain capillaries, covering almost the 99% of abluminal wall, and to the neuron plasma membrane. In this context, they also have a major role in the regulation of cerebrovascular tone and neurovascular coupling (Filosa, Morrison, Iddings, Du, & Kim, 2016).

The regulation of NVU is also affected by pericyte activity. These cells show stem cell-like properties and are embedded within the basement membrane of capillaries, precapillary arterioles and postcapillary venules, and directly communicate with endothelial cells and other pericytes. Over the last decades different functions have been assigned to pericytes: (1) blood vessel formation and vessel maintenance, (2) angiogenesis, (3) permeability of BBB, (4) clearance of cellular debris and Aβ peptide in AD, (5) immune cells entry, (6) neurovascular coupling and participation in blood flow regulation, and (7) neuroinflammation (Sweeney, Ayyadurai, & Zlokovic, 2016). Pericytes are able to communicate directly with endothelial cells through gap junctions and via junctional complexes located to peg—socket contacts at sites where the basement membrane is absent. This interaction is important for the integrity and maintenance of the basement

membrane of the vessel wall and allows pericytes to participate in the cerebral blood flow regulation (McConnell et al., 2017).

Pericytes and astrocyte end-feet are structurally associated at the capillary level, enveloping the outer endothelial wall, generating an anatomical assembly maintained by the basal lamina (Giannoni et al., 2018). Pericytes also play a major role in sprouting angiogenesis, induced by the activation of endothelial cells via growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), placental growth factor, and hypoxia-inducible factor (HIF)-1α (Sweeney et al., 2016).

Other cells contacting the NVU also play important roles in BBB dynamics. For example, microglia and perivascular macrophages represent the first barrier to pathogens and contact the other cells of the NVU. In a mutual way, neurons are able to induce vasodilation and vasoconstriction, regulating the NVU dynamics, by secreting specific vasoactive factors (McConnell et al., 2017).

Since the NVU plays a key role in the metabolic supply to the brain, this structure is under active investigation for a possible role in neurodegenerative diseases including AD. Recent imaging studies have in fact indicated the presence of a disturbed capillary flow pattern and deteriorated microvascular hemodynamics in AD patients (Nielsen et al., 2017). Moreover, a longitudinal study performed in an AD murine model by MRI technique suggests that the reduction observed in middle-aged animals is probably caused by an impaired vasoactivity of capillaries and arterioles, which is not directly correlated with the amount of Aβ deposition in parenchyma nor blood vessel walls (Zerbi et al., 2013).

In fact, an early impairment of NVU during early and preclinical stages of AD emerged from recent imaging studies, pointing out the contribution of vascular dysfunction in very early preclinical stages, and not only in AD progression (Kapasi & Schneider 2016).

Blood–brain barrier in Alzheimer's disease

Healthy BBB protects neurons from substances, cells, and drugs present in the systemic circulation, thus regulating and maintaining homeostasis in the CNS.

BBB breakdown in AD precedes amyloid plaque deposition, neurodegeneration, and brain atrophy, and a BBB dysfunction leading to an increased permeability to circulating molecules was also found in mild cognitive impairment (MCI) patients (Sweeney, Sagare, & Zlokovic, 2018). The causes of barrier disaggregation are not completely understood. Recent data suggest an age-dependent deterioration of the BBB during normal aging, and a more accelerated degradation in patients with MCI (Zenaro, Piacentino, & Constantin, 2017). Age-related BBB deterioration is promoted by cerebrovascular dysfunction and vascular pathologies, as observed in systemic diseases, like cardiovascular diseases, hypercholesterolemia, and diabetes where also cognitive alterations and neuronal loss are observed. In AD, cerebral amyloid angiopathy and Aβ deposition in the tissue promote inflammation (Fig. 26.5) and consequently BBB permeabilization

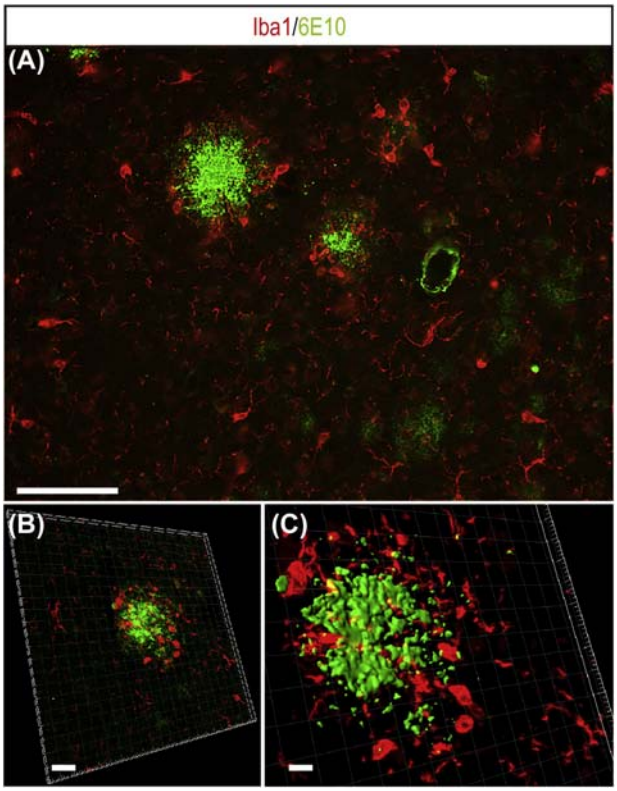

Figure 26.5 ***Amyloid plaque deposition in Tg2576 mouse hippocampus.*** Panels show representative images of immunolabeling of hippocampus of 18-month-old Tg2576 mouse. Images were acquired by epifluorescence (A) or laser confocal microscope (B) elaborated by IMARIS software (C). Slice were stained for 6E10 (green), an antibody marking the full human APP and Aβ fragment, showing the plaque deposition, and for Iba1 (red) a marker for activated microglia. Scale bar in A: 50 μm; B: 20 μm; C: 10 μm. Figure shows original pictures not published elsewhere. *Iba1*, ionized calcium-binding adapter molecule 1.

(Sweeney et al., 2018). According to the so-called "two-hit vascular hypothesis of AD" (Zhu, Raina, Perry, & Smith, 2004), damage to brain microcirculation (hit one) can occur in aging brain as a result of genetic risk factors, environmental factors, lifestyle, or vascular risk factor, thus initiating a cascade of events (including BBB breakdown, hypoperfusion, accumulation of toxic products and inflammatory cells in brain tissue), which directly damage neurons. This modified environment contributes to the second hit, i.e., the accumulation of Aβ in brain parenchyma and the alteration of its clearance, also promoting neurodegeneration (Kisler, Nelson, Montagne, & Zlokovic, 2017).

The extravasation of molecules and cells from systemic circulation to brain parenchyma also alters the structure and the function of the NVU, impairing the neurovascular coupling. Several molecular and cellular alterations have been described in BBB

components during AD. For example, Aβ accumulation modifies expression of TJs and adherent junctions among brain microvessel endothelial cells (Zenaro et al., 2017). These modifications were also found in different animal models for amyloid pathology (Montagne et al., 2017; Yamazaki & Kanekiyo 2017) and in postmortem brains of patients with CAA and AD (Zenaro et al., 2017).

Moreover, endothelial cells of brain microvessels show atrophy and smaller cellular size compared to healthy age-matched subjects, possibly due to reduction of Aβ efflux from the brain. Brain endothelium actively transports Aβ from parenchyma to systemic circulation, and, in AD patients there is a correlation between Aβ accumulation and reduced expression of transporters (Sweeney et al., 2018). Finally, brain capillary endothelial cells also express the glucose transporter GLUT1, which is reduced in AD patients (Zenaro et al., 2017).

Modification of the extracellular matrix, basal membrane composition and structure also occur consistently in AD patients. Basement membrane results in thicker brain capillaries of AD patients and the expression level of different ECM components results in increased frontal and temporal cortex of patients with subclinical AD (Yamazaki & Kanekiyo 2017). In addition, Aβ stimulates expression of MMP-2 and MMP-9, which contributes to TJ cleavage and degrades endothelial basal lamina, triggering BBB dysfunction.

Astrocytes in AD

The most investigated role of astrocytes in AD is the contribution to plaque deposition and turnover, and the associated inflammation. In fact, (1) reactive astrocytes are an integral part of Aβ plaques in AD patients; (2) astrocytes are involved in plaque progression and secondary plaque development (3) after death of Aβ-loaded astrocytes due to peptides phagocytosis (Fig. 26.4; Imbimbo, Solfrizzi, & Panza, 2010; Sivilia et al., 2013). However, the emerging evidence of the importance of microvascular components in the preplaque phase of AD, and the role of astrocytes in NVU regulation, are leading to a redefinition of the astrocytes' role in microvascular dysfunction in AD (Tarantini, Tran, Gordon, Ungvari, & Csiszar, 2017).

Astrocytes undergo several changes during AD progression in a region-specific manner. A decrease in mean volume, surface area, and protoplasmic process has been described in several mouse models of AD, before plaque deposition, such as the disruption of the astrocyte end-foot function (Price, Norris, Sompol, & Wilcock, 2018). An age-dependent intracellular Aβ accumulation is observed in astrocytes with a peak at the preclinical phase of the disease *(Giuliani* et al. *personal communication)*. Moreover, intraneuronal Aβ is linked to reduced astrocyte glycolytic capacity and reduction in NVU function, altering the overall oxidative neuronal microenvironment (González-Reyes et al., 2017). At this stage, changes in astrocyte NVU coupling, astrocyte retraction and swelling, and decrease in GLUT1 and lactate transporters expression also have been observed (Merlini, Meyer, Ulmann-Schuler, & Nitsch, 2011).

Aβ deposits in microvessels seem to contribute to the astrocyte dysfunction (Zenaro et al., 2017), also impacting on the astrocyte contribution to the Aβ clearance, and alteration of several molecules produced by astrocytes has been described (Park, Kook, Park, & Mook-Jung, 2014; Yang et al., 2012). Thus, vascular amyloid may reduce the dynamic range of vessels throughout astrocyte stimulation, before disease manifestation and even in patients that never develop AD.

Pericytes in AD

Accumulating clinical and experimental evidence indicates that an alteration in pericyte structure, molecular phenotype and function occurs in AD, contributing to the BBB breakdown and decreased cerebral blood flow (CBF; Sweeney et al., 2016). For example, AD subjects are affected by significant loss of pericytes in hippocampus and cortex compared to control patients, correlating with the severity of BBB degradation (Zenaro et al., 2017). An injury in pericyte associated to the BBB was also observed in the hippocampus of MCI patients (Montagne et al., 2015). An age-dependent reduction in cerebral microcirculation is also evident in pericyte-deficient mice, and the BBB deteriorates leading to neurodegeneration and cognitive impairment (Sagare et al., 2013). Furthermore, sPDGFRβ, a marker of pericyte injury, is increased in MCI patients compared to control subjects, while no other signs of endothelial or neural damage, or tau and Aβ pathology, are observed (Montagne et al., 2015), suggesting a microcirculation defect (Sweeney et al., 2016). In fact, mouse models with partially disrupted PDGF-BB/PDGFRβ signaling develop a progressive and rapid vascular phenotype with reduction in pericyte coverage and numbers and reduction in capillary length in several brain regions (Nikolakopoulou, Zhao, Montagne, & Zlokovic, 2017).

The underlying cause for the loss of pericytes in AD patients is not yet completely understood, but some studies are focusing on the presence of Aβ. The loss of pericytes found in AD patients is associated with increased Aβ depositions (Sengillo et al., 2013). In support, studies in transgenic APP mice have found correlation between Aβ load and pericyte number. Moreover, as demonstrated by in vitro studies, Aβ is able to influence the survival of cultured pericytes; $A\beta_{1\text{-}40}$ seems to be particularly toxic (Schultz et al., 2018), while $A\beta_{1\text{-}42}$ oligomers increase the production of reactive oxygen species (ROS) by pericytes (Zenaro et al., 2017). Furthermore, there is evidence that the accumulation of Aβ reduces CBF not only through the development of cerebral amyloid angiopathy but also by inducing chronic vasoconstriction and interfering with autoregulation and neurovascular coupling (Miners, Schulz, & Love, 2018).

Impact of hypoxia/hypoxemia on neuronal function in AD

Clinical and preclinical data suggest that the gradual decline in brain oxygen supply in aging, and chronic brain hypoperfusion can participate in the onset of clinical symptoms

in AD, affecting both the gray and white matter (Zhao & Gong 2014). Moreover, the possible impact of conditions impairing oxygen supply and glucose utilization, are regarded as possible risk factors, although supporting data are still disputing (Seitz, Reimer, & Siddiqui, 2013).

As already described, converging evidences suggest that the microvascular dysfunctions in AD leads to a reduction in cerebral perfusion and energy deficits, causing tissue damages when this hypoperfusion exceed the metabolic demand. Not surprisingly, different processes and pathways contributing to these pathological mechanisms overlap and interact each other, involving Aβ accumulation (Ashok, Ajith, & Sivanesan, 2017). In fact, processes in the cascade of events triggered by hypoxia and leading to cell damage, are influenced by Aβ (Guglielmotto, Tamagno, & Danni, 2009) (Fig. 26.6).

Hypoxia/hypoxemia and neurons

The brain is particularly vulnerable to hypoxia, due to its energetically expansive mechanisms, such as synapses, which require 30%–50% of cerebral oxygen (Mukandala, Tynan, Lanigan, & O'Connor, 2016). Hypoxia/ischemia leads to neuronal death, affecting oxidative phosphorylation and ATP production, and subsequent dysfunction of ATP-dependent ion transport pumps, calcium overload, and mitochondrial dysfunctions, activating caspases-dependent and independent mechanisms of cell death induction and necrosis (Northington, Chavez-Valdez, & Martin, 2011). Moreover, this process leads to a metabolic adaptation of neurons for different sources of energy, such as glutamate, glutamine, and GABA (Gamma-aminobutyric acid), and excitotoxicity mechanisms. Necrotic death and autophagy are also associated with acute hypoxia/ischemia through the calcium influx, activating calpain-mediated cell death processes (Descloux, Ginet, Clarke, Puyal, & Truttmann, 2015).

The NVU, and in particular astrocytes, are involved in all these processes, playing a fundamental role in AD progression (González-Reyes et al., 2017). In vitro data indicates that hypoxia stimulates APP processing, increases Aβ generation in neuronal cells, astrocytes, and vascular endothelial cells. Moreover, HIF-1α, the transcription factor involved in the cellular adaptations to hypoxia, activates both β and γ secretases, increasing the Aβ production (Salminen, Kauppinen, & Kaarniranta, 2017). Thus, due to the intrinsic toxicity of amyloid fragment intracellular accumulation, the increased production of Aβ may itself cause an increased neuronal vulnerability.

Although hypoxia/ischemia processes and APP metabolism influence each other, a clear link leading to neuronal damage is missing. However, it has been demonstrated that primary neuronal cultures isolated from Tg2576 mice, producing and accumulating human Aβ in the cytoplasm, are more vulnerable to oxygen-glucose deprivation (OGD). Moreover, blocking Aβ production restores the increased vulnerability, proving a direct link to metabolism impairment damage and Aβ intracellular accumulation (Baldassarro

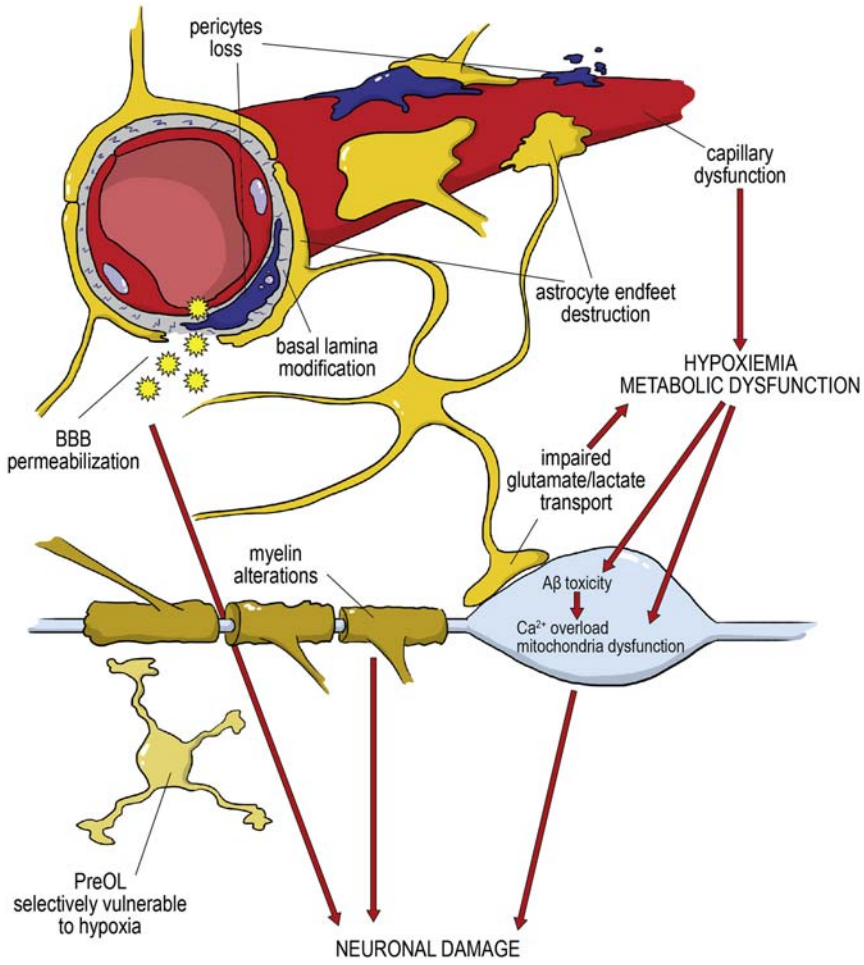

Figure 26.6 ***Neurovascular unit alterations in Alzheimer disease.*** BBB permeabilization and basal lamina modifications, together with capillaries dysfunction and pericytes loss, contributing to hypoxemia environment onset. Moreover, astrocyte end-feet destruction and impaired glucose/lactate transport lead to metabolic dysfunctions. These noxious stimuli increase overproduction of Aβ, which exert intrinsic toxicity and, in a mutual way, increases neurons vulnerability to hypoxia and metabolic challenges. These mechanisms, together with myelin alterations and PreOL sensitivity to hypoxia, contribute to neuronal damage. Figure shows original drawing not published elsewhere. *BBB*, blood–brain barrier; *HIF1α*, hypoxia-inducible factor 1 alpha; *PreOL*, pre-oligodendrocyte.

et al., 2017). Moreover, dysregulation in gene expression of VEGFa and its receptors has been described as dysregulated in both primary cortical and neural stem cell–derived neurons isolated from this mouse model (Baldassarro et al., 2013, 2017).

Moreover, alteration in NVU processes and dynamics, leading to a metabolic disfunction, is linked to the increased secretion of inflammatory factors, leading to an increased

Aβ production. These data point out again the microvascular dysfunction in a key position in the pathological cascade in the disease progression (Rius-Pérez, Tormos, Pérez, & Taléns-Visconti, 2018).

Hypoxia/hypoxemia and myelin-forming cells

OLs, the myelin-forming cells in the CNS, have specific roles in the NVU, providing metabolic, trophic, and mechanic support to axons (Barateiro, Brites, & Fernandes, 2016). OLs are cells very sensitive to different noxious stimuli, showing a decrease of 27% in aging brain, accentuated in AD patients (De Strooper & Karran 2016), as described by the decreased levels of the main OL proteins, in both white and gray matter of postmortem AD brains (Liu & Zhou 2013). Moreover, extensive evidence from imaging studies indicates that white matter signal abnormalities are quite common in older individuals as well as patients with dementia, also suggesting a key role in vascular events (Riphagen et al., 2018).

The link between AD pathology and OL loss is not clear, however, myelin and OL alterations occur before Aβ plaque appearance in animal models of the disease. In fact, myelin disruption has already been described by Alois Alzheimer, and other studies have demonstrated that myelin injury precedes the onset of cognitive impairment. Moreover, these cells express APP mRNA and protein, secrete $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ fragments, and Aβ is described as toxic for OLs, inducing white matter damage in vivo and cytotoxicity in vitro (Barateiro et al., 2016).

Both OLs and their precursors (oligodendrocyte precursor cells, OPCs), the cells responsible for myelin turnover and OL replacement in adult CNS, are also very sensitive to hypoxia.

The injury pattern depends on the severity of the impairment and on the developmental stage of the brain, showing a marked vulnerability in neonatal stages (Jablonska et al., 2012). Moreover, vulnerability depends also on maturation; the most susceptible stage is PreOLs (late OPCs), followed by early OPCs and mature OLs, which implies death of PreOLs and subsequent failure of myelination during the injury progression (Back et al., 2002).

Hypoxia affects DNA stability, induces microvascular alteration and oxidative stress, and increases iron levels (Nasrabady, Rizvi, Goldman, & Brickman, 2018). In particular, in vitro experiments showed that OGD in primary OPCs induces PreOL death by intracellular Ca^{2+} overload, mitochondrial damage, and ROS generation (Cai, Ma, Li, Tian, & Li, 2016). This ROS production plays a key role in hypoxia-induced cell damage, because of low content of antioxidant (in particular glutathione), and high consumption of oxygen and ATP of these cells. These radicals induce lipid peroxidation, impair protein and acid nucleic formation, and also promote membrane disruption (Barateiro et al., 2016).

Moreover, OL injury due to hypoxia and metabolic challenge is characterized by an early excitotoxic-oxidative cascade, caused by the reduction of the high-energy

phosphate metabolism. In fact, this leads to an increase in lactic acid, and cell membrane ionic transport failure that, combined with cytoskeleton destruction, causes depolarization and excessive release of glutamate. This condition is also worsened by the depleted glutamate reuptake caused by the reduced glucose availability (Rocha-Ferreira & Hristova, 2016).

Beyond the pathological mechanisms directly correlated to hypoxia and metabolic stress, hypoxia also induces an inflammatory response that promotes damaged cells and debris removal, and stimulates repair processes (Rocha-Ferreira & Hristova, 2016). However, it is well described how an inflammatory local environment is detrimental to OPC differentiation and myelin injury repair (Fernández, Baldassarro, Sivilia, Giardino, & Calzà, 2016).

Key facts of neurovascular unit

- The brain represents only 2% of the total body mass in humans but consumes about the 20% of oxygen and 25% of glucose of the whole body; the synaptic function requires 30%–50% of cerebral oxygen.
- The neurovascular unit (NVU) is the complex and tightly controlled structure providing continuous connection between brain metabolic demand and blood supply participating in the blood–brain barrier regulation.
- The term "blood–brain barrier" was first proposed by M. Lewandowsky in 1900.
- Astrocytes are the physical bridge between capillaries and neurons and astrocytes end-feet and cover 99% of abluminal surface of brain capillaries.
- "Small-vessel disease" consists of all the alterations of the NVU leading to impairment of the neurovascular coupling.

Summary points

- This chapter focuses on the role of microvascular dysfunctions in onset and progression of Alzheimer's disease (AD).
- Different components of the neurovascular unit (NVU) seem to be altered in early stages of AD.
- Early stages of the disease are linked to Aβ intracellular accumulation.
- Astrocytes are the main physical bridges between microvessels and neurons, and their alterations in AD are seen as the major trigger of the metabolic dysfunction.
- NVU alterations produce hypoxia/hypoxemia and metabolic challenges to neurons, leading to neuronal dysfunctions and cell death.
- Microvessel alterations affect also oligodendrocytes and oligodendrocyte precursor cells, which are sensitive to metabolic dysfunctions, showing emerging evidence of their involvement in AD progression.

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CHAPTER 27

Neuropeptides and neurolipids: what they are and how they relate to Alzheimer's disease

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List of abbreviations

ACh Acetylcholine
AD Alzheimer's disease
AEA Anandamide
BFCN Basal forebrain cholinergic neurons
CB_1 Type-1 cannabinoid receptor
CB_2 Type-2 cannabinoid receptor
CCK Cholecystokinin
CNS Central nervous system
CRF Corticotropin-releasing factor
eCB Endocannabinoid
FAAH Fatty acid amide hydrolase
Gal Galanin
GPCR G protein-coupled receptor
hAPP Human amyloid precursor protein
LPA Lysophosphatidic acid
MALDI-IMS Matrix-assisted laser desorption ionization-imaging mass spectrometry
NK Neurokinin
NL Neurolipid
NP Neuropeptide
NPY Neuropeptide Y
PNS Peripheral nervous system
S1P Sphingosine-1-phosphate
$S1P_1$ Sphingosine-1-phosphate receptor subtype one
SP Substance P
SST Somatostatin
TRPV Transient receptor potential channel
2-AG 2-arachidonoylglycerol
Δ^9-THC Δ^9-tetrahydrocannabinol

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Mini-dictionary of terms

Imaging mass spectrometry An analytical technique that allows the digital visualization of the spatial distribution of molecules from a mass spectrum following a laser-induced ionization of a tissue slice.

Neuropeptides Polypeptide chains usually composed of between 2 and 50 amino acids that can function in the brain with agonistic and neuromodulatory properties.

Neurolipids Endogenous lipid-based signaling molecules derived from membrane lipid precursors with agonistic and neuromodulatory properties.

Cannabinoid A chemical compound that binds to and activates cannabinoid receptors. Depending on the origin of the cannabinoids, they can be divided into phytocannabinoids (produced by *Cannabis* spp), endocannabinoids (i.e., released by animal cells), and synthocannabinoids (chemically synthesized).

Orphan receptor Cloned receptors whose endogenous ligands need to be described.

Lysophospholipid Membrane phospholipid-derived molecules, which lack one acyl group following the hydrolysis driven by different phospholipases.

Introduction

Neuropeptides (NPs) and neurolipids (NLs) are endogenous molecules with neuromodulatory properties.

NPs are polypeptide chains usually composed of 2–50 amino acids that can function in the central nervous system (CNS) as hormones or neurotransmitters modulating other systems. To date, approximately 100 NPs have been described as being released by nerve endings and are capable of modulating biological functions. NPs are usually coreleased with other neurotransmitters such as biogenic amines. This coexistence of biogenic amines and neuropeptides was first demonstrated in endocrine cells; since then, this coexistence has been shown to be a common and widespread process in the CNS.

On the other hand, NLs or endogenous lipid-based signaling molecules fine-tune cellular communication mainly through G protein-coupled receptor (GPCR) with enzymatic machinery for their biosynthesis and degradation, constituting a complex signaling network (Figs. 27.1 and 27.2). The activation of multiple signaling systems (e.g., dopaminergic, cholinergic, glutamatergic, serotonergic, or GABAergic) is able to evoke the synthesis and release of NLs, e.g., endocannabinoids (eCBs) in the CNS.

Neuropeptides

NPs act as cotransmitters in the nervous system, along with other peptides and/or nonpeptidic neurotransmitters, modulating processes such as trophic functions. NPs are particularly relevant when the nervous system is stressed, altered, or affected by an impairment or disease. This neuromodulation is responsible for the selectivity of NP actions. The anatomical localization pattern of the NP is very selectively based on brain area, resulting in specific effects mediated by NPs. NP signaling is commonly mediated by GPCRs, although they can also recognize tyrosine kinase receptors.

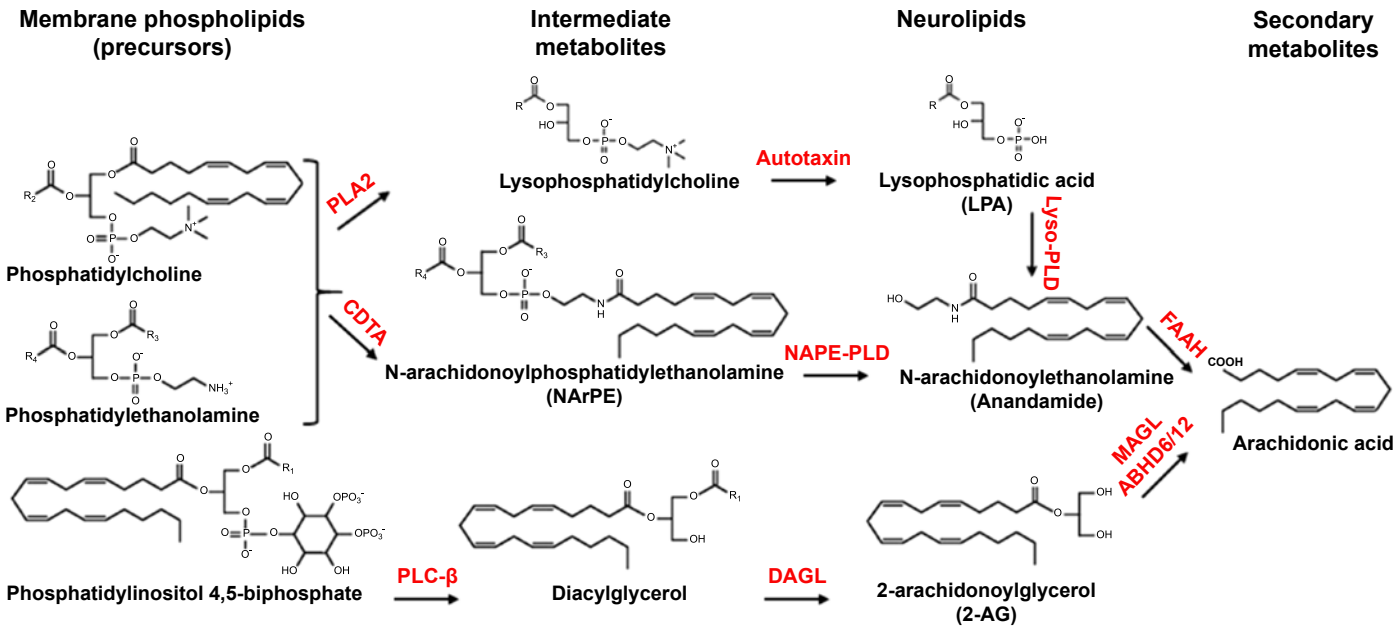

Figure 27.1 ***Metabolic pathways for endocannabinoids and lysophosphatidic acid.*** Synthesis and degradation of neurolipids lysophosphatidic acid (LPA), anandamide, and 2-AG, including membrane phospholipid precursors, synthesis and degradation enzymes, and intermediate and secondary metabolites. Note that the degradation of the endocannabinoids, anandamide, and 2-arachidonoylglycerol (2-AG) leads to the release of the proinflammatory molecule arachidonic acid. The dysregulated enzymatic activity described in Alzheimer's disease may contribute to poor disease outcomes.

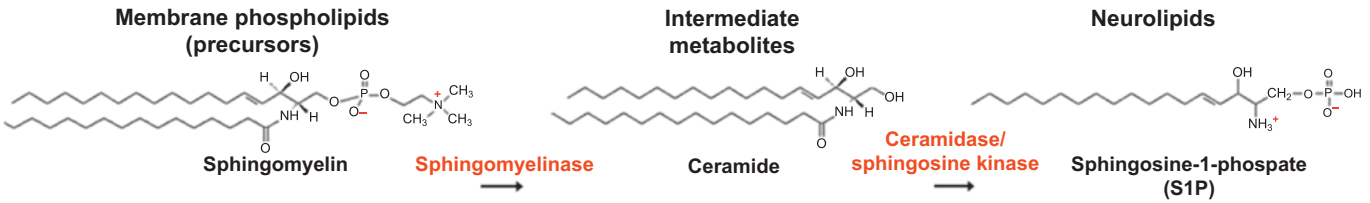

Figure 27.2 ***Synthesis pathway of sphingosine-1-phosphate.*** Synthesis pathway for neurolipid sphingosine-1-phosphate (S1P), including membrane phospholipid precursors and enzymes.

NPs are involved in the regulation of many physiological responses, including regulation of pain, sleep, stress, memory, and learning. To date, about 20 NPs have been directly related to neurochemical alterations in AD, but only a few of them present conclusive results. Despite the important role of NPs as neuromodulators, studies analyzing NPs and their receptors in AD are scarce and sometimes contradictory. Most mainly focus on receptors for galanin, opioids, substance P (SP), and other compounds, such as neuropeptide Y (NPY), corticotropin-releasing factor (CRF), somatostatin (SST), or cholecystokinin (CCK) receptors (Table 27.1).

Table 27.1 Changes in neuropeptides and neuropeptide receptor levels in the brain and CSF of Alzheimer's disease patients and models of the disease.

	AD patient or models of the disease	NP concentration (brain area)	Receptor density (brain area)
Galanin	AD patient	↑ BF ↑ Cx	↑ Hpc ↑ Hyp ↑ Amyg
	APP/PS1 mice	↑ Cx ↑ Hpc	
	3xTg-AD mice		↑ Activity Hyp
Opioid peptides	AD patient	↑ Total (CSF) ↓ β-endorphin (CSF) ↑ Enkephalin (DG) ↑ Dynorphin (Cx) ↑ Hemorphin (TCx)	↑ Total (Amyg) ↑ Total (Hpc) ↑ Total (FrCx) ↑ κ receptor (CP) ↓ μ receptor (DG and Ent) ↓ δ receptor (DG and Ent)
Substance P	BFCN lesion model rat		↓ μ receptor (Cx, hpc) ↑ δ receptor (Cx)
	AD patient	↓ Cx ↑↓ CSF	↓ B
	hAPP (human APP overexpressing mice)	↑ Hpc (around senile plaques) ↑ Cx regions (astrocytes)	
Neuropeptide Y	AD patient	↓ Cx ↑ SI =/↓ CSF	↓ TCx ↓ Hpc
	PDAPP mice	↑ Cx, hpc	
	APP23 mice	↑ Cx, hpc	

Continued

Table 27.1 Changes in neuropeptides and neuropeptide receptor levels in the brain and CSF of Alzheimer's disease patients and models of the disease.—cont'd

	AD patient or models of the disease	NP concentration (brain area)	Receptor density (brain area)
	PS1/APP mice	↓ Hpc	
Corticotropin releasing factor	AD patient	↓ Cx ↓ CSF	↑ Cx areas
Somatostatin	AD patient	↓ Cx	↓ Total (FrCx) ↓ SST2,4 and 5 (Cx) ↑ SST3 (Cx) ↓ SST1 (FrCx)
	APP/PS1 mice Thy-Tau22 mice	↓ Hpc	↓ SST3 (EPLA)
Cholecystokinin	AD patient PDAPP mice APP23 mice	↓ Cx ↑ Hpc ↑ Hpc	

Amyg, amygdala; *B*, basal nucleus; *BF*, basal forebrain; *CP*, caudate and putamen; *CSF*, cerebrospinal fluid; *Cx*, cerebral cortex; *DG*, dentate gyrus; *Ent*, entorhinal cortex; *EPLA*, external plexiform layer of the accessory olfactory bulb; *FrCx*, frontal cortex; *Hpc*, hippocampus; *Hyp*, hypothalamus; *TCx*, temporal cortex.

Galanin

Galanin (Gal) is a neuropeptide composed of 29 amino acids (30 in humans) and is widely distributed in the CNS with neurotrophic and neuroprotective actions, in addition to its role in attention, memory, and learning. The galaninergic system seems to be intimately related to the modulation of the cholinergic pathway that innervates cortical and hippocampal areas from the basal forebrain. In AD, the galaninergic system has been described as hypertrophied in brain areas involved in cognitive functions, e.g., around the basal forebrain cholinergic neurons (BFCNs) of AD patients. Gal levels also increased in the cortex of AD patients. Gal receptor density increased in the hippocampus, hypothalamus, amygdala, and cortical internal layers from postmortem samples of patients with advanced AD (Counts, Perez, Ginsberg, & Mufson, 2010; Rodríguez-Puertas, Nilsson, Pascual, Pazos, & Hökfelt, 1997). The functional relationship between Gal and the cholinergic systems has been widely analyzed. Gal modulates spatial learning and memory in behavioral tests and inhibits ACh release in the ventral hippocampus and cerebral cortex (Fisone et al., 1987). In contrast, Gal increases ACh release in the hippocampus when administered locally in the diagonal band area (Elvander et al., 2004). The total number of cholinergic neurons decreased in Gal KO mice, indicating a role of Gal in cholinergic system consolidation (O'Meara et al., 2000). Different transgenic murine models of AD have shown increased Gal receptor activity in the hypothalamus or in Gal levels (Manuel, Lombardero, LaFerla, Giménez-Llort, & Rodríguez-Puertas, 2016; Mufson, Counts, Perez, & Binder, 2005).

Opioid peptides

In addition to the known role of the opioid system in the control of pain perception, some dynorphins play an important role in learning and memory control. The opioid system mediates its effects through four kinds of opioid receptors: δ, κ, μ, and nociceptive receptors. All of them are coupled to G proteins and are activated by different endogenous peptides such as enkephalins, endorphins, dynorphins, and hemorphin. Opioid receptors are broadly distributed both in the CNS, preferentially located in limbic areas, and in the peripheral nervous system. In general terms, the CNS of AD patients shows high concentrations of total opioids (Cai & Ratka, 2012). The endogenous opioids exert their function in AD presumably by regulating other neurotransmitters, such as ACh or norepinephrine release, or by disrupting GABAergic and glutamatergic neurotransmission. Opioid receptors are differently regulated in AD depending on the area and type of receptor analyzed. Thus, opioid receptors are upregulated in the amygdala, hippocampus, and frontal cortex of AD patients; some authors have described an increase in the putamen (and caudate), probably by accounting for the increase in κ receptor binding sites, while other authors reported a decrease in the density for both κ and δ receptor subtypes in the putamen (Barg et al., 1993; Hiller, Itzhak, & Simon, 1986). Moreover, other studies showed a lower density of μ and δ opioid receptors in the dentate gyrus of the hippocampus and entorhinal cortex (Jansen, Faull, Dragunow, & Synek, 1990). In the rat BFCN-lesion model of AD, an increase in δ receptor density in the cortex and in κ receptor density in the cortex and hippocampus were reported. However, μ receptor density decreased in the same brain areas (Ofri, Fan, Simon, & Hiller, 1992).

Substance P

Substance P (SP) is an 11 amino acid neuropeptide belonging to the tachykinin family. Three subtypes of neurokinin receptors (NK1, NK2, and NK3) mediate the biological actions of SP. The most abundant subtype in the CNS is NK1, which is present in the hippocampus, hypothalamus, and amygdala. Although there are many studies about SP levels in AD, the data is contradictory; a general decrease in SP levels in the cortex has been described, but this is not in agreement with the CSF levels (Peineau, Rabiant, Pierrefiche, & Potier, 2018). Immunoreactivity assays showed a marked loss for both SP and NK receptors in AD (Kowall et al., 1993). Regarding memory processes, SP seems to have an important role in learning, following central or systemic administration. In transgenic mouse models of AD, such as mice overexpressing the human amyloid precursor protein (hAPP), SP has been found in amyloid plaques (Willis et al., 2007).

Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid peptide broadly distributed in the striatal and limbic brain areas but is also present in the hippocampus and cerebral cortex. NPY is

involved in the control of multiple physiological processes and in a wide range of mental illnesses including anxiety, depression, and nociception. NPY receptors are GPCRs and five of them have already been cloned (Y1, Y2, Y4, Y5, and Y6). The expression of NPY seems to be reduced in the cortex and increased in the substantia innominata in AD patients, but there is not a consensus regarding the levels of NPY in CSF, which show disparate results. Regarding the density of receptors in AD, autoradiographic studies showed a reduction in the binding sites of [^{3}H]NPY in the temporal cortex and hippocampus, but animal models of the disease also showed an increase in the NPY levels in the hippocampal area. NPY administration alleviates learning and memory impairments in the PS1xAPP mouse model (Duarte-Neves, Pereira de Almeida, & Cavadas, 2016).

Corticotropin-releasing factor

Corticotropin-releasing factor (CRF) is a neuropeptide composed of 41 amino acids. It is the main regulatory element of the stress response in the hypothalamic-pituitary-adrenal axis regulating anxious behavior and enhancing alertness. Two subtypes of CRF receptors have been described: CRF1 and CRF2. At the central level, CRF is found in areas directly related to the stress response but also in cognitive processes. Alterations in the CRF system have been described in AD, from reductions in CRF immunoreactivity in the cortex and CSF of AD patients to increase in CRF receptor densities in cortical areas. Indeed, the decrease of CRF levels in the CSF correlates with the severity of the cognitive impairment. Stress-mediated activation of CRF1 receptors is able to improve cognition in the AβPP/PS1 mouse model of genetic AD (Scullion, Hewitt, & Pardon, 2013). However, in the PSAPP transgenic mice model, antagonism of CRF receptors seems to mitigate cognitive deficits (Zhang et al., 2016).

Somatostatin

Somatostatin (SST) is a peptide with two bioactive forms, SST-14 and SST-28. In the brain, it is preferably located in the hypothalamus, preoptic area, and spinal cord. Five subtypes of SST receptors have been identified—SST1 to SST5—and all of them are GPCR. SST's main functions are to inhibit the secretion of hormones in the pituitary gland; however, SST also mediates cognitive processes in the hippocampus and cortex. In AD patients, the cortical SST deficits correlate with a decrease in ACh synthesis (Jiménez, Astarloa, & Morales, 1991). In this sense, APP/PS1 mice expressing Aβ show impairment in learning and memory related to the dysfunction of SST-expressing interneurons in the hippocampus, which in turn receives cholinergic inputs (Schmid et al., 2016). SST is not only a reduced neuropeptide in AD but SST receptors are also downregulated, mainly for the SST2, SST4, and SST5 subtypes. SST3 receptors are increased in the cortex of AD patients. Studies using the nonsubtype selective radioligand [^{125}I]Tyr11SRIF14 have described a significant reduction of binding sites in the frontal and

temporal cortices of patients with AD, whereas SST1 subtype is specifically vulnerable in the frontal cortex (Burgos-Ramos et al., 2008). Concerning SST receptors in AD models in THY-Tau22 mice, a model for tau aggregation, only a slight decrease in SST3 was detected in the external plexiform layer of the olfactory bulb (Martel et al., 2015).

Cholecystokinin

Cholecystokinin (CCK) is a neuropeptide made of 33 amino acids; however, multiple isoforms exist, with the octapeptide being the most abundant in the CNS. The main identified function of CCK in the CNS is the modulation of anxiety, learning, and memory. Both receptors for CCK (CCKA and CCKB) are GPCR. CCK has been found to be decreased in the cerebral cortex of AD patients, where receptor densities were unmodified (Löfberg, Harro, Gottfries, & Oreland, 1996; Mazurek, & Beal, 1991). In the basal forebrain lesion model of AD in rats, the activation of CCK receptors via administration of CCK8 prevented the degeneration of cortical cholinergic neurons (Sugaya, Takahashi, & Kubota, 1992). In transgenic models of the disease, such as the PDAPP and APP23 models, an increase in immunoreactivity for CCK in certain areas of the hippocampus has been described (Diez et al., 2003).

Table 27.1 summarizes the main modifications of neuropeptides in AD. In addition to neuropeptide systems, other signaling and neuromodulatory systems have been more recently identified, including neurolipids such as the endocannabinoids.

Neurolipids

The NLs are particularly abundant and widely distributed in the CNS, further supporting their broad role in the modulation of CNS neurotransmission and control of neuronal network activity. NLs are synthesized through the cleavage of specific membrane phospholipid precursors involving several pre- and postsynaptic enzymatic processes and can diffuse within the membrane or be released to act as autocrine or paracrine mediators, respectively (Figs. 27.1 and 27.2). Lipidomic studies contribute to our understanding of these complex signaling systems. The recent development of the matrix-assisted laser desorption ionization-imaging mass spectrometry (MALDI-IMS) technique has contributed to the identification of the anatomical distribution of specific lipid species (Fig. 27.3). Some examples of NL receptors are those activated by arachidonic acid derivatives, e.g., cannabinoid receptors (CB_1/CB_2), but also lysophosphatidic acid receptors (LPA_1-LPA_6) and sphingosine-1-phosphate receptors ($S1P_1$—$S1P_5$) (Fig. 27.4). The transient receptor potential channel TRPV1 and some orphaned GPCR are also recognized by NLs (e.g., GPR18, GPR55, and GPR119), and some of them could have a relevant role in AD. The physiology mediated by NL is becoming particularly relevant in neurodegenerative diseases, because these molecules are able to integrate structural, metabolic, and signaling functions.

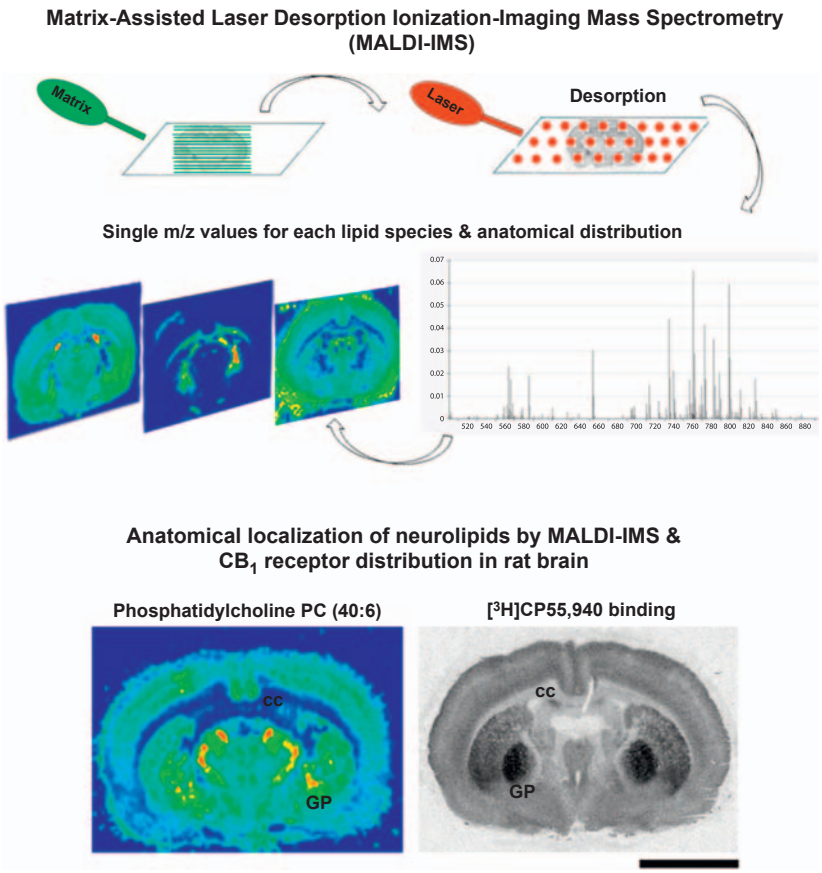

Figure 27.3 ***MALDI-IMS schematic diagram.*** Matrix-assisted laser desorption ionization-imaging mass spectrometry (MALDI-IMS) allows for the anatomical localization of lipids, revealing the distribution of phospholipid precursors and neurolipids (bottom left). The simultaneous use of radioligand-binding autoradiography (bottom right) allows for the comparison of the receptor distribution for a specific neurolipid. Phosphatidylcholine PC (40:6) shows a similar pattern of abundance and distribution (left) to the CB_1 receptor (right) and could be a plausible source for the synthesis of endocannabinoids. *cc*, corpus callosum; *GP*, globus pallidus. Scale bar = 0.5 mm.

Cannabinoids

The synthesis of the eCB (Fig. 27.1), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), is on demand, and neuronal damage triggers eCB release to induce presumable protective effects. The CB_1 cannabinoid receptors (see the distribution of CB_1 receptors in Figs. 27.3 and 27.4) have been analyzed in excitotoxicity models (Kim, Won, Mao, Jin, & Greenberg, 2006; Stella, Schweitzer, & Piomelli, 1997). Excitotoxicity contributes to the neurodegenerative processes and memory impairment described in AD (Sonkusare, Kaul, & Ramarao, 2005), and the CB_1 receptor-mediated modulation of glutamatergic

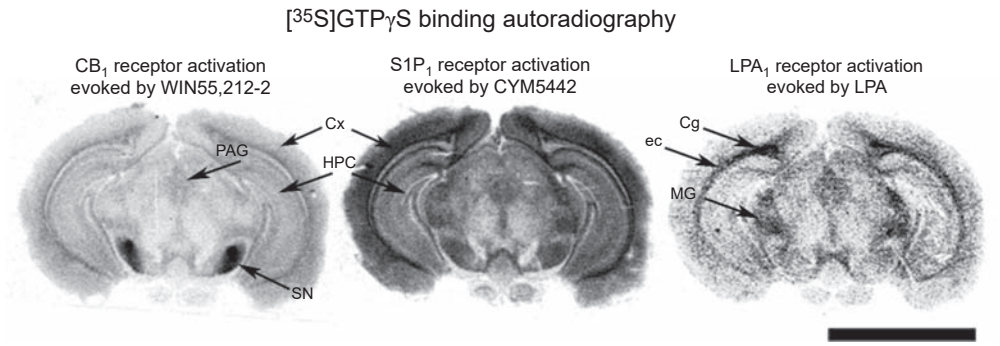

Figure 27.4 ***Neurolipid receptor-mediated activity in the mouse brain.*** Autoradiographic distribution of the functional coupling of cannabinoid receptor subtype 1 (CB_1), sphingosine-1-phosphate receptors subtype 1 ($S1P_1$), and lysophosphatidic acid receptors subtype 1 (LPA_1) in consecutive mouse brain sections obtained in the coronal plate (−3.16 mm from Bregma). Note the differences in the abundance of CB_1 and $S1P_1$ receptors distributed in gray matter, and LPA_1 receptors, distributed in white matter. *Cg*, cingulum; *Cx*, cortex; *ec*, external capsule; *HPC*, hippocampus; *MG*, medial geniculate nucleus; *PAG*, periaqueductal gray; *SN*, substantia nigra. Scale bar = 0.5 mm (Unpublished results from the authors).

neurotransmission may confer neuroprotective effects. The antagonist of NMDA receptors, memantine, is used for the treatment of moderate to severe AD, and the synthetic cannabinoid agonist, HU-210, also inhibits NMDA receptors (Nadler, Mechoulam, & Sokolovsky, 1993). Therefore, CB_1 receptor activation may represent a therapeutic approach to attenuate the excitatory/inhibitory imbalance described in AD. Changes in CB_1 receptor-mediated signaling have been described in the 3xTg-AD triple transgenic mouse model of AD (Llorente-Ovejero et al., 2018; Manuel et al., 2016). Chronic administration of low doses of Δ^9-tetrahydrocannabinol (Δ^9-THC) in old rodents improved learning and memory by enhancing the expression of synaptic proteins (Bilkei-Gorzo et al., 2017).

CB_1 receptors are abundantly expressed in the human CNS in areas related to learning and memory processes. Westlake, Howlett, Bonner, Matsuda, & Herkenham (1994), observed that the binding of the CB_1/CB_2 radioligand, [^{3}H]CP55,940, was reduced in the cortex of AD patients, but other research groups found similar cortical and hippocampal CB_1 receptor levels (Mulder et al., 2011). Additional studies showed decreased levels of signaling mediated by CB_1 in cortical areas (Basavarajappa, Shivakumar, Joshi, & Subbanna, 2017). The first quantitative in vivo PET imaging study of CB_1 receptor density using [^{18}F]MK-9470 showed no differences when compared to healthy volunteers (Ahmad et al., 2014). Further studies were focused on the observation of dynamic changes during the progression of AD according to the Braak stages. Autoradiographic studies using [^{125}I]SD7015 revealed increased levels of CB_1 receptor expression in the frontal cortex in the early stages of AD and a decline during the later stages (Farkas et al., 2012). A detailed autoradiographic study showed increased levels of [^{3}H]

CP55,940 binding in the frontal cortex, hippocampus, and caudate and putamen during the middle stages of AD but no changes in the early stages, which demonstrated that CB_1 receptor density inversely correlated with tau accumulation. Moreover, the functionality of CB_1 receptors also revealed a similar modulation of hippocampal CB_1 receptor activity to that observed in [^{3}H]CP55,940 binding and increased cortical CB_1 receptor activity during the initial stages of AD, preceding the increase of CB_1 receptor density (Fig. 27.5) (Manuel, González de San Román, Giralt, Ferrer, & Rodríguez-Puertas, 2014).

In contrast, CB_2 receptors are irrelevant in the CNS during normal physiological conditions, but increased levels have been described in AD patients together with an increased density and activity of FAAH (AEA-degrading enzyme), consistent with decreased levels of AEA, probably related to inflammatory processes due to increased arachidonic acid levels (Basavarajappa et al., 2017; Jung et al., 2012). There are also inconsistent results related to the regulation of CB_2 receptors in AD, but a low CB_2 receptor density was identified following the preclinical evaluation by PET neuroimaging in AD patients (Ahmad et al., 2016).

Clinical data demonstrated modest beneficial effects of nabilone or dronabinol (analog of Δ^9-THC) on some of the behavioral symptoms of AD. A clinical trial including 15 patients with a diagnosis of probable AD resulted in weight gain and a

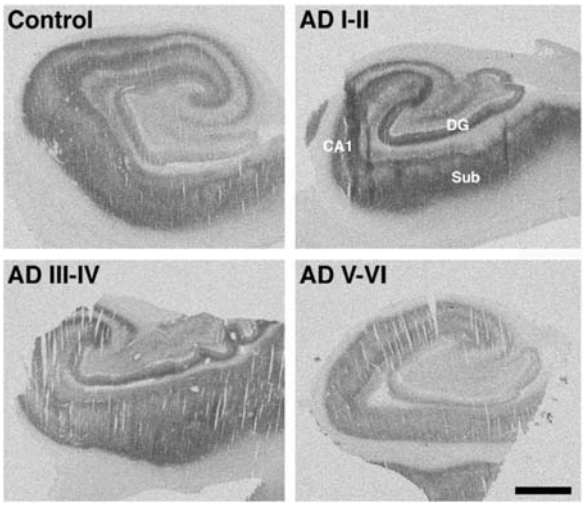

Figure 27.5 ***Cannabinoid receptor activity during AD progression.*** Density of WIN55.212-2–stimulated [^{35}S]GTPγS binding corresponding to representative slices of human brain hippocampus from representative control and Alzheimer's disease (AD) patients at different Braak's stages: AD I-II, AD III-IV, and AD V-VI (D). The functionality of cannabinoid receptors increased during the initial stages and decreased during the more advanced stages. Scale bar, 2.5 mm. *(Reprinted from Manuel, I., González de San Román, E., Giralt, M. T., Ferrer, I., & Rodríguez-Puertas, R. (2014). Type-1 cannabinoid receptor activity during Alzheimer's disease progression.* Journal of Alzheimer's Disease, 42*(3), 761–766, Copyright (2014), with permission from IOS Press." The publication is available at IOS Press through https://doi.org/10.3233/JAD-140492.)*

decrease in the severity of disturbed behavior after 6 weeks of dronabinol treatment. Another clinical trial involving eight patients with dementia reported reduced nighttime agitation and behavioral disturbances. Clinical trials with Δ^9-THC in patients with dementia revealed beneficial effects and the absence of side effects when higher doses were orally administered for longer periods (van den Elsen et al., 2015, 2017). However, there is no evidence of cannabinoid-based improvement in dementia. Clinical trials have evaluated the safety and the efficacy of nabilone and dronabinol on agitation and aggression in AD (https://clinicaltrials.gov/).

Sphingosine-1-phosphate receptors in AD

Sphingosine-1-phosphate (S1P) receptors are GPCRs that mediate a wide range of cellular responses and are widely and abundantly distributed in the CNS (Fig. 27.4). Increasing evidence has reported that S1P-signaling system activation improved learning and memory deficits in animal models with cognitive impairment. Low expression of sphingosine kinase-1 (S1P-synthesizing enzyme), together with enhanced expression of S1P lyase (S1P-degrading enzyme) lead to decreased S1P levels, which are more pronounced in aging females (Ceccom et al., 2014; Couttas et al., 2014, 2018). Particularly, the S1P receptor subtype 1 ($S1P_1$) has been defined as a crucial modulator of immune cell migration and hemostasis (Matloubian et al., 2004). Fingolimod (Gilenya®), a nonselective $S1P_1$ agonist that was approved for the treatment of multiple sclerosis, was able to decrease the release of Aβ40/Aβ42 in neurons expressing hAPP (Takasugi et al., 2013). Furthermore, the administration of fingolimod or SEW2871 (specific $S1P_1$ agonist) to a mouse model overexpressing Aβ alleviated memory impairment by attenuating neuroinflammation (Asle-Rousta, Kolahdooz, Dargahi, Ahmadiani, & Nasoohi, 2014; Aytan et al., 2016). The modulation of the S1P signaling system will help to evaluate its immunomodulatory and cerebral homeostatic properties, contributing to an increased therapeutic repertoire for AD treatment.

Lysophosphatidic acid receptors in AD

Little is currently known about the role of lysophosphatidic acid (LPA) receptor-mediated signaling in neurodegenerative diseases despite its involvement in crucial biological mechanisms, e.g., cell proliferation or neuronal processes sprouting. LPA's synthesis pathways and distribution in the CNS are being identified (Figs. 27.1 and 27.4). The autotaxin enzyme that drives the synthesis of LPA, together with LPA levels, are enhanced in AD (Zhang et al., 2017). In experimental models of AD, LPA leads to increased production of Aβ through PKC-mediated upregulation of β-secretase expression (Shi, Dong, Cui, & Xu, 2013). However, other authors demonstrated that the administration of gintonin (LPA-receptor-activating ligand) to transgenic AD mice reduced amyloid plaque deposition, increased hippocampal neurogenesis, and alleviated

memory impairment (Hwang et al., 2013). Therefore, further research on the role of LPA-mediated signaling is needed to evaluate the appropriate use of LPA-related compounds in AD.

Orphan receptors

Different NLs, including free fatty acids, are being identified as endogenous neurotransmitters for orphan receptors. GPR3, GPR6, or GPR17 are orphan GPCRs present in brain areas related to the pathogenesis of AD and participate in the APP processing. In addition, GPR55 and GPR159 modulate learning and memory processes. Deorphaning these receptors and their role in neurological disorders will contribute to the identification of novel therapeutic strategies.

Key facts of neuropeptides

- Neuropeptides are polypeptide chains that can function in the central nervous system as hormones, neurotransmitters, or as molecules with neuromodulatory properties.
- Neuropeptides coexist with classical neurotransmitters and use the same machinery for signal transduction, but both their synthesis and release differ from small classical neurotransmitters.

Key facts of neurolipids

- Neurolipids are endogenous lipid-based signaling molecules derived from membrane precursors synthesized on demand, which fine-tune neurochemical and behavioral processes.
- The enzymatic machinery for their biosynthesis and degradation and their receptors constitute a complex lipid-signaling network.

Key facts of neuropeptides and neurolipids in Alzheimer's disease

- Neuropeptides and neurolipids govern "classical" neurotransmission from a higher level of complexity and dysregulated signaling mediated by these bioactive compounds, which has been described in Alzheimer's disease.

Summary points

- Neuropeptide- and neurolipid-mediated signaling governs several neurochemical processes and behavioral outcomes, including neuromodulation, neuroinflammation, glial proliferation, learning, and memory.

- Some neuropeptides, such as galanin, corticotropin-releasing factor, somatostatin, and opioid peptides, are modified in AD and are intimately related with some hallmarks of the disease.
- The described alterations of neuropeptides in AD seem to go in different directions because of their different and heterogeneous actions and interactions on other neurotransmitters.
- The endocannabinoid and sphingosine-1-phosphate signaling systems represent promising pharmacological targets for alleviating the cognitive symptoms of AD.
- Drugs targeting neurolipid systems approved for other neurodegenerative diseases may contribute to the development of new therapies for AD.

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CHAPTER 28

Neurotransmitter receptors in Alzheimer's disease: from glutamatergic to cholinergic receptors

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List of abbreviations

AChE acetylcholinesterase
AD Alzheimer's disease
AMPA α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
Aβ amyloid-beta
BFCN basal forebrain cholinergic neurons
cAMP cyclic adenosine monophosphate
ChAT choline acetyltransferase
CNS central nervous system
GABA gamma-aminobutyric acid
GPCR G protein-coupled receptor
HACU high-affinity choline uptake or transporter
KA kainate
LTD long-term depression
LTP long-term potentiation
mAChR muscarinic receptors
nAChR ligand-gated ion nicotinic receptors
nbM nucleus basalis of Meynert
NMDA N-methyl-D-aspartate
PrPc prion proteins
ROS reactive oxygen species

Mini-dictionary of terms

Allosteric modulator A chemical compound or biomolecule that is able to specifically modify the activity of a receptor by interacting with a receptor site other than the active site, usually potentiating the agonist action of the molecules with an affinity for that active site.

Autoradiography Radioligand binding technique used for tissue slices to obtain microscopic anatomical information of the densities of binding sites for drugs.

Autoreceptors The receptors for a given neurotransmitter that are localized at the same cell (neuron) responsible for the synthesis of its endogenous neurotransmitter, usually controlling or inhibiting the signal transmission.

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Basal forebrain A rostrobasal area that includes one of the main groups of cholinergic cells in the brain, including the nucleus basalis of Meynert that innervates cortical and subcortical areas in the main pathway for control of learning and memory in mammals. This area is specifically damaged in Alzheimer's disease patients and is thought to be responsible for the clinical symptoms of dementia.

Catecholamines In a neuroscience context, this refers to neurotransmitters that share a chemical structure including a catechol group together with an amine that are synthesized from tyrosine. This includes noradrenaline, serotonin, and dopamine.

High-affinity choline transporter A transmembrane protein of presynaptic cholinergic localization responsible for the uptake of choline, constituting the rate-limiting step in the synthesis of acetylcholine.

Ionotropic receptors The receptors for neurotransmitters consisting of transmembrane proteins with a molecular structure of different subunits forming a channel permeable for ions, which is different from the active site.

Metabotropic or G protein-coupled receptors Seven-transmembrane domain proteins with high affinity for specific endogenous ligands. They initiate the intracellular cascade of signaling mediated by activation of heterotrimeric G proteins.

Organotypic cultures Parts of an organ or a whole organ from complex tissue in which different types of cells interact and grow under controlled conditions.

Radioligand A chemical compound or drug labeled with a radioisotope (e.g., tritium) that is commonly used in pharmacodynamic experiments to calculate the density of binding sites and other pharmacological parameters such as affinity.

Introduction

The cholinergic system, including the different metabotropic muscarinic and ionotropic nicotinic receptors, constitutes the most vulnerable neurotransmitter system in Alzheimer's disease (AD), specifically at the initial stages of the disease and in the pathways responsible for learning and memory processes, which arise from the basal forebrain innervation of the subcortical and cortical brain areas (Davies & Maloney, 1976; Lang & Henke, 1983; Probst, Cortés, Ulrich, & Palacios, 1988). However, other systems and their receptors are also modified during the advance of the disease. The implicated receptors range from those controlling neuronal excitation or inhibition that in turn are regulated by the receptors for monoamines, to neuromodulation by neuropeptides and neurolipids, the latter being described in a subsequent chapter.

Glutamatergic and GABAergic receptors

The effects of glutamate are mediated by ionotropic (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate [AMPA], N-methyl-D-aspartate [NMDA], kainate [KA]) or metabotropic (mGlu1-8) receptors. Amyloid-beta (Aβ) has been shown to disrupt the glutamate uptake capacity and calcium signaling by astrocytes, thus modifying the cooperative relationship with neurons in the modulation of cognitive functions and contributing to excitotoxicity in AD (Acosta, Anderson, & Anderson, 2017). Synaptic plasticity is very sensitive to Aβ, which increases extracellular glutamate concentration in hippocampal slices and inhibits long-term potentiation (LTP)

(Li et al., 2009). Both micromolar Aβ oligomers and glutamate can cause rapid phosphorylation of tau in dendrites and local depletion of mitochondria and subsequently induce the loss of spines in cultured hippocampal neurons (Zempel, Thies, Mandelkow, & Mandelkow, 2010). Keeping in mind that there is an early loss of glutamatergic neurons in cholinergic pathways in AD, such as in the hippocampus, and that there is a disruption of synaptic transmission before the loss of synapses, the role of the glutamatergic receptors in the factors that contribute to neurodegeneration in AD has been thoroughly studied.

The AMPA receptor is responsible for most of the excitatory transmission in the brain, mediating synaptic plasticity. AMPA receptor trafficking is challenged in the aging brain, and its deregulation can be linked to neurological disorders such as AD (Jurado, 2018). Aβ oligomers increase extracellular glutamate concentration in hippocampal slices and inhibit LTP (Li et al., 2009). Both micromolar Aβ oligomers and glutamate can also cause rapid phosphorylation of tau in dendrites, inducing loss of spines in cultured hippocampal neurons (Zempel et al., 2010). AMPA receptor-mediated desensitization in the presence of the allosteric modulator cyclothiazide prevents the Aβ oligomer-induced reduction of postsynaptic current, thus inhibiting glutamate uptake (Alberdi et al., 2010). The loss of hippocampal dendritic spines in the transgenic mouse model, Tg2576-APP, is related to the decrease of AMPA receptor-mediated synaptic transmission and results in memory impairments caused by Aβ oligomers via caspase-3 activity (D'Amelio et al., 2011; Cavallucci et al., 2013).

In patients with AD, a reduction in AMPA receptors in hippocampal areas has been demonstrated by autoradiography. Indeed, accumulation of hyperphosphorylated tau in dendritic spines has been implicated in the disruption of AMPA (and NMDA) receptor trafficking (Hoover et al., 2010).

The NMDA receptor has long been implicated in excitotoxicity leading to neurodegeneration in the later stages of AD. NMDA receptor-mediated transmission is modulated by an increase in extracellular glutamate levels mediated by Aβ oligomers (Li et al., 2009). Choi (1995), suggesting that the activation of NMDA receptors could be one of the major causes of neuronal death in AD. Organotypic culture studies of cholinergic areas, including the entorhinal cortex and hippocampus, have shown that Aβ oligomers are responsible for the dysregulation of Ca^{+2} influx through NMDA receptors. These changes provoke mitochondrial depolarization and reactive oxygen species (ROS) generation. Antagonists of NMDA receptors reduce deleterious events in mitochondria, demonstrating that they are mediators of mitochondrial dysfunction induced by Aβ oligomers (Alberdi et al., 2010).

NMDA receptor-dependent LTP in the hippocampus has been widely described by electrophysiology studies in different AD models. The induction of LTD in the CA1 region of the hippocampus requires activation of NMDA receptors (Malenka & Bear, 2004). LTP is impaired in AD and is dependent on NMDA receptors, suggesting that the antagonist memantine could protect neurons from degeneration, thus

maintaining learning and memory capacity. Memantine and the inhibitors of AChE, donepezil, galantamine, and rivastigmine have been approved for the treatment of AD.

The group I of glutamate metabotropic receptors, mGluR (mGluR1/R5), are also implicated in LTD. Aβ-enhanced LTD depends on mGluR1/R5 and involves p38 MAPK and caspase-3, but not NMDA receptor activity (Chen et al., 2013). The mGluR5 that is widely distributed in the cortex and hippocampus could have a relevant role in the cognitive impairment associated with AD. Interestingly, mGluR5 has been postulated to be a co-receptor for prion proteins (PrPc) and Aβ oligomers. Its antagonist, MTEP, and the genetic ablation of this receptor are protective in AD mice (Hamilton et al., 2016). The group II and III of mGluR are predominant on the presynaptic terminals of glutamatergic and GABAergic neurons, behaving as glutamate autoreceptors. Activation of group III has been described to be neuroprotective in both *in vitro* and *in vivo* models of AD (Domin et al., 2014). The activation of mGluR7 protects neurons of the basal forebrain by reducing NMDA-currents. Therefore, both the excitotoxicity and damage in the BFCN induced by Aβ toxicity are reduced (Gu et al., 2014).

The two known subtypes of GABAergic receptors are localized in the central nervous system (CNS): $GABA_A$ and $GABA_B$, being the ionotropic $GABA_A$ receptor the most widely studied. AD-related loss of cholinergic and glutamatergic fibers could result in an alteration in the expression of certain $GABA_A$ receptor subunits, but, in general, the vulnerability of GABAergic neurons and their receptors is hardly observed. The majority of studies on the $GABA_A$ receptor in AD have utilized binding techniques with radiolabeled ligands such as [^{3}H]-flumazenil and [^{3}H]-GABA. These studies have demonstrated a moderate reduction of $GABA_A$ in the brains of patients with AD (Chu, Penney, & Young, 1987; Vogt, Crino, & Volicer, 1991).

In contrast, an alternative splicing of $GABA_B$ receptors is upregulated in frontal and temporal cortical samples of AD patients (Massone et al., 2011). This alternative splicing is triggered by inflammatory processes, leading to altered $GABA_B$ signaling and increased secretion of Aβ (Khedr, Ahmed, Darwish, & Ali, 2011). Studies in animal models of AD show increased $GABA_B$-mediated GIRK current and expression in the hippocampus, which may mediate cognitive deficits. The administration of $GABA_B$ antagonists restores normal learning and memory behavior in a transgenic mouse model of Down syndrome (Kleschevnikov et al., 2012) (Fig. 28.1).

Monoamine receptors

The receptors for monoamines, including those for catecholamines such as noradrenergic and dopaminergic receptors, as well as others such as serotonergic receptors, are also affected during the progression of AD.

Noradrenergic receptors

One of the most consistent observations regarding the status of noradrenergic neurotransmission in AD patients is an important loss of noradrenergic neurons at one of the main

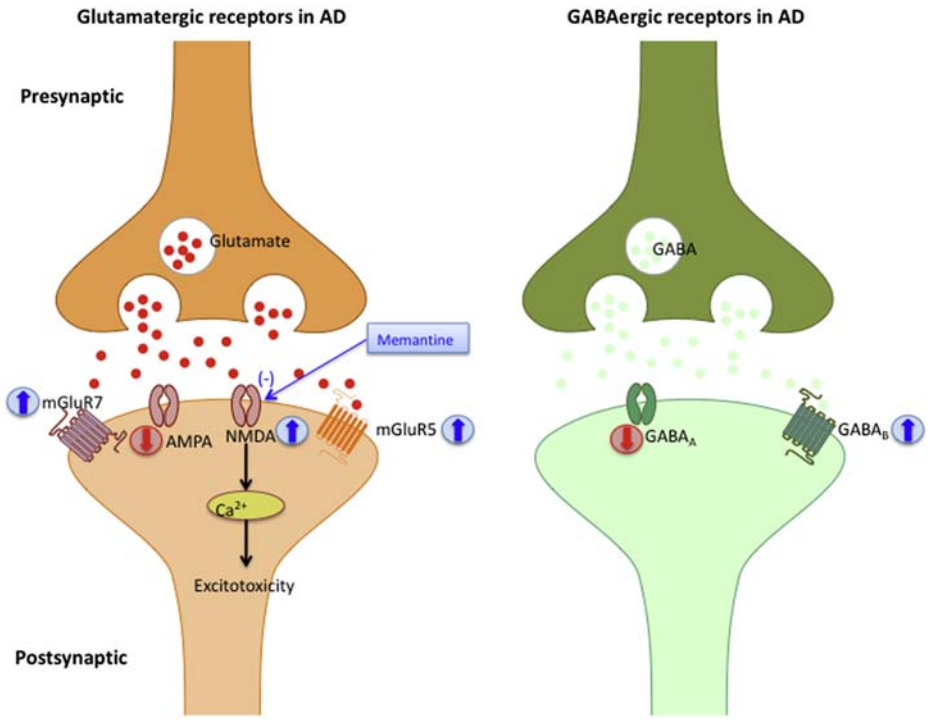

Figure 28.1 ***Glutamatergic and GABAergic receptors in AD.*** The different subtypes of glutamatergic and GABAergic receptors have been analyzed in Alzheimer's disease (AD) patients and/or in experimental models, including *in vitro* studies or animal models. The AMPA ionotropic receptors are downregulated in AD, but the NMDA receptors are overactive, increasing the intracellular Ca^{2+} influx and subsequent excitotoxic processes. Memantine is an FDA-approved drug for the treatment of AD that antagonizes NMDA receptors. The $GABA_A$ ionotropic receptor is downregulated, and the $GABA_B$ metabotropic is upregulated.

noradrenergic nuclei of the brain, the locus coeruleus, which is involved in learning and memory processing, the sleep-wake cycle and synaptic plasticity. This loss probably depends on the degeneration of the cholinergic innervation of the locus coeruleus in AD (Strong et al., 1991). The damage of noradrenergic neurons in the locus coeruleus impairs working memory and hippocampal neurogenesis (Coradazzi et al., 2016).

In the case of the regulation of noradrenergic receptors, genetic polymorphisms that increase the risk of developing AD have been identified for both the alpha and beta subtypes of adrenoceptors. There is high variability in the results reporting modifications in the density of noradrenergic receptors in the brain of AD patients. Consequently, the proposed treatments targeting adrenergic receptors are also very different. Nevertheless, according to the observation that both α_2 and β_2 noradrenergic receptors are increased in the prefrontal cortex and hippocampus of AD patients, the beta-blockers, mainly for the β_2 subtype, have been assayed in brain cell cultures treated with Aβ, transgenic mice models or patients, but with diverse and sometimes opposite outcomes (Kalaria et al., 1989; Yu et al., 2011).

Dopaminergic receptors

The dopaminergic system has also been extensively studied in AD, but often in relation to other neuropsychiatric symptoms concomitant to dementia, such as psychosis or depression. Therefore, selective compounds for the different dopaminergic receptors have been proposed. Agonists for the D_2 subtype have been identified as the preferable candidates to develop dopaminergic drugs acting at dopaminergic receptors with therapeutic potential in AD (Reeves, Mehta, Howard, Grasby, & Brown, 2010).

Serotonergic receptors

Thirteen serotonergic receptor subtypes have been identified. The modulation of different subtypes has been described in AD patients, but the 5-HT_6 and 5-HT_4 are especially singled out for research and development of new treatments for cognitive deficits. The activation of 5-HT_4R, which is present in both CNS and peripheral tissues, improves the central cholinergic neurotransmission and favors the nonamyloidogenic pathway in APP processing. The procognitive properties of 5-HT_4R agonists (e.g., RS67333) have been successfully assayed in transgenic mouse models of AD, and other similar compounds have reached stage II clinical trials but have been discontinued (Giannoni et al., 2013). On the other hand, 5-HT_6R antagonists, such as dimebolin, idalopirdine, intepirdine, or SUVN-502, have reached clinical trials alone or in combination with AChE inhibitors. Latrepirdine (Dimebon), which was initially identified as a histaminergic H_1/H_2 receptor agonist with potential therapeutic properties for AD, has been shown to also share an affinity for 5-HT_6R, but the clinical trials failed (Cano-Cuenca, Solís-García del Pozo, & Jordán, 2014). The 5-HT_3-specific antagonist, tropisetron, has also shown potential therapeutic effects for AD patients (Hashimoto, 2015). Furthermore, 5-HT depletion could be involved in the loss of the melatonin receptor-mediated activity and the consequent alterations in sleep and circadian rhythm observed in AD patients (Wu et al., 2003).

In aged transgenic mouse models of AD with cortical Aβ plaques, $GABA_A$, NMDA, AMPA, kainate, adrenergic, and 5-HT receptor densities are affected at a similar age as cholinergic markers, showing a general effect of amyloid proteins on neurotransmission (Klingner et al., 2003) (Fig. 28.2).

Cholinergic receptors

The loss of basal forebrain cholinergic neurons (BFCNs) in the nucleus basalis of Meynert (nbM, or Ch4 cells) is one of the main hallmarks of AD (Davies & Maloney, 1976). The loss of cortical cholinergic innervation in AD can be attributed to the loss of BFCN present in the nbM (Whitehouse et al., 1982). A significant decrease in presynaptic high-affinity choline transporter was also found in the hippocampus, entorhinal and frontal cortex of AD brains (Rodríguez-Puertas, Pazos, & Pascual, 1994).

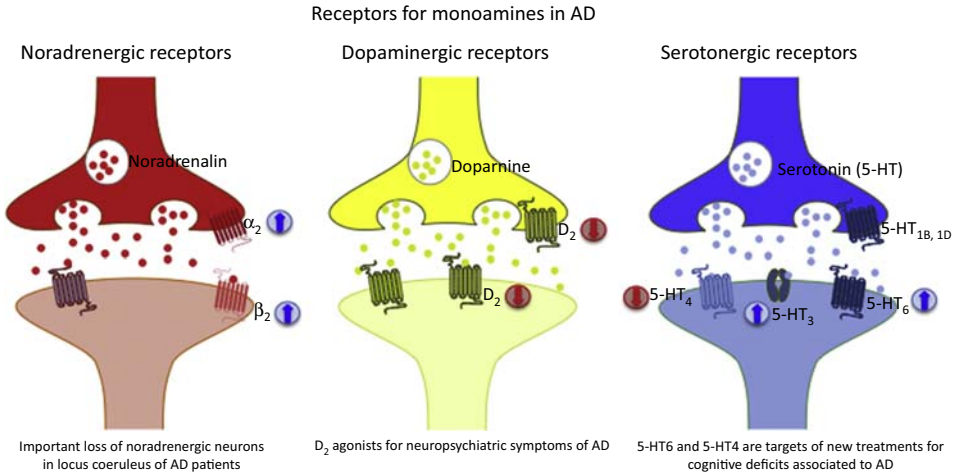

Figure 28.2 ***Receptors for monoamines in AD.*** The regulations of the main receptors for monoamines are illustrated. The autoreceptors α_2 and postsynaptic β_2 for noradrenalin have been found to be increased in cortical areas, probably as a compensation for the locus coeruleus nucleus impairment described in Alzheimer's disease (AD) patients. The most consistent finding for the dopaminergic system in AD is the impairment in the D_2 subtype with both presynaptic (high form) or postsynaptic (low) possible localizations. Therefore, D_2 agonists may reduce some of the concomitant neuropsychiatric symptoms of AD. The procognitive properties of 5-HT_4 serotonergic receptor agonists have reached stage II clinical trials, and 5-HT_6 antagonists have also reached clinical trials alone or in combination with acetylcholinesterase (AChE) inhibitors. The 5-HT_3-specific antagonist, tropisetron, has also shown therapeutic effects in AD patients.

Neuroimaging techniques using PET markers of ChAT and AChE activity, or cortical glucose metabolism, demonstrate a significant reduction of cortical cholinergic function in AD patients (Herholz et al., 2004). These findings support the hypothesis that damage to the integrity of the BFCN at the nbM may underlie the cognitive deficits associated with AD.

BFCN degeneration modulates the levels of ACh, ChAT, and AChE, as well as the cholinergic receptors located in projecting areas. The two classes of cholinergic receptors, the G protein-coupled metabotropic muscarinic receptors (mAChR) and the ligand-gated ion nicotinic receptors (nAChR) are differentially regulated in AD. Most studies have found a decrease of nicotinic receptors in AD. However, binding studies using muscarinic agonists or antagonists and histochemical studies show either decreased levels, unchanged levels, or even increased levels of mAChR in AD (Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016).

Nicotinic acetylcholine receptors in AD

Different studies suggest that brains from AD patients present reduced densities of binding sites for nicotinic ligands such as [^{3}H]ACh, [^{3}H]nicotine, [^{3}H]methylcarbamylcholine,

and [^{125}I]α-bungarotoxin, in comparison with brain samples from age-matched patients. The partial loss of nAChR in AD brains has been confirmed by autoradiographic methods, showing reductions in nicotinic binding (and the distribution of [^{3}H]nicotine, [^{3}H]epibatidine, and [^{3}H]cytosine) throughout the cortical layers of the temporal cortex, the presubiculum and parahippocampal gyrus in AD patients, which usually correlates with a decrease in ChAT activity (Kása, Rakonczay, & Gulya, 1997).

The detection of nicotinic receptor-specific mRNA sequences in brain slices by *in situ* hybridization shows a differential distribution of mRNA for the α_3- and α_4-subunits. However, no alterations in the localization of the α_4-subunit-expressing neurons have been detected in the cortical regions of AD patients (Schröder et al., 1991).

The nAChR subtypes present in the CNS, such as α_7, have been described to be modified in AD patients, and a nicotine transdermal patch is in phase II clinical trials.

Muscarinic acetylcholine receptors in AD

The first studies analyzing the muscarinic acetylcholine receptors in AD described a general decrease in the cortex and hippocampus (Quirion et al., 1989). The M_1, M_3 and M_5 subtypes of mAChR are considered to have a postsynaptic localization, and the M_2 is the presynaptic autoreceptor. M_1 mAChR is usually found preserved in AD patients (Araujo, Lapchak, Robitaille, Gauthier, & Quirion, 1988; Giacobini, 1990), but a dramatic loss of the M_2 mAChR subtype has been observed in the cortex and hippocampus, which may be explained by the loss of BFCN during the disease (Nordberg, Alafuzoff, & Winblad, 1992; Reinikainen, Soininen & Riekkinen, 1990; Rodríguez-Puertas, Pascual, Vilaró, & Pazos, 1997). Cortical M_4 mAChRs have been found to be preserved in AD patients (Rodríguez-Puertas et al., 1997; Fig. 28.3).

The first studies measuring activated receptors analyzed radioligands bound in the presence of guanine nucleotides to describe the loss of high-affinity agonist binding to M_1 mAChR in the frontal cortex of patients with AD (Flynn, Weinstein & Mash, 1991). The signaling of mAChR through Gαq/11 proteins activating PLCβ coupling was impaired in AD (Jope, Song, & Powers, 1997). [^{35}S]GTPγS binding to G proteins (Fig. 28.4) combined with immunoblot analysis of G protein subunits also revealed that the receptor-mediated activation of G proteins is reduced in the brain cortex of AD patients (Wang & Friedman, 1994).

The M_2 mAChR subtype plays a crucial role in cognitive processes as demonstrated in knockout mice (Tzavara et al., 2003). Hippocampal muscarinic activation, through the high-affinity M_2 mAChR subtype, induces muscarinic long-term potentiation as a mechanism of neuronal plasticity (Segal & Auerbach, 1997).

Finally, a relationship between cholinergic muscarinic activation and amyloid precursor protein processing has been demonstrated (Nitsch, Slack, Wurtman, & Growdon, 1992). The activation of PKC and MAPK signal transduction pathways diminishes the

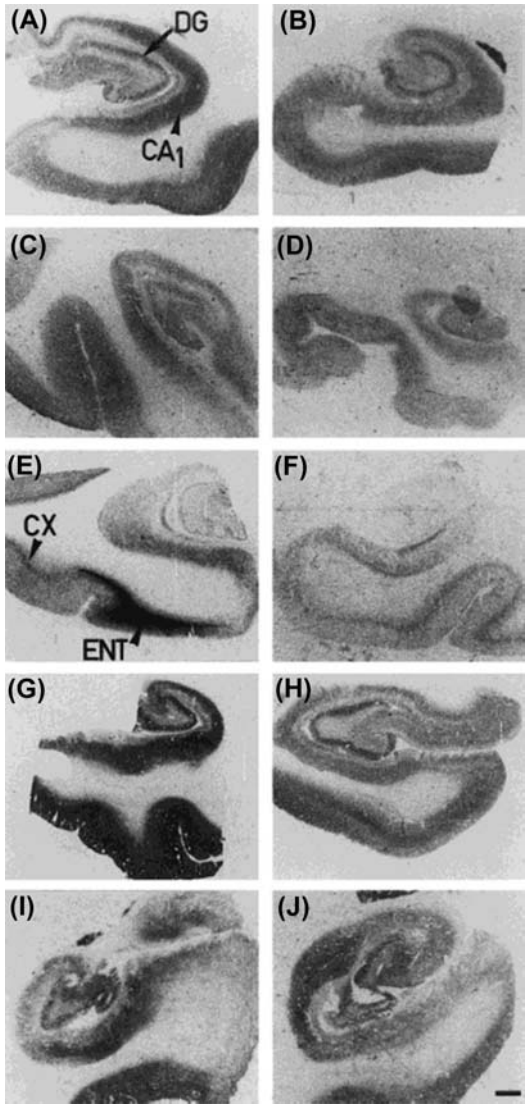

Figure 28.3 ***Autoradiography of muscarinic receptor subtypes in the hippocampus of controls (A, C, E, G, I) compared to AD patients (B, D, F, H, J).*** All five subtypes of muscarinic receptors (mAChR) labeled with [^{3}H]NMS (A,B), M_1 (C,D), M_2 (E,F), M_3 (G,H), and M_4 (I,J). Note moderate reductions in the densities of total MR, M_1, and M_3 in Alzheimer's disease (AD) brains. The M_2 were markedly reduced, but M_4 were preserved. Bar = 2 mm. *CA1*, pyramidal layer of the CA1 field at the hippocampus; *CX*, temporal cortex; *DG*, dentate gyrus; *ENT*, entorhinal cortex. *(Reprinted from Rodríguez-Puertas, R., Pascual, J., Vilaró, T., & Pazos, A. (1997). Autoradiographic distribution of M1, M2, M3, and M4 muscarinic receptor subtypes in Alzheimer's disease.* Synapse, *26(4), 341–350, Copyright (1997), with permission from IOS Press. The publication is available at Wiley Online Library through https://doi.org/10.1002/(SICI)1098-2396(199708)26:4<341::AID-SYN2>3.0.CO;2-6.)*

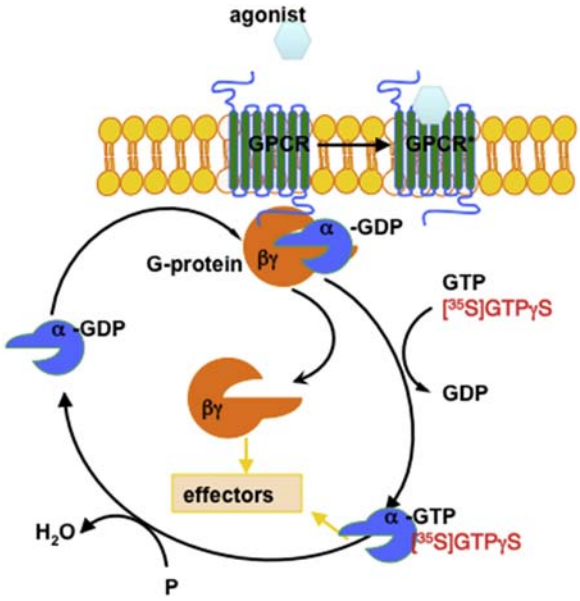

Figure 28.4 ***[^{35}S]GTPγS binding to G proteins.*** The activity mediated by GPCR can be analyzed by the quantification of the binding of the [^{35}S]GTPγS in the presence of specific agonists that induce a conformational change in the receptor to the activated molecular form (GPCR*). Then, the heterotrimeric G-protein interchanges the GDP for GTP (or [^{35}S]GTPγS in the in vitro assay) and dissociates to a βγ dimer and the α-subunit linked to GTP, both of which are able to modify the activity of different signaling effectors modifying intracellular second messengers such as AMPc. Then, the GTPase activity dissociates the GTP from the α-subunit and the G-protein heterotrimer is recycled in the GDP-linked inactive conformation.

generation of Aβ, which in turn induces the uncoupling of M_1 mAChR from G proteins (Kelly et al., 1996). This uncoupling of M_1 mAChR in the neocortex has been associated with dementia severity and reductions in PKC activity and NMDA receptor density, contributing to postsynaptic cholinergic dysfunction in AD (Tsang et al., 2007).

M_1 mAChR agonist and positive allosteric modulators (e.g., TAK-071) are in clinical trials alone or in combination with AChE inhibitors. The AChE inhibitors, donepezil, rivastigmine, and galantamine, have been approved for the treatment of AD to increase the cholinergic signaling.

Fig. 28.5 illustrates cholinergic synapse regulation in AD, and Table 28.1 summarizes the major findings in the regulation of neurotransmitter receptors in the AD.

Key facts about glutamate in Alzheimer's disease

- Amyloid-β disrupts glutamate uptake capacity and calcium signals, modulating cognitive functions and contributing to excitotoxicity in Alzheimer's disease.

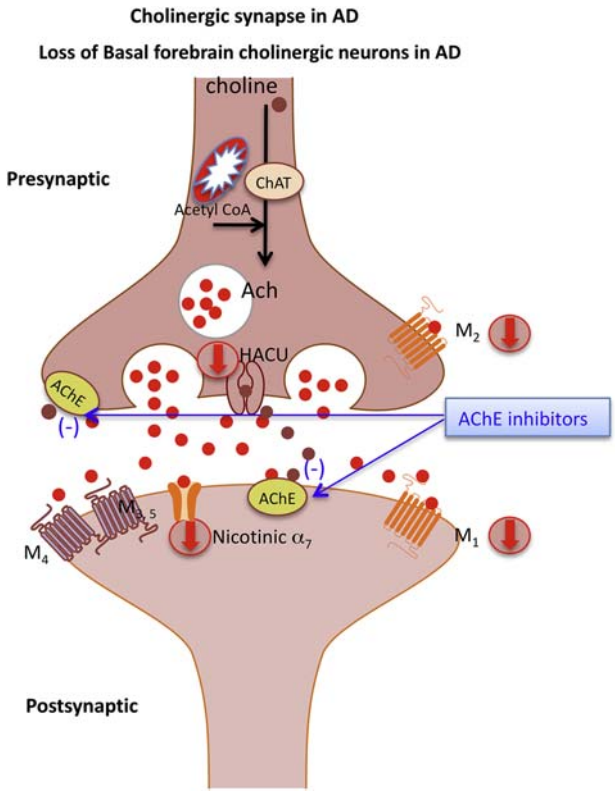

Figure 28.5 ***Cholinergic synapse in AD.*** The basal forebrain cholinergic neurons are specifically vulnerable during the progression of Alzheimer's disease (AD). The first clinical symptoms of dementia are a consequence of the impairment of the cerebral cholinergic pathway that innervates cortical areas from the nucleus basalis of Meynert (nbM or Ch4) in the basal forebrain. The hippocampal and cortical cholinergic synapses are specifically damaged, including a downregulation of presynaptic autoreceptors (M_2), and postsynaptic such as the M_1 muscarinic and the α_7 nicotinic receptors. The AChE inhibitors, donepezil, rivastigmine, and galantamine, have been approved for the treatment of AD to potentiate the cholinergic signaling.

- The NMDA receptor has long been implicated in the excitotoxicity that leads to neurodegeneration in the later stages of Alzheimer's disease.
- The NMDA antagonist, memantine, has been approved for the treatment of Alzheimer's disease alone or in combination with the inhibitors of acetylcholinesterase, donepezil, galantamine or rivastigmine.
- Activation of metabotropic receptors of glutamate, mainly of the $mGluR_7$ subtype, protects basal forebrain cholinergic neurons, probably by reducing NMDA currents.

Table 28.1 The most relevant adaptations of neurotransmitter receptors in AD.

Receptor		Regulation	Patients/model of AD
AMPA		• ↓ signaling • ↓ signaling in HPC • Loss of dendritic spines • ↓ receptors in HPC	Pyramidal neurons treated with Aβ Tg2576-APP Patients with AD
NMDA		• ↑ extracellular glutamate • ↑ activity • Inhibition of LTP • Ca^{+2} influx dysregulation • ROS generation • mitochondrial dysfunction	Hippocampal slices from rodent models of AD Culture of rat cortical neurons; entorhinal-hippocampal organotypic slices
Group I mGlu (mGluR5)		• ↑ signaling • ↑ neurotoxic signaling • ↑ pathology • ↓ cognition	APPswe, PS1ΔE9 or 3xTg-AD mice
Group III mGlu (mGluR7)		• ↑ activation • ↑ neuroprotective • ↓ glutamatergic transmission • ↓ LDH release	In vitro (cortical and hippocampal cell cultures); in vivo models of AD; rodent BFCN neurons
$GABA_A$		• Downregulation	Postmortem samples from patients with AD
$GABA_B$		• ↑ B1 subunit • ↑ alternative splicing • ↑ GIRK current • ↑ expression in HPC	frontal and temporal cortical samples of AD patients; Down Syndrome mice model (Ts65Dn mice)
Noradrenergic receptors		• Loss of noradrenergic neurons in LC • ↓ neurogenesis in HPC • ↑ expression α_2 and β_2 in HPC	Autopsied brains of AD patients; young adult rats with selective immunotoxic ablation of LC; brain cells cultures treated with Aβ; Tg mouse models
Dopaminergic receptors (D2 subtypes)		• preserved or decreased	Mild-moderate AD with neuropsychiatric symptoms
Serotonergic receptors ($5HT_4$)		• improved cholinergic neurotransmission • favor the nonamyloidogenic pathway	Transgenic mouse models with AD
nAChR		• partial loss in cortical layers of the temporal cortex, the presubiculum and parahippocampal gyrus • ↓ ChAT activity • modification of α_7-subunit	Patients with AD
mAChR	M1 M2 M4	• ↓ Frontal cortex • loss in cortex and hippocampus • ↓ LTP • conserved	

The table summarizes the main physiological modifications or regulations identified for several neurotransmitter receptors in different in vitro or in vivo animal models and/or in Alzheimer's disease (AD) patients. *ChAT*, choline acetyltransferase; *HPC*, hippocampus; *LC*, Locus coeruleus; *Tg*, transgenic.

Key facts about GABA receptors in Alzheimer's disease

- The analysis of [^{3}H]-flumazenil and [^{3}H]-GABA-binding sites indicates a downregulation of $GABA_A$ receptors in Alzheimer's disease.

Key facts about monoaminergic receptors in Alzheimer's disease

- The damage of noradrenergic neurons in the locus coeruleus impairs working memory and hippocampal neurogenesis.
- The dopaminergic system has been analyzed in Alzheimer's disease in relation to neuropsychiatric symptoms concomitant to the dementia, such as psychosis or depression.
- 5-HT_4R agonists and 5-HT_6R serotonergic antagonists have been assayed for their potential therapeutic properties in Alzheimer's disease.

Key facts about the cholinergic system in Alzheimer's disease

- The loss of cortical cholinergic innervation in Alzheimer's disease can be attributed to the loss of basal forebrain cholinergic neurons.
- Both classes of cholinergic receptors, muscarinic and nicotinic, are differentially regulated in Alzheimer's disease.

Summary points

- Uptake capacity and calcium signaling in astrocytes is disrupted by amyloid-β, contributing to excitotoxicity.
- A decrease in AMPA receptor-mediated synaptic transmission is related to loss of hippocampal dendritic spines and cognitive impairment in animal models of Alzheimer's disease.
- Autoradiography studies show a reduction in AMPA receptors in hippocampal areas of patients with Alzheimer's disease.
- The Ca^{2+} influx dysregulation through NMDA receptors contributes to excitotoxicity, leading to neurodegeneration in the later stages of Alzheimer's disease.
- NMDA receptor-dependent LTP in the hippocampus is impaired in Alzheimer's disease.
- A moderate reduction of $GABA_A$ receptors may mediate the cognitive impairment in Alzheimer's disease patients.
- The α_2 and β_2 subtypes of adrenergic receptors have been found to be increased in the brains of Alzheimer's disease patients.
- Agonists for the D_2 dopaminergic receptor subtype have been identified to have therapeutic potential for Alzheimer's disease.

- A modulation of different subtypes of serotonergic receptors has been described in Alzheimer's disease patients, including the 5-HT_{1A}, 5-HT_{2C}, 5-HT_6, and 5-HT_4.
- A significant loss of M_2 muscarinic cholinergic receptors has been observed in the cortex and in the hippocampus of Alzheimer's disease patients.

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CHAPTER 29

Aβ42-α7-like nicotinic acetylcholine receptors and Alzheimer's disease

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List of abbreviations

3x Tg AD mice triple transgenic Alzheimer's disease mice
AD Alzheimer's disease
APOE apolipoprotein E
$A\beta_{42}$ amyloid-β_{1-42}
FLNA filamin A
MCI mild cognitive impairment
NMDAR N-Methyl-D-Aspartate glutamatergic receptor
RAGE advanced receptor for advanced glycation end products
TLR4 toll-like receptor 4
α7-like nAChR α7-like nicotinic acetylcholine receptor
α7nAChR α7-nicotinic acetylcholine receptor

Mini-dictionary of terms

Amyloid-β_{1-42} ($A\beta_{42}$)-α7 nicotinic acetylcholine receptor-like (α7-like nAChR) complex $A\beta_{42}$ binds to α7nAChR with high affinity that leads to piling of additional $A\beta_{42}$ molecules on α7nAChRs to form $A\beta_{42}$-α7nAChR complexes in the brain. In the lymphocytes, $A\beta_{42}$ interacts with a mixture of α7nAChR and CHRFAM7A, a α7nAChR chimeric gene product (termed α7-like nAChR) to form $A\beta_{42}$-α7-like nAChR complexes. These $A\beta_{42}$-α7nAChR complexes internalized into cells to become intracellular aggregates.

Coimmunoprecipitation A procedure with which a set of physically associated proteins are precipitated using a specific antibody and antibody binder, protein A or G conjugated with agarose by centrifugation. For example, $A\beta_{42}$ is coimmunoprecipitated with α7nAChRs by anti- $A\beta_{42}$ antibodies.

Filamin A (FLNA) Filamin A is a 280-KDa scaffold protein that exists as dimer with its carboxyl end tethered to cell membranes and the rest protruding intracellularly. FLNA regulates actin dynamics and is associated with dozens of receptors and signaling molecules. $A\beta_{42}$ binding to α7nAChRs recruits FLNA to α7nAChR and thereby facilitates aberrant α7nAChR signaling. FLNA with altered conformation is prevalent in Alzheimer's disease brains.

Synaptosomes They are circular structures of the truncated neuronal terminals artificially produced following homogenization in hypertonic solution such as buffered 0.32 M sucrose and centrifugation. They are often used to study functions of neurons such as receptor signaling and neurotransmitter release.

Tau phosphorylation Tau is a microtubule-associated protein primarily located in axons. The function of tau is regulated by phosphorylation. Phosphorylation of tau on its 30+ phosphorylation sites is mediated

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by various kinases. The phosphorylated tau levels are elevated in AD and other neurodegenerative disorders, and its presence in cerebrospinal fluid indicates neurodegeneration.

Neurofibrillary lesions These are conditions in which the function of neuronal processes are compromised. This is primarily caused by the persistently heightened tau phosphorylation. The elevated phosphorylated tau eventual spreads from axons to dendrites, causing transport arrest and truncation of neuronal processes.

3x Tg AD mice Mice carrying mutated amyloid precursor protein (APP_{SWe}), presenilin 1 ($PS1_{M146V}$), and tau (tau_{P301L}) genes show increased amyloid plaques and heightened phosphorylated tau and are used as an Alzheimer's disease model.

Introduction to toxicity of soluble Aβ

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder causing dementia without effective disease-modifying treatment. The severity of neurodegeneration and cognitive impairments in AD positively correlate with soluble amyloid-β (Aβ), especially $Aβ_{42}$, rather than amyloid plaque in the brain, suggesting that soluble Aβ is a key AD pathogenic factor that inflicts neuronal damage (Haass & Selkoe, 2007; McLean et al., 1999; Naslund et al., 2000). Aβ′s neurotoxic effect is supported by extensive data collected from various in vitro and in vivo systems. Aβ dysregulates membrane fluidity possibly by its interactions with cholesterol, a critical membrane component that controls membrane fluidity (Kremer, Pallitto, Sklansky, & Murphy, 2000; Peters et al., 2009; Wood, Eckert, Igbavboa, & Müller 2003). Aβ and its soluble oligomers cause intracellular Ca^{2+} dyshomeostasis by acting like ionophores (Shirwany, Payette, Xie, & Guo, 2007). Soluble Aβ can also alter Ca^{2+} influx via ligand- and/or voltage-gated channels such as directly modulating NMDA receptors. This disruption of Ca^{2+} influx diminishes mitochondrial function and survival of neurons, including the cholinergic neurons linked to cognitive impairment (Alberdi et al., 2010; Ferreira et al., 2013, p. P520; Niidome et al., 2009; Parri, Hernandez, & Dineley, 2011; Selkoe, 1999). Aβ also impacts synaptic activity, neurotransmission, neuronal signaling, and receptor function and trafficking (Mucke & Selkoe, 2012; Reinders et al., 2016; Shankar et al., 2007; Snyder et al., 2005). However, the neurotoxic effects reported in these and many other studies utilize exceedingly high (μM or barely sub-μM) Aβ concentrations, which are an order of magnitude higher than levels detected in AD transgenic models and AD brains. Most importantly, these neurotoxic effects from high concentrations of exogenous Aβ are rather nonspecific.

In searching for soluble Aβ targets, earlier reports indicate that Aβ interacts with the advanced receptor for advanced glycation end products (RAGE) expressed ubiquitously throughout the body to promote Aβ disposal (Chaney et al., 2005; Yan, Stern, & Schmidt, 1997). Enhancement of RAGE signaling in microglia exacerbates an inflammatory response leading to neuronal dysfunction and increased Aβ accumulation (Fang et al., 2010). In addition, Aβ can induce cell destruction by binding scavenger

receptors expressed in microglia and macrophages by generating reactive oxygen species (Wilkinson & El Khoury, 2012). However, the expression pattern, distribution and function of RAGE and scavenger receptors and their low affinity (μM) for Aβ cannot logically explain AD pathophysiology such as deafferentation and degeneration of cholinergic and pyramidal neurons (D'Andrea, Nagele, Wang, Peterson, & Lee, 2001; Selkoe, 1999). These data again strongly suggest there are neuronal receptors for soluble Aβ, particularly the more pathogenic $A\beta_{42}$.

α7nAChR as the soluble Aβ target driving AD pathogenesis

The α7nAChR is abundantly expressed in brain regions innervated by basal forebrain cholinergic neurons (Breese et al., 1997; Perry et al., 1992) and also highly correlated with the distribution and density of amyloid plaques in AD. The relevance of α7nAChR to amyloid plaques rich in $A\beta_{42}$ is further supported by in vitro studies showing that increased α7nAChR levels increase $A\beta_{42}$ aggregates, amyloid plaques, and cell death (Nagele, D'Andrea, Anderson, & Wang, 2002). Critically, $A\beta_{42}$ and α7nAChRs colocalize in cortical and hippocampal neurons in brains of AD patients and (to a lesser extent) aged subjects, as well as in amyloid plaques (Nagele et al., 2002; Wang, D'Andrea, & Nagele, 2002; Wang, Lee, D'Andrea et al., 2000). These data together strongly suggest that surface α7nAChR is an important neuronal target for soluble Aβ. By binding to α7nAChRs, Aβ, especially $A\beta_{42}$, piles onto α7nAChRs to cause receptor dysfunction and subsequent internalization of the $A\beta_{42}$-α7nAChR complexes, leading to the intraneuronal $A\beta_{42}$ aggregates abundant in brains of AD transgenic models and patients (D'Andrea et al., 2001, 2002; Gouras et al., 2000; Nagele et al., 2002; Wang et al., 2010; Wang, Lee, D'Andrea et al., 2000; Wang, Lee et al., 2017). The accumulation of the $A\beta_{42}$-α7nAChR complexes could disrupt axonal/dendritic transport and cause energy dyshomeostasis, leading to lysis of neurons and eventual formation of amyloid plaques (Nagele et al., 2002). Importantly, $A\beta_{42}$-α7nAChR interaction induces $A\beta_{42}$ toxic signaling to cause tau phosphorylation and neurofibrillary lesions that exacerbate $A\beta_{42}$ toxicity, form neurofibrillary tangles, and promote neurodegeneration (Wang et al., 2009, 2010; Wang, Lee et al., 2017; Wang, Li, Benedetti, & Lee, 2003). Moreover, α7nAChR is a primary target for $A\beta_{42}$ and the $A\beta_{42}$-α7nAChR interaction is a key event in Aβ-driven AD pathogenesis. The critical role of α7nAChR in AD pathogenesis is further supported by attenuated cognitive impairment and synaptic abnormalities following α7nAChR deletion in AD transgenic mice (Dziewczapolski, Glogowski, Masliah, & Heineman, 2009).

$A\beta_{42}$ binding to α7nAChR was first demonstrated by a reciprocal immunoprecipitation technique using specific antibodies against $A\beta_{42}$ and α7nAChR. In hippocampal membranes of postmortem brains from matched pairs of neurologically normal and sporadic AD cases, endogenous $A\beta_{42}$ proteins coimmunoprecipitated with α7nAChRs

but not with other nicotinic receptor subtypes (Wang, Lee, D'Andrea et al., 2000). Importantly, AD brains had 15- to 20-fold higher levels of the $A\beta_{42}$-α7nAChR complex (Wang, Lee, D'Andrea et al., 2000). This selective and stable $A\beta_{42}$-α7nAChR binding was confirmed in vitro by incubating cell membranes containing α7nAChRs with exogenous $A\beta_{42}$ (and to a lesser extent, $A\beta_{40}$) but not other peptides (Wang, Lee, D'Andrea et al., 2000; Wang, Lee, Davis et al., 2000). Later, the $A\beta_{42}$-α7nAChR was demonstrated by $A\beta_{42}$ incubation of rodent neuronal cells, brain slice cultures, and postmortem human brain tissues (Tong, Arora, White, & Nichols, 2011; Wang et al., 2009, 2010, 2012, Wang, Lee et al., 2017; Wang, Trocmé-Thibierge, et al., 2017; Wang, Lee, D'Andrea et al., 2000). The fact that $A\beta_{42}$-α7nAChR complexes survived detergent treatments and multiple washes indicated the high-affinity binding of this interaction. Binding studies using intact α7nAChR-expressing SK-N-MC cells (Wang, Lee, D'Andrea et al., 2000) or cells and membranes prepared from rodent or human brains illustrated that $A\beta_{42}$ binds α7nAChRs with femtomolar affinity (Wang et al., 2012; Wang, Lee, D'Andrea et al., 2000; Wang, Lee, Davis et al., 2000). Subsequent studies showed that multiple, additional $A\beta_{42}$ monomers, dimers, or small oligomers appear to bind α7nAChRs, albeit at about 10-fold lower affinity (Wang et al., 2009).

Importantly, $A\beta_{42}$ binding to α7nAChRs in AD transgenic mice and in AD postmortem brain induces recruitment of filamin A (FLNA) to α7nAChRs and toll-like receptor 4 (TLR4) to enable $A\beta_{42}$'s toxic signaling and drive neuroinflammation (Wang et al., 2012, Wang, Lee et al., 2017). The FLNA-targeting AD therapeutic candidate PTI-125 has revealed that the FLNA-α7nAChR interaction enables $A\beta_{42}$'s tight binding to α7nAChRs and subsequent toxic signaling (Wang et al., 2012). Disrupting FLNA's linkage to α7nAChR via PTI-125 reduces $A\beta_{42}$'s affinity for α7nAChRs by at least 1000-fold, resulting in lower levels of $A\beta_{42}$-α7nAChR complexes and even releasing $A\beta_{42}$ molecules from them (Wang et al., 2012; Wang, Lee et al., 2017). $A\beta_{40}$ also induces FLNA recruitment to α7nAChRs, although with 10-fold higher concentrations and to a markedly lesser extent (Wang et al., 2012). This finding is in accordance with $A\beta_{40}$'s 1000-fold lower potency versus $A\beta_{42}$ in inhibiting α7nAChR-regulated function (Lee & Wang, 2003). The differential efficacy between $A\beta_{42}$ and $A\beta_{40}$ in inducing FLNA recruitment to α7nAChRs suggests that C-terminal amino acids of Aβ play a regulatory role in the Aβ-α7nAChR interaction and in $A\beta_{40}$'s reversibility of binding (Lee & Wang, 2003).

Identification of the critical Aβ- and α7nAChR-binding domains for $A\beta_{42}$-α7nAChR interaction

Because $A\beta_{42}$ binding to α7nAChRs leads to intraneuronal accumulation of $A\beta_{42}$-α7nAChR complexes, neurodegeneration, and amyloid plaque formation (Nagele et al., 2002), one therapeutic strategy is to prevent or disrupt the $A\beta_{42}$-α7nAChR

interaction. Understanding the interaction domains of Aβ and α7nAChR is an obvious first step. The putative $A\beta_{42}$-binding domain to α7nAChR was first evidenced by incubation of membranes prepared from α7nAChR-expressing SK-N-MC cells with exogenous $A\beta_{42}$ and various Aβ peptide (blocking) fragments prior to solubilization and immunoprecipitation with anti-$A\beta_{42}$. $A\beta_{12-28}$ robustly reduced $A\beta_{42}$-α7nAChR complexes, indicating that the $A\beta_{42}$-binding domain resides within amino acids 12—28 of Aβ (Espinoza-Fonseca, 2004; Wang, Lee, D'Andrea et al., 2000). The interaction site of $A\beta_{42}$ was further defined using selective Aβ fragments to block the $A\beta_{42}$-α7nAChR association in synaptosomes prepared from postmortem human control frontal cortex. In these experiments, $A\beta_{42}$-α7nAChR formation by incubating with $A\beta_{42}$ was blocked by $A\beta_{15-20}$ but not by $A\beta_{1-11}$, $A\beta_{25-35}$, $A\beta_{29-40}$, or $A\beta_{37-43}$ (Wang et al., 2009). Interestingly, $A\beta_{15-20}$ is also reported to be critical for $A\beta_{42}$ oligomerization (Austen et al., 2008).

To elucidate the critical α7nAChR domain involved in the interaction with $A\beta_{42}$ binding, selective aromatic amino acid residues in the mouse α7nAChR agonist-binding site were changed by site-directed mutagenesis, and tyrosine 188 was found to be critically important. Mutations of tyrosine188 to serine and phenylalanine almost completely abolished the $A\beta_{42}$-induced presynaptic α7nAChR Ca^{2+} response and modestly reduced the $A\beta_{42}$-α7nAChR association (Tong et al., 2011). The critical α7nAChR interaction site with $A\beta_{42}$ was further defined by testing various fragments of the α7nAChR extracellular (EC) domain as decoy peptides in blocking $A\beta_{42}$-α7nAChR complex formation in rat synaptosomes incubated with $A\beta_{42}$. The α7nAChR EC domain containing amino acids 184—196 robustly inhibited the $A\beta_{42}$-α7nAChR interaction, thus identifying the extreme carboxyl terminus of the α7nAChR EC domain as the Aβ-binding site (Fig. 29.1). Furthermore, substituting tyrosine 188 and/or tyrosine 195 to either phenylalanine or arginine abolished the inhibitory effect on the $A\beta_{42}$-α7nAChR interaction. Fig. 29.1 and Tong's data both indicate that tyrosine 188 in the α7nAChR EC is pivotal for $A\beta_{42}$ binding. Discrepancies regarding the magnitude of the $A\beta_{42}$-α7nAChR interaction blockade and tyrosine 195 may be related to the experimental approaches utilized and subtle changes in EC structure. The identification of the pivotal binding regions in Aβ and α7nAChR responsible for the high-affinity $A\beta_{42}$-α7nAChR interaction may facilitate development of therapeutic agents that reduce this pathological interaction of $A\beta_{42}$.

$A\beta_{42}$-α7nAChR interaction in AD and normal aging in brain and lymphocytes

AD pathologies such as fibrillar Aβ deposits are often found in cognitively normal subjects without signs of neurodegeneration (Bennett et al., 2006). The abundance of Aβ aggregates appears to increase with advancing age and *APOEε4* gene dose (Armstrong

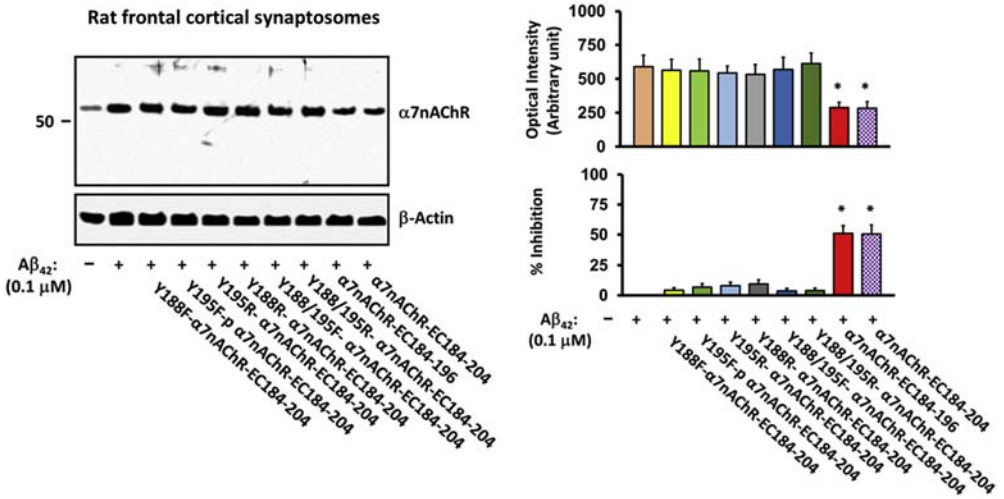

Figure 29.1 ***Specific tyrosine residues of the α7nAChR extracellular domain are critical to the Aβ$_{42}$-α7nAChR interaction.*** The critical Aβ$_{42}$-binding domain in α7nAChR was determined by incubating rat cortical synaptosomes simultaneously with Aβ$_{42}$ and peptide fragments homologous to specific fragments of α7nAChR extracellular (EC) ligand-binding domain: 184–204: SERFYECCKEPYPDVVTFTVTM; 184–196: SERFYECCKEPYP with or without substitutions for key tyrosine residues. Acting as a decoy for the α7nAChR protein, both α7nAChR-EC 184–204 and α7nAChR-EC 184–196 robustly blocked the Aβ$_{42}$-α7nAChR interaction. Substituting tyrosine 188 and/or tyrosine 195 with phenylephrine (F) or arginine (R) abolished the inhibition of the Aβ$_{42}$-α7nAChR association. N = 4 *$P < .01$ versus Aβ$_{42}$-exposed synaptosomes.

et al., 1996; Reiman et al., 2009). The Aβ aggregates and hyperphosphorylated tau protein that are markedly elevated in brains of AD transgenic mice (e.g., 3× AD transgenics) are also detectable in wild-type E129 mice along with related pathophysiology and cognitive abnormalities (Wang, Lee et al., 2017). These data suggest that normal age-related brain dysfunction may be at least partially attributable to Aβ-induced, AD-like pathology.

Extensive ex vivo and in vivo data have shown that Aβ, and especially Aβ$_{42}$ in monomeric, dimeric or oligomeric form, interacts with α7nAChRs to enable a sustained, aberrant α7nAChR signaling cascade. Hence, Aβ$_{42}$-α7nAChR interaction can lead to impaired synaptic activity, intraneuronal Aβ$_{42}$ aggregates, and dysfunctional α7nAChRs, resulting in core AD pathology, and ultimately, cognitive impairment (Dziewczapolski et al., 2009; Liu, Kawai, & Berg, 2001; Pettit, Shao, & Yakel, 2001; Wang et al., 2009, 2010, 2012; Wang, Lee et al., 2017). These data suggest that the Aβ$_{42}$-α7nAChR interaction is a key upstream event in AD pathogenesis (Dziewczapolski et al., 2009; Inestrosa et al., 2013; Medeiros et al., 2014; Ondrejcak et al., 2012; Wang et al., 2009, 2010, 2012; Wang, Lee et al., 2017).

As in the brain, higher levels of $A\beta_{42}$-bound α7-like nAChRs were detected in lymphocytes derived from AD subjects. Interestingly, this linkage in lymphocytes positively correlates with *APOE*ε4 gene levels (Wang et al., 2012; Wang, Trocmé-Thibierge et al., 2017). The $A\beta_{42}$-α7-like nAChR complexes in lymphocytes appear to contain both α7nAChR and CHRFAM7A, the product of a α7nAChR chimeric gene (Araud et al., 2011). The parallelism between $A\beta_{42}$-α7nAChR association in brains and the $A\beta_{42}$-α7-like nAChR interaction in lymphocytes suggests that $A\beta_{42}$-α7-like nAChR complex levels in lymphocytes may be used to estimate levels of these complexes in brain and the magnitude of AD pathology in AD and normal aging.

To validate that the $A\beta_{42}$-α7nAChR association increases with advancing age, $A\beta_{42}$-α7nAChR complexes were isolated from frontal cortices and lymphocytes of wild-type E129 and 3x Tg AD mice at 5, 10, and 15 months of age using an established coimmunoprecipitation protocol (Wang et al., 2009, 2010, 2012; Wang, Lee et al., 2017). A markedly higher level of $A\beta_{42}$-α7nAChR complexes (also containing FLNA) was found in frontal cortices from 3x Tg AD mice compared to wild types (Fig. 29.2A). In addition, levels of this FLNA-$A\beta_{42}$-α7nAChR complex increased with age in both transgenics and wild types (Fig. 29.2A). Remarkably, at 15 months, the level of FLNA-$A\beta_{42}$-α7nAChR complexes in the wild-type mice was approximately 40% of the 3x Tg AD level (Fig. 29.2B). These data clearly show that FLNA-$A\beta_{42}$-α7nAChR complexes and AD pathology increase during normal aging (Wang, Lee et al., 2017). Illustrating that these protein complexes in lymphocytes may be a reliable predictor of brain pathology, lymphocytes from the same mice showed a similar age-dependent increase in FLNA-$A\beta_{42}$-α7-like nAChR complexes.

To assess whether these findings in mice (Fig. 29.2) translate to humans, the levels of $A\beta_{42}$-α7nAChR complexes in postmortem frontal cortices from 11 pairs of cognitively normal and AD, matched for age, gender, and postmortem intervals (<10 h), were determined. While the abundance of $A\beta_{42}$-α7nAChR complexes in nondemented controls strongly correlated with age (linear correlation coefficient of 0.7080; $P < .0012$), this age-dependent increase was modest. In contrast, there was no correlation between $A\beta_{42}$-α7nAChR complex levels and age in AD cases ($r^2 = 0.0908$, $P = .3678$), despite the fourfold higher abundance of $A\beta_{42}$-α7nAChR complexes in AD compared to nondemented controls (Fig. 29.3). The $A\beta_{42}$-α7-like nAChR complex levels in lymphocytes from these 13 pairs of non-*APOE*ε4 age- and gender-matched cognitively normal controls and AD subjects were also examined. As in postmortem human frontal cortices from control subjects, the $A\beta_{42}$-α7-like nAChR complex levels in control lymphocytes positively correlated with age ($r^2 = 0.4977$, $P<.0071$) (Fig. 29.4). In contrast to the dramatic increase in AD brain levels of $A\beta_{42}$-α7nAChR, the $A\beta_{42}$-α7-like nAChR complex levels in AD lymphocytes were only modestly higher than in control lymphocytes. Again, there was no correlation with age in these $A\beta_{42}$-α7-like nAChR complexes in lymphocytes from AD patients ($r^2 = 0.0838$, $P = .3374$).

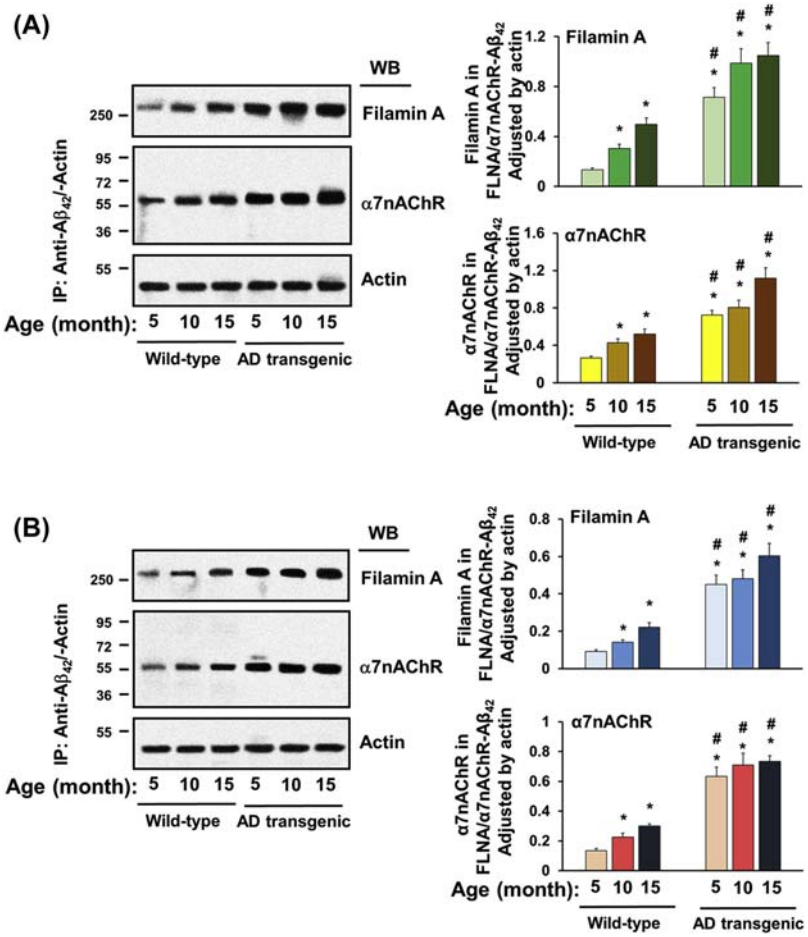

Figure 29.2 ***Age effect on endogenous Aβ$_{42}$-α7nAChR-filamin A complex levels in brain and lymphocyte.*** The effect of age on the endogenous Aβ$_{42}$-α7nAChR-filamin A (FLNA) complex levels was determined by coimmunoprecipitation with anti-Aβ$_{42}$ antibodies in frontal cortical synaptosomes (A) and lymphocytes (B) from wild-type and 3 × AD transgenic (Tg) mice at 5, 10, and 15 months of age. N = 4 *$P < .01$ versus 5-month-old wild type. #$P < .01$ compared 3x Tg with wild type of same age group.

It is unclear whether the increased Aβ$_{42}$-α7-like nAChR complex level in lymphocytes from AD patients plays any role in AD pathology or simply represents a surveillance function on brain activity by immune cells. Amyloid activates the immune system to promote an inflammatory reaction and possibly prevent harmful autoimmune activities (Wang & Zhang, 2018). Considering that transferring lymphocytes from senescent mice to young mice resulted in poorer learning by the young mice with senescence-like serum-brain reactivity (Lal, Bennett, Bennett, Forster, & Nandy, 1986), it is possible that the heightened Aβ$_{42}$-α7-like nAChR in lymphocytes indicates a dysfunctional immune system that may contribute to AD neuropathology.

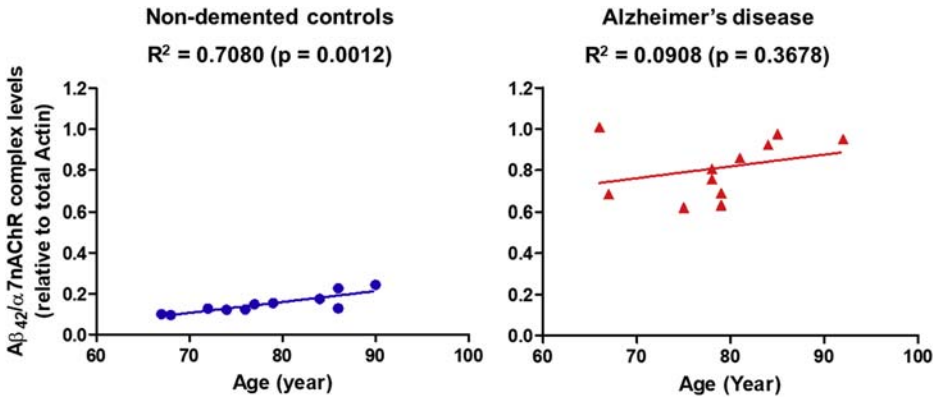

Figure 29.3 ***Age effect on endogenous Aβ$_{42}$-α7nAChR complex levels in postmortem human frontal cortices from cognitively normal controls and AD cases.*** The correlation between age and the abundance of Aβ$_{42}$-α7nAChR complexes was analyzed by linear regression separately in 11 paired nondemented cognitive control and AD cases. The Aβ$_{42}$-α7nAChR complex is defined by the levels of α7nAChRs in the anti-Aβ$_{42}$ antibodies immunoprecipitates normalized by β-actin immunoreactivity as the immunoprecipitation/loading controls. The Aβ$_{42}$-α7nAChR complexes increase with advancing age in controls ($R^2 = 0.7080$, $P = .0012$) but not in AD cases ($R^2 = 0.0908$, $P = .3678$) although ADs have fourfold-higher abundance of Aβ$_{42}$-α7nAChR complexes.

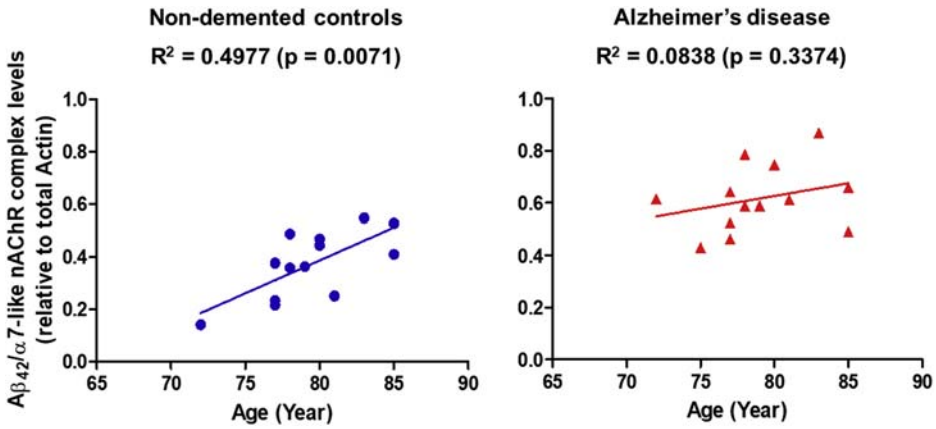

Figure 29.4 ***Age effect on endogenous Aβ$_{42}$-α7nAChR complex levels in lymphocytes from nondemented control and AD non-APOEε4 carriers.*** The correlation between age and the Aβ$_{42}$-α7nAChR complex levels was analyzed by linear regression separately in 13 paired nondemented cognitive control and AD cases who are not *APOE*ε4 carriers. The Aβ$_{42}$-α7nAChR complex is defined by the levels of α7nAChRs in the anti-Aβ$_{42}$ antibodies immunoprecipitates normalized by actin immunoreactivity as the immunoprecipitation/loading controls. The Aβ$_{42}$-α7nAChR complexes increase with advancing age in controls ($R^2 = 0.4977$, $P = .0071$) but not in AD cases ($R^2 = 0.0838$, $P = .3374$) although ADs have higher Aβ$_{42}$-α7nAChR complex levels.

Reducing $A\beta_{42}$'s binding to α7nAChR as a disease-modifying therapeutic approach in AD and cognitively compromised elderly

The femtomolar-binding affinity of $A\beta_{42}$ for α7nAChR indicates that the formation of $A\beta_{42}$-α7nAChR complexes is rapid with an extremely slow off rate (Wang, Lee, D'Andrea et al., 2000; Wang, Lee, Davis et al., 2000). It is therefore predictably challenging to prevent $A\beta_{42}$ from engaging α7nAChRs in $A\beta_{42}$-rich environments and initiating α7nAChR-dependent toxic signaling. Therapeutics directly interfering with this interaction must compete with the ultra-high-affinity binding of $A\beta_{42}$ or completely remove all $A\beta_{42}$. The initial ultra-high-affinity $A\beta_{42}$-α7nAChR interaction also leads to subsequent picomolar affinity binding (piling) of multiple $A\beta_{42}$ molecules onto α7nAChRs, sustaining the toxic signaling (Nagele, D'Andrea, Lee, Venkataraman, & Wang, 2003; Wang et al., 2009). The $A\beta_{42}$ toxic signaling through α7nAChR leads to heightened tau phosphorylation, internalization of $A\beta_{42}$-α7nAChR complexes, cell destruction, and formation of amyloid plaques (Nagele et al., 2003; Wang et al., 2009). Hence, agents that attenuate the $A\beta_{42}$-α7nAChR interaction can potentially shorten $A\beta_{42}$ signaling, thereby reducing neurofibrillary lesions, neuronal death, and amyloid plaque in brains.

The $A\beta_{42}$-α7nAChR interaction can be reduced by dramatically lowering or eliminating $A\beta_{42}$. While this goal can be achieved using inhibitors of β- and/or γ-secretases (Dovey et al., 2001; Sankaranarayanan et al., 2008), none of the secretase inhibitors have achieved AD therapeutic efficacy, suggesting that a nearly complete elimination of $A\beta_{42}$ is needed to prevent the high-affinity $A\beta_{42}$-α7nAChR interaction. Another strategy is to disrupt the $A\beta_{42}$-α7nAChR interaction by directly competing with $A\beta_{42}$ for its α7nAChR-binding sites, since selective Aβ fragments such as $A\beta_{12\text{-}28}$ and $A\beta_{15\text{-}20}$ reduce $A\beta_{42}$-α7nAChR complex levels (Wang, Lee, D'Andrea et al., 2000; Wang et al., 2009). Competing with $A\beta_{42}$'s femtomolar binding, the α7nAChR partial agonist S24795 was shown to reduce the $A\beta_{42}$-α7nAChR interaction and to remove some of the $A\beta_{42}$ from existing $A\beta_{42}$-α7nAChR complexes (Wang et al., 2009, 2010). Similarly, a peptide homologous to the nicotine-binding site on α7nAChR was shown to reduce $A\beta_{40}$-evoked inhibition of carbamylcholine-induced current in PC12 cells (Nery et al., 2013). These studies provide a rationale that selective α7nAChR-targeting agents may be able to reduce $A\beta_{42}$-α7nAChR coupling by binding to α7nAChRs.

Because FLNA's interaction with α7nAChR is critical to $A\beta_{42}$'s toxic signaling and high-affinity binding to α7nAChR, targeting FLNA represents an alternative approach (Wang et al., 2012). By binding FLNA, small molecule PTI-125 reverses FLNA's altered conformation and linkage to α7nAChR and prevents $A\beta_{42}$'s signaling via α7nAChR (Wang, Li et al., 2017). As mentioned above, PTI-125 reduces $A\beta_{42}$'s binding affinity for α7nAChR by at least 1000-fold, removing $A\beta_{42}$ from existing $A\beta_{42}$-α7nAChR complexes (Wang et al., 2012). Reducing this $A\beta_{42}$-α7nAChR interaction via

PTI-125 reduced tau hyperphosphorylation, augmented receptor function, enhanced synaptic plasticity, reduced Aβ aggregates, and neurofibrillary lesions, and improved cognition. These multiple beneficial effects by an agent that disrupts $A\beta_{42}$'s binding to α7nAChR is additional evidence that this toxic interaction is a key upstream event in AD pathology. Therapeutic agents that reduce the $A\beta_{42}$-α7nAChR interaction should slow AD pathogenic progression, preserve brain function, and improve cognition.

Key facts of amyloid-β in Alzheimer's disease

- A small percentage of Alzheimer's disease (AD) patients with heightened production of amyloid-β (Aβ) peptides relative to other AD patients show accelerated neurodegeneration and dementia.
- Amyloid-β (Aβ) peptides with 38—43 amino acids are the product of sequential cleavage of amyloid precursor protein by β- and ɣ-secretases and are a primary pathogenic factor in AD.
- Among Aβ peptides, $A\beta_{1-42}$ ($A\beta_{42}$) has the greatest propensity to aggregate and is the most toxic to neurons.
- Amyloid plaques are the remnants of dead neurons overly burdened with Aβ aggregates.
- Aβ can cause neurofibrillary lesions and axonal transport arrest leading to neurodegeneration by promoting phosphorylation of the microtubule-associated protein tau.
- With a femtomolar-binding affinity, the α7 nicotinic acetylcholine receptor (α7nAChR) is the only known high-affinity neuronal protein target of soluble Aβ monomers or small oligomers.

Summary points

- This chapter focuses on amyloid-β (Aβ) interaction with α7 nicotinic acetylcholine receptor (α7nAChR) in Alzheimer's disease (AD) pathogenesis by identifying critical $A\beta_{42}$-binding domain in α7nAChR and age effects.
- $A\beta_{42}$ forms complexes with α7nAChRs in brains and α7-like nAChR, a mixture of α7nAChR and CHRFAM7A, a α7nAChR chimeric gene product in lymphocytes.
- By binding to α7nAChR, Aβ, especially $A\beta_{42}$, recruits filamin A (FLNA) and initiates toxic signaling resulting in neurofibrillary lesions as well as piling of additional $A\beta_{42}$ molecules on α7nAChR leading to dysfunctional of cells, neurodegeneration, and amyloid plaque formation.
- The tyrosine residues in the carboxyl end of the α7nAChR extracellular ligand-binding domain is the critical element for $A\beta_{42}$ binding to α7nAChRs.

- The levels of $A\beta_{42}$-α7nAChR and $A\beta_{42}$-α7-like nAChR complex that include filamin A in brain and lymphocyte, respectively, are higher in AD and increase with advancing age.
- Breaking up $A\beta_{42}$-α7nAChR and $A\beta_{42}$-α7-like nAChR complexes should help slow down $A\beta_{42}$-induced AD pathologies and possibly reserve brain function and cognition.

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CHAPTER 30

Synaptosomal bioenergetic defects in Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
APP amyloid precursor protein
Aβ amyloid β
BHI bioenergetic health index
CAA cerebral amyloid angiopathy
CJD Creutzfeldt–Jakob disease
CRC cell respiratory control
FBD/FDD familial British dementia/familial Danish dementia
FDG-PET fluor deoxyglucose-positron emission tomography
FTD frontotemporal dementia
GSS Gerstmann-Sträussler-Scheinker
N.D. not determined
PrP prion promoter
PS1 presenilin 1
RCR respiratory control ratio
SRC spare respiratory capacity
WT wild-type

Mini-dictionary of terms

Cell respiratory control (CRC) It is considered the best general measure of mitochondrial function in cell populations and reports all the parameters obtained with the Seahorse respirometer.

Spare respiratory capacity (SRC) It is a parameter obtained with the Seahorse respirometer and indicates the capability of the cell to respond to an increase of the energy demand. It can be an indicator of cell fitness or flexibility.

State 3 Also known as *"active respiration,"* reflects mitochondrial oxygen consumption (*ng-at O/min/mg protein)* in the presence of oxidizable substrates + ADP. It represents the maximum physiological O_2 consumption coupled to ATP production.

State 4 Also known as *"resting respiration,"* reflects mitochondrial oxygen consumption (*ng-at O/min/mg protein)* in the presence of oxidizable substrates without ADP.

Synaptosome It is a subcellular organelle isolated from brain tissue by homogenization and centrifugation-mediated fractionation. It comprises a resealed presynaptic terminal containing mitochondria surrounded by a physiological cytoplasm and plasma membrane.

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Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia in the aging population. It is expected that the number of people with AD worldwide will rise as the population ages, becoming an important public health concern as older portions of the population grow worldwide. Changes in the brain begin years before any clinical signs of the disease appear. At the clinical level, three different categories can be established: presymptomatic, moderate AD and severe AD. From a neuropathological level, it is considered a progressive neurodegenerative disorder, characterized by the progressive deposition of aggregated proteins—amyloid β (Aβ) and hyper-phosphorylated tau—and hippocampal synaptic loss, leading to the onset of behavioral and cognitive impairments. While the molecular basis underlying this kind of dementia remains a significant challenge, synaptic mitochondrial dysfunction appears to be a critical factor at the early stage of AD pathogenesis.

In this chapter, we explore the relationship between AD and neuronal bioenergetics, and the impact of synaptic mitochondrial bioenergetics on cognitive performance in transgenic animal models of AD. Furthermore, we speculate that targeting synaptosomal bioenergetic defects could slow or prevent the neurodegenerative process and restore neuronal function.

Pathological hallmarks of Alzheimer's disease

AD accounts for 50%–60% of all dementia cases (Barker et al., 2002; Ferri et al., 2005). Only 5% of the cases are attributable to familial mutations in three specific genes: amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2). From a histopathological standpoint, AD brain displays extra- and intraneuronal deposits of Aβ peptide, intraneuronal aggregates of hyperphosphorylated tau protein, and synaptic loss.

The term *amyloid* refers to a self-assembled protein with a typical fibrillar morphology when analyzed under transmission electron microscopy or atomic force microscopy. Fibrils are usually 8–12 nm wide, nonbranching and very long (several microns). Monomers that conform the amyloid protein acquire a β-sheet periodicity resulting in a typical "cross-β conformation." Tissues containing amyloid have specific dyeing properties when stained with Thioflavin S (green fluorescence viewed under a fluorescent microscope). At the biophysical level, soluble precursors (small peptides, folded proteins) undergo intermediate structures (oligomers, protofibers) before turning into an insoluble amyloid. Different factors are involved in amyloid formation *in vivo*, including (1) the intrinsic property of the amyloid precursor (primary structure), (2) local concentration, and (3) interactions with ions, proteins, or polysaccharides. A variety of intra- and extracellular proteins/peptides may form amyloid deposits. Human amyloidoses are classified as systemic, vascular and localized. Amyloidoses of the central nervous system include very rare diseases (cerebral angiopathy, prion diseases, British and Danish dementia) as well as those that are quite common, such as Parkinson's disease, Alzheimer's disease, Down syndrome, and frontotemporal dementia (Table 30.1).

Table 30.1 Human amyloidosis of the central nervous system.

	Neurodegenerative diseases with amyloid deposition					
	Alzheimer's		**Parkinson's**	**FTD**	**FBD/FDD**	**Prionosis (CJD/GSS)**
Amyloid		p-tau	α-synuclein	p-tau	ABri/ADan	PrP^{sc}
Affected brain areas	• Hipp. • Cortex	• Hipp. • Cortex	• Substantia nigra	• Hipp. • Cortex	• Cortex • Cerebellum	• Cortex • Cerebellum
Subcellular deposition	• Intraneuronal (cholinergic) • Extraneuronal (senile plaque) • CAA	• Intraneuronal (cholinergic)	• Intraneuronal (cholinergic)	• Intraneuronal (cholinergic)	• Extraneuronal • CAA	• Extraneuronal (spongiform plaque)

CAA, cerebral amyloid angiopathy; *CJD*, Creutzfeldt-Jakob disease; *FBD/FDD*, familial British dementia/familial Danish dementia; *FTD*, frontotemporal dementia; *GSS*, Gerstmann-Sträussler-Scheinker; *Hipp.*, hippocampus; *PrP*, prion promoter.

Alzheimer's disease and brain bioenergetics

During the last few decades, experimental and clinical data have strongly suggested different cellular mechanisms that ensure neuronal functional integrity as contributors for AD pathology at early stages, such as energy metabolism dysfunction (Kapogiannis & Mattson, 2011; Leuner, Muller, & Reichert, 2012). Neurons require large amounts of energy to meet the energy needs of memory formation and consolidation (Belanger, Allaman, & Magistretti, 2011; Schubert, 2005), and a prominent characteristic in AD patients is the inability to consolidate long-term memory, resulting in progressive memory deterioration in the elderly (Hampel et al., 2011). Large projection neurons with relatively long axons are the most damaged ones in AD. This type of neuron possesses high energy requirements and metabolic rates, making its functionality dependent on glucose availability and utilization.

It was not until the 1970s that alterations in mitochondrial ultrastructure were observed for the first time by electron microscopy in AD brains (Johnson & Blum, 1970; Wisniewski, Terry, & Hirano, 1970). Years later, *in vivo* glucose uptake studies by fluorodeoxyglucose-positron emission tomography (FDG-PET) indicated a topographic pattern of reduced glucose uptake in the parietal, temporal and posterior cingulate cortex in AD patients, that is evident even in early stages of the disease (Jagust, Reed, Mungas, Ellis, & Decarli, 2007). Furthermore, FDG-PET has been validated as a biomarker with prognostic value, since the largest decreases in glucose uptake correlate with more severe cognitive impairment throughout the continuum from normal cognition to mild cognitive impairment, and finally dementia (Minoshima et al., 1997).

After these findings, the concept of brain metabolic dysfunction as a potential component of the disease gained interest (Blass & Zemcov, 1984; Hoyer, 1993; Swerdlow et al., 1994). The efforts that followed were focused on trying to decipher the reasons for the reduced glucose uptake: decreased glucose transport through the blood–brain barrier (Marcus & Freedman, 1997), lower energy requirement due to reduced synaptic activity, and alterations in the activity of enzymes related to metabolism (Blass & Zemcov, 1984) were among the proposed hypotheses. In fact, shortly after that, deficiencies in glycolytic enzymes (Meier-Ruge, Iwangoff, & Reichlmeier, 1984), pyruvate dehydrogenase complex (Sorbi, Bird, & Blass, 1983), α-ketoglutarate dehydrogenase complex (Gibson et al., 1988), and some others enzymes from the tricarboxylic acid cycle were reported (Gibson, Starkov, Blass, Ratan, & Beal, 2010). Oxidative and nitro-oxidative protein damage and lipid peroxidation were also observed in areas where neurofibrillary tangles and amyloid plaques were present (Perry et al., 2000).

Furthermore, it has been suggested that mitochondria play a role in the mechanism by which Aβ triggers synaptic dysfunction and neurodegeneration. In this regard, the notion that both Aβ and APP can accumulate within mitochondria in AD brains was crucial (Fernandez-Vizarra et al., 2004; Hansson Petersen et al., 2008).

A potential impact of synaptic mitochondrial dysfunction on the development of the AD phenotype is an attractive hypothesis because synaptic terminals are very sensitive to bioenergetic defects due to their high energy demand, and synaptic loss is well accepted as both a cause of cognitive impairment and hallmark of AD pathogenesis.

Synaptic mitochondrial bioenergetics

The brain makes up only 2% of the body's mass but consumes 20% of its resting energy production. Brain function depends critically on an adequate energy supply, which is provided in the blood in the form of oxygen and glucose (Attwell & Laughlin, 2001). Energy is used mainly by neurons to maintain active processes important for synaptic transmission, such as reversal of ion movements following the opening of ion channels, vesicle recycling, replenishment of vesicles with neurotransmitters, and activation of downstream signaling cascades following Ca^{2+} entry.

In the adult brain, energy in the form of ATP is almost entirely generated by the complete oxidation of glucose. Mitochondria provide ~93% of the ATP generated (Sokoloff, 1960), with only ~7% coming from glycolysis, and also sequester and buffer cytoplasmic Ca^{2+}. Therefore, their localization in neurons can influence rapid ATP supply, Ca^{2+} homeostasis, and local regulation of Ca^{2+}-mediated neural signaling and plasticity (Knott, Perkins, Schwarzenbacher, & Bossy-Wetzel, 2008; MacAskill & Kittler, 2010; Mattson, Gleichmann, & Cheng, 2008).

Consistent with this, mitochondria are preferentially localized to pre- and postsynaptic sites (~1 mitochondrion on either side of most synapses) where ATP is consumed and Ca^{2+} enters (Chang, Honick, & Reynolds, 2006; Macaskill et al., 2009; Sakata & Jones, 2003). Although mitochondria from the cell body may produce some ATP that diffuses to the nerve terminal as phosphocreatine (Kuiper, Oerlemans, Fransen, & Wieringa, 2008), long-distance energy transport probably does not contribute much energy at the synapse. Instead, regional mitochondria in axons probably supply most of the energy at the synapse (Verstreken et al., 2005). Since mitochondria are formed at the soma, they are transported for long distances around neurons by kinesin and dynein motors, moving on microtubule tracks at approximately 0.3−1 μm/s (MacAskill, Atkin, & Kittler, 2010).

It was shown that the vesicle scission phase of endocytosis requires high energy levels (Heidelberger, 2001; Pathak et al., 2015). Indeed, attenuating endocytosis may help neurons preserve ATP when needed, as synaptic vesicle recycling likely uses much of the ATP at the synapse (Rangaraju, Calloway, & Ryan, 2014). In contrast, vesicle release from the readily releasable pool requires little ATP but depends on Ca^{2+} (Holz, Bittner, Peppers, Senter, & Eberhard, 1989; Pathak et al., 2015). Although endocytosis is far more sensitive to decreased energy than exocytosis, some ATP is still needed to facilitate exocytosis, presumably in part due to the ATPase activity of the

N-ethylmaleimide-sensitive factor, which is required before vesicle fusion (Kuner, Li, Gee, Bonewald, & Augustine, 2008).

Since cognitive function is tightly related to metabolic state, insufficient ATP may contribute to early disease-related changes in synaptic transmission (Keating, 2008; Yasuda, Nakata, Choong, & Mochizuki, 2013) even before synapses are lost. In this regard, defects in vesicle recycling have been linked to impairment in short-term synaptic plasticity, memory and cognition (Murthy & De Camilli, 2003).

Involvement of "synaptopathy" in Alzheimer's disease pathogenesis

Several experimental evidence supports the hypothesis that AD is a spine pathology (Selkoe, 2002; Sivanesan, Tan, & Rajadas, 2013) and that soluble Aβ oligomers are causative of AD synaptopathy. *In vitro* studies have shown that Aβ oligomers induce the formation of pore-like structures on the plasmatic membrane (Arispe, Rojas, & Pollard, 1993; Lashuel, Hartley, Petre, Walz, & Lansbury, 2002) and thus interfere with glutamatergic transmission. Moreover, it was reported that Aβ oligomers decrease the number of AMPA and NMDA receptors as well as the expression of PSD-95 protein, a postsynaptic density marker (Roselli et al., 2005). Thus, Aβ oligomers would be the culprits of the reduced strength and plasticity of excitatory synapses (Chapman et al., 1999; Walsh et al., 2002), basically as a result of structural alterations. However, a stereological analysis of the CA1 hippocampal region of transgenic rats that accumulate discrete amounts of Aβ oligomers, and do not develop senile plaques, has revealed that in contrast to control animals, both the number of synapses/mm^3 and PSD-95 protein levels remain unaltered (Martino Adami, Galeano, et al., 2017; Martino Adami, Quijano, et al., 2017). Together, this evidence suggests that the underlying intracellular mechanisms mediated by Aβ oligomers and involved in synaptic functionality may be related to synaptic mitochondrial dysfunction, rather than to synaptic structural alterations.

Even though experimental data fully demonstrated that neuronal mitochondrial dysfunction is a pathological hallmark of AD, employing isolated brain mitochondria possesses some limitations, because preparations include mitochondria from both neurons and glia, and the environment and substrate availability (artificial buffers with saturating levels of substrates) differ considerably from the physiological ones (Gerencser et al., 2012). In this context, the use of isolated nerve terminals (synaptosomes) offers some advantages (Table 30.2).

The synaptosome is an isolated nerve terminal or varicosity whose axonal attachment or attachments have been pinched off by shearing forces during homogenization, which then reseal to form synaptosomes. The postsynaptic density often remains attached, presumably because of the transsynaptic protein complexes that physically link the two compartments. The enrichment of synaptic fractions from homogenized brain tissue was first performed in the late 1950s (Hebb & Whittaker, 1958) and validated in the 1960s (Gray & Whittaker, 1962) (Fig. 30.1).

Table 30.2 Impact of the type of sample in the study of brain bioenergetic parameters.

	Biological material	
	Isolated mitochondria	**Intact synaptosomes**
Advantages	• Quick isolation • High-yield purification • Reagents and substrates can be added directly • Methods are generally very well established	• Mitochondria are exclusively neuronal • Presynaptic metabolism, mitochondrial function, plasma membrane excitability, receptors, ion channels, and machinery for the exocytosis and reuptake of neurotransmitters is preserved • Mitochondria exist surrounded by a physiological cytoplasm and a plasma membrane
Disadvantages	• Preparations include mitochondria from both neurons and glia • Environment and substrate availability depart considerably from the physiological	• Time-consuming purification • Low-yield purification • Many reagents and substrates are synaptosome-impermeable

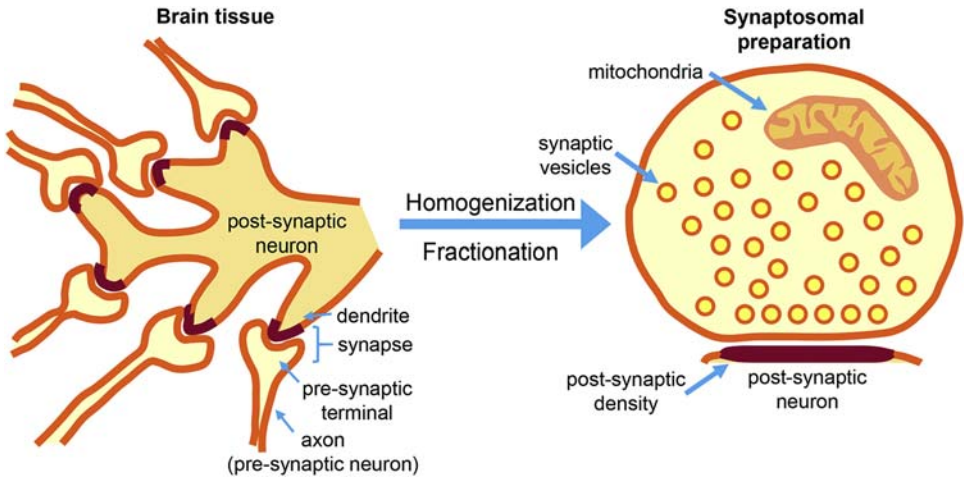

Figure 30.1 ***Schematic representation of synaptosome generation.*** In brain tissue, dendrites of post-synaptic neurons interact with the pre-synaptic terminal of a pre-synaptic neuron to generate what is known as a synapse. Synaptosomes are generated by homogenization followed by fractionation (on the basis of density). The synaptosome is a subcellular fraction of an isolated synapse containing mitochondria and synaptic vesicles.

Synaptosomes largely preserve the metabolism, mitochondrial function, plasma membrane excitability, receptors, ion channels, and machinery for the exocytosis and reuptake of neurotransmitters characteristic of the intact presynaptic terminal *in situ* (Nicholls, 1993, 2003). A major attraction of synaptosomes is that they represent the simplest possible mammalian "mini-cell" in which mitochondria exist, surrounded by a physiological cytoplasm and a plasma membrane. In this way, synaptosomes are expected to resemble the mitochondrial phenotype *in vivo* more closely than isolated mitochondria. Finally, they also possess the considerable advantage, in contrast to primary neuronal cultures, that preparations can be made from experimental animals of any age, thus allowing critical age-related changes to be monitored.

Synaptosomal bioenergetics has been monitored in several studies using conventional oxygen electrode chambers, which is a technique with limited sensitivity. Recently, this restriction has been solved with the availability of Seahorse respirometer technology (Choi, Gerencser, & Nicholls, 2009), which allows the estimation of basal respiration, mitochondrial ATP turnover, proton leak, and spare respiratory capacity (SRC) (Brand & Nicholls, 2011) by the sequential addition of oligomycin, a protonophore, and antimycin A. A list with the bioenergetic parameters that can be determined *in vitro* is detailed in Table 30.3.

Seahorse technology has been exploited to investigate the hypothesis that a restriction in SRC is a manifestation of bioenergetic dysfunction in AD models (Choi et al., 2012). SRC reflects the ability of mitochondria to meet increased energy demand with increased respiration and is considered a primary factor in defining the survival of neurons under stress (Brand & Nicholls, 2011).

Synaptosomal bioenergetic defects in Alzheimer's disease transgenic rodent models

Even though it has been reported in multiple *in vitro* studies that Aβ is toxic for isolated mitochondria (Guo, Ersoz, Butterfield, & Mattson, 2000; Moreira, Santos, Moreno, Rego, & Oliveira, 2002), only a few have assessed mitochondrial bioenergetic decline in synaptosomes, which are expected to resemble the mitochondrial phenotype *in vivo* more closely than isolated mitochondria. In the following section, published results of transgenic mouse and rat models of AD will be discussed.

J20 mice

J20 mice (C57BL/6 × DBA/2 F2 strain) overexpress human APP cDNA with the Swedish double mutation (K670N/M671L) and the Indiana mutation ($APP_{Swe/Ind}$) under the platelet-derived growth factor promoter, and exhibit Aβ deposits and synaptic loss as early as 5–7 months of age (Mucke et al., 2000). It has been reported that mitochondria in digitonin-permeable synaptosomes from 4-month-old transgenic

Table 30.3 Bioenergetic parameters obtained by in vitro determinations.

Instrument	Biological preparation	Parameter	Definition
Oxygraph (clark-type oxygen electrode)	Isolated mitochondria, permeated cells and synaptosomes	State 4	Respiration in the absence of ADP (resting respiration)
		State 3	Respiration in the presence of ADP (maximum physiological O_2 consumption coupled to ATP production).
		RCR	Regulation of the rate of oxidative phosphorylation by ADP level
Extracellular flux analyzer (Seahorse)	Intact cells and synaptosomes	Basal respiration	Energetic demand of the cell under baseline conditions
		ATP synthesis-linked respiration	Respiration associated with ATP synthesis that contributes to meeting the energetic needs of the cell
		Proton leak	Remaining basal respiration not coupled to ATP production
		Maximum respiration	Maximum rate of respiration that the cell can achieve
		Nonmitochondrial respiration	Oxygen consumption corresponding to cytosolic enzymes
		SRC	Capability of the cell to respond to an energetic demand as well as how closely the cell is to respiring to its theoretical maximum
		Coupling efficiency	Fraction of basal mitochondrial oxygen consumption used for ATP synthesis
		CRC	The best general measure of mitochondrial function in cell populations. Reports in a single experiment all the parameters obtained in seahorse respirometer.
		BHI	Composite mitochondrial profile for a selected cell type

BHI, bioenergetic health index = *SRC* × *ATP synthesis-linked respiration/nonmitochondrial respiration* × *proton leak*; *CRC*, cell respiratory control; *RCR*, respiratory control ratio; *SRC*, spare respiratory capacity.

mice showed increased mitochondrial permeability transition, decline in both respiratory function and cytochrome *c* oxidase activity, and increased mitochondrial oxidative stress (Du et al., 2010). However, intact cortical and hippocampal synaptosomes from fully symptomatic J20 mice (6 months of age) showed no bioenergetic defects and maintained respiratory capacity, membrane potential and intracellular calcium handling capacity, as well as age-matched wild-type (WT) control mice, under both resting and stress conditions (Choi et al., 2012).

Tg2576

Tg2576 mice are one of the most widely used transgenic mouse models overexpressing human APP_{Swe} (C57BL/6 strain). In this model, APP695 with the Swedish mutation is driven under the control of hamster prion promoter, allowing transgene expression in the forebrain regions and spinal cord (Hsiao et al., 1995). Tg2576 mice develop thioflavin S−positive Aβ plaques at around 10−12 months of age along with other pathological changes. Tg2576 mice also produce oligomeric Aβ, which seems to be more toxic than fibrillar or aggregated Aβ in plaques (Lesne et al., 2006). Even though it has been reported that whole brain mitochondria from 6-month-olds exhibited impaired state 3 and uncoupled respiration (Gillardon et al., 2007), cortical synaptosomes from fully symptomatic Tg2576 mice (16 months of age) showed no bioenergetic defects (Choi et al., 2012).

Amyloid precursor protein/presenilin 1

APP/PS1 mice (C3H/HeJ x C57BL/6J strain) overexpress mouse/human chimeric APP695 with the Swedish mutation and human mutated PS1 (A246E) under the control of mouse prion promoter. These mice exhibit an increased $A\beta_{42}/A\beta_{40}$ ratio, Aβ plaques at 9 months of age, and dystrophic neurites and gliosis at 12 months of age (Borchelt et al., 1997). Cortical synaptosomes from fully symptomatic transgenic mice showed no bioenergetic defects at 9 months of age but exhibited increased basal respiration and proton leak, as well as decreased coupling efficiency and cell respiratory control (CRC) at 14 months of age, although this finding was not replicated in age-matched hippocampal synaptosomes (Choi et al., 2012).

5xTg

5xTg mice (C57BL/6 × SJL F1 strain) overexpress high levels of APP with the Swedish, Florida (I716V) and London (V717I) mutations, as well as double-mutated PS1 (M146L; L286V). These mice rapidly develop severe amyloid pathology. They accumulate high levels of intraneuronal $A\beta_{42}$ around 1.5 months of age, with amyloid deposition rapidly following at around 2 months of age. Plaques spread throughout the hippocampus and cortex by 6 months of age. Gliosis also begins at around 2 months of age, developing

in parallel with plaque deposition. Synapse degeneration is also observed at approximately 4 months of age (Oakley et al., 2006). It was recently reported that mitochondria from digitonin-permeable synaptosomes from 4- and 9-month-old transgenic mice exhibit decreased state 3 respiration in the presence of glutamate and malate (Wang et al., 2016). However, bioenergetic properties of permeable synaptosomes depart substantially from those of intact ones.

McGill-R-Thy1-APP

McGill-R-Thy1-APP rats (HsdBrI:WH Wistar strain) harbor the human APP751 transgene with the Swedish and Indiana mutation under control of the murine Thy1.2 promoter. Transgenic animals accumulate Aβ intraneuronally from postnatal week 1; only homozygous rats display plaque deposition from 6 months of age, and microgliosis and dystrophic neurites from 12 months of age (Leon et al., 2010). Lately, it has been reported that hippocampal synaptosomes from 6-month-old hemizygous rats, which mimic early AD (Galeano et al., 2014), exhibit decreased SRC and CRC (Martino Adami, Galeano, et al., 2017; Martino Adami, Quijano, et al., 2017) in contrast to what has been found in synaptosomes from AD mouse models.

A comparison of brain bioenergetics parameters described in different rodent transgenic animal models of AD can be found in Table 30.4. The discrepancies between findings with mouse isolated mitochondria and mouse intact synaptosomes could be explained by the possibility that isolated mitochondria are exposed to Aβ during homogenization, and the detected deficiencies are the result of an acute Aβ exposure. In addition, free mitochondria in buffers with saturating levels of substrates will exhibit a different bioenergetic profile than mitochondria within synaptosomes, which are surrounded by cytosol and plasma membrane (Gerencser et al., 2012). This difference may include redistribution of control over respiration, and therefore the same process deficit will result in a different overall decline in respiration (Brand & Nicholls, 2011). Furthermore, the fact that synaptosomal bioenergetic defects are detected only in rat models of AD could imply that rats are physiologically, genetically and morphologically more similar to humans.

Modulation of synaptosomal bioenergetics in hemizygous McGill-R-Thy1-APP rats

Few reports have attempted to assess whether dysfunctional synaptosomal bioenergetics have a negative impact on cognition. When hemizygous McGill-R-Thy1-APP rats were administered pyrroloquinoline quinone, an antioxidant compound (Stites, Mitchell, & Rucker, 2000), from weaning to 6 months of age, SRC in hemizygous animals was restored to WT control levels. This restoration was accompanied by prevention of the cognitive deficits displayed by hemizygous rats, suggesting that synaptosomal

Table 30.4 Determination of brain bioenergetic parameters in different rodent transgenic animal models of Alzheimer's disease.

		Alteration of bioenergetic parameters			
		Synaptosomes		Isolated mitochondria	
Animal model	**Strain**	**Intact**	**Digitonin-permeable**		**References**
J20	C57BL/6 × DBA/2 F2 (mouse)	No bioenergetic defects detected	• ↑ mitochondrial permeability transition • ↓ respiratory function • ↑cytochrome c oxidase activity • ↑ mitochondrial oxidative stress	N.D.	Du et al., 2010 Choi et al., 2012
Tg2576	C57BL/6 (mouse)	No bioenergetic defects detected	N.D.	↓ state 3	Gillardon et al., 2007 Choi et al., 2012
APP/PS1	C3H/HeJ x C57BL/6J (mouse)	• ↑ basal respiration • ↑ proton leak • ↓ coupling efficiency • ↓ CRC	N.D.	N.D.	Choi et al., 2012
5xTg	C57BL/6 × SJL F1 (mouse)	N.D.	↓ state 3	N.D.	Wang et al., 2016
McGill-R-Thy1-APP	HsdBrI:WH wistar (rat)	• ↓ SRC • ↓ CRC • ↓ complex I activity	N.D.	• ↓ state 3 • ↓ state 4 • ↑ H_2O_2 • ↑ TBARS	Martino Adami, Galeano, et al., 2017; Martino Adami, Quijano, et al., 2017

CRC, cell respiratory control; *N.D.*, not determined; *SRC*, spare respiratory capacity; *State 3*, active respiration; *State 4*, resting respiration; *TBARS*, thiobarbituric acid reactive substances, indicative of accumulation of lipid oxidation products.

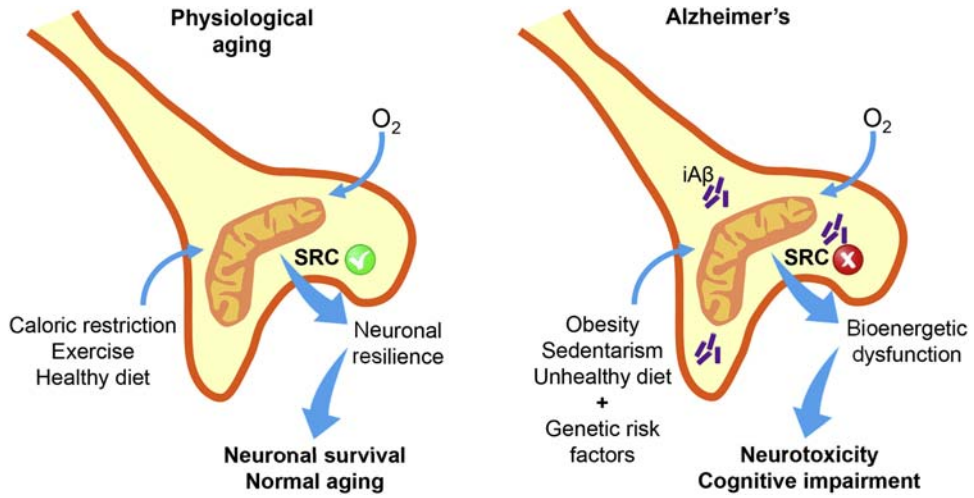

Figure 30.2 ***Induction of mitochondrial functionality may be a strategy for the treatment of synaptic metabolic dysfunction in early Alzheimer's disease.*** During physiological aging, mitochondria from presynaptic terminals consume oxygen for respiration, resulting in normal spare respiratory capacity (SRC) to sustain active processes important for synaptic transmission. Caloric restriction, exercise, and a healthy diet promote mitochondrial functionality, neuronal survival, and normal cognition. By contrast, in the Alzheimer's brain, Aβ deposits at the presynaptic terminal may interact with mitochondria to impair oxygen consumption, resulting in decreased SRC. Obesity, a sedentary lifestyle, and an unhealthy diet, in addition to genetic risk factors, promote bioenergetic dysfunction, neurotoxicity and cognitive impairment.

bioenergetic defects are associated with cognitive impairment (Martino Adami, Galeano, et al., 2017; Martino Adami, Quijano, et al., 2017). This concept was reinforced when hemizygous McGill-R-Thy1-APP rats were fed, also from weaning to 6 months of age, a high-fat/high-sugar diet, a well-known cause for metabolic dysregulation. In this case, hemizygous animals exhibited a reduced bioenergetic health index and worsened cognitive impairment (Martino Adami, Galeano, et al., 2017; Martino Adami, Quijano, et al., 2017). A schematic representation of mitochondrial functionality as a potential strategy for the treatment of synaptic bioenergetic dysfunction in early AD is shown in Fig. 30.2.

Key facts of brain bioenergetics and Alzheimer's disease

- The brain makes up only 2% of the body's mass but consumes 20% of its resting energy production.
- In the adult brain, mitochondria provide ~93% of the ATP generated, with only ~7% coming from glycolysis.
- Neurons require large amounts of energy to meet the energy needs of memory formation and consolidation.

- AD is the first cause of dementia and represents a serious medical problem that severely affects social, sanitary, and economic aspects of human life and its populations.
- The pathophysiological process of AD is thought to begin many years before the appearance of dementia symptoms.
- Early diagnostic methods or specific treatments are not available.
- *In vivo* glucose uptake studies in AD patients show a topographic pattern of reduced neuronal metabolism that is evident even at early stages of the disease.

Summary points

- This chapter focuses on the impact of presynaptic energy dysfunction on cognitive functioning at early stages of AD.
- Transgenic models of AD provide insight into bioenergetics dysfunction that occurs in vivo at early stages of the disease.
- Therefore, the utility of animal models as a platform for understanding the earlier biological basis of AD and its pharmacologic manipulation is reinforced.
- The harmful impact of unhealthy diet, obesity, sedentary lifestyle, and genetic risk factors on brain bioenergetics and ultimately disease progression in a transgenic model of AD, supports the notion that targeting synaptosomal bioenergetic defects may be a potential strategy to slow or prevent the neurodegenerative process and restore neuronal function.

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CHAPTER 31

Limitations of amyloid imaging in Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
DMT disease-modifying therapy
LBD Lewy body dementia
MCI mild cognitive impairment
NFT neurofibrillary tangles
PET positron emission tomography

Mini-dictionary of terms

Amyloid-beta protein plaques Concretions formed by smaller protein fragments (amyloid-beta peptides or oligomers) that aggregate to become insoluble protein deposits between neurons (extracellular)
False negative When the result of a diagnostic test incorrectly indicates that an abnormality is absent
False positive When the result of a diagnostic test incorrectly indicates that an abnormality is present
Mild cognitive impairment In neurodegenerative diseases, the stage between age-related cognitive decline and dementia when impairments do not interfere with daily function
Neuritic versus diffuse beta-amyloid plaques Neuritic plaques contain amyloid-beta and neuronal fragments with tau as well as astrocytes and microglia, whereas diffuse plaques only contain amyloid-beta.
Phenocopy For sporadic medical conditions, when a patient demonstrates a pattern of signs and symptoms that are identical to a disease but biologic evidence for that condition is absent

Introduction

For more than a century, the two characteristic neuropathological hallmarks of Alzheimer's disease (AD)—extracellular deposits of plaque consisting of amyloid-β protein and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein—could be verified accurately only by postmortem assessment. Now nearly 20 years past, the discovery that positron emission tomography (PET) radioligands could be developed that reliably bind to fibrillar amyloid-β (amyloid) neuritic plaque was groundbreaking for AD research (Mathis, Mason, Lopresti, & Klunk, 2012). Amyloid-PET imaging has accelerated the understanding of AD and related

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neurodegenerative disorders causing cognitive impairment, has provided dementia specialists valuable information to make a more specific diagnosis and enhance management in patients with mild cognitive impairment (MCI) and possible AD (Grundman et al., 2013), and has helped investigators select more appropriate candidates for AD therapeutic trials. As with any diagnostic test, however, appreciation of the additional value of amyloid-PET imaging should be a measured one with due consideration given to its limitations. The primary aim of this chapter is to outline some of these limitations in the different contexts in which this imaging modality has been found to be valuable.

Amyloid imaging for diagnosis of early or mild dementia

The neuropathologic diagnosis of AD requires moderate or frequent amyloid-β neuritic plaque deposition (hereafter referred to as elevated amyloid pathology). Amyloid-PET radiotracers have been validated to detect elevated amyloid pathology using the modified Consortium for Establishing a Registry for AD scoring method as a truth standard (Mirra et al., 1991) through prospective postmortem studies leading to approval for clinical use. The specificities, or reliability that a normal scan excludes the presence of elevated amyloid pathology, range between 88% and 100%, and sensitivities, or ability for amyloid imaging to detect elevated amyloid pathology, range between 88% and 98% (Clark et al., 2012; Curtis et al., 2015; Sabri et al., 2015). Because the neuropathologic diagnosis of AD is not made when NFTs are absent or confined to the transentorhinal region—i.e., not extending to neocortical brain regions (called Braak stages III and IV)—an amyloid-PET scan demonstrating elevated amyloid pathology cannot alone establish a diagnosis of AD. The package inserts of the approved, commercially available amyloid-PET radiotracers emphasize this limitation.

The radiologic assessment of amyloid imaging for clinical use is based on detection of areas of binding of F-18 amyloid radiotracers to cerebral cortical fibrillar amyloid, superficial to the expected confluent nonspecific deep and subcortical white matter labeling that is an inherent property of these tracers, causing a loss of normal cortical grey–subcortical white matter differentiation (Fig. 31.1). When two or more cortical areas demonstrate this loss of contrast, the scan may be interpreted as positive for elevated amyloid. The approved assessment is visual, and the reported result is binary; the scan is either positive or negative for elevated amyloid pathology. Quantitative assessments of amyloid deposition are utilized in imaging research studies; several different methods are available, but none are approved for clinical use. Nonetheless, evidence from these studies can be applied to more adequately interpret the clinical significance of amyloid imaging results.

Adequate interpretation of the clinical significance of positive and negative amyloid-PET scan results requires a review of the prevalence of positive amyloid-PET scans and

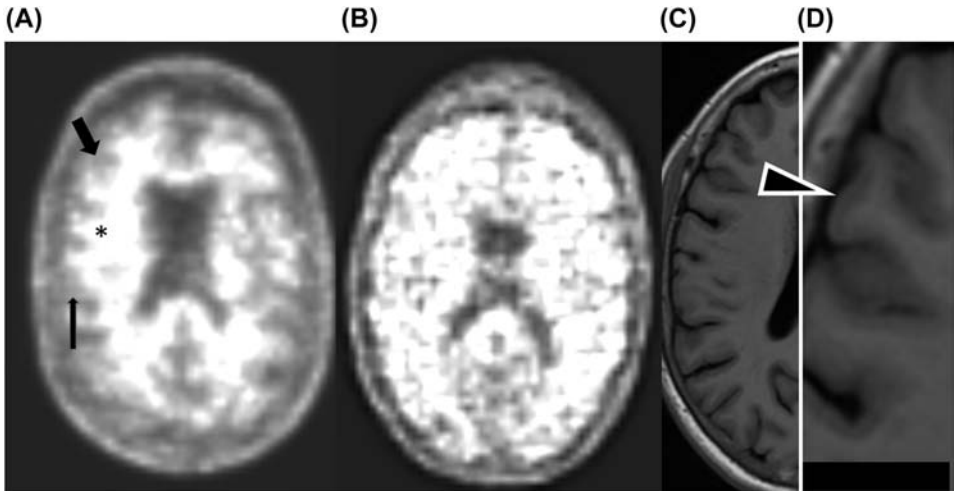

Figure 31.1 ***Visual assessment of an amyloid-PET scan: absence or presence of cortical gray matter binding.*** (A): Negative amyloid-PET scan. Only nonspecific white matter binding is present with finger-like projections of subcortical white matter (*) but no appreciable uptake in gray matter (*arrows*), with intact gray–white matter differentiation indicating no to sparse β-amyloid plaque deposition. (B): Diffuse pattern of increased tracer labeling in cortical gray matter with loss of gray–white matter differentiation consistent with moderate to severe β-amyloid pathology. (C and D): Axial T1-FLAIR MRI sequence at same level to illustrate normal cortical gray–subcortical white anatomy: ribbon of cortical gray matter with subcortical white matter interdigitation (*arrowhead*).

the severity of amyloid burden in different populations in relation to the basic epidemiology of AD:

- With advancing age, the prevalence of positive amyloid-PET scans (the proportion relative to negative scans, also referred to as percentage of amyloid-PET positivity) in cognitively unimpaired individuals increases, and relatively sharply after age 75 (Villemagne et al., 2013), which is in line with neuropathologic studies showing that a subset of individuals with histopathologic evidence of AD remain nondemented during life (SantaCruz et al., 2011). In the very elderly, the prevalence of amyloid-PET positivity approaches 50% (Jansen et al., 2015). The proportion of elderly nondemented individuals at autopsy without evidence for any neuritic plaque deposition (sparse, low, moderate, or frequent) is uncommon; "exceptional agers," age $\geq$ 85, without evidence of AD pathology are rare (Vemuri et al., 2017).
- The prevalence of amyloid-PET positivity is higher—about 90%–95%—in younger AD dementia patients (<70), compared with elderly AD patients, when AD is defined by clinical criteria—about 80% (Ossenkoppele et al., 2015). The density and distribution of amyloid plaques is greater in younger AD patients than in elderly AD dementia patients (Tellechea et al., 2018). While on the surface this finding may seem paradoxical, a plausible explanation is that younger-onset AD patients have more cognitive reserve, leading to a longer period of downstream effects of toxic

species of soluble amyloid (which may include a longer period of fibrillar cortical amyloid deposition) before cognitive symptoms develop (Marshall, Fairbanks, Tekin, Vinters, & Cummings, 2007).

- The incidence of new clinical diagnoses of AD increases with age (Satizabal et al., 2016), but the ability to discriminate between subjects with and without dementia diminishes. The relationship between dementia and AD pathology (including NFTs) is strong until about 75 years of age (Savva et al., 2009) and then considerably weaker above age 80 (Vandenberghe, Adamczuk, Dupont, Laere, & Chetelat, 2013).

Amyloid-PET positivity in older dementia patients is less specific for AD as the primary cause of the impairment compared with younger patients. In those older than 75–80, positivity may be related to patient age, be of no clinical significance in relation to symptomatology, or signify AD as a comorbid condition in a mixed dementia, not necessarily the primary etiology. Other neurodegenerative disorders, such as Lewy body dementia (LBD), are not infrequently associated with elevated amyloid pathology. Various quantitative methods of measuring amyloid binding suggest that LBD is associated with greater amyloid deposition than in idiopathic Parkinson's disease (Donaghy, Thomas, & O'Brien, 2015) and lower tracer binding compared with that in AD patients, but a binary visual assessment does not differentiate between AD and DLB adequately.

Although rare, false-positive amyloid-PET results do occur (Landau, Horng, Fero, Jagust, & Alzheimer's Disease Neuroimaging, 2016). Although amyloid radiotracers have higher binding affinity to neuritic amyloid plaques, binding to diffuse plaques, if extensive, has been found in DLB patients and in cerebral amyloid angiopathy related to binding to cortical blood vessel amyloid (Ikonomovic, Fantoni, Farrar, & Salloway, 2018).

In most circumstances, a negative amyloid-PET scan result excludes AD and can help differentiate between AD and a frontotemporal dementia when the presentation is similar. The exception is in an older dementia patient in whom after a comprehensive evaluation there is a high pre-PET probability of AD (Bergeron, Ossenkoppele, & Laforce, 2018). After about age 75, and rising more sharply after age 80, the predictive value of a negative scan to exclude AD decreases. As a hypothetical example, based on an approximate sensitivity to detect elevated amyloid pathology of 92%, were a clinician to order amyloid imaging routinely on elderly (age >80) patients whose estimated likelihood of having AD is high (80%), the probability a negative PET scan that excludes AD decreases to about 60%—i.e., the possibility that elevated amyloid pathology is present despite a negative scan approaches 40% (Fig. 31.2). The patients in this situation may meet the core clinical criteria for probable AD, however, and the use of amyloid imaging may be considered inappropriate (Johnson et al., 2013).

A negative amyloid-PET scan should also be interpreted carefully when structural brain imaging reveals focal cerebrovascular lesions. Autopsy studies have shown that the coexistence of lacunes and larger infarcts may result in a higher likelihood of a clinical diagnosis of AD when AD pathology burden is low compared with when vascular

	80% estimate to have AD = 160 patients	20 % estimate that not AD-dementia = 40 patients
	160 patients + 16 patients with Comorbid Age-Related elevated amyloid pathology = 176 patients Truth standard **(+)**	BUT: About 40% may have comorbid Age-related elevated amyloid pathology 40% X 40 patients ≈ 16 non-AD dementia individuals 24 patients without elevated amyloid pathology, Truth standard **(-)**
PET scan Positive Based on 92 % sensitivity	176 X 0.92 = 162 scans True Positive	1 scan False Positive
PET Scan Negative (97% specificity)	176 X 0.08 (false negative rate is 1-92%) = **14 False Negative scans**	24 X 0.97 ≈ 23 **23 True Negative scans**

Figure 31.2 ***Predictive value of a negative amyloid-PET scan to exclude the diagnosis of AD in the elderly (age $\geq$ 80).*** Hypothetical example if obtaining amyloid imaging routinely on 200 elderly early dementia patients in whom the clinician estimates pre-PET probability of AD being present as approximately 80% to show that a normal amyloid-PET scan may not exclude AD in about 40% of cases. Negative Predictive Value $= \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Negatives}} = \frac{2_3}{3_8} \approx 61\%$. *Probability amyloid-PE-negativity excludes AD is about 61%, meaning probability of AD being present, despite PET negativity is close to 40%.*

lesions are absent; when AD pathology burden is moderate or high, the presence of vascular lesions does not influence the (considerably higher) likelihood that a clinical diagnosis of AD will be made during life (Dodge et al., 2017). Since amyloid imaging is not expected to detect low or sparse neuritic amyloid plaque, a negative scan in a dementia patient with these vascular lesions may signify an AD "phenocopy," possibly with a slower clinical course (Ossenkoppele et al., 2015)

Advanced cortical atrophy may influence ability to visualize loss of gray–white matter contrast. When extensive white matter pathology is present, the normal nonspecific radiotracer binding in subcortical white matter may be diminished, with regional signal reductions (Schmidt et al., 2015). Incorrect visual interpretation of PET images in these situations may be minimized using structural imaging, brain CT, or MRI to understand the extent of the atrophy and subcortical white matter microvascular change.

Amyloid imaging for diagnosis and prognosis of mild cognitive impairment

Assuming accurate, a negative amyloid-PET scan reliably excludes AD as the etiology of cognitive impairments in MCI patients. Because amyloid neuritic plaque is still accumulating, albeit at a decelerating rate, in patients with MCI due to AD

(Villemagne et al., 2011), the fact that a negative scan can reliably exclude AD may at first seem counterintuitive. Amyloid-beta deposition, however, is an early, upstream pathologic event, most likely reflecting the burden of toxic amyloid-beta peptide oligomers, starting several years prior to early symptoms of AD (Selkoe, 2008), and evidence increasingly suggests that cognitive symptoms are not a direct result of amyloid accumulation (Hyman, 2011). By the time symptoms and object cognitive impairment are evident, more directly the result of downstream pathology—elevated tau and neuronal and synaptic loss—an MCI patient can be expected to have elevated amyloid pathology on PET imaging when AD is the primary etiology accounting for the condition (Fig. 31.3).

Disclosure of normal amyloid imaging results after information conveyed that the scan does not show elevated amyloid burden could include qualifications that a non-AD neurodegenerative disorder may be present, or in younger individuals with a longer expected lifespan, a practitioner could counsel that AD could develop as a separate condition a decade or more later (Grill et al., 2017).

The diagnostic value of amyloid imaging for MCI patients is closely tied to its prognostic value given that the clinical diagnosis of probable AD requires functional impairments related to subsequent cognitive decline that interfere with carrying out daily tasks. Amyloid-PET positivity is associated with cognitive decline but as a single

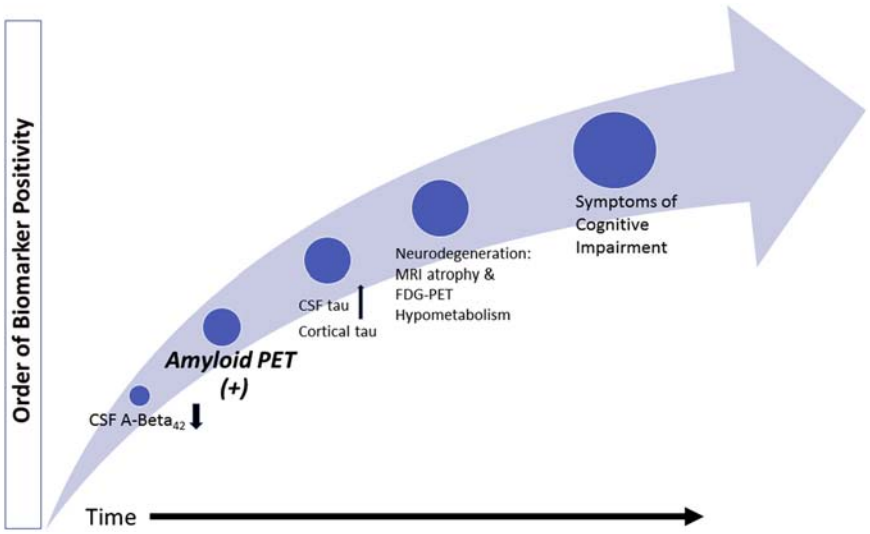

Figure 31.3 ***Model of order of biomarker positivity in Alzheimer's disease over time.*** A biologic model to show the chronology of pathologic events in Alzheimer's disease. Amyloid-PET imaging shows elevated brain amyloid early on, along the continuum of degeneration. *A-Beta$_{42}$*, a toxic amyloid-beta peptide oligomer; *CSF*, cerebrospinal fluid; *FDG*, fluorodeoxyglucose; *PET*, position emission tomography.

biomarker of AD has weaker ability to predict when an individual patient may progress or convert to AD dementia compared with combining with a biomarker of brain degeneration, such as fluorodeoxyglucose-PET (Prestia et al., 2015). Individual studies and meta-analyses assessing the probability and rate of conversion of MCI to AD demonstrate that amyloid-PET-positive MCI patients are more likely to progress to a dementia stage of AD compared with amyloid-PET-negative subjects, but the prognostic value of amyloid positivity is more limited than amyloid negativity (Ma et al., 2014). Amyloid negativity predicts high likelihood of stability (very low probability of conversion to AD) "up front," regardless of follow-up length, whereas ability of amyloid positivity to predict conversion to AD improves with longer follow-up. Quantitative assessment of amyloid deposition may better predict progression (Ben Bouallegue, Mariano-Goulart, Payoux, & Alzheimer's Disease Neuroimaging, 2017).

By the time a practitioner considers amyloid imaging for some individuals with MCI of uncertain etiology, subtle or more obvious functional impairment may already have emerged, patients meet the clinical criteria for probable AD (Morris, 2012), and the limitations of amyloid imaging for early or mild dementia may be appropriate to consider.

Impact of amyloid imaging on patient management

Both amyloid-positive and amyloid-negative PET results influence clinical decision-making, leading to meaningful changes in diagnosis, diagnostic confidence, and management. The results can help a practitioner select appropriate pharmacologic treatments for symptoms, avoid unnecessary testing, and tailor approaches to counseling and planning for the future based on a better understanding of the clinical course with a more specific etiologic diagnosis (Fantoni, Chalkidou, O'Brien, Farrar, & Hammers, 2018). A large Medicare-sponsored clinic-based trial is comparing the dementia specialist's intended management, as if amyloid imaging was not available, with treatment decisions made after scan results are known. Preliminary results indicate that management decisions change in about two-thirds of cases (Rogers, 2017). Another recent prospective study has compared the management decisions taken over 1 year by a group of treating physicians who received amyloid imaging results immediately (the "information group") with the decisions made by a control group of physicians who did not receive the PET amyloid status until the end of the study. Changes in patient management were greater in the information group, but no significant difference was observed in cognitive change from baseline or health outcomes at 1 year when patient groups were compared (Pontecorvo et al., 2017). In the absence of an effective disease-modifying therapy (DMT) for AD, which helps slow neurodegeneration as well as cognitive and functional decline, whether these refinements will improve long-term

patient outcome remains to be seen. When a specific therapy for early AD is proven to be safe and effective to help slow neurodegeneration and cognitive decline, use of amyloid imaging in clinical practice may change substantially. Irrespective of the mechanism of action of a DMT, a practitioner may need to determine degree of amyloid burden before deciding who should receive what will almost certainly be an expensive treatment with potential side effects. A limitation of utilization may include the relatively high cost of amyloid imaging (Insel et al., 2016), financially straining a health care system's financial resources, with a potential impact on equitable access (Witte et al., 2015).

Radiologic diagnosis for mild cognitive impairment or early dementia

The required binary visual assessment may pose a challenge for the radiologist when confronted with equivocal or indeterminate scan images (Weidman et al., 2017) or when an intermediate degree of amyloid deposition may be present (Morris et al., 2016). Lack of a clear distinction between normal and positive findings may also be due to technical factors, such as timing of scan acquisition, inadequate resolution, head angle, motion artifact, and scanner function (Trembath, Newell, & Devous, 2015). The proficiency of the radiologist can influence accuracy of the result (Weidman et al., 2017). Even for a trained and experienced nuclear medicine physician, there will occasionally be a false-positive or false-negative result. Furthermore, in studies comparing the visual "read" with a quantitation of cortical amyloid deposition, multiple well-trained and experienced readers had to reach a consensus for a scan to be classified as positive or negative. The "real world" practice of the clinician ordering amyloid imaging is naturally different—a single radiologist will be reading the scan. In some memory centers, dementia specialists may review the scan images themselves but may not have access to a quantitative assessment for comparison when scans appear to be of lower quality, or a potentially misleading radiologic diagnosis is suspected, in equivocal or intermediate cases (Morris et al., 2016).

While the visual assessment and quantification of amyloid have been shown to have a similar accuracy (Schreiber, Landau, Fero, Schreiber, & Jagust, 2015), they could be complementary to each other—i.e., combined they may be more accurate than either method alone (Yamane et al., 2017, reference below). Even for the proficient radiologist, availability of a quantitative measurement in visually equivocal cases would likely be more adequate than relying solely on the binary read (Bullich et al., 2017). Even if a quantitation method is approved for an amyloid-beta radiotracer, the additional knowledge, equipment, and software required to determine a quantitative measurement may be too onerous to implement and utilize efficiently in daily radiologic practice.

Subjective cognitive decline

As a group, patients who present to memory centers with cognitive concerns starting less than 5 years prior, but without deficits on examination and meeting criteria for subjective cognitive decline, have been shown to be at higher risk for AD (Eckerstrom et al., 2017). Amyloid-PET positivity cohorts may be associated with a higher report of subjective concerns compared with amyloid-negative participants enrolled in longitudinal aging studies (Amariglio et al., 2015), but a practitioner could consider a confounding factor, that amyloid positivity is greater in those with family history of AD, and that history may heighten concern in patients with subjective symptoms, triggering them to seek medical attention.

Presently, amyloid imaging for individuals without objective cognitive impairment is investigational and used in prevention trials but presently does not have a clinical purpose (Johnson, 2013). Standard of care may change should an effective preventative DMT for individuals at higher risk of AD become available, but at what age (e.g., midlife vs. older adulthood) amyloid imaging should be performed may remain poorly defined. Amyloid positivity could be associated with more imminent disease risk than a positive genetic test result (e.g., within the next few years vs. at some point in one's lifetime), but the precise timeframe for conversion probably depends on several other variables whose predictive values are not currently well defined (Roberts, Dunn, & Rabinovici, 2013).

Therapeutic trials: patient selection and tracking disease progression

Before amyloid-PET imaging was incorporated as a screening procedure, AD therapeutic trials enrolled subjects based on clinical criteria for probable AD, which has been shown to be inaccurate for the pathologic diagnosis of AD (Beach, Monsell, Phillips, & Kukull, 2012). First implemented in trials to verify target engagement of antiamyloid monoclonal antibodies, amyloid imaging results demonstrated that in a fair number of participants, significant amyloid burden was absent, confirming the inadequate accuracy of using only clinical criteria (Liu et al., 2015). Studies of amyloid imaging in patients recruited to more recent trials show that nearly half of MCI patients and about 25% of mild dementia patients are amyloid-PET negative (Sevigny, Suhy, et al., 2016).

A positive amyloid-PET scan at study entry may be associated with cognitive worsening (Rowe et al., 2013) but does not predict when MCI and early AD patients or cognitively unimpaired trial participants in prevention trials will measurably decline. Furthermore, the severity of amyloid deposition is a weak indicator of where along the continuum of AD neurodegeneration an individual trial participant may be at the start of a trial, and the sites of amyloid accumulation do not correlate well with the pattern of symptomatology. A composite assessment at study baseline has included other factors combined with amyloid imaging or cerebrospinal fluid analysis, such as apolipoprotein-E genetic status, participant age, and baseline cognitive/function

composite scores. Tau burden, propagation, and topography correlate more closely with disease stage and clinical symptoms (Brier et al., 2016).

For cognitively unimpaired participants in AD prevention trials, further research is needed to understand more precisely the emotional impact of learning of an amyloid imaging result, negative or positive (Mozersky, Sankar, Harkins, Hachey, & Karlawish, 2018). The significance of amyloid positivity and negativity at an individual level will vary, and what he or she experiences after disclosure may be a dynamic process, as comprehension of scan results changes over time. The biologic concept of resilience, that some individuals cope with AD pathology well and remain cognitively normal despite moderate to elevated amyloid burden, can be part of the disclosure (Arenaza-Urquijo & Vemuri, 2018).

The primary objective of most MCI and early AD trials is to evaluate the efficacy of drug therapies in slowing both cognitive and functional impairment. Serial amyloid imaging has limited utility for tracking clinical disease progression, given that the rate of amyloid accumulation is decelerating in MCI patients, reaches a plateau in early dementia patients, and severity of amyloid deposition does not correlate with disease stage. Amyloid imaging is useful in assessing how effectively various concentrations of infusions of antibodies selectively targeting aggregated species of amyloid-beta reduce cortical amyloid burden (target engagement). A dose—response relationship for some these study drugs has been demonstrated (Sevigny, Chiao, et al., 2016)

This review of limitations of amyloid imaging is summarized in tabular format (Table 31.1). Key limitations of positive and negative amyloid-PET results are delineated and can serve as an initial reference guide.

Table 31.1 Limitations of amyloid imaging in Alzheimer's disease.

	Positive Amyloid-PET scan (Amyloid-PET positivity)	**Negative Amyloid-PET scan (Amyloid-PET negativity)**
Early dementia	• As a single biomarker, cannot establish a diagnosis of AD • In elderly individuals, less specific for AD as primary etiology • False-positive scans: DLB, CAA	• Predictive value to exclude AD diminishes after about age 75 • Vascular dementia may mimic AD or present with AD phenotype • Advanced cortical atrophy may influence radiotracer binding affinity
Mild cognitive impairment	• As a single biomarker, cannot establish a diagnosis of AD • As a single biomarker, weak indicator of future clinical course • Early dementia limitations may apply in some cases with emerging functional impairment	• Non-AD dementia at an early stage could be present • In younger individuals, AD could develop as a separate condition decade or more later • May be more difficult to interpret if amyloid deposition is intermediate—higher risk false negative

Table 31.1 Limitations of amyloid imaging in Alzheimer's disease.—cont'd

	Positive Amyloid-PET scan (Amyloid-PET positivity)	Negative Amyloid-PET scan (Amyloid-PET negativity)
Impact on disease management	• Evidence lacking that refined treatment improves patient outcome • Limited utilization without an effective DMT • Limited utilization based on cost, unproven economic benefit	• Evidence lacking that refined treatment improves patient outcome • Limited utilization without an effective DMT • Limited utilization based on cost, unproven economic benefit
Radiologic diagnosis	• Technical factors (e.g., head motion/tilt, timing of acquisition) may affect scan quality • Lack of proficiency/experience of radiologist may reduce accuracy in equivocal cases • Quantitative + visual assessment more accurate than either alone, but quantitative not approved for clinical use and not available to most radiologists	• Technical factors (e.g., head motion/tilt, timing of acquisition) may affect scan quality • Lack of proficiency/experience of radiologist may reduce accuracy in equivocal cases • Quantitative + visual assessment more accurate than either alone, but quantitative not approved for clinical use, and not available to most radiologists
Subjective cognitive decline	• No clinical indication or purpose presently • Inadequate to predict when an individual will develop cognitive impairment, if ever	• No clinical indication or purpose presently • Does not necessarily mean will not develop elevated amyloid pathology in one's lifetime
Therapeutic trials	• Neither severity nor topography of elevated amyloid deposition an adequate indicator of disease stage at trial entry or when measurable decline will occur • Serial imaging does not track clinical disease progression • Prevention trial participants: further research needed to understand emotional impact of knowing positive amyloid status	• Prevention trial participants: Further research needed to understand psychological impact of knowing negative amyloid status

Limitations of a positive and negative amyloid-PET scan results, in different patient populations and in different contexts, in a cross-referenced format. *AD*, Alzheimer's disease; *CAA*, cerebral amyloid angiopathy; *DLB*, dementia with Lewy bodies; *DMT*, disease-modifying therapy; *PET*, positron emission tomography.

Key facts of amyloid imaging

- Amyloid imaging is a nuclear medicine neuroimaging modality that can create a picture of beta-amyloid accumulation in a person's brain using PET technology.
- Beta-amyloid radiotracers are molecules labeled with a radioactive isotope that bind to specific amyloid proteins in the brain with high affinity.
- PET scans are carried out within 2 hours of injection of the radiotracer.
- A PET camera detects the radioactivity being emitted, measuring the rate of retention of the binding and radioactivity in several brain regions.
- The measurements allow for both a visual and quantitative assessment of beta-amyloid deposition.

Summary points

- This chapter focuses on the limitations of beta-amyloid (amyloid) imaging in AD.
- Amyloid PET can accurately detect elevated amyloid pathology, one of the two essential features of AD.
- Negative or normal amyloid imaging results are helpful to exclude the diagnosis of AD in many cases but should be interpreted with caution in some circumstances.
- Positive amyloid imaging results leave open the possibility that AD is the primary etiology of cognitive impairment but alone are not sufficient to make an accurate diagnosis.
- Both positive and negative amyloid imaging results influence and help tailor clinical management of patients with early dementia or cognitive impairment, but these refinements have not been proven to improve health outcomes.
- The binary radiologic interpretation of amyloid-PET scans can sometimes be difficult when amyloid deposition is intermediate in severity or technical factors compromise scan quality.
- Amyloid imaging for patients without objective cognitive impairment is investigational, with no clinical utility presently.
- For therapeutic drug trials, a negative amyloid scan helps exclude MCI and early dementia patients who do not have Alzheimer's, but a positive scan neither indicates disease stage precisely nor when trial participants will measurably decline.

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CHAPTER 32

Linking gradient echo plural contrast imaging metrics of tissue microstructure with Alzheimer disease

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List of abbreviations

AD Alzheimer disease
AT(N) amyloid, tau, neuronal damage
Aβ amyloid β
BOLD blood oxygen level dependent contrast
CDR clinical dementia rating
CSF cerebrospinal fluid
GEPCI gradient echo plural contrast imaging
GRE gradient recalled echo
MPRAGE magnetization prepared rapid gradient echo
MRI magnetic resonance imaging
SUVR standardized uptake value ratio

Mini-dictionary of terms

Alzheimer disease (AD) A disease affecting brain cells leading to their malfunction and consequent devastating disability. Memory loss is one of the earliest clinical signs of AD.

Brain damage in AD It is believed that the brain pathology in AD is characterized by accumulation of extracellular senile plaques (composed of amyloid β peptide) and intracellular neurofibrillary tangles (composed of tau protein), leading eventually to cell death.

Gradient echo plural contrast imaging (GEPCI) An advanced MRI-based technique allowing evaluation of the brain cells' health condition. It has a potential for early diagnosis of AD-related cell damage that is not visible with currently used clinical MRI methods.

Magnetic resonance imaging (MRI) Broadly available medical modality allowing to visualize structure of brain and other organs and their pathological changes.

R2** and R2t* GEPCI-measured quantitative parameters that can serve as surrogate biomarkers sensitive to accumulation of senile plaques (*R2**) and cellular damage (*R2t**).

Pathological changes in the brains of people with Alzheimer disease (AD) are known to start decades before appearing of clinical symptoms (Bateman et al., 2012; Benzinger et al., 2013; Jack et al., 2010; Sperling et al., 2011). Hence, earlier identification of AD brain pathology is important for potential disease-modifying interventions.

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Recent NIA-AA Research Framework (Jack et al., 2018) introduced a biological AT(N) (Jack et al., 2016) definition of Alzheimer disease that is based on three biomarkers: β-amyloid (Aβ) deposition, pathologic tau, and neurodegeneration (N). In this approach, Aβ and tau are identified by positron emission tomography (PET) and/or cerebrospinal fluid (CSF) biomarkers, while neurodegeneration assessment is largely based on direct MRI measurements of brain atrophy (Frisoni, Fox, Jack, Scheltens, & Thompson, 2010; Frisoni et al., 2015; Jack et al., 2018). As well, CSF total tau (Blennow, Hampel, Weiner, & Zetterberg, 2010), and PET FDG hypometabolism (Vos et al., 2016) can also be related to neuronal damage (Jack et al., 2018).

Gradient echo plural contrast imaging—biophysical and biological background

The recently introduced advanced version of the gradient echo plural contrast imaging (GEPCI) approach (Ulrich & Yablonskiy, 2016) demonstrated that the AD-related neuronal brain tissue damage can be identified even in the brain regions initially spared of atrophy (Zhao et al., 2017). This is an important finding that opens a door to even earlier diagnosis of tissue damage in AD than the volumetric measurements of tissue loss. It can add important information to the (N) component of the AT(N) classification. GEPCI measurements also demonstrated a strong association with direct PET amyloid measurements, suggesting a potential for MRI-based information on the A component of the AT(N) classification.

The MRI-based GEPCI technique provides quantitative in vivo high-resolution 3D measurements of several brain-tissue-specific relaxation properties (GEPCI metrics) of the gradient-recalled echo (GRE) MRI signal. GEPCI is based on a (1) 3D GRE MRI sequence with multiple gradient echoes (currently available from most MRI scanner manufacturers), (2) theoretical model of GRE signal relaxation properties (Yablonskiy, 1998; Yablonskiy & Haacke, 1994), and (3) a set of acquisition and postprocessing methods that allow minimization of artifacts related to macroscopic magnetic field inhomogeneities (Yablonskiy, Sukstanskii, Luo, & Wang, 2013), and physiological fluctuations (Wen, Cross, & Yablonskiy, 2014).

While the GRE signal decay is usually expressed in terms of the total transverse decay rate constant $R2^*$ ($=1/T2^*$):

$$S(TE) = S(0)\cdot\exp\left(-R2^*\cdot TE\right)\cdot F(TE) \tag{32.1}$$

the GEPCI algorithm (Ulrich & Yablonskiy, 2016) allows disentanglement of tissue-cellular-specific ($R2t^*$) and magnetic-susceptibility-related contributions to the total GRE MRI signal decay:

$$S(TE) = S(0)\cdot\exp\left(-R2t^*\cdot TE\right)\cdot F_{susc}(TE)\cdot F(TE) \tag{32.2}$$

In these equations, S(0) is the GRE signal intensity amplitude, *TE* is the gradient echo time, function $F(TE)$ accounts for the adverse effects of macroscopic magnetic field inhomogeneities (that can be accounted for using a voxel spread function (VSF) method (Yablonskiy et al., 2013)), and function $F_{SUSC}(TE)$ accounts for the magnetic susceptibility contributions to the GRE signal decay (Yablonskiy, 1998; Yablonskiy & Haacke, 1994).

The tissue-cellular-specific (*R2t**) MRI relaxation parameter depends on the environment of water molecules (the main source of MRI signal), such that higher concentrations of proteins, lipids, and other constituents of cell-building materials of biological tissue cellular matrix (sources of MR signal relaxation) lead to higher relaxation rate constants (Zhao et al., 2017; Zhao, Wen, Cross, & Yablonskiy, 2016). Moreover, assessment of correlations between GEPCI measurements and gene expression profiles in the human brain demonstrated that the GEPCI *R2t** metric is specifically related to the cellular composition of the human cortex (Wen, Goyal, Astafiev, Raichle, & Yablonskiy, 2018). Hence, GEPCI metrics can serve as surrogate markers of brain tissue cellular composition and integrity and disease-related tissue damage. Previous studies demonstrated that compared to healthy control brains, GEPCI metrics substantially change with distinct patterns in multiple sclerosis (MS) (Luo, Yablonskiy, Hildebolt, Lancia, & Cross, 2014; Sati, Cross, Luo, Hildebolt, & Yablonskiy, 2010; Wen et al., 2015; Yablonskiy, Luo, Sukstanskii, Iyer, & Cross, 2012) and in some psychiatric diseases (Mamah et al., 2015).

In AD the GEPCI *R2** metric (sensitive to magnetic susceptibility effects) increases with accumulation of amyloid, whereas the GEPCI *R2t** metric decreases with loss of neuronal density (Zhao et al., 2017).

GEPCI *R2t** measurements are sensitive to neuronal damage and correlate with cognition and disease progression

Clearly, the brain atrophy is a secondary process following the neuronal damage and death. Indeed, Price et al. (2001) established by an autopsy study that cognitive decline does not start until the onset of neuronal loss. They found that there was no significant decrease in neuron number or volume with age in the healthy nondemented group and little or no difference between the healthy and preclinical AD groups. Substantial decreases were found in the very mild AD group in neuron number (35% in the entorhinal cortex, and 46% in hippocampus) and volume (28% in the entorhinal cortex, and 29% in hippocampus). Greater damage was observed in the hippocampus in the severe AD group. One of the important conclusions from these data is an outpacing decrease in neuronal number in the hippocampus as compared to hippocampal volume, suggesting decreased neuronal density in the remaining tissue. Since GEPCI *R2t** is sensitive to neuronal density, this decrease can be revealed by decreased GEPCI *R2t**. Indeed, the data in reference (Zhao et al., 2017) demonstrated that the early AD pathology in the

hippocampus can be detected by GEPCI *R2t**, thus confirming the role of *R2t** as a surrogate biomarker of cellular integrity.

Fig. 32.1 shows examples of MPRAGE and GEPCI images obtained from three participants representing healthy control, preclinical, and mild AD groups (see Table 32.1 for definition of groups). The red contour (MPRAGE) and yellow contour (GEPCI *R2t**) outline hippocampal area. In all cases, T1 MPRAGE images show small atrophy progressing from healthy to AD group. Gradually decreased GEPCI *R2t** suggests altered tissue integrity even in the preserved hippocampal area. One should keep in mind that GEPCI metrics (*R2** and *R2t**) are quantitative and provide information on tissue integrity based on comparison with healthy control measurements, thus uncovering tissue damage that might not be (and usually is not) simply visible on images. Tissue damage in the hippocampus can also be clearly seen as hypointense signal on *R2t** maps in Fig. 32.1.

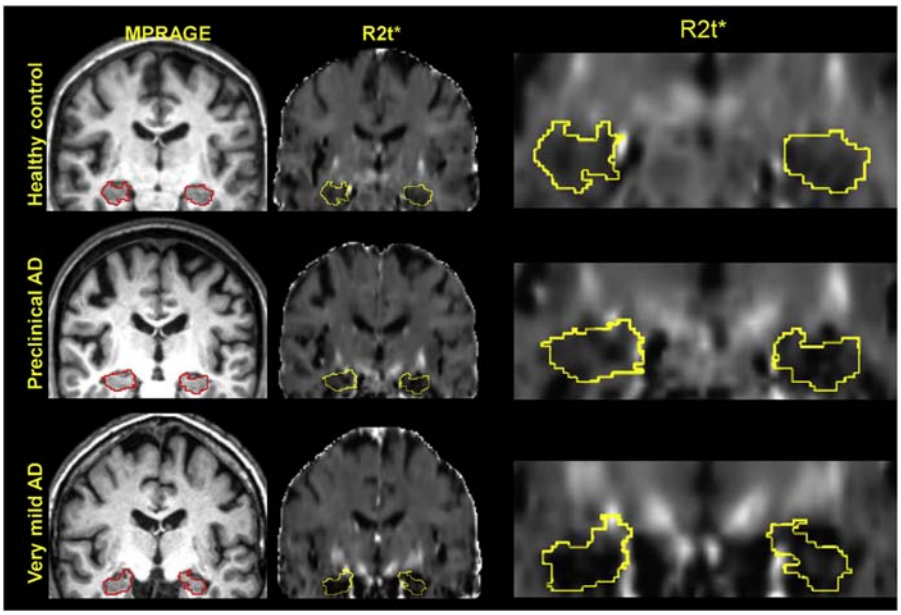

Figure 32.1 Examples of MPRAGE and GEPCI *R2t** images. Data obtained from three participants: upper row – 69-year-old female representing healthy control group (CDR = 0, amyloid negative); middle row – 72-year-old male representing preclinical AD group (CDR = 0, amyloid positive); lower row – 69-year-old male representing mild AD (CDR = 0.5, amyloid positive). Thin contours outline hippocampal area determined by FreeSurfer segmentation. In all cases, MPRAGE images show small atrophy progressing from healthy to AD group. Decreased GEPCI *R2t** suggests altered tissue integrity even in the preserved hippocampal areas. Images in the right column represent enlarged data of hippocampal areas from the middle column. Quantitative comparison of *R2t** values in three groups is presented in Fig. 32.4. *(Modified from Zhao, Y., Raichle, M. E., Wen, J., Benzinger, T. L., Fagan, A. M., Hassenstab, J., et al. (2017). In vivo detection of microstructural correlates of brain pathology in preclinical and early Alzheimer disease with magnetic resonance imaging.* NeuroImage, 148*, 296–304. https://doi.org/10.1016/j.neuroimage.2016.12.026.)*

Table 32.1 GEPCI metrics corresponding to different stages of AD progression.

AD stage	Description	GEPCI metrics
Healthy	CDR 0, amyloid (−)	*R2** normal, *R2t** normal
Preclinical AD	CDR 0, amyloid (+)	*R2** increased, *R2t** normal
Very mild AD	CDR 0.5 or 1, amyloid (+)	*R2** increased, *R2t** decreased

Detail comparison between GEPCI *R2t** data in the hippocampus and psychometric tests is shown in Fig. 32.2 along with the group comparison among healthy control, preclinical AD, and mild AD participants. The data allow drawing several important conclusions.

Firstly, results in the first column of Fig. 32.2 show that changes in the hippocampal *R2t** correlate with cognitive performance. The data are from 34 participants that were

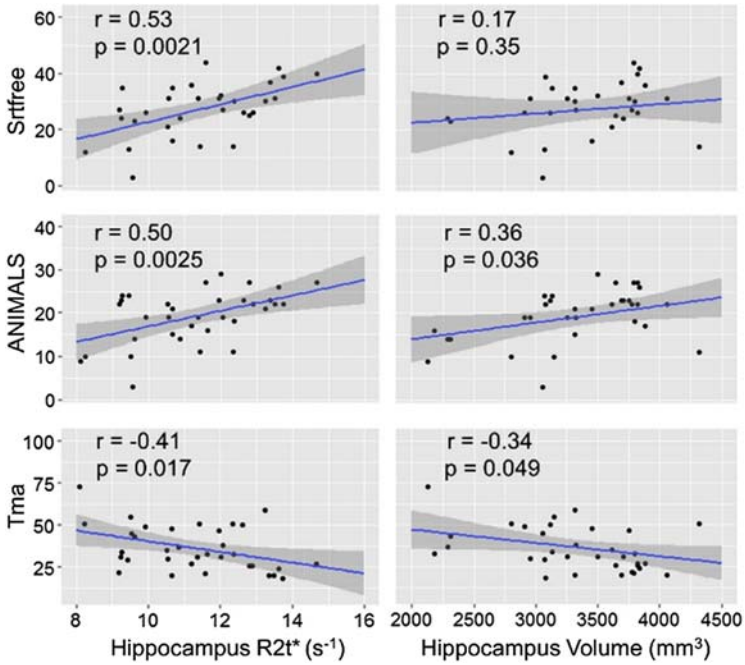

Figure 32.2 Cognitive performance based on hippocampal data. Left column: correlation between cognitive tests performance and hippocampal *R2t**; Right column: correlation between cognitive tests and hippocampal volume. Cognitive measures included Free and Cued Selective Reminding Test (Srtfree), Animal Naming (ANIMALS), and Trail Making Test Part A completion time (Tma). Note that higher scores correspond to good performance on Srtfree and ANIMALS and worse performance on Tma. Each point represents a single participant (n = 34). Shaded areas represent 95% confidence intervals of linear fits (*solid lines*). Pearson correlation coefficients (r) and *P* values are shown in the left upper corners. *(Modified from Zhao, Y., Raichle, M. E., Wen, J., Benzinger, T. L., Fagan, A. M., Hassenstab, J., et al. (2017). In vivo detection of microstructural correlates of brain pathology in preclinical and early Alzheimer disease with magnetic resonance imaging.* NeuroImage, 148*, 296–304. https://doi.org/10.1016/j.neuroimage.2016.12.026.)*

characterized either as cognitively normal or having mild cognitive impairment.As presented in the scatter plots in Fig. 32.2, the hippocampal *R2t** was associated with the free recall condition of the Free and Cued Selective Reminding Test (Srtfree; $r = 0.53$, $p = 0.002$), with the total score from the Animal Naming test (ANIMALS; $r = 0.50$, $p = 0.0025$), and with the Trail Making Test Part A completion time (Tma; $r = -0.47$, $p = 0.017$).

Secondly, considerably weaker correlations were found between hippocampal volume and cognitive performance (Fig. 32.2, second column), suggesting that the integrity of the remaining hippocampal tissue (characterized by *R2t**) is a more important parameter of hippocampal pathology than the hippocampal volume.

No significant correlation was found between cognitive performance and tissue *R2** (biomarker for amyloid burden) or CSF amyloid biomarker $A\beta_{42}$. This result is in agreement with the known dissociation between PiB-defined amyloid plaques and cognitive performance—at least 30% of people with significant amyloid burdens in their brains are cognitively normal (Morris et al., 2009). This comparison further confirms the important role of *R2t** as a surrogate marker of tissue neuronal integrity.

It is important to stress that GEPCI *R2t** biomarker is substantially different from volumetric MRI biomarkers. The data show that the progressive hippocampal volume atrophy is a characteristic process of a normal ageing (Price et al., 2001; Raz et al., 2004). Several other studies have also shown that there is essentially no neuronal loss in the hippocampus and entorhinal cortex with aging but substantial cell and volume loss in AD (Gomez-Isla et al., 1996; Juottonen, Lehtovirta, Helisalmi, Riekkinen, & Soininen, 1998; Kordower et al., 2001; West, 1993; West, Coleman, Flood, & Troncoso, 1994). These results are consistent with GEPCI measurements showing that GEPCI *R2t** metric remains either constant or increases with normal ageing (Zhao et al., 2016). It is the AD pathology that leads to decreased *R2t** metrics. Hence, GEPCI *R2t** surrogate marker can distinguish normal from abnormal brain cellular microstructure.

GEPCI *R2** metric provides a surrogate measure of Aβ accumulation in the brain

While the primary role of the Aβ peptide in the development of Alzheimer disease is now almost universally accepted (Jack et al., 2018), only PET-based measurements currently allow in vivo quantitative estimate of human brain amyloidosis. Data obtained in (Zhao et al., 2017) demonstrate that GEPCI *R2** metric has a potential to provide a surrogate measure of Aβ accumulation in the brain. This is especially important since amyloid accumulation in the brain represents the earliest changes in the course of Alzheimer disease.

The correlation analysis between GEPCI measurements of *R2** relaxation rate constant and amyloid PET measurements (using PiB standardized uptake value ratio [SUVR]) revealed positive correlations in most cortical brain regions (Zhao et al., 2017). Examples of the correlations between GEPCI *R2** and PET Aβ SUVR measurements

are shown in Fig. 32.3. The data demonstrate significant correlations not only in the areas of high amyloid accumulation (e.g., precuneus) but also in the areas of medial temporal lobe (MTL), such as the parahippocampal cortex and the fusiform cortex. Remarkably, the strongest and most significant correlation exists in the parahippocampal cortex, the area of low amyloid accumulation in early AD stages. This shows the high sensitivity of GEPCI *R2** metric to Aβ accumulation in this very important area of pathological changes in early AD.

The observed association between *R2** and amyloid burden in the brain can be attributed to the additional GRE signal relaxation due to accumulated phase incoherence resulting from magnetic susceptibility difference between amyloid plaques and surrounding brain tissue. This effect can be described in the framework of the theoretical model of Yablonskiy & Haacke (1994) that was previously validated on phantoms (Yablonskiy, 1998) and in vivo (He, Zhu, & Yablonskiy, 2008). This model can account for different types of magnetic susceptibility "disturbers," including veins with deoxygenated blood (so-called BOLD effect (Ogawa, Lee, Kay, & Tank, 1990)) and also amyloid plaques that can cause *R2** signal decay (Maier et al., 2015). Since no correlation exists between amyloid accumulation and BOLD effect (Vlassenko et al., 2010), we can conclude that the correlation between amyloid accumulation and *R2** that is found in AD patients (Zhao et al., 2017) is mostly related to magnetic susceptibility effects created by amyloid plaques. A hypothesized iron presence in amyloid plaques (Duyn, 2012; Meadowcroft, Connor, Smith, & Yang, 2009) could lead to additional sensitivity of GEPCI *R2** metric to Aβ.

While most MRI applications to AD are focusing on AD-related volumetric measurements of brain atrophy, a few studies attempted to directly identify amyloid plaques in postmortem specimens (Benveniste, Einstein, Kim, Hulette, & Johnson, 1999) or mice models (Chamberlain et al., 2009; Lee, Falangola, Nixon, Duff, & Helpern, 2004; Maier et al., 2015; Wengenack et al., 2011), though the latter methods require high-field scanners, long imaging time and have not yet been translated to human studies. On the other hand, results shown above demonstrate that the GEPCI technique allows in vivo detection of Aβ in a human brain with a short imaging time (about 10 min) on a clinical commercial MRI scanner.

Brain patterns of GEPCI *R2** and *R2t** metrics allow distinguishing among healthy control, preclinical, and mild AD groups

Based on the above results we can see that GEPCI *R2** increase is caused by accumulation of senile plaques formed by amyloid β peptide, whereas GEPCI *R2t** decrease reflects loss of cellular integrity. Table 32.1 summarizes the results in the following classification of disease progression based on GEPCI *R2** and *R2t** metrics.

The data in Fig. 32.4 show that *R2** in the parahippocampus can distinguish between normal control and preclinical groups, whereas *R2t** in hippocampus can identify mild

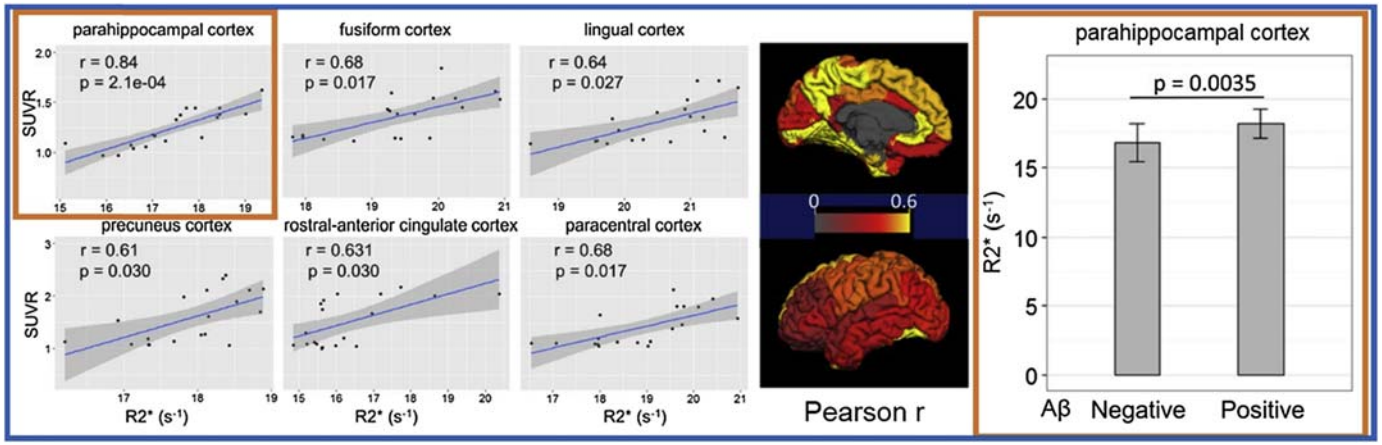

Figure 32.3 Relationships between PET PiB Aβ SUVR (dimensionless) and GEPCI *R2** (s^{-1}) relaxation rate constant sensitive to magnetic susceptibility effects. Plots show examples of correlation in several brain regions. Each point represents a single participant (only 19 participants had PET Aβ measurements with PiB). Shaded areas represent 95% confidence intervals of the linear fits (*solid lines*). Pearson correlation coefficients *r* and *P* values (corrected for multiple comparison using false discovery rate over all cortical regions) are shown in the left upper corners. The surface maps in the middle represent Pearson *r* in all cortical areas. The image segmentation is based on the FreeSurfer software. The bar graph on the right shows group comparison based on the *R2** measurements in the parahippocampus. Bars represent mean values and error bars are standard deviations. Data show significant differences between participants (independent of CDR) with negative (n = 15) and positive (n = 19) amyloid status. *(Modified from Zhao, Y., Raichle, M. E., Wen, J., Benzinger, T. L., Fagan, A. M., Hassenstab, J., et al. (2017). In vivo detection of microstructural correlates of brain pathology in preclinical and early Alzheimer disease with magnetic resonance imaging.* NeuroImage, 148, *296–304. https://doi.org/10.1016/j.neuroimage.2016.12.026.)*

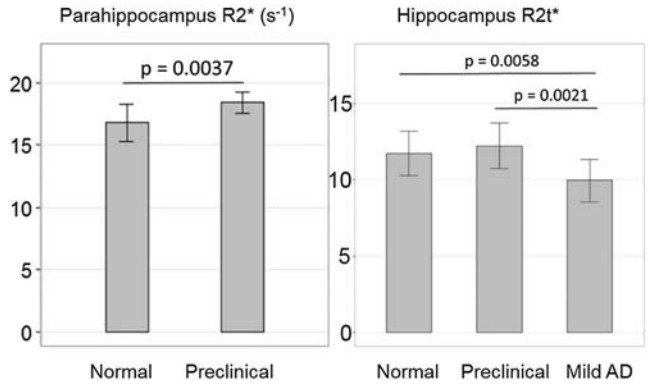

Figure 32.4 Group comparison based on the *R2** and *R2t** measurements in two regions of the medial temporal lobe. Bars are mean values, error bars are standard deviations. Three groups are shown: normal control (CDR = 0, amyloid negative, n = 13), preclinical (CDR = 0, amyloid positive, n = 10), and mild AD (CDR = 0.5 or 1, n = 11). Left panel shows significant *R2** (surrogate marker of amyloid) differences between normal and preclinical group. Right panel shows significant *R2t** (surrogate marker of neuronal integrity) differences between preclinical and AD groups. *(Modified from Zhao, Y., Raichle, M. E., Wen, J., Benzinger, T. L., Fagan, A. M., Hassenstab, J., et al. (2017). In vivo detection of microstructural correlates of brain pathology in preclinical and early Alzheimer disease with magnetic resonance imaging.* NeuroImage, 148*, 296–304. https://doi.org/10.1016/j.neuroimage.2016.12.026.)*

AD group. This further supports the hypothesis that GEPCI *R2t** measurements can significantly contribute to identifying the N component of AT(N) classification.

Importantly, GEPCI metrics vary across the brain even in healthy control participants (Zhao et al., 2016), which reflects variation in brain cellular content. The changes in GEPCI metrics due to the AD progression are also expected to vary across the brain as the onset of neuronal damage appears in the medial temporal lobe (that should manifest as a decrease in GEPCI *R2t** metrics) while the initial amyloid plaques show up outside of the medial temporal lobe (that should manifest as an increased *R2** metrics). It is also important that the pattern of GEPCI metrics changes associated with AD is significantly different as compared to other diseases. An example of the comparison in GEPCI metrics variation between GEPCI and MS is shown in Fig. 32.5 using GEPCI Barcode method proposed in Jie Wen et al. (2015).

Conclusions

GEPCI in vivo measurements obtained on a clinical MRI scanner provide information on brain AD-related pathology in human participants. Since MRI is a widely available technology, the GEPCI technique has great potential for widespread use in tracking early brain pathology and evaluation of new disease-modifying therapies. GEPCI data are quantitative, reproducible, and MRI scanner independent, thus allowing multicenter applications.

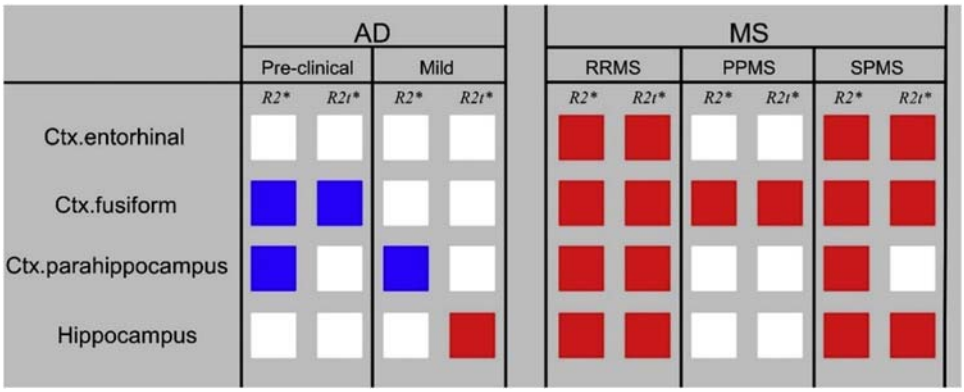

Figure 32.5 GEPCI Barcode comparing AD and multiple sclerosis (MS) results in the medial temporal lobe. *Blue (red) squares* show brain regions with statistically significant ($P < .05$) increased (decreased) GEPCI *R2** and *R2t** metrics as compared to normal control participants. *White squares* represent data in the normal range. RRMS, PPMS, and SPMS are MS subtypes: relapsing remitting, primary, and secondary progressive, correspondingly. Increased *R2** values (blue) indicate accumulation of amyloid. Decreased *R2t** values (red) indicate neuronal damage. The patterns of GEPCI metrics values and spatial distribution are clearly distinct between all five disease groups, suggesting that GEPCI barcode can identify the disease type and the stage. *(MS data are from Wen, J., Yablonskiy, D. A., Luo, J., Lancia, S., Hildebolt, C., & Cross, A. H. (2015). Detection and quantification of regional cortical gray matter damage in multiple sclerosis utilizing gradient echo MRI.* NeuroImage: Clinical, 9*, 164–175.)*

Summary points

- MRI-based GEPCI technique provides a new approach for the in vivo evaluation of AD-related tissue pathology in the preclinical and early symptomatic stages of AD.
- GEPCI quantitative measurements demonstrated significant differences between all the groups of participants: normal, preclinical and mild AD.
- Data suggest higher sensitivity of GEPCI *R2t** measurements to tissue neuronal loss as compared to standard volumetric measurements.
- GEPCI quantitative metric *R2t** (tissue cellular integrity) in the hippocampus demonstrated much stronger correlations with psychometric tests than the hippocampal atrophy.
- GEPCI is based on a multigradient-echo MRI sequence available from most MRI manufacturers.
- GEPCI data are quantitative, reproducible, and MRI scanner independent, allowing multicenter applications for AD diagnostics, and the evaluation of disease-modifying therapies.

Key facts (areas of interest)

- Alzheimer disease (AD) is a devastating illness affecting millions people worldwide.
- AD-related brain damage starts decades before onset of clinical symptoms.

- We still do not know what triggers brain damage in AD.
- Most drug trials to date fail to provide meaningful impact on disease progression, most likely because they start too late in disease progression when brain damage is already extensive.
- For drug trials to become more effective, we need to develop diagnostic methods that can identify AD brain damage at the very initial stages of the disease.

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CHAPTER 33

Hypertensive disorders during pregnancy and later dementia: is there a connection?

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List of abbreviations

AD Alzheimer's disease
aHR adjusted Hazard ratio
BMI body mass index
CI confidence interval
CVD cardiovascular disease
HPDs hypertensive disorders of pregnancy
IUGR intrauterine growth restriction
MRI magnetic resonance imaging
OR odds ratio
PE preeclampsia
PRES posterior reversible encephalopathy syndrome
RR relative risk
WML cerebral white-matter lesion

Mini-dictionary of terms

Eclampsia Tonic and clonic seizures with an incidence in western countries of 3–5 cases/10,000 pregnancies. Hypertension is not mandatory for a diagnosis. An eclamptic fit might be the first symptom of HPD, and this should be considered in all pregnant patients with loss of consciousness.

Hemolysis, elevated liver enzymes, and low platelets A severe form of preeclampsia occurring in less than 1% of all pregnancies. Hypertension is not mandatory for a diagnosis. Takes a serious and very rapid course.

Intrauterine growth restriction Impaired fetal growth. One-third of all cases of IUGR are caused by HPD. IUGR is connected with fetal death, caesarean section, cerebral palsy, and long-term consequences for the child such as cognitive impairment, CVD, and type 2 diabetes.

Placental disease All complications during pregnancy with impaired placental function. This includes HPD as well as complications without hypertension but with symptoms from placental impairment such as placental abruption, IUGR, and some forms of spontaneous preterm birth.

Posterior reversible encephalopathy syndrome Reversible neuroimaging findings and subcortical edema without infarction in the white matter of the brain diagnosed in patients with eclampsia, renal insufficiency, immunosuppression, or hypertension. Seizures, headache, confusion, and visual abnormalities are often present.

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Introduction

Hypertensive disorders are common during pregnancy, and women with such a history are also at greater risk of developing hypertension and cardiovascular disease (CVD) later in life. Certain forms of hypertensive disorders of pregnancy (HPDs) are also associated with cerebral white-matter lesions (WMLs) and cognitive decline. CVD, cerebral WMLs, and cognitive decline are all also linked to Alzheimer's disease (AD) and dementia. Genetic similarities between AD and preeclampsia (PE) have recently been identified, and this leads to the question of whether HPDs might be associated with dementia.

Hypertensive disorders during pregnancy

Classification

HPDs, which affect around 10% of all pregnant women, include hypertension existing before pregnancy as well as hypertension diagnosed after 20 gestational weeks, both with and without proteinuria (Magee, Pels, Helewa, Rey, & von Dadelszen, 2015). Thus the symptoms, treatments, and prognoses differ, and when discussing long-term effects it must be kept in mind that it is possible that we are dealing with several different diseases (Vatten & Skjaerven 2004). All of these might end in PE, which is the condition most studied. The classification of HPD is shown in Table 33.1.

Table 33.1 Various types of hypertensive disorders during pregnancy along with their definition and typical course.

Condition	Definition	Course
Chronic hypertension	Hypertension diagnosed before pregnancy or before 20 gestational weeks	Risk for intrauterine growth restriction and risk for superimposed preeclampsia
Gestational hypertension	Hypertension diagnosed after 20 gestational weeks	If significant proteinuria occurs, the diagnosis is preeclampsia
Superimposed preeclampsia	Addition of significant proteinuria in women with chronic hypertension	See below
Mild preeclampsia	Blood pressure 140/90 or above. Significant proteinuria	Usually mild. Might affect fetal size. Progression to severe preeclampsia might follow
Severe preeclampsia	Blood pressure ≥160/110 plus significant proteinuria or renal, liver, cerebral, or coagulation disturbances	Risk for fetal growth restriction, cerebral edema, stroke, and convulsions as well as liver and coagulation disturbances

Hypertension is defined as blood pressure ≥140/90 mm Hg on two occasions at least 4 h apart. Significant proteinuria is defined as ≥300 mg/24 h. Preeclampsia is sometimes divided into early-onset or late-onset if diagnosed before or after gestational week 34, representing severe and mild preeclampsia, respectively. Lately it has been proposed that proteinuria should not always be mandatory for a diagnosis of preeclampsia (Brown et al., 2018).

Risk factors for preeclampsia

A number of factors have been linked to increased risk for PE. Factors linked to vascular dysfunction include chronic hypertension, pregestational diabetes, obesity, and preexisting renal disease. Factors linked to obstetric history include 10 years or more since last delivery, prior PE, nulliparity, assisted reproductive techniques, and multiple gestations. Genetic factors include being African American or having a family history of preeclampsia.

Some studies include maternal age over 40, whereas others don't (Paré et al., 2014; Steegers, von Dadelszen, Duvekot, & Pijnenborg, 2010). Low socioeconomic status is connected with greater risk (Silva et al., 2008), whereas cigarette smoking lowers the risk (Alpoim et al., 2016).

Pathophysiology of preeclampsia

The pathophysiology of PE remains unclear, although it is known that the placenta, but not a fetus, is necessary for the development of PE. In a normal pregnancy, cytotrophoblasts invade the spiral arteries of the uterus, and the muscular vessels are widened to allow for the increase in blood volume during pregnancy. This process ensures adequate perfusion of the placenta. In many cases of PE, the migration of cytotrophoblasts is altered such that the invasion is shallow and the spiral arteries remain narrow, resulting in insufficient placental perfusion and subsequent ischemia (Fig. 33.1) (Fisher, 2015).

The maternal tolerance for fetal antigen exposure might also be involved in the development of PE, and insufficient exposure to paternal antigen increases the risk for PE. Normal pregnancy requires an altered inflammatory response. This inflammatory response is even more pronounced in PE and might be associated with induced production of various factors such as reactive oxygen species and other immune factors that further interfere with placental function (Harmon et al., 2016; Powe et al., 2011).

The two-stage model is the most widely accepted hypothesis behind the pathophysiology of PE. Stage 1 represents impaired trophoblast invasion causing placental hypoxia and the release of antiangiogenic factors. Stage 2 represents the ensuing vascular dysfunction causing clinical manifestations of the disease (Roberts & Hubel, 2009). Recently, however, it was claimed that vascular dysfunction is the cause of the placental dysfunction rather than the result (Thilaganathan, 2016) (Fig. 33.2).

Cardiovascular disease after hypertensive disease during pregnancy

An abundance of epidemiologic evidence indicates an association between PE and CVD.

However, it was not until 2011 that the American Heart Association added PE, gestational diabetes, and fetal growth restriction as risk factors for CVD (Mosca et al.,

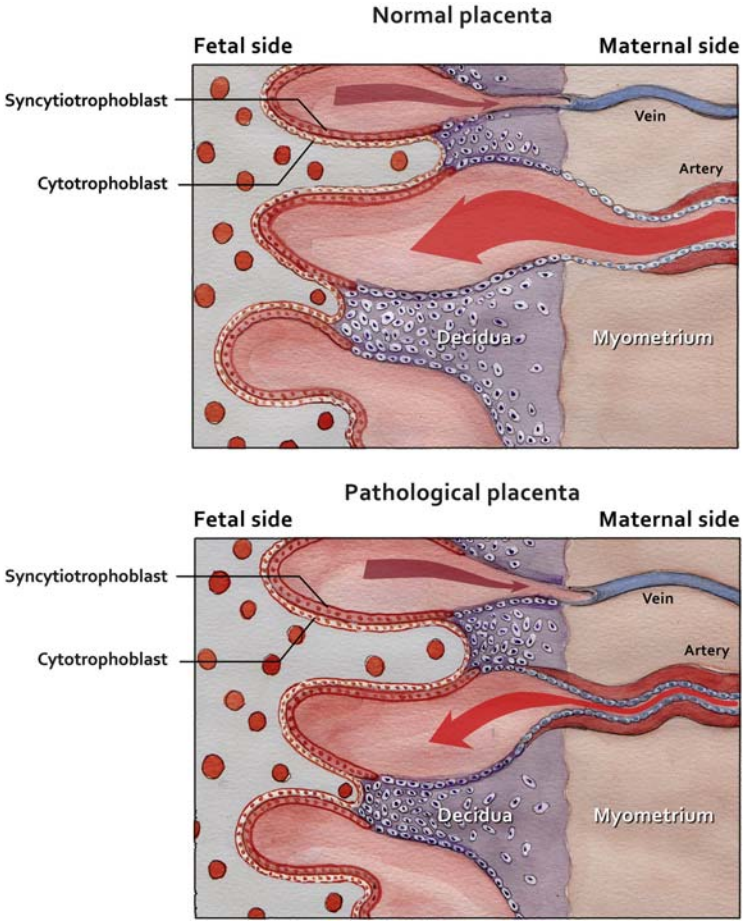

Figure 33.1 ***Normal and abnormal trophoblast invasion in early pregnancy.*** During normal pregnancy, trophoblasts change phenotype and invade the spiral arteries, thus creating low-resistance vessels that enable adequate perfusion of the placenta. In pregnancies with impaired placental function, this does not occur and the spiral arteries continue to be high-resistance vessels.

2011), and they are still not included in scores used to evaluate future risk for CVD (Goh, Welborn, & Dhaliwal, 2014). Several systematic reviews and meta-analyses show that the risk for hypertension, ischemic heart disease, and stroke is doubled or tripled after a previous episode of PE, and the risk is further increased with longer follow-up, recurrent PE, and the severity of PE (Table 33.2).

The reason for why CVD is linked to PE is not clear. Constitutional factors as well as the harmful effects of the preeclamptic episode might contribute (Sattar & Greer, 2002) (Fig. 33.3). CVD and PE have similar profiles in terms of gene expression (Sitras, Fenton,

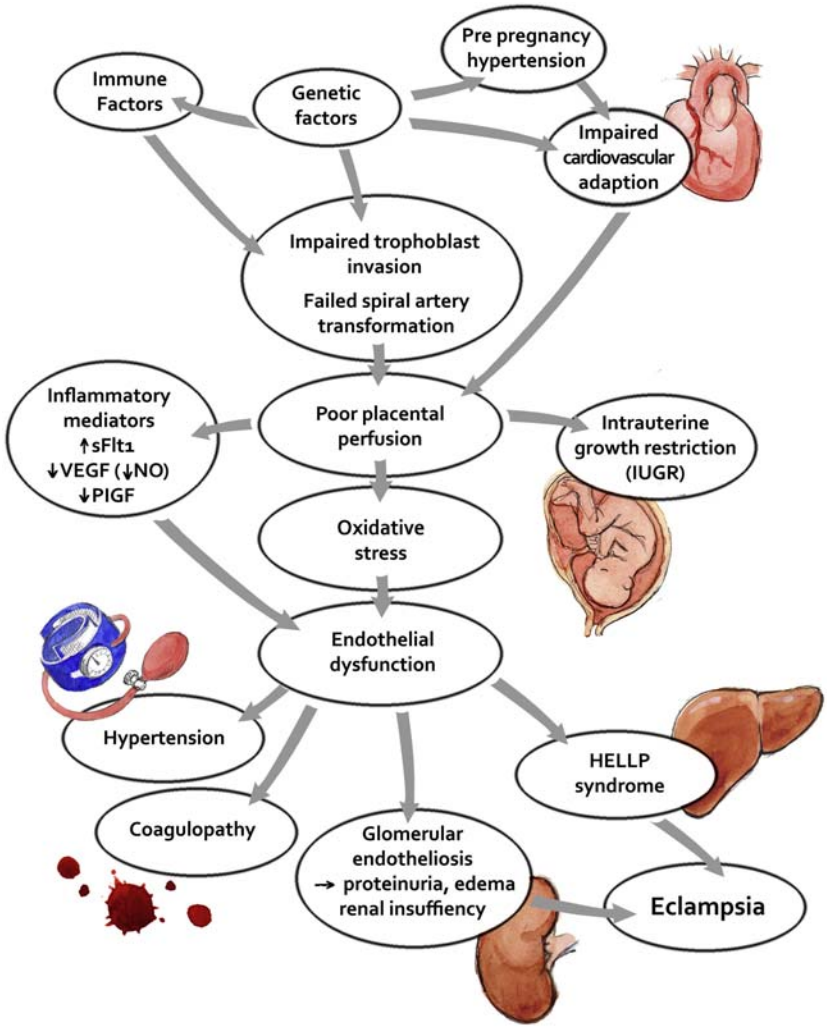

Figure 33.2 ***Stage 1 and 2 models of the pathogenesis of preeclampsia.*** Preeclampsia is usually considered a two-stage disorder. The first stage consists of impaired trophoblast invasion leading to an underperfused placenta, oxidative stress, and endothelial dysfunction. The second stage is the maternal syndrome consisting of hypertension, proteinuria, liver dysfunction, coagulation disturbances, and occasionally seizures and death.

& Acharya, 2015), and women with cardiovascular risk factors before becoming pregnant have a higher risk for PE (Magnussen et al., 2007). Biomarkers affecting vascular growth and endothelial function differ in women with PE and normal pregnancy, and there are differences before and after pregnancy. Some of these biomarkers are also affected in nonpregnant individuals with CVD (Powe et al., 2011).

Table 33.2 Hypertensive disorders during pregnancy and later hypertension, heart disease, and stroke.

Author	Type of study	Inclusion criteria	Exposure	Outcome	Number of participants	Method	Results	Results Hyper-tension	Results Cardiac disease	Results Cerebrovascular disease	Comments
Bellamy, Casas, Hingorani, & Williams (2007)	SR MA	Retrospective and prospective cohort studies	PE	CVD Cancer	3,488,160 women, of whom 198,252 had PE	Embase Medline 1960–2006	25 articles included	RR 3.70 (95% CI (2.70–5.05)	Ischemic Heart Disease RR 2.16 (95% CI 1.86–2.52)	RR 1.81 (95% CI 1.45–2.27)	Smaller studies reported larger effect for hypertension
Mcdonald, Malinowski, Zhou, Yusuf, & Devereaux (2008)	SR MA	Case-control and cohort studies	PE	CVD	16,175 women with PE 2,259,576 women with unaffected pregnancies	Medline 1966–2006) embase 1980–2006)	15 articles included		4 case-control studies OR 2.47, 95% CI 1.22–5.01 10 cohort studies RR 2.33, 95% CI 1.95–2.78	1 case-control study OR 2.6, 95% CI 1.5–4.3 6 cohort studies RR 2.03, 95% CI 1.54–2.67	Several episodes of preeclampsia indicated higher risk
Brown et.al. (2013)	SR MA	Case-control cohort studies	PE Eclampsia	CVD HT		Medline Embase until 2012	43 articles included in the meta-analysis	RR 3.13, 95% CI 2.51–3.89	CVD OR 2.28, 95% CI 1.87–2.78	OR 1.76%, 95% CI 1.43–2.21	Substantial heterogeneity for hypertension
Wu et al. (2017)	SR MA		PE	CVD Stroke	6.4 million, of whom 258,000 had PE	Medline Embase 2005–15	22 articles included		Heart failure RR 4.19, 95% CI, 2.09–8.38 Coronary heart disease RR 2.50, 95% CI 1.43–4.37	RR 1.81; 95% CI, 1.29–2.55	

CI, confidence interval; *CVD*, cardiovascular disease; *HT*, hypertension; *MA*, meta-analysis; *OR*, odds ratio; *PE*, preeclampsia; *RR*, relative risk; *SR*, systematic review.

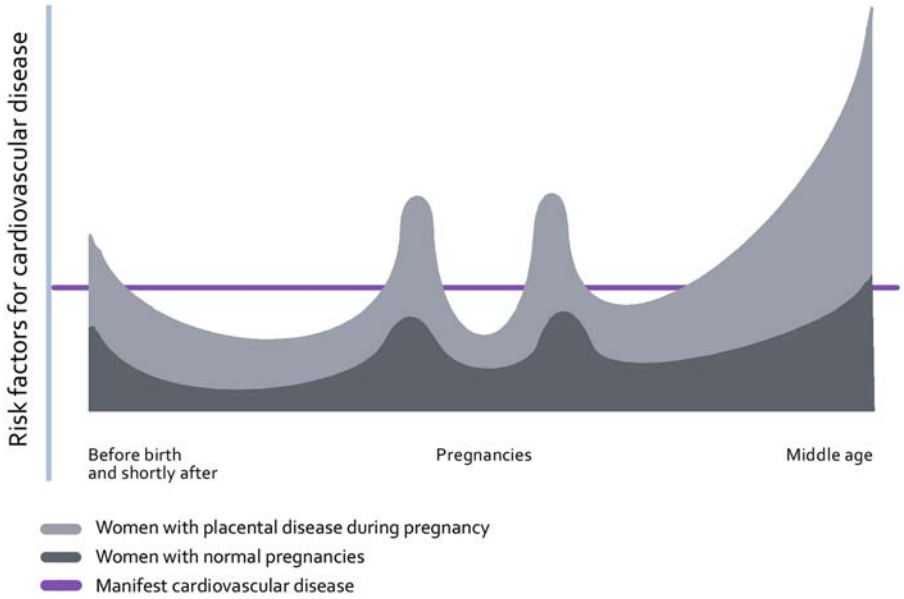

Figure 33.3 ***Development of cardiovascular disease (CVD) in relation to intrauterine life, course of pregnancy, and age.*** Risk factors for CVD (y-axis) accumulate over a person's lifetime (x-axis) and might result in manifest CVD during pregnancy. Although symptoms of disease often disappear post-partum, they tend to return with age. *(Figure adapted from Sattar, N., & Greer, I. A. (2002). Pregnancy complications and maternal cardiovascular risk: Opportunities for intervention and screening?* BMJ, 325*(7356), 157–160.)*

Genetic and epigenetic similarities between Alzheimer's disease and preeclampsia

Genetic and epigenetic factors have been linked to PE and late onset of AD. STOX1 is a transcription factor associated with PE and abundantly expressed in cases of AD that result in increased amyloid-beta protein precursor processing, and the expression of STOX1 is correlated with the severity of disease (van Abel et al., 2012; van Dijk et al., 2010). Transthyretin, a transporter of thyroxine and retinol, is dysregulated in PE and AD in a similar fashion (Kalkunte et al., 2013).

It has been proposed that PE, just like AD, is a protein-misfolding disorder. The urine of individuals with such disorders and of women with PE exhibits congophilia (an affinity for Congo red), and the Congo-positive protein aggregates in women with PE include beta amyloid. These and other protein aggregates have also been found in the placentas of women with PE (Buhimschi et al., 2014, Cheng, Nakashima, & Sharma, 2016). Placental gene expression in PE is to a certain extent similar to gene expression in AD (Sitras et al., 2009).

Cerebral blood flow during preeclampsia

Studies of cerebral blood flow using transcranial Doppler techniques showed that autoregulation was impaired in women with PE in comparison with women with gestational hypertension and normal pregnancies (van Veen et al., 2015). When using magnetic resonance imaging (MRI) to study blood flow in the same groups, no differences were detected (Nelander, Hannsberger et al., 2018; Nelander, Wikstrom et al., 2018).

Posterior reversible encephalopathy syndrome

Posterior reversible encephalopathy syndrome (PRES) was first described more than 20 years ago. Neuroimaging showed reversible subcortical edema without infarction in the white matter of the brain in patients with renal insufficiency, immunosuppression, hypertension, or eclampsia. Findings were accompanied by seizures, headaches, confusion, and visual abnormalities (Hinchey et al., 1996).

Three possible mechanisms have been described leading to extravasation of fluid and edema: (1) rising blood pressure along with breakdown of cerebral autoregulation, (2) endothelial dysfunction affecting the blood–brain barrier, and (3) focal vasospasm.

The incidence of PRES is not known (Postma, Bouma, Ankersmit, & Zeeman, 2014; Postma, de Groot, Aukes, Aarnoudse, & Zeeman, 2014; Postma, Slager, Kremer, de Groot, & Zeeman, 2014), but for diagnosis, MRI is the gold standard (Roth & Ferbert 2011). The neuroimaging findings are identical regardless of the underlying condition (Roth & Ferbert 2009). PRES has also been diagnosed in women with neurological symptoms and PE (Mayama et al., 2016).

Differential diagnoses include severe cerebrovascular disorders such as stroke or thrombosis. Swift control of blood pressure and seizures so that secondary infarct or hemorrhage is avoided is mandatory (Cozzolini et al., 2015).

Short-term prognosis for PRES seems to be linked to the underlying condition. In a meta-analysis of six studies and 448 patients with varying conditions as well as PE and eclampsia (112 patients), the pregnancy complications were associated with better outcome in the absence of hemorrhage compared with other conditions (Chen et al., 2018).

Stroke and preeclampsia/eclampsia

The risk of thrombosis is increased in all pregnant women due to increased coagulability. The risk of stroke is estimated to be 30 cases per 100,000 pregnancies, and this increases by about five-fold in women with HPD. Compromised cerebral blood flow along with impaired autoregulation might contribute to cases of PRES (McDermott, Miller, Rundek, Hurn, & Bushnell, 2018), and cerebral infarctions have been seen in up to one-fourth of women with eclampsia (Zeeman, Fleckenstein, Twickler, & Cunningham, 2004).

The risk of stroke is also increased for several years after pregnancies with PE/eclampsia, and several meta-analyses have estimated the relative risk/odds ratio to be around 2 (Table 33.2). Again, the increased risk might be due to constitutional vascular factors that cause both PE and stroke, metabolic factors, or the harmful vascular effects of PE (McDermott et al., 2018).

Other signs of neurological damage in preeclampsia/eclampsia

Plasma concentrations of neuronal proteins produced in the brain are higher during pregnancy prior to diagnosis in women who develop PE, and they remain elevated for up to 1 year postpartum (Bergman et al 2016, 2018). By magnetic spectroscopy resonance, cerebral osmolytes have been shown to be altered in pregnant women compared with those of nonpregnant women, and glutamate levels were also lower in those with PE, which might be of importance in the case of risk for seizures (Nelander, Hannsberger et al., 2018; Nelander, Wikstrom et al., 2018). Cerebral levels of magnesium were also shown to be lower in women with PE using the same method (Nelander et al., 2017). Magnesium sulfate is an efficient treatment for hypertension and seizures occurring in patients with PE/eclampsia.

Cognitive functioning and previous preeclampsia/eclampsia

Normal pregnancy does not seem to affect subjective cognitive functioning as measured with validated questionnaires. No difference was found in parous women with normal pregnancies and nulliparous women, and functioning was not related to the number of cerebral WMLs (Postma, Bouma et al., 2014; Postma, de Groot et al., 2014; Postma, Slager et al., 2014).

Self-reported cognitive function has been shown to be affected in previously eclamptic and preeclamptic women several years after the index pregnancy (Aukes, Wessel, Dubois, Aarnoudse, & Zeeman, 2007; Postma, Bouma et al., 2014; Postma, de Groot et al., 2014; Postma, Slager et al., 2014). However, women with previous PE/eclampsia also scored higher on tests for depression and anxiety in comparison with women with normal pregnancies, and this could have interfered with the results (Postma, Bouma et al., 2014; Postma, de Groot et al., 2014; Postma, Slager et al., 2014).

Cerebrovascular reactivity was reduced for up to 35 years after previous PE, which might indicate impaired cerebral microcirculation and increased risk for cognitive decline (Barnes et al., 2018).

Cognitive function studied 35−40 years after PE showed a trend for mild cognitive impairment or dementia in women with previous PE compared with those who had normotensive pregnancies (20% vs. 8%, $P = .10$), and more domains were affected among the PE group ($P = .03$). Women with PE also had higher body mass index

(BMI) and more often had hypertension, which could have affected the results (Fields et al., 2017).

In a systematic review and meta-analysis of 13 studies concerning PE and cognitive decline, the authors concluded that previous PE is associated with subjective cognitive symptoms, but such an association could not be confirmed using standard tests for neurocognitive function. They also noted that few of the included studies were of high quality (Elharram, Dayan, Kaur, Landry, & Pilote, 2018).

Cerebral white-matter lesions

Cerebral WMLs are hyperintensive findings diagnosed with MRI located in the white matter of the brain. They are considered one of the signs of small-vessel disease in the brain, which is associated with stroke and cognitive decline (Pantoni, 2010).

WMLs have been independently associated with cognitive decline and are predictive of cognitive decline and dementia (Jokinen et al., 2012). There is also a correlation between increased numbers of WMLs in the brain and cognitive decline (LADIS Study Group, 2011).

WMLs can also be seen in healthy women, and normal pregnancy does not seem to affect the number of cerebral WMLs. No difference was found in the number of lesions (22% vs. 19%) in 81 parous women with normal pregnancies and in 65 nulliparous women (Postma, Bouma et al., 2014; Postma, de Groot et al., 2014; Postma, Slager et al., 2014). In a follow-up of 73 women with previous PE after a mean of 5 years, WMLs were found in 34% of cases and 21% of normotensive parous controls ($P = .04$). Hypertension at follow-up as well as a history of early-onset PE was independently related to WMLs (Aukes et al., 2012).

Women with a familial predisposition for hypertension were followed up at around the age of 60 with neurocognitive assessment and MRI. A self-reported pregnancy questionnaire was used, and 201 out of 1279 (16%) were diagnosed with previous HPD. Women with previous HPD performed poorer on cognitive assessments and had smaller brain volumes even after adjusting for age, race, education, BMI, smoking, current hypertension, hypertension duration, and family history of hypertension (Mielke et al. 2016).

Hypertensive disorders of pregnancy and dementia

The risk for CVD after HPD, especially PE, has been thoroughly investigated, but the risk for dementia has been much less studied, and we could only find five relevant articles (Table 33.3).

In the study by Abheiden et al. (2015), HPDs were not associated with AD. The results were adjusted for age and BMI. Limitations were study size (250 participants),

Table 33.3 Hypertensive disorders during pregnancy and later dementia.

Author	Country	Type of study	Inclusion criteria	Exposure	Outcome	Number of participants	Method	Results
Abheiden et al. (2015)	Netherlands	Retrospective case–control study	Cases: women diagnosed with AD Controls: partners of AD patients	Hypertensive disorders of pregnancy	Hypertensive disorders of pregnancy in women with dementia	118 cases 139 controls	Medical records for inclusion criteria Paper survey or telephone interview (exposure and outcome) (help from family was allowed)	Present AD was not associated with later reported hypertensive disorder of pregnancy
Theilen et al. (2016)	USA (Utah)	Retrospective cohort Population-based database	Women with deliveries 1939–2012	Hypertensive disorders of pregnancy	Cause of death	60,580 exposed 123,140 unexposed	Birth certificates Inpatient records	Increased risk for Alzheimer's disease
Nelander et al. (2016)	Sweden	Cohort study	Twin registry Born 1958 or earlier	Hypertensive disorders of pregnancy	Dementia, CVD, and stroke	3232	Interviews (exposure) national registers (outcome)	No increased risk for dementia, only for CVD and stroke
Andolf, Sydsjo, Bladh, Berg, & Sharma (2017)	Sweden	Nationwide population-based registry study	All women giving birth between 1973 and 1975	Hypertensive disorders of pregnancy	In-hospital diagnosis of CVD, vascular dementia, or dementia between 1987 and 2009	In total 284,598, of whom 10,769 had a diagnosis of HPD	Swedish medical birth register linked to the national patient register, and the total population register	Increased risk for vascular dementia in women with previous hypertension and proteinuria during pregnancy

Continued

Table 33.3 Hypertensive disorders during pregnancy and later dementia.—cont'd

Author	Country	Type of study	Inclusion criteria	Exposure	Outcome	Number of participants	Method	Results
Basit et al. (2018)	Denmark	Nationwide register based cohort study	All women with at least one live birth or stillbirth between 1978 and 2015	Preeclampsia	Dementia	1,178,005	Danish medical birth register linked to the national patient register, the civil registration system, and the causes of death register	Increased risk for especially vascular dementia, modest association with AD

CVD, Cardiovascular disease.

self-reported pregnancy history or reported by family members, and controls who were partners of patients with AD.

The second article (Theilen et al., 2016) reports an investigation of death certificates from the Utah population-based database. More than two million pregnancies were available, and 2.9% were diagnosed with HPD.

Women with a history of PE had a significantly increased risk of death from AD (aHR 4.02, 95% CI 1.08−14.99) than those without, and the corresponding figure for women with previous gestational hypertension was aHR 3.44 (95% CI 1.004−11.82). These results were adjusted for infant's sex, gestational age at delivery, maternal and paternal education, maternal race/ethnicity, and maternal marital status, but not for BMI or later hypertension.

A potential limitation is that the study population was relatively homogenously white and lean, which may explain the low rate of HPD (2.9%).

The third article (Nelander et al., 2016) is a study from the Swedish Twin registry on 3065 women. Women over 65 were screened for cognitive impairment and were excluded if the test was positive.

Fourteen percent reported that they had hypertension during at least one pregnancy. These women were followed up from the interview until 2010 in national registries for diagnoses of dementia, CVD, and stroke. Rates of dementia were 7.6% versus 7.4% ($P = .37$) between women who reported a history of HPD and those who did not (the aHR of dementia was 1.19 (95% CI 0.79−1.73)). Increased risks for CVD were found after adjusting for education, smoking, and BMI at the time of the interview.

The main limitation is that pregnancy history was self-reported. The inclusion of twins only makes this cohort special, and the high rate of HPD (14%) indicates that they might have a different disease pattern. Dementia often starts with a period of cognitive decline, and because women with cognitive decline were excluded at the time of the interview, the reported rate of dementia might be too low.

In the study by Andolf et al. (2017), all women who gave birth in Sweden between 1973 and 75 were included, and the aHR for vascular dementia after hypertension and proteinuria during pregnancy was 4.94 (CI 1.21−20.16). No increase was seen after preeclampsia or for other types of dementia. The results were adjusted for the mother's age at birth, attained educational level in 1985, marital status, origin (Nordic/non-Nordic), and cardiovascular disease. Since 1973−75 the definition of preeclampsia has changed; hypertension and proteinuria are now diagnosed as preeclampsia. The absolute risk was very low; only 88 women had vascular dementia, and 608 were diagnosed with dementia out of 284,598 total. Only inpatients were included. The women were in their early sixties, so the follow-up might have been too short.

The fifth article (Basit, Wohlfahrt, & Boyd, 2018) is a large study on more than a million women in Denmark giving birth between 1978 and 2015. Both inpatients and outpatients were included. aHR was 1.53 (CI 1.26−1.85) for overall dementia, 3.46

(CI 1.97−6.10) for vascular dementia, 1.45 (1.05−1.99) for AD, and 1.40 (1.08−1.83) for unspecified dementia. Results were adjusted for maternal birth year, parity, region of most recent delivery, cardiovascular disease, stroke, chronic kidney disease, hypertension, and diabetes, but not BMI.

Key facts about preeclampsia

- Preeclampsia is a form of HPD that affects 3%−7% of all pregnant women.
- It is a global problem with high maternal and neonatal morbidity and mortality.
- The cause is unclear, but several theories have been put forward.
- Symptoms vary, swift diagnosis is often difficult, and disease progression can be rapid.
- No effective treatment except delivery is available.
- High risk for fetal growth restriction and induced preterm birth.
- In early gestation, the decision to deliver is difficult, and risks for the mother and the fetus must be balanced.

Summary points

- Hypertensive disorders of pregnancy (HPD) affect around 10% of women and are associated with later hypertension, cardiovascular disease, cerebral white-matter lesions and cognitive decline, all of which are conditions that are also linked to Alzheimer's disease (AD) and dementia.
- There are genetic similarities between HPD and AD and dementia.
- Studying whether HPD increases the risk for dementia is difficult for a number of reasons.
- Long-term follow-up is necessary.
- The criteria for the diagnosis of HPD have varied over time, so comparison of medical records and registries from different periods might lead to incorrect conclusions.
- The criteria for diagnoses of different types of dementia might be questionable.
- Much data are self-reported and might thus be subject to recall bias.
- Many patients suffer from dementia long before diagnosis.
- Most available studies testing the association between HPD and AD and dementia have limitations, use different methods, and have come to different conclusions, but a recent large high-quality study showed an increase in vascular dementia, confirming a previous, smaller study.
- Regarding the biological plausibility of a link between preeclampsia and dementia and recent results, it is possible that women with a history of preeclampsia may benefit from screening for early signs of dementia.

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CHAPTER 34

Unraveling the contributions of sleep dysfunction to Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
Aβ amyloid beta
CSF cerebrospinal fluid
EEG electroencephalography
ISF interstitial fluid
NREM non−rapid eye movement
OSA obstructive sleep apnea
REM rapid eye movement
TBI traumatic brain injury

Mini-dictionary of terms

Actigraphy A method for quantifying activity patterns
Glymphatic flow A waste clearance system optimized during sleep that fosters the removal of metabolites and soluble proteins from the brain including amyloid beta
Insomnia A sleep disorder characterized by difficulty sleeping
Obstructive sleep apnea A sleep disorder characterized by recurrent episodes of cessation of breathing due to upper airway occlusion
Polysomnography Is the gold-standard method for evaluating sleep determined by electroencephalography in concert with other physiological processes
Rapid eye movement sleep Characterized by irregular, sharply peaked eye movements determined by electrooculogram and accompanied by dreaming, low-amplitude electroencephalography, and paralysis of musculature
Slow-wave sleep Also known as deep sleep (comprises stage 3 of NREM sleep) electroencephalographically defined by high-amplitude slow waves

Introduction

Sleep is a dynamic process defined as a reversible state of perceptual uncoupling and unresponsiveness from the external environment (Carskadon & Dement, 2005; Dement, 2005). Cortical electroencephalography (EEG) in a polysomnography protocol is used to

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identify and define stages of sleep. Sleep can be classified into two broad stages, non–rapid eye movement (NREM) and rapid eye movement (REM). NREM sleep comprises three stages ranging from light sleep to slow-wave sleep (also called deep sleep). Periods of NREM and REM alternate in a cyclic fashion every 90–100 min, occurring approximately four to five times per night (Carskadon & Dement, 2005; Kryger, Roth, & Dement, 1989).

Sleep–wake regulation occurs as part of a diffuse rather than focal network in the brain. The ascending reticular activating system extends from the medulla and pons onto fiber tracts innervating thalamic nuclei and forebrain cholinergic systems. The mechanisms underpinning sleep and wake regulation are best theorized by the two-process model of sleep regulation. The model suggests that two systems govern sleep, the homeostatic process and an internal 24-h circadian rhythm (Borbely, 1982). Sleep is homeostatically regulated such that sleep propensity increases with elapsed time awake, subsequently dissipating during sleep (Borbely, 1982). The homeostatic process "counts" the length of time an individual has been asleep or awake and is a predictor of "sleep pressure"; the longer an individual stays awake, the greater the buildup in sleep pressure (Dijk & Lockley, 2002). The circadian rhythm is driven by the suprachiasmatic nucleus and synchronized by environmental light–dark exposure utilizing luminescence information from photosensitive retinal ganglion cells. This 24-h cycle for sleep and wake propensity overlies the homeostatic process.

In normal aging, alterations in circadian and sleep homeostatic systems occur and have shown to phase advance circadian rhythms, increase sleep disturbances, and change a variety of subjectively measured sleep–wake parameters (e.g., increased fatigue) (Floyd, Medler, Ager, & Janisse, 2000). Sleep architecture changes with aging (Fig. 34.1**),** leading to increased nighttime awakenings, increased time spent in light sleep (i.e., increased stages 1 and 2), and overall diminishment of slow-wave and REM sleep (Mander, Winer, & Walker, 2017).

Sleep–wake alterations are also pronounced in dementia, including Alzheimer's disease (AD) dementia, and may stem from the accumulation of AD pathology in the brain's sleep–wake centers, neurodegeneration, medical comorbidities, and medication use. Social and environmental changes, including altered light–dark exposure and impaired light perception may contribute to sleep–wake disturbance in dementia. Disruption to sleep can also impair cognitive function acutely, thereby amplifying AD symptoms. However, newer evidence linking sleep to soluble protein clearance supports a bidirectional relationship between sleep dysfunction and AD (Ju, Lucey, & Holtzman, 2014). Unraveling the relationship between sleep dysfunction, AD genesis, and incident dementia is important for informing novel sleep-based therapies aimed at preventing or delaying dementia onset. This review discusses mechanisms that may implicate poor sleep in the initiation and progression of AD. We then summarize sleep disturbances in dementia and review the evidence for sleep as a risk factor for cognitive decline and future AD dementia from prospective cohort studies.

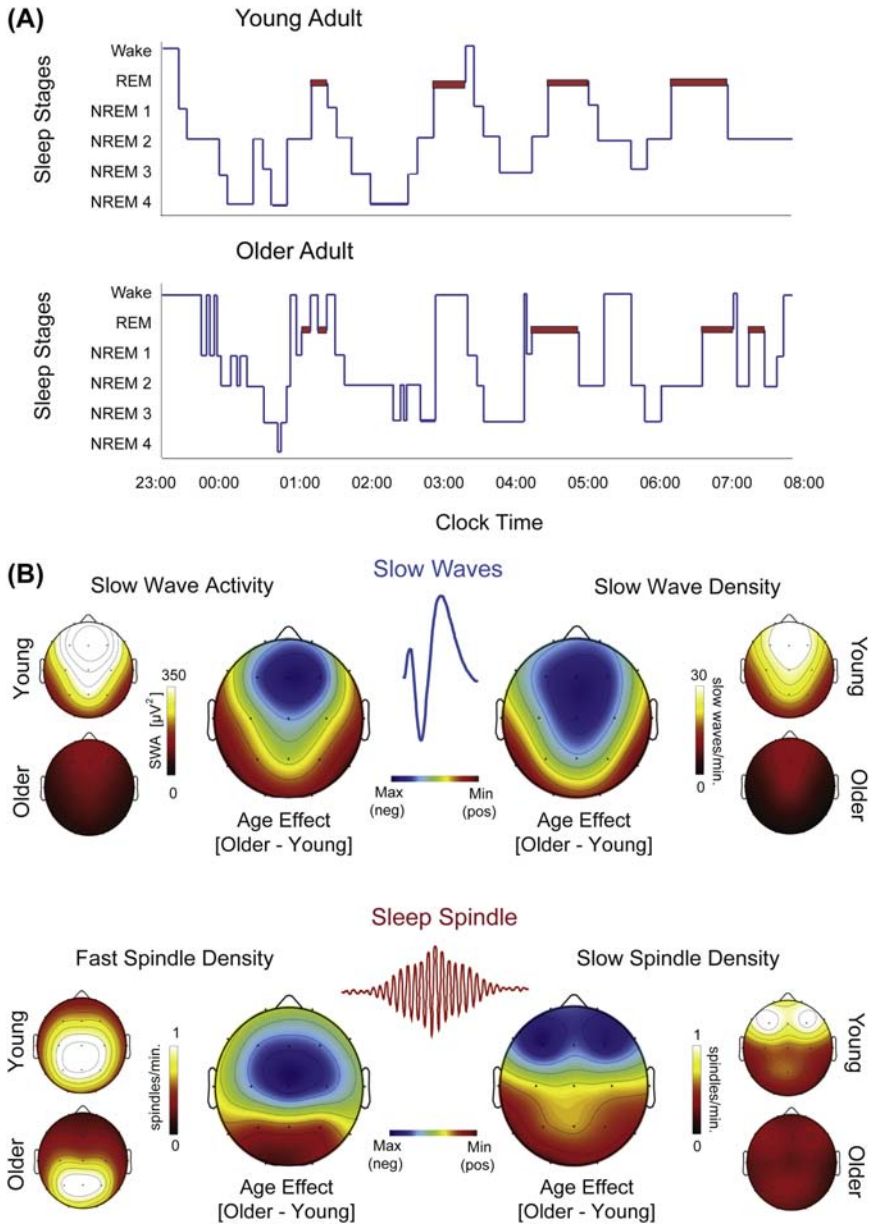

Figure 34.1 Sleep architectural reductions occurring as part of aging process. (A) Prototypical sleep stage architecture across a 9 h sleep period in a younger adult (top) and an older adult (bottom) using classic sleep-staging criteria. (B) Upper: representative topographical head plots of electroencephalography-quantified differences between younger and older adults in slow-wave activity (left upper) and density (right upper). *(Reproduced with permissions from Mander, B. A., Winer, J. R., & Walker, M. P. (2017). Sleep and human aging.* Neuron, *94(1), 19–36.)*

Pathological protein species and sleep

Sleep facilitates glymphatic clearance of amyloid-beta (Aβ): A pathological hallmark of AD is the accumulation of Aβ plaques. One of the theories explaining the pathogenic aggregation of Aβ posits deficiencies in the removal of interstitial metabolic wastes (Lim, Gerstner, & Holtzman, 2014). The human brain lacks an anatomically defined lymphatic vascular system responsible for metabolic waste clearance, and only very recently have there been descriptions of how the brain's "rinse cycle" might occur. A pseudolymphatic perivascular route most active during sleep may be responsible for waste clearance in the brain. The "glymphatic" system, aptly named after the system's dependence on specialized astro*glial* aquaporin-4 channels, propels metabolic waste clearance through convective exchanges of cerebrospinal fluid (CSF) and interstitial fluid (ISF) (Iliff et al., 2012). Glymphatic perfusion occurs along perivascular routes; CSF influxes along penetrating arteries, and convective flow from arterial pressure propagates ISF drainage along veins (Fig. 34.2). Critically, metabolic waste clearance is most efficiently driven by slow-wave sleep (Xie et al., 2013).

In a landmark study conducted by Xie et al. (2013), slow-wave sleep in rodents was associated with a 60% increase in interstitial space resulting in enhanced Aβ clearance via glymphatic activation. Chronic sleep deprivation or altered sleep architecture may disrupt clearance of metabolic waste through glymphatic suppression, thereby exacerbating Aβ

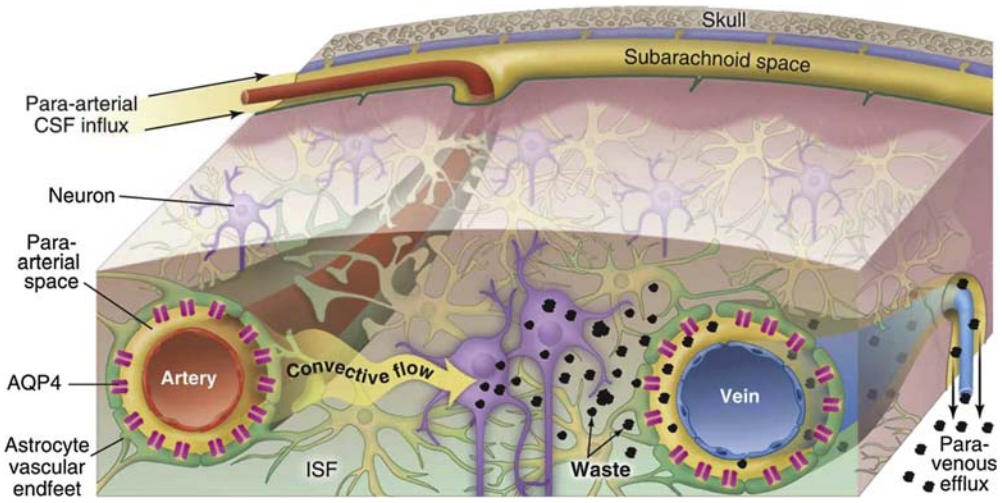

Figure 34.2 Mechanisms involved in glymphatic system activation. Convective glymphatic fluxes of cerebrospinal fluid (CSF) and interstitial fluid (ISF) propel the waste products of neuron metabolism into the paravenous space, from which they are directed into lymphatic vessels and ultimately return to the general circulation for clearance by the kidney and liver. *(Reproduced with permissions from Nedergaard. 2013.* Science 340*(6140):1529–1530.)*

accumulation, particularly in late adulthood given recent experimental findings indicating a significant decrease in glymphatic perfusion in aging mice (Kress et al., 2014). These findings have been confirmed in preclinical experiments involving sleep restriction. Acute and chronic sleep deprivation are associated with a significant rise in ISF Aβ accumulation (Gerstner et al., 2017; Tabuchi et al., 2015). However, an important limitation that requires further consideration is the use of *exogenously* administered Aβ to examine the function of glymphatic activity during sleep. It is not known whether endogenously aggregated Aβ is cleared and mediated by sleep. Furthermore, no studies to date have directly assessed the glymphatic system in AD patients. Nonetheless, preliminary findings from human studies generally mirror preclinical results.

In humans, Bernardi et al., (2016) found that 24 h of sleep restriction with demanding cognitive task training was associated with decreased ventricular volumes, indicative of decreased glymphatic perfusion and interstitial space. This was normalized following sleep rebound. Shokri-Kojori et al. (2018) expanded on neuroanatomical findings and provided direct evidence for the impact of sleep deprivation on Aβ accumulation in humans. Relative to baseline, one night of sleep deprivation increased Aβ aggregation in the hippocampus and thalamus measured using ^{18}F-florbetaben uptake on positron emission tomography imaging. These results dovetail findings in cognitively normal adults revealing that worse subjective sleep quality and generalized self-reported sleep dysfunction are associated with lower CSF Aβ and greater AD pathology (Sprecher et al., 2017). It is theorized that in humans, lower Aβ in CSF is reflective of Aβ aggregation in neuronal tissue, suggestive of suppressed glymphatic clearance of Aβ peptides (Spies, Verbeek, van Groen, & Claassen, 2012). In patients with obstructive sleep apnea (OSA) caused by nocturnal obstructions of the upper airway and characterized by sleep fragmentation and oxygen desaturation, OSA severity predicted a fall in CSF Aβ levels over 2 years (Sharma et al., 2018). However, in healthy adults, suppression of slow-wave sleep was associated with greater CSF Aβ levels (Ju et al., 2017). These findings may reflect the impact of excitatory neuronal activity (i.e., nocturnal arousal from slow-wave sleep disruption) on acute endogenous Aβ secretion in CSF.

Sleep—wake dysfunction and tau pathology: Hyperphosphorylated tau proteins forming neurofibrillary tangles are a pathognomonic feature of AD and are more strongly associated with cognitive impairment when compared with Aβ (Nelson et al., 2012). While the majority of authors researching sleep and AD have focused on the potential for glymphatic activation to clear Aβ, recent findings reveal a potentially similar effect for tau proteins. This was first observed in rodent models of traumatic brain injury (TBI) (Iliff et al., 2014). A prior history of TBI has been associated with a late-life diagnosis of AD, although the proposed mechanisms have always been controversial (Ramos-Cejudo et al., 2018). Following TBI, glymphatic system activity was reduced by 60% and persisted for more than 1 month postinjury (Iliff et al., 2014). Furthermore, genetic knockout of glymphatic-dependent astroglial aquaporin-4 channels exacerbated

glymphatic suppression and increased interstitial phosphorylated tau pathology, neurodegeneration, and neuroinflammatory markers after injury (Iliff et al., 2014).

In rodent models of AD pathology, chronic sleep restriction of 6 h/day increased cortical insoluble tau twofold compared with that of controls (Di Meco, Joshi, & Praticò, 2014). In a comparable experiment, sleep restriction of 4 h/day for 8 weeks was associated with increased fraction of insoluble tau (Di Meco et al., 2014). In humans, nocturnal sleep architectural dysfunction is associated with orexinergic system dysregulation and tau-mediated neurodegeneration (Liguori et al., 2014). Overexpression of orexin, a neuropeptide that regulates arousal, parallels sleep dysfunction and may be directly related to CSF total tau and tau phosphorylation (Osorio et al., 2016). In healthy humans, worse at-home sleep efficiency was associated with increased CSF tau (Ju et al., 2017). These findings highlight the neurobiological impact of chronic sleep deprivation that may modulate the development of AD neuropathology.

The bidirectionality between sleep dysfunction, Aβ, and tau pathology: Whereas sleep dysfunction may lead to increases in brain Aβ and tau, AD pathology may further the progression of sleep dysfunction. In *Drosophila* models of AD, a bidirectional effect of Aβ burden and sleep dysfunction was reported such that increased Aβ was associated with sleep fragmentation, and chronic sleep deprivation directly elevated Aβ levels (Gerstner et al., 2017; Tabuchi et al., 2015).

Distinct patterns of tau phosphorylation have been reported in sleep-specific brain regions. Sleep-regulating structures within the ascending arousal system are affected by tau pathology before cortical tau or amyloid deposition. One area of relevance is the locus coeruleus, a nucleus within the brain stem's dorsal pontine tegmentum. The locus coeruleus is one of the first structures in which tau pathology appears in AD; locus coeruleus noradrenergic neuronal loss is extensive, with density of these neurons reflective of expedited cognitive decline (Wilson et al., 2013). These findings also extend to the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus, regions critical in sleep regulation that also exhibit tau pathology (Dugger, Tu, Murray, & Dickson, 2011). Tau pathology in sleep—wake structures has been shown to affect sleep architecture directly. In $PLB1_{Tau}$ mice models, forebrain mutant human tau model expression explicitly suppresses NREM sleep and affects EEG amplitude compared with effects in combined tau and Aβ PBL1 models (Jyoti, Plano, Riedel, & Platt, 2015).

The longitudinal assessment of sleep, glymphatic activity, Aβ, and tau in large cohorts is needed to corroborate and expand translation from animal models to humans. Furthermore, it is unclear whether sleep-mediated glymphatic clearance of metabolites is present and equally efficient in humans. Direct assessment of the glymphatic system in humans may further elucidate the mechanistic role of sleep dysfunction and Aβ deposition. Prospective studies are also needed to tease apart the extent to which sleep dysfunction predicts the emergence and progression of AD pathology and vice versa.

Sleep dysfunction and Alzheimer's dementia

Sleep pathology is pronounced in people with a clinical diagnosis of AD: Sleep changes are common with aging and are pronounced in AD dementia. Epidemiological studies suggest that nearly half of all AD patients experience impaired sleep (Moran et al., 2005). Sleep deficits observed in AD include abnormalities in sleep architecture, sleep disorders, and a worsening of subjective sleep quality. Caregivers are also burdened by patient sleep–wake disturbances, which are a significant risk factor for early institutionalization (Pollak, Perlick, Linsner, Wenston, & Hsieh, 1990). Indeed, the severity of sleep disturbance increases with the progression of AD dementia (Pat-Horenczyk, Klauber, Shochat, & Ancoli-Israel, 1998). The underlying cause of sleep pathology in AD is multidimensional and bidirectional: neuronal loss to sleep–wake regulating networks (e.g., ascending arousal system) common in AD pathogenesis may directly impair sleep function, and psychiatric or medical comorbidities (e.g., depression, pain) may contribute to sleep–wake changes.

Sleep architectural disturbances are more severe in AD patients than in age-matched controls (Benca, Obermeyer, Thisted, & Gillin, 1992). Sleep efficiency, a ratio of time spent asleep versus in bed, is markedly decreased in AD patients, likely a result of increased sleep-onset latency and nocturnal awakenings. Total sleep time is also reduced in patients with AD, and together with increased wake after sleep-onset may increase daytime irritability and somnolence and contribute to cognitive impairment. Objective assessment and discrimination of NREM sleep stages, particularly slow-wave sleep, are problematic in AD patients due to low-amplitude (0.5–2 Hz) and topographically diffuse EEG activity during both sleep and wake (Bliwise, 2004). Nonetheless, a majority of studies report marked AD-related decreases in slow-wave sleep, sleep spindles, and K complexes (Hassainia, Petit, Nielsen, Gauthier, & Montplaisir, 1997; Prinz et al., 1982). Interestingly, REM sleep duration is disrupted in AD patients despite being preserved in normal aging. Specifically, REM alterations focally affect parieto-temporal and frontal regions and are likely reflective of cholinergic dysfunction within the brain stem and forebrain (Hassainia et al., 1997; Montplaisir, Petit, Gauthier, Gaudreau, & Décary, 1998; Peter-Derex, Yammine, Bastuji, & Croisile, 2015). REM abnormalities have therefore been proposed as a potential physiological marker of AD (Prinz et al., 1982).

Cholinergic denervation is also associated with REM behavior disorder (RBD), a parasomnia characterized by loss of usual nocturnal muscle atonia and "acting out" of vivid dreams. RBD is pathognomic of Lewy body dementia and α-synucleinopathies. That is, patients with a diagnosis of RBD *will* develop an α-synucleinopathy if they live long enough (Postuma et al., 2009). While RBD beyond α-synucleinopathies is rare, cases of RBD in AD have been reported and may be associated with disinhibition of cholinergic mesopontine neuronal loss from locus coeruleus neurodegeneration

(Schenck, Garcia-Rill, Skinner, Anderson, & Mahowald, 1996). Thus, the presence of RBD in clinical AD may be indicative of AD comorbid with α-synucleinopathy dementia subtypes (Boeve, Silber, & Ferman, 2004).

Sleep-disordered breathing is common in patients with AD. Approximately 40%–70% of patients with AD experience more than five apneas/hypopneas per hour of sleep, suggestive of mild OSA (Hoch, Kupfer, Houck, Berman, & Stack, 1986; Reynolds et al., 1985). Patients with AD and OSA also exhibit significant dysfunction in sleep architecture compared with non-AD OSA controls (Cooke et al., 2006). Furthermore, OSA is associated with increases in Aβ (Sharma et al., 2018). The pathophysiological mechanisms underlying the relationship between sleep apneas and AD remain merely speculative; neurodegeneration to respiratory centers within the brainstem may directly contribute to sleep-related breathing disorders, while OSA may contribute to dementia by causing ischemic brain injury and sleep disruption that could contribute to cognitive impairment (Peter-Derex et al., 2015). In this manner, sleep disturbances may be both a cause and a consequence of AD dementia. The following sections review the evidence for poor sleep as a risk factor for AD dementia or cognitive decline.

Subjective sleep and the risk of cognitive decline and dementia: Numerous studies have examined aspects of self-reported sleep with respect to cognitive decline and dementia. Cross-study comparisons are hampered by heterogeneous methods for sleep measurement and differences in the quality of dementia case ascertainment. Despite these limitations, long sleep duration has consistently been associated with increased risk of dementia; however, definitions of long sleep duration vary between studies (Ohara et al., 2018; Virta et al., 2013; Westwood et al., 2017). Compared with those of long sleep duration, findings for short sleep duration and risk of dementia are less consistent. In the Framingham Heart Study, although long sleep duration (>9 h vs. ≤9 h) was associated with an approximate doubling of dementia risk over the next 10-years, short sleep duration (<6 h) was not (n = 2457); however, it did correlate with poorer cognitive performance (Westwood et al., 2017). Such studies raise the question of whether persons who have always been long sleepers are at increased risk of dementia or whether prodromal AD drives changes in sleeping habits. The study by Westwood et al. suggests the latter; associations between long sleep duration and incident dementia were driven by persons with mild cognitive impairment at baseline and by persons transitioning to becoming long sleepers in old age (Westwood et al., 2017).

In a sample of older Swedish adults, persons self-reporting a decrease in sleep depth or duration were twice as likely to develop AD dementia after 9 years of follow-up (Hahn, Wang, Andel, & Fratiglioni, 2014). However, findings were no longer significant after adjusting for depressive symptoms, suggesting that mood may mediate this relationship. Self-reported poor sleep quality was associated with accelerated decline in executive function over a mean of 3.4 years of follow-up in 2822 cognitively intact

community-dwelling older men from the Osteoporotic Fractures in Men Study (Blackwell et al., 2014). However, the community-based Rotterdam study (N = 4835) did not find any associations between subjective sleep measures and risk of dementia over 13 years of follow-up (Lysen et al., 2018).

Objectively measured sleep and dementia risk: A number of large cohorts have used actigraphy (activity monitoring) to provide information on sleep continuity and risk of dementia. The Osteoporotic Fractures in Men Study reported that poorer sleep efficiency and greater sleep fragmentation (i.e., increased bouts of wakefulness) were linked to a higher risk of clinically significant cognitive decline over a mean of 3.4 years (Blackwell et al., 2014). For example, persons with sleep efficiency less than 70% (vs. ≥70%) and wake after sleep onset ≥90 min (vs. <90 min) displayed 1.6 and 1.5 times higher odds, respectively, of clinically significant decline in executive functioning. Similarly, the Rush Memory and Aging Project reported that persons with high sleep fragmentation (90th percentile) over 10 consecutive days of actigraphy had a 1.5-fold risk of developing AD dementia over a mean follow-up of 6 years (Lim, Kowgier, Yu, Buchman, & Bennett, 2013).

Unlike polysomnography, actigraphy does not record EEG that precludes sleep staging, and actigraphy has poor specificity for determining wakefulness (Marino et al., 2013). In the only existing study to use polysomnography to examine the association between sleep architecture and the risk of incident dementia, the Framingham Heart Study identified reduced REM sleep as a risk factor for incident AD dementia up to 18 years later (Pase et al., 2017). Effect sizes were large, with each percentage reduction in REM sleep associated with a 9% increase in dementia risk. Stages of NREM sleep were not associated with dementia risk. In the population-based prospective Osteoporotic Fractures in Men Study, men with the lowest quartile of REM sleep (vs. highest quartile) experienced more than twice the annual rate of cognitive decline (modified mini-mental state examination) over a mean follow-up of 3.4 years (Song et al., 2015). Similar to the results of Pase and colleagues, slow-wave sleep was not associated with cognitive decline. Thus, despite interest surrounding the role of slow-wave sleep in glymphatic flow and Aβ clearance, cohort studies identify shorter REM rather than slow-wave sleep as a predictor of cognitive decline and dementia.

Sleep disorders and risk of cognitive decline and dementia: A meta-analysis combining study-level estimates from three prospective cohorts reported that the presence of insomnia and sleep-disordered breathing were each associated with 1.5 and 1.2 times higher relative risk of AD dementia, respectively (Shi et al., 2020). However, two of the three studies on insomnia relied on self-report, and one of the studies on sleep-disordered breathing relied on snoring symptoms. Consequently, such results may be influenced by recall bias and the fact that not all persons may have good insight into their sleep. Although not included in the meta-analysis, the Study of Osteoporotic Fractures showed that older women with untreated moderate-to-severe OSA were 85% more

likely to have mild cognitive impairment and dementia as a combined outcome after 4.7 years of follow-up (Yaffe, Laffan, Harrison, & et al., 2011). In contrast, the Atherosclerosis Risks in the Community Study did not find consistent evidence for an association between OSA and the risk of dementia or cognitive decline over approximately 15 years of follow-up in a large community sample (Lutsey et al., 2016, 2018). Such research highlights how little is known about the role of sleep disorders in the development of dementia. Concerning sleep-disordered breathing, research is needed to establish the thresholds of OSA severity best associated with future dementia.

Perspectives

Difficulties with sleep are often secondary to treatable causes such as illness, disturbed circadian rhythms, or medication use (Ancoli-Israel & Ayalon, 2006). However, different problems with sleep require different treatments. Poor sleep quality has long been linked to impairments in cognitive function, at least in the short term, with research now attempting to unravel the associations between sleep and dementia. Studies on sleep and AD pathology have implicated disturbed slow-wave sleep in the short-term accumulation of Aβ, whereas prospective studies with clinical endpoints have failed to link slow-wave sleep to the incidence of dementia or cognitive decline. Rather, such studies suggest an association of long sleep duration, sleep fragmentation, and lower REM sleep with a higher risk of cognitive impairment and dementia. Clarifying the specific aspects of sleep that relate to incident dementia is the first step toward the development of sleep interventions to reduce dementia risk.

Research on sleep and AD remains in its infancy with an explosion of recent interest. AD has a long preclinical phase, making it challenging to ascertain directionality between sleep dysfunction and the genesis and progression of AD in humans. Further well-powered community-based cohort studies are needed to ascertain whether sleep disturbances are associated with the development and progression of preclinical AD as well as the onset of clinical dementia, which could identify clearer targets for intervention. Given the failure of amyloid-lowering drugs to impact clinical dementia, research into mechanisms linking sleep and AD beyond Aβ should be a priority. However, definitive evidence for sleep dysfunction as a modifiable risk factor for AD dementia will need to come from randomized controlled trials. The SAVE trial, which showed that continuous positive airway pressure did not reduce cardiovascular events in OSA, serves as a reminder that treating putative risk factors does not always have the intended protective effect (McEvoy et al., 2016). Combined with the failure of antiamyloid therapies, such results should encourage continued research into the effects of poor sleep on AD risk but also suggest caution in extrapolating beyond preclinical or observational data.

Key facts about the regulation of sleep and wake

- The mechanisms underpinning sleep and wake regulation include a homeostatic process and an internal 24-h circadian rhythm.
- Sleep is homeostatically regulated such that sleep pressure increases with elapsed time awake and dissipates during sleep.
- The circadian system allows sleep to occur at similar times from day to day.
- The circadian system is bilaterally rooted in the suprachiasmatic nucleus of the anterior hypothalamus.
- The human suprachiasmatic nucleus generates an internal sleep–wake rhythm approximately 24.2 h in length.
- Sleep–wake cycles are governed by interactions between the circadian and homeostatic processes, the light–dark cycle (which transmits light cues to the suprachiasmatic nucleus), and feedback from the sleep–wake cycle.

Summary points

- The relationship between sleep disturbance and AD dementia may be bidirectional.
- Sleep disturbances are highly prevalent in AD dementia.
- Sleep has been linked to enhanced glymphatic flow, assisting with the clearance of metabolic waste and Aβ.
- Wakefulness and slow-wave sleep disruption are associated with acute increases in Aβ levels.
- However, cohort studies have linked lower REM sleep rather than slow-wave sleep to a higher risk of cognitive decline or future dementia.
- As sleep is modifiable, future research is needed to unravel the specific aspects of sleep that most strongly associate with the development of preclinical and clinical AD dementia.

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PART III

Behaviour and psychopathology

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CHAPTER 35

Overview of behaviors in dementia

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List of abbreviations

BPSDs behavioral and psychological symptoms of dementia
ISB inappropriate sexual behavior
NPSs neuropsychiatric symptoms

Mini-dictionary of terms

Challenging behavior multifaceted, frequent unpredictable behavior lasting for long periods and resulting in distress to the individual exhibiting the behavior and/or their caregivers

Circadian rhythm the body's 24-h internal clock that determines an individual's alertness and sleepiness in regular cycles

Inappropriate sexual behavior inappropriate sexual behavior exhibited verbally or physically as intimacy or disinhibition that is not consistent with the individual's personality prior to dementia

Neuropsychiatric symptoms clustered behaviors including but not limited to confusion, appetite and eating abnormalities, aggression (both verbal and physical), agitation, anger, psychosis (delusions and hallucinations), paranoia, irritability, apathy, hoarding, nighttime behaviors (sundowning), wandering, repetitive questioning, withdrawing, sexually inappropriate behaviors, motor disturbances, and depressive behaviors including suicidal ideations

Sundowning syndrome behavior associated with increased agitation, restlessness, and confusion usually occurring in the evening and nighttime hours. This syndrome is routinely noted as dementia progresses to the middle stages and is sensitive to inadequate or disturbed sleep and circadian rhythm alteration

Introduction

Dementia is an umbrella term given to neurocognitive diseases that cause overall decline in global and cognitive functioning (Kverno & Velez, 2018; Marshall & Hale, 2017). Brain injuries can also cause dementia (Alzheimer's Association, 2018; Kverno & Velez, 2018). Dementia is considered to occur when two core mental functions are significantly impaired (Fig. 35.1). These core functions include memory, focus and attention ability, language and communication, judgment and reasoning ability, and visual perception (Alzheimer's Association, 2018). Dementia diagnoses encompass many different forms (Fig. 35.2): Alzheimer's disease, Lewy body dementia, vascular dementia, frontal lobe dementia, Parkinson's, Creutzfeldt-Jakob, and Wernicke-Korsakoff syndrome (Butcher, 2018; Marshall & Hale, 2017). Each type of dementia manifests and progresses in

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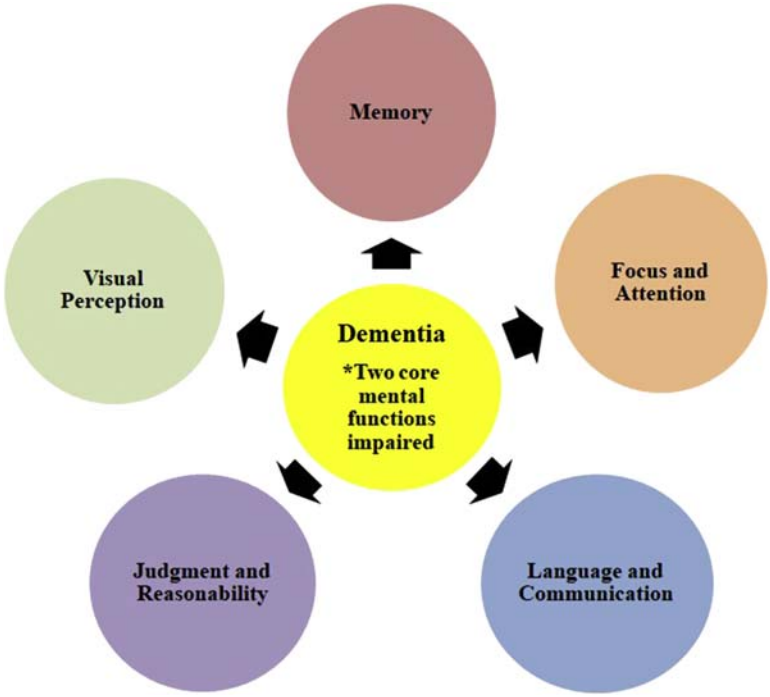

Figure 35.1 Core mental functions associated with dementia diagnoses.

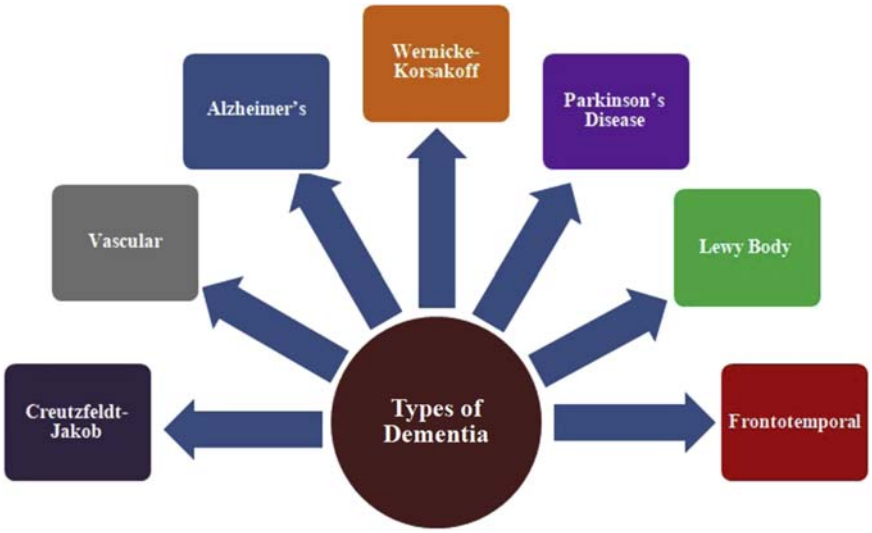

Figure 35.2 Types of dementia.

different ways and is dependent on the type of brain cell damage and region of the brain affected (Alzheimer's Association, 2018; Butcher, 2018). Dementia does not occur as a normal aspect of aging (Marshall & Hale, 2017). The statistics surrounding dementia are astonishing. Over 30 million persons are affected globally, and the number is projected to increase to over 100 million by 2050 (Spector, Orell, Charlesworth, & Marston, 2016). The majority of people affected by dementia are more than 65 years of age. These people may be cared for by family or extended care facilities (Kvaal, Engedal, & Ulstein, 2013). The majority of individuals with dementia progress to a moderate or severe form of the disease resulting in a combination of cognitive and functional decline, communication impairments, neuropsychiatric symptoms, and comorbid medical conditions (Ballard et al., 2018). There is no known cure for this debilitating disease (Butcher, 2018).

In dementia, changes in the brain become neurotoxic, kill nerve cells, affect the person's ability to function, and adversely affect neurotransmitters in the brain (Nazarko, 2011), resulting in challenging behaviors. These behaviors are defined as challenging because caring for individuals with dementia can be stressful for family caregivers as well as professionals (Gitlin, Kales, Lyketsos, & Plank, 2012). Challenging behaviors as a term is relatively new and encompasses previous terms and descriptions such as disruption in behavior, neuropsychiatric symptoms (NPSs), and behavioral and psychological symptoms of dementia (BPSDs) (Zwijsen et al., 2014). Almost every person with dementia will exhibit a challenging behavior (Fig. 35.3). These behaviors correlate with the person's inability to connect with social interactions (Zwijsen et al., 2014), and the behaviors continue to evolve over the span of the disease (Zwijsen et al., 2014). This insidious disease process prevents the individual from making sense of the circumstances within their environment, resulting in an increased risk for falls, accidents, and issues with skin integrity (Kemerer, 2018). Behaviors associated with dementia are often perceived as being impulsive or childlike (Ferman, Smith, & Melom, 2018). Dementia patients have little insight into their behaviors (Gitlin et al., 2012). Their behavior becomes their form of communication (Ferman, Smith, & Melom, 2018).

Neuropsychiatric Behaviors in Dementia

*May Exhibit Alone or in Clusters

Confusion	Appetite Disturbance	Verbal and Physical Aggression
Agitation	Anger	Psychosis
Paranoia	Irritability	Apathy
Hoarding	Night-time behaviors	Wandering
Repetitive Questions	Sexual Inappropriateness	Withdrawing
Motor Disturbance	Depressive Behavior	Suicidal Ideations

Figure 35.3 Neuropsychiatric behaviors in dementia.

Challenging behaviors

Challenging behavior is described as any behavior that is unpredictable, frequent, lasts for long periods, and causes distress to the individual or others, including caregivers (Nazarko, 2011). These behaviors often are multifaceted and can be attributed to changes in the environment, chronic comorbid conditions, or medication (Daly, Bay, Levy, & Carnahan, 2015). Environmental factors affecting behaviors of dementia include over-stimulation, understimulation, routine changes, and demands of functional ability (Kales, Gitlin, & Lyketsos, 2015). Undetected illness has also been associated with behavioral and psychological symptoms of dementia (Kales et al., 2015). Aggressive behavior can be a manifestation of pain (Kales et al., 2015). Personality characteristics may have a direct correlation with the development of psychological and behavioral symptoms of dementia and the treatment of those behaviors (Kales et al., 2015).

Behaviors are generally associated with types of dementia. The neuropsychiatric symptoms of dementia include confusion, appetite and eating abnormalities, aggression (both verbal and physical), agitation, anger, psychosis (delusions and hallucinations), paranoia, irritability, apathy, hoarding, nighttime behaviors (sundowning), wandering, repetitive questioning, withdrawing, sexually inappropriate behaviors, motor disturbances, and depressive behaviors including suicidal ideations (Daly et al., 2015; Kales et al., 2015). These behaviors often occur in clusters (Gitlin et al., 2012; Kales et al., 2015).

It is common for individuals with vascular dementia to experience depression as well as urinary urgency, frequency, and incontinence. Emotional responses are often stronger than what is considered usual behavior (Butcher, 2018; Gitlin et al., 2012). Hallucinations are more common with Lewy body dementia than with Alzheimer's dementia (Kales et al., 2015). Behaviors such as disinhibition (social and sexual inappropriateness), apathy, and wandering are more closely associated with frontotemporal dementia (Kales et al., 2015). Alzheimer's dementia symptoms include anxiety and depression in the early stages that continue to worsen as the disease progresses (Kales et al., 2015). Apathy is the most frequently seen behavior in most types of dementia (Gitlin et al., 2012). In the moderate to severe stages of the disease, delusions, hallucinations, and aggression are seen. These behaviors tend to be episodic (Kales et al., 2015).

Cognitive changes

A decline in cognitive function may be the precipitator of the challenging behaviors in dementia patients. A decline in memory function is a primary warning sign of cognitive decline (Morbidity and Mortality Weekly Report [MMWR], 2013). Dementia routinely has characteristic symptoms that include memory loss (short or long term), poor judgment, aphasia, and difficulty performing activities of daily living (Staedtler & Nunez, 2015). The patient often exhibits fluctuations in cognition, attention, and alertness

resulting in a loss of focus, memory, ability to learn, and language capabilities (MMWR, 2013). These fluctuations may last a few minutes to a few hours (Hamdy, Kinser, Lewis, Kendall-Wilson, & Whalen, 2017). This loss of cognitive decline negatively affects quality of life and functioning for dementia patients.

Agitation and aggression

Agitation and aggression are common among dementia patients, especially those that have progressed to moderate or severe dementia (Ballard et al., 2018; Costa et al., 2018; Spector et al., 2016). Forty to sixty percent of dementia patients will experience agitation. It remains one of the most distressing symptoms, increases caregiver burden, and increases the possibility of institutionalization (Costa et al., 2018). Aggressive behavior can often be associated with internal factors such as depression, psychosis, and pain, or a combination of those factors (Kales et al., 2015; Van der Mussele et al., 2015). It may also occur because of thirst, hunger, or tiredness. An adverse effect to medication may also trigger agitation and aggressive behavior (Alzheimer's Association, 2018). As the disease progresses, the patient's ability to process external stimuli and express needs decreases. This creates higher levels of frustration, anxiety, and agitation (Kales et al., 2015). Agitation encompasses a wide range of affective, verbal, and motor disturbances (Costa et al., 2018). Emotional distress, increased psychomotor activity, wandering, irritability, vocally disruptive issues, and refusal of care is a manifestation of the internal distress the person with dementia is unable to communicate to others (Gitlin et al., 2012). Often, behaviors exhibited may be totally out of character for the person prior to the stages of dementia. These character changes may lead to discontent with relationships, especially with caregivers (Spector et al., 2016). Caregivers often feel disconnected and frustrated by their inability to maintain the same type of relationship they enjoyed with the person prior to the diagnosis of dementia (Alzheimer's Association, 2018).

Although the most frequent disturbances associated with agitation manifest as restlessness, cursing, aggressive actions, hyperactivity, refusal of care exhibited by combativeness, and repetitive yelling (Costa et al., 2018), it may also manifest as uncontrollable bouts of laughter and mood lability (Van der Linde et al., 2013). Agitation with aggression may occur with an increased level of distress and decreased quality of life for the dementia patient. Caregivers often express feelings of increased burden caring for the person with dementia (Ballard et al., 2018).

Categories of agitation

Four categories of agitation exist: aggressive physical, nonaggressive physical, aggressive verbal, and nonaggressive verbal (Van der Mussele et al., 2015). Patients with agitation have been shown to display more severe behavioral symptoms or challenging behaviors

including the symptoms of depression (Van der Mussele et al., 2015). Agitation and aggressive behaviors may escalate due to stress caused by too much stimuli, lack of stimuli, and/or environmental changes (Kales et al., 2015). Aggression and agitation are frequently difficult behaviors to manage clinically (Staedtler & Nunez, 2015).

Sundowning syndrome/wandering

Increased agitation, restlessness, and confusion in the evening hours and throughout the night are referred to as sundowning syndrome (Ferman et al., 2018). This syndrome is distinguished by increased anxiety, asking repetitive questions, increased tearfulness, increased hallucinations or delusions, pacing, wandering, attempts to "go home," and worsening confusion (Forbes, 2011). Activities of daily living such as toileting may become more difficult in the evening hours (Forbes, 2011). These behaviors will begin in middle stages of dementia and escalate as dementia progresses (Forbes, 2011). Inadequate or disturbed sleep patterns and circadian rhythm alterations have been shown to affect sundowning (Forbes, 2011; Ooms & El-Ju, 2016). The person affected by dementia may exhibit for months or years when in midstage dementia, but these behaviors tend to cease as function declines (Forbes, 2011).

Loss of role identity during the routinely busy evening hours has been attributed as one reason why behaviors escalate at the end of the day (Forbes, 2011). Other reasons include the person feeling hungry and being unable to express this need. Sundowners may be predicated by the person being tired or pain medication that is no longer effective (Forbes, 2011). Disturbed sleep patterns due to poor sleep/wake patterns also effect sundowner's syndrome (Forbes, 2011).

Another common behavior expressed in relation to anxiety or distress in dementia is wandering. The wandering term covers behaviors including aimless movement without a detected purpose (Cipriani, Lucetti, Nuti, & Danti, 2014). The person with dementia is unsettled in some way and begins to wander as an expression of something they can perform (Andrews, 2017). Pacing and restlessness may result from discomfort or an unmet need such as toileting. The person may be uncomfortable with the temperature or noise of the space (Andrews, 2017). Wandering may also be due to boredom (Ferman et al., 2018). The main psychosocial factors potentially affecting a propensity for wandering behavior are a lifelong pattern of coping with stress, previous work roles, and places associated with comfort and security (Cipriani et al., 2014). Assessing the rationale of the wandering may be difficult due to communication difficulties in dementia. Negative outcomes of wandering may be accidents, injury, and getting lost as well as malnutrition, weight loss, fatigue, and sleep disturbances (Cipriani et al., 2014).

Sleep impairment

Sleep impairment in dementia patients is pervasive and contributes to negative health outcomes as well as decreased quality of life for these persons (Petrovsky et al., 2018).

Ninety percent of people with Lewy body dementia and Parkinson's dementia suffer from sleep disturbances (Petrovsky et al., 2018). These disturbances in sleep patterns often result in the person requiring professional care and nursing home placement (Ferman et al., 2018). Circadian rhythms are affected in normal aging; however, it is much worse in dementia due to neurodegeneration in the affected areas of the brain that regulate sleep (Ooms & El-Ju, 2016). Sleep impairment includes poor sleep and increased night awakening that may include an increased level of restlessness or anxiety (Petrovsky et al., 2018). Dementia patients may also exhibit an increased level of drowsiness during daytime hours. Daytime naps can exacerbate nighttime sleep impairment (Petrovsky et al., 2018). Sleep impairment without proper treatment causes emotional distress, increased rates of depression and apathy, increased cognitive decline, increased rates of challenging behaviors, sundowning, and overall functional decline including morbidity and mortality (Petrovsky et al., 2018). Hypersomnia due to loss of orexinergic neurons (Ooms & El-Ju, 2016) may also be present with dementia patients (Desai, Schwartz, & Grossberg, 2012).

Inappropriate sexual behavior

Sexuality is a basic human need, and this need does not change with a diagnosis of dementia. Sexual interest may not change as dementia patients progress in age and may last well into the eighth decade of life (Cipriani, Ulivi, Danti, Lucetti, & Nuti, 2015). There are two types of inappropriate sexual behavior (ISB), intimacy and disinhibited (Cipriani et al., 2015). ISB may be displayed in dementia in any or all of the following ways: sex talk, sexual acts, and implied sexual acts (Cipriani et al., 2015) The most common form of this behavior involves the use of inappropriate language of sexual content not consistent with the patient's predementia personality (Cipriani et al., 2015). Other behaviors that include touching, grabbing, exposing genitals, and masturbating in view of other persons are examples of sexual acts. Requesting additional genital care and viewing of pornographic material define implied sexual acts (Cipriani et al., 2015). Men exhibit more physically aggressive activities while women exhibit more verbal behaviors (Cipriani et al., 2015; Desai et al., 2012).

The prevalence of some type of sexual disinhibition can be as high as 25% in dementia patients, and there is no correlation with the stage of disease progression (Cipriani et al., 2015). This challenging behavior can be witnessed in mild to severe dementia stages (Cipriani et al., 2015). ISB may also be seen in the early stages of frontotemporal dementia (Cipriani et al., 2015). A higher prevalence of ISB is seen in nursing facilities as opposed to community-dwelling dementia patients (Cipriani et al., 2015). As a dementia patient's cognition and judgment decline, challenging, sexually inappropriate behaviors may rise (Cipriani et al., 2015). One description of inappropriate dementia-related sexual behaviors is reported as overt acts associated with increased libido or disinhibited sexual acts directed at oneself or other persons, while another describes this behavior as a graphic verbal or physical act of sexual content (Cipriani et al., 2015).

Hoarding

Although hoarding behavior is not as frequently seen as other behaviors of dementia, it does occur and is primarily in frontotemporal dementias such as Alzheimer's disease (Bicer Kanat, Altunoz, Kirici, Bastug, & Ozel Kizil, 2016). Studies have shown that hoarding behavior associated with dementia affects 22%–36% of that population (Bicer Kanat et al., 2016). This compulsion to collect and keep unnecessary items that are unusable can result in a restriction in living space that results in environmental hazards such as fall and fire hazards as well as breeding areas for insects and germs (Bicer Kanat et al., 2016). Hoarding associated with dementia is referred to as an agitation behavior, because it is often accompanied by disinhibition causing the patient to take and hide things that do not belong to them (Bicer Kanat et al., 2016).

Psychiatric symptoms associated with dementia

Psychiatric symptoms are quite common in those with dementia. Sixty to 98 percent of people with dementia experience NPSs (Daly et al., 2015; Kverno & Velez, 2018). These symptoms can include depression, apathy, psychosis, wandering, and sleep impairment (Van der Linde, Stephan, Matthews, Brayne, & Savva, 2013). When clustered, the behaviors may be known as NPSs or BPSDs and are representative of cognitive decline (Van der Linde et al., 2013).

Psychosis

Psychosis may exhibit as hallucinations, delusions, and illusions. Hallucinations are sensory experiences without a trigger and should be differentiated from delusions (Hamdy et al., 2017). Hallucinations can be quite distressing and include hearing voices, seeing things or persons that are not present, smells, or gustatory hallucinations (Van der Linde et al., 2013). They are complex and are often visual, well formed and detailed, featuring deceased persons, children, or animals (Hamdy et al., 2017). Less frequently, hallucinations may be unformed, where the patient sees flashing lights or different colors, or hears noises (Hamdy et al., 2018). In early stages of disease, hallucinations will be frightening. They may quickly flee, and the patient may still have the ability to realize that they are hallucinating; yet as the disease progresses, the patient may begin to believe the hallucinations are real (Hamdy et al., 2017). Fear of hallucinations can intensify other challenging behaviors such as anxiety and aggression (Hamdy et al., 2017).

Delusions/illusions

Delusions are strong, unsubstantiated beliefs despite evidence to the contrary (Hamdy et al., 2018). These false beliefs can include feelings of persecution and irrational thoughts that someone is attempting to harm them or is laughing at them (Van der Linde et al.,

2013). Paranoid delusions tend to occur with late dementia and may include ideas of spousal infidelity, theft, and intruders (Handy et al., 2017). This paranoia is difficult to manage. Due to impairments in memory and attention span, frequent reassurances may be beneficial (Hamdy et al., 2018).

Illusions are misperceptions of an object or a situation. Present items may be perceived as animals wanting to harm the patient—e.g., a snake. Illusions are more common in evening hours when lighting is lower or glares occur (Hamdy et al., 2018). Visual and physical disabilities that may impair the ability to see or hear may heighten these illusions (Hamdy et al., 2018). Visual difficulties such as cataracts, macular degeneration, or unclean glasses can create illusions (Hamdy et al., 2018). Other physical deficiencies such as tinnitus may be interpreted as conversations from others. As circadian rhythms are interrupted, hallucinations, delusions, and illusions may become more prominent in the evening hours (Hamdy et al., 2018).

Depression

Dementia and depression are the two most commonly occurring conditions in adults over the age of 65 and often are comorbid (Kverno & Velez, 2018). Late-life depression is a predisposing symptom of dementia (Kverno & Velez, 2018). Apathy is the most common NPS to occur in dementia and may occur alone or with major depression (Kverno & Velez, 2018). Apathy is the continued loss of motivation or lack or interest in usual activities (Kverno & Velez, 2018). It can manifest as less affection toward loved ones and social withdrawal with detachment (Desai et al., 2012; Kverno & Velez, 2018). Apathy is often the most difficult behavior for caregivers because of the loss of personal contact with a loved one (Gitlin et al., 2012). Additionally, patients with depression may experience lack of motivation to perform day-to-day activities and may suffer from loss of interest in usual activities and continual feelings of sadness (Marshall & Hale, 2017). Major depression is diagnosed after a persistence of depressive symptoms and loss of pleasure over a period of 2 weeks or longer. It encompasses a depressed mood as well as three additional symptoms that may include appetite changes resulting in weight loss or gain, changes in sleep patterns including insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, loss of energy, diminished thought processing, inability to concentrate, indecisiveness, and recurrent thoughts of self-harm or suicide (Kverno & Velez, 2018). Patients should continually be observed for signs and symptoms of suicidal ideations.

Older persons who attempt suicide do so more intently, choose more deadly methods, and are more successful than younger persons (Cipriani, Vedovello, Lucett, Di Fiorino, & Nuti, 2013). Suicide risk is highest at the dementia stage where patients become distressed at loss of independence and the feeling of becoming a burden on others (Cipriani et al., 2013). The probability of suicide increases with physical illness,

functional impairment, a previous suicide attempt, and social isolation (Van Orden & Conwell, 2015). In dementia patients, self-poisoning is the most common method of suicide followed by drowning and hanging (Cipriani et al., 2013). Passive ideations include voicing that life is no longer worth living, significant depressive symptoms, and wishing for death to come (Van Orden & Conwell, 2015). Active suicidal ideations include writing a plan to end life, giving treasured items away, discussing a plan to end life, and having the means to end life. Both passive and active suicidal ideations can lead to suicide in the dementia patient, and they should not be ignored (Van Orden & Conwell, 2015).

Conclusion

Dementia is a progressive disease that causes decline in both global and cognitive functions (Fig. 35.4). This decline results in the patient's lack of ability to effectively communicate their needs to others. Challenging behaviors develop in an effort to communicate and result in stressful situations for all involved in the patient's care. These symptoms are manifested as confusion, appetite and eating abnormalities, aggression

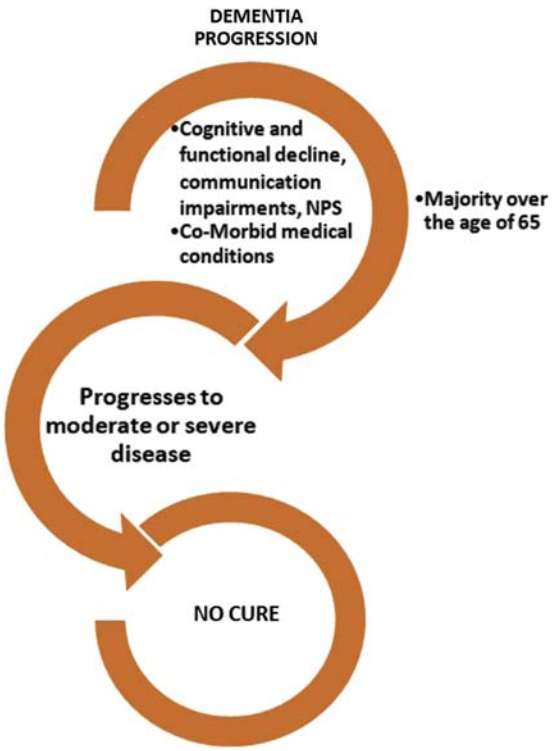

Figure 35.4 Dementia progression.

(both verbal and physical), agitation, anger, psychosis (delusions and hallucinations), paranoia, irritability, apathy, hoarding, nighttime behaviors (sundowning), wandering, repetitive questioning, withdrawing, ISBs, motor disturbances, and depressive behaviors including suicidal ideations. While many challenging behaviors occur in clusters, some behaviors may be episodic. Due to the patient's declining condition, they are often unaware of their behaviors.

Key facts for behavioral disturbances

- Almost every person with dementia will develop a challenging behavior due to neurocognitive decline.
- In dementia, two core mental functions are impaired.
- Challenging behavior is described as any behavior that is unpredictable, frequent, lasts for long periods, and causes distress to the individual or others.
- Behaviors can occur in clusters or may be episodic in appearance.
- How behavior presents is directly related to the amount of brain cell damage and the area of the brain affected.

Summary points

- Dementia is a progressive neurological disease leading to the development of challenging behaviors.
- Behaviors may be documented or known as BPSDs, ISB, or NPSs.
- Patients experiencing common symptoms of dementia including fluctuations in cognition, attention, loss of focus, memory, ability to learn, and language capabilities can exhibit challenging behaviors.
- The type of challenging behavior may be dependent on the type of dementia the person exhibits.
- Behaviors may manifest as confusion, appetite abnormalities, aggression (both verbal and physical), agitation, anger, psychosis (delusions and hallucinations), paranoia, irritability, apathy, hoarding, nighttime behaviors (sundowning), wandering, repetitive questioning, withdrawing, sexually inappropriate behavior, motor disturbances, and depressive behaviors including suicidal ideations.

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CHAPTER 36

Delirium superimposed on dementia: a clinical challenge from diagnosis to treatment

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Mini-dictionary of terms

Activities of daily living (ADLs) ADLs is a term used in health care to refer to people's daily self-care activities. Common ADLs include bathing, hygiene, dressing, transferring, feeding, and continence.

Drug reconciliation The process of ensuring that a hospital patient's medication list is as up-to-date as possible.

Endocrinopathies Endocrinopathy is commonly used as a medical term for a hormone problem. Common endocrinopathies include hyperthyroidism and hypothyroidism.

Hypoxia Hypoxia is a condition in which the body or a region of the body is deprived of adequate oxygen supply at the tissue level.

Subdural hematoma Subdural hematoma is a type of hematoma usually associated with traumatic brain injury.

Urinary retention Urinary retentionis the inability to empty the bladder completely.

Introduction

Delirium is defined as an acute neuropsychiatric disorder characterized by a disturbance in attention and awareness, which develops over a short period of time, with additional disturbance in cognition that is not explained by a preexisting cognitive impairment. Importantly, delirium is generally triggered by medical causes, pain and/or drug administration or withdrawal (Inouye, Westendorp, & Saczynski, 2014). When delirium occurs in the context of a preexisting dementia, it is defined as delirium superimposed on dementia (DSD). In this chapter, we will review the current evidence on epidemiology, diagnosis, and prevention-treatment of patients with this condition.

The epidemiology of delirium in the context of dementia

The prevalence of DSD varies according to the studies and to the tools used to diagnose delirium in this population. It was estimated that 35.6 million people lived with dementia worldwide in 2010, with the prevalence expected to nearly double every 20 years, to 65 million in 2030 and 115 million in 2050 (Prince et al., 2013). DSD should therefore be

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considered as a key priority for health care providers. The prevalence of DSD in institutionalized patients ranges from 1.4% to 70% (de Lange, Verhaak, & van der Meer, 2013), while the prevalence in community and hospital populations ranges from 22% to 89% (Fick, Agostini, & Inouye, 2002). The occurrence of DSD is associated with worsening of cognitive and functional status, higher mortality rates, and higher health care costs when compared to patients with dementia alone (Bellelli et al., 2007; Fick et al., 2002; Fick, Steis, Waller, & Inouye, 2013; Morandi et al., 2014; Sampson, Blanchard, Jones, Tookman, & King, 2009). Additionally, DSD leads to a high formal and informal caregiver distress (Morandi et al., 2015a, 2015b). About half of the patients with DSD are able to remember being confused, and they report an overall "fair amount" of distress related to the delirium episode (Morandi et al., 2015a). Higher levels of distress were reported in association with memories of anxiety/fear, delusions, restlessness, hypokinesia, and deficit in orientation. Interestingly, the distress level is described to be moderate among caregivers and mild among health care staff caring for patients with DSD (Morandi et al., 2015b).

Almost 20 years ago, Inouye described the role of predisposing and precipitating risk factors for delirium. Vision impairment, severe illness, dehydration, and preexisting cognitive impairment were recognized as predisposing risk factors for its occurrence (Inouye, 1999). However, only recently have studies specifically shown how delirium acts in the context of cognitive impairment and how delirium can indeed worsen a preexisting dementia (Davis et al., 2012, 2015, 2017). First, in a longitudinal cohort study on very old persons, delirium has been shown to lead to accelerated and significant decline in cognitive performance; additionally people who already had dementia before experiencing delirium seemed to worsen the severity of dementia afterward (Davis et al., 2012). Subsequently, another investigation has shown the direct relationship between cognitive function, evaluated with the Mini-Mental State Examination (MMSE)—and delirium risk. The results showed that for every MMSE point lost, risk of incident delirium increased by 5% (Davis et al., 2015). Finally, Davis further analyzed how delirium might act in the context of dementia and especially according to the degree of the neuropathology of dementia (Davis et al., 2017). The combination of delirium and the pathologic process of dementia resulted in the greatest decline; individuals with delirium and more dementia pathologic burden have the fastest cognitive decline.

The under recognition of delirium in the context of dementia

Despite the fact that DSD is well documented in terms of prevalence and associated outcomes, this condition is under recognized in 60% of the cases (Oh, Fong, Hshieh, & Inouye, 2017). Several reasons can explain this shortcoming.

First, dementia has been reported as a significant risk factor for delirium under recognition along with hypoactive delirium in persons aged 80 years and older

(Inouye, Foreman, Mion, Katz, & Cooney, 2001). The risk of under recognition by nurses increased with the number of risk factors present, from 2% (0 risk factors) to 6% (1 risk factor), 15% (2 risk factors), and 44% (3 or 4 risk factors) (Inouye et al., 2001). Second, dementia is frequently under recognized in hospitals, leading to a high risk of delirium under recognition or misclassification (Sampson et al., 2009). This is relevant since under detection might indeed increase the risk of mortality and lack of recognition could lead to ineffective communication with patients and relatives (Kakuma et al., 2003). Third, many physicians do not use a tool for delirium assessment in their routine clinical practice. This attitude is likely to be the main reason for delirium under recognition, as previously shown (Bellelli et al., 2015). Other issues might indeed be related to be absence of a specific tool for DSD, which can help in differentiating delirium from dementia and especially in persons with preexisting severe dementia (Morandi et al., 2012).

It is imperative to alert health care providers to the diagnosis of DSD and to the available tools for its diagnosis.

The diagnosis of delirium in the context of dementia

The *Diagnostic and Statistical Manual of Mental Disorders-5* (*DSM-5*) is currently the gold standard for delirium assessment (American Psychiatric Association, 2013). However, it does not provide specific criteria on how to assist clinicians or researchers with the diagnosis of DSD (Morandi et al., 2016). Dementia and delirium have different characteristics (Table 36.1), which might help in differentiating between these two conditions.

The Confusion Assessment Method (CAM) has been widely used since 1990 for delirium assessment in the older population (Inouye et al., 1990). It was originally developed and validated in older patients admitted to a medical/geriatric ward. Of these persons, 12 (21%) had dementia and 9 had DSD. The sensitivity and specificity of the delirium tool in the entire sample ranged from 94% to 95%, respectively, but specific measures for those with dementia were not reported. A subsequent validation study of a German translation of the CAM included a high percentage (85%) of patients with dementia, but the test characteristics in this subgroup were not reported (Hestermann et al., 2009). The CAM requires specific training before being used in order to obtain reliable results. A recent review highlighted how, overall, the CAM has moderate to high sensitivity, and high specificity (Shi, Warren, Saposnik, & Macdermid, 2013).

The 4AT is a relatively new tool that was created to overcome some of the existing issues on delirium diagnosis, including lack of cognitive assessment, the requirement of training, and also the time required for the assessment (https://www.the4at.com) (Fig. 36.1) Indeed the 4AT does not require specific training to be used, it takes less than 2 min to be administered, and it also includes a measure of cognitive impairment. It has been validated in acute geriatric and rehabilitation wards showing a sensitivity of

Table 36.1 Differential diagnosis between delirium and dementia.

	Delirium	Dementia
Onset	Sudden	Slow
Duration	Day to weeks	Years
Reversibility	Fluctuating	Persistently progressive
Variation at night	Almost always worse	Worse
Level of consciousness and orientation	Fluctuates, disoriented	Impaired, worsening in the last stages
Attention and memory	Inattention and poor short-term memory	Attention retained, but loss of short-term memory
Cognition	Focal to global cognitive deficits	Global cognitive failure
Psychotic symptoms	May have hallucinations (mostly visual), delusions, and illusions	Less frequent
Speech	Often incoherent words	Difficulty finding words
Other disorders or physical symptoms	Comorbidities often present	Comorbidities often present
Electroencephalogram	Generalized diffuse slowing	Variable

The table provides important characteristics to differentiate delirium from dementia. Three important features are the onset, duration, and reversibility. In fact, delirium develops over a short period of time, lasts for hours or days, and is reversible. Conversely, dementia develops progressively over years and it is not reversible. There are other cognitive characteristics that might help in differentiating delirium from dementia, including the degree of inattention and memory impairment. Attention is usually preserved in the early to moderate stages of dementia, while it is always impaired in delirious patients. Finally, psychotic symptoms including hallucinations or delusions might be present in both conditions but are not mandatory for the diagnosis of delirium or dementia.

89.7% and a specificity of 84.1% (Bellelli et al., 2014). A 4AT score of 0 indicates the absence of dementia or delirium, a score of 1−3 suggests a possible cognitive impairment but not delirium, and a score $\geq$4 is strongly suggestive of delirium. A subanalysis of the 4AT in patients with dementia showed a sensitivity of 94% and a specificity of 64.9% for delirium detection. The 4AT has been shown to be effective in other settings, including palliative care and emergency departments (Baird & Spiller, 2017; O'Sullivan et al., 2018). When used in the emergency department, the 4AT accurately excludes delirium and dementia in older patients (O'Sullivan et al., 2018). Interestingly, in the context of hospice admission, a recent quality improvement study showed how the implementation of the 4AT was well received both for delirium and cognitive impairment screening (Baird & Spiller, 2017).

Other supporting instruments have been proposed, given the difficulties in diagnosing delirium especially in the advanced stages of dementia. In fact, recent studies have reported the importance of motor fluctuations for the detection of delirium, given that delirium is not an isolated mental disorder but can affect motor fluctuation as well (Adamis, Treloar, Gregson, Macdonald, & Martin, 2011; Bellelli et al., 2011; Morandi,

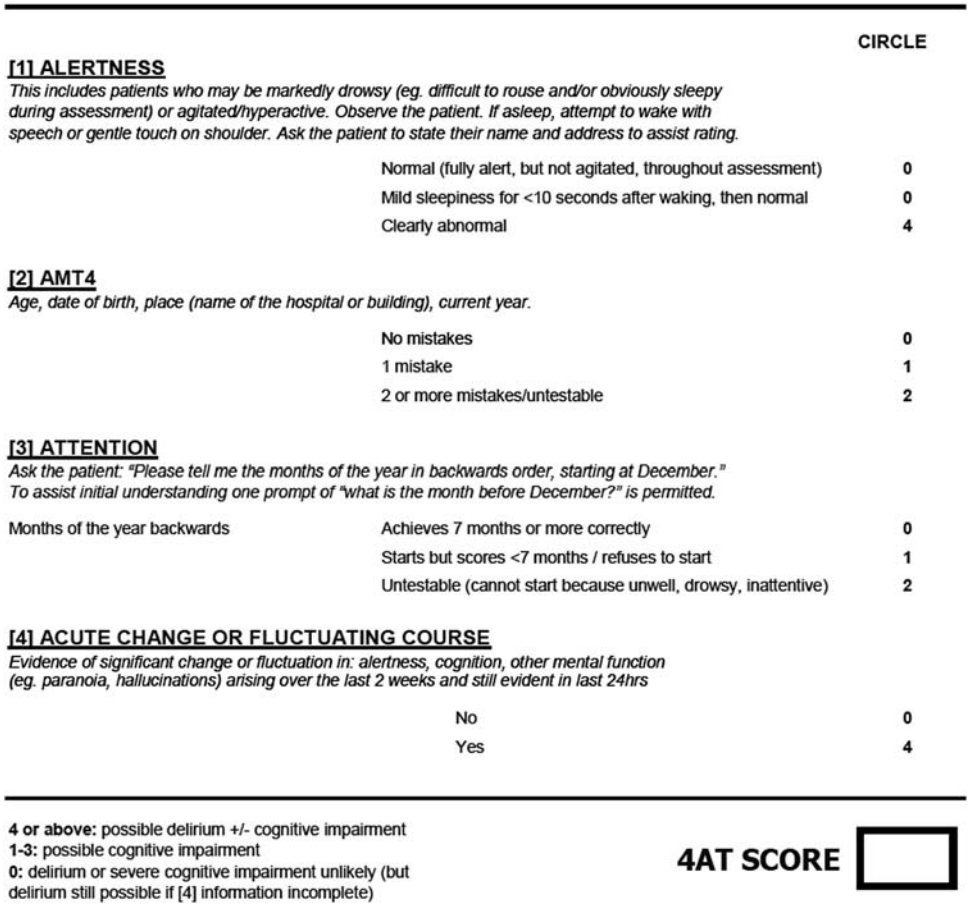

Figure 36.1 ***4AT assessment test for delirium.*** A score of 4 or more *suggests* delirium but is not diagnostic; more detailed assessment of mental status may be required to reach a diagnosis. A score of 1−3 suggests cognitive impairment and more detailed cognitive testing and informant history taking are required. A score of 0 does not definitively exclude delirium or cognitive impairment; more detailed testing may be required depending on the clinical context (https://www.the4at.com).

Han, et al., 2016). A study comparing patients divided in four groups (with delirium alone, with dementia alone, with delirium superimposed on dementia, and with neither delirium nor dementia) found that when delirium develops, and especially DSD, there is a significant decline in motor functions (Bellelli et al., 2011). In line with this observation, the Richmond Agitation and Sedation Scale (RASS) (Table 36.2) (Ely et al., 2003; Sessler et al., 2002), a scale originally used to monitor the level of consciousness in intensive care unit (ICU) patients, might provide key information on motor fluctuations given its potential to identify motor subtypes of delirium. The scale has been recently modified and adapted for its use outside the ICU (i.e., the modified RASS or m-RASS) (Chester, Beth

Table 36.2 The modified Richmond Agitation and Sedation Scale (m-RASS) (Chester et al., 2012).

Score	m-RASS
+4	**Combative:** No attention; overtly combative, violent, immediate danger to staff
+3	**Very agitated:** Very distractible; repeated calling or touch required to get or keep eye contact or attention; cannot focus; pulls or removes tube(s) or catheter(s); aggressive; fights environment, not people
+2	**Slightly agitated:** Easily distractible; rapidly loses attention; resists care or uncooperative; frequent nonpurposeful movement
+1	**Restless:** Slightly distractible; pays attention most of the time; anxious, but cooperative; movements nonaggressive or vigorous
0	**Alert and calm:** Pays attention; makes eye contact, aware of surroundings; responds immediately and appropriately to calling name and touch
−1	**Wakes easily:** Slightly drowsy; eye contact >10 s; not fully alert, but has sustained awakening; eye opening/eye contact to voice >10 s
−2	**Wakes slowly:** Very drowsy; pays attention some of the time; briefly awakens with eye contact to voice <10 s
−3	**Difficult to wake:** Repeated calling or touch required to get or keep eye contact or attention; needs repeated stimuli (touch or voice) for attention, movement, or eye opening to voice (but no eye contact)
−4	**Can't stay awake:** Arousable but no attention; no response to voice, but movement or eye opening to physical stimulation
−5	**Unarousable:** No response to voice or physical stimulation

The m-RASS is a brief (<30 s) inpatient screening measure of mental status that could be administered serially. The m-RASS is highly specific for delirium screening in geriatric and emergency department wards and especially in the context of dementia.

Harrington, & Rudolph, 2012). Different studies showed how the RASS/m-RASS is highly specific for delirium screening in geriatric and emergency department wards and especially in the context of dementia (Chester et al., 2012; Han et al., 2015; Morandi, Han, et al., 2016; Tieges, McGrath, Hall, & Maclullich, 2013). Finally, a newly developed nursing tool named RADAR might help in the screening for delirium, especially in the detection of the hypoactive subtype (Voyer et al., 2015). The RADAR includes three questions that should be answered by the nurses while administering drugs to the patient: (1) Was the patient drowsy? (2) Did the patient have trouble following your instructions? (3) Were the patient's movements slowed down? A RADAR screening is considered positive when at least one item is checked "Yes." When compared with DSM-IV-TR criterion-defined delirium, RADAR had a sensitivity of 73% and a specificity of 67% (Voyer et al., 2015).

Therefore, the current evidence suggests that in patients with dementia, and especially in the advances stages of dementia, it might be useful to use tools that are not only

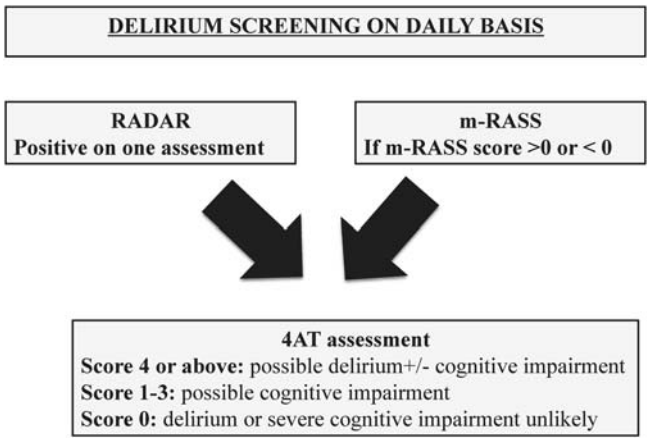

Figure 36.2 ***Assessment of delirium in patients with comorbid dementia.*** The figure describes a possible approach to increase the detection of delirium. Nurses can screen for delirium using either the RADAR or/and the m-RASS. The RADAR includes three questions, which should be answered by the nurses while administering the drugs to the patient: (1) Was the patient drowsy? (2) Did the patient have trouble following your instructions? (3) Were the patient's movements slowed down? A RADAR screening is considered positive when at least one item is checked "Yes" (Voyer et al., 2015). The modified-Richmond Agitation and Sedation Scale (m-RASS) was created from the RASS, a scale originally used to monitor the level of consciousness in intensive care unit (ICU) patients (Chester et al., 2012). The m-RASS scores range from −5 (unarousable) to +4 (combative).

evaluating attention and other cognitive deficits but also motor performance and changes. It could be hypothesized to screen patients for delirium using the RADAR and/or the m-RASS followed by a 4-AT assessment to increase the ability to detect DSD (Fig. 36.2).

Prevention and treatment of delirium in dementia

There is current evidence that delirium can be prevented using nonpharmacological multicomponent intervention. Indeed, the NICE guidelines and consensus statements provide support and recommendation for nonpharmacological intervention as a first approach for prevention and treatment (Bellelli et al., 2018; Excellence, 2010).

The first study that showed a significant reduction in delirium incidence was published almost 20 years ago (Inouye et al., 1999). The multicomponent intervention focused on six specific areas, which are well-known risk factors for delirium in the elderly: (1) cognitive impairment, (2) sleep deprivation, (3) immobility, (4) visual impairment, (5) hearing impairment, and (6) dehydration. The study showed for the first time a 40% reduction in the odds of delirium in the intervention group compared to the usual care (matched odds ratio, 0.60; 95% confidence interval: 0.39 to 0.92). Several other investigations were conducted in subsequent years and the evidence for use of

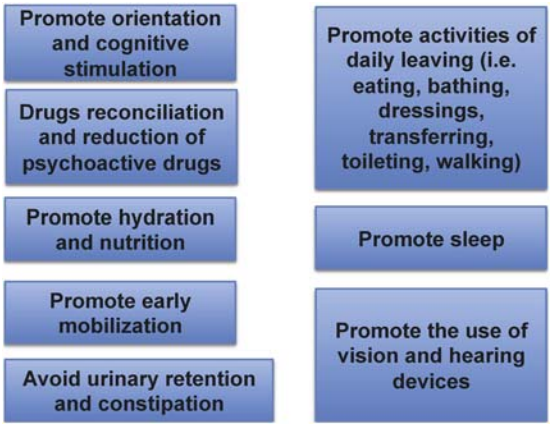

Figure 36.3 ***Multicomponent and interdisciplinary interventions for delirium prevention.*** The interventions should focus on reorientation (e.g., using calendars, watches) and cognitive stimulation (e.g., sudoku), drugs reconciliation and reduction of psychoactive drugs, promotion of sleep, early mobilization, adequate hydration and nutrition, avoiding urinary retention and constipation, and use of vision and hearing devices. An interdisciplinary team involving geriatricians or other medical clinicians, nurses, physiotherapists, occupational therapists, speech therapists, nutritionists, clinical pharmacists, and social workers should carry out the multicomponent intervention.

the multicomponent intervention has gained further strength (Abraha et al., 2016; Hshieh et al., 2015; Siddiqi et al., 2016). There is currently strong evidence of the reduction in the incidence of delirium using a multicomponent intervention compared to usual care (relative risk, RR, 0.69, 95% CI 0.59–0.81) (Abraha et al., 2016; Siddiqi et al., 2016). The findings are similar for patients admitted to surgical and medical wards (Abraha et al., 2016; Siddiqi et al., 2016). These interventions have been proven to be effective in a recent meta-analysis regardless of the ward type or dementia rates (RR 0.73, 95% CI 0.63–0.85) (Martinez, Tobar, & Hill, 2015). The multicomponent intervention adopted includes reorientation, drug reconciliation and reduction of psychoactive drugs, promotion of sleep, early mobilization, adequate hydration and nutrition, and use of vision and hearing devices (Fig. 36.3). An interdisciplinary team involving geriatricians or other medical clinicians, nurses, physiotherapists, occupational therapists, speech therapists, nutritionists, clinical pharmacists, and social workers should carry out the multicomponent intervention. There is specific emerging evidence on the role of occupational therapy in the management of patients with delirium (Alvarez et al., 2017; Schweickert et al., 2009) and especially in those with delirium and dementia (Pozzi et al., 2017). An occupation-based treatment of a person with delirium should have the following goals: (1) improve the autonomy and/or the involvement in everyday basic activities of daily living; (2) adapt the environment to the need of the person suffering of delirium; (3) favor a proactive presence of the family; and (4) select the best devices in order to safeguard an appropriate posture in bed, on the chair, and/or in wheelchair.

Once delirium has occurred, nonpharmacological intervention can still be effective in decreasing the severity of delirium and improving the medium-term cognitive functions

(Cerveira, Pupo, Dos Santos, & Santos, 2017). The presence of delirium is the expression of an underlying cause and often there is more than one cause. It is imperative to search and treat the underlying causes. Different acronyms are available to help clinicians systematically review the possible causes of delirium (Fig. 36.4).(Flaherty et al., 2003) Without assiduous attention to this step, often neglected, the patient will not improve (Inouye et al., 2014). Next, removal or reduction of anticholinergic drug agents should be considered in every patient. Preliminary data in older patients admitted to an acute geriatric ward suggest that melatonin might be useful in the prevention of delirium (Al-Aama et al., 2011).

Finally, there is currently little evidence for pharmacological prevention and treatment of delirium. There is no single drug that has shown to be effective in the treatment of DSD. Despite this notion, antipsychotics are generally and largely used to relieve the positive symptoms (i.e., agitation and sleep disorders) of delirium in patients with preexisting dementia. This approach could be deleterious. Indeed, antipsychotic medications have not been proven to prevent delirium or to reduce its duration or severity (Neufeld, Yue, Robinson, Inouye, & Needham, 2016). In palliative care, medicated patients might even experience worse symptoms of delirium (Agar et al., 2017). Therefore, antipsychotics should only be given to patients who have distressing symptoms and whose behavior means their safety or the safety of those around them is compromised. Clinicians have to consider short-term treatment (usually for 1 week or less haloperidol or olanzapine), starting at the lowest clinically appropriate dose and titrating cautiously according to symptoms (Bellelli et al., 2018; Excellence, 2010). Simultaneously, there has to be a thorough evaluation and treatment of the underlying causes of delirium.

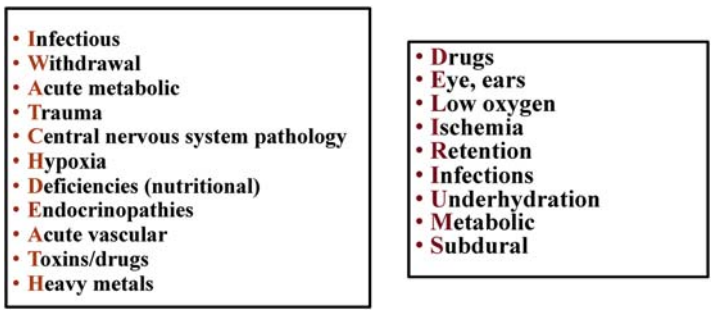

Figure 36.4 ***Delirium acronyms.*** Different acronyms are available to help clinicians systematically analyze the possible causes of delirium. Delirium is often multifactorial and it is imperative to not miss any of the possible causes in order to increase the chances of quickly resolving the presence of delirium.

Key facts of delirium

- Delirium is a risk factor for the development of dementia and worsening of dementia
- Delirium and dementia are often under recognized
- Under recognition of delirium leads to adverse clinical outcomes
- It is imperative to use tools to diagnose delirium in dementia patients
- Nonpharmacological and multidisciplinary interventions are effective in reducing delirium
- There is no current scientific evidence on the use of antipsychotics to improve delirium in patients with dementia

Summary points

- Delirium is an acute brain dysfunction and when it occurs in the context of dementia is named delirium superimposed on dementia (DSD).
- Dementia itself is an important risk factor for delirium, and delirium is a known risk factor for newly developed dementia or worsening of dementia.
- DSD is associated with adverse clinical outcomes, including worsening of functional status, higher mortality rates, and higher health care costs.
- Delirium often is under recognized and dementia is an important risk factor for under recognition.
- The diagnosis of DSD, especially in the advanced stages of dementia, is challenging, and clinicians along with validated tools for delirium assessment can use additional evaluations, including changes in motor performance.
- Delirium can be prevented using multicomponent interventions carried out by a multidisciplinary team targeting predisposing and precipitating risk factors for delirium.
- Once delirium has occurred, nonpharmacological interventions can still be effective in decreasing the severity of delirium and improving the medium-term cognitive function. The presence of delirium is the expression of an underlying cause. It is imperative to search for and treat the underlying causes.
- Antipsychotics should only be used in patients with severe distressing symptoms and whose behavior means their safety or the safety of those around them is compromised.

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CHAPTER 37

Self-consciousness deficits in dementia

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List of abbreviations

DMN default mode network
bvFTD behavioral variant of frontotemporal dementia
SC self-consciousness

Mini-dictionary of terms

Consciousness Consciousness is a mental act, a biological phenomenon through which we form a sense of our life and that of others.
Consciousness of others It is the consciousness the self has of other congeners.
Default mode network It is a broad neural network that seems to assure the coherence of self, and it works at rest states when the subject is focused on himself (meditation, daydreaming, etc.).
Humanistic neuropsychology This is the science that studies the biological basis of behavior focused on the unrepeatable perspective of the patient in order to promote one's general well-being.
Self-consciousness It is the knowledge that the subject has of his/her own state of consciousness, being the most distinctive feature of our human condition.

Introduction

The topic of consciousness is a classic one in the history of psychology. In fact, psychology began to spread as an experimental science supported by its definition as the science of consciousness. William James (1890) defined consciousness as an inner sensation of knowing characterized by being selective, continuous, personal, and related to other objects different from it. Consciousness is a mental act, a biological phenomenon through which we form a sense of our life—self-consciousness (SC)—and of the personal identity of others, and thus is inexorably linked to the concept of subjectivity. In this sense, consciousness coexists with "mind" (Zeman, 2001).

Ortega and Gasset (1983) provided differentiation between two "being conscious" states. On one hand, "being aware" refers to those states of being awake and lost when one is asleep or anesthetized (Edelman and Tononi, 2002). On the other hand, the "conscious being" is able to perceive oneself objectively without losing subjectivity, the private interpretation of oneself. This paradox of consciousness implies the integration of cognition and emotion. Thus, consciousness has several levels of complexity

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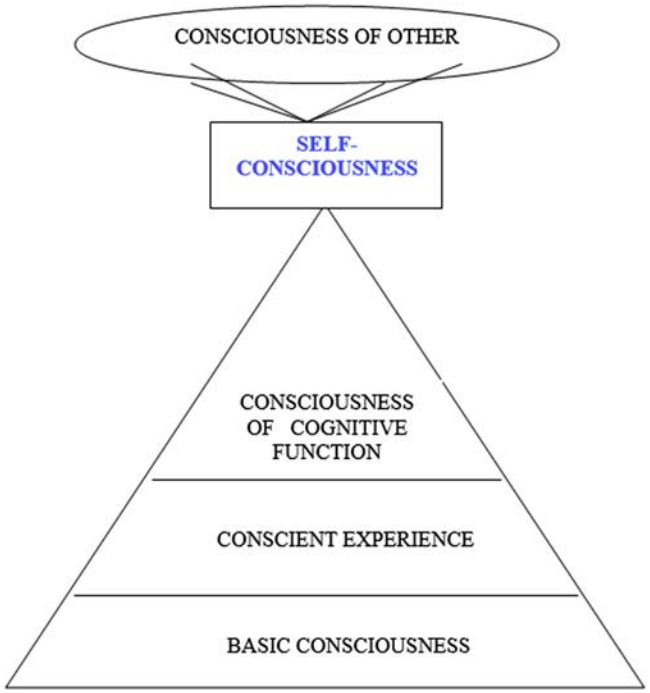

Figure 37.1 ***Levels of consciousness.*** Consciousness has several levels of complexity from the simplest consciousness to the highest hierarchy of complexity of consciousness, such as those of self and other persons.

(Fig. 37.1) that have been formed ontogenetically and phylogenetically, and they have some similarity with the functional units distinguished by Luria (1973):

(a) Level 1: basic consciousness, a state of alertness linked with the reticular activating system, thalamus, limbic system, basal ganglia, and prefrontal cortex as well as selective attention dependent on the right posterior parietal cortex, lateral pulvinar, and superior colliculus;
(b) Level 2: conscious experience related to thalamocortical networks;
(c) Level 3: awareness of specific cognitive function dependent on basal and retrorolandic circuits;
(d) Level 4: SC; and
(e) Level 5: consciousness of others related to social cognition.

In regard to the neural correlate of consciousness, most authors consider thalamocortical circuits the essential neurobiological bases of consciousness, although Mesulam (1985) emphasized the role of the parietal lobe, particularly the heteromodal cortex or angular and supramarginal areas. Several authors (Koch, 2004; Llinás, 2001) defend the hypothesis of network participation that connects the thalamus to the cortex, while

others such as Edelman and Tononi (2002) suggest the hypothesis of a "dynamic nucleus" due to a distributed neuronal process with intervention of the thalamocortical system. In addition, Schacter (1989), based on two types of knowledge, conscious (or explicit) and unconscious (or implicit), proposed an explanatory model of consciousness called DICE—"Dissociable Interactions and Conscious Experience"—postulating a system that produces conscious experience.

Self-consciousness

We would place SC, which is also called reflective consciousness or self-awareness, at the highest and most sublime level of human capacities. SC is the knowledge of a subject's own state of consciousness and is thus the most sublime mental act of a human being. The individual can thus separate from his own perception, realize that he is in the process of perceiving, and thus deduce the reality of existence, or in Descartes's words, "I think, therefore I am." For Stuss and Benson (1994), SC is a human attribute that allows not only for SC but also for one to grasp one's position in the social environment. Its functions would be to control conscious experiences, represent current experiences in relation to previous ones, and use acquired knowledge to guide decision-making for the future. In this sense, SC is related to executive functions. For these functions to take place, a highly evolved phylogenetically and ontogenetically cerebral substrate is needed, which is the prefrontal cortex, where von Economo neurons are located (Cairns-Smith, 1996).

Additionally, the most important historical antecedent of the neurobiological bases of SC (see Table 37.1) is probably A. Luria (1973), who assigned consciousness to the third functional unit, located primarily in the activity of the frontal lobes (Das, Kar, & Parrilla, 1996; Grafman, Partiot, & Hollnagel, 1995; Posner & Raichle, 1994). Damasio (2003) considers consciousness as not exclusively an experience of knowledge or a product of the brain but as the sum of genetics, a unique personal history, experiences, a social

Table 37.1 Possible neural correlates of self-consciousness.

Authors/studies	Neurobiological bases of self-consciousness
Luria (1973)	Third functional unit
Posner and Raichle (1994)	Frontal lobes
Grafman et al. (1995)	Frontal lobes
Das et al. (1996)	Frontal lobes
Damasio (2003)	Prefrontal cortex
Gil (2007)	Frontal supramodal associative cortex
Huang et al. (2016)	Default mode network

Over the years, the authors have argued several neurobiological bases of self-consciousness, but the frontal lobe seems to be the critical neural system.

context, and a specific culture. He differentiated between central consciousness (similar to conscious experiences) and extended consciousness. SC is a consequence of the ability to retain records of conscious experiences and the ability to reactivate them to generate a sense of personal, individual, and subjective perspective of "being myself who knows" (Damasio, 2003; Vogeley et al., 2004). In addition, Damasio argues that the critical neural system for SC is particularly found in the prefrontal cortex, because this area is highly convergent. This zone receives signals from all the sensorial regions where conscious experiences are formed as well as from bioregulatory sectors of the brain, representing categorizations of previously lived situations with their corresponding specific emotional valences. For Damasio, SC is a process of coordinated activation of personal identity memories located in a ubiquitous network more localized in the convergence zones situated in the temporal and frontal superior cortex as well as in the subcortical nuclei (such as those of the amygdale). Therefore, he believes that without conscious experience there could be no SC, because SC allows the existence of our historical continuity: who I am, where I come from, and where I am going.

Gil, Fargeau, and Jaafari (2011) also describes the crucial participation of the frontal supramodal associative cortex in SC related to the heteromodal associative frontal cortex where information arrives from areas involved in memory, language, and perceptual functions—to finally synthesize them. In this similar way, recent studies have observed that the default mode network (DMN) seems to assure the coherence of self (Huang, Obara, Davis, Pokorny, & Northoff, 2016) and works at rest states when the subject is self-focused (meditation, daydreaming, etc.). The DMN is a broad neural network with components that converge with key associative areas (Buckner, Andrews Hanna, & Schacter, 2008) defined essentially by the ventral midtemporal zone, the posterior zone of the cingulum, A39, and the frontoorbital and frontoventral cortex.

Therefore, SC is an emergent property of the brain, and its level of complexity depends essentially on personal history and its representation in the neural system. Consciousness can be the object of scientific study (Delacour, 1995; Zeman, 2001), and the search for SC deficits in a subject requires us to consider which aspects of SC are altered or preserved. Thus, several authors have considered SC multifactorial (Delacour, 1995, Fig. 37.2). It is the consciousness of body-morphological characteristics and its position and mobilization in space (Schore, 1994). SC is also the consciousness of perceptions (Edelman and Tononi, 2002), the consciousness of our state of mind (Nezlek, 2002); as well, it is the consciousness of one's own history, of one's autobiography (Zeman, 2001). Therefore, it is inseparable from memory, because the identity of each human is being built up and crystallizing little by little (Schacter, 1989). SC is awareness of our own projects (Stuss & Benson, 1994) or of the future (Tulving, 2002). Finally, it is the conscience of morality that allows the individual to make judgments about their thoughts and actions (Gil et al., 2001) and to act in a complex social world with knowledge of oneself and others (Damasio, 2003). In a strict sense, SC deficit would imply that

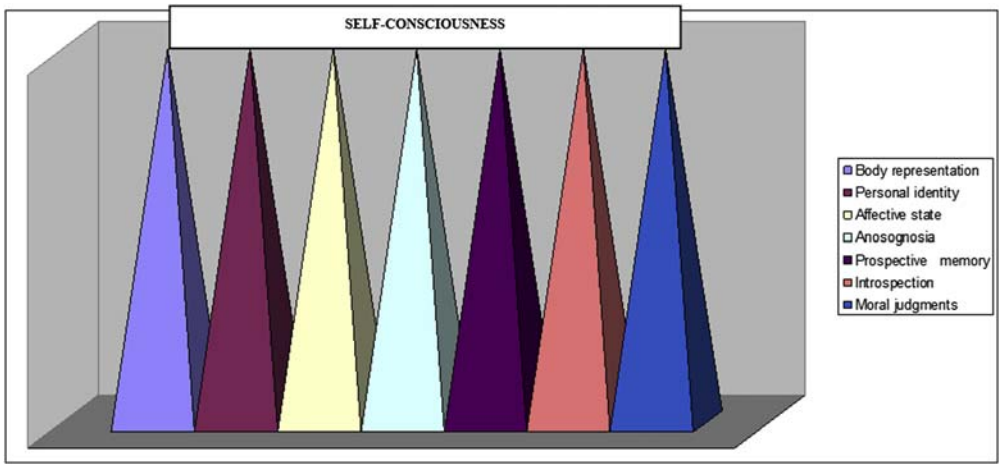

Figure 37.2 ***Aspects of self-consciousness.*** Self-consciousness is an emergent property of the brain, and its neural representation will depend essentially on different aspects, such as personal identity, metacognition, affective state, body representation, prospective memory, introspection, and moral judgment.

the individual does not go beyond one's own "being" (Gil et al., 2001) without achieving the the awareness to "be himself, knowing that he exists" (Ortega & Gasset, 1983).

The relationship between SC and memory is considered the fundamental role played by memory, and in particular autobiographical memory in the construction and permanence of the self. Autobiographical memory has been considered a long-term mnesic system that serves to encode, store and retrieve a set of representations in which the self is the central theme or axis, allowing the construction and maintenance of our personal identity and therefore playing a fundamental role in SC (Duval, Eustache, & Piolino, 2007; Fargeau et al., 2010; Piolino, 2008). The autobiographical memory has two components, one being semantic and referring to the general representations of personal events, and the other being episodic and referring to personally lived events, which is very concrete and specific temporally and spatially (Conway, 2005; Piolino, 2008). Overall, autobiographical memory seems to be especially supported by network connections in automatic or spontaneous mode—DMN—and the hippocampus (De Brigard, Nathan Spreng, Mitchell, & Schacter, 2015). This has led to the distinction between noetic consciousness associated with the semantic component, the feeling of familiarity or "knowing" and autonoetic awareness related to the episodic component with reactivation of the self-memory of lived events, of reexperiencing experiences (Gardiner, 2001; Tulving, 2002). Over the whole of one's life, SC gathers a set of biological tributaries of perceptions, thoughts, projects, and actions (our experiences) in a cultural context that generate and express the permanence and coherence of one's "self" and, therefore, of the human being. Thus, it could be considered the "sedimentation or crystallization" of the riverbed

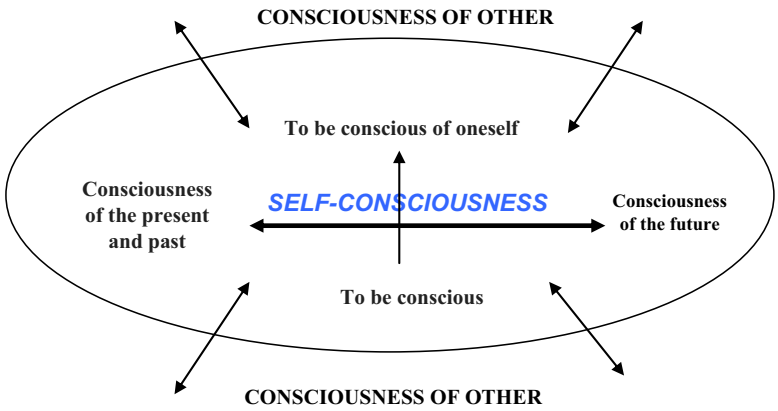

Figure 37.3 ***Crystallization of self-consciousness.*** Self-consciousness is progressively built and maintained over three axes: a) from below to above: from "to be conscious" up to "to be conscious of oneself"; b) from back to front: from the awareness of one's own experiences (present and past) to planning the future; and c) from inside to outside: from consciousness of self to consciousness of the other congener. Unpublished data.

of our autobiologic-biographic river in relation to other persons from which SC is emerging and is generated in three different sedimentation axes (Fig. 37.3):

(a) from below to above—from "to be conscious" up to "to be conscious of oneself" (SC);

(b) from back to front—from the awareness of one's own experiences (present and past) to planning the future; and

(c) from inside to outside—from consciousness of self to consciousness of the other congener (social cognition).

In this way and thanks to brain maturation and the fruit of individual development (experience; biography), the subject achieves a representation of oneself (SC) and others (Arroyo-Anlló, Chamorro Sanchez, Ortiz, & Gil, 2017).

Self-consciousness in neurodegenerative diseases

One of the neuropathologies that causes a progressive disintegration of SC is Alzheimer's disease (AD). AD is a neurodegenerative disease essentially characterized by progressive cognitive deterioration that preferentially affects the regions of the medial temporal lobe also essential in emotional processes. It causes memory and learning disorders as well as difficulties in interaction between the self and the world due to language disorders, apraxias, agnosias, etc. It is also associated with behavioral and emotional alterations throughout the disease, with a loss of autonomy of activities of daily life, decrease in the quality of life of the patient and his family, and alteration of social interactions. In this way, AD raises the question of the deterioration of SC clearly and in an exemplary way.

As has been already pointed out (Gil, 2007), most studies of consciousness in AD are essentially confined to the anosognosia of cognitive deficits (Gil, 2007; Rankin, Baldwin, Pace-Savitsky, Kramer, & Miller, 2005; Starkstein, Brockman, Bruce, & Petracca, 2012). According to Starkstein et al. (2012), anosognosia is related to the degree of cognitive deficiency and apathy, though for López, Becker, Somsak, Dew, and Dekorsdy (1993) it is also associated with frontal functions.

Thus, Gil et al. (2001) proposed a neuropsychological study of the different aspects of SC in a group of 45 patients with AD of mild or moderate severity. For this, a questionnaire was prepared— the SC questionnaire—that allows for evaluation of seven different aspects (Table 37.2): personal identity, metacognition, affective state, body representation,

Table 37.2 Questions assessing each aspect (A) of self-consciousness from the self-consciousness questionnaire.

A1- Personal identity	
- What is your name (surname and first name)? - Have you had a job? What was it? - What is the first name of your spouse (or partner)? - What is your mother's first name?	
A2- Anosognosia	
- Why have you come to see me? - Do you have any health problems that prevent you from leading a normal life? - Have you got any problem with your memory?	
A3- Affective state	
- Do you feel happy or unhappy? Why?	
A4- Body representation	
- Would you say that you are fair- or dark-haired? - Are you now sitting, standing or lying down?	
A5- Prospective memory	
- What are you planning to do shortly or tomorrow?	
A6- Introspection	
- If you had to live your life over again, is there anything you would like to change?	What?
A7- Moral judgments	
- Is it a good thing or a bad thing to tell a lie? Why? - Is it a good thing or a bad thing to give some money or some food to someone who starving?	Why?

Self-consciousness questionnaire of Gil et al. (2001) assesses seven aspects of Self-consciousness, using several questions for each aspect; for instance, four concern personal identity aspect.

prospective memory, introspection, and moral judgment. The SC questionnaire showed a high interobserver validity (Kendall's correlation: 0.96, $P < .0001$) as well as a high reliability evaluated by test–retest (Spearman correlation: 0.73, $P < .0001$). The results indicated that AD clearly produces SC deficits but not a total abolition of it, and therefore it cannot be said that these patients are not fully aware of SC. Analysis of the data obtained showed no significant correlations between total score on the SC questionnaire and educational level, age, or duration of the disease. However, a significant correlation was found between total score on the SC questionnaire and dementia severity, and in addition, frontal alterations were correlated at the threshold of significance with the SC score. The different aspects of SC were not altered homogeneously. On one hand, the most deteriorated factor in AD was awareness of cognitive disturbances followed by factors on moral judgment and prospective memory. A significant correlation was found between anosognosia and dementia severity as well as frontal disorder. On the other hand, the least disturbed aspects of SC were personal identity and mental representation of the body. Anosognosia was the most commonly observed SC deficit and the mostly studied in AD (Vasterling, Seltzer, & Watrous, 1997).

This same team assessed SC in another AD group (Arroyo-Anlló, Poveda Díaz, & Gil, 2013). They found that AD induced heterogeneous impairment of SC, and hence different aspects of SC were not impaired to the same degree. Nevertheless, both studies showed a similar SC impairment profile. Therefore, the results of both works show that AD clearly induced a heterogeneous deterioration of SC requiring convergence of multiple neural networks.

In terms of Bergson's theory of consciousness (1966), the most important deficiency of AD could be considered the inability to maintain "attention to life" consecutively and simultaneously. Thus, the most basic aspects of consciousness do not sufficiently nurture some of its most elaborate levels, breaking the supercontrolled monitoring of cognition and human behavior (Kircher and Leube, 2004). In this way, Gil, Ornon, Arroyo-Anlló, and Bonnaud, (2002) considered that the disturbance of SC could represent the axis of AD deterioration. Consciousness requires the synthesis of information from innumerable neural networks located in the brain, areas that participate in the processing of sensory data, memory, and emotional life management.

Furthermore, the work of Fargeau et al. (2010) studied the self who is manifested or expressed in one's environment, thanks to the three elements of self differentiated by James (1890) in a group of patients with AD. James identified three elements of self: (1) the material self where the body is the central element, (2) the social self who designates the way in which one acts socially, and (3) the spiritual self who is expressed by moral, political, philosophical, and similar opinions. These elements shape the identity profile of a person, recognized by another due to behavior, preferences in dress, opinions, and the like. Thus, it can currently be heard in the familial context about AD patients: "do not recognize my relative …," "he looks like another person …," because SC

deficits are manifested by changes in dressing style, social presentation, political or religious ideologies, etc. They observed that most patients showed at least one impaired element of self, the most common being the social self, and a 1/5 of the 47 patients manifested the three altered elements of self. In addition, they suggested that the explanatory variables of self-deterioration were the semantic aspects of autobiographical memory and apathy.

Concerning frontotemporal dementias, to our knowledge very little research has been carried out to study SC in frontal dementias except two particular studies (Arroyo-Anlló et al., 2016; Arroyo-Anlló, Turpin Boston, Fargeau, Orgaz Baz, & Gil, 2017). Nevertheless, several studies have analyzed anosognosia in frontotemporal dementias (Mendez & Shapira, 2011; Rosen, 2011). Arroyo-Anlló et al. (2016), Arroyo-Anlló, Turpin Boston, et al. (2017) studied SC in patient groups of bvFTD, which is characterized more by changes in personal, social, and emotional behavior together with a loss of insight rather than cognitive deterioration. These recent works found that the most altered SC factors in patients with bvFTD were anosognosia, affective state, and moral judgment as well as the less altered factors, consciousness of personal identity and body representation. This same profile in SC with other patient groups with bvFTD was observed in a second investigation in which they compared SC in patients with bvFTD and AD (Fig. 37.4). Thus, the patients with bvFTD showed greater deterioration of SC aspects related to frontal functions. The bvFTD patients were more profoundly anosognosic than the AD patients. In this sense, affective state self-assessment in the bvFTD group was also poorer than that of the AD group, as many previous studies had already observed in AD (Neary et al., 1998; Zanetti et al., 1999) and in bvFTD (Hornberger et al., 2014; Rosen et al., 2014). Nevertheless, some studies have not revealed differences in insight between FTD and AD (Banks & Weintraub, 2008; Eslinger et al., 2005). They also observed that the bvFTD group showed a more important impairment in the introspection aspect than in the AD group. These findings are of similar thinking to James (1890) and Ricoeur (2013) regarding the "maintenance of Self," or ipseity, because frontal patients currently have many difficulties in choosing among options and in maintaining their self as seen from the changes of their usual behavior (e.g., aggressiveness, lack of respect for social norms, etc.). In addition, the study of Arroyo-Anlló, Turpin Boston et al. (2017) observed a very important impairment in the moral judgment aspect in the bvFTD group as compared with the AD group. In this sense, most studies addressing frontal dementias using mind theory tasks have found deficits in social cognition (Le Bouc et al., 2012). In general, these altered aspects of SC in the bvFTD group reflect the classical clinical characteristics of FTD, such as changes in personal and social conduct as well as early and emotional blunting (Gregory et al., 1999). It suggests that those SC deficits are more related to behavioral aspects and orbitofrontal functioning and suggesting the involvement of the DMN (Andrews-Hanna, Irving, Fox, Spreng, & Christoff, 2017; Arroyo-Anlló, Turpin Boston et al., 2017). Also, Miller et al. (2001) found a relation between self and nondominant cerebral function in patients with frontotemporal dementia using SPECT imaging.

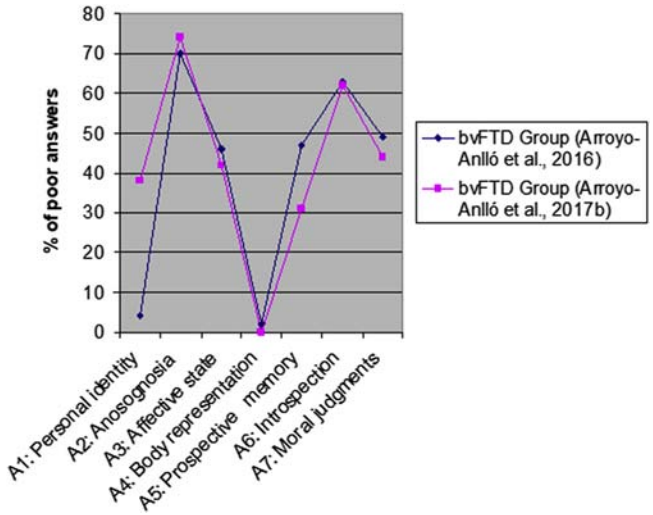

Figure 37.4 ***Common impairment profile of self-consciousness in behavioral variant of frontotemporal dementia.*** In our knowledge, the only two works studying self-consciousness (Arroyo-Anllo et al., 2016; Arroyo-Anlló, Turpin Boston, et al., 2017) in the behavioral variant of frontotemporal dementia (fvFTD) showed a similar impairment profile of self-consciousness, consisting of anosognosia, affective state, introspection and moral judgment as the most altered SC factors. *(Reprinted data from Arroyo-Anlló, E. M., Turpin Boston, A., Fargeau, M. N., Orgaz Baz, B., & Gil, R. (2016). Self-consciousness in patients with behavioral variant frontotemporal dementia.* Journal of Alzheimer's Disease, 49, *1021–1029; Arroyo-Anlló, E. M., Turpin Boston, A., Fargeau, M. N., Orgaz Baz, B., & Gil, R. (2017). Self-consciousness in Alzheimer's disease and frontotemporal dementia.* Journal of Alzheimer's Disease, 55, *1437–1443 with permission from Journal of Alzheimer's disease. IOS Press. The publication is available at IOS Press through https://doi.org/10.3233/JAD-160770 and https://doi.org/10.3233/JAD-150821.)*

Enhancing elements of self-consciousness in neurodegenerative diseases

Alteration of autobiographical memory has been related to diminution of the sense of self (El Haj, Gandolphe, Gallou, Kapogiannis, & Antoine, 2017) due to limited access to the knowledge that shapes and helps maintain personal identity throughout one's past, present, and future and therefore one's SC. Considering the devastating consequences of the deterioration of autobiographical memory in SC, the positive effect of autobiographical memories rich in emotional content evoked directly/automatically (Conway, 2005) by sensorial stimuli as familial odor or music could be used to reestablish/reinforce the permanence and coherence in self, because as Bergson (1966) said "consciousness is first memory" and someone does not know if he lives if he does not experience that he is living in his present life. Thus, some studies have attributed the nonvoluntary nature of autobiographical memories evoked by music in comparison with intentional memories in a group of patients with AD (Arroyo-Anlló et al., 2013; El Haj et al., 2017; Irish et al. 2006).

We believe that neuropsychological research, particularly in neurodegenerative diseases, is evolving toward a humanistic paradigm, because the studies that are currently being carried out on dementias have more of the patient's accompaniment of improving their well-being, of caring and not of curing. We seek to empower the patient, and that is where SC and the power of emotion come into play. Thus, focusing on emotion, we focus on something essential in life that can be stimulated by daily activities such as smell, taste, and music, because although the patient is unable to identify/name the smell, dish, or musical piece, they can experience emotions, relive situations, find the emotions that contribute to and recognize it. From this perspective of "care," neuropsychology also has an essential role to play, since it can scientifically prove that even temporarily, certain protocols exalt memory, their affective state, and their personal identity—that is, their SC.

A study by Arroyo-Anlló et al. (2013) initiated the paradigm of humanistic neuropsychology, the search for SC-enhancing and SC-facilitating elements in dementia, as the basis for the regulation of human behavior and the promotion of one's general well-being. The first work under this perspective was released at the end of 2013, in which they presented the results of the positive effect of family music on SC thanks to its emotional power in a group of patients with AD. The results observed were that musical intervention through familiar songs for patients with AD significantly stabilized or improved the aspects of SC except those of prospective memory and introspection.

On the other hand, empirical evidence has suggested that odors are a stimulus with great capacity to recover episodic autobiographical memories (El Haj et al., 2017; Herz, 2011; Larsson, Willander, Karlsson, & Arshamian, 2014). However, to our knowledge there is not any work that examines the effects of familiar odors evoking autobiographical memories on SC, even less so for patients with AD.

The study of sensory perceptibility in neurodegenerative diseases should be considered under humanistic perspectives focused on the unrepeatable perspective of the patient due to its beneficial influence on the state of mood, mental concentration, personal history, and the permanence of the self in the broadest sense. We hope that these reflections can open the way to research focused on the person within a new future paradigm of neuropsychology, *humanistic neuropsychology*.

Key of facts of self-consciousness

- SC is an emergent property of the brain that is considered one's ability to understand one's own states of consciousness.
- SC can be the object of scientific study.
- The search for SC deficits in a subject requires to consider which aspects of self-consciousness are altered or preserved.
- Several authors have considered SC to be multifactorial.
- Crucial participation is required of the prefrontal cortex in SC.
- Very few works have studied SC in neurodegenerative diseases.
- Dementias clearly induce an alteration of SC, but not its total abolition.

Summary points

- This chapter focuses on SC in dementia, which is the knowledge a subject has of his/her own state of consciousness.
- SC is considered a multifactorial concept formed by personal identity, metacognition, affective state, body representation, prospective memory, introspection, and moral judgment.
- Dementia heterogeneously affects the different SC factors.
- Studies have shown a similar impairment profile of SC in AD.
- The most deteriorated factor in AD was awareness of cognitive disturbances, followed by factors on moral judgment and on prospective memory.
- The patients with behavioral variant of frontotemporal dementia showed greater deterioration of self-consciousness aspects related to frontal functions than in AD.
- It suggests that crucial participation of the prefrontal cortex in self-consciousness and the involvement of the DMN in self-consciousness emergence and construction.
- The role played by autobiographical memory is considered as essential for the construction and permanence of the self and thus as a potential enhancer element of SC.

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CHAPTER 38

Attentional impairments to novel images in dementia

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List of abbreviations

AD Alzheimer disease
ChEI cholinesterase inhibitor
CPT Conners' Continuous Performance Test
DMN default mode network
DS Wechsler Adult Intelligence Scale-Digit Span
MCI mild cognitive impairment
VPC visual-paired comparison

Mini-dictionary of terms

Anterior cingulate cortex A region in the cingulate cortex that connects the prefrontal cortex and the limbic system. The anterior cingulate cortex is involved in attention allocation, top-down and bottom-up processing, reward anticipation, decision-making, and error detection.

Bottom-up processing "Data-driven" perception, based on the processing of sensory stimuli.

Donepezil An acetylcholinesterase inhibitor, used to improve cognitive functioning in dementia patients through increasing acetylcholine tone in the central nervous system.

Galantamine An acetylcholinesterase inhibitor and allosteric activator of the nicotinic acetylcholine receptor, used to treat the cognitive symptoms of dementia.

Mild cognitive impairment An intermediate stage of cognitive impairment beyond what would be expected from normal ageing, however, below the threshold for clinical dementia.

Mini-Mental State Examination A 30-point questionnaire used to measure cognitive impairment in clinical and research settings. The Mini-Mental State Examination tests five areas of cognitive function: attention, calculation, orientation, recall, and language. Lower scores are indicative of cognitive impairment.

Nucleus basalis of Meynert A group of neurons that provide most of the cholinergic tone in the cerebral cortex.

Prefrontal cortex A region of the frontal lobe implicated in planning, decision-making, executive function, and attention.

Selective attention The ability to attend to specific sensory stimuli, while ignoring other stimuli.

Top-down processing "Cognition driven" perception, based on the processing of stimuli through application of preexisting knowledge.

Visual-paired comparison task A computerized cognitive test that measures participants' tendency to attend to novel visual stimuli when presented with both repeated and novel stimuli.

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Alzheimer disease (AD) is primarily characterized by deficits in memory and learning, however, attentional impairments are frequent in these patients (McGuinness, Barrett, Craig, Lawson, & Passmore, 2010). Symptoms of attentional impairments include difficulties reading, finding a face in a crowd, and operating a motor vehicle (Fig. 38.1) (Rizzo, Anderson, Dawson, Myers, & Ball, 2000). These deficits in attention represent a diminished ability to process information (McGuinness et al., 2010), a reduced or biased field of view (Redel et al., 2012), and/or hindered performance on visual search tasks (Landy et al., 2015). Compared to healthy controls, Rizzo et al. (2000) reported that patients with AD have poorer sustained, divided, and selective attention, in addition to reduced visual processing speed. Even in prodromal stages of dementia, such as mild cognitive impairment (MCI), deficits in attention are detectable (McLaughlin, Anderson, Rich, Chertkow, & Murtha, 2014; Okonkwo, Wadley, Ball, Vance, & Crowe, 2008). Studies have also shown that attention is associated with disease severity, as scores progressively decline from healthy controls to MCI, and further in AD patients (Belleville, Chertkow, & Gauthier, 2007; Redel et al., 2012).

Attentional impairments are also predictive of greater cognitive decline (Chau et al., 2017) and conversion from MCI to AD (Silveri, Reali, Jenner, & Puopolo, 2007). Evidence has suggested that attentional abilities toward novel stimuli, specifically, may

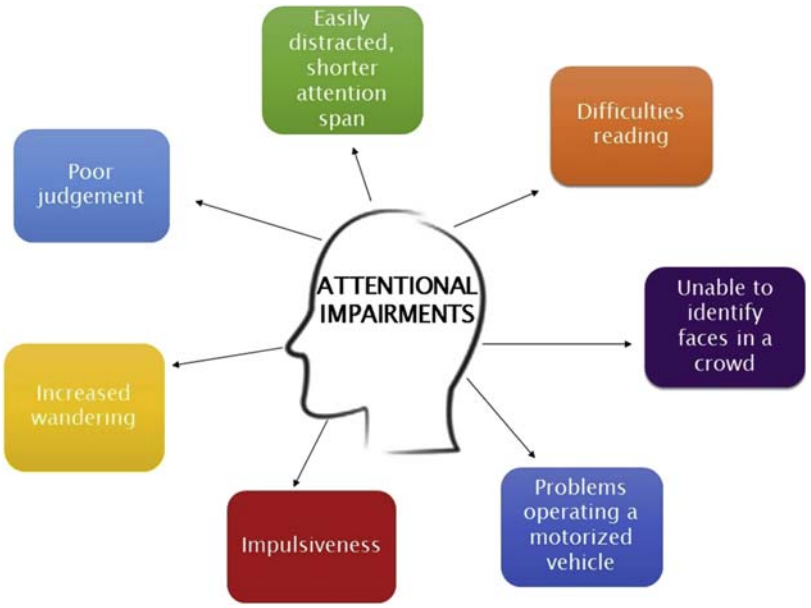

Figure 38.1 ***Frequently reported symptoms of attentional impairments in dementia.*** Examples of attentional impairments in dementia may include increased wandering, poor judgment, shorter attention span, difficulties reading, inability to identify faces in a crowd, problems operating a motorized vehicle, and impulsiveness.

also be able to differentiate between cognitively healthy and impaired people (Chau, Herrmann, Eizenman, Chung, & Lanctot, 2015; Crutcher et al., 2009). Novel stimuli may include characters, images, letters, digits, and two- and three-dimensional shapes that are presented upside-down or mirror reversed (Reicher, 1976; Shen & Reingold, 2001; Wang, Cavanagh, & Green, 1994). When faced with novel stimuli, cognitively healthy individuals redirect their attention toward the novel stimulus, however, AD patients demonstrate significantly reduced attention toward novel stimuli (Chau et al., 2015). Daffner, Mesulam, Cohen, and Scinto, (1999) suggested that diminished attention to novelty in patients with AD may in part be due to behavioral disturbances, likely caused by disruptions in neural systems that modulate engagement and maintain attentional bias toward novel stimuli (Daffner et al., 1999). This chapter aims to comprehensively discuss (a) how attentional impairments toward novel images are measured; (b) correlates of attentional impairments toward novel images; (c) putative mechanisms underlying attentional impairments toward novel stimuli, and (d) the pharmacological treatment of attentional impairments in dementia.

Measuring attentional impairments toward novel images in dementia

There are several verbal, written, and computerized tests used to assess attention in patients with dementia (Table 38.1). One of the most widely used tests of auditory attention is the Wechsler Adult Intelligence Scale- Digit Span (DS) test, a cognitive assessment where participants are read a series of numbers, then asked to recite the numbers in

Table 38.1 Attentional tests commonly used in Alzheimer disease studies.

Test	Format of test	Attentional measurement
Digit span	Verbal	Auditory attention
Stroop test	Verbal	Selective attention, cognitive interference
Symbol cancellation task	Written	Visual selective attention, visual search
Trail making Test B	Written	Attentional set shifting
Trail making Test A	Written	Visual attention, visual search
Attention battery of the cognitive drug research computerized assessment system	Computerized	Focused attention, selective attention, sustained attention
Visual-paired comparison task	Computerized	Visual selective attention, novelty preference, visual search
Conners' continuous performance test	Computerized	Sustained attention

The included attentional tests are organized by format and describe which attentional domain is measured.

the same (DS forward) or reverse (DS backward) order. The DS forward task is said to measure auditory attention, while the DS backward task is more reflective of working memory capacity (Choi et al., 2014). Additionally, tests of visuospatial attention such as the Symbol Cancellation Task, and Trail Making Tests A and B are used to assess visual selective attention in AD (Bossers, van der Woude, Boersma, Scherder, & van Heuvelen, 2012; Foldi et al., 2005). The Stroop Test measures inhibitory processing and selective attention using congruent and incongruent conditions (Spieler, Balota, & Faust, 1996). Participants are instructed to read the names of colors printed in black ink and the name of different color patches in the congruent condition. Conversely, in the incongruent condition, color-words are printed in an inconsistent color (e.g., the word "red" is printed in green ink), thereby measuring the ability to inhibit cognitive interference. Computerized tests of attention such as the Attention Battery of the Cognitive Drug Research Computerized Assessment System have also been used to measure focused, selective, and sustained attention in AD patients with diagnoses ranging from mild to severe (Galvin et al., 2008). Conners' Continuous Performance Test (CPT) is another computerized test of sustained attention that has been used in studies with mild- to moderate-AD patients and MCI (Newhouse et al., 2012; White & Levin, 1999). The CPT is a 14-minute test where participants view a screen that rapidly shows images of a single letter, the participants are instructed to press a computer key each time they see an "X," while refraining from pressing the key when presented with other letters (White & Levin, 1999). Throughout the test, commission errors, omission errors, and variability in reaction time are measured as indicators of attentiveness and sustained attention (White & Levin, 1999).

More recently, infrared eye tracking technology has been investigated as a tool to objectively measure selective attention to novel visual stimuli in patients with dementia. Known as the visual-paired comparison (VPC) task, this computerized behavioral assay assesses preference for novel stimuli by measuring the relative time spent fixating on novel and repeated images (Chau et al., 2015). In the VPC task, subjects are seated comfortably within viewing distance of a monitor, with their head resting on a chin rest to prevent head movement (Chau et al., 2015). Visual scanning parameters are measured using a binocular infrared eye tracking system, which consists of both an infrared light source as well as an infrared sensitive camera (Chau et al., 2015). Before beginning the test, subjects undergo a 9-point calibration phase in which participants follow a moving target on the computer screen to accurately map the subject's gaze positioning to the calibration points (Crutcher et al., 2009). Each trial consists of two phases, a familiarization phase, followed by a test phase (Fig. 38.2). During the familiarization phase, subjects are shown a set of novel images, presented side by side on the monitor for a standardized amount of time. This familiarization phase is followed by a standardized delay interval where subjects are presented with a blank screen. After the delay interval, subjects enter the testing phase where they are presented with a combination of novel and

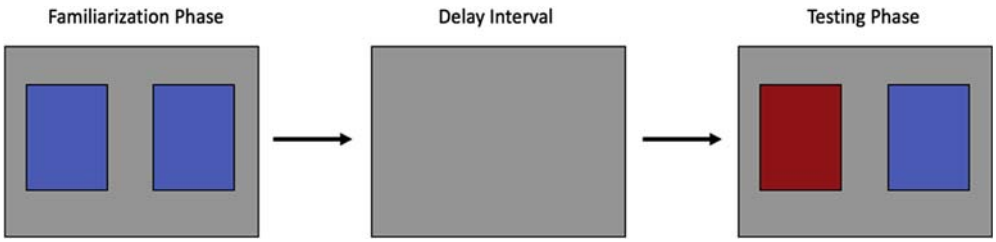

Figure 38.2 ***Schematic representation of one trial on the visual-paired comparison task.*** One trial of the visual-paired comparison task is shown. First, two novel images (blue) are shown during the familiarization phase, followed by a delay interval (blank screen). In the testing phase, one familiar image (blue) and one novel image (red) are shown together, and the participant's novelty preference is assessed.

repeated images for a standardized amount of time. During this phase, several outcomes can be measured including relative fixation time (how much time is spent fixating on the image divided by the total time), fixation duration (how much time was spent fixated on each image), and pupil diameter. Previously, pupil constriction and dilation patterns have been shown to differ between AD patients and healthy adults during a visual search task, indicating that measures of pupil diameter may be able to distinguish between AD and cognitively normal participants (Dragan et al., 2017). The degree of novelty preference shown by the participant can be calculated by subtracting the relative fixation time for repeated and novel images (Chau et al., 2017).

Studies have reported that the VPC task includes several benefits compared to other tests of selective attention and memory. Firstly, the VPC task requires limited language comprehension or motor activity, and thus can be used to measure attention across a diverse range of ages, languages, and populations (Richmond, Sowerby, Colombo, & Hayne, 2004). VPC task scores have also been shown to correlate with scores on the DS and the standardized Mini-Mental State Examination (sMMSE), which includes components of selective attention and executive function. Chau et al. (2015) propose that the VPC task may present a nonverbal, less cognitively demanding method of assessing selective attention. Furthermore, the parameters of the VPC task can be modified to best suit the intended research goal and population. Changing the number of images per slide has been shown to alter the ability of the VPC task to distinguish between healthy controls and MCI. In the standard VPC task, with two images per slide, studies have been unable to distinguish between healthy and cognitively impaired participants (Crutcher et al., 2009). Conversely, Chau et al. (2015) showed participants four images per slide (two novel and two repeated images), which was effectively able to distinguish MCI patients from healthy controls. Researchers attributed the increased sensitivity of this test to the heightened competition for attention that the four image task provides (Chau et al., 2015). Additionally, the length of the familiarization phase, delay interval,

and test phase can be modified. A VPC study in undergraduate university students found that the magnitude of novelty preference increased with increasing familiarization time and decreased with increasing delay interval (Richmond et al., 2004). Additionally, Crutcher et al. (2009) found that with a 2 second delay interval, MCI patients did not demonstrate a difference in novelty preference compared to healthy and neurological controls, however, when the delay interval was increased to 2 minutes, MCI patients spent significantly less time fixating on the novel images (Crutcher et al., 2009). This suggests that the length of the delay interval can be modified in order to differentiate between different levels of cognitive impairment. Lastly, the ability of the VPC to measure multiple outcomes simultaneously may improve its utility as an objective diagnostic test for dementia. In a study by Lagun et al. (2011), researchers demonstrated that an automatic classification algorithm combining measures of novelty preference, fixation duration, number of refixations, and saccade orientation was successful at distinguishing between MCI and healthy age-matched controls with a significantly higher degree of sensitivity and specificity than novelty preference alone (Lagun et al. 2011). In the dementia population, verbal communication becomes increasingly difficult as the disease progresses, therefore this nonverbal and less cognitively demanding tool may provide a more optimal method to assess attention.

Correlates of attentional impairments in dementia and prodromal stages

In patients with MCI, attentional impairments are associated with poorer disease severity and are predictive of conversion to AD (Fig. 38.3). Silveri et al. (2007) followed 28 MCI patients over the course of 1 year, and found that those who converted to AD had lower

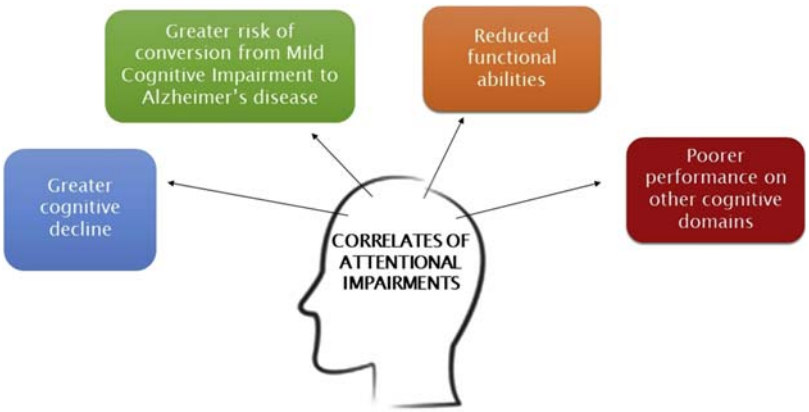

Figure 38.3 ***Correlates of attentional impairments in cognitively impaired populations.*** Attentional impairments are often correlated with greater cognitive decline, greater risk of conversion from mild cognitive impairment to Alzheimer disease, reduced functional abilities, and poorer performance on other cognitive domains.

scores at baseline on tasks exploring attention and executive function compared to patients who did not convert to AD (Silveri et al., 2007). Similarly, researchers from the Berlin Aging Study (Rapp & Reischies, 2005) followed a large cohort of healthy participants over a period of 4 years, measuring attention, executive function, learning, recall, and conversion to AD. Tests of attention and executive function, together with learning and recall, were significantly greater predictors of incident AD than age, gender, and education. In line with those findings, Van Dam et al. (2013) found that, compared to healthy controls, MCI patients demonstrated less activation in the prefrontal and anterior cingulate cortices—neural areas responsible for executive control and function of the human attentional system (Van Dam et al., 2013).

Specific attentional impairments to novel images have also been useful in predicting impending cognitive decline within dementia and prodromal populations (Fig. 38.3). Using the VPC task, researchers could predict those amnestic MCI patients who would progress to AD based on VPC scores up to 3 years prior to a change in clinical diagnosis. A low performance score on the VPC task (<50%) was not only predictive of conversion from healthy control to cognitively impaired but also of conversion from MCI to AD. Additionally, those authors reported that reduced time spent on novel images measured by visual attention scanning technology was associated with a greater decline in sMMSE scores in AD patients (Chau et al., 2017). Together, those findings suggest that novelty preference or attention to novel images may be predictive of disease progression in cognitively impaired populations.

Attentional impairments have also been shown to have a negative impact on other cognitive domains and functional abilities in individuals with dementia (Liu, McDowd, & Lin, 2004) (Fig. 38.3). Liu et al. (2004) reported that AD subjects who omitted more targets on the symbol cancellation test, a measure of visuospatial attention, demonstrated more deficits on subtests of the Behavioral Inattention Test, a measure of daily life task performance (e.g., telephone dialing, menu reading, article reading, map navigation, etc.). Evidence also suggests that selective attentional impairments are related to the motor vehicle accident rates and can differentiate safe versus unsafe drivers in dementia populations (Duchek, Hunt, Ball, Buckles, & Morris, 1998; Parasuraman & Nestor, 1991). Deficits of attention in early AD have also been correlated with poorer performance in other cognitive domains, including executive function and memory (Simone & Baylis, 1997). Baudic et al. (2006) reported that impairments of executive function in very mild AD patients preceded the disturbance of sustained attention, language (oral comprehension, verbal abstraction, and naming) and constructional abilities (Baudic et al., 2006). In line with those findings, Belleville et al. (2007) reported a significant correlation between impairments in recall and divided attention in MCI patients (Belleville et al., 2007). In another study, Fernandez et al. (2018) analyzed eye movement behaviors in relation to short-term memory tests. AD participants were asked to identify changes across two consecutive arrays of bicolored objects whose features (i.e., colors) are

either remembered separately (i.e., unbound colors), or combined with an integrated object (i.e., bound colors). Patients with mild AD showed deficits in selective memory accompanied by significant impairments in their eye movements only when they processed bound colors (Fernandez et al., 2018). Those findings suggest that selective and divided attentional impairments negatively affect memory in patients with mild AD.

Putative mechanisms underlying attentional impairments in dementia

Mechanisms underlying attentional impairments in dementia and prodromal stages are likely multifactorial. Abnormalities in two attention-related functional networks have been implicated as causes of attentional impairments in prodromal dementia and AD (Li et al., 2012; Zhang et al., 2015) (Fig. 38.4). These networks, known as the dorsal and ventral attention networks, are damaged and continue to degrade as the disease progresses. The dorsal attention network is responsible for the endogenous attention orienting ("top-down") process and is bilaterally centered on the intraparietal sulcus and frontal eye fields. The ventral attention network is responsible for the exogenous attention reorienting ("bottom-up") process and includes the right lateralized temporal-parietal junction and ventral frontal cortex (Li et al., 2012). Zhang et al. (2015) reported decreased functional connectivity in the dorsal attention network, but preserved activity in the ventral attention network in patients with MCI, and impaired functional anatomy for both "top-down" and "bottom-up" processing in AD patients. However, in another study, Li et al. (2012) revealed disrupted dorsal attention network connectivity and preserved ventral attention network in AD patients, indicating impairment of a "top-down" and relatively intact "bottom-up" attentional processing mechanism. Differences in findings between these studies may reflect variations in recruitment

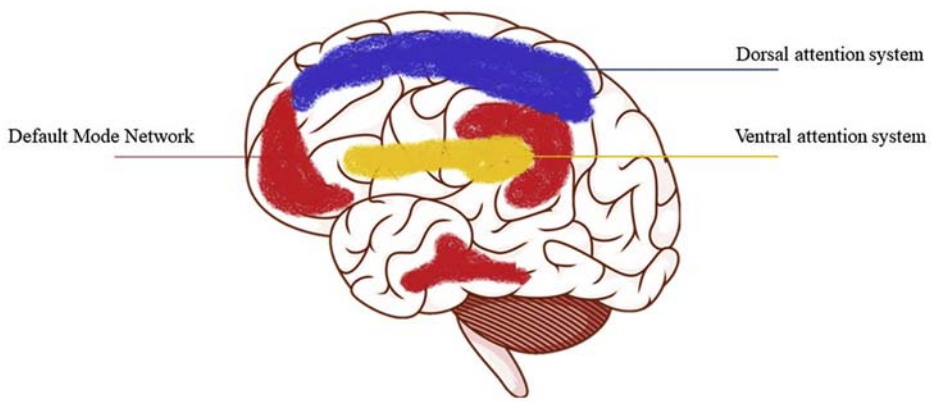

Figure 38.4 ***Putative disrupted networks underlying attentional impairments in dementia.*** Schematic of brain networks that may be dysregulated in patients with dementia who have attentional impairments.

criteria and data processing, as the participants enrolled in the study by Zhang et al. (2015) were 5 years older and data were processed using independent component analysis compared to seed-based regions. In addition to network connectivity abnormalities, impaired structural integrity within specific lobes of the brain has been shown to affect visual attention. Reduced connectivity from the right middle frontal gyrus to the left superior parietal cortex was associated with reduced regional gray matter volume, contributing to impairments in "top-down" attentional control in patients with prodromal AD (Neufang et al., 2011).

Another network that has been implicated in attentional impairment is the default mode network (DMN) (Fig. 38.4). The DMN includes overlapping structures from the previously discussed dorsal and ventral attention networks, such as frontoparietal midline structures, parts of the lateral temporal and parietal cortices, and the medial temporal lobe (Greicius, Srivastava, Reiss, & Menon, 2004; Sorg et al., 2007). In AD, plaque deposition accumulates in areas of the DMN and is associated with functional connectivity disruptions (Drzezga et al., 2011; Sperling et al., 2009). Moreover, in prodromal AD, studies have found a close spatial correspondence between plaque deposition and functional connectivity disruption within attention networks, resulting in impaired performance on selective attention tasks (Neufang et al., 2011, 2014). Those studies strongly suggest that even in prodromal stages, the DMN and specifically frontoparietal attention networks are disrupted and related to impaired attention.

Less is known about the specific mechanisms underlying attentional impairments to novelty. Research has suggested that attentional impairments to novel stimuli may be due to abnormalities of specific neurotransmitter processes, including the cholinergic and dopaminergic neurotransmitter systems (Rangel-Gomez & Meeter, 2016). Cholinesterase inhibitors (ChEIs), such as donepezil, galantamine, and rivastigmine, are approved antidementia drugs that enhance acetylcholine tone in the central nervous system. Recordings from cells in the primate basal forebrain, an area rich in cholinergic neurons that provides major cholinergic innervation to the neocortex, have shown increased neural response to novel stimuli (Wilson & Rolls, 1990). In rodents, lesioning the nucleus basalis of Meynert with cholinergic excitotoxins has resulted in attentional impairments that can be reversed with nicotine, a nicotinic acetylcholine receptor agonist, and ChEIs (Muir, Everitt, & Robbins, 1995; Muir, Page, Sirinathsinghji, Robbins, & Everitt, 1993). In healthy young controls, ChEIs and the dopamine precursor, levodopa, have also been shown to modulate response to novel stimuli (Bunzeck, Guitart-Masip, Dolan, & Duzel, 2014). Bunzeck et al. (2014) measured repetition suppressions, defined as the difference in activity between novel and repeated stimuli in 48 healthy human subjects, and found that galantamine was associated with repetition enhancement, whereas levodopa attenuated repetition suppressions (Bunzeck et al., 2014). This suggests that both cholinergic and dopaminergic neurotransmission are involved in different aspects of the novelty response.

Pharmacological treatment of attentional impairments in dementia

In relation to the aforementioned role of acetylcholine in attentional processes and widespread use of ChEIs to treat AD, a number of studies have investigated the effect of ChEIs on attention in dementia patients (Table 38.2). It has previously been suggested that ChEIs may primarily improve global cognition through improving attention (Brousseau, Rourke, & Burke, 2007). Bracco et al. (2014) found that mild-moderate AD patients who were prescribed ChEIs for 1 year declined in performance on tests of processing speed, semantic processing, and verbal episodic memory, while tests of verbal and visuospatial attention, as well as executive functioning, remained stable over the course of treatment (Bracco, Bessi, Padiglioni, Marini, & Pepeu, 2014). Notably, patients in that study with mild AD showed significant improvements in visuospatial attention following 1 year of ChEI treatment (Bracco et al., 2014). As attention is frequently impaired in patients with dementia and MCI, and is associated with greater disease progression, those results suggest that early treatment may be particularly important in attenuating the early decline of attentional processes. Additionally, two parallel pilot studies comparing the effects of two different ChEIs, donepezil and galantamine, showed that galantamine may be more efficacious in improving scores in selective, focused, and sustained attention, as measured by the attention battery of the Cognitive Drug Research Computerized Assessment System (Galvin et al., 2008). While both drugs are ChEIs, galantamine is also an allosteric modulator of the nicotinic acetylcholine receptor, which has previously been associated with attentional impairments in AD (Galvin et al., 2008; Kadir, Almkvist, Wall, Langstrom, & Nordberg, 2006).

Nicotine, a selective nicotinic acetylcholine receptor agonist, has been studied for its effects on attention in dementia. In MCI patients, 6 months of treatment with transdermal nicotine patches significantly improved performance on the CPT (Newhouse et al., 2012). Furthermore, both acute subcutaneous treatment and chronic transdermal nicotine treatment significantly improved scores on tasks of visual sustained attention in mild- to moderate-AD patients (Jones, Sahakian, Levy, Warburton, & Gray, 1992; White & Levin, 1999). Those studies suggest that nicotine administration may compensate for the reduced nicotinic acetylcholine receptor density seen in AD patients and enhance attentional performance (Nordberg & Winblad, 1986).

Alternatively, the role of monoamine modulation has also been explored in improving attention in AD. A randomized placebo-controlled trial showed that 6 weeks of treatment with methylphenidate, a psychostimulant that increases central norepinephrine and dopamine, significantly improved selective attention in mild- to moderate-AD patients (Lanctot et al., 2014). Altogether, those findings further lend support to the roles of both acetylcholine and dopamine in controlling attention.

Improvements in attention also have important implications for functional abilities in AD patients. A combined cohort and case-control study found that administration of

Table 38.2 Summary of studies assessing the effects of different drugs on attention in patients with dementia.

First author (Year)	Drug	Study design	Duration	Study population	Treatment protocol	Primary outcome measure	Primary findings
Jones et al. (1992)	Nicotine	Single-blind, placebo-controlled trial	2 weeks	22 patients with mild to moderate Alzheimer disease (clinical dementia rating scale stage 1−2), 24 healthy young adult controls, 24 healthy elderly controls	Each participant engaged in seven test sessions, where they were administered no drug, or subcutaneously administered placebo, 0.4 mg nicotine, 0.6 mg nicotine, or 0.8 mg nicotine prior to administration of cognitive tests	Rapid visual information processing task, delayed response matching to location-order task	Nicotine treatment significantly improved sustained visual attention, measured by rapid visual information processing task and delayed response matching to location-order task, with the largest effect in Alzheimer disease patients.
White and levin (1999)	Nicotine	Double-blind, placebo-controlled crossover study	4 weeks	8 patients with mild to moderate Alzheimer disease (mini-mental State examination: 10−26)	Patients were treated with transdermal nicotine patches (5 mg/day or 10 mg/day) for 16 h/day, for 4 weeks. Patients were then treated with transdermal placebo patches for a 4-week phase. The two phases were separated by a 2-week washout period	Conners' continuous Performance Test	Chronic nicotine treatment significantly improved sustained attention as measured by a reduction in errors and reaction time on the conners' continuous Performance Test.

Continued

Table 38.2 Summary of studies assessing the effects of different drugs on attention in patients with dementia.—cont'd

First author (Year)	Drug	Study design	Duration	Study population	Treatment protocol	Primary outcome measure	Primary findings
Galvin et al. (2008)	Galantamine and donepezil	Two parallel trials; (A) double-blind, randomized, group pilot study comparing galantamine and donepezil in patients with mild to moderate Alzheimer disease, (B) rater-blinded, open-label, randomized, group study comparing the effects of galantamine and donepezil in patients with mild to severe Alzheimer disease	(A) 8 weeks (B) 52 weeks	(A) mild to moderate Alzheimer disease (mini-mental state examination: 10–24). (B) moderate to severe Alzheimer disease (mini-mental State examination: 5–22).	(A) 2-week placebo run in phase, followed by randomization into donepezil (5–10 mg) or galantamine (4–8 mg) group. (B) patients were randomized to either donepezil (5–10 mg) or galantamine (4–12 mg).	Cognitive drug research battery measures of attention	Both drugs attenuated decline in performance on attention tasks. Galantamine improved attention early in patients with mild to moderate Alzheimer disease.

Daiello et al. (2010)	Donepezil or galantamine	Combined observational open-label cohort study and case control study	3 months	24 patients with untreated early Alzheimer disease 35 demographically matched Alzheimer disease patients under stable cholinesterase inhibitor treatment. (case-control study)	Patients with untreated early Alzheimer disease began treatment with a cholinesterase inhibitor for 3 months, 22 patients treated with donepezil (mean dose 9.5 mg/day), 2 patients treated with galantamine (16 mg/day).	Simulated driving task, visual search task, maze task	Cholinesterase inhibitor treatment improved performance on driving simulator task, visual search task, and maze task.
Newhouse et al. (2012)	Nicotine	Double-blind randomized placebo-controlled trial	6 months	74 patients with MCI (mini-mental State examination: 24–30).	34 patients treated with transdermal nicotine patch (15 mg/day) for 6 months, 33 treated with placebo.	Conners' continuous Performance Test	Nicotine induced a significant improvement in attention as measured by the conners' continuous Performance Test. Secondary measures of attention and memory also showed significant improvements.

Continued

Table 38.2 Summary of studies assessing the effects of different drugs on attention in patients with dementia.—cont'd

First author (Year)	Drug	Study design	Duration	Study population	Treatment protocol	Primary outcome measure	Primary findings
Bracco et al. (2014)	Galantamine, donepezil and physostigmine	Cohort study	12 months	121 patients with Alzheimer disease (mini-mental State examination ≥17) beginning cholinesterase inhibitor treatment for the first time.	93 patients treated with donepezil (5−10 mg/day), 19 patients treated with rivastigmine (4.6 −6.0 mg/day), 9 patients treated with galantamine (8.0 mg/day). Treatment was continued for the study duration.	Corsi tapping Test, Wechsler Adult intelligence Scale-Digit Span, short story immediate recall	Corsi tapping Test, Wechsler Adult intelligence Scale- Digit Span, and short story immediate recall scores remained stable over 1 year with cholinesterase inhibitor treatment. In patients with mild Alzheimer disease, scores on the corsi tapping Test improved.

Lanctot et al. (2014)	Methylphenidate	Randomized double-blind placebo-controlled trial	6 weeks	60 mild- to moderate-Alzheimer disease patients (mini-mental State examination: 10–26) with apathy (Neuropsychiatric inventory Apathy≥4)	Patients were administered placebo or 10 mg methylphenidate twice daily.	Wechsler Adult intelligence Scale- Digit Span forward	Methylphenidate improved scores on tests of selective attention.
Park et al. (2017)	Galantamine	Open label trial	16 weeks	1516 patients with Alzheimer disease and cerebrovascular disease (Korean mini-mental State examination: 10–25)	Administered galantamine twice daily (8 mg), escalated to 16 and 24 mg at 4 week intervals.	Attention Questionnaire Scale	Attention Questionnaire Scale score significantly improved over 16 weeks of galantamine treatment.

Summary of studies measuring the effect of different treatments on attention in dementia.

ChEI significantly improved visual search performance on a computerized test of selective attention, as well as improving performance on a driving simulation task, which was attributed to improvements in attention (Daiello et al., 2010). Additionally, a large open-label study of 1512 patients with combined AD and cerebrovascular disease found that 16 weeks of treatment with galantamine significantly improved scores on the Attention Questionnaire Scale, a caregiver rated scale that assesses attention in real-life situations (Park et al., 2017). Given that patients with dementia have significant attentional impairments (Dong et al., 2013), optimizing treatments for attentional impairments in this population is an important factor in improving functional capacity.

Key facts of the visual-paired comparison task

- The visual-paired comparison task tests visual selective attention through measuring participants' tendency to reorient attention toward novel stimuli.
- The visual-paired comparison task has been used in depression, eating disorders, infants, and dementia.
- The visual-paired comparison task measures attention without verbal or motor input from the participants, thus is ideal for measuring attention in patients with verbal communication difficulties.
- In dementia patients, longitudinal decline in performance on the visual-paired comparison task is associated with cognitive decline.
- Poorer scores on the visual-paired comparison task are early markers for cognitive impairment and predict subsequent progression to Alzheimer disease.

Summary points

- Attentional impairments are frequent in patients with mild cognitive impairment and Alzheimer disease
- Using the visual-paired comparison task, novelty preference can be objectively measured in patients with cognitive impairment
- Attentional impairments to novel stimuli are predictive of cognitive worsening, disease progression, and reduced functional abilities
- Damage to the dorsal and ventral attention networks, reduced neural structural integrity, and disrupted neurotransmitter processes are putative mechanisms underlying attentional impairments in dementia
- Pharmacological treatment with cholinesterase inhibitors, nicotine, and methylphenidate have either demonstrated maintained or improved attention in patients with dementia

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CHAPTER 39

Frontal lobe syndrome and dementias

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List of abbreviation

AD Alzheimer disease
bvFTD frontotemporal dementia—behavioral variant
DLB dementia with Lewy bodies
DLPFC dorsolateral prefrontal cortex
FBI Frontal Behavior Inventory
FDG fluoro-deoxy-glucose
FLS frontal lobe syndrome
FTLD frontotemporal lobar degeneration
PDD Parkinson's disease dementia
PET positron emission tomography
ToM theory of mind
VPFC ventral prefrontal cortex
VPFC ventral prefrontal cortex

Mini-dictionary of terms

Apathy Loss of motivation, which can arise accompanied by decrease in the cognitive and emotional aspects of goal-directed behavior. Specifically, apathy can be "emotional-affective" when there is inability to create the necessary link between emotional-affective responses and the behavior. Cognitive apathy refers to difficulties in planning actions and using working memory abilities and set-shifting. Autoactivation apathy causes the failure to self-activate thoughts or actions with respect to a relatively spared ability to generate behavior. Apathy is often due to prefrontal lesions or dysfunctions.

Environmental dependency phenomena Patients tend to imitate the movements of the examiner even when they have not been asked to do so, and continue emulating despite being requested to stop.

Executive Functions Set of processes that include: the ability to plan and evaluate effective strategies related to a specific purpose related to problem-solving skills and cognitive flexibility; attentional control, referring to the ability to inhibit interfering stimuli and to activate the relevant information; the inhibitory control and decision-making processes that support the selection of the functional response and the modification of the response (behavior) in relation to the change of the environmental contingencies (e.g., reinforcement); working memory, which refers to cognitive mechanisms that allow the online maintenance and manipulation of the necessary information for the execution of complex cognitive operations such as language and reasoning.

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Metacognitive abilities Abilities in the following: predicting or planning actions in the future; in monitoring/self-evaluating actions in the past, to learn to move away from the immediate present and in projecting forward and backward in time, as well as controlling the mental paths used to process information, make decisions, solve problems, and be aware of the possible strategies for coping with situations.

Social cognition Refers to the processing of social information in the brain that underlies abilities such as the detection of others' emotions and responding appropriately to these emotions.

Theory of mind The capacity to make inferences about other peoples' mental states and motivations.

Introduction

Cognitive deficits due to damage or disease affecting frontal lobe systems are well known, and they may result in a frontal syndrome attributable to dysfunction of the frontal lobe circuits (Bonelli & Cummings, 2007). Frontal lobe lesions determined by progressive degenerative or acute pathologies can disrupt multiple circuits because of their close proximity to one another. Frontal lobe syndrome (FLS) includes both cognitive and behavioral disorders (Stuss & Alexander, 2007). Cognitive deficits mainly encompass language impairments and executive disorders (dysexecutive syndrome), while behavioral changes primary concern social and affective processes (frontal syndrome) (Cummings & Miller, 2007). Although the terms "frontal syndrome" and "dysexecutive syndrome" are often used interchangeably, several studies have pointed out that the terms should be distinguished (i.e., Krause et al., 2012) as frontal pathology does not always lead to executive impairments and dysexecutive syndrome does not fully explain some specific behavioral disorders associated with frontal lobe lesions (Alvarez & Emory, 2006). Besnard et al. (2018) tried to disentangle the specificity of the two syndromes and found that patients assigned to the "behavioral" group differed from the patients assigned to the "dysexecutive" group because of environmental dependency phenomena (e.g., behavioral disorders triggered by social interaction), by confirming sociobehavioral deficits without executive impairment in case of frontal lobe lesions.

In this vein, the classical view of FLS as a merely dysexecutive or behavioral disorder has been drastically modified in the last few years by reports of social cognition impairments in a high proportion of subjects presenting with frontal lobe dysfunction (Zaki & Ochsner, 2012) (Fig. 39.1). Disturbances of social cognition are early and salient features of many major psychiatric diseases, brain injury, and neurodegenerative disorders. The neural correlates are linked for example to abnormalities in the orbitofrontal cortex (social inappropriateness, hypersexuality, and compulsive gambling) (Beer John, Scabini, & Knight, 2006) or in the dorsomedial prefrontal cortex (processing of social information and decision-making) (Henry, Von Hippel, Molenberghs, Lee, & Sachdev, 2016). These observations are particularly relevant in the light of the phenotypic syndrome of behavioral variant of frontotemporal dementia (bvFTD), a clinical syndrome resulting from an underlying complex neuropathological process named frontotemporal lobar

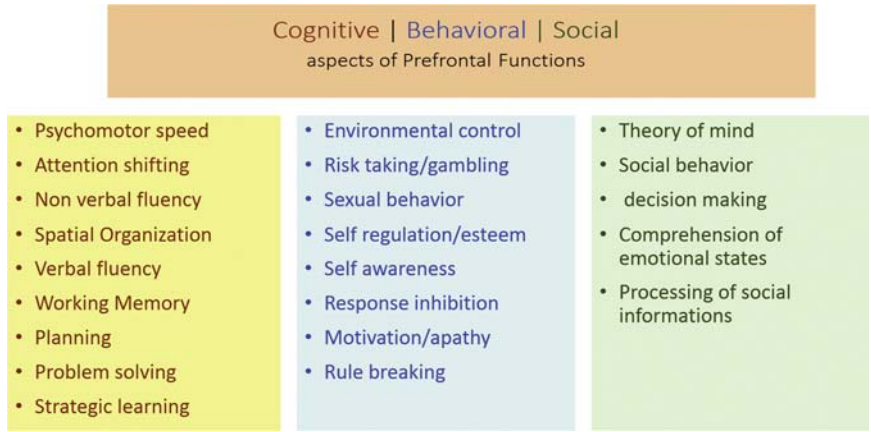

Figure 39.1 Overview of main cognitive, behavioral, and social aspects processed by frontal lobes.

degeneration (FTLD) (Mackenzie et al., 2010), in which the core symptoms are progressive changes in personality and behavior with emotional and motivational blunting, and impairment in social conduct. Nonetheless, an FLS is also reported in other neurodegenerative conditions; i.e., Alzheimer disease (AD) (Godelfroy et al., 2010). In this chapter, we provide a brief guide to the main cognitive, behavioral, and affective deficits that can arise from an FLS from the point of view of the usefulness in differentiating among the principal forms of cortical dementias that have relative specific frontal lobe symptoms. We discuss other dementias associated with movement disorders, such as Parkinson's disease. We also supply a quick look at the neuroepidemiology of FLS, which needs further studies in order to assess the correct prevalence and avoid difficulties in the recognition of the pattern of symptoms.

Neuroanatomy of the main frontal lobe circuits

The frontal lobes are within the brain regions anterior to the central sulcus and fill around one-third of the whole cerebral cortex. The frontal cortex can be divided into four main areas: precentral cortex, premotor cortex, prefrontal cortex, and orbitofrontal cortex. According to Alexander (1986), at the frontal lobes levels, multiple frontal–subcortical circuits can be described. This model provided comprehensive reviews of five neuroanatomical circuits connecting regions of the frontal lobes with subcortical structures, such as the striatum, globus pallidus, and thalamus and has specific target regions in the frontal lobes, including: the supplementary motor area, the frontal eye fields, the dorsolateral prefrontal cortex, the orbitofrontal cortex, and the medial frontal cortex. These areas are involved in motor dysfunctions (e.g., release of primitive reflexes, incontinence), executive dysfunctions (e.g., disorganization, inflexibility, loss of hypothesis generation and testing, impaired working memory), reduction in drive and motivation, and

disinhibition (impulsivity, disinhibition, poor social judgment). A schematic representation of the basic structure of frontal-subcortical circuits can be found in Cummings (1993).

The prefrontal cortex, together with its underlying subcortical regions, is interconnected with the major sensory and motor systems of the brain. These areas represent the most anterior regions of the frontal lobes. Anatomically, the prefrontal cortex is usually divided into the following regions: the dorsolateral prefrontal cortex (DLPFC), the ventral prefrontal cortex (VPFC), and the medial frontal cortex. The DLPFC originates in the lateral, anterior frontal lobe, projecting to the dorsolateral caudate nucleus, which sends fibers to the substantia nigra and the globus pallidus. These areas are then connected to the dorsal thalamic nuclei.

The VPFC subcortical circuit arises in the orbitofrontal cortex projecting to the caudate nucleus, the globus pallidus and substantia nigra, and the thalamic nuclei. Furthermore, the VPFC is connected with the DLPFC, the amygdala, and the temporal pole. The VPFC is also interconnected with limbic nuclei involved in emotional processing and in the stimulus-reward associations (Rolls, 2017).

The medial—frontal circuit begins in the anterior cingulate cortex. It contains the nucleus accumbens, the globus pallidus and substantia nigra, and the thalamic nucleus. The medial—frontal circuit has interconnections with DLPFC and the amygdala; it also receives input from the ventral tegmental area. The frontal poles have been considered to be involved in processes that define us as human, as they have a prominent role not only in consciousness, self-awareness but also in social cognition abilities (i.e., theory of mind, ToM) (Stuss, 2001).

Frontal lobe syndromes

Building upon the neuroanatomical work described above, Cummings (1993) proposed a model linking the three fronto—striato—thalamic circuits to the three clinically observable frontal behavioral syndromes (Table 39.1). Overall, the networks beginning in the DLPFC have been associated with executive cognitive dysfunction; the networks arising in the VPFC have been associated with disorders of self-regulation, such as the syndrome of frontal disinhibition; and the medial—frontal circuit including the anterior cingulate circuit has been associated with disorders of activation, spontaneous behavior, and motivation, resulting in syndromes such as apathy.

Table 39.1 Overview of anatomical areas, cognitive functions and the corresponding syndromes.

Brain circuit	Function	Syndrome
Dorsolateral	Cognition	Disorganized
Orbitofrontal	Emotion	Disinhibited
Mesial frontal	Motivation	Apathetic

Executive functions refer to the set of mental processes necessary for the elaboration of adaptive cognitive-behavioral schemes in response to new and challenging environmental conditions (Hoffman, 2013). These mechanisms are able to optimize performance in situations that require simultaneous activation of different cognitive processes (Logie, 2016). These functions appear particularly critical when response sequences must be generated and organized and when new action programs must be formulated and executed (Fig. 39.1). The clinical pictures deriving from the dysfunction of the executive processes characterize the dysexecutive syndromes. Several studies in the literature agree on considering the prefrontal cortex the main neural substrate of these functions. Patients with lesions restricted to this network typically present decreased fluency, perseveration, difficulty shifting set, poor recall/retrieval of information, reduced mental control, limited abstraction ability, and poor response inhibition but intact perception, calculation, language ability, and storage of memories. Moreover, the dorsolateral network receives information from all three frontal–subcortical circuits, allowing the integration of information coming from the external world with cognitive and emotional states of the individual (Fig. 39.2).

Traditionally, the ventral orbitofrontal region plays an important role in the social behavior. Neuroimaging studies have differentiated the contribution of the different regions of the orbitofrontal cortex in the mediation of the different components of behavior and executive functions (Murray, O'Doherty, & Schoenbaum, 2007). The results of these studies show that the orbitofrontal regions are particularly involved in decision-making processes and in the ability to modify behavior based on changes in environmental contingencies (e.g., reinforcement). Lesions in these areas, in fact, produce difficulty in metacognitive, sociocognitive, emotional, and reward processing (i.e., disinhibition, impulsivity, deciding in an advantageous way for themselves and to respect social norms) (Bechara, 2000; Peters & D'Esposito, 2016). Behavioral self-regulation disorders are shown in numerous case studies of patients with pathology in

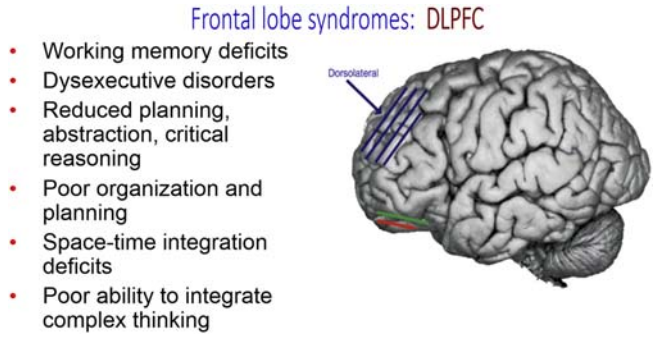

Figure 39.2 Neuroanatomy and clinical syndrome of dorsolateral prefrontal cortex.

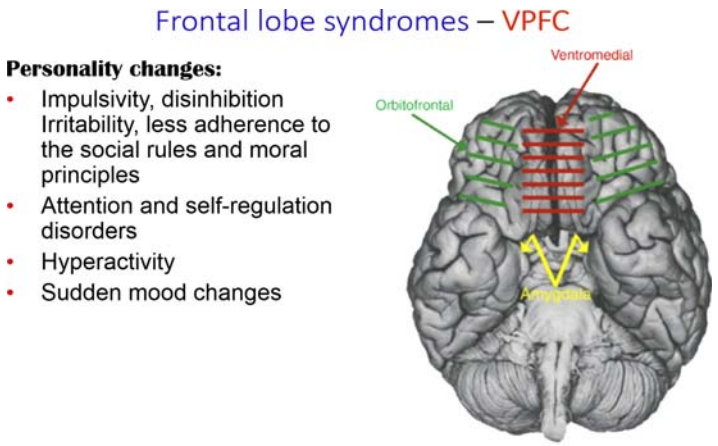

Figure 39.3 Neuroanatomy and clinical syndrome of ventral prefrontal cortex.

this circuit (i.e., Ardila, 2013). Moreover, recent studies indicate that this brain region is particularly involved in the ability to decide when the external situation has characteristics of low structuring, i.e., when it is necessary to base oneself on a subjective feeling of correctness—"feeling of rightness" (Elliott, Dolan, & Frith, 2000) (Fig. 39.3).

The medial frontal–subcortical circuit is involved in motivation. Lesions specific to this network may produce apathy, lack of motivation, decreased interest, engagement with the environment, and poor behavioral maintenance. Furthermore, the anterior cortex of the cingulum appears involved in the attentive processes that allow the activation of useful information and the inhibition of interfering stimuli (Fig. 39.4).

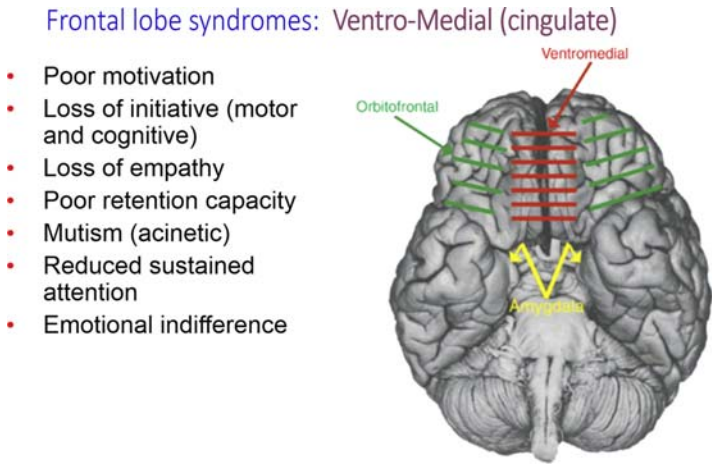

Figure 39.4 Neuroanatomy and clinical syndrome of ventral-medial prefrontal cortex.

Neuropsychological assessment

Several neuropsychological tests have been developed to assess frontal lobe functions. Among many of the batteries used to evaluate executive functions, a selection of specific tests can be adopted. The most useful tasks to assess executive functions could be the fluency tasks that have been often proposed as they examine the ability to maximise the number of responses under constraint of time and restricted search conditions (words or figures) while avoiding response repetition. Moreover, inhibitory functioning can be assessed by the Stroop Test or the Hayling Test of sentence completion; abstraction and reasoning using a subtest of the Behavioral Assessment of the Dysexecutive Syndrome, the Progressive Colored Matrices, the Wisconsin Card Sorting Test, and the Brixton Test. The behavior alterations can be measured using informant-based questionnaires like the Frontal Behavioral Inventory. Since social cognition and emotion recognition can be also impaired due to a frontal lobe dysfunction, tests like the Reading the Mind in the Eyes test or the Ekman faces have been used to quantify this difficulties.

Frontal lobe syndrome in neurodegenerative diseases

Several studies showed that the frontal lobes are particularly vulnerable to the ageing processes of the brain (i.e., Jagust, 2013). Neuroimaging data on brain morphology suggest that normal ageing mainly involves cortical and subcortical structures and also the white matter fibers of the frontal lobes. Despite some methodological concerns, numerous neuropsychological studies indicate a particular susceptibility of the executive functions to the normal ageing process (Fjell, Sneve, Grydeland, Storsve, & Walhovd, 2016; Lustig and Jantz, 2015). These data have been proven by studies using various cognitive tasks and finding a prominent deficit of activation of the DLPFC (i.e., Toepper et al., 2014). Cerebral atrophy (e.g., a decrement in the size of the cells) is a phenomenon frequently reported both in anatomopathological and neuroradiological studies, and it is more pronounced in the frontal subcortical areas (Habes et al., 2016), however, the relationships between atrophy and cognitive functioning of the elderly are not yet fully clear. Many neurodegenerative diseases can present an involvement of the frontal lobes, and the related functions, not only in an intermediate or advanced stage of the disease but also in a very early phase. Dementias with a prevalent involvement of the frontal lobes can cause damage of the subcortical frontal circuits involved in the regulation of cognition, emotions, and behavior.

Alzheimer disease

AD is a clinical-pathological entity accompanied by specific neuropathological changes, which usually presents as a distinct phenotype characterized by cognitive and behavioral impairment. The development of cognitive deficits in AD typically reflects the temporal neuropathological changes. In the earliest stages of AD, patients can exhibit episodic memory loss, subtle executive problems, and lexical-semantic deficits (such as anomia or semantic paraphasias).

Attentional and executive deficits may arise as the disease progresses through frontal and temporal areas; when cognitive deficits become more prominent patients may exhibit a prominent dysexecutive syndrome (Salmon & Bondi, 2009). During the intermediate phase of the disease, deficits of executive functions (such as problem-solving, planning, and abstraction) are associated to the neuropsychiatric symptoms. Neuropsychiatric symptoms such as apathy, disinhibition, psychosis, vagrancy, and social withdrawal are common during this stage (Kales, Gitlin, & Lyketsos, 2014). Over the years, additional studies found that not all patients with AD had the same symptomatology and that in the initial stages some patients had symptoms typical of intermediate or late stages of the disease.

Moreover, a less common presentation of AD pathology can be the nonamnestic, focal cortical syndromes. Almost one-third of pathologically verified AD subjects present with other clinical syndromes (Alladi et al., 2007): posterior cortical atrophy, primary progressive aphasia, and behavioral manifestations similar to the bvFTD, e.g., the frontal variant of AD. The latter form is the less prevalent among the variants and was identified by Johnson, Head, Kim, Starr, and Cotman, (1999) when he noticed some forms of AD that had at their onset mainly frontal deficits (in planning, problem solving, judgment, abstraction, and cognitive flexibility) (Mez et al., 2013). The spectrum of the frontal variant also includes behavioral disorders characterized by disinhibition, apathy, and compulsiveness. These clinical characteristics make nontrivial the differential diagnosis with the bvFTD. A recent study by Ossenkoppele et al. (2015) compared the characteristics of a large group of patients presenting with the frontal variant of AD and with bvFTD. They collected, for most of them, neuropathological data or at least biomarker examinations (i.e., amyloid PET). The authors concluded that the phenomenological description of the two syndromes is not completely explicative in the diagnostic criteria for bvFTD (e.g., Rascovsky et al., 2011). In fact, the neuropsychological tests of executive functions seem to be not useful for the differential diagnosis, while a major sensitivity has been revealed by the FDG-PET and the amyloid-PET.

Frontotemporal dementia

FTD is a clinical entity encompassing a spectrum of neurodegenerative diseases with heterogeneous clinical presentations determined by an underlying complex neuropathological process named FTLD. FTD is characterized by heterogeneity at the clinical, pathological, and genetic levels. The disease has an insidious onset and progresses slowly with a duration that usually goes from 10 to 14 years. Being a very heterogeneous pathology, FTD includes two major clinical manifestations (Rabinovici & Miller, 2010), language variants (e.g., primary progressive aphasia, PPA) and a behavioral variant (bvFTD). The cognitive deficits in FTD may occur in conjunction with other neurological features, such as motor neuron disease (parkinsonian syndromes, that is, corticobasal

degeneration and progressive supranuclear palsy) (Kertesz & Munoz, 2002). Overall, atrophy of FTLD mainly involves frontal and temporal lobes, independently of histopathological lesions.

Concerning the language variants due to FTD clinical syndromes, two main clinical phenotypes of PPA have been proposed: nonfluent variant and semantic variant. They can be distinguished by the different profiles of cognitive and speech/language deficits and by a supportive pattern of atrophy on imaging (Gorno-Tempini et al., 2011). The correct classification of these variants requires the use of ad hoc speech/language and nonlanguage neuropsychological tests to define the whole profile (i.e., Battista et al., 2017, 2018; Catricalà et al., 2017).

Among the different phenotypic pictures, a frontal syndrome is prominent in the behavioral form due to the involvement of orbitofrontal areas, the limbic system, the anterior cingulate cortex, amygdala, ventrolateral frontal, and ventromedial frontal areas (Snowden, Neary, & Mann, 2007), which causes the onset of early alterations. bvFTD begins with an insidiously progressive alteration in personality and behavior, ranging from the deterioration of social conduct, loss of insight and emotional inhibition, to inertia, apathy, disinhibition, perseverative behavior, hyperorality, and hyperphagia (Rascovsky et al., 2011). In particular, the damage that also involves the amygdala determines the three specific symptoms of disinhibition, stereotypies, and a grade of greed (Rolls, 2017). Patients presenting with a prevalent atrophy of the right frontal lobe show a dyscontrol of impulses, aggression, financial impudence, impulsivity, and lack of empathy; while patients with a prevalent left frontal lobe atrophy present more linguistic disorders (Mychack, Kramer, Boone, & Miller, 2001).

In general, patients with bvFTD exhibit difficulties in the ability to attribute mental states to one's self and to others (i.e., ToM), and they are often not aware of their cognitive and behavioral abnormalities, linked to a deficit in the domain of social cognition (Adenzato, Cavallo, & Enrici, 2010). However, behavioral alterations are not uniform. Some patients show hyperactivity, while others may be completely apathetic and inert with a total lack of initiative and poor reaction to external stimuli. In other patients, compulsive characteristics prevail, such as stereotyped, ritualistic behaviors with respect to personal hygiene, dressing, and food.

At the first neuropsychological examination, patients may initially not experience major difficulties in executive functions, as revealed by the scores obtained from specific cognitive tests. In fact, executive tests are especially sensitive to the damage of the DLPFC while in these patients the atrophy initially affects the orbitofrontal region and its connections (Cerami et al., 2014). Therefore, patients with bvFTD fail in the ability to make decisions based on reward and that require control of impulsivity. Executive deficits may appear in the course of the disease, such as planning and reasoning impairments (Hornberger, Piguet, Kipps, & Hodges, 2008). To conclude, the usefulness of a social cognitive neuroscience approach in bvFTD patients is clear nowadays, thus, neuropsychological assessments should take into consideration this aspect.

Dementia with lewy bodies

DLB is the second most common neurodegenerative pathology after AD in subjects over age 65, typically characterized by senile age-onset around 65 years, with a slightly higher prevalence in males (McKeith, 2007). In general, the evolution of the disease is quite rapid. DLB patients usually exhibit a subtle onset with a progressive pattern, characterized by considerable fluctuations, so that in the same day the patient may seem completely normal and respond correctly to the questions, but the same patient can present a severe and marked distractibility, almost to confusion. This cognitive fluctuation can be daily but also within days. The diagnosis arises in the presence of a clear dementia syndrome, with pronounced variations in attention associated with recurrent visual hallucinations, generally well structured, and extrapyramidal motor disorders (McKeith et al., 2005). DLB symptoms begin with neuropsychiatric clinical manifestations, such as delusions, visual hallucinations, apathy, and anxiety, sometimes accompanied by typically frontal manifestations, such as verbal disinhibition and hyperactivity (McKeith et al., 2005).

With respect to AD patients, DLB clinical manifestations are more related to executive dysfunction, i.e., difficulties of imitations, abstract reasoning, verbal fluency, and a lot of perseverations (Nation, Salmon, & Bondi, 2014). Furthermore, many patients may show a combination of cortical and subcortical neuropsychological deficits, with marked attention disorders, relevant subcortical frontal dysfunctions, and also visuospatial disorders. In particular, short-term verbal memory deficits may be present in the early phase of the disease; some studies have detected the presence of a frontal lobe and hippocampal atrophy (Aarsland, Ballard, & Halliday, 2004), so that the deficit of the executive functions could be determined by a dysfunction of the frontohippocampal projections. Probably the localization of the Lewy bodies especially in the anterior cingulate cortex and in the inferior temporal cortex determines the onset of deficits not only of perceptive type but also of attentive and executive type (Collerton, Burn, McKeith, & O'Brien, 2003).

Parkinson's disease

Parkinson's disease (PD) is probably the subcortical pathology in which executive function processes have been more studied. PD is a neurodegenerative syndrome whose neuropathological bases are primarily constituted by a neuronal depletion of the pars compacta of the black substance with consequent dopaminergic deafferentation of the nigrostriatal pathway (Gelb, Oliver, & Gilman, 1999). The dopaminergic depletion observed also in the ventral tegmental area of the midbrain determines a total dysregulation of the three main dopaminergic systems of the brain: beyond the nigrostriatal pathway, the mesolimbic and mesocortical circuits are involved (Gelb et al., 1999).

According to this evidence, cognitive dysfunction in PD may be a consequence of lesions not only in the primary motor circuit but also in a number of pathways from

the basal ganglia to the prefrontal cortex. Dopamine depletion in the lateral VPFC and the DLPFC networks has been proposed as a possible mechanism of cognitive impairment in PD.

The probability of developing a frank cognitive decline appears to be estimated around 40% (Emre, 2003), inducing a PD with dementia (PPD). Risk factors seem to be the presence of familiarity for dementia, advanced age at the onset of the disease, and a greater severity of extrapyramidal symptomatology, as well as the development of mental confusion and psychotic disorders following the administration of levodopa. PPD seems to be characterized by a progressive dysexecutive syndrome, with three main components: inhibition and switching, working memory, and sustained and selective attention. Therefore, a variety of executive function tasks can be impaired in these patients, leading to deficits in the ability to plan, organize, and regulate behavior (Sawamoto et al., 2008). Importantly, the impairment of executive functions that characterizes most of PD patients from early stages of the disease is not directly due to a neuropathology of prefrontal cortex but to reduced dopaminergic striatal stimulation, disrupting the functioning of frontostriatal networks. Many researchers have focused their attention on the study of executive functions whose alteration constitutes the dominant neuropsychological finding in individuals with PD without dementia. The tests considered sensitive to an alteration of these functions, such as the Wisconsin Card Sorting Test, the Tower of London, the Trail Making and the Stroop, essentially require the implementation of mechanisms of cognitive flexibility and the ability to organize and monitor the chosen strategies to solve the task. At these tests, patients with PD generally achieve impaired performance by showing a pattern similar to that of patients with lesions of the frontal lobes (Sawamoto et al., 2008). As the disease progresses, the orbital circuit becomes impaired by the dopamine depletion, probably resulting in an impairment of related behavioral aspects, such as cognitive and emotional apathy (Prange et al., 2018).

Epidemiology

Few population-based studies have investigated the prevalence and the phenotypical characteristics of FLS among older subjects. A previous epidemiological was performed in the age group older than 85 years by using a neuropsychological examination and a semistructured interview (Gislason et al., 2003). In this study, the estimated prevalence of FLS was 19%, moreover, 3% of these subjects fulfilled the criteria of bvFTD. However, in this study, the neuropsychological examination did not include a comprehensive assessment of some components of executive functions and social cognition abilities, which recently have been shown to be very important aspects of FLS. Standardized neuropsychological measures may be more efficient compared to semistructured clinical interviews and may provide more accurate findings in terms of diagnosis. Recently, we collected preliminary data in a population-based study in Southern Italy

(65y+): The Great-Age Study (Lozupone et al., 2018). In order to define FLS, the neuropsychological assessment focused on social cognition, behavioral impairments, and executive function dysfunctions. The neuropsychological examination included the Frontal Behavior Inventory (FBI) for detecting behavioral symptoms, and standardized tests for executive functions and social cognition. We adopted two algorithms: the first, broad (presence of dysfunction in at least one domain); and the second, narrow (presence of behavioral symptoms—FBI > 11—plus executive dysfunction or social cognition deficits). Our preliminary results showed that among 112 subjects, FLS was present in 36% of subjects using the broad algorithm and in 7% using the narrow algorithm (Battista, Piccininni, Tortelli, Panza, & Logroscino, 2018).

Conclusion

Frontal lobe areas and their implications on the cognitive, behavioral, and emotional sphere have represented an elective field of study from the very first neuropsychological studies on patients suffering from focal frontal lobe lesions. Overall, the frontal lobe areas are functionally very plastic and do not correspond to strict organizational diagnostic criteria for each syndrome. Syndromic scenarios in the course of neurodegenerative pathology reflect the involvement of these areas and show a high variability within the same pathology. Future diagnostic criteria that account for several neuropsychological and behavioral test batteries could be identified by the combinations of specific features that most reliably and accurately classify patients based on neuropsychological scores. A recent meta-analytic review (Yuan & Raz, 2014) analyzed 31 studies, including a total of 3272 participants, and found a strict correlation between the volumes of prefrontal areas, the thickness of the cortex, and specific neuropsychological tests for executive functions in healthy individuals. The further use of machine learning algorithms, as reported by several studies, may improve the identification of specific measures, to this aim (e.g., Battista, Salvatore, & Castiglioni, 2017). This approach could be very useful for clinical practice, since FLS, if investigated in all its components (cognitive, behavioral, and emotional), is a quite frequent phenotype in older-aged individuals.

Key facts of frontal lobe syndrome

Frontal lobe syndrome can be observed in diverse conditions such as psychiatric diseases and neurologic disorders. In the last 20 years, the interdisciplinary knowledge from neuroimaging, neuropsychological and animal models, led to a detailed description of the clinical syndrome and to increasing insight in functions and circuits. In dementias, frontal lobe syndrome can present several pictures, depending on the cerebral networks affected by the atrophy.

Summary points

- This chapter provides an overview of the frontosubcortical circuits, related clinical syndromes, and the main neurodegenerative diseases.
- Frontal lobe syndrome is a term that has been used in many ways over the years, thus there are numerous alternative interpretations of it.
- Few population-based studies have investigated the prevalence and the phenotypical characteristics of frontal lobe syndrome among the elderly.
- Syndromic scenarios in the course of neurodegenerative pathology reflect the involvement of frontal areas and show a high variability within the same pathology.
- Neuropsychological and behavioral tests can help in the differential diagnosis of the neurodegenerative diseases with predominant frontal dysfunction.

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CHAPTER 40

The stigma of dementia

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List of abbreviations

AD Alzheimer's disease
ADRC Alzheimer's Disease Research Center
BPSDs behavioral and psychological symptoms of dementia
DAC dementia awareness campaign
GP general practitioner
MLT modified labeling theory

Mini-dictionary of terms

Courtesy stigma negative perceptions attributed to the caregivers of patients with stigmatized conditions, often leading to increased caregiver burden.
Behavioral and psychological symptoms of dementia Mood and personality changes presenting early in the progression of dementia. They are often misunderstood by friends and family and are difficult for caregivers to manage.
Instrumental activities of daily living (IADL) Activities requiring a higher level of cognitive functioning that are often necessary for fully independent living.
Promotora A community member trained by medical experts to disseminate health information in a culturally relevant manner.
Vascular dementia A form of dementia resulting from atherosclerotic blockages of vessels to the brain that result in cellular hypoxia.

Introduction

Rates of dementia continue to rise steadily, with recent estimates of Alzheimer's disease (AD) approaching 11% of the US population (Hebert, Weuve, Scherr, & Evans, 2013). By 2050 the incidence rate is expected to double, yielding a new case of AD every 33 s and up to a million cases per year (Alzheimer's Association, 2013). Globally, 46.8 million individuals were living with AD in 2015 (Prince, 2015). The financial cost of treating dementia in the US is substantial, with estimated healthcare costs in 2013 nearing $203 billion excluding contributions by families, friends, and other unpaid caregivers (Alzheimer's Association, 2013). The burden of disease associated with dementia calls for a concerted effort by physicians, caregivers, and community members to review and improve upon the current management of and approach to treatment.

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A topic gaining increasing attention in the context of dementia is stigma. Stigma is defined as a set of negative beliefs and attitudes, often discriminatory in nature, toward a specific group of individuals (Herrmann et al., 2018). While the literature on dementia-related stigma is limited, there is evidence to suggest that stigma impedes proper patient care, leading to poorer outcomes for individuals with dementia (Herrmann et al., 2018). Several misconceptions regarding dementia have led to negative associations with the condition. Many view memory loss as a natural part of aging, while others see dementia purely as a mental illness (Gove, Downs, Vernooij-Dassen, & Small, 2016). Researchers surveying population attitudes toward individuals with dementia found a substantial amount of associated negative emotion (Kessler & Schwender, 2012). In the UK and US, dementia is perceived as one of the most feared conditions, second only to cancer (Kessler & Schwender, 2012). Forced to imagine being diagnosed with dementia, many individuals over the age of 75 suggested that life would no longer be worth living (Lawton et al., 1999).

Dementia is impacting healthcare systems around the world, and negative attitudes toward the condition often manifest differently across cultural subgroups (Prince, 2015). In the West, a variety of cultural beliefs are contributing to the growing stigma (Herrmann et al., 2018). While the reasons may differ, the results are often similar, with increasing levels of social isolation and decreased patient access to resources. One study exploring dementia among minority communities in the UK revealed specific cultural-based stigma toward both patients and caregivers (Mackenzie, 2006). Caregivers of Eastern European descent were often noted as keeping their relative's dementia diagnosis private, believing that publicizing the diagnosis would lead to condemnation from community members (Mackenzie, 2006). Within this community, many caregivers emphasized the need to carry the burden of care alone, rarely expecting support from family or friends (Mackenzie, 2006). The tendency to socially isolate patients in an effort to avoid feeling stigmatized is believed to stem from a historical background of trauma and violence. Sharing such a diagnosis might evoke negative emotions in fellow community members and is thus generally avoided (Mackenzie, 2006). The result is social isolation for both patients and their caregivers, leading to diminished access to desperately needed resources (Mackenzie, 2006).

A similar situation is encountered in South Asian communities. Caregivers within this community allude to religious-based cultural beliefs that depict patients with dementia as possessed by evil spirits (Mackenzie, 2006). Often this negative perception regarding patients extends to their caregivers, separating whole families from the community. Faced with the fear of stigmatization, families often resort to hiding relatives with dementia, isolating the patient socially, which also has the effect of reducing access to resources. In the Chinese American population, exceedingly delayed diagnoses and treatment have been documented among community members (Woo, 2017a, 2017b). Individuals in this population are much less likely to utilize mental health services (Zheng, Chung, &

Woo, 2016). This is in part due to cultural barriers to access coupled with an underlying stigma toward mental illness within the community (Woo, 2017a, 2017b). Dementia is also a concern in the Latino community. Due to high rates of cardiovascular disease, Latinos are currently 1.5 times more likely to develop Alzheimer's or vascular dementia (Alzheimer's Association, 2017). They are currently the fastest-growing population in the US, with the potential for a substantial burden of disease (Alzheimer's Association, 2017). Both of these groups face limited access to educational resources and support due to language barriers that only further augment the prevailing cultural stigma.

Stigma is also a concern on a global scale, especially in developing countries with limited literacy levels (Faure-Delage et al., 2012). One study explored perceptions of dementia in the Republic of Congo, where no term for dementia exists within the population's language (Faure-Delage et al., 2012). Among community members, dementia was recognized based on specific symptom patterns (Faure-Delage et al., 2012). While primarily associated with old age and hardship, the use of witchcraft and evil intentions were attributed to those with the condition (Faure-Delage et al., 2012). While community members demonstrated understanding of the illness, being in close contact with such an individual was nonetheless considered to have negative repercussions (Faure-Delage et al., 2012). It is important to continue exploring the cultural perceptions surrounding dementia in order to work toward tangible solutions that address stigmatization within the community.

Location also can affect the amount of stigma that patients with dementia encounter. Patients in urban settings are often susceptible to a greater degree of stigma relative to those in more rural settings (Burgener et al., 2015). This may be due to a stronger sense of community among rural populations, which could mean increased support and acceptance (Burgener et al., 2015). Similarly, in assisted living facilities, increased stigmatization is directed at residents with dementia despite being among a community of peers (Burgener et al., 2015). Evidently, the fear of developing dementia encourages unaffected residents to disassociate themselves, alienating those with the condition (Burgener et al., 2015). These findings reveal that social isolation is often less dependent on the concentration of individuals in close proximity and more concerned with the support and openness afforded by surrounding community members.

Stigma: theory and development

Stigma has seen a significant transformation throughout the course of history, initially directed primarily at social deviants and only recently encompassing individuals with physical and mental illness (Bos et al., 2013). Today, stigma is recognized as a means of discrediting the identity of an individual or group within society, thereby creating widespread social disapproval (Bos, Pryor, Reeder, & Stutterheim, 2013). Stigma is furthermore dependent on social interaction, as no inherent stigma resides in an

individual outside of a social context (Bos et al., 2013). In order for stigma to develop, two criteria must be met—the recognition that a difference exists among a specific group and the devaluation of the group based on that difference (Dovidio, Major, & Crocker, 2000). In the context of medical illness, its stigma may have served an evolutionary purpose. Historically, separating and isolating sick individuals might have protected community members from contracting an illness (Kurzban and Leary, 2001). While this approach may appear reasonable in cases of communicable disease, its implicit manifestation within the context of behavioral disorders serves only to perpetuate disdain and mistreatment.

Stigmatizing beliefs are by no means limited to dementia and have long been recognized as an important factor in mental illness. Modified labeling theory (MLT), developed by Link, expanded on the idea that labeling an individual with a psychiatric diagnosis could negatively impact their health (Scheff, 1966). MLT built upon the work of Scheff, who noticed specific behavioral changes in individuals after receiving a psychiatric diagnosis (Scheff, 1966). Once labeled, responses from the surrounding community would reinforce an individual's behavior, eventually causing them to adopt the role of "mentally ill patient" (Scheff, 1966). Link expanded this theory, asserting that stigma develops both on an individual level, as an internal cognitive process, and through exposure to external behaviors in the environment (Link, Cullen, Struening, Shrout, & Dohrenwend, 1989). Through media and socialization with others, individuals form an idea of how mental illness is perceived in society. Once diagnosed, these same individuals begin to self-attribute these negative perceptions (Link et al., 1989). According to Link, both patients and society ultimately conclude that stigma devalues an individual's inherent worth and leads to inevitable discrimination (Link et al., 1989).

Beliefs regarding negative community attitudes are likely to lower a patient's self-esteem and negatively impact social interaction (Link et al., 1989). As a result, these individuals face a diminishing support network and decline in upward social mobility (Link et al., 1989). These findings are further supported by Scheff's "deference-emotion system," which argues that individuals derive their self-esteem based on the amount of respect they perceive from their contemporaries (Mackenzie, 2006). Similarly, perceived condemnation by community members contributes to an individual's sense of shame and negative self-worth (Mackenzie, 2006). The theories proposed by Scheff and Link serve as important paradigms. They exemplify how stigma forms both in the community and on a personal level, providing valuable insight into the consequences stigma can have on patient wellness.

The development of stigma

Several symptoms inherent in the presentation of dementia may contribute to the stigma toward individuals with the condition. This bias often develops even before dementia is

formally diagnosed. It has been well documented that several behavioral and psychological symptoms of dementia (BPSDs) present early in the disease process, often before classic neurocognitive signs (Smith-Gamble et al., 2002). These personality changes often manifest as early as 2 years prior to a dementia diagnosis (Smith-Gamble et al., 2002). Changes to personality often vary but may include an increase in apathy, neuroticism, and eccentricity as well as a decrease in extroversion and agreeableness (Smith-Gamble et al., 2002). These early changes in personality are closely related to premorbid personality structure (Welleford, Harkins, & Taylor, 1995). One study investigating the relationship between dementia and psychiatric comorbidity found that patients with higher premorbid agreeableness presented with increased hallucinations and aggressiveness, while those with increased neuroticism were more likely to present with delusional ideation (Low, Brodaty, & Draper, 2002). Additionally, patients that exhibited increased premorbid dependence showed increased social withdrawal during their disease course (Gould and Hyer, 2004). Thus the progression of dementia leads to an exaggeration of the personality traits present before disease onset (Welleford et al., 1995). These personality changes are less associated with age and more closely related to a decrease in functional activity level (Gao, Dolan, Hall, & Hendrie, 2000). Because neuropsychiatric symptoms of dementia often develop insidiously, they can have a large impact on negative external biases directed toward patients. This is exacerbated by the fact that individuals without formal experience with dementia are often unaware of the behavioral manifestations of the disease (Hooker et al., 2002, pp. P453–P460).

One of the primary groups affected by BPSDs are family caregivers. Studies have demonstrated a strong correlation between caregiver distress and the severity of neuropsychiatric symptoms (Storti, Quintino, Silva, Kusumota, & Marquesm, 2016). Interestingly, increases in caregiver stress directly parallel worsening neuropsychiatric symptoms, while decreases in independent activities of daily living showed no such correlation (Aneshensel, Pearlin, Mullan, Zarit, & Whitlatch, 1995). Often, the emphasis in dementia morbidity is on cognitive deterioration, with much less attention being paid to the behavioral aspects of the disease (Hooker et al., 2002, pp. P453–P460). In caregivers unaware of this presentation, these behaviors may go unrecognized as symptoms and lead to increased levels of stress (Hooker et al., 2002, pp. P453–P460). This inevitable increase in caregiver stress, and the inability to properly cope, may result in negative emotions by caregivers toward patients with dementia.

Perceived lack of reciprocity in patients with dementia is another major contributor to the development of stigma among health providers and the population at large. General reciprocity can be understood as recognizing a helpful or beneficial act and responding in a reciprocal manner (Adams and Sharp, 2013). The ability to reciprocate can increase the quality of care an individual receives, whereas a perceived lack of reciprocity may diminish an individual's social value (Kurzban and Leary, 2001). Patients with dementia are often perceived as lacking the ability to recognize, show interest in,

or even acknowledge others (Gove, Small, Downs, & Vernooij-Dassen, 2017; Woo and Mehta, 2017). This can often lead to caregivers feeling underappreciated. However, reciprocal behaviors demonstrated by patients with dementia often go unrecognized by caregivers (Vernooij-Dassen, Leatherman, & Rikkert, 2011). Subtle displays of reciprocity are often overshadowed by caregiver expectations of a patient's premorbid level of social functioning (Graham and Bassett, 2006). This perceived lack of reciprocity in patients with dementia also extends to primary care physicians. When asked about their personal experience with such patients, general practitioners often refer to a lack of "meaningful presence" and a decreased sense of sincere social contact (Gove et al., 2017).

Many also report a diminished return on social investment due to the perceived inability of patients to respond appropriately to family members (Gove et al., 2017; Woo, 2017a, 2017b). The notion that patients with dementia are not fully present contributes to a belief that any attempt to socially engage the patient is a wasted effort. This can lead to increased avoidance and social isolation by extended family and friends (Gove et al., 2017). It is especially detrimental in the case of close family members who are responsible for major decisions of care. Additionally, dementia patients may be perceived as a burden to society and a major financial investment (Gove et al., 2017). This is especially true in a society that values hard work and independence (Gove et al., 2017). While several of these negative perceptions regarding dementia are based on late-stage presentations of the disease, they are often generalized to all patients with dementia regardless of disease severity (Gove et al., 2017). Furthermore, while there is a general understanding that patients are not responsible for their disease, the perception that patients with dementia lack the ability to reciprocate on an emotional level encourages stigmatization nonetheless (Gove et al., 2017).

In the general population, pervasive misconceptions regarding normal aging and the clinical manifestations of dementia have further contributed to the stigma facing patients. Many unfamiliar with dementia believe that memory loss is a natural process of aging (Banerjee, 2010). Among those aware of the symptoms associated with dementia, there is a tendency to believe that very little can be done medically for those with such conditions (Banerjee, 2010). These misunderstandings surrounding dementia, a product of lacking public awareness about the condition, largely contribute to late presentations and missed diagnoses (Banerjee, 2010).

Stigma among the general population is further exacerbated by inaccurate depictions in popular film and media. Films may depict individuals with dementia in a sensationalized fashion, distorting popular perceptions. Researchers analyzing several popular films over the last two decades with themes centering on dementia found that the condition is often overly romanticized (Gerritsen, Kuin, & Nijboer, 2014). The challenges and burdens facing both patients and caregivers were vastly underrepresented (Gerritsen et al., 2014). As a consequence, viewers may not realize the degree of burden facing this population and might respond with less empathy and support (Gerritsen et al., 2014).

Films also depict moments of full lucidity in patients with dementia, misrepresenting disease progression and offering unrealistic expectations (Gerritsen et al., 2014). It remains to be seen whether more accurate depictions of dementia might evoke greater understanding and support for those with the condition (Gerritsen et al., 2014). As it is, false depictions may very well be contributing to the underlying stigma observed toward patients with dementia and their caregivers.

Repercussions of stigma

Misguided beliefs, reinforced by subtle behavioral symptoms and decreased public awareness, have resulted in a multitude of negative repercussions for individuals with dementia. One repercussion with a lasting impact on both patients and caregivers is the delay in timely diagnosis by primary care physicians. Recent estimates reveal that more than 60% of individuals with dementia remain undiagnosed (Lang et al., 2017). Misinformation regarding the "typical" presentation of dementia among general practitioners (GPs) is a contributing factor causing physicians to mistake early signs of dementia as part of the normal aging process (Lang et al., 2017). GPs are often under the impression that a lack of reciprocity is inherent to the presentation of dementia (Gove et al., 2017). In less experienced physicians, this stereotype, which is often only true in advanced dementia, is used to screen for the disease in the general patient population (Gove et al., 2017). Even when physicians are able to recognize early signs of the disease, physicians may be reluctant to officially diagnose a patient due to the inherent negative associations (Gove et al., 2016). While not all physicians subscribe to the negative perceptions that stigmatize dementia, most physicians are aware of these perceptions in the general population. This may dissuade them from wanting to "label" a patient before absolutely necessary (Gove et al., 2016). While the delay in diagnosis affects all patients with dementia, some individuals may be at a higher risk for remaining undiagnosed. Several factors have been implicated, with lower educational level and nonwhite ethnicity being the most significant contributors (Amjad et al., 2018).

Whatever the reason may be for delayed diagnosis, the consequences are salient. In older adults with probable dementia, the likelihood of engaging in unsafe activities is significantly higher for individuals without a formal diagnosis (Amjad, Roth, Samus, Yasar, & Wolff, 2016). Several instrumental activities of daily living may be compromised in early-stage dementia including cooking, driving, and managing finances (Arrighi, Gélinas, McLaughlin, Buchanan, & Gauthier, 2013). These activities must be properly addressed with patients, ideally around the time a diagnosis is made. In undiagnosed patients, safety is not appropriately assessed, placing these individuals at increased risk for injury and poor health outcomes (Amjad et al., 2016). Undiagnosed patients report a higher incidence of unattended doctor visits, self-management of finances, meal preparation, and driving (Amjad et al., 2016). Without proper awareness of their

deficits and the necessary resources to manage activities of daily living, patients are at a higher risk for exploitation of finances, increased annual falls, and medication-related hospital admissions (Amjad et al., 2016). Early detection and diagnosis of dementia plays an important role in future planning for both caregivers and patients, preventing future safety risks and additional health complications (Amjad et al., 2018).

However, late diagnoses alone are not the only consequence a negative perception of dementia can have on an individual. In fact, while a formal diagnosis may reduce certain risk factors for patients, it can have a negative impact on an individual's self-esteem and social support structure (Burgener et al., 2015). Patients with dementia report numerous negative interactions with friends and family as well as coworkers and healthcare providers (Alzheimer's Association, 2008). Many voice concern that they feel misunderstood and misrepresented (Alzheimer's Association, 2008). This may stem from a generalized fear of dementia in the general population that results in avoidance of these individuals and inevitably pushes them toward social isolation (Sutherland, 2010). Patients with dementia express a common trend whereby people begin to act differently toward them, at times even avoiding them (Sutherland, 2010). These feelings are especially notable in individuals with a higher level of cognitive functioning who develop early signs of dementia (Burgener et al., 2015). These individuals are often more socially engaged, and thus the higher social demands required often reveal early pathological deficits (Burgener et al., 2015). Faced with expectations necessitating a high level of cognitive function, these patients are met with negative perceptions and social isolation earlier in their disease progression (Burgener et al., 2015). This only emphasizes the fact that many individuals with dementia continue to display keen insight and social awareness throughout the early stages of the disease, thus exacerbating the emotional repercussions associated with stigma (Clare et al., 2012).

Future developments

Despite the prevalence of stigma surrounding dementia as well as obstacles to proper care and support for patients and caregivers, the future remains bright. Work is being done to change negative attitudes within communities at home and abroad through education, scientific research, and technological innovation.

Groups are working tirelessly toward increasing the level of dementia education within their respective communities. This is especially significant in communities with underlying cultural biases and a lack of educational resources. In the Chinese American population, community members are raising awareness through health fairs, seminars, and culturally relevant educational materials (Woo, 2017a, 2017b). Media-based education distributed through radio, television, and web-based platforms has shown great success (Woo, 2017a, 2017b). One example is "Radio Beneath the Sky," a Cantonese

radio broadcast in Los Angeles that created a dementia awareness program designed to specifically engage Chinese American community members (Woo, 2017a, 2017b).

Similarly, the Chinese Phoenix Television Station created a series on AD in Cantonese, which was subsequently uploaded to YouTube (Woo, 2017a, 2017b). Both programs yielded fairly successful results and provided invaluable feedback on how to improve media-based awareness campaigns in the future (Woo, 2017a, 2017b; Zheng and Woo, 2017). Emphasis was placed on the importance of utilizing stories about real-life individuals when providing education about dementia (Woo, 2017a, 2017b). An appeal to pathos was found to be highly effective in educating community members as compared with facts and figures alone. Another important finding revealed by these awareness campaigns is the importance of culturally relevant educational materials (Woo, 2017a, 2017b). Oftentimes the lack of awareness about dementia in immigrant communities is less a result of general disinterest and more a product of inaccessibility to coherent educational materials (Zheng et al., 2016).

Stanford University's Alzheimer's Disease Research Center (ADRC) chose to approach the issue of dementia awareness in the Latino community by creating and implementing a dementia awareness campaign (DAC). Collaborating with a local community health advocacy organization, the group aimed to educate community member intermediaries known as promotoras (Askari, Bilbrey, Garcia Ruiz, Humber, & Gallagher-Thompson, 2017). Promotoras received education on the topic of dementia from the ADRC and then disseminated that information within their respective neighborhoods (Askari et al., 2017). This allowed community members with very little knowledge about the condition to access culturally relevant and coherent information (Askari et al., 2017). This approach to raising awareness aims to tackle the recurring theme of cultural and language barriers that too often impede accurate transfer of information in minority communities. Although performed on a small scale, the results of this study were hugely successful in both training promotoras and increasing awareness in the community (Askari et al., 2017). The DAC has continued to run successfully in East Palo Alto, and plans have been made to expand the program to other nearby communities in the Bay Area (Askari et al., 2017).

The theoretical foundation utilized by the Stanford ADRC is based on contemporary research in the field of outreach and engagement in minority populations. The group made sure to hire "culturally competent" staff members, healthcare professionals with a similar demographic to the target community (Askari et al., 2017). This is important, as it cultivates a sense of trust among community members, increasing their willingness to listen and comply with health recommendations (Askari et al., 2017). Another important implementation was of staff with specialized knowledge in the field of dementia who could provide the most up-to-date, patient-specific knowledge (Askari et al., 2017).

Community-based awareness programs have also achieved success on a much larger scale. In Japan, a 10-year government-funded public awareness project successfully

altered the Japanese word for dementia in order to remove its derogatory quality (World Health Organization, 2012). The change was implemented into administrative, academic, and media channels, demonstrating patient solidarity while equally acting as a nationwide awareness program (World Health Organization, 2012). The Japanese government simultaneously created a network of seminars across the country to educate citizens about dementia, which 2.4 million citizens had already attended by 2011 (World Health Organization, 2012).

National campaigns utilizing media outlets have also been successful in the UK and Brazil. The UK designed a campaign featuring everyday citizens with dementia in an effort to normalize the condition (World Health Organization, 2012). As a response to qualitative research revealing growing fears of the condition and avoidance of affected individuals, the campaign depicted citizens with the condition under the headline "I have dementia, I also have a life" (World Health Organization, 2012). The Brazilian Alzheimer's Association also successfully utilized media outlets, creating a television campaign to raise awareness and funds with the help of a well-known Brazilian actress (World Health Organization, 2012). Calls to the association helpline increased from 1000 to 2500 per month, an increase directly attributable to the television campaign (World Health Organization, 2012).

In Australia, a government-funded program created a campaign aimed at educating citizens about lifestyle modifications to reduce dementia risk factors (World Health Organization, 2012). "Mind your Mind," which was initiated in 2005, promoted seven factors found to reduce future risk of dementia: diet, tobacco and alcohol use, exercise, and several others (World Health Organization, 2012). Educational sessions were created for the general public and coupled with a modern approach utilizing web-based mobile applications to connect directly with users (World Health Organization, 2012). More recently, an awareness project in the city of Kiama created educational seminars with the added dimension of featuring actual patients with dementia as seminar educators (Phillipson et al., 2019). The idea was based on earlier research that emphasized that increasing the amount of direct communication with dementia patients could lead to decreased avoidance of the condition among community members (Phillipson et al., 2019).

Promotion of dementia awareness has also been achieved in the political sphere. The US Alzheimer's Association led a grassroots effort to mobilize constituents to pressure their respective congressperson into supporting dementia-friendly legislation (World Health Organization, 2012). This eventually led to the Alzheimer's Project Act, signed by President Obama in 2011, that aimed to address the dementia epidemic on a national scale (World Health Organization, 2012).

Another promising development is the steady growth of "dementia friendly communities," which work to facilitate a supportive environment in which individuals with dementia can live and maintain their independence (Wiersma & Denton, 2016).

These communities, many of which have appeared across the UK as well as the rest of Europe, are based in a more rural setting and offer social, emotional, and instrumental support (Wiersma & Denton, 2016). They also offer local facilities for comprehensive dementia care (Wiersma & Denton, 2016). These communities allow for full integration of individuals with dementia into a nurturing community and are a tangible example of the continued campaign to end the associated stigma.

Much work must still be done in order to rid dementia of its negative associations and offer patients the proper support and respect they deserve. However, there is ample evidence to suggest that positive change is on the horizon. Physicians, caregivers, and community members must continue working together to educate and advocate on behalf of patients with dementia. Only then will patients receive the proper attention, care, and support required for a rewarding and satisfying future.

Key facts of the national Alzheimer's Project Act

- National legislation signed by president Barack Obama in 2011
- A national plan to increase funding for dementia research while expanding services and care for patients and their caregivers
- Created an advisory council with leading experts in the field working together to formulate a national action plan
- Set a national goal to effectively prevent and treat dementia by 2025 by accelerating treatment development
- International coalition formed among governments to address dementia globally

Summary points

- As dementia rates rise, finding sustainable ways to effectively manage the condition is of great importance.
- Negative perceptions of dementia, often based on misinformation, are major detriments to proper care.
- Stigmatizing beliefs surrounding dementia differ across cultures but often result in similar consequences for patients and caregivers.
- Behavioral and psychological symptoms of dementia are often misunderstood by the general population and are a major contributor to stigma.
- Stigmatizing beliefs often lead to delayed diagnosis by physicians, leading to patient participation in dangerous activities and thus increased injury burden.
- Encouraging progress is being made toward raising awareness and educating the general population about dementia.

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CHAPTER 41

Delusions in dementias

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List of abbreviations

AD Alzheimer disease
bvFTD frontotemporal dementia-behavioral variant
CAA cerebral amyloid angiopathy
DLB dementia with Lewy bodies
DLPFC dorsolateral prefrontal cortex
FTLD frontotemporal lobar degeneration
LBD Lewy body disease
NPS neuropsychiatric symptoms
PDD Parkinson's disease dementia
PJS Peutz-Jeghers syndrome
SVD subcortical arteriosclerotic leukoencephalopathy
WMH white matter hyperintensities

Mini-dictionary of terms

Braak stages: A neuropathological diagnosis of AD based upon assessment of two 100 mm sections (at hippocampal formation and occipital neocortex) processed according to the silver-iodate technique. Distinctive differences in the topographical distribution pattern of the neurofibrillary lesions enable the observer to assign a given autopsy case to one of six stages.

Capgras delusion: Patients believe that a familiar person is someone else, has been reduplicated, or is an imposter.

Cerebral amyloid angiopathy: One form of vascular pathology, defined as deposits of amyloid in the vessel walls that increase risk of hemorrhage and ischemia.

De Clerambault syndrome or erotomania: The delusional belief that one is secretly loved by another.

Delusion: Is a belief that is clearly false and that indicates an abnormality in the affected person's content of thought characterized by certainty (held with absolute conviction), incorrigibility (not changeable by compelling counterargument or proof to the contrary), and impossibility or falsity of content.

Othello syndrome or delusional jealousy: Is a set of irrational thoughts and emotions, with extreme or unacceptable behavior, in which the dominant theme is the concern with the sexual partner's infidelity not based on concrete evidence.

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Schneiderian first-rank symptoms: Are symptoms that people with psychosis may experience, for example, hallucinations, hearing voices, and thinking that other people can hear their thoughts; these symptoms correctly identify people with schizophrenia 75%–95% of the time.

The delusion of theft: Is most closely linked to the erroneous conviction that an intruder periodically enters the home, which, in such cases, absolves caregivers from accusations of being thieves.

The phantom boarder syndrome: Patients believe their house is inhabited by unwelcome guests.

Introduction

Many patients with dementia of various etiologies experienced delusional symptoms during the course of illness (Bassiony & Lyketsos, 2003) (Fig. 41.1). Delusions in dementia are associated with adverse outcomes such as aggression, caregiver stress, and earlier institutionalization (Fischer, Bozanovic-Sosic, & Norris, 2004). Alzheimer disease (AD) accounts for 60%–70% of cases of dementia. Current estimates indicate 17% of people aged 75–84 years in the United States have AD, and the disease costs the country $277 billion per year (Alzheimer's Association, 2018). AD is often complicated by neuropsychiatric symptoms (NPS), which occur in one-third of patients at an early stage of the disease (Burns, Jacoby, & Levy, 1990; Paulsen et al., 2000). Delusions appearing with their disabling and persistent features is among the most common NPS characterizing AD course (Fischer et al., 2004) (Table 41.1). Regardless, with current estimates of over 5 million Americans affected by AD and estimates of greater than 13 million affected by 2050 (Alzheimer's Association, 2018), AD with delusions would currently be the

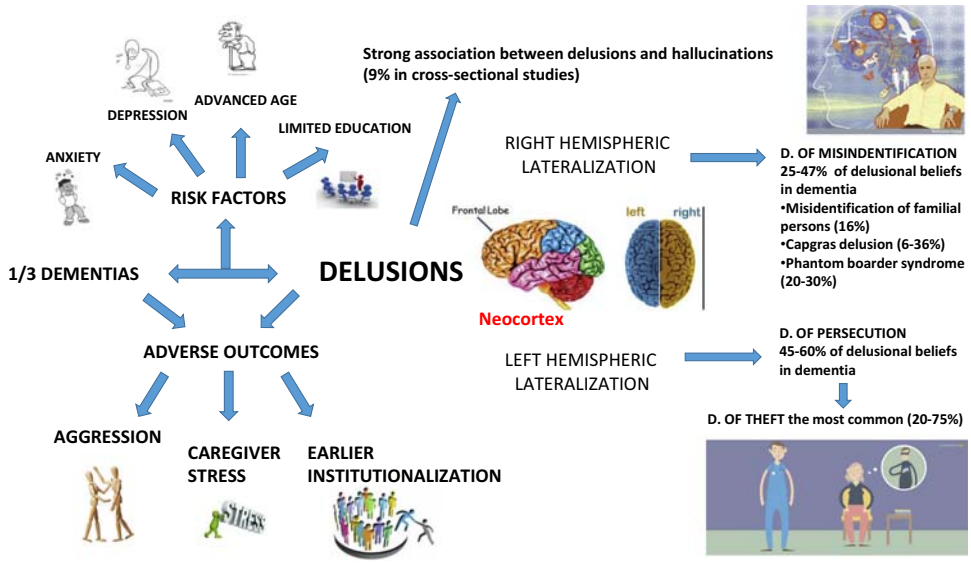

Figure 41.1 Neuroanatomy, categories, risk factors, and adverse outcomes of delusions in dementias. Frequency rates of delusions in dementias with various etiologies. *AD*, Alzheimer disease; *bvFTD*, frontotemporal dementia-behavioral variant; *DLBs*, dementia with Lewy bodies; *FTLD*, frontotemporal lobar degeneration; *VaD*, vascular dementia.

Table 41.1 Risk factors of delusions in dementia.

References	Risk factors	Principal results
Bassiony et al. (2002)	Depression	Delusions, but not hallucinations, were closely associated with depression in dementia.
Kotrla et al. (1995), Nambudiri, Teusink, Fensterheim and Young, (1997), Sala, Francescani, Muggia and Spinnler (1998)	Age	In some (but not all) studies of dementia, psychosis was associated with older age.
Flynn, Cummings and Gornbein (1991),Kotrla et al. (1995)	Educational level	Studies of AD have reported that delusions are associated with less education.
Gormley and Rizwan (1998), Kotrla et al. (1995), Sala et al. (1998)	Gender	The studies of association with gender are ambiguous, but the majority are inclined toward an association with the female gender.
Deutsch, Bylsma, Rovner, Steele, & Folstein (1991)	Ethnicity	African Americans with AD were more likely to have delusions, compared to Caucasians.
Vik-Mo et al. (2018)	Advanced neuropathology	Psychosis has been found to be associated with more severe AD and Lewy body pathology in patients with AD and cerebrovascular disease-related vasculopathy.
Paulsen et al. (2000)	Frontal lobe dysfunction	Studies have shown impaired frontal lobe metabolism, higher density of senile plaques in the frontal cortex, and deficits in frontosubcortical circuits.
Flynn et al. (1991), Jeste et al. (1992), Paulsen et al. (2000)	Cognitive decline	Some studies showed a relationship with more rapid and severe cognitive decline, while others showed only mild differences.

Continued

Table 41.1 Risk factors of delusions in dementia.—cont'd

References	Risk factors	Principal results
Leroi et al. (2003), Ostling and Skoog (2002)	Hearing and vision impairments	Significant risk factors for delusions in dementia include impaired hearing but not impaired vision, for whom results are contrasting.
Idiaquez, Sandoval and Seguel (2002), Nilsson (2004)	Antihypertensive medication; antiparkinsonian drug	Use of antihypertensive medication is a risk factor for delusions; among the risk factors for developing delusion in dementia in LBD (DLB and PDD), the psychotic phenomenon triggered by changes in antiparkinsonian drug therapy should be taken into account.
Ostling and Skoog (2002)	Myocardial infarction and congestive heart failure	Delusions in dementia are associated with myocardial infarction and with acute phase of coronary disease.
Holmes et al. (1998)	Selective neuronal populations; specific neurotransmitter systems	Selective loss of different neuronal populations (i.e., locus coeruleus), alterations of specific neurotransmitter systems (serotonin, noradrenalin).
Hollingworth et al. (2012)	Genetic risk	Delusions show heritability up to 61% and have been proposed as a marker for a disease subtype suitable for gene mapping efforts.
Kotrla et al. (1995)	Social behavior	Asociality was the only negative symptom associated with delusion in dementia.

second most prevalent psychotic disorder (after schizophrenia) in the United States, and may soon be the most prevalent (Murray et al., 2012).

Visual hallucinations in AD are more common than auditory hallucinations and, if accompanied by a fluctuating course or extrapyramidal signs, may suggest dementia with Lewy bodies (McKeith et al., 2017). Lewy body disease (LBD) is an umbrella term covering Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB), two neurodegenerative dementias together affecting as much as 6% of all individuals older than 65 (Rongve, 2013), with a high degree of clinical and pathological overlap

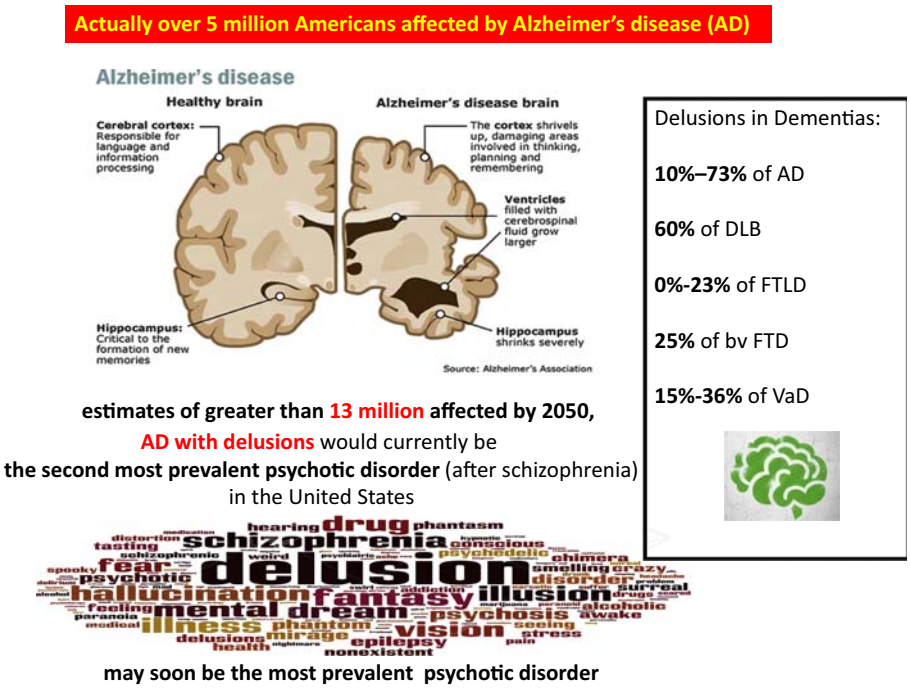

Figure 41.2 Frequency rates of delusions in dementias with various etiologies. *AD*, Alzheimer disease; *bvFTD*, frontotemporal dementia-behavioral variant; *DLBs*, dementia with Lewy bodies; *FTLD*, frontotemporal lobar degeneration; *VaD*, vascular dementia.

with AD and no disease-modifying treatment (Mueller et al., 2017). Phenomenologically, psychotic symptoms are indistinguishable between PDD and DLB, but the frequency of occurrence is greatest in the latter (Marsh, 2004). Visual hallucinations are the most frequent symptoms in DLB, and they have been identified as one of the core features in the clinical diagnostic criteria; systematized delusions also occur, but are less frequent and are regarded as supportive features (Nagahama et al., 2007). The aim of the present chapter was to shed light on the relationship among delusions and the most frequent forms of dementia of various etiologies in terms of epidemiology, risk factors, neuroanatomy, neurochemistry, neurobiology, and relationship to cognition.

Epidemiology

Some recent studies show that approximately one-third of dementia patients suffer from delusions (Bassiony & Lyketsos, 2003) (Fig. 41.2). Prevalence rates are comparable among dementia types, except for frontotemporal dementia (FTD), a clinical entity encompassing a spectrum of neurodegenerative diseases with heterogeneous clinical presentations determined by an underlying complex neuropathological process named frontotemporal lobar degeneration (FTLD). In fact, in FTD, delusional symptoms are generally regarded as an uncommon feature (Hodges et al., 2004). In early observational studies, delusions were reported in 10%–73% of patients with AD (Rao & Lyketsos 1998). It has been

established that delusions occur more commonly in up to 60% of patients with DLB and less commonly in FTD cohorts, where it ranges from 0% to 23% (Hodges et al., 2004; Omar et al., 2009). However, recent studies showed that delusions, hallucinatory behavior, and suspiciousness were present in one-fifth of behavioral FTD (bvFTD) patients, whereas negative psychotic symptoms such as social and emotional withdrawal, blunted affect, and formal thought disorders were more frequently present (Gossink et al., 2017). Delusions have received even less attention in vascular dementia (VaD), and although the studies have limited sample size, the prevalence rates of delusion in VaD range from 15% to 36%, with persecutory delusion being the most common (25%) (Tsai, Hwang, Yang, & Liu, 1997).

Delusions of persecution make up approximately 45%–60% of delusional beliefs in dementia. The most common is the delusion of theft (20%–75% of persecutory delusions) (Holt & Albert, 2006) wherein the patient believes that others are stealing his own property; instead, the delusion of "One's house is not one's own" is less common (7%–17%) (Holt & Albert, 2006). Although AD patients have been reported to experience "systemized" delusions such as suspicions of theft, infidelity, and persecution (Gauthier et al., 2010), they are also a commonly reported symptom "supportive" of a diagnosis of DLB and PDD (McKeith et al., 2017). The strong association between delusions and hallucinations emerged in a cross-sectional case-control study where 9% of patients (30 out of 342 patients) experienced both delusions and hallucinations (Bassiony & Lyketsos, 2003).

Several types of delusions for several types of dementia

Delusions can be categorized within two subgroups: delusions of misidentification, associated with auditory and visual hallucinations (Cook et al., 2003), and delusions of persecution. Delusions of persecution make up about 45%–60% of delusions in dementia (Webster & Grossberg, 1998). Rates are comparable by dementia type (Bianchetti et al., 1992; Webster & Grossberg, 1998). Delusions of misidentification make up 25%–47% of delusions in AD (Binetti et al., 1995; Burns et al., 1990). Unlike schizophrenia, delusions in AD are typically not bizarre or complex, and Schneiderian first-rank symptoms are rare (Jeste & Finkel, 2000). Delusions of misidentification and hallucinations appear earlier and with greater frequency in DLB than in other forms of dementia (Ballard et al., 2004). Nagahama and colleagues used factor analysis to classify psychotic symptoms in DLB; they found that hallucinations, misidentification experiences, and delusions were independent symptom domains (2007).

Misidentification of familiar persons (in which patients insist that familiar persons are not who they really are), the Capgras delusion, and the phantom boarder syndrome make up the majority of delusional misidentifications at 16% (Harwood et al., 1999), 6%–36% (Cohen-Mansfield, Taylor, & Werner, 1998), and 20%–30% (Harwood et al., 1999) of

delusions of misidentification, respectively. The rates of delusional jealousy or Othello syndrome in dementia found in the study of Tsai et al. (1997) amounted to 15.8%. Secondary erotomania or De Clerambault syndrome can occur in the context of organic disorders such as dementia (Cipriani, Logi, & Di Fiorino, 2012). To the best of our knowledge, only a few cases have been reported in AD (Brüne & Schröder, 2003) and VaD (Heinik, Aharon-Peretz, & Hes, 1991), and one in FTD and motor neuron disease (Olojugba, de Silva, Kartsounis, Royan, & Carter, 2007). Brüne and Schröder (2003) reported a case of VaD in which erotomania emerged in the early stage of the underlying disorder.

Risk factors

Known risk factors for delusions include depression and anxiety, advanced age, and limited education (Bassiony & Lyketsos, 2003), while the role of gender and ethnicity is less clear (Kotrla et al., 1995) (Table 41.2). Predominant symptom onset is usually in the seventies. It appears to be an association of delusions with advanced neuropathology, selective frontal lobe dysfunction, preserved intellect, and rapid cognitive decline (Fischer et al., 2004). Significant risk factors include also impaired hearing but not impaired vision (Bassiony & Lyketsos, 2003), use of antihypertensive medication, myocardial infarction, and congestive heart failure (Ostling & Skoog, 2002).

Among the risk factors for developing delusion in dementia in LBD (DLB and PDD), the psychotic phenomenon triggered by changes in antiparkinsonian drug therapy should be taken into account (Nilsson, 2004), although the exact relationship with

Table 41.2 Clinical features of dementias.

Alzheimer's disease	Dementia with lewy bodies/Parkinson's disease dementia	Frontotemporal dementia	Vascular dementia
Memory loss	Hallucinations	Personality change	Vascular risk factors
Aphasia	Parkinsonism	Executive dysfunction	Frontal deficits
Apraxia	Fluctuations	Aphasia (fluent/nonfluent)	Neurological signs
Agnosia	Attention/executive dysfunction	Disinhibition	Neuroimaging findings
Executive dysfunction	Visuospatial impairments	Early onset	Acute onset, stepwise decline
Activities of daily living impairments		Family history	
Gradual onset, progressive decline			

Behavioral features: aggression, agitation, psychosis (hallucinations, delusions, misidentification), depression, apathy.

antiparkinsonian medication is unclear. Some findings suggest that disease-related factors, in interaction with medications, account for psychotic features, rather than medication alone (Fenelon, Mahieux, Huon, & Ziegler, 2000). Another hypothesis is that denervation hypersensitivity of mesolimbic and mesocortical dopaminergic receptors predisposes patients to a hypersensitivity response that manifests as psychosis (Ravina et al., 2007).

Previous studies have shown that NPS in AD were highly heritable (Bacanu et al., 2005), increased AD familial risk, and showed significant genome-wide linkage (Hollingworth et al., 2012), providing evidence that genetic variation does contribute to delusion risk. The possible hypotheses for psychopathology in AD involve selective loss of different neuronal populations (i.e., locus coeruleus), alterations of specific neurotransmitter systems (serotonin, noradrenalin), and genetic risk factors (Holmes, Arranz, Powell, Collier, & Lovestone, 1998).

Hallucinations also were associated with increased dementia severity, NPS, and a lifetime history of hallucination-evoking disease (such as depression and sensory impairment), but not with age or gender (Linszen et al., 2018). Older patients suffering from hallucinations often live alone, are unmarried or without children, tend to be African American, and have a lower level of education (Cook et al., 2003; Ostling & Skoog 2002). Cohen et al. (1993) suggested that women may suffer more frequent multiple NPS, for example, delusions and hallucinations or delusions and aggression. Finally, Friston (2010) proposed that beliefs (both normal and abnormal) arise through a combination of innate or endowed processes, learning, experience, and interaction with the world.

Neuroanatomy and neuroimaging

Neuroimaging and behavioral studies suggest a frontotemporal localization of delusions in the elderly, with right hemispheric lateralization in delusional misidentification and left lateralization in delusions of persecution (Holt & Albert, 2006). Comparisons of SPECT imaging in dementia patients with and without delusions of theft and persecutions have highlighted that frontal cortex is principally involved in these symptoms (Nagahama, Okina, Suzuki, & Matsuda, 2010). Binetti et al. (1995) compared a group of 24 AD and multiinfarct dementia patients' delusions with nondelusional controls. Delusion content in the group was split in simple persecutory beliefs/delusions of misidentification. The same authors found the presence of isolated frontal white matter hyperintensities (WMH) to be independently associated with active delusions. More recent studies showed that AD patients with delusions had significantly greater right frontal WMH volumes than those without (Anor et al., 2017). Other studies found contrasting results. Mega et al. (2000) found significant hypoperfusion of left and right dorsolateral prefrontal cortex (DLPFC) and left paralimbic regions in 10 delusional AD patients compared with 10 AD patients without delusion and hallucinations matched for AD severity and severity of other NPS.

Despite the preferential involvement of the frontal lobe in delusions, several studies focused on temporal lobe atrophy characteristic of the early AD clinical phase. It was not clear how to separate the effect of temporal lobe abnormalities from that of cortical atrophy in dementia associated with delusions (Holt & Albert, 2006). An early SPECT study of 16 AD patients with mixed persecutory and misidentification delusions and 29 matched AD patients without delusions found bilateral hypoperfusion of superior and inferior temporal lobes in subjects with delusions (Starkenstein et al., 1994). This area of hypoperfusion corresponds with location of the fusiform face area and parahippocampal place area, which show increased activation after viewing faces and physical locations, respectively. Frith and Frith (2001), for instance, proposed that prefrontal and parietotemporal parts of the neocortex are involved in mental state attribution and emotion recognition, both aspects of social cognition being critically involved in the formation of delusional beliefs.

Starkenstein et al. (1994) suggested potential disruption of these regions as possible determinants of house misidentification, reduplicative paramnesia for houses, and Capgras delusion. In this regard, case studies suggested that right frontoparietal infarcts may determine the onset of Capgras delusion in dementia (Forstl et al., 1994; Staff et al., 2000). Right frontal hypoperfusion is also found in nurturing syndrome (Venneri et al., 2000), phantom boarder syndrome (Jenkins et al., 1997), and mirror sign (Breen, Caine, & Coltheart, 2001). Staff et al. (2000) found this hypoperfusion to include Brodmann's areas 9 and 10, which they hypothesize may suggest a failure of episodic memory retrieval. Delusions of misidentification, more common by far in AD than in other delusional syndromes, alone show a loss of cell count in CA1 regions of hippocampus (Jenkins et al., 1997). In another study, changes in WMH, primarily in the frontal and parieto-occipital regions, were suggested to contribute to the development of delusional misidentification in patients with AD (Lee et al., 2006).

At least 30% of Othello syndrome cases in the literature showed a neurological basis for delusion of infidelity, although its biological basis is not fully understood (Cipriani, Vedovello, Nuti, & di Fiorino, 2012). Several case reports have suggested that the right frontal lobe is the neuroanatomical correlate for delusional jealousy (Luaute, Saladini, & Luaute, 2008). It is hypothesized that focal damage to the right hemisphere and frontal lobes may play an important part in the genesis of "content-specific delusions" due to the role of the right hemisphere in producing the experience of familiarity and the role of the frontal lobes in correcting misperceptions on the basis of new information. This model highlights the dual effects of loss of function due to damage of the right hemisphere and release of inhibition due to hyperactivity of the intact left hemisphere (Devinsky, 2009). This pathogenetic model is valid both for LBD and AD. A postmortem study using radioligand-binding assays to quantify muscarinic receptors in AD showed that muscarinic M2 receptor density was higher in Brodmann's area 11 (orbitofrontal cortex) in those patients who experienced delusion compared to those without delusion (Lai et al., 2001).

Neurobiology

Perhaps the most compelling evidence that AD with delusions has a specific biology from AD without psychosis is the finding that the risk for psychosis in AD is transmitted in families. To demonstrate it, an odds ratio for psychosis of 3.2 was found in siblings of AD with delusions subjects who were both affected with AD (Sweet, Nimgaonkar, Devlin, Lopez, & DeKosky, 2002). The estimated heritability of psychosis in AD amounted to 61% (Bacanu et al., 2005). Although more than 20 studies have evaluated whether carrying one or more ε4 alleles of the apolipoprotein E (APOE) gene may increase risk for delusions in AD, a recent report analyzing a large cohort with uniform and standardized criteria for diagnosing both AD and psychosis, available through the National Alzheimer's Disease Coordinating Center uniform data set, found no association of APOE ε4 alleles with delusion AD (DeMichele-Sweet, Lopez, & Sweet, 2011) (Fig. 41.3). More recently, it has been suggested that a poly-T repeat sequence polymorphism in translocase of outer mitochondrial membrane 40 homolog, TOMM40, which is in linkage disequilibrium with APOE, may explain some of the association of APOE with AD risk (Chu et al., 2011).

The first genome-wide association study of AD with psychotic symptoms was recently reported (Hollingworth et al., 2012). Among the most significant Single Nuclear Polymorphisms (SNPs) in the AD with delusions versus AD without psychosis analysis was

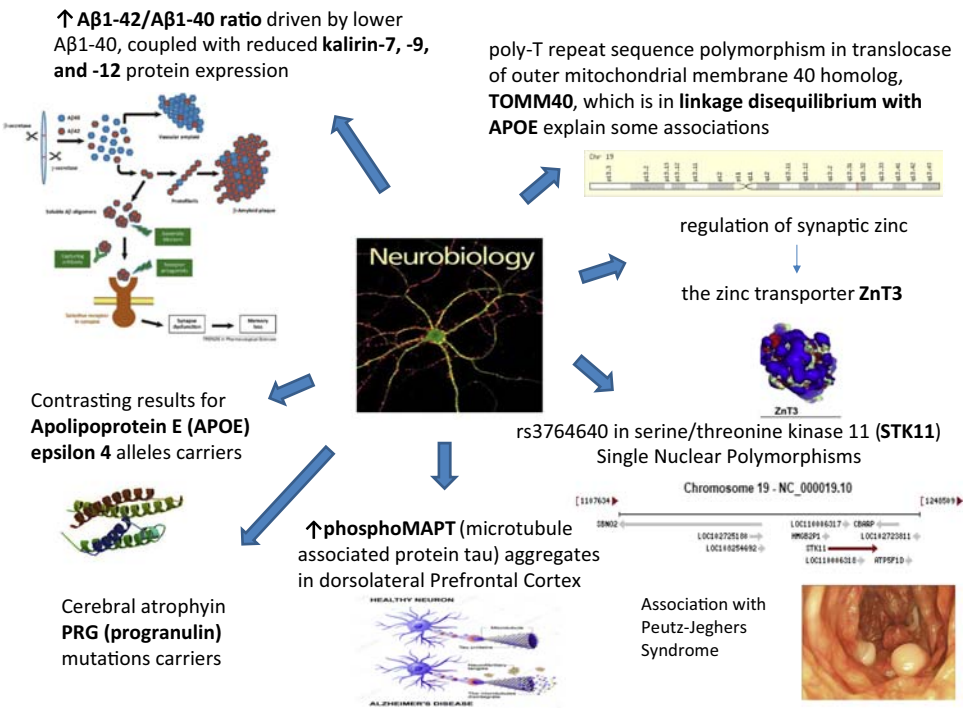

Figure 41.3 Neurobiology and genetics of delusions in dementia. *Aβ*, amyloid-β; *APOE*, apolipoprotein E.

rs3764640 in serine/threonine kinase 11 (STK11). Although STK11 deletions are present in Peutz-Jeghers syndrome (PJS), one case with an unusually large STK11 deletion has been described in which PJS, mental retardation, and schizophrenia co-occurred (Kam et al., 2006). Moreover, polymorphisms in gene coding for dopamine receptors has been associated with delusions, specifically, homozygous carriers of the DRD3 1 allele, are more likely to experience delusions (Holmes et al., 2001).

Several studies have investigated whether delusions in AD associate with more severe fibrillar amyloid-β (Aβ) pathology, in the form of neuritic plaques, but results are contrasting. Some neuropathological studies emphasize the important roles of plaques and tangle density in AD plus delusion, suggesting that frontal plaques and tangles are associated with delusions (Farber et al., 2000). Soluble Aβ induces loss of dendritic spine synapses through impairment of long-term potentiation. But Murray et al. (2012) provide a foundation for identifying a novel pathway in the pathogenesis of delusions in AD: an increased $A\beta_{1-42}/A\beta_{1-40}$ ratio driven by lower $A\beta_{1-40}$, coupled with reduced kalirin-7, -9, and -12 protein expression, isolates' additive and potentially related processes that may underlie the enhanced synaptic disruption in delusions in AD.

In contrast to studies of fibrillar Aβ, one of the studies to evaluate microtubule associated protein tau (MAPT) pathology found some evidence of increased indices of pathologic MAPT aggregation in psychosis in AD (Farber et al., 2000). The contribution of MAPT to AD with delusions was further highlighted in a recent study that found no increased spread of phosphorylated microtubule-associated protein tau but increased concentrations of phospho-MAPT aggregates in DLPFC in these subjects (Murray et al., 2013). Kawakami et al. (2014) suggested that there may be a connection between increased levels of tau in the nucleus accumbens and delusions in a cohort of tangle-predominant dementia cases; however, they did not pursue this observation in depth.

Current evidence indicates that the presence of comorbid Lewy body pathology in AD may contribute to psychosis, although by no means can the occurrence of psychosis in AD be attributed principally to Lewy body pathology. Nevertheless, Lewy body pathology may contribute in some cases, especially in individuals with neocortical stage Lewy body pathology (Ballard et al., 2004).

Interestingly, Zn^{2+} has been shown to promote phosphorylation and aggregation of tau. Recent studies showed an association between the regulation of synaptic zinc by the zinc transporter ZnT3 and delusions (Whitfield, Francis, Ballard, & Williams, 2018). The overlap between FTD and primary psychiatric disorders has been brought to light by reports of prominent NPS in FTD-related genetic mutations, particularly among progranulin (GRN) carriers (Le Ber et al., 2008). Psychotic symptoms correlated mainly with gray matter (GM) atrophy in the anterior insula, left thalamus, cerebellum, and cortical regions including frontal, parietal, and occipital lobes in GRN mutations carriers (Sellami et al., 2018). Histopathologically, in FTD delusions have been most frequently associated with ubiquitin-positive, tau-negative neuronal inclusions (with or without evidence of motor neuron disease) (Hodges et al., 2004). From recent postmortem studies, subcortical arteriosclerotic leukoencephalopathy (small vessel disease [SVD]-related pathology) and

cerebral amyloid angiopathy (CAA), but not specific vascular pathologies, were found associated with psychosis in moderate dementia (Vik-Mo, Bencze, Ballard, Hortobágyi, & Aarsland, 2018).

Delusions and cognition

In a review by Ropacki and Jeste (2005), the severity of cognitive impairment showed a significantly positive association with the presence of NPS of dementia in patients with AD in 20 studies, and no association in 10 studies. Delusions in dementia typically begin early in the course of illness, and dissipate as the disease reaches moderate to severe severity (Bassiony & Lyketsos, 2003). This pattern also holds for VaD (Binetti et al., 1995). Delusions of misidentification appear at a somewhat later age than persecutory delusions and reflect greater cognitive decline. Patients suffering from delusions of misidentification showed lower Mini-Mental State Examination (MMSE) scores at onset and higher Blessed Dementia Scale ratings than matched nondelusional demented patient controls (Forstl et al., 1994).

The majority of studies found comparable (Tsai et al., 1997) or higher (Binetti et al., 1995) MMSE scores in AD patients with persecutory delusions when compared with matched AD patients without delusions. Persecutory symptoms showed to require a threshold level of preserved cognitive function to sustain. In a recent study, as compared with AD patients without delusions, it was revealed that AD patients with delusions showed higher dementia severity, and higher impairment in cognitive and depressive symptoms, and in several neuropsychiatric domains, and this appeared to be associated with higher multidimensional impairment, including deficits in executive functioning, and increased risk of mortality (D'Onofrio et al., 2016).

Although most studies utilized measures of global cognition, several reports that have evaluated cognitive domains suggested a frontal localization of the greater cognitive deficits in AD with delusion, with working memory particularly affected (Paulsen et al., 2000). A number of studies now indicate that more rapid cognitive deterioration begins before onset of psychosis, during prodromal and early stages of AD, subsequently manifesting as frank psychotic symptoms. For example, greater cognitive dysfunction was already present in the earliest stages of AD, preceding the onset of psychosis by at least 1–2 years (Weamer et al., 2009). However, clinical studies clearly indicated that the most rapid increase in rates of psychosis occurs during the transition from mild cognitive symptoms to early and middle stages of cognitive impairment (Ropacki & Jeste, 2005), corresponding roughly to Braak stages III to V (Mukaetova-Ladinska et al., 2000).

Cognitive impairment has been considered a necessary component in the development of delusions in PDD (Ffytche et al., 2017). The rate of impairment was higher in cases with combined hallucinations and delusions than in cases with isolated delusions. We speculate that cognitive impairment may be less important for delusion formation than for hallucinations in PDD psychosis.

There is a suggestion that those with PDD, especially with impulse control disorders, may have an increased rate of "jumping to conclusions," a cognitive characteristic that may lead to early acceptance of delusional explanations (Djamshidian et al., 2012).

Conclusions

Further studies are needed to focus on clarifying clinical-pathological and neuropsychological correlations to guide more rational psychopharmacological and psychoeducational interventions to alleviate the distressing delusional ideation in different forms of dementia (Fig. 41.4). Interestingly, the neuropsychological profile of both CAA and SVD is characterized by decreased processing speed, which is also found in psychotic diseases, and previous episode of psychosis is by far the strongest risk factor for development of psychosis in AD (Fischer et al., 2016). Thus, vascular pathology and reduced information processing speed may be risk factors of psychosis in dementia that are modifiable by cardiovascular disease prevention (Vik-Mo et al., 2018). Epidemiological and clinical studies showed evidence for increased peripheral inflammatory markers in psychosis spectrum disorders (Radhakrishnan, Kaser, & Guloksuz, 2017), and systemic inflammation is known to contribute to cerebrovascular pathologies and cognitive impairment.

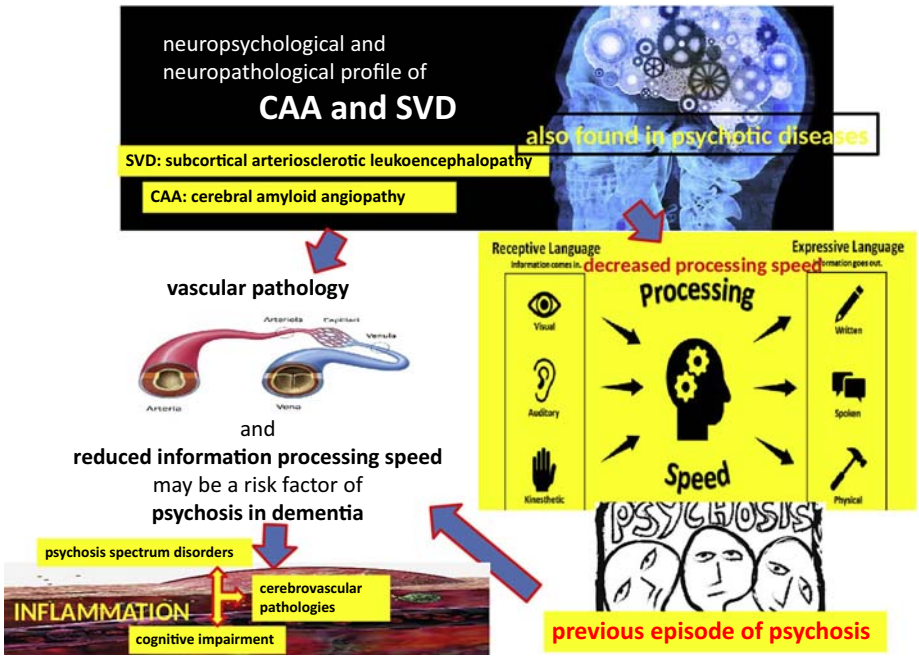

Figure 41.4 New vascular mechanisms for new therapeutic perspectives; inflammation may be a possible link between vascular pathology and psychosis in dementia.

Key facts of organic psychosis

- Organic or secondary psychosis can be seen in diverse conditions such as toxic/metabolic disorders, neurodegenerative disease, and stroke.
- For abnormal belief creation, the right lateral prefrontal cortex is a pivot in a neural network that includes the basal ganglia and limbic system and receives inputs from midbrain dopamine neurons.

The role of dopamine in signaling salience supports that aberrant dopamine signaling is the first stage of delusion formation.

- Everyday events become imbued with a sense of importance, and this sets the emotional context or mood for a delusional interpretation of their meaning.
- The content and complexity of delusions depends on whether there is cortical damage, its degree, and location.
- In dementia, which is associated with widespread cortical damage and intellectual impairment, delusions are simple, unelaborated, and persecutory.
- Greater impairments of cerebral cortical synapses, particularly in dorsolateral prefrontal cortex, may contribute to the pathogenesis of psychosis in Alzheimer disease phenotype.

Summary points

- Common delusions in Alzheimer disease (AD) patients include delusions of persecution, infidelity, abandonment, or that deceased individuals (e.g., parents) are still living.
- Although certain behavioral manifestations of frontotemporal dementia (FTD), such as abulia or rituals, are frequent, delusional symptoms are generally regarded as an uncommon feature of FTD.
- Psychotic symptoms are also frequent in vascular dementia, although they were not significantly more common than amongst patients with AD.
- The presence of psychosis in AD clearly demarcates a more severe phenotype of AD.
- Psychosis in AD is most closely associated with exaggerated reductions of gray matter volume, blood flow, and glucose metabolism in neocortex rather than in medial temporal lobe structures.
- Tau pathology and reduced levels of the synaptic zinc transporter Znt3 are associated with agitation and delusions in dementia with Lewy bodies (DLB) and AD.
- It is not possible to differentiate or compare differences of delusional jealousy across the various type of dementia or distinguish the syndrome in demented patients from the syndrome in other psychiatric disorders, although it is predominantly related to DLB and AD.
- When the dementia advances and one grows progressively less self-aware, the increased cognitive defect interferes with the formation of a coherent delusion.

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CHAPTER 42

Linking motor speech function and dementia

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List of abbreviations

AD Alzheimer's disease
AOS apraxia of speech
bvFTD behavioral variant frontotemporal dementia
DLB dementia with Lewy bodies
FTD frontotemporal dementia
lvPPA logopenic variant primary progressive aphasia
MND motor neuron disease
MSD motor speech disorder
nfvPPA nonfluent agrammatic primary progressive aphasia
PAOS progressive apraxia of speech
PCA posterior cortical atrophy
PPA primary progressive aphasia
PPAOS primary progressive apraxia of speech
svPPA semantic variant primary progressive aphasia

Mini-dictionary of terms

Agrammatism A feature of aphasia (acquired language impairment) where people speak in sentences that lack grammatical markers and function words. For example, "The boy is climbing on the chair" becomes "Boy … climb … chair."

Apraxia of speech In adults, an acquired neurological speech disorder that affects a person's ability to plan the motoric movements for speech. The distinguishing features of AOS include distorted sound substitutions and additions, increased pauses between syllables, slow speech rate, and repeated attempts to achieve a consistent speech-based movement.

Dysarthria An acquired MSD resulting from damage to the nervous system. The disorder is caused by weakness, paralysis, or incoordination of the speech musculature.

Phonemic speech errors Occurs when a phoneme (speech sound without corresponding motor plan) is inserted, deleted, or substituted within a word. Phonemic errors are clearly articulated and do not result from an MSD.

Phonetic speech errors Distortions or inaccurate productions of speech sounds. Phonetic errors result from disorders of motor speech (AOS or dysarthria).

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Introduction

Aims of this review

Here we review what is known about motor speech impairment in the most common forms of dementia. Motor speech production is discussed in the context of spoken expression, with a focus on dysarthria and apraxia of speech. We describe the characteristics of motor speech disorders (MSDs) in Alzheimer's disease (AD—amnestic, language, and visuoperceptual presentations), frontotemporal dementia (FTD—behavioral, language, and speech onsets), and dementia with Lewy bodies. While most forms of dementia result in markedly reduced expression in severe disease stages (Feldman & Woodward, 2005; Neary et al., 1998), this review will focus on speech impairments in mild to moderate stages of disease. In doing so, we aim to describe the early changes to speech that are important for diagnosis and monitoring progression.

Motor speech as a component of verbal expression

It is important to consider motor speech production (articulation, respiration, phonation, resonance) within the broader context of verbal expression. Levelt (1989) provides a model of information processing that describes three stages of verbal expression: conceptualization, formulation, and articulation.

The first stage, conceptualization, describes the process by which a speaker conceives of their intended message. This is a preverbal stage that requires the speaker to identify the purpose and contents of a message (Levelt, 1989). The second stage, referred to as the formulator, involves the process by which a preverbal message derives a linguistic structure. This process can be subdivided into multiple stages and includes grammatical encoding, which involves selection of lexical meanings (words), identification of grammatical information, and ordering of words (syntax; Bock & Levelt, 1994). The final process within the formulator stage is phonological encoding, which involves retrieval of the phonological forms of words, such as the number of syllables and patterns of emphasis (lexical stress). Phonological encoding also designates the speech sounds (phonemes) required to produce a word (Levelt, 1989). It is not until the conclusion of this complex process that the phonological codes produced by the formulator are converted into motoric programs (phonetic encoding) to be realized by the motor speech musculature at the articulatory level of the model (Levelt, 1989).

Dementia can manifest in a breakdown of verbal expression at each stage of the model. Cognitive deficits may impact the conceptualization level, leading to a variety of forms of disordered communication, such as repetition of content, disorganized flow of ideas, or aspontaneity (Neary et al., 1998; Savundranayagam, Hummert, & Montgomery, 2005). In contrast, impairments at the formulator stage lead to errors of word selection or grammar. Breakdown at the articulator stage causes disordered motor speech and may involve impaired conversion of phonological codes to phonetic plans—apraxia of speech (AOS)—or impaired execution of phonetic plans (dysarthria).

Dysarthria and apraxia of speech

The disorders of motor speech can be characterized as either AOS or dysarthria (see "Key facts" for a detailed definition). AOS is an impairment of planning and programming of speech and can be attributed to the phonetic encoding component of Levelt's model of information processing (Levelt, 1989; Ziegler, 2008). Unlike dysarthria, AOS is not the culmination of weakness, slowness, or incoordination of speech musculature (Ziegler, Aichert, & Staiger, 2012). Dysarthria is a disorder of the execution and control of motor plans (Ziegler, 2008). Dysarthria itself may be subclassified into several different forms, each characterized by a different collection of speech features (Darley, 1969). The traditional description of these dysarthria types include flaccid dysarthria resulting from lower motor neuron damage; spastic dysarthria caused by bilateral damage to upper motor neurons (UMNs); unilateral UMN dysarthria associated with unilateral UMN damage; ataxic resulting from damage to the cerebellum and its connections; and hypokinetic and hyperkinetic dysarthrias caused by abnormalities of the basal ganglia control circuit (Darley, 1969). A combination of dysarthria types is termed mixed dysarthria (Darley, 1969) and is common (compared with clearly defined isolated forms) in complex neurological disease.

The characteristic features of AOS that assist in differential diagnosis from dysarthria include hesitancy, repeated attempts for accurate placement of speech articulators, and self-corrections (Ziegler et al., 2012). Furthermore, AOS causes inconsistent speech sound substitutions in contrast to the consistent distortions of target sounds observed in dysarthria (Strand, Duffy, Clark, & Josephs, 2014).

Alzheimer's disease

Amnestic presentation

MSDs are not part of the diagnostic criteria for amnestic AD (Dubois et al., 2007). While spastic dysarthria has been reported in at least two instances of familial early-onset AD (Moretti et al., 2004; Rudzinski et al., 2008), neither dysarthria nor AOS is a common feature of the disease (Dubois et al., 2007; McKhann et al., 2011). Instead, researchers have described language deficits that affect the verbal expression of people with amnestic AD, such as word-finding difficulty and impaired naming (Savundranayagam et al., 2005; Taler & Phillips, 2008). Pragmatic components of language, such as digressing from the topic of conversation or speaking for excessive length, have also been reported (Ripich, 1994; Savundranayagam et al., 2005).

Language presentation (logopenic variant primary progressive aphasia)

The language presentation of AD is characterized by a prominent disturbance in verbal expression and is also often classified as the logopenic variant of primary progressive aphasia (PPA), or lvPPA (Gorno-Tempini et al., 2004). Individuals with lvPPA typically present with deficits in phonological processing that manifest as word-finding difficulties and

difficulty repeating phrases (Gorno-Tempini et al., 2011). Secondary features include a slower rate of speech, phonemic speech errors, and dysfluencies referred to as false starts (words partially produced that are subsequently corrected or abandoned) and hesitations (Ash et al., 2013; Gorno-Tempini et al., 2004; Wilson et al., 2010). These errors relate to the phonological encoding stage of speech production (Levelt, 1989) and are not regarded as motor speech impairments.

An important consideration for the diagnosis of MSDs is the presence or absence of phonemic errors, as they can be difficult to differentiate from the phonetic (motor speech) errors observed in AOS. Phonemic speech errors are hypothesized to occur at the phonological encoding stage of Levelt's model (1989). Phonemic errors may be omissions (e.g., "dog" → "do"), additions (e.g., "dog" → "drog"), or substitutions (e.g., "dog" → "mog") of speech sounds. Phonemic speech sound errors are clearly articulated and are not caused by impaired motor planning, execution, or control. Phonemic speech errors are a secondary diagnostic feature of lvPPA (Gorno-Tempini et al., 2011); however, they may not be particularly prominent, and researchers who have rated the severity of phonemic errors have found them to be questionable/mild to moderate in their cohorts (Croot, Ballard, Leyton, & Hodges, 2012; Josephs et al., 2014).

Although cohorts of lvPPA have been rated as having subtle speech impairments, they do not significantly differ from healthy control groups on ratings of AOS or dysarthria (Brambati et al., 2015; Mandelli et al., 2014; Poole, Brodtmann, Darby, & Vogel, 2017). The overt presence of motor speech impairment in a patient with suspected lvPPA would therefore indicate an alternative diagnosis (Gorno-Tempini et al., 2004; Mandelli et al., 2014; Poole et al., 2017).

Visuospatial presentation (posterior cortical atrophy)

Posterior cortical atrophy (PCA) is characterized by a decline in visual processing, including a space perception deficit, simultanagnosia, object perception deficit, and constructional dyspraxia (Crutch et al., 2017). These primary features are seen in the absence of memory, language, and motor speech impairments in the early disease stages (Crutch et al., 2017). Investigations of language in PCA have reported impairments of nonword and sentence repetition, auditory-verbal span, naming, and speech rate (Crutch, Lehmann, Warren, & Rohrer, 2013). These features are similar to those observed in lvPPA but subtler (Crutch et al., 2013; Magnin et al., 2013). The logopenic aphasia sometimes observed in PCA is thought to be contingent on atrophy of the left-sided temporoparietal junction due to the absence of logopenia in people with PCA associated with right-hemisphere atrophy (Magnin et al., 2013).

The aphasic features of PCA relate to functions associated with more posterior language regions of the brain, resulting in deficits in word retrieval and auditory processing (Crutch et al., 2013). The absence of motor speech impairment in PCA and lvPPA is likely related to the confinement of atrophy to posterior brain regions, thereby sparing frontal motor speech regions (Gorno-Tempini et al., 2004; Magnin et al., 2013).

Frontotemporal dementia

Behavioral variant frontotemporal dementia

Behavioral variant FTD (bvFTD) is characterized by a progressive impairment of executive function and personality change relating to disinhibition and loss of empathy (Neary et al., 1998). Atypical speech production was reported for between 57% and 74% of people with bvFTD in two broad studies of disease characteristics (Diehl & Kurz, 2002; Mendez, Joshi, Tassniyom, Teng, & Shapira, 2013). The most comprehensive study of motor speech in bvFTD identified speech impairment that was perceptible to the listener in 75% of participants (Vogel et al., 2017). The most common abnormality was reduced speech rate followed by increased pauses during speech and preference for short phrases over lengthier and more complex sentences (Vogel et al., 2017). In addition to these changes to contemporaneous speech, individuals with bvFTD presented with difficulty during syllable repetition tasks where speakers are asked to repeat the syllables "pataka" as quickly and clearly as possible (Vogel et al., 2017). Listener-based perception of speech changes was supported by objective acoustic measures of speech rate and syllabic rate production (Vogel et al., 2017). Data suggest that a timing-based motor speech impairment is present in some individuals with bvFTD in the absence of any overt signs of dysarthria or AOS, such as slurring of speech (Vogel et al., 2017).

The distinction between these timing deficits and the development of dysarthria is of clinical importance, as pathological and genetic links between bvFTD and motor neuron disease (MND) have been established (Devenney, Vucic, Hodges, & Kiernan, 2015). As many as 10%–15% of people with bvFTD later develop motor impairments sufficient to meet the criteria for MND (Burrell, Kiernan, Vucic, & Hodges, 2011; Giordana et al., 2011). Speech impairment in MND affects speech rate (consistent with bvFTD); however, it also causes prominent articulatory errors, decreased respiratory capacity, strained voice, and increased nasal resonance (Tomik & Guiloff, 2010). When observed collectively, these features are commonly referred to as a mixed spastic-flaccid dysarthria resulting from degradation of both upper and lower motor neurons (Tomik & Guiloff, 2010).

Semantic variant primary progressive aphasia

The semantic variant of PPA (svPPA) is a language-onset form of FTD (Gorno-Tempini et al., 2011). SvPPA is the clinical diagnosis given to impaired semantic memory manifesting as disordered confrontation naming and single word comprehension (Gorno-Tempini et al., 2011). Typically, these features are accompanied by loss of object knowledge and surface dyslexia and dysgraphia (Gorno-Tempini et al., 2011). Dysarthria and AOS are not typically observed in svPPA (Ash et al., 2013; Botha et al., 2015; Gorno-Tempini et al., 2004; Mandelli et al., 2014; Miller et al., 2013; Wilson et al., 2010), and the formal diagnostic criteria list the absence of motor speech disturbance as a secondary diagnostic feature (Gorno-Tempini et al., 2011). Despite this, speech fluency can be reduced due

to word-finding difficulties in conversation (Ash et al., 2013). The presence of MSD in an individual with suspected PPA would therefore suggest an alternative diagnosis as svPPA (Duffy, Strand, & Josephs, 2014; Poole et al., 2017).

Nonfluent/agrammatic variant of primary progressive aphasia

The second language-onset form of FTD is classified as the nonfluent/agrammatic variant of PPA (nfvPPA; Gorno-Tempini et al., 2011). The prominent features of nfvPPA are AOS and difficulty producing the grammatic markers of expressive speech (agrammatism; Gorno-Tempini et al., 2011). NfvPPA can be diagnosed based on agrammatism without AOS; however, average AOS severity ratings indicate that patients are often considered mild to moderate (Croot et al., 2012; Gorno-Tempini et al., 2004; Ogar, Dronkers, Brambati, Miller, & Gorno-Tempini, 2007; Wicklund et al., 2014). The key speech features indicating AOS in nfvPPA include a slower rate of speech, less prosodic variation, and phonetic speech errors (Ash et al., 2013; Croot et al., 2012; Grossman et al., 2013; Knibb, Woollams, Hodges, & Patterson, 2009). Abnormalities of lexical stress (i.e., the relative emphasis placed on each syllable within a word), can be useful in distinguishing the speech impairment in nfvPPA from that of lvPPA (Ballard et al., 2014). Excess and equal stress is a common feature of nfvPPA, which describes increases in the duration and loudness of syllables that ordinarily carry relatively less emphasis in English (Ballard et al., 2014). For example, the first and final syllables of the word "banana" carry less emphasis than the second syllable; however, speakers with AOS increase the loudness and duration of the first and final syllables so that there is less variation between the syllables (Ballard et al., 2014; Vergis et al., 2014). An objective measure of lexical stress based on loudness and duration of syllables has a strong association with expert judgment of the presence of AOS and can reliably differentiate nfvPPA from lvPPA (Ballard et al., 2014).

Dysarthria has also been described in nfvPPA. Estimates of the prevalence of dysarthria range from 18% to 60% (Caso et al., 2014; Ogar et al., 2007). The dysarthric features of nfvPPA include impairments of prosody (monotone speech), phonation (a strained and/or breathy quality with poor initiation), increased nasal resonance, and slower speech rate (Caso et al., 2014; Ogar et al., 2007), broadly fitting the categories of spastic or hypokinetic dysarthria. Dysarthria in nfvPPA has been consistently rated more severely by expert listeners than in logopenic and semantic forms of progressive aphasia (Miller et al., 2013; Rabinovici et al., 2008; Wilson et al., 2010). Features of dysarthria are therefore perceptible in nfvPPA and can be considered a feature of the syndrome that is present in some but not all cases (Caso et al., 2014; Ogar et al., 2007).

AOS in nfvPPA is associated with gray matter degradation in the middle and inferior frontal gyrus, premotor area, insula, and cingulate of the left hemisphere (Ballard et al., 2014; Grossman et al., 2013; Gunawardena et al., 2010; Rohrer, Rossor, & Warren, 2010). These regions are widely recognized as important for speech production and frequently result in AOS when damaged by stroke (Graff-Radford, Jones, & Graff-Radford, 2014; Hillis et al., 2004).

Progressive apraxia of speech

Syndromes dominated by degenerative AOS have been described as both progressive apraxia of speech (PAOS) and primary progressive apraxia of speech (PPAOS). These disorders should be discussed alongside the PPA spectrum of disorders, as they possess clinical features (AOS and agrammatism) like those of nfvPPA. While a presentation of progressive AOS with minimal agrammatic aphasia can be considered to meet diagnostic criteria for nfvPPA (Gorno-Tempini et al., 2011), some authors have suggested that PAOS is a more appropriate diagnosis in cases where the most prominent and early feature is progressive apraxia and not aphasia (Josephs et al., 2013). The distinction between PAOS and PPAOS is essentially the presence or absence of language impairment (agrammatism), with the term PPAOS reserved for those with an isolated motor speech impairment (Brodtmann, Pemberton, Darby, & Vogel, 2016; Josephs et al., 2013).

AOS is necessarily present in all patients diagnosed with this condition (Josephs et al., 2006). One study has indicated that AOS in PAOS may have more prominent impairments of prosody (syllable segmentation and increased intersegment durations) compared with AOS observed in nfvPPA (Josephs et al., 2013). Spastic and mixed spastic-hypokinetic forms of dysarthria have been recorded in five cases of PAOS (Josephs et al., 2013). Longitudinal studies of PPAOS indicate that dysarthria can emerge in later stages of the disease, with spastic dysarthria being the most common (characteristics such as strained voice quality, monoloudness, and monotone), followed by hypokinetic dysarthria (reduced loudness, monotone, rapid speech rate, and reduced stress; Duffy et al., 2015; Josephs et al., 2014).

Speech deficits of PAOS have been associated with gray matter atrophy and hypometabolism of the inferior frontal gyri, precentral cortex, and supplementary motor area (Josephs et al., 2012, 2013) as well as white matter connections to these regions (Whitwell et al., 2013). Articulatory deficits are thought to be associated with function of the supplementary motor area, precentral gyrus, and cerebellar crus bilaterally, whereas prosodic deficits are linked to more focal atrophy of the supplementary motor area and right superior cerebellar peduncle (Utianski et al., 2018).

PPAOS and PAOS can develop at markedly different rates, with some cases retaining a syndrome dominated by AOS without cognitive change for many years, and others developing a progressive supranuclear palsy syndrome with a more rapid degeneration (Brodtmann et al., 2016; Josephs et al., 2014).

Dementia with lewy bodies

Dementia with Lewy bodies (DLB) is a form of dementia that accounts for up to 23% of dementia cases (Jones & O'brien, 2014). It involves a progressive cognitive decline that interferes with social or employment opportunities (McKeith et al., 2017). Core clinical features of DLB include fluctuating cognitive abilities, well-formed and recurrent visual hallucinations, REM sleep behavior disorder, and parkinsonism (McKeith et al., 2017). Disordered or incoherent speech is an often-cited feature of fluctuating cognition and

can be used to distinguish DLB from AD (Ferman et al., 2004; McKeith et al., 2017). Speech rates of people with DLB are often slower than those of age-matched healthy controls as well as people with Parkinson's disease (Ash et al., 2012). Disordered speech of people with DLB may be due to poor executive level ordering of speech manifesting as an inability to correctly sequence a narrative during connected speech (Ash et al., 2011). Poor sequencing of ideas and difficulty maintaining a narrative theme have been associated with poor neuropsychological scores in the domains of executive functioning (Ash et al., 2011). These findings support the notion that disordered speech in DLB results from an impairment of executive functioning caused by damage to the ventral frontal cortex (Ash et al., 2011) rather than an impairment of motor speech per se (Ash et al., 2012).

The emergence of parkinsonism may be an additional source of speech abnormalities in DLB. Parkinsonian speech features, such as reduced speech volume, prosodic variation, and increased pause length between utterances (Ash et al., 2012; Sachin et al., 2008), are observed in approximately 60%–70% of people with DLB in addition to executive dysfunction (Ballard et al., 1997; McKeith et al., 2017; Müller et al., 2001). The median time between onset of cognitive symptoms and the emergence of observable dysarthria in a group of people with pathologically confirmed DLB was 42 months (Müller et al., 2001). The most common features of dysarthria in DLB are reduced speech volume (hypophonia) and monotonic speech (Müller et al., 2001).

Conclusion

Assessment of motor speech impairments is important for the diagnosis of dementias as well as monitoring for the emergence of secondary syndromes and disease progression. MSDs are rare in AD. Despite this, a clear understanding of phonetic and phonemic speech errors is required for differentially diagnosing lvPPA from nfvPPA. Motor speech impairments are most common in the frontotemporal dementias, firstly because AOS is a key feature of nfvPPA and PAOS, and secondly because subtle motor speech impairments are common in bvFTD. Finally, speech in DLB may deteriorate with progression due to the emergence of dysarthria related to parkinsonism.

Key facts about dysarthria

- Dysarthria is a collective name for a group of speech disorders caused by acquired impairments in the neuromuscular control of speech.
- Traditionally, there are six types of dysarthria as well as mixed dysarthria, which involves features from two or more types of dysarthria.
- Flaccid dysarthria results from weakness caused by lower motor neuron damage. Distinguishing features are breathy voice, short phrases, increased nasal resonance, and imprecise articulation.

- Spastic dysarthria is caused by spasticity resulting from bilateral UMN damage. Distinguishing features are strained voice, monotonicity, and slow rate.
- Ataxic dysarthria is due to incoordination caused by damage to the cerebellum. Distinguishing features are irregular articulatory errors, equal and excessive stress on syllables, and inappropriate variation of pitch and loudness.
- Hypokinetic dysarthria is due to rigidity and bradykinesia resulting from impairment of the basal ganglia control circuit. Distinguishing features are reduced loudness, rapid speech rate, sound repetitions, and reduced stress.
- Hyperkinetic dysarthria results from involuntary movements associated with impairment of the basal ganglia control circuit. It is characterized by unpredictable movements of the speech mechanism.
- Unilateral UMN dysarthria is caused by unilateral damage to the UMNs. Distinguishing features are hoarse voice, imprecise articulation, and slow rate.

Summary points

- The review describes the relative impact of MSD in AD, FTD, PPA, and DLB.
- MSDs are not part of the phenotype of amnestic AD.
- Speech errors in lvPPA are phonemic in nature, and clear evidence of an MSD may indicate an alternative diagnosis.
- Verbal expression in PCA is similar in nature to that of lvPPA, and MSDs are uncommon.
- Subtle abnormalities of motor speech timing are apparent in bvFTD.
- MSDs are not present in svPPA.
- AOS is a core (though nonessential) diagnostic criteria for nfvPPA and may be observed with concomitant dysarthria.
- PAOS and PPAOS are defined by the presence of AOS, and concomitant dysarthria is common.
- Primary speech abnormalities in DLB relate to executive dysfunction. Parkinsonian MSD may emerge with the progression of DLB.

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CHAPTER 43

Spatial navigation and Alzheimer's disease

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List of abbreviations

AD Alzheimer's disease
CA cornu ammonis
MCI mild cognitive impairment
MEC medial entorhinal cortex

Mini-dictionary of terms

Allocentric World-centered frame of reference (i.e., position of objects relative to other objects in the environment; body-position independent)
Egocentric Body-centered frame of reference (i.e., position of body relative to objects in the environment; body-position dependent)
Grid cell Neuron that fires when an animal enters several locations in an environment arranged in a hexagonal grid
Head direction cell Neuron that fires when an animal's head is pointed in a specific direction
Place cell Neuron that fires when an animal enters specific locations in an environment
Spatial disorientation A state of misalignment between an individual's true and estimated position within the environment
Spatial navigation The act of using environmental and internal sensory cues to move within an environment

Introduction

Spatial disorientation, or a disruption in the ability to orient oneself within an environment, is an early symptom of Alzheimer's disease (AD) (Henderson, Mack, & Williams, 1989; Monacelli, Cushman, Kavcic, & Duffy, 2003). This symptom may lead to behaviors such as wandering or getting lost (McShane et al., 1998; White, & Montgomery, 2015). Prominent attributes of spatial disorientation in AD include impaired recognition of spatial features (e.g., environmental landmarks) or the inability to determine the position of an object relative to oneself (Guariglia & Nitrini, 2009). Studies of spatial navigation have been employed both in human subject experiments and using rodent models of AD. Generally, spatial navigational impairments have been shown to progressively increase over time, and deficits are apparent even in the

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preclinical phase (Allison, Fagan, Morris, & Head, 2016). In addition, the first indications of AD-related pathology have been identified in regions involved in spatial processing (Braak & Braak, 1995; Thal, Rüb, Orantes, & Braak, 2002). Although such pathology is difficult to detect through noninvasive techniques, the study of spatial navigation may serve as an important early behavioral marker of AD.

This chapter will discuss spatial disorientation and spatial navigation in AD, focusing on cortical-limbic functions associated with the generation of the spatial representations critical for accurate navigation. Although the precise mechanisms of spatial disorientation in AD are currently unknown, we will review how functional differences observed in spatial navigation circuitry have the potential to underlie spatial navigation deficits observed in individuals with AD. In the sections below, we first discuss how spatial navigation performance is measured in human research followed by a description of some recent work utilizing rodent models of AD. Finally, we summarize key findings in electrophysiological research that point to disruption of spatial representations in animal models with AD-like pathology. The chapter concludes with an evaluation of future considerations for understanding spatial navigation deficits in AD.

Neural representations and spatial navigation

Brain regions involved in the processing of spatial information succumb to AD-related pathology early on in disease progression (Braak & Braak, 1995; Kalus, Braak, Braak, & Bohl, 1989). Such circuitry consists of cortical-limbic structures, including the parahippocampal gyrus, entorhinal cortex, parietal cortex, and limbic thalamic nuclei (Fig. 43.1). The neural activity in these regions has been found to support spatial navigation (Moser, Moser, & McNaughton, 2017), and more broadly is proposed to be involved in the formation of a cognitive map, or a set of neural processes that create map-like representations of the environemnt (Tolman, 1948; O'Keefe & Nadel, 1978). The hippocampus, in particular, contains a subset of neurons, called "place cells," that fire maximally in specific locations in each environment (O'Keefe & Nadel, 1978). Outside the hippocampus, other specialized cell types have been discovered (Fig. 43.1B–G), including cells that fire relative to a given head direction (e.g., head direction cells) (Taube, Muller, & Ranck, 1990), along a tessellating grid pattern (e.g., grid cells) (Hafting, Fyhn, Molden, Moser, & Moser, 2005; Sargolini et al., 2006), along the border of an environment (e.g., border cells) (Solstad, Boccara, Kropff, Moser, & Moser, 2008), at a given body position relative to an object (e.g., egocentric cells) (Wang et al., 2018; Wilber, Clark, Forster, Tatsuno, & McNaughton, 2014), or modulated by a specific route or action through the environment (Nitz, 2006) (see Fig. 43.1 for cell type examples).

There is evidence that primate brains, including humans, contain similar spatial cell types. In vivo recordings in human surgical patients or nonhuman primates have identified evidence of grid cells in the medial entorhinal cortex (MEC) (Jacobs et al., 2013) and head

(A)

Area	Functional Cell Types	Panel
ATN	HD	C
PoS	HD	C
Hip	Place	B
EC	Grid, Border, HD	D,E,F
RSC	HD, Bidirectional HD	C,F
PPC	Route-centric, HD, Egocentric	G,C

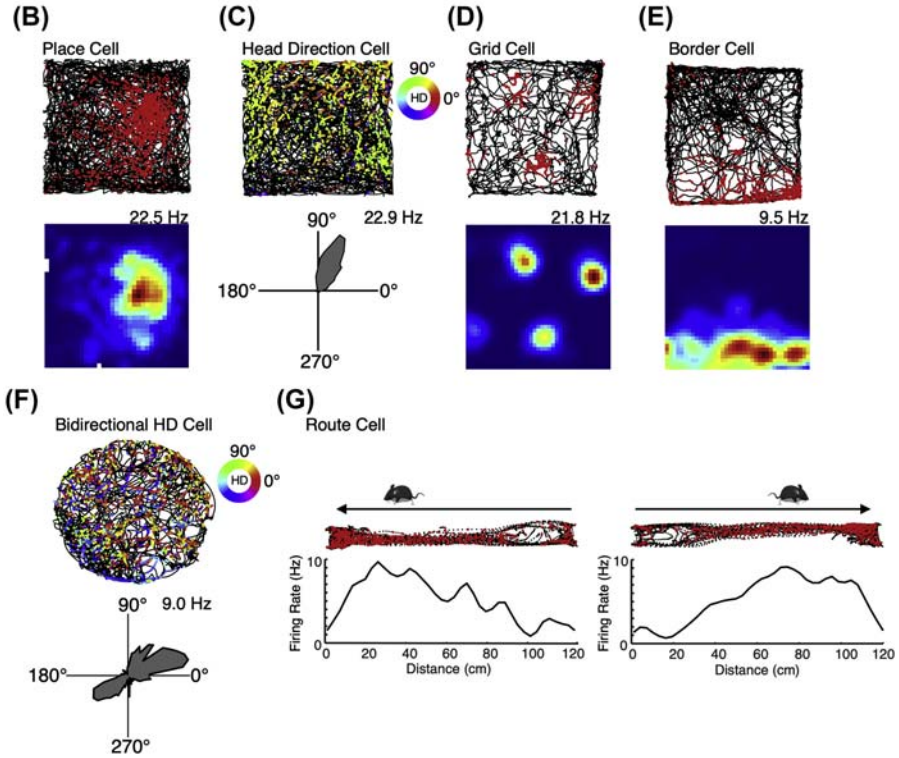

Figure 43.1 Spatial navigation circuit loci. (A) Table describing the major loci of the spatial navigation circuit implicated in Alzheimer's disease and the major functional cell types that reside there. (B) Place cell. Top—an overhead view of animal's path throughout a recording session is indicated with black. Action potentials from a single cell are indicated with red. Note the clustering of red in an area. Bottom—heat map that represents the cell's firing rate (peak rate labeled on the top right) in action potentials per second with red indicating higher and blue indicating lower firing rates. Note that the darkest red is in the same position as the clustering in the above plot. (C) Head direction cell. Top—similar layout to that o (B). However, each action potential is now color-coded by the animal's head direction at the time the cell fired. Note the dominate green/yellow color. Bottom—polar plot demonstrating the cell's preferred firing direction and firing rate. (D) Grid cell. Note the gridlike firing formation. (E) Border cell. Note that the cell fires maximally at the south border. (F) Bidirectional head direction cell. Note that the cell has two major preferred firing directions. (G) Route cell. In this plot, the animal made consecutive laps on a linear track. The laps split by running direction (left and right). The lower plots represent the tuning curves generated from the two running directions. Note that the tuning curves mirror each other and ramp up in firing rate as the animal moves toward the end of the track, indicating that this cell may encode information about the route from right to left and from left to right. (B–G) All cells were obtained from unpublished data sets with the exception of panels D and E from Winter et al., (2015). Mouse in panel G designed by Freepik.

direction cells in the presubiculum (Robertson, Rolls, Georges-Francois, & Panzeri, 1999). Additionally, using noninvasive functional magnetic resonance imaging (fMRI) in humans, gridlike signals were found to emerge in the MEC during virtual navigation (Doeller, Barry, & Burgess, 2010). Furthermore, perceived directional correlates have been observed in the medial parietal cortex and medial temporal lobe (Baumann & Mattingley, 2010; Vass & Epstein, 2013). Importantly, these spatial representations are modulated by both internal (e.g., head translation or feedback from motor units or motor efferent copy) and external (vison or olfaction) sensory information (Moser, Moser, & McNaughton, 2017). Lastly, it is essential to note that damage to brain regions containing these spatial signals results in profound impairments in the ability to navigate accurately (Aggleton, Pralus, Nelson, & Hornberger, 2016; Clark & Harvey, 2016). Thus, neurodegenerative changes in these regions may indicate potential threats to successful spatial navigation (Aggleton et al., 2016).

Spatial navigation circuitry and structural change in Alzheimer's disease

Functional differences have been observed in cortical-limbic regions involved in spatial navigation of individuals at various stages of AD. Mild cognitive impairment (MCI), particularly the amnesic subtype (aMCI), has been considered the prodromal form of AD (Petersen et al., 2009). Sousa, Gomar, Goldberg, and the Alzheimer's Disease Neuroimaging Initiative (2015) assessed whether regional volume and glucose metabolism was predictive of spatial disorientation in MCI and AD. The results indicated that thickness of the entorhinal cortex, hippocampus, and middle-inferior temporal cortex, and glucose metabolism of the latter two regions, were predictive of how well subjects could orient within their environment. Furthermore, individuals with MCI who exhibited increased disorientation were more likely to progress to AD. Accurate navigation ability has also been found to correspond to changes in hippocampal and parietal cortex volumes in individuals with MCI or AD (DeIpolyi, Rankin, Mucke, Miller, & Gorno-Tempini, 2007). More recently, Kunz et al. (2015) found that young apolipoprotein E, allelic variant 4 carriers, who are at elevated risk for developing late-onset AD, exhibited reduced grid-like representations in the entorhinal cortex despite having performance equal to that of control subjects in a virtual spatial navigation task. Interestingly, carriers also had increased activity in the posterior hippocampus, indicating either a compensatory mechanism or further evidence of budding dysregulation of the circuit. Grid-like representations may correspond to the integrity of entorhinal function in spatial navigation, as these representations have been shown to significantly predict spatial navigation performance (Kunz et al., 2015). Overall, differential structural and functional changes in cortical-limbic spatial processing loci of individuals with MCI or AD are predictive of spatial disorientation and poor spatial navigation performance.

Measuring spatial navigation in human subjects

Understanding how spatial navigation deficits manifest in AD may be just as important as elucidating neural changes that result from AD pathology, as emerging evidence suggests that spatial navigation deficits serve as an important behavioral marker for diagnosing AD (Coughlan, Laczó, Hort, Minihane, & Hornberger, 2018). Spatial orientation abilities can be measured through self-report surveys (Hegarty, 2002; Kozlowski & Bryant, 1977). Use of two-dimensional tests, such as map drawing or landmark identification, can be used to assess subject recall of experienced spatial navigation or to assess visuospatial processing (Allison, Fagan, Morris, & Head, 2016; Monacelli et al., 2003). Importantly, physical or virtual navigation tasks can reveal how individuals use different types of environmental features or landmarks when navigating. One example of a physical navigation task, called the hidden goal task (Fig. 43.2), allows for evaluation of participant ability to navigate to a hidden goal location in either an egocentric or allocentric reference frame. For instance, participants can solve this task using an egocentric frame of reference by executing a sequence of actions (e.g., turn right) relative to the start point (Fig. 43.2B). Alternatively, participants can navigate by using an allocentric frame of reference in which distant visual cues (cues hanging along the testing room walls) are

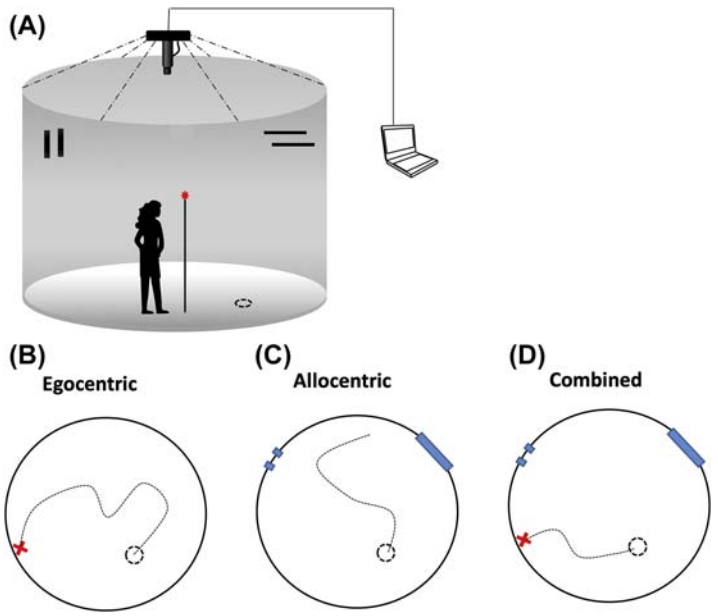

Figure 43.2 Diagram of the hidden goal task adapted from Laczó et al. (2010). (A) In this task, participants locate a goal location while holding a trackable LED indicator. The movement of participants is tracked with an overhead camera and the participant's route is then analyzed offline using a computer. (B–D) Participants undergo testing in three conditions using egocentric cues (start position indicated with a red X), allocentric (blue shapes represent wall hangings), or both to locate the goal location (dashed circle).

used to accurately triangulate the goal location (Fig. 43.2C). Outcome measures such as total distance traveled or proximity of the end location to the goal location are used to assess performance in each condition. The controlled environment provided by the hidden goal task allows for the evaluation of navigation by limiting the types of navigationally relevant spatial features. This may allow for the formation of precise yet ecologically limited inferences on how certain reference frames pertain to navigation performance but may not be reflective of how subjects navigate in a complex environment. The route learning task addresses this by evaluating how subjects navigate in a hospital or city setting (Cherrier & Mendez, 2001; DeIpolyi et al., 2007). Generally, subjects are exposed to a route within an environment and are tested on several spatial domains (e.g., dead-reckoning, landmark location, and route-learning) either during or after navigating the route. Importantly, this test usually allows for extraneous variables to be included, such as people or objects. The use of these tests in Alzheimer's populations has demonstrated navigational impairments that become progressively worse over time (Cherrier & Mendez, 2001; DeIpolyi et al., 2007).

Spatial frames of reference and navigation in Alzheimer's disease

Navigational impairments in AD may correspond to the decreasing ability to use allocentric or egocentric frames of reference during navigation (see Fig. 43.3 for a general description). Individuals with prodromal forms of AD first exhibit impairments in allocentric reference frame processing. DeIpolyi et al. (2007) assessed individuals with MCI or AD in the route learning task compared with cognitively typical controls. Notably, MCI and AD groups could recognize a landmark that was along the route but were worse at remembering the position of the landmark relative to other spatial features. A similar impairment was observed during testing in the hidden goal task. Hort et al. (2007) found that individuals with MCI were less precise in finding the goal location when only allocentric cues were present, a finding that has been replicated in subsequent studies (Laczó et al., 2019, 2010). More recently, individuals with aMCI or dementia were assessed in a virtual Y-maze task and a physical navigation task (Parizkova et al., 2018) (Fig. 43.4). Results indicated that 67% of the aMCI and 94% of the dementia group preferentially used an egocentric strategy to complete the Y-maze task (see Fig. 43.4C). In addition, individuals with aMCI who preferred the egocentric strategy had worse performance in allocentric reference frame navigation compared with individuals with aMCI who preferred the allocentric strategy for the Y-maze (Fig. 43.4B). Thus, allocentric impairments are evident in MCI and become worse over time. Indeed, individuals with AD show increased impairments in using environmental features from either

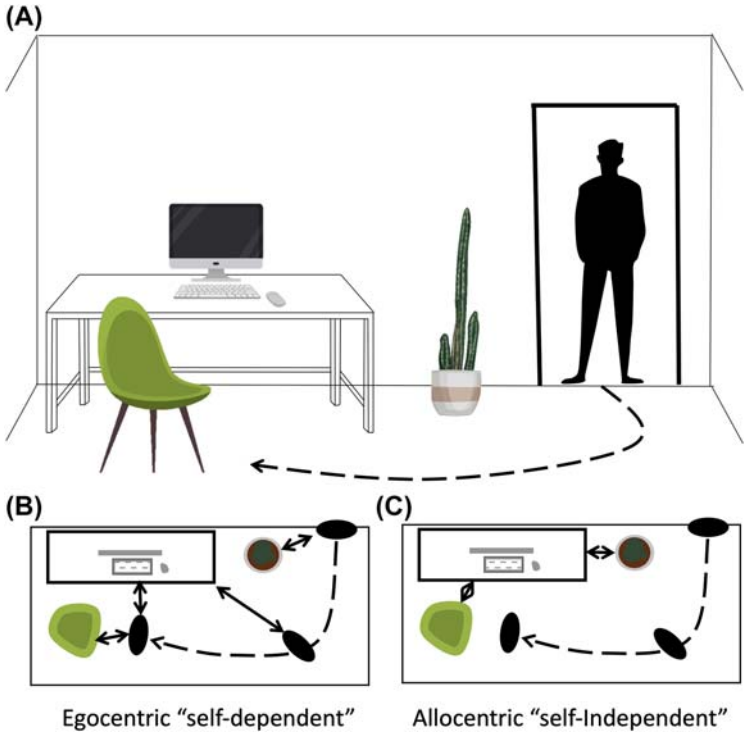

Figure 43.3 Allocentric and egocentric frames of reference. This figure demonstrates the difference between the allocentric and egocentric reference frames. (A) A person entering their office may use the position of objects relative to oneself (B, egocentric) or the position of objects relative to each other (C, allocentric) to estimate their position while walking to the chair. Plant designed by N. Dmitrova (natalka_dmitrova), Freepik. Computer including keyboard and mouse designed by Freepik. Chair designed by macrovector/Freepik.

reference frame. For example, individuals with AD are worse at orienting within the route or locating a specific position on the route during physical navigation (Cherrier & Mendez, 2001; Cushman & Duffy, 2007; Monacelli et al., 2003). The severity of spatial navigation impairments has been hypothesized to result from a worsening ability to transition from using the two reference frames during navigation. Burgess, Trinkler, King, Kennedy, and Cipolotti (2006) suggested that animals utilize egocentric information to self-localize within an allocentric frame of reference and vice versa (see Clark, Simmons, Berkowitz, & Wilber, 2018 for in-depth review). Morganti, Stefanini, & Riva (2013) found that individuals with AD were worse at using a top-down view map to navigate through the city in virtual reality. This study suggests that allocentric-egocentric translation might be disrupted in AD. (Morganti, Stefanini, & Riva, 2013).

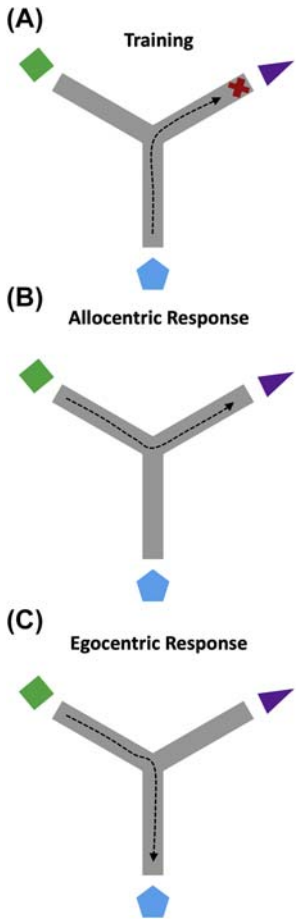

Figure 43.4 Y-maze strategy types. (A) Following training on the Y-maze using consistent start and goal positions (A), the participants are given a probe task where the start location is changed (B–C). (B) Participants can have an allocentric response where they correctly use the room cues to locate the goal. (C) Alternatively, participants can have an egocentric response (e.g., turn right) that is independent from room cues.

Assessment of spatial navigation in rodent models of Alzheimer's disease

Animal models have served an integral role in elucidating the neurobiological mechanisms that underlie spatial disorientation in AD. There are many different rat and mouse models that have been created to assess aspects of AD-like pathology (Götz, Bodea, & Goedert, 2018), and many exhibit spatial navigation impairments (Grøntvedt et al., 2018; Lester, Moffat, Wiener, Barnes, & Wolbers, 2017; Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014). One of the most commonly employed

tests of spatial memory is the Morris water task (MWT) (Morris, 1984). In this task, animals are placed in a pool of cloudy water and trained to swim to a hidden escape platform. Animal models of AD-like pathology tend to exhibit poor performance on general measures of the MWT, including swim latency, path distance, and proximity to the platform location (Janus, 2004; Brody & Holtzman, 2006; Berkowitz et al., 2018). Recently, a longitudinal assessment using a detailed path analysis revealed that TgF344-AD rats less precise swim movements when completing the MWT. Over time, TgF344-AD animals will transition to less direct swim movements (Fig. 43.5). In addition to MWT impairments, other studies have also demonstrated that these rats progressively develop impairments in spatial reversal learning (Cohen et al., 2013; Rorabaugh et al., 2017).

Neural representations of space in rodent models of Alzheimer's disease

Several studies have investigated the impact of AD-like pathology on spatial representations, but the studies have been largely focused on the impact of AD pathology on hippocampal place cell function (Cacucci, Yi, Wills, Chapman, & O'Keefe, 2008;

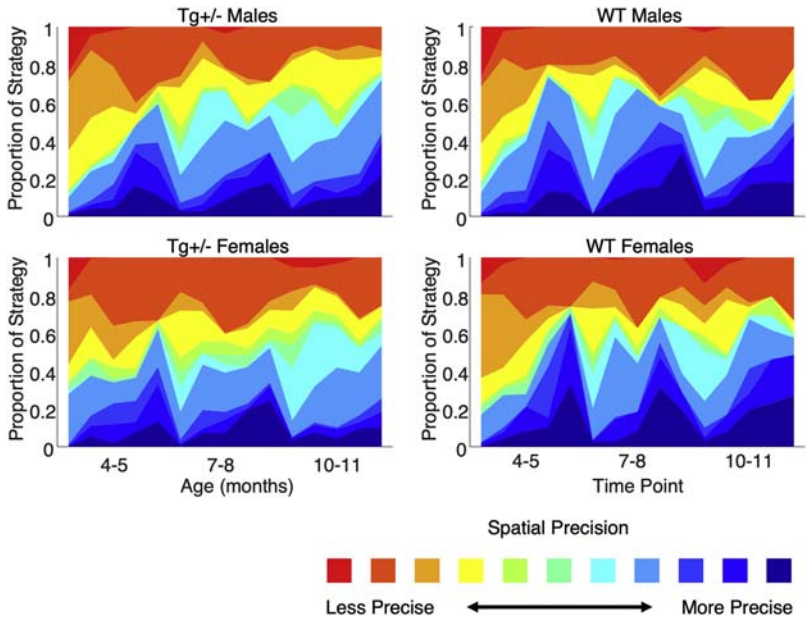

Figure 43.5 Strategy preference figure and path description from Berkowitz et al. (2018). Relative proportion of swim movements over days at each age of testing. Area plots represent the proportion of all movements across days and age points. Note that Tg animals show fewer spatially precise strategies compared to wild-types at 7–8 and 10–11 months of age.

Galloway et al., 2018; Mably, Gereke, Jones, & Colgin, 2017; Zhao, Fowler, Chiang, Ji, & Jankowsky, 2014). Generally, place cells have been shown to convey less precise spatial information (example cell shown in Fig. 43.1B). Zhao et al. (2014) found that, while place cells recorded from wild-type mice became more spatially precise over repeated exposures to a novel environment, spatial precision of place cells in APP mice did not improve. It is possible that synaptic plasticity disruption may underlie place cell instability in animal models with AD-like pathology. Amyloid toxicity has been linked to impaired synaptic plasticity (Rowan, Klyubin, Cullen, & Anwyl, 2003), while reduced synaptic plasticity has been shown to contribute to the formation of less precise place fields and decreased phase-locking to slow gamma (Kitanishi et al., 2015). Indeed, Mabley et al. (2017) found similar, albeit more severe, results when assessing place cell firing in 3xTg mice. Place cells recorded from 3xTg mice were less stable within and between sessions and exhibited less spatially precise firing. Importantly, these cells also showed a decrease in phase-locking to slow gamma (25–55 Hz) (for phase-locking example, see Fig. 43.6A). The TgF344-AD rat model shows disruption of long term potentiation in the dentate and decreased basal transmission of CA3-CA1 (Smith & McMahon, 2018). Interestingly, Galloway et al. (2018) found that CA2/CA3 place cells conveyed less spatial information, whereas CA1 cells were similar to that of wild-type rats. In addition, elicited theta-slow gamma phase amplitude coupling (Fig. 43.6B–F) is also impaired in TgF344-AD rats at 12 months of age (Stoiljkovic, Kelley, Stutz, Horvath, & Hajós, 2018). Thus, it is possible that synaptic plasticity changes may be underlying the decreased spatial precision of hippocampal place cells in animal models with AD-like pathology.

Alterations in oscillatory activity in the entorhinal cortex may also contribute to downstream impairments in hippocampal place cell firing. The entorhinal cortex is the largest input into the hippocampus (Witter, Wouterlood, Naber, & Van Haeften, 2006). Layers II and III of the MEC send projections to dentate and CA1, respectively. Pyramidal cells in layers II/III MEC show reduced phase-locking to both slow (30–50 Hz) and fast (55–100 Hz) gamma in an the APP mouse model (Nakazono, Jun, Blurton-Jones, Green, & Igarashi, 2018). However, altered phase-amplitude coupling between theta and fast gamma occurred prominently in layer III (Nakazono et al., 2018). Additionally, a mouse model of tauopathy shows grid cell dysfunction at 30+ months of age when pathological load of hyperphosphorylated tau is extensive (Fu et al., 2017). Although these two studies indicate a relationship between AD pathology and entorhinal dysfunction, more evidence is required to further elucidate the extent of entorhinal contributions to hippocampal place cell dysfunction. Further, the impact of AD-related pathology on other cellular correlates of navigation (Fig. 43.1) remain to be investigated.

In summary, electrophysiological findings suggest possible uncoupling of the trisynaptic circuit. Synaptic plasticity disruption, possibly linked to amyloid toxicity (Rowan et al., 2003), may influence the expression of unstable place representations, changes in phase-locking, and reduced theta-gamma coupling. AD-related changes in the MEC may also contribute to reduced theta–fast-gamma coupling in the hippocampus, but more studies

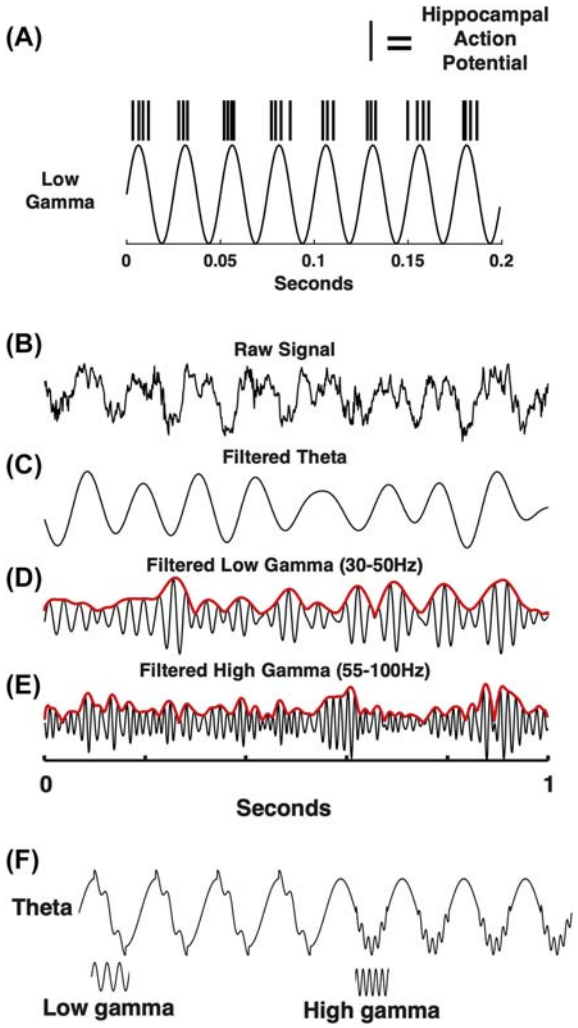

Figure 43.6 Oscillatory activity. (A) Phase-locking. Hippocampal cells are known to fire consistently at particular phases of low gamma (30–50 Hz). (B) Raw hippocampal cornu ammonis 1 local field potential. (C) The signal from A filtered for theta (4–12 Hz). (D) The signal from A filtered for low gamma (30–50 Hz) with the signal's envelope shown in red. (E) The signal from A filtered for high gamma (55–100 Hz) with the signal's envelope shown in red. (F) Example of how low and high gamma can nest within hippocampal theta frequency, with low gamma on the downward slope and high gamma nested within the troughs of theta.

are needed to clarify this relationship. Notably, Colgin et al. (2009) found that CA1 slow gamma may be entrained by CA3, while CA1 fast gamma may be entrained by the MEC. Evidence of theta–gamma coupling disruption in the hippocampus of animals with AD-like pathology may indicate an information flow disruption in the trisynaptic circuit could underlie changes in spatial representation in AD.

Summary and conclusions

Spatial disorientation is a powerful early indicator of AD. Early on in disease progression, the circuitry involved in the processing of spatial features exhibits evidence of AD-related pathology as well as functional differences as measured by fMRI. Specifically, the entorhinal cortex and hippocampus exhibit differential function in individuals with AD relative to healthy controls. Importantly, these changes have been shown to predict performance on spatial navigation tasks (Sousa, Gomar, Goldberg, & ADNI, 2015). Individuals with prodromal AD or aMCI show impairments when having to use cues within an allocentric reference frame but could use egocentric information (routes or body-centered cues) during navigation. Ultimately, individuals with AD show severe navigational impairments in either reference frame (Vlček & Laczó, 2014). Spatial deficits are also observed in animal models with AD-like pathology, and detailed assessments of behavior reveal that rats with AD-like pathology develop progressively worse performance when using distal cues during navigation (Berkowitz et al., 2018). Investigation into the possible mechanisms of spatial navigation deficits in animal models of AD has shown that spatial representations may be unstable over time. Place and grid cells recorded from animals with AD-like pathology exhibit less precise spatial firing (Fu et al., 2017; Mably et al., 2017; Zhao et al., 2014). Evidence of dysfunction is also observed at the cellular population level. In particular, evaluation of local field potentials in vivo has shown suppression of theta–gamma phase-amplitude coupling in the hippocampus (Stoiljkovic et al., 2018). Overall, both human and animal studies suggest a decoupling of spatial navigation circuitry in AD, leading to deficits in using allocentric and egocentric reference frames during navigation.

Key facts

- Spatial disorientation is an early symptom of AD.
- Activity patterns in corticolimbic structures indicate that spatial features, such as place information or heading direction, are represented in rodent and primate brains.
- Noninvasive imaging, such as fMRI, may be used to determine whether spatial representations are disrupted in individuals with AD.
- Physical and virtual navigational tasks can be used to assess navigational impairment in individuals with AD.
- Spatial navigation can be tested in rodents by using mazes, such as the Morris water task.

Summary points

- This chapter reviews spatial disorientation and spatial navigation in AD.
- Spatial disorientation or spatial navigation impairment is a promising behavioral biomarker for AD.
- Spatial representations are disrupted in individuals with AD and predict spatial navigation performance.
- Navigation within an allocentric reference frame is impaired in prodromal or early AD, which may be due to impairments in egocentric-to-allocentric transformations.
- Rodent studies assessing AD-like pathology find that animals are impaired in spatial navigation.
- Single-unit recordings in rodents with AD-like pathology suggest that cells that code for place are less precise in conveying spatial information.
- Oscillatory activity indicates a potential disruption in the flow of information within the hippocampus of rodents with AD-like pathology.

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CHAPTER 44

Violence and dementia

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List of abbreviations

AD Alzheimer' disease
BPSD behavioral and psychological symptoms of dementia
bvFTD behavioral variant of frontotemporal dementia
CAG cytosine–adenine–guanosine trinucleotide
COMT catechol-O-methyltransferase monoamine oxidase
DA dopamine
DbH dopamine beta-hydroxylase
DLB dementia with Lewy bodies
DMS delusional misidentification syndrome
EEG electroencephalogram
FTD frontotemporal dementia
HTR2B hydroxytryptamine receptor 2B
MAO monoamine oxidase
OS Othello syndrome
PET positron emission tomography
RBD rapid eye movement sleep behavior disorder
SPECT single-photon emission computerized tomography
VaD vascular dementia
5-HIAA 5-hydroxyindoleacetic acid
5-HT 5-hydroxytryptamine

Mini-dictionary of terms

Aggression It is an observable behaviour—not a thought or feeling. Aggression is an umbrella term; it includes physical, verbal, or sexual interactions that are considered to be negative, aggressive, or intrusive.

Antipsychotics Also known as neuroleptics, are used as treatment for hallucinations and delusions. They are considered therapeutic options for treating violent behavior.

Brain structural abnormalities in violent persons Damage to specific brain areas is linked with a lack of empathy or remorse and guilt for their acts. Poor impulse control can be the result of such abnormalities. People with dementia have impaired impulse control and so react more impulsively.

Crime An act that is prohibited by law and punished by the state. A behavior can be criminal without having involved a violent action.

Delusion A false belief held with extraordinary conviction, impervious to other experiences and compelling counterarguments. Delusional thoughts are common in patients with Alzheimer disease and other dementias. The association of delusions with violent behavior has been observed.

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Dementia Acquired and persistent disorders characterized by deficits in multiple cognitive domains that are severe enough to interfere with everyday functioning. The degree of deterioration represents a decline from the patient's previous level of function.

Frontotemporal dementia A range of clinically and pathologically heterogeneous conditions that primarily affect the frontal and temporal lobes of the brain, causing problems with personality, behavior (including violent acts), semantic memory and language.

Hallucination A perception without an object is the classic definition of a hallucination. For example, visual hallucinations are experienced when one sees something where nothing actually exists. Violent behavior may be associated with auditory hallucinations.

Psychosis Term used to describe a number of psychopathological symptoms. There is evidence of association between the presence of psychotic symptoms such as delusions/hallucinations and violence.

Violence Extreme form of behavior with the immediate intent to cause serious injury or death. A behavior does not have to cause actual harm to be classified as violent, e.g., the failed attempt to kill must be considered a violent act.

Introduction

Alois Alzheimer, in his original manuscript, stressed the prominence of behavioral disturbances in the patient's behavior (Cipriani, Dolciotti, Picchi, & Bonuccelli, 2011). Behavioral and psychological symptoms of dementia (BPSD), such as psychosis, repetitive questioning, and aggression, occur in most patients sooner or later, almost regardless of the level of cognitive disability. These symptoms provoke a significant level of psychological stress and burden on the caregivers and a considerable cost on the health system because they are the first cause of the institutionalization. Demented patients are often vulnerable and fragile, but in rare cases, they can become violent. In the introduction to this chapter, in which we review the phenomenon of violence and dementia, we describe a case that we directly observed. AG, an 89-year-old retired industrialist, presented to our department with clinical features of a chronic, progressive multidomain cognitive decline and behavioral changes as in cases of Alzheimer disease (AD). His wife, very ill with heart disease, was being looked after by a caregiver, a handsome 45-year-old woman. AG, who fell in love with the caregiver, asked her for sexual attentions, first with gallantry, then with haughtiness and arrogance. In response to the umpteenth refusal, the man became violent, trying to force himself into the woman's bedroom and kicking and punching the neighbors who had come to his house because they heard shouting. After admission to hospital, AG seriously injured one staff member and required physical restraint on several occasions. Subsequently, the patient was transferred to a nursing home where, in one circumstance, he tried to push a resident out of the window.

Defining violence

The definition of a "violent behavior" is not univocal because it depends on neurobiological, cultural, and environmental factors and their crossed interactions (Table 44.1). The World Health Organization (1996) defines violence as, "The intentional use of

Table 44.1 Definitions of violence.

Violence definitions	References
The intentional use of physical force or power, threatened or actual, against oneself, another person, or against a group or community, that either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment, or deprivation.	Krug et al. (2002). *World report on violence and health*. Geneva: World Health Organization
Violence refers to the threat or use of physical force with the intention of causing physical injury, damage, or intimidation of another person. Verbal and psychological abuse are not included in the definition of violence.	Elliott et al. (1998). *Violence in American Schools*. New York: Cambridge University Press.
Actions of a physical, psychological, sexual, or economic (e.g., being accused by the assailant of stealing money or things from him/her) nature leading to actual harm or to an increased risk of harm.	Aström et al. (2002). *Scandinavian Journal of Caring Sciences, 16*(4), 66–72
An act carried out with the intention of, or perceived as having the intention of, physically hurting another person.	Straus et al. (1980). *Behind closed doors: Violence in the American family*. New York: Anchor Books
Violence is physical aggression at the extremely high end of the aggression continuum, such as murder or aggravated assault.	Anderson et al. (2002). *Annual Review of Psychology, 53*, 27–51
Violence is the use of physical force, verbal abuse, threat, or intimidation that can result in harm, hurt, or injury to another person.	Harwood (2017). *The Journal of the Royal College of Physicians of Edinburgh, 47*(2), 94–101
Violence is defined as any act of actual physical aggression involving physical contact.	Shah (1995). *International Journal of Geriatric Psychiatry, 10*, 887–891

physical force or power, threatened or actual, against oneself, another person, or against a group or community, that either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment or deprivation." Some researchers consider violence as an aggressive behavior (Allen & Anderson, 2017); however, treating these phenomena as synonymous may create miscommunications and confusion not only in the scientific community but also in general opinion. Most agree on the following definition of violence: a life-threatening form of aggression that has serious physical damage as a goal (Anderson & Bushman, 2002; Bushman & Huesmann, 2010). Some authors have conceptualized aggressive and violent behaviors as a continuous phenomenon, where aggression is one extreme and it consists of relatively minor acts, while violence is the other extreme and it consists of major acts (Allen & Anderson, 2017). Thus, all violent behavior is aggression, but most aggression is not violence.

Violence and neurobiology

All of us have the capacity to be violent, but most people can control this force. Aggression and violence are frequently associated with specific cognitive impairments and emotional reactivity, such as deficit in cognitive inhibitory control, in the understanding the consequences of behavior and reduced emotional responses (Blake & Grafman, 2004). However, the neurobiological basis of violence is poorly understood. It is clear that many neurobiological causes and correlates exist, and that these interact both with each other and with nonneurobiological factors. Predisposition for violent behavior can be considered a phenotype resulting from the combination of some genetic variations and peculiar triggers in the environment (e.g., socioeconomic disadvantage, acquired brain damage, substance abuse, and psychological stress) (Cipriani, Lucetti, Danti, Carlesi, & Nuti, 2016). Antisocial and violent behavior in males may be weakened by functional polymorphism in the monoamine oxidase (MAO) A gene in patients with early childhood maltreatment (Caspi et al., 2002). A stop codon in HTR2B, apparently exclusive to Finnish people and widely expressed in frontal lobe, was linked to severe impulsivity (Bevilacqua et al., 2010). Moreover, multiple markers within candidate genes, such as the ones involved in dopamine turnover (COMT, MAO A and B, and DbH), and many of their combinations, are involved in aggressive behavior Grigorenko et al. (2010). Data from PET studies with 6-L-[18F]fluorodopa show that low levels of subcortical dopamine are linked with irritability (Laakso et al., 2003). Low cerebrospinal fluid (CSF) concentrations of the serotonin metabolite 5-HIAA, to be linked to low serotonin concentrations in brain tissue, have been found in many violent offenders (Virkkunen, Nuutila, Goodwin, & Linnoila, 1987). Kandel and Freed (1989) wrote a comprehensive review of the association between specifically violent criminal behavior and frontal lobe dysfunction. More recently, the focus of attention has been pointed on dysfunction both in frontal and in temporal cortices and their subcortical connections. A network including orbitofrontal cortices, amygdala, and somatosensory and insular brain areas and their projections to peripheral nervous system has been specifically associated with anger and violent behavior (Bechara, Damasio, & Damasio, 2000). Survivors of head injuries coming from the Vietnam Head Injury Study, with severe lesions (i.e., penetrating wounds) to frontal lobes, showed more aggressive and violent behaviors with respect to the other survivors with nonfrontal head injuries (Grafman et al. 1996). Damage to the prefrontal cortex has been correlated also with psychopathy, as it is the key area for inhibition, judgment and planning, emotional reactivity, empathy, prosocial behavior, and ability to learn from own mistakes and punishment (Koenigs, 2012). Case reports have documented the presence of violence and aggression in patients with temporal lobe epilepsy (Grant, Koziorynska, Lushbough, Maus, & Mortati, 2013) and an increasing slow-wave activity in the temporal lobe in offenders who commit a murder (Gatzke-Kopp, Raine, Buchsbaum, & LaCasse, 2001). Impulsive behavior has

been correlated with hyperactivity of the limbic system, especially when triggered by anger stimuli (Siever, 2008), as an expression of reduced top-down regulation of frontal cortices. HMPAO-SPECT study has shown evidence of abnormalities in the prefrontal cortex and the temporal lobes in impulsive violent offenders (Soderstrom, Tullberg, Wikkelsö, Ekholm & Forsman, 2000) in absence of MRI lesions. An FDG-PET study showed that individuals who committed impulsive murders exhibited lower subcortical prefrontal activity and higher activity in the temporal lobe when compared to control subjects (Raine et al., 1998).

Dementia and violence

People suffering from dementia have been studied as victims of violence but also as perpetrators (Cipriani & Danti, 2014). Indeed, dementing illnesses can cause dysfunction of the neural structures involved in inhibitory control, moral judgments, interpersonal conduct, and comprehension of social norms (Cipriani, Borin, Vedovello, Di Fiorino, & Nuti, 2013; Damasio, Tranel ,& Damasio, 1990). The exact prevalence of the phenomenon of perpetrators of violence in demented patients is unknown; nonetheless, in some reports it is noteworthy (up to 40% both psychogeriatric inpatients and in community-based referrals) and it is ascribed above all to males over 65 years old (O'Callaghan, Richman, & Majumdar, 2010). Although it had a small sample size, a study showed that half of 14 elderly psychiatric patients had been diagnosed with dementia before they attempted or committed homicide (Ticehurst, Ryan, & Hughes, 1992). Furthermore, some studies pointed out that there were missing reports. Violent behavior has to be reported by caregivers, and many individual and cultural factors contribute to neglect it; often the burden of such a behavior impedes the institutionalization, in other cases it provokes abuse from caregivers (Jackson & Mallory, 2009). The literature has pointed out also a lack of distinction between severe violence and milder outbreaks in reports of in-care settings. Professional experience of the senior staff may be a crucial factor in diminishing incidents of violent behavior of patients (Åström, Bucht, Eisemann, Norberg, & Saveman, 2002). However, other studies showed a prevalence of physical assault by demented patients on care workers from 42% to 68% (Stutte, Hahn, Fierz, & Zúñiga, 2017; Isaksson, Sandman, Åström, & Karlsson, 2008) despite staff personnel professional experience in dealing with violent behavior.

What factors are associated with violence?

Violence often complicates nursing care, and most incidents of violence in dementia care occur in the morning or in the evening when the caregiver is required to work in close contact with the resident, providing personal care, e.g., helping the resident with meals and personal hygiene (Åström et al., 2004). Deutsch and Rovner (1991) found that AD patients who were more physically violent were more dependent on others regarding

oral hygiene, dressing, and toilet needs. Any activity that involves the invasion of personal space increases the risk for assault; violence-prone patients have a body buffer zone four times larger than those patients not prone to violence (Negley & Manley, 1990). Chou, Kaas and Richie (1996) found that aggressiveness and violent behavior among older residents is related to physical illness, side effects of drugs, and mental instability. Such behavior was also related to previous history of aggressiveness, excessive stimuli, and the quality of relationship between the caregiver and the resident. People with chronic pain had an increased risk for violent behavior (Fishbain, Cutler, Rosomoff & Steele-Rosomoff, 2000). The influence of gender on violence in elderly people is unclear. Some authors (Isaksson, Graneheim, Åström, & Karlsson, 2011) found three factors independently associated with physically violent behavior: male gender, antipsychotic treatment, and decline in orientation. Other studies confirmed that violence is associated with male gender (Beck et al., 1998; Lyketsos et al., 1999) although some researchers have reported that women exhibit more violence (Serper et al., 2005). There is a positive correlation between greater dementia severity and the prevalence of violent behavior (Isaksson et al., 2011), but some authors have suggested that the link is modest (Cooper, Mungas & Weiler, 1990). Other triggers for violence are reduced vision and/or hearing, changes in the environment, excessive noise or activity, and locked doors (Enmarker, Olsen & Hellzen, 2011; Kunik et al., 2010). In one study, married patients and those who lived with family were overrepresented in the group of violent patients; the prevalence of violent behavior in the sample of geriatric patients with dementia decreased after admission to the locked unit, showing the impact of situational factors on violence (Haller, Binder & McNiel, 1989). Cohen (2004) suggests that the following antecedent factors increase the risk for homicidal behavior in persons with dementia: history of previous violence or "other-directed" behaviors, history of alcohol abuse, paranoid symptoms and other psychotic symptoms, psychotic depression, history of catastrophic reactions, traits such as low frustration tolerance and aggressiveness, and military/law enforcement/firefighter history (Table 44.2).

Violent behavior in different types of dementia

AD is a progressive neurodegenerative condition marked by cognitive impairment that significantly interferes with baseline daily functioning and frequently involves behavioral disturbances. It is the most common form of dementia among older people. Nearly all brain functions, including memory, movement, language, judgment, and behavior, are eventually affected. The symptoms of AD are generally mild to start with, but worsen over time. It is well recognized that, in some circumstances, AD patients can become violent persons. Newspapers reported the case of Homer Castor, an 87-year-old Colorado man who suffered from AD who was arrested for allegedly beating to death Mr. Propp, a 76-year-old fellow dementia patient at the nursing home where both

Table 44.2 Factors associated with violence in people with dementia.

Triggers of violence	References
Situations involving personal care	Åström et al. (2004). *Scandinavian Journal of caring sciences, 18*(4), 410–416
Quality of relationship between the caregiver and the patient	Chou et al. (1996). *Journal of gerontological nursing, 22*(11), 30–38.
Decline in orientation	Isaksson et al. (2008). *Journal of Clinical Nursing, 18*, 972–980.
Psychoses	Tsai et al. (1997). *The Kaohsiung journal of medical sciences, 13*(10), 639–642; Cipriani et al. (2012). *Psychiatry and clinical neurosciences, 66*(6), 467-473.
Depression	Lyketsos et al. (1999) *The American Journal of Psychiatry, 156, 66–71*
History of alcohol abuse	Cohen (2004). *Journal of Mental Health and Aging, 10*, 83–86.
Pain	Fishbain et al. (2000). *Pain Medicine, 1*, 140–155
Physical illness	Chou et al. (1996). *Journal of Gerontological Nursing, 22*(11), 30–38
Visual and hearing impairment	Enmarker et al. (2011). *International Journal of Older People Nursing, 6*(2), 153–162; Kunik et al. (2010). *The Journal of Clinical Psychiatry, 71*(9),1145–1152.
Influence of gender	Beck et al. (1998). *Gerontologist, 38,* 189–198; Lyketsos et al. (1999). *The American Journal of Psychiatry, 156*, 66–71.
Changes in the environment	Enmarker et al. (2011). *International Journal of Older People Nursing, 6*(2), 153–162.
Military veterans	Orengo et al. (2008). *American Journal of Alzheimer's Disease and Other Dementias, 23*(3), 227–232.
Premorbid aggressiveness	Kunik et al. (2010). *Journal of Clinical Psychiatry, 71*(9), 1145–1152
Dementia severity	Isaksson et al. (2011). *Aging and Mental Health, 15*(5), 573–579

men lived. Castor told police officers he thought Propp had tried to beat him up in the middle of the night and was pretending to be asleep when he assaulted him. About one-third of outpatients with AD are suggested to present violent behavior (Reisberg, Borenstein, Salob & Ferris, 1987). Male gender and presence of dyspraxia were reported to increase the likelihood of violent behavior (Eastley & Wilcock, 1997). Frontotemporal dementias (FTDs) are a group of neuropathologically heterogeneous neurodegenerative disorders with distinct clinical phenotypes. Terminology for FTD has evolved over time. The clinical presentation of FTD was described as early as the 19th century, initially most

comprehensively by Arnold Pick, who lent his name to the historical designation of the entire FTD spectrum as Pick's disease. It affects the frontal and anterior temporal regions, especially the ventromedial prefrontal cortex, orbitofrontal cortex, and anterior temporal regions. About half of cases present with behavioral changes (bvFTD), and the remainder present with language decline (primary progressive aphasia) characterized either by impaired speech production (progressive nonfluent aphasia) or by impaired word comprehension and semantic memory (that is, memory for meaning) (semantic dementia). The core features of the usual behavioral variant FTD (bvFTD) are alterations in social conduct and emergence of a variety of abnormal behaviors such as impulsive, rash, or careless actions. For example, a patient tried to run over a police officer with his car when being pulled over and another one tried several times to strangle a sleeping patient and to hit another patient with an iron pipe (Liljegren, Landqvist Waldö, & Englund, 2018). Liljegren et al. (2018) performed research with the objective to investigate the prevalence of physical aggression among patients with dementia of different types and to analyze potential differences. The physical aggression (PA) was prevalent both in the early and in late stages of the disease, although almost half of the physically aggressive FTD patients exhibited PA during the first half of their disease, compared with the AD patients with PA. Furthermore, the FTD patients' behavior seemed more brutal and unprovoked when comparing to the violent acts in the AD group. Violence is commonly reported in individuals with Huntington disease (HD). It is an inherited genetic, autosomal dominant, neurodegenerative disorder caused by cytosine–adenine–guanosine trinucleotide repeat expansion on gene codifying for huntingtin protein. The characteristic triad of symptoms and signs includes a progressive chorea, neuropsychiatric manifestations such as emotional and behavioral disturbances, and dementia. Researchers have described populations in which 5%–18% of the patients were convicted of various sorts of crime, including cases of murder (Dewhurst, Oliver, & McKnight, 1970; Reed & Chandler, 1958). Alcohol-drinking dementia patients tended to commit violent crimes. Kim et al. (2011) suspected that alcohol-related crimes took place in an early stage of dementia as a result of hallucinations, delusions, and executive dysfunction caused by inebriation, whereas patients without alcohol consumption committed a crime in a later stage when dementia had progressed considerably and they had lost most of their socioeconomic and familial support. A peculiar case is represented by violent behavior in association with violent dream content as a feature of rapid eye movement sleep behavior disorder (RBD). RBD is an interesting clinical condition characterized by recurrent dream enactment behavior that includes movements mimicking dream content and accompanied by the absence of normal REM muscle atonia (Högl, Stefani & Videnovic, 2018). Dreams often involve chases or attacks by animals or humans and motor activity varies from simple limb jerks to complex motor behavior, with potential injuries to the patient or bed partner (Trotti, 2010). There is a close relationship between RBD and degenerative neurological conditions. In fact, it may precede cognitive decline in dementia with Lewy bodies (DLB) and it occurs

frequently in autopsy-confirmed cases compared with non-DLB (76% vs. 4%) (Ferman et al., 2011). Uchiyama et al. (1995) described the case of a patient with RBD suffering from DLB who began experiencing nocturnal episodes during which he hit his wife on the head and threw objects that were available near his bed.

Violence and psychotic symptomatology

Episodes of violence have been associated with positive psychotic symptoms such as delusions and hallucinations. Dealing with the psychopathology of hallucinations may be an argument of forensic relevance. For example, they have been described as risk factors for physically violent behavior. Prominent examples are the so-called imperative hallucinations (commanding auditory hallucinations) that have been implicated in some cases of physical assault. Such voices are often very distressing and some people are unable to resist complying with the commands. Some researchers (Erkwoh, Eming-Erdmann & Willmes, 2001) studied command hallucinations and concluded that imperative hallucinations may have an impact on the individual's behavior. The predictors of dangerous actions were identifying the hallucinated voice, being affected by emotions after hallucinations, and disregarding the voice as being real. These psychopathological features are described in dementia syndromes too (Rubin, Drevets, & Burke, 1988). Delusional thoughts are a source of serious distress for patients, and, in many cases, these thoughts increase the burden of caregivers. Particularly, many persons with dementia of various etiologies experience paranoid delusions during the course of illness. Paranoid thoughts are suspicious in nature, ideas of persecutions, beliefs in imaginary intruders, or thinking that others are stealing one's belongings. For the patient, the presence of delusions can result in increased violent behavior. The delusional misidentification syndromes (DMSs) are psychopathologic phenomena in which a patient consistently misidentifies persons, places, objects, or events. The category of DMS is sometimes characterized by hostility toward misidentified objects and, subsequently, it can lead to significant danger of physical harm to others (Silva, Leong, & Weinstock, 1992). For example, Taj et al. (Tsai, Hwang, Yang, Liu, & Lo, 1997) described a 70-year-old man with a diagnosis of vascular dementia (VaD). This patient declared that his wife had the same appearance, but was a double, and he showed occasional violence toward her. The violence became exaggerated in the preceding 2 months leading up to hospitalization and he even set fire to his home in order to kill his wife. Othello syndrome (OS) is a psychotic disorder characterized by delusion of infidelity or jealousy. The danger presented by OS individuals ranges from serious verbal threats to homicidal acts. Persons with OS can also harm themselves. Regarding the degree of dangerousness, demented patients suffering from OS pose a significant societal problem in terms of potential violence, especially in domestic situations, but literature describing the problem in demented patients is limited to individual cases (Cipriani, Vedovello, Nuti, & di Fiorino, 2011). (Ticehurst, Gale & Rosenberg (1994) described the case of a 78-year-old man

admitted to a psychogeriatric ward after allegedly trying to kill his wife. He said she had provoked him with infidelity. He detailed how his wife had provoked him by having her dress up high when the electrician called and by leaving the curtains open when she changed at night. He had inferred by various looks from the neighbor that they were party to this exhibitionism and may have been involved sexually with his wife. His Mini-Mental State Examination score was 13 out of 30. There was cerebral atrophy on cranial CT scan. He was diagnosed as suffering from AD. Kim et al. (2011) described five patients who had been diagnosed with alcohol-related dementia or VaD, and committed violent crimes including murder, attempted murder, arson, and assault; two patients attempted to kill their neighbors because they wrongly thought the neighbors were trying to seduce their wives; another two assaulted pedestrians because of persecutory delusion.

Management

Nonpharmacologic interventions

There are a multitude of nonpharmacologic interventions to treat agitation and aggression in dementia (Table 44.3). However, their evidence for reducing violence is much more limited and not conclusive (Rampling et al., 2016). Despite the promising results from studies, there is little controlled evidence that nonpharmacologic interventions in fact work, and they are often difficult to implement in real-world settings (Nowrangi, Lyketsos, & Rosenberg, 2015).

Table 44.3 Nonpharmacologic treatment of violent behavior.

Nonpharmacologic treatments	References
Music therapy	Ledger et al. (2007). *Aging and Mental Health*, *11*(3), 330–338.
Pet therapy	Filan et al. (2006). *International Psychogeriatrics*, *18*(4), 597–611.
Light therapy	Lyketsos et al. (1999). *International Journal of Geriatric Psychiatry*, *14*(7), 520–525.
Aromatherapy	Scuteri et al. (2017). *Evidence-Based Complementary and Alternative Medicine*, *2017*.
Multisensorial stimulation techniques	Livingston et al. (2005). *American Journal of Psychiatry*, *162*(11), 1996–2021.
Reality orientation	Patton (2006). *Journal of Clinical Nursing*, *15*(11), 1440–1449.
Reminiscence therapy	Gonzalez et al. (2015). *International Psychogeriatrics*, *27*(10), 1731–37.
Cognitive behavioral therapy	Spector et al. (2012). *Trials*, *13*(1), 197.

Pharmacologic interventions

The decision to start a patient on medication for BPSD is based on a judicious consideration of risks and benefits. Drug treatments for the control of violence and extreme agitation should be used to calm the person with dementia and reduce the risk of violence and harm, rather than treat any underlying psychiatric condition; the lowest effective dose should be used (National Collaborating Center for Mental Health, 2007). Historically, antipsychotics have been prescribed for treating psychosis as well as acutely violent and agitated behavior. Among pharmacologic options, atypical antipsychotic medications generally have replaced conventional antipsychotics and have been considered preferred pharmacologic treatments for behavioral disturbances associated with dementia (Alexopoulos et al., 2005) (Table 44.4). Second-generation antipsychotics include clozapine, olanzapine, risperidone, quetiapine, ziprasidone, and aripiprazole. However, prescribing an antipsychotic to reduce violent acts in dementia is a difficult decision. In fact, in 2005, the US Food and Drug Administration warned of an increased risk of death in elderly patients with dementia treated with these drugs based on analyses of randomized, placebo-controlled trials (averaging 10 weeks in duration). The use of antipsychotics continues to be controversial. They should be considered within the context of medical need and the efficacy and safety as an alternative. Their use for dementia is precarious, but on occasion, their use is within reasonable clinical practice, especially given the dearth of alternatives (Table 44.5). Expert consensus suggests that the use off-label of antipsychotics can be appropriate when

Table 44.4 Pharmacologic treatment of violent behavior.

Pharmacologic interventions	References
Typical and atypical antipsychotics	Schneider et al. (2006). *American Journal of Geriatric Psychiatry, 14*(3), 191–210.
Trazodone	Sultzer et al. (2001). *Journal of the American Geriatrics Society, 49*(10), 1294–1300.
Cholinesterase inhibitors	Masterman et al. (2004). *Primary Care Companion to the Journal of Clinical Psychiatry, 6*(3), 126.
Memantine	Wilcock et al. (2008). *The Journal of Clinical Psychiatry, 69*(3), 341–348.
Citalopram	Leonpacher et al. (2016). *American Journal of Psychiatry, 173*(5), 473–480.
Gabapentin, lamotrigine, and topiramate	Gallagher et al. (2014). *Drugs, 74*(15), 1747–755.
Carbamazepine	Olin et al. (2001). *American Journal of Geriatric Psychiatry, 9*(4), 400–405.
Sodium valproate	Sival et al. (2004). *International Journal of Geriatric Psychiatry, 19*(4), 305–312.

Table 44.5 Recommendations on the use of antipsychotics.

Recommendations for the use of antipsychotics
Assess the frequency of violent behavior, degree of risk, and potential triggers
Do not use antipsychotics unless the person is severely distressed or there is an immediate risk of harm to them or others
Violent behaviors that put the patient or others at risk should be treated urgently
Involve family caregiver in the process of antipsychotic prescription considering risks versus benefits
Consider cardiovascular risk factors, when initiating antipsychotics
If used, they should be prescribed at low dosages and for short periods
Remember: atypical antipsychotics are associated with fewer extrapyramidal symptoms than conventional antipsychotics
Consider medication cessation or reduction, if appropriate and rationale

nonpharmacologic approaches fail to adequately control behavior to minimize the risk of violence and reduce patient distress (Salzman et al., 2008). Use of anticonvulsant agents such as valproic acid has become a mainstay in the treatment of these behaviors, but similarly carries considerable risk (Mizukami et al., 2010). The data for carbamazepine are conflicting, and both tolerability and kinetic concerns limit its use (Gallagher & Herrmann, 2014).

Key facts of violence and dementia

- Multiple markers within candidate genes, such as the ones involved in dopamine turnover (COMT, MAO A and B, and DbH) are involved in aggressive behavior.
- Low cerebrospinal fluid concentrations of the serotonin metabolite 5-HIAA have been found in many violent offenders.
- A network including orbitofrontal cortices, amygdala, and somatosensory and insular brain areas and their projections to peripheral nervous system has been associated with anger and violent behavior.
- The following antecedent factors increase the risk for homicidal behavior in persons with dementia: previous violent behavior, alcohol abuse, paranoid symptoms, psychotic depression, catastrophic reactions, low frustration tolerance and aggressiveness, and military/law enforcement/firefighter.
- Expert consensus suggests that the use off-label of antipsychotics can be appropriate when nonpharmacologic approaches fail to adequately control behavioral to minimize the risk of violence.

Summary points

- Some authors conceptualized aggressive and violent behaviors as being on a continuum of severity with relatively minor acts of aggression (e.g., pushing) at the low end of the spectrum and violence (e.g., homicide) at the high end.
- Violent behavior associated with dementia is relatively rare but is always serious because the person is generally unable to control his emotions and has limited insight and poor judgment.
- Any activity that involves the invasion of personal space increases the risk violence.
- Other triggers for violence are the presence of psychotic symptomatology, excessive stimuli, chronic pain, and changes in the environment.
- Expert consensus suggests that the use of antipsychotics can be appropriate when nonpharmacologic approaches fail to adequately control violent behavior.

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CHAPTER 45

Factors contributing to protection and vulnerability in dementia caregivers

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List of abbreviations

AD Alzheimer's disease
EE expressed emotion
SOC sense of coherence

Mini-dictionary of terms

Expressed emotion As a critical indicator of emotional climate in care dyads and refers to caregiver criticism, hostility, and emotional overinvolvement.

Familism Stresses the sense of belonging to family, shared mutual dependence, and the importance of familial obligations.

Neuropsychiatric symptoms Broadly similar to "behavioral and psychological symptoms of dementia" and include, for example, depression, delusions, hallucinations, agitation, insomnia, apathy, or withdrawal.

Resilience It refers to adaptive capacities or a positive trajectory of functioning despite caregiving challenges.

Self-efficacy in caregiving Defined as beliefs about the capacity of successfully performing desirable actions to cope with caregiving demands.

Sense of coherence Coined by A. Antonovsky to describe a dispositional tendency that promotes well-being under stressful situations, including comprehensibility, manageability, and meaningfulness.

The stress process model Proposed by Pearlin and colleagues (1990), articulates various primary (e.g., problematic behaviors of the care-recipient) and secondary (e.g., family conflicts) stressors whose effects are mediated by caregiver personal resources and social support.

Joseph is an 82-year-old retired tailor living with his wife Maria (75 years old). Their only daughter (Anna, 45 years old, divorced) works as a nurse in another town. This is their realistic fictional story.

Joseph was diagnosed with dementia 2 years ago and is now on an anticholinesterase inhibitor. He displays periods of tearfulness, low motivation, and psychomotor slowness. He would not keep with his medication were it not for Maria, who actually struggles with him on account of this. Joseph was never "fond of pills." Recently, the situation worsened—incontinent and increasingly dependent, he has episodes of unfounded, irrational jealousy and keeps saying to everyone "my wife is a whore; she only cares about having fun." Joseph also has angry outbursts, and it

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becomes almost impossible to calm him down, as he interprets all explanations or reassurance in paranoid ways. Unpredictably, this behavior alternates with apathy and withdrawal.

Maria never accepted her husband's condition, searching for some magic solution. First, she developed "doctor shopping" and eventually sought advice from traditional healers. She avoided opportunities to confide to her closest ones, seeming to cope astonishingly "well", a smile on her face, always hoping for "a cure."

In recent months, she has experienced despair and is often sad and unable to relax and enjoy the few pleasurable moments she might have. She has even started to wake up early in the morning in anguish, fearing that she must "face another day." Talking to her daughter, Anna, she is hardly able to confide her feelings of loneliness and loss along with a deep sense of injustice and revolt. "Nothing is the same now, he turned out to be another person . . . what a shame to hear all these things throughout the day, fearful of what the neighbors might hear. I wish God could take me or take us both, this life is not worth living." Nonetheless, she manages to fulfill all her duties at home only to find herself guilty if she stops for a moment to look after the house and after Joseph himself. Anna suggests contacting the same GP or neurologist again and avoiding more second opinions. She also insists that home support would help ease Maria's burden ("it's difficult to face this, but perhaps Father would not mind being in a nursing home temporarily").

Maria keeps talking about the need to be relieved, but she cannot find an acceptable way out and refuses her daughter's suggestions: "I must bear this burden, that's what's expected of me; if I sent him elsewhere, what would people think? And he would hate to have strangers at home, fussing around. Besides, he would talk lots of nonsense to them." Anna has recently noticed a different tone in her mother's comments: "He has always had a bad temper; he's selfish and cold …now I realize my whole life was wasted." In fact, Maria sounds increasingly critical of Joseph's behaviors, even those that would not seem that disrespectful to her. From time to time she is even openly hostile toward him. Notwithstanding, she remains deeply involved in providing care, controlling all that concerns medications and diapers and refusing to take a break (her few friends are to the point of giving up on offering help).

Anna, on the other hand, remains committed to helping her parents. Despite her challenging job and difficulty negotiating days off to do so, she never misses an opportunity to be there or at least to give them a call. Her worries are many, including about the future, and her efforts to change these family issues are frequently unsuccessful. But she remains optimistic in a balanced, realistic way, probing positive alternatives: " … this may be tough to hear, but there must be ways of making it better. Positive things happened, anyway … always felt my parents were authoritarian and cold, but now their frailty somehow moves me, making me closer to them no matter the physical distance … ."

Anna has no signs of burnout, although she was actually depressed years ago after her divorce. She then attended psychotherapy for months and had to count on her friends' support, but eventually recovered. Anna often recalls her feelings of loss from that time, including meaningful recollections of how she surpassed them. All this seems to provide her with a stronger sense of purpose throughout her caregiving pathway.

Introduction

Joseph is one of the 50 million persons around the world living with some form of dementia. Their journey is often not traveled alone but together with family members. As the

person's condition deteriorates, one or more relatives may assume the caregiving role and take on increasing responsibilities over time. The job is often so demanding and exhausting that caregivers are notorious for ignoring self-care. Brodaty et al. (2014) found over half of a caregiver sample reporting a high burden of care and approximately 22% experiencing increases in their burden from moderate to severe levels across 12 months. Over time, caregiver burden implies elevated risks for physical and psychological morbidity. A meta-analysis found that compared with noncaregivers, dementia caregivers reported higher stress, lower subjective well-being, and poorer physical health (Pinquart & Sörensen, 2003a). A review addressing the prevalence of clinically significant depression and anxiety among caregivers of persons with Alzheimer's disease (AD) reported overall rates of 34% and 44%, respectively (Sallim, Sayampanathan, Cuttilan, & Ho, 2015). This confirms a greater risk of psychiatric morbidity among dementia caregivers. Again compared with noncaregivers, an increased risk of hypertension was found among dementia caregivers (Shaw et al., 1999). Stress-related increase in cortisol levels can contribute to hyperinsulinemia, obesity, and inflammation (Von Känel et al., 2012), further exacerbating caregiver risk of incident coronary heart disease (Von Känel et al., 2008). Notably, although the link between caregiving and increased mortality was not always found (Roth, Brown, Rhodes, & Haley, 2018), greater self-reported caregiving stress seems to be a robust predictor of caregiver mortality. *Dementia caregiving* has been coined as a natural experiment of extreme stress, with caregivers being the hidden victims of dementia.

We should therefore make sense of the stressors for caregivers along with protective factors against the detrimental effects of caregiving demands. In this chapter, we first examine the stressors in dementia caregiving and the influence of cultural contexts, and then analyze factors promoting caregiver strengths (see Fig. 45.1). These are related to resilience, self-efficacy, sense of coherence (SOC), adaptive coping, and emotion-regulation skills.

Stressors in dementia caregiving

The majority of people with dementia are cared for in the community by family members (and occasionally friends and neighbors), providing informal (unpaid) care sometimes on a nearly round-the-clock basis. As dementia has a chronic, progressive course, it typically demands great personal attention and problem-solving skills on the part of the caregiver. This relates not only to cognitive problems but also, and mostly, to behavioral and mood disturbances (e.g., apathy, depression, anxiety, repetitive behaviors, irritability, aggressiveness), psychotic symptoms, superimposed *delirium*, or deterioration in physical functioning (Cheng, 2017). As our case illustrates, Joseph displayed delusional jealousy along with angry outbursts, apathy, and withdrawal. Neuropsychiatric symptoms in general, and disruptive behaviors in particular (e.g., agitation/aggression, irritability, disinhibition, and aberrant motor behavior) are more predictive

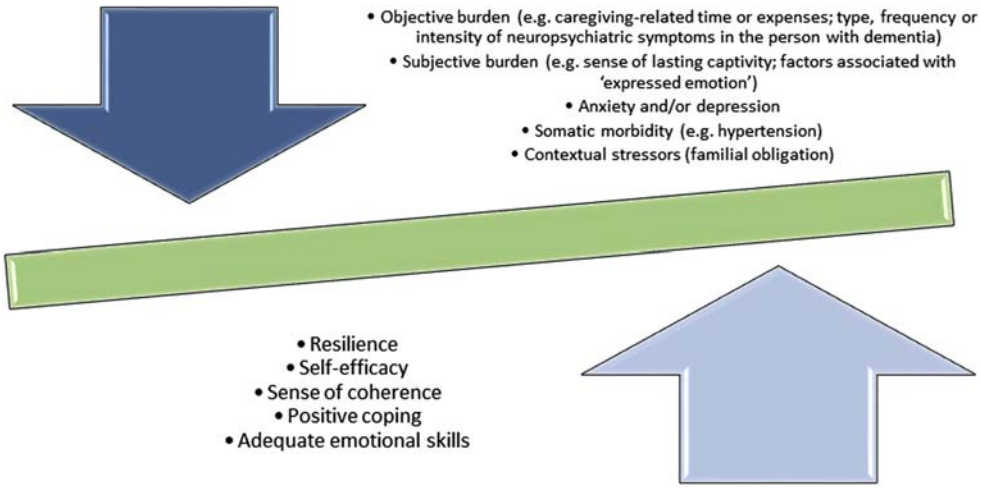

Figure 45.1 Stress-related and protective factors among dementia caregivers.

of caregiver burden and depression than the patient's cognitive symptoms. Besides the impact on emotional connections between caregiver and care-recipient, behavioral disturbances also interfere with care routines (e.g., resisting bath), further adding to caregiver difficulties (Cheng, 2017). As dementia progresses, more time must be spent caring for activities of daily living.

Although countries vary in terms of support provided to family caregivers, the stress they face is indeed universal. In a sample of 1133 primary caregivers in South Korea, over one-third reported putting in more than 8 h/day to support a relative with dementia (Park, Sung, Kim, Kim, & Lee, 2015). By comparison, a European study of 1181 caregivers found a nearly dose-dependent relationship between dementia severity and caregiving hours, with 20%, 39%, and 50% of caregivers spending over 10 h per day to take care of someone with mild, moderate, and severe dementia, respectively (Georges et al., 2008). This suggests that most caregivers in the community are physically tied to the caregiving situation regardless of the socioeconomic (and cultural) context they are in. Needless to say, the number of hours providing care is a well-established contributor to caregiver burden and depressive symptoms (Pinquart & Sörensen, 2003b), although a distinction between objective and subjective components of caregiver burden must also be made.

Indeed, a sense of lasting captivity is a common feeling among caregivers (Givens, Mezzacappa, Heeren, Yaffe, & Fredman, 2014), often accompanied by activity restriction (Mausbach et al., 2011) and lack of self-care, as in Maria's example. There are other ramifications when taking up the role of a caregiver, such as role conflicts, making sacrifices, disturbed sleep, and dealing with decision dilemmas or family disputes. Caregivers frequently feel a loss of control over their lives as well as over the care-recipient's behaviors. Helplessly, they grieve over the loss of their loved one, now seemingly a stranger, to dementia. Despite

how much sacrifice caregivers have made, many, especially adult children, blame themselves for not having done more or when something wrong happens (Losada et al., 2010; Losada, Márquez-González, Peñacoba, & Romero-Moreno, 2010).

Out of frustration, caregivers may also episodically express anger and even abusive language (Coon et al., 2003), only to afterward feel regretful or ashamed about the way they have acted. The sense of guilt and self-contempt may further contribute to depressive cognitions/symptoms (Losada et al., 2010; Losada, Márquez-González, Peñacoba, & Romero-Moreno, 2010). A related construct is expressed emotion (EE), describing unhelpful caregiver behaviors and emotions that have a potential influence on health outcomes, generally interpreted as arising from difficult interpersonal interactions in chronic disease (Safavi, Berry, & Wearden, 2017). EE could also be viewed as an emotion-regulation strategy by which negative emotions aroused by caregiving are projected to the care-recipient. When EE was first studied in families of people with schizophrenia, it was characterized by criticism, hostility, and emotional overinvolvement toward the care-recipient (Brown & Rutter, 1966). In the context of dementia caregiving, some researchers have suggested that EE is more likely to consist of criticism and hostility rather than emotional overinvolvement (e.g., Yu, Kwok, Choy, & Kavanagh, 2016), mainly because caregivers are usually adult children or spouses instead of parents (as in schizophrenia). However, as illustrated in our case, dementia caregivers can display emotional overinvolvement (e.g., overprotective behaviors) as well. Anyway, higher EE in terms of hostility and criticism was associated with greater perceived burden, higher depression, and poorer self-reported health in caregivers (Li & Lewis, 2013). As in schizophrenia, criticism and hostility may signal caregiver exhaustion (Maria, in our example, also started to experience depressive symptoms) and proneness to give up. A recent meta-analysis reported that high-EE caregivers, despite being a minority, show greater vulnerabilities to caregiving burden, depression, more dysfunctional coping, less social support, and a poorer relationship with the care-recipient (Safavi et al., 2017). They also tended to attribute dementia-related problems to the person themselves as potentially controllable issues. It is now a strong working hypothesis that the unfavorable attitude underlying a caregiver's EE may elicit more neuropsychiatric symptoms in the care-recipient that could in turn exacerbate the caregiver's EE (Gonçalves-Pereira, Marques, & Grácio, 2017).

The influence of cultural values

Furthermore, cultural values may determine how caregivers take up the caregiving role as well as the stress they face (Knight & Sayegh, 2010). For example, in Asian cultures with a strong tradition of filial responsibility for parents, adult children are more likely to serve as the primary caregivers even when care-recipients have living spouses. On a broader level, research has suggested, contrary to common expectation, that caregiving in collectivistic cultures may be more stressful than in individualistic cultures. Collectivistic cultural

values such as familism may place caregivers at higher risk by demanding their sacrifice and devotion. Such values may also predispose a caregiver to dysfunctional thoughts such as blaming oneself for not trying hard enough or for feeling frustrated by the care-recipient (Losada et al., 2010; Losada, Márquez-González, Peñacoba et al., 2010)

As illustrated by our Maria, devoted caregivers may become inflexible about what is good for the care-recipient, setting yardsticks that make it difficult for others to follow and exerting tremendous pressure upon themselves and those around them. Complicating the matter are the often unfulfilled expectations, under the influence of familism, that other family members should share the responsibilities (Knight & Sayegh, 2010). When such deeply held wishes are not met, consequences can be substantial. For instance, Chinese caregivers were found to sever ties with relatives (including 40% of their biological children, even very young), leading to strikingly restricted support systems (in this study, network members totaled three for spouse caregivers and five for adult child caregivers) and gross dissatisfaction with the support they received (Cheng, Lam, Kwok, Ng, & Fung, 2013b). The authors attributed this to disappointment and emotional detachment arising when expected support from close relatives does not materialize as well as to family conflicts concerning the patient's care. In collectivistic cultures, individuals may shoulder the responsibilities of caregiving for the collective well-being of the family and end up isolated and disappointed when no family support is received whatsoever. More research is needed to find out how culture and society affect caregiver coping and adaptation.

Caregiver protective factors

Despite the detrimental influences of caregiving stress, many caregivers have reported some benefits of caregiving, such as personal growth, a sense of fulfillment, and enhanced bonding with the care-recipient (e.g., Cheng, Mak, Lau, Ng, & Lam, 2015; Yu, Cheng, & Wang, 2017), even coexisting with hardship in dealing with dementia-related problems (Cheng, Lam, Kwok, Ng, & Fung, 2013a; Gonçalves-Pereira et al., 2010). The stress process model by Pearlin et al. (1990) articulates various primary (e.g., problematic behaviors and functional impairments of the care-recipient) and secondary (e.g., family conflicts, role strain, financial difficulty) stressors whose effects on outcomes are mediated by caregiver personal resources (e.g., mastery, optimism, and coping skills) and social support. Variants of this model, most notably the "appraisal model" (Lawton, Moss, Kleban, Glicksman, & Rovine, 1991), highlight the importance of subjective appraisals in explaining why caregivers respond differently to similar situations. Indeed, by altering caregiver appraisal toward positive gains, a benefit-finding intervention developed by Cheng and colleagues was found to reduce caregiver burden and depressive symptoms in two randomized controlled trials (Cheng, Fung, Chan, & Lam, 2016; Cheng et al., 2017). These findings suggest that instead of the pervasive single focus on adversity, attention

should be paid to caregivers' personal strengths and positive experiences. There is no doubt that some of these strengths are present prior to assuming the caregiving role, as somehow "constitutional." However, evidence on positive aspects of caregiving (Cheng et al., 2015) and posttraumatic growth (Leipold, Schacke, & Zank, 2008) suggests that some strengths could also be fostered through caregiving. In the current chapter, we would also like to focus on a variety of protective factors that have been widely studied in dementia caregiving research, including resilience, self-efficacy, SOC, and emotion-regulation strategies.

Resilience is one of the contributing factors to individual differences in health outcomes when facing similar caregiver situations. Characterized by adaptive capacities or a positive trajectory of functioning, resilience enables caregivers to adapt to a rather stressful situation reasonably well (Dias et al., 2015). Note that being resilient does not mean that one experiences no stress or difficulties; in fact, emotional pain and distress are common and widely accepted among resilient individuals, which may even lead to better adaptions to later stresses (Rutter, 2006). This was the case with Anna, as illustrated above. Meanwhile, instead of an "extraordinary" trait only found among certain people, resilience refers to a repertoire of behaviors that can be developed in anyone. As identified by a systematic review (Dias et al., 2015), resilience factors include task-focused coping strategy, optimism, self-efficacy, internal locus of control, commitment to life, psychological flexibility, and social support. In short, resilience is a constellation of qualities with potentially protective effects. However, most research on caregiver resilience has focused on a single factor such as coping (Wilks, Little, Gough, & Spurlock, 2011), with little attention paid to synthesizing various inner strengths and resources. Trying to fill this gap and inspire future research, we hereby integrate the research on caregiver personal qualities that buffer against the negative impacts of caregiving. Although we still do not understand these strengths as much as we do the vulnerability factors, they are likely outgrowths of the challenges of caregiving, embedded in reflections of underlying personality factors.

Self-efficacy was found to mitigate the detrimental effects of caregiving on mental health. Self-efficacy in caregiving was defined as one's beliefs about his or her capacity of successfully performing desirable actions to cope with caregiving demands (Rabinowitz, Mausbach, & Gallagher-Thompson, 2009). It serves as a mindset that channels caregiver appraisal and coping into positive styles (Steffen et al., 2018). Caregivers with high self-efficacy are more likely to tackle challenging tasks (e.g., managing disruptive behaviors), master the complexity of providing care for someone with dementia, and develop new sets of skills when the old ones become inadequate. Different domains of self-efficacy have been examined, including self-efficacy in obtaining respite, responding to disruptive behaviors, and controlling upsetting thoughts (Steffen et al., 2018). Self-efficacy in dealing with disruptive behaviors was found to mitigate the effect of a patient's memory and behavioral problems on caregiver depression (Rabinowitz et al., 2009). Other studies

have supported the moderating role of self-efficacy in controlling the caregiver's own upsetting thoughts. Cheng et al. (2013a,b) found that when confronted with more neuropsychiatric symptoms, caregivers with high self-efficacy in controlling upsetting thoughts reported more gains and less burden. Romero-Moreno et al. (2011) also found that self-efficacy in controlling upsetting thoughts was associated with less depression and anxiety. It appears that self-efficacy can reduce caregiver vulnerability to burden and depressive symptoms while enhancing resilience through positive gains. Self-efficacy probably triggers the implementation of adaptive self-regulation strategies curbing automatic dysfunctional thoughts, thus attenuating the detrimental impacts on mental well-being. In our case, Joseph's daughter apparently believes that she will be able to conciliate her demanding job with helping her parents by relying on her own strengths. This relates to self-efficacy but also to a sense of meaning and purpose in life that leads us to the following point.

SOC describes a dispositional tendency or resource that promotes successful coping and maintenance of well-being under stressful situations. SOC consists of three components: comprehensibility, manageability, and meaningfulness (Antonovsky, 1987). It was found that with higher SOC, caregivers reported lower burden (Orgeta & Sterzo, 2013), less anxiety and fewer depressive symptoms (Välimäki, Vehviläinen-Julkunen, Pietilä, & Pirttilä, 2009), and more adaptive coping (Andrén & Elmståhl, 2005). SOC can also influence caregivers' appraisal and coping processes. Meaning-making could be viewed as a coping mechanism (Pearlin, Mullan, Semple, & Skaff, 1990) through which caregivers interpret the caregiving situation as stressful yet worthwhile. Indeed, by testing a regression model including resilience, optimism, and SOC, Sutter et al. (2016) found that independent of the effects of trait resilience and optimism, SOC was associated with reduced burden and increased satisfaction (Sutter et al., 2016). An exploratory study in couples suggested that good relationship quality, either current or prior to the onset of dementia, may cultivate a caregiver's SOC by enhancing "manageability" (problems become bearable) and "meaningfulness" (difficulties are viewed as challenges) (Marques & Gonçalves-Pereira, 2016). While associations between caregiver SOC and relationship quality in dementia are under exploration, SOC is acknowledged as potentially health-promoting in different contexts (Lindström & Eriksson, 2006). For a brief review on the role of SOC in dementia caregiving and the related working hypothesis, see Marques & Gonçalves-Pereira (2014), Gonçalves-Pereira et al. (2017).

As a manifestation of resilience, self-efficacy, and SOC, **positive coping strategies** should be considered in more detail (see Fig. 45.2). Research on **coping** usually focuses on two main types of strategies, emotion-focused and problem-focused. The former refers to the cognitive processes lessening emotional distress, including avoidance, distancing, and selective attention. Problem-focused strategies aim instead at changing the problem itself, including active coping, seeking instrumental support, or planning. Classically, problem-solving subsumes six stages (defining the problem, generating

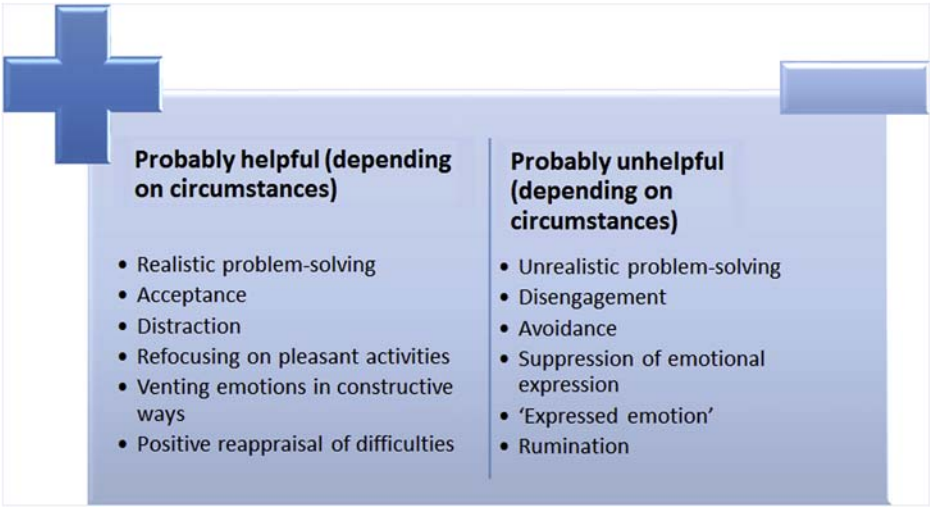

Figure 45.2 Examples of coping strategies and emotional skills among dementia caregivers.

alternative solutions, weighing costs and benefits, choosing solutions, implementing solutions, and reevaluating). Both strategies can be beneficial, depending on circumstances, but most empirical findings suggest problem-focused strategies (e.g., instrumental coping) and acceptance (a particular emotion-focused strategy) are more adaptive and associated with, for example, greater positive affect and adjustments (Kneebone & Martin, 2003). Distraction was also found to be associated with lower levels of "negative" EE (i.e., more positive remarks and fewer criticisms) among caregivers (Bledin, MacCarthy, Kuipers, & Woods, 1990). Furthermore, interventions that train caregivers to use positive reappraisal (i.e., reinterpreting the situation in a positive light) have been effective in reducing caregiver burden and depressive symptoms (Cheng et al., 2016; Cheng, 2017). What is perhaps key to successful coping is how individuals perceive the situation as manageable, construct positive beliefs about themselves and the situation, and drive realistic problem-solving along with a positive outlook.

On the other hand, certain styles—e.g., passive, avoidance, and disengagement coping—were associated with more burden, anxiety, and depressive symptoms among caregivers (Cooper, Balamurali, & Livingston, 2007). In our case, Maria exemplifies that rigidly sticking to problem-solving strategies when the "problem" (as defined) is unsolvable (dementia has no cure) leads to frustration and negative outcomes. She simultaneously turns down opportunities related to adaptive emotion-focused coping—e.g., sharing her feelings and relying on emotional support.

Recent research has paid increasing attention to a coping style called **experiential avoidance**, referring to the unwillingness to remain in contact with particular private experiences (e.g., bodily sensations, emotions, and thoughts), and to take steps to modify

the form or frequency of these events and the contexts that occasion them (Losada, Márquez-González, Romero-Moreno, & López, 2014). Experiential avoidance incorporates strategies such as avoidant coping, distancing, and suppression by which caregivers avoid getting in touch with their thoughts and feelings, usually negative, about the situation. Three dimensions of this construct were described in the caregiving context: active avoidant behaviors, intolerance of negative thoughts and emotions toward the care-recipient, and apprehension concerning negative internal experiences related to caregiving. In particular, greater caregiving demands were related to higher apprehension levels, while overall experiential avoidance was positively correlated with anxiety, dysfunctional thoughts, and alexithymia (Losada et al., 2014).

On the contrary, and returning to our case, Anna (the daughter) has balanced problem-focused and emotion-focused coping in adaptive ways throughout her life path. She seems able to positively reframe part of her (or her family's) difficulties ("positive things happened, anyway"), and this is being fostered by what might be interpreted as a trend toward optimism (dispositional optimism, a trait characteristic that is also protective, Rasmussen, Scheier, & Greenhouse, 2009).

In addition to coping, caregiver **emotional skills** (or lack of them) are also important predictors of well-being (or burden and distress). Those using inappropriate emotion-regulation strategies such as suppressing the open expression of negative emotions are more prone to experience negative emotions inwardly and report emotional exhaustion (Bassal, Czellar, Kaiser, & Dan-Glauser, 2016). On the other hand, emotion-regulation strategies also help to explain the effects of perceived stress in positive emotions (Yildiz, 2017). In particular, "internalizing" dysfunctional emotional regulation strategies (e.g., rumination) would lead to increased negative affect, while more functional strategies (e.g., refocusing on pleasant aspects) were associated with increased positive affect.

Concluding remarks and future research

After reviewing caregiver sources of stress and positive personal resources influencing the outcomes of caregiving, several observations can be made. First, both objective stressors and subjective burden can be detrimental to caregivers as determinants of mental or physical problems. Second, recent findings suggest that cultural factors such as familism can aggravate caregiving stress. Third, despite all the risks described, numerous persons can still respond adaptively to caregiving demands, often attaining personal growth in the long term. To explain this discrepancy in caregiving outcomes, we discussed a range of protective factors, including resilience, self-efficacy, SOC, adaptive coping, and emotion-regulation strategies. With considerable overlap, these constructs share a complex interplay rather than unidirectional "causal" relationships. Furthermore, as a manifestation of individual qualities, caregiver coping style determines the strategies adopted in specific situations, further influencing health outcomes.

Meanwhile, other dispositional attributes not covered in this chapter may also play significant roles in caregiver health. For example, related personality traits—e.g., high extraversion and agreeableness as well as low neuroticism—were found to be associated with lower caregiver burden (Melo, Maroco, & de Mendonça, 2011) Also, though only briefly mentioned here, emotional dispositions—e.g., optimism—could also be important personal strengths predicting caregiver well-being (Sutter et al., 2016). Finally, most of these findings were obtained in families of people with AD and caregivers of people with other dementia subtypes (e.g., frontotemporal dementia) may differ. Future studies should compare the protective and risk factors for different groups of caregivers.

With a better understanding of determinants in dementia caregiving, more insights can be acquired for screening high-risk caregivers and developing intervention programs to promote protective factors. Adopting family-oriented approaches to complement those that are person-centered in health or social services may help reinforce resilience not only in informal caregivers but systemically in the family or other close networks as a whole (Gonçalves-Pereira, 2017).

Key facts of dementia caregiving

- Compared with noncaregivers, dementia caregivers generally report higher stress, lower subjective well-being, and poorer physical health.
- Over one-third of caregivers have clinically significant depression and/or anxiety.
- Caregivers generally find disruptive behaviors (e.g., agitation/aggression, irritability, disinhibition, and aberrant motor behavior) more disturbing than other neuropsychiatric symptoms.
- Not all caregivers fare poorly; some harbor strengths that enable more positive functioning.

Summary points

- Sources and impacts of caregiving stress and burden are reviewed.
- Caregiving is associated with physical and mental disorders.
- Caregiving stress is related to neuropsychiatric symptoms of dementia, longer caregiving hours, lasting captivity, EE, and familism.
- Protective factors include resilience, self-efficacy, SOC, adaptive coping, and emotional skills.
- The protective factors of caregivers may lead to positive outcomes for caregiving.

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PART IV

Diet, nutrition and environment

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CHAPTER 46

Nutritional status of dementia and management using dietary taurine supplementation

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List of abbreviations

AD Alzheimer disease
DHA docosahexaenoic acid
EPA eicosapentaenoic acid
KDRIs Dietary Reference Intakes for Koreans
MMSE-DS Mini Mental State Examination-Dementia Screening
PUFA polyunsaturated fatty acid
UN United Nations
WHO World Health Organization

Mini-dictionary of terms

CAN-pro 4.0 This was a program developed by the Korean Nutrition Society for the purpose of nutritional evaluation of individuals or groups. It analyzes the nutrients of all foods consumed by individuals.

Day care facility It is a facility where patients only stay during the day, unlike long-term care facilities. It is mainly used by patients with mild dementia.

Long-term care facility This is a facility where patients live 24 h a day, such as geriatric hospital or nursing home. It is mainly used by patients with severe dementia.

MMSE-DS This is an easy and simple dementia screening questionnaire and is the most widely used method of assessing cognitive function. It consists of a total of 19 questions and 30 points. It is evaluated relative to the criteria score, and a high score means that the cognitive function is good.

Taurine index It is a score of the past intake of taurine-containing foods and supplements. The index assesses a score according to the taurine content of the food and supplements (one to three points) and scores it with a five-point scale of the frequency of intake. Multiplying the two scores is the taurine index.

Introduction

Dementia is increasing due to a rapidly ageing global population (Mio et al., 2013). Although the most powerful risk factor for dementia is age, dementia is not the result of the normal ageing process and is thus hard to predict. Early onset dementia, which begins before the age of 65, now constitutes about 9% of total dementia cases (World Health Organization, 2017a).

Genetics, Neurology, Behavior, and Diet in Dementia
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Dementia is a chronic disease caused by various brain dysfunctions (Alzheimer's Disease International, 2017). Alzheimer' disease (AD), the most common neurodegenerative disease, accounts for 60%—70% of dementias (Barberger-Gateau et al., 2007). In addition to AD, there are various forms of dementia such as vascular dementia, alcoholic dementia, and frontal dementia, but they are difficult to distinguish or often develop simultaneously (World Health Organization, 2017a).

According to the World Health Organization, about 50 million people worldwide now suffer from dementia, and nearly 60% live in low- and middle-income countries (World Health Organization, 2017a). As Fig. 46.1 shows, dementia is more prevalent in women than in men in most countries. In particular, more than 60% of dementia patients were found to be women in Canada (65%), Mexico (64%), and South Africa (75%). There are a large number of dementia cases in China, which accounts for 20% of all dementia patients worldwide. It is estimated that the prevalence of dementia will greatly increase by 2030 in all countries (Martin, Adelina, Martin, Maelenn, & Maria, 2016; World Health Organization, 2017b).

Currently, there is no treatment to eliminate the progress of dementia (Okubo et al., 2017). Despite decades of study, the fundamental mechanism of dementia is still not perfectly understood, and various treatments are being studied in clinical tests (Virginia, Chiara, Marta, & Patrizia, 2017). It is necessary to obtain accurate evidence-based information of the nutritional status of dementia in patients in order to achieve successful management of the disease. Therefore, the purpose of this review is to deal with the nutritional problems of dementia and management strategies using dietary taurine supplementation.

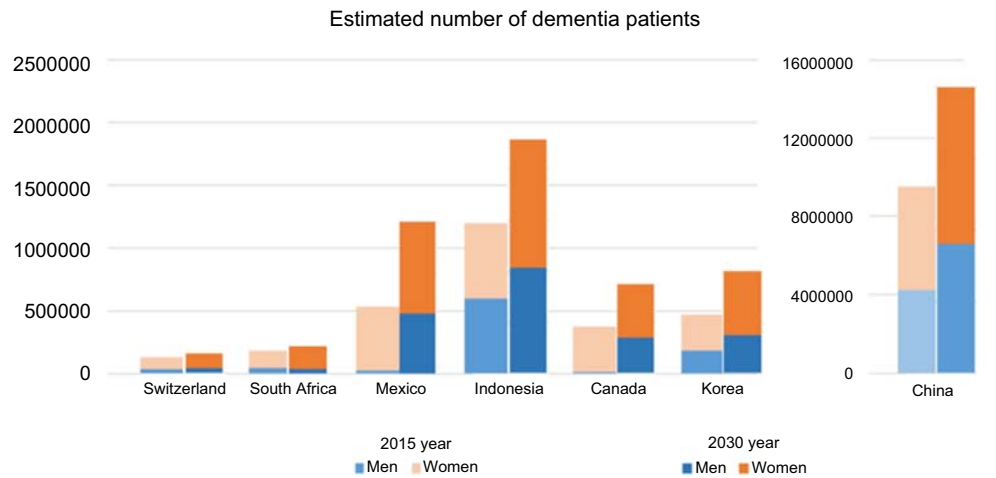

Figure 46.1 Estimated number of dementia patients in the World Alzheimer report. The number of dementia patients (men and women) in 2015 is compared with the estimated number of dementia patients in 2030. China is shown separately in the graph because of the large estimated number of dementia patients.

Nutritional problems of dementia

Most dementia patients have nutritional and dietary problems, and nutritional deficiency (malnutrition) is common (Christina et al., 2010). Elderly with dementia gradually develop trouble in putting food into their mouths, chewing, and swallowing (Lee & Song, 2012). Dementia is associated with a lack of psychological and emotional stability, and patients show specific behaviors such as refusing to eat or overeating (Jung, Lee, Kim & Chang, 2008). Previous studies have shown that elderly with dementia show problematic eating habits such as decreased or increased food intake, changes in intake frequency, improper use of meal utensils, and altered food choices (Gabriele et al., 2016).

Nutritional disorders and weight loss are common in the early stage of dementia (Holm & Soderhamn, 2003). Previous studies have shown that patients with mild cognitive impairment and dementia suffer from greater malnutrition or lower nutritional intake than normal cognitive patients, and nutritional status is worse in older patients (Burns, Johnson, Watts, Swerdlow & Brooks, 2010; Giuseppe et al., 2009). Weight loss is associated with reduction of food intake, such as eating disorders and loss of appetite, as well as severity of dementia, but the cause is still unknown (Emiliano et al., 2013; Holm & Soderhamn, 2003).

Previous studies reported that dementia patients aged 70–75 years who underwent at least a 10% decrease in weight were at a significantly higher risk of death over the next 5 years than patients with a stable weight and who showed less than 5% weight loss (Day, Rothenberg, Sundh, Bosaeus & Steen, 2001). Weight loss in AD patients may be associated with disease progression and is a predictor of mortality, depending on the severity and progress (White, Pieper & Schmader, 1998).

The nutrient intake of dementia patients typically decreases as the disease becomes more severe (Holm & Soderhamn., 2003; Orsitto et al., 2009). Fig. 46.2 shows the status of nutrients intake in long-term care facilities where patients with severe dementia are mostly hospitalized and day care facilities where mild dementia patients are living. The nutrient intake of the elderly with dementia in long-term care facilities was lower than that in day care facilities, and most nutrients including energy were lower than the recommended standard (KDRIs). It was also found that the elderly with dementia in both facilities had very low intake of both vitamin D and magnesium (Kim, 2017). As shown in the study, the elderly with dementia may have loss of self-eating ability within about 8 years; mild dementia patients in day care facilities are also likely to gradually develop nutritional imbalances, therefore, nutritional management for them will be continuously necessary (Volicer et al., 1987).

Elderly people with dementia suffer from dehydration, which is a dangerous factor and is reported as the second leading cause of death in elderly people with dementia (Lee et al., 2012). Drinking a moderate amount of water is a good way to promote blood circulation in the brain. In particular, the elderly tend to suffer from chronic dehydration as the thirst regulatory function of the hypothalamus declines, and elderly people with dementia are more likely to be dehydrated since they are unaware of their need for water (Han, 2000).

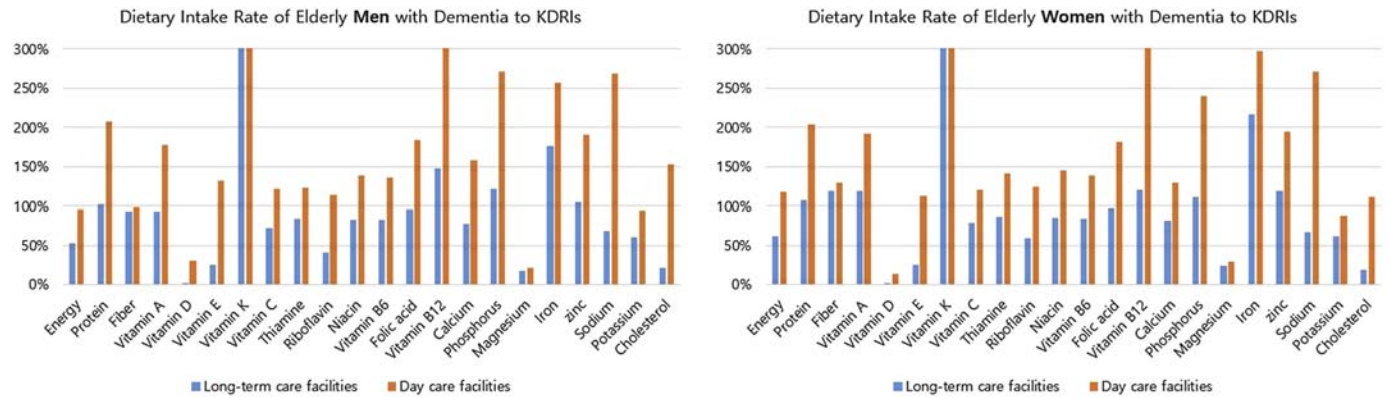

Figure 46.2 Dietary intake rate of the subjects to KDRIs. This graph shows the daily nutrient intake rate in the elderly with dementia in long-term care facilities and day care facilities. It was analyzed using the CAN-pro 4.0.

It was previously reported that anemia is a risk factor for cognitive decline and dementia (Hong et al., 2013). Chronic hypoxia associated with anemia partially promotes cognitive decline by accelerating the accumulation of β-amyloid in the brain (Jeong et al., 2017). The elderly with anemia are at 49% higher risk of dementia than the elderly without anemia. As anemia causes the brain to lose oxygen, it is important to determine the cause of anemia quickly since nerve cells can become damaged, resulting in cognitive impairment and dementia (Hong et al., 2013).

It is very important to satisfy the nutritional needs of dementia patients and detect early nutritional problems (Bulent, Omer, Gulistan, Nilgun, & Karan, 2010; Emiliano et al., 2013). Although there is no perfect treatment for dementia, positive and healthy food intake can improve brain health and reduce the risk of dementia (Alzheimer's Disease International, 2017). However, eating disorders, such as insufficient food intake of dementia patients, increases the physical and psychological burden on the caregiver and family as well as the patient (Gabriele et al., 2016; Lee et al., 2012). The families (caregivers) of dementia patients are referred to as 'invisible second patients," and they often have poor quality of life in addition to mental health (Brodaty & Donkin, 2009). However, nutritional intake of dementia patients is almost entirely dependent on the caregiver. Therefore, systematic management by the caregiver has a positive effect on the nutritional status of dementia patients (Han, 2000; Jung et al., 2008).

Nutritional management of dementia

There are ongoing efforts to determine which foods should be eaten or avoided in order to prevent dementia. This review focuses on antioxidant vitamins and dietary fatty acids, which have been widely studied in relation to dementia. Of particular interest, taurine has been studied in the context of nutritional management of dementia (Barberger-Gateau et al., 2007).

Antioxidant vitamins

Oxidative damage and stress caused by free radicals has been detected in the brains of AD patients. Oxidative damage contributes to the degeneration of nerve cells and hinders structural and functional homeostasis, making it difficult to maintain brain function (Luchsinger, Tang, Shea, & Mayeux, 2003). Oxidative stress promotes accumulation of β-amyloid, which is known to produce reactive oxygen species that are toxic to neurons (Misonou, Morishima-Kawashima & Ihara, 2000).

Administration of antioxidant nutrients is expected to reduce the degree of oxidative damage and stress and delay the reduction of cognitive function in AD. Many studies have shown that antioxidant intake decreases cognitive impairment due to ageing as well as the onset of dementia (Cho, 2006; Alzheimer's Disease International, 2017). Antioxidants protect cells from neurotoxins caused by β-amyloid and delay cognitive

deterioration in patients with severe dementia (Cho, 2006). More than 3000 elderly Japanese people living in Hawaii have shown a reduced risk of vascular dementia as a result of vitamins C and E supplementation (Masaki et al., 2000). In a study on 4000 people aged 45 years or older, supplementation with vitamins C and E reduced the risk of AD (Zandi et al., 2004). Thus, supplementation of antioxidant vitamins is associated with reduced onset of AD by potentially reducing the deposition or toxicity of β-amyloid, which results in death of nerve cells (Luchsinger, Tang, Shea & Mayeux, 2003).

Antioxidant vitamins such as vitamins A, C, and E are typical antioxidant nutrients that can respond to oxidative stresses in AD, and they have potential positive effects on nerve damage (Luchsinger et al., 2003). Retinol, the active form of vitamin A, protects the cell membrane against free radicals and inhibits production of fatty acid oxidation or lipid peroxides (Mettlin, 1984). Vitamin C, a typical water-soluble antioxidant, has the ability to directly remove reactive oxygen species (Frei, Stocker & Ames, 1989). Vitamin E is a typical lipid-soluble antioxidant that is known to inhibit lipid peroxidation by eliminating reactive oxygen species (Handelman, Packer & Cross, 1996; Packer, 1991). Although these antioxidant vitamins play important independent roles, they increase antioxidant function by interacting with each other to eliminate oxidative stress (Niki, Noguchi, Tsuchihashi & Gotoh, 1995). A previous study on elderly men reported that when vitamins E and C were supplemented together, cognitive decline was abrogated (Masaki et al., 2000).

Antioxidant vitamins are recommended to be consumed moderately and frequently with foods (vegetable oil, margarine, nuts, apples, melons, berries, avocado, etc.) rather than with high doses of medicines (Central Dementia Center, 2013). Dietary antioxidant intake reduces the risk of stroke associated with high-risk factors in AD by reducing oxidative damage caused by free radicals, and it has the beneficial effect of suppressing neurodegeneration (Jose & Richard, 2004; Okubo et al., 2017). These foods may be more important for the elderly with high antioxidant needs. However, the elderly have fewer chances of eating raw vegetables due to the effects of physical ageing such as tooth loss and reduced digestion ability. There is great concern about antioxidant and vitamin deficiency in foods since many modern foods are cooked with excessive heat treatment, which may destroy nutrients (Han, 2000).

Dietary fatty acids

Cerebral tissue is mainly made of lipids, and docosahexaenoic acid (DHA) is the most abundant omega-3 polyunsaturated fatty acid (PUFA) in the cerebrum (Cho, 2006). Omega-3 fatty acids are the main components of brain cell membrane phospholipids, and they affect the growth and synaptic formation of nerve cells. Fatty acids are involved in interactions between neurons and thus affect cognitive function. They have antiinflammatory characteristics, which can explain their long-term protective effect against

dementia (Linschee et al., 1994, pp. 47–88). Previous studies have shown that the concentration of DHA in the hippocampus of the brain and spinal of AD patients is lower than that of normal people (Prasad, Lovell, Yatin, Dhillon & Markesbery, 1998; Soderberg, Edlund, Alafuzoff, Kristensson & Dallner, 1992).

Intake of lipids (fatty acids) is known to be associated with risk of dementia through various mechanisms such as cardiovascular disease, arteriosclerosis, thrombosis, inflammation, brain development, and accumulation of β-amyloid (Cho, 2006). In particular, omega-3-PUFAs, including EPA and DHA, have shown positive results that can reduce the risk of dementia through many studies as a calibrating factor for cognitive decline or the onset of dementia (Engelhart et al., 2002; Kalmijn et al., 1997).

A previous study on the elderly in Japan showed that a diet rich in plant foods and fish is positively related to cognitive function. Consumption of green vegetables, legumes, seaweed, mushrooms, potatoes, fruits, fish, and green tea can prevent ageing-related cognitive decline (Okubo et al., 2017). Previous studies showed that omega-3 fatty acids in fish inhibit β-amyloid accumulation in the brain and reduce risk of dementia (Barberger-Gateau et al., 2007; Jin & Jeon, 1999; Kalmijn, 2000). A mouse model study showed that the level of β-amyloid was reduced by more than 70% in rats that consumed a high amount of DHA compared to rats that consumed a lower amount DHA (Lim et al., 2005). There is a positive relationship between memory and intake of fish and shellfish, and the elderly with dementia have been recommended to eat fish containing omega-3 fatty acids rather than saturated fat (Jung, Lee & Kim, 2008).

In PAQUID (Personnes Age'es QUID) cohort studies, risk of dementia was found to be significantly reduced in a 7-year follow-up on regular fish consumers, which could be attributed to the effect of omega-3 fatty acids (PUFA) (Barberger-Gateau et al., 2007). Omega-3 fatty acids contained in fish oil can protect blood vessels as well as reduce inflammation of the brain (Luchsinger, Tang, Shea & Mayeux, 2002). If DHA intake is low, onset of dementia including AD, is high. Intake of DHA is effective in improving symptoms of patients with severe dementia. In addition, high intake of total fat, saturated fat, and cholesterol increased the onset of dementia, whereas risk of dementia decreased in the group that consumed a lot of fish (Cho, 2006).

Diets known to have positive effects on cognitive decline or prevention of dementia are based mainly on dietary patterns than single nutrient or foods. In particular, the Mediterranean diet recommends high intake of fruits, vegetables, fish, nuts, and legumes and low intake of saturated fats and high-fat dairy products such as red meat and butter (Mio et al., 2013). In many Western countries, previous studies have shown that the Mediterranean diet prevents dementia, and higher intake of vegetables, fruits, and fish lower risk of dementia. However, it is not desirable to apply the Mediterranean diet pattern in general since it may not be common or not accessible for Asians (Mio et al., 2013).

High intake of calories and fat can increase oxidative stress, cause cardiovascular disease, and are associated with risk of AD (Jose & Richard, 2004). Saturated fatty acids

and cholesterol increase the risk of vascular dementia by increasing the risk of cardiovascular disease and arteriosclerosis (Cho, 2006). In a 4-year follow-up study in the United States, high-calorie and low-calorie groups were examined to determine the relationship between AD onset and calorie intake. Those who in the high-calorie group were at a 1.5-fold higher risk of developing AD than those in the low-calorie group. This shows that eating less may help prevent dementia (Central Dementia Center, 2013; Luchsinger et al., 2002). As a result, it is desirable to reduce intake of saturated fat, lower risk of vascular disease, and increase consumption of monounsaturated and polyunsaturated fats and fish (Luchsinger et al., 2002).

Taurine

Taurine, a sulfur-containing free amino acid, is present in retina, skeletal muscle, and the heart, and especially in the brain at high concentrations (Kendler, 1989). Taurine is not only harmless to the human body but also plays a diverse and important physiological role in promoting growth and development of skeletal muscle cells, maintaining the immune system, development of the retina and nervous system, antioxidant activity, fatigue recovery, and blood pressure stabilization (Grimble, 2006). It plays an important role in brain function and is known to be effective in neuroprotection and cognitive improvement in various types of dementia (Barthel et al., 2001). When hypoxic or oxidative stress is applied to nerve tissue, taurine inhibits the toxic effects of excitatory neurotransmitters (Grimble, 2006). It was reported that Alzheimer's patients have 25% lower taurine concentration in the central nervous system compared to normal people (Alom, Mahy, Brandi & Tolosa, 1991).

Taurine is known to be safe, and one of the characteristics that differentiates it from other amino acids is that reduction of absorption rate, growth inhibition, and other side effects were not reported even if it is consumed in excess. Also, it easily penetrates the blood–brain barrier, and it is effective even when consumed in foods (Yoon, Choi & Shin, 2015).

Taurine is mostly obtained from seafood such as shellfish and fish. Taurine content among seafood was mostly high in mollusks such as webfoot octopus, beka squid, and long-arm octopus (around 1300, 700, and 600 mg taurine per 100 g, respectively) and shellfish such as ark shell, little neck clam, and hard-shelled mussel (around 1100, 900, and 800 mg taurine per 100 g, respectively). It had a high content in pacific herring and Pacific Ocean perch among fishes (around 400 mg taurine per 100 g, respectively). However, they may differ depending on the habitat and the timing of the catch (Kim, Kim & Moon, 1999). Also, taurine is weakened with high heat and may lose its content during the cooking and drying process. Thus, it is desirable to minimize the taurine loss in the processing of seafood with a high amount of taurine on the skin such as squid (Cho et al., 2000). In addition, taurine is contained in health supplements such as energy drinks and tablets, so it is easy to consume.

In 2014, the Korea Institute of Science and Technology reported improvement of mild dementia in AD-induced mice using cognitive function behavior tests. Taurine was orally administered and was shown to activate glial cells in the brain, restoring memory and learning ability to normal levels (Kim et al., 2014). Taurine was able to reduce cerebral cortical inflammation, which is a symptom of AD progression, as well as the amount of β-amyloid in the hippocampus of the brain. This result could be attributed to the activation of glial cells, which are associated with memory, and supports the specific association between taurine and dementia (Luchsinger et al., 2002). Previous studies reported insufficient taurine levels in the brains of AD patients, and that taurine was shown to have a neuroprotective effect on local cerebral ischemia in rats (Alom, Mahy, Brandi & Tolosa, 1991).

This author investigated that the relationship between taurine and cognitive function by measuring past and present intakes of taurine-containing foods in the elderly with dementia, including AD, and normal elderly. There was no difference in present taurine intake between the elderly with dementia and normal elderly, but in the taurine index, which means past taurine intake, it showed that the elderly with dementia had significantly lower intake than normal elderly (Fig. 46.3). Also, positive association was shown between taurine index and total score of cognitive function. As a result, past taurine intake was shown to have a positive effect on the present cognitive function of the elderly, which implies taurine-rich foods may be recommended to be consumed to prevent dementia (Bae, Gao, Kim & Chang, 2017). Previous study has investigated the effects of dietary taurine supplementation on cognitive function of the elderly with dementia by providing 3 g of taurine once a day for 4 weeks. As a result, the total MMSE-DS score, which means cognitive function after dietary taurine supplementation, increased (Fig. 46.4), especially subscores for "place replacement" and "judgment and abstract thinking" increased

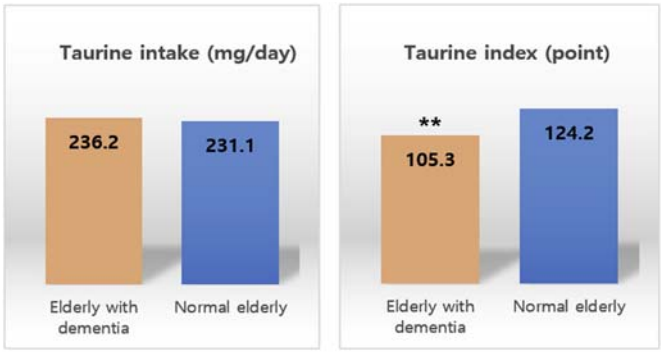

Figure 46.3 Taurine intake of present and past. Taurine intake means present taurine intake by 24-h recall and analysis using CAN-pro 4.0. Taurine index means past taurine intake by scoring the intake frequency of taurine-containing foods.

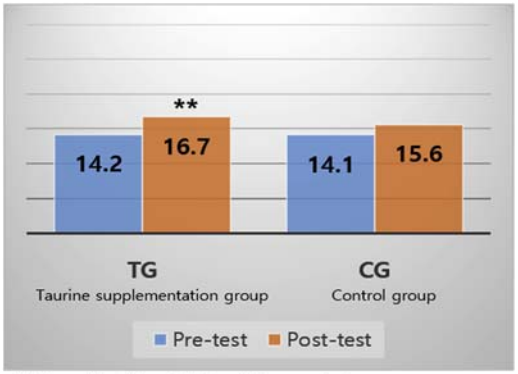

Figure 46.4 Changes of MMSE-DE total scores TG and CG. There was no difference in MMSE-DS scores in the two groups before taurine supplementation, but only TG showed a significant increase after the 4-week supplementation.

(Bae, Gao, Cha, Chang & Kim, 2018). These studies showed that dietary taurine supplementation has a positive effect on the cognitive function of the elderly with dementia and may be a good nutrient for the prevention and treatment of dementia.

The concentration of taurine in the body is determined by the balance between its intake, biosynthesis, and excretion. If the taurine level is insufficient, its reabsorption in the kidneys is increased and excretion reduced. On the other hand, if taurine is too abundant, it is excreted into the urine or bile (Hayes, 1985). According to one study, taurine excretion was reported to have a significant relationship with dementia. Although the taurine contents of dietary intake are almost same, comparison of the amount of taurine excretion between the elderly with dementia and normal elderly receiving the same meals found that taurine was excreted more in the urine of the elderly with dementia (Gao, Bae, Chang & Kim, 2017). Based on this result, the higher urinary excretion of taurine can be an impending sign of dementia, and studies on taurine excretion in the elderly with dementia is considered to be more necessary in the future. The evaluation method in this study might be useful for the diagnosis or prevention of dementia, and it could potentially be a biomarker for dementia.

Conclusion

Nutrition—to be exact, a nutritious diet—is important in the etiology and prevention of cognitive decline and functional damage. Proper nutritional intake directly promotes brain function and neurological health and can prevent or delay neurodegenerative diseases such as dementia (Stewart et al., 2005). As the prevalence of dementia is low in the elderly who have adhered to dietary guidelines for preventing dementia, proper nutritional intake should be the basis for improving quality of their life (Cho, 2006; Kang et al., 2014).

Nutritional management of dementia is difficult to study due to various issues related to the disease, and there are many parts to be studied. As discussed, it is necessary that varied

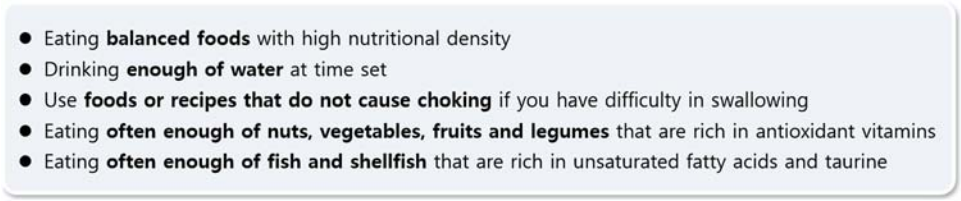

Figure 46.5 Dietary guidelines for dementia. Based on the topics dealt with in this chapter, dietary guidelines for dementia patients are suggested.

and suitable intake of nutrients is maintained to prevent excessive weight loss. Also it is necessary to evaluate the patient's nutritional status periodically, and conduct timely nutritional intervention (Han, 2000). There is no complete method to treat dementia yet, but adequate nutrition and eating habits improve physical status in all kinds of dementia (Cho, 2006). Therefore, it is suggested to follow dietary guidelines for dementia, as shown in Fig. 46.5. In the future, more sophisticated nutritional support strategies should be developed to relieve the suffering of dementia patients and their families (Han, 2000).

Summary points

- Most dementia patients have dietary problems and nutritional deficiency. It is especially severe in the later stages of dementia.
- Antioxidant vitamins such as vitamins A, C, and E are typical antioxidant nutrients that can respond to oxidative stresses in AD, and they have potential positive effects on nerve damage.
- It is desirable to reduce intake of saturated fat, and increase consumption of monounsaturated and polyunsaturated fats and fish.
- Dietary taurine supplementation has a positive effect on the cognitive function of the elderly with dementia and may be a good nutrient for the prevention and treatment of dementia.
- There is no complete method to treat dementia yet, but it is necessary to evaluate the patient's nutritional status periodically, and conduct timely nutritional intervention.

Key facts about taurine

- Taurine is the most widely distributed β − free amino acid in the body.
- Taurine is colorless and relative tasteless.
- Taurine is water soluble and does not dissolve in alcohol or ether.
- Taurine plays various and important roles in the human body, such as maintaining the immune system, developing the retina and nervous system, and activating antioxidants.
- The main source of taurine is animal foods such as fish, shellfish, and meat.
- Taurine was found to be a safe nutrient after testing in laboratory animals and humans for safety.

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CHAPTER 47

Selenium and Alzheimer's disease

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Mini-dictionary of terms

Apolipoprotein E (ApoER2) is a gene with three alleles (ε2, ε3, and ε4). The presence of the ε4 allele is the most important genetic risk for Alzheimer's disease (AD), as one copy of the allele can increase the risk by 2 to 3 times, and two copies can increase the risk by 12 times.

Ferroptosis Programmed cell death pathway dependent on iron and characterized by the presence of lipid peroxidation.

Selenocysteine Identified as the 21st amino acid and is analogous to cysteine with a selenol moiety replacing the thiol.

Selenoproteins Proteins characterized by the translational incorporation of selenocysteine. To date, 25 selenoproteins have been identified in humans.

Selenoprotein P The main selenium transporter in the body, is also responsible for selenium delivery to the brain through interaction with ApoER2.

Selenoproteome Entire set of selenoproteins.

Introduction

Selenium was discovered in 1817 by a Swedish physician and chemist, Jöns Jacob Berzelius. This element was found to be essential to normal health in 1957 when Schwarz and Foltz evidenced that selenium prevented necrotic liver damage in mice (Schwarz & Foltz, 1957). Almost 20 years later, Flohe, Gunzler, & Schock (1973) confirmed the importance of selenium in humans by identifying it as an essential cofactor of glutathione peroxidase (GPx).

Diet is the principal source of selenium, and selenium intake reflects its concentration in the soil where crops and fodder are grown. Selenium concentration can vary from 0.1 to 2 mg/kg depending on geology and environmental compartments (Jones et al., 2017). Seleniferous soils have been identified in parts of China, Canada and the United States (Combs, 2001). Conversely, selenium-poor soils have been detected in New Zealand, Denmark, Finland, parts of Brazil, Russia, and China. As seleniferous areas are less widespread throughout the world, it is estimated that one of seven people is selenium deficient due to low selenium concentration in the soil (Jones et al., 2017). Brazil nuts are the most concentrated selenium food source (Cardoso, Duarte, Reis, & Cozzolino, 2017), although meats, seafood, cereals, grains, milk, and dairy products may also provide reasonable amounts depending on soil conditions (Rayman, 2012).

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Selenium deficiency is associated with several diseases, such as cancer, cardiovascular and immune system disorders, reproduction and thyroid function impairment, and neurodegenerative diseases (Cardoso, Roberts, Bush, & Hare, 2015; Rayman, 2012).

Selenium metabolism

Selenium plays its different roles in the human body through selenoproteins, characterized by the presence of at least one selenocysteine (Sec) residue, recognized as the 21st amino acid. To date, 25 selenoprotein-encoding genes have been identified in mammals, although it is believed that more will be characterized in the future (Cardoso et al., 2015).

As the first step of selenoprotein synthesis, dietary selenium—which can be either organic (mainly selenomethionine and selenocysteine) or inorganic (selenate [SeO_4^{2-}] and selenite [SeO_3^{2-}])—is converted to selenide (Se^{2-}), which is used to synthesize selenophosphate ($SePO_3^{3-}$) for incorporation into proteins via a unique tRNA pathway. Sec is synthesized by a specific tRNA (RNAt[Ser]Sec) that presents a seryl (Ser) residue replaced by Sec. RNAt[Ser]Sec codifies a UGA codon that is usually identified as a stop codon to integrate selenium into the selenoprotein amino acid sequence. Sec is incorporated into the nascent polypeptide chain, where there is a stem—loop structure known as the Sec insertion sequence (SECIS) element at the 3' untranslated region. This SECIS element recruits substrate-binding protein, which captures a specific elongation factor of SeCys and its cognate RNAt[Ser]Sec tRNA (Labunskyy, Hatfield, & Gladyshev, 2014) (Fig. 47.1).

The human selenoproteome comprises five forms of GPx, three thioredoxin reductases, three iodothyronine deiodinases, and selenoprotein P (SelenoP), among others. These proteins have different biological functions involved in numerous processes, such as immune system homeostasis, thyroid hormone metabolism, spermatogenesis, antioxidant defense, and redox state regulation. For some selenoproteins, biological functions are yet to be discovered. Table 47.1 summarizes the main selenoprotein functions.

Selenium and the brain

Selenium is vital for the brain despite its small concentration (~2.3% of total body selenium). In a selenium-deficient state, the brain is the last organ to be depleted; it is also the first to revert to normal levels when selenium status becomes replete. This demonstrates the importance of this trace element for brain homeostasis (Nakayama, Hill, Austin, Motley, & Burk, 2007). Indeed, severe selenium deficiency or deficient selenium supply to the brain causes irreversible brain damage as demonstrated by various animal models (Cardoso et al., 2015). The main explanation for the importance of selenium to brain homeostasis is the vulnerability of this organ to oxidative stress due to its high oxygen consumption as well as the elevated content of polyunsaturated fatty acids and transition metals (Jomova, Vondrakova, Lawson, & Valko, 2010).

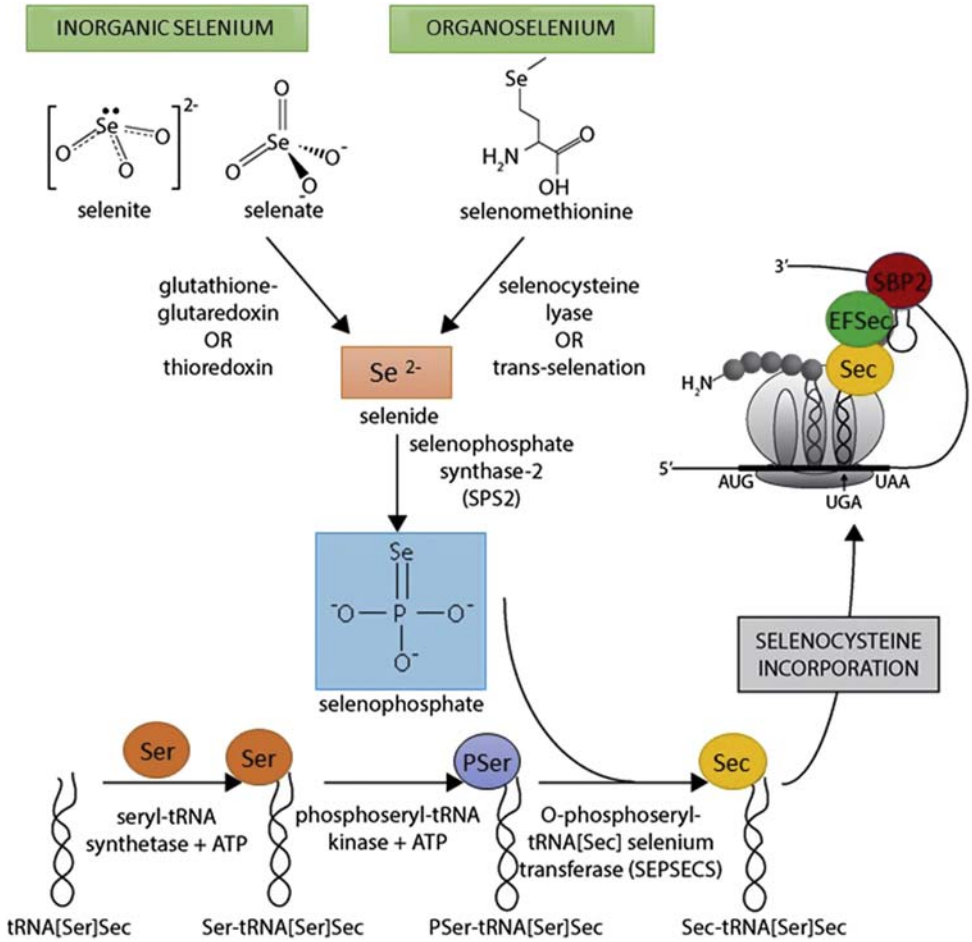

Figure 47.1 Selenium metabolism. Dietary selenium is converted into selenide (Se^{2-}), which is used to synthesize selenophosphate ($SePO_3^{3-}$) for incorporation into proteins via a unique tRNA pathway. Selenocysteine is synthesized by a specific tRNA (RNAt[Ser]Sec) that presents a seryl (Ser) residue replaced by Sec. RNAt[Ser]Sec codifies a UGA codon, usually identified as a stop codon, to integrate selenium into the selenoprotein amino acid sequence. Selenocysteine is incorporated in the nascent polypeptide chain where there is a stem–loop structure known as Sec insertion sequence (SECIS) element located at the 3′ untranslated region. This SECIS element recruits substrate-binding protein (SBP2), which captures a specific elongation factor of SeCys (EFSec) and its cognate RNAt[Ser]Sec tRNA. *EFSec*, sec elongation factor; *SBP2*, sec insertion sequence binding protein-2; *Se*, selenium; *Sec*, selenocysteine; *Ser*, seryl. *(Adapted from Cardoso, B. R., Roberts, B. R., Bush, A. I., & Hare, D. J. (2015). Selenium, selenoproteins and neurodegenerative diseases.* Metallomics, *7(8), 1213–1228.)*

Selenium delivery to the brain is accomplished through the binding of SelenoP to the surface of apolipoprotein E receptor 2 (ApoER2) present in the blood–brain barrier, brain capillary endothelial cells, and choroid plexus epithelial cells. This receptor facilitates selenium transport in an as-yet-unknown chemical form into the brain.

Table 47.1 Human selenoprotein functions (Benstoem et al., 2015; Mangiapane, Pessione, & Pessione, 2014).

Selenoprotein	Biological function
GPx1, GPx2, GPx3, GPx4, GPx6, SelenoP, SelenoK, SelenoR, SelenoW, SelenoH, SelenoM	Antioxidant defense
TrxR1, TrxR2, TrxR3, SelenoO	Redox signaling
SPS2	Selenocysteine synthesis
Seleno15, SelenoN, SelenoM	Protein folding
SelenoP	Metal detoxification/transport and storage
GPx4	Structural protein in sperm
SelenoI	Phospholipid biosynthesis
SelenoH	Transcription factor
SelenoN	Calcium signaling, role in muscle formation
SelenoR	Reduction of oxidized methionine residues groups
SelenoS	Removal of misfolded proteins
SelenoT	Calcium mobilization
DIO1, DIO2, DIO3	Thyroid metabolism
SelenoV	Unknown function

DIO, iodothyronine deiodinase; *GPx*, glutathione peroxidase; *Seleno*, selenoprotein; *SPS*, selenophosphate synthetase; *TrxR*, thioredoxin reductase.

Once in the brain, astrocytes and possibly other glial cells uptake selenium for SelenoP synthesis, which is released to be transported to neurons via the apoER2-mediated pathway (Burk et al., 2014). Considering the known genetic association between the *APOE* ε4 allele and increased risk for Alzheimer's disease (AD) (Heffernan, Chidgey, Peng, Masters, & Roberts, 2016), it has been hypothesized that this intricate mechanism of interaction between ApoER2 and SelenoP to deliver selenium to the brain might have repercussions for AD pathogenesis. In support of this hypothesis, Cardoso, Hare et al. (2017) reported lower selenium concentration in the frontotemporal area of *APOE* ε4 carriers than in noncarriers, suggesting that the presence of this allele alters the expression of ApoER2, leading to decreased interaction with SelenoP and subsequent attenuated selenium delivery to the brain. In this sense, different lines of evidence connect low selenium levels to the development and progression of AD. Several studies in cells, animals, and humans have associated a lack of selenium with cognitive decline, although a consensus has not yet been achieved.

Experimental studies

Selenium is mostly concentrated in the gray matter, as revealed in rats and confirmed in human brains, where the highest concentration was observed in the putamen, parietal inferior lobule, and occipital cortex (Ramos et al., 2015). Although there is a low concentration of selenium in the central nervous system, a multitude of selenoproteins have

already been identified in the brain (Hoppe et al., 2008). The essentiality of selenoprotein synthesis for the central nervous system is demonstrated by ablation of selective elenoproteins in brain of animal models. As per the results of these experiments, animals presented severe neurological dysfunction characterized by seizures, ataxia, and impairment of neuron development (Wirth et al., 2010).

In addition to the antioxidant role of some selenoproteins, other pathways are known to be reliant on selenium. Selenium treatment induced modulation of calcium homeostasis in an animal model of traumatic brain injury (Nazıroğlu, Senol, Ghazizadeh, & Yürüker, 2014), and SelenoP was demonstrated to be required for normal synaptic transmission (Peters, Hill, Burk, & Weeber, 2006). Additionally, SelenoP counteracts transition metal dyshomeostasis in the brain by preventing metal-mediated amyloid beta $(A\beta)_{1-42}$ aggregation and subsequent free radical generation (Du et al., 2013; Du, Wang et al., 2014) as well as inhibiting aggregation of tau protein induced by Cu^{+}/Cu^{2+} (Du, Wang et al., 2014). Selenoprotein M enhances the activation of ERK signaling, which induces a decrease in tau phosphorylation, α-secretase, and γ-secretase activity and an increase in β-secretase (Yim et al., 2009). Glutathione peroxidase 4, the most abundant selenoprotein in the brain, was recently identified as a regulator of ferroptosis, a newly identified form of programmed cell death dependent on iron that causes lipid peroxidation in high rates (Dixon et al., 2012; Dixon & Stockwell, 2014). Ferroptosis was characterized in different brain areas, such as the hippocampus (Dixon et al., 2012) and forebrain (Hambright, Fonseca, Chen, Na, & Ran, 2017), indicating the relevance of this cell death pathway for neurodegeneration. In order to inhibit ferroptosis, it is hypothesized that maximization of GPx4 activity by increasing selenium delivery to the brain delays or even prevents neuronal loss (Cardoso, Hare, Bush, & Roberts, 2017; Ingold et al., 2018) (Fig. 47.2).

Sodium selenate reduced tau hyperphosphorylation in different mice models and neuroblastoma cells by activation of phosphatase 2A (Corcoran et al., 2010; van Eersel et al., 2010). The other inorganic selenocompound, selenite, decreased the activity of γ-secretase through activation of the ERK−MAPK pathway, which led to decreased Aβ formation in human embryonic cells (HKE293) (Tung et al., 2008). Organic selenocompounds also have promising effects on AD pathogenesis. Selenomethionine treatment for triple transgenic AD model mice decreased production and deposition of Aß, down-regulation of ß-secretase levels, enhanced activity of selenoenzymes and increased selenium levels in the hippocampus and cortex (Zhang et al., 2016). The organoselenium compound p,p′-methoxyl-diphenyl diselenide reverted memory loss, decreased oxidative stress, and normalized acetylcholinestarase activity in a sporadic dementia model (Pinton, Bruning, Sartori Oliveira, Prigol, & Nogueira, 2013). Selenium-methyl-selenocysteine reduced oxidative stress, neuroinflammation, and generation of Aβ as well as attenuated hyperphosphorylation of tau in a triple-transgenic AD model, improving spatial learning and memory deficits (Xie et al., 2018).

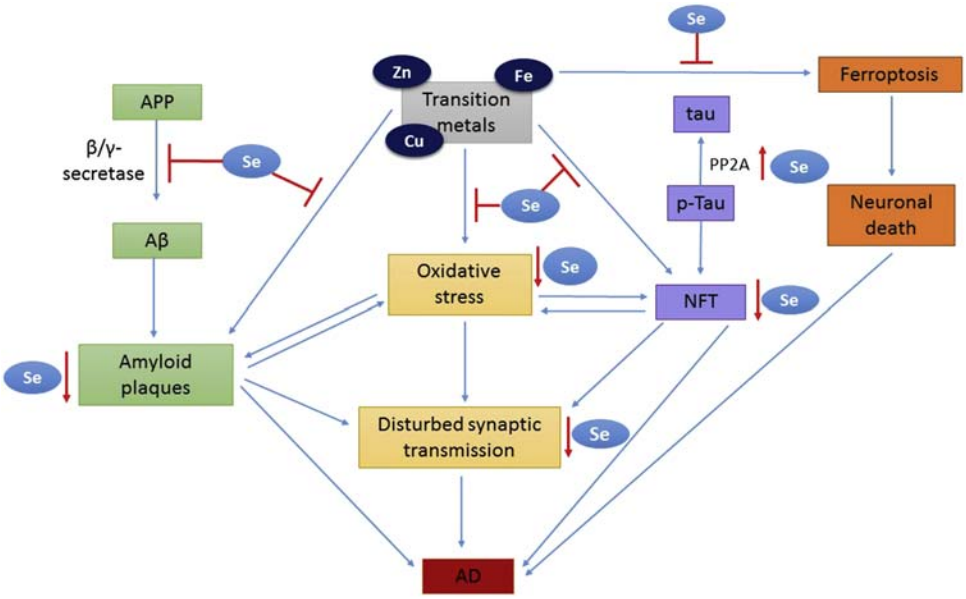

Figure 47.2 Molecular pathways of selenium in Alzheimer's disease pathogenesis prevention. *Red arrows*: selenium or selenoproteins either enhancing or inhibiting the pathway; *green boxes*: amyloidogenesis; *yellow boxes*: oxidative stress and synaptic damage; *purple boxes*: neurofibrillary tangles formation; *orange boxes*: ferroptosis. *Aβ*, amyloid-β; *AD*, Alzheimer's disease; *APP*, amyloid peptide precursor; *NFT*, neurofibrillary tangle; *p-Tau*, phosphorylated tau. *(Adapted from Du, X., Wang, C., & Liu, Q. (2016). Potential roles of selenium and selenoproteins in the prevention of Alzheimer's disease.* Current Topics in Medicinal Chemistry, 16*(8), 835–848.)*

Human studies

Evidence from human studies indicates a negative association between selenium status and cognitive performance in older adults. Cross-sectional analysis from the EVA study, a cohort conducted in France with 1166 people aged 60–70 years, revealed a 58% increase in the odds ratio of cognitive decline in patients with selenium plasma concentration in the first quartile (<75.8 μg/L), compared with a mean of 86.9 μg/L (Berr, Balansard, Arnaud, Roussel, & Alperovitch, 2000). After 9-year follow-up, findings confirmed that low plasma selenium was associated with an increased risk of cognitive decline (Akbaraly et al., 2005). Similarly, a study conducted in China with 2000 participants aged 65 or older showed a positive association between selenium concentration in nails (long-term selenium marker) and cognitive scores. Additionally, they observed that carriers of the *APOE* ε4 allele had lower selenium concentration than that of noncarriers (Gao et al., 2009). Cardoso, Bandeira, Jacob-Filho, & Cozzolino (2014) assessed selenium status in plasma (short-term selenium marker) and erythrocytes (medium-term selenium marker) and reported that AD patients were more selenium deficient than mildly cognitively impaired elderly and healthy controls in a Brazilian population. Corroborating these observations, a meta-analysis of 12 case–control/observational studies with controls

and AD patients showed that plasma selenium was decreased in Alzheimer's patients, while the lack of available studies with erythrocytic and cerebrospinal fluid markers precluded further conclusions (Reddy, Bukke, Dutt, Rana, & Pandey, 2017). These studies using different markers suggest that chronic deficiency correlates with cognitive decline and increased risk of AD.

Assessment of selenium and selenoproteins in the AD brain provides inconsistent results. Cornett, Markesbery, & Ehmann (1998) observed higher selenium concentration in amygdala but not in the other six areas of AD brains compared with control subjects. Additionally, selenium was associated with neurofibrillary tangle severity in two cortical areas (Morris, Brockman, Schneider et al., 2016). In contrast, Cardoso, Hare et al. (2017) observed lower selenium concentration in the AD frontotemporal brain area compared with controls, although the presence of allele APOE ε4 was an independent strong factor associated with reduced selenium concentration. SelenoP was found to be increased in the choroid plexus and cerebrospinal fluid of Alzheimer's patients (Rueli et al., 2015) and colocalized with senile plaques and neurofibrillary tangles (Bellinger et al., 2008). These findings suggest an attempt to increase selenium delivery to the brain to counteract the disease pathogenesis. It has been hypothesized that rather than total selenium concentration in the brain, the Sec/Cys ratio in selenoproteins is decreased in the Alzheimer's brain. Thus, even though SelenoP might be increased in the AD brain, it is not optimized to deliver selenium for other selenoproteins' syntheses (Cardoso, Hare et al., 2017). Furthermore, it is speculated that the capacity of incorporating selenocompounds into selenoproteins is of great relevance for brain homeostasis (Vinceti et al., 2017).

Studies that aimed to use selenium as a strategy to prevent AD are limited and controversial. A large-scale primary prevention study (PREADViSE), using selenomethionine (200 μg/day) either isolated or associated with alpha-tocopherol (400 IU/day), did not show any evidence of a preventive effect in older men (Kryscio et al., 2017). In contrast, supplementation of one kernel of Brazil nut (*Bertholletia excelsa*) (*ca* 288.75 μg/day) to mildly cognitively impaired older adults significantly improved cognitive performance in verbal fluency and constructional praxis tests after 6 months of intervention (Cardoso et al., 2016). These two studies enrolled different populations regarding selenium status: while participants of PREADViSE were predominantly selenium-replete, the study with Brazil nuts was conducted in a selenium-deficient population. Such a difference in selenium status might have affected the response to treatment, indicating that selenium supplementation would not have any further impact on the cognitive status of selenium-replete individuals.

Conclusions

The noticeable role of selenium for brain homeostasis comprises the participation of selenoproteins in redox balance maintenance, mitochondrial dynamics, regulation of Ca^{2+} channels, and modulation of neurogenesis. More research is required, however, to elucidate the potential role of selenium in strategies for the prevention and treatment of AD.

Key facts of selenium

- Selenium was recognized as essential to human health only in 1957. Before that, it was associated with toxicity.
- Selenium status is directly associated with soil selenium content. In seleniferous areas, populations are more prone to selenium toxicity, while in selenium-poor regions, populations are more vulnerable to deficiency that can be either severe and acute or subclinical and chronic.

Summary points

- Selenium mitigates AD pathogenesis.
- Selenium delivery to the brain is dependent on ApoER2.
- AD patients tend to present lower selenium circulating levels.
- Selenium deficiency is associated with higher risk of AD.
- Response to selenium treatment might be associated with baseline status.

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CHAPTER 48

Linking adiponectin and obesity in dementia

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List of abbreviations

AD Alzheimer's disease
AdipoR1 adiponectin receptor 1
AdipoR2 adiponectin receptor 2
ADPN adiponectin
AMPK 5′AMP-activated protein kinase
Aβ beta-amyloid
Aβ-42 Amyloid-β protein fragment 1−42
BBB blood−brain barrier
BMI body mass index
CNS central nervous system
CSF cerebrospinal fluid
fAd full-length form of adiponectin
gAd globular adiponectin
HMW high molecular weight
IL interleukin
LOAD late-onset Alzheimer disease
MCI mild cognitive impairment
MCP-1 monocyte chemoattractant protein 1
MD mixed dementia
NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells
PAI-1 plasminogen activator inhibitor-1
Phospho-tau phosphorylated tau protein
PPAR peroxisome proliferator-activated receptor
RBP-4 retinol-binding protein-4
SFRP5 secreted frizzled-related protein 5
T-cad T-cadherin
Tau tau protein
TNF-α tumor necrosis factor α
VaD vascular dementia

Mini-dictionary of terms

Adipokines Cell-signaling polypeptides such as cytokines, acute phase reactants, inflammatory mediators, hormones, and other chemical messengers secreted by white adipose tissue. Adipokines regulate numerous physiological functions such as appetite, energy expenditure, insulin sensitivity and secretion,

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fat distribution, lipid and glucose metabolism, endothelial function, blood pressure, hemostasis, neuroendocrine functions, and immunity.

Blood–brain barrier A set of physical and biochemical features that create a selective border between blood vessels and nerve tissue. Is designed to protect the nervous system against harmful agents and allow selective transport of the substance from the blood into the cerebrospinal fluid.

Body mass index A measure of body fat calculated from a simple formula in which body weight in kilograms is divided by the height in meters raised to the power of the other (kg/m^2).

Mini-Mental State Examination (MMSE) A clinical scale designed to measure cognitive impairment. MMSE consists of 30 questions or tasks allowing for quantitative assessment of various aspects of cognitive functioning.

White adipose tissue The main site of energy storage in the form of triglycerides. Is also recognized as an endocrine organ that produces a wide variety of highly bioactive substances called adipokines.

Introduction

Age-related dementia is a condition characterized by progressive deterioration of memory and other cognitive functions and behavior. With the general increase in the life span, disability due to cognitive impairment and dementia is expected to become the main health and social problem in elderly if no effective therapy is developed (Reitz & Mayeux, 2014). According to the World Alzheimer Report (2015), over 46 million people live with dementia worldwide. This number will almost double every 20 years and is estimated to increase to 131.5 million by 2050 (World Alzheimer Report, 2015).

Alzheimer disease (AD) and vascular dementia (VaD) constitute the vast majority of the age-related dementias. AD is a progressive neurodegenerative disorder that is defined by the neuropathological hallmarks of extracellular beta-amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles of hyper-phosphorylated forms of tau protein. These pathological changes result in the irreversible loss of neurons and synapses, particularly in the cortex and hippocampus. Vascular dementia is a heterogeneous group of brain disorders where cognitive decline is attributable to a wide range of cerebrovascular pathologies. With age an increasing prevalence of coincident cerebrovascular pathology with AD with additive effects of both pathologies on cognitive decline was observed (Iadecola, 2013; Reitz & Mayeux, 2014).

The majority of all cases of Alzheimer disease are late onset and sporadic in origin. Late-onset AD (LOAD) is a multifactorial disorder in which both genetic predisposition and environmental factors contribute to the pathogenesis of the disease. Main known risk factors for LOAD are age, family history of dementia, and the presence of apolipoprotein E ε4 allele. Many recognized vascular risk factors for ischemic heart disease or stroke (obesity, diabetes or insulin resistance, hypertension, and dyslipidemia) have all been found to increase the risk of developing not only vascular dementia but also AD (Ischi & Iadecola, 2016; Reitz & Mayeux, 2014; Song, Lee, Park & Lee, 2014).

The incidence of both obesity and dementia increases with age. It seems important to recognize the contribution of adiposity to age-related cognitive decline, which may help

in the prevention and possible therapy of dementia. This review summarizes recent studies that have contributed to understanding the potential association between obesity, adiponectin, and development of dementia.

Obesity as risk factor of dementia

The prevalence of obesity has been increasing in the Western countries during recent years, and obesity has become the major health problem associated with many comorbidities. The World Health Organization reported that worldwide more than 1.9 billion adults were overweight in 2016, and of these over 650 million were obese (World Health Organization, 2017).

Obesity is a condition characterized by an excessive accumulation of fat in adipose tissue in the form of triglycerides. Clinically obesity is defined by measurements of body mass index (BMI) or waist circumference and waist-to-hip ratio. Accumulating evidence suggests that obesity (particularly abdominal obesity) contributes to chronic inflammation, characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways and leading to the development of insulin resistance and metabolic disorders. Obesity is associated with elevated risk for various diseases including diabetes, cardiovascular and cerebrovascular disease, gastrointestinal disorders, as well as certain types of cancer (Awada, Parimisetty, & d'Hellencourt, 2013; Chakrabarti et al., 2015).

So far the impact of obesity on cognition has been related to the effects of hyperinsulinemia and insulin resistance in the brain. It was shown that impaired brain insulin signaling might increase Aβ toxicity, tau phosphorylation, oxidative stress, and neuroinflammation, leading to neurodegeneration and synaptic loss, which are responsible for cognitive decline (Chen & Zhong, 2013; Letra, Santana, & Seiça, 2014). There is evidence from animal studies that obesity also has insulin-independent influence on cognitive impairment development (Letra et al., 2014).

Several epidemiological studies performed in different ethnic groups demonstrated the association of midlife obesity with the increased risk of late-life cognitive decline and dementia that is independent of other known vascular risk factors. Stronger associations have been observed in women than in men (Emmerzaal, Kiliaan, & Gustafson, 2015).

On the contrary in most studies, late-life obesity or overweight has been associated with a reduced risk for developing cognitive decline and dementia (Emmerzaal, Kiliaan, & Gustafson, 2015). The weight loss often precedes the cognitive symptoms in AD and other dementias and positively correlates with disease morbidity and mortality (Ischi & Iadecola, 2016).

Several studies have reported that BMI and central obesity are also related to underlying brain pathologies, such as temporal atrophy, white matter changes, and blood–brain barrier (BBB) disturbances, which are more pronounced in late life and AD (Gustafson, 2010; Parimisetty et al., 2016).

Adipose tissue as an endocrine organ

Recent findings indicate that white adipose tissue, previously considered as an energy storage tissue, is at present recognized as a major endocrine organ, secreting a wide variety of highly bioactive substances called adipokines. Adipokines includes dozens of cell-signaling polypeptides such as cytokines, acute phase reactants, inflammatory mediators, hormones, and other chemical messengers (Table 48.1). Adipokines exert pleiotropic effects on different tissues and regulate numerous physiological functions such as appetite, energy expenditure, insulin sensitivity, fat distribution, lipid and glucose metabolism, endothelial function, blood pressure, neuroendocrine functions, and immunity (Blüher & Mantzoros, 2015; Deng & Scherer, 2010; Gustafson, 2010; Parimisetty et al., 2016).

It is considered that adipokines are much more important in the brain than previously thought. These molecules are not only produced by the adipose tissue but can also be expressed in the central nervous system (CNS) where receptors for these factors are present. Some adipokines have the ability to cross the BBB and therefore carry out its action on the brain. Moreover, the disturbance of the BBB,

Table 48.1 Overview of main adipokines.

		Function			
Adipokine	**Obesity**	**Glucose/ energy homeostasis**	**Proinflammatory**	**Antiinflammatory**	**Vascular**
Adiponectin	↓	✓		✓	✓
Angiotensinogen	↑				✓
Apelin	↑				✓
IL-1β	↑		✓		
IL-6	↑		✓		✓
IL-8	↑		✓		
IL-10	↑			✓	
IL-18	↑		✓		
Leptin	↑	✓	✓		
MCP-1	↑		✓		
Omentin	↓	✓		✓	
PAI-1	↑				✓
Resistin	↑		✓		
RBP-4	↑	✓			
SFRP5	↑	✓			
TNFα	↑		✓		✓
Vaspin	↑	✓			
Visfatin	↑	✓			✓

Table presents an overview of selected adipokines, levels in obesity, and their main functions. *IL*, interleukin; *MCP-1*, Monocyte chemoattractant protein 1; *PAI-1*, plasminogen activator inhibitor-1; *RBP-4*, retinol-binding protein-4; *SFRP5*, Secreted frizzled-related protein 5; *TNFα*, tumor necrosis factor alpha.

common in dementia, enhances central crossing of these adipose-derived compounds (Letra et al., 2014).

The association between adipokines and dementia is largely unexplored, despite published epidemiological data supporting associations between obesity and various types of dementia and Alzheimer disease. It is suggested that CNS adipokines could regulate neuroinflammation and oxidative stress, which are two major physiological processes involved in neurodegeneration and are associated with many chronic neurodegenerative diseases (Parimisetty et al., 2016).

Individuals with abdominal obesity exhibit a dysregulation of adipokines production. Most of the adipokines, such as leptin, TNF-α and IL-6, are upregulated in obese states and promote obesity-inducible metabolic and cardiovascular diseases. In contrast, there are a smaller number of adipokines that exert beneficial actions on obese complications with antiinflammatory properties (e.g., adiponectin). Thus, the imbalance in the production of proinflammatory and antiinflammatory adipokines under conditions of obesity could contribute to the development of obesity-linked disorders (Table 48.2) (Blüher & Mantzoros, 2015; Ouchi, Ohashi, Shibata, & Murohara, 2012).

Adiponectin

Adiponectin (ADPN) is one of the most important adipokines released by the adipose tissue. Human adiponectin is a 28–30 kDa protein that comprises 244 amino acids. ADPN is a cytokine that is exclusively secreted by adipocytes and is the most abundant adipokine in circulation, representing 0.01% of total serum proteins in humans. The physiological levels of adiponectin are generally higher in females and decrease with

Table 48.2 Adipokine dysfunction in obesity.

	Adipokines	Metabolic and inflammatory status	Diseases
Normal weight	Proinflammatory adipokines ↓ Antiinflammatory adipokines ↔	Energy balance Metabolic homeostasis Vascular homeostasis Immune homeostasis	Protection against obesity-linked disorders
Obesity (adipocyte hypertrophy, macrophage infiltration)	Proinflammatory adipokines ↑↑ Antiinflammatory adipokines ↓	Positive energy balance Insulin resistance ↑ Inflammation ↑ Vascular dysfunction ↑	Diabetes Atherosclerosis Hypertension Dyslipidemia Nonalcoholic fatty liver disease Carcinogenesis

Table presents the altered adipokine secretion in obesity and its contribution to the development of obesity-linked disorders.

age in both sexes. It is considered as an adipose tissue-specific protein, though very small amounts can be synthetized by other cell types. Unlike most adipocyte-derived factors, which increase with excess body fat mass, circulating adiponectin levels decrease with increasing central adiposity (Letra, Rodrigues, Matafome, Santana, & Seiça, 2017; Parimisetty et al., 2016; Song et al., 2014; Thundyil, Pavlovski, Sobey, & Arumugam, 2012; Turer & Scherer, 2012).

Although in humans the physiological levels of adiponectin are 1000-fold lower in cerebrospinal fluid (CSF) than in serum, Une et al. (2011) reported a good positive correlation between CSF and serum ADPN concentration, suggesting that a fraction of circulating adiponectin levels may cross the BBB and exert their biologic effects in the CNS. Contrary, Waragai et al. (2016) found the discrepancy between CSF and serum adiponectin levels—ADPN was significantly decreased in CSF of patients with AD, as compared to MCI and controls, despite elevation of ADPN in serum. To date it is still not fully explained where CSF adiponectin is produced and how it circulates in the brain.

ADPN circulates in the body in its full-length form (fAd) or as a proteolytic fragment that corresponds to a globular domain (gAd) but also as different oligomers—trimers, hexamers, or as high-molecular-weight (HMW) forms. The regulation of adiponectin expression and secretion of its oligomers into the circulation is not yet well explained (Ng & Chan, 2017; Thundyil et al., 2012).

Adiponectin exerts its effects by binding to specific receptors. Three ADPN receptors have been identified: transmembrane AdipoR1 and AdipoR2 and a recently discovered cell surface protein called T-cadherin (T-cad). The binding of adiponectin to its receptors can lead to activation of signaling cascades of AMPK, p38-MAPK, PPAR-α, and NF-κB, dependent on the specific localization of these receptors. AMPK acts as the main downstream effector of ADPN. It was shown that adiponectin-adipoR1-AMPK activation provides various beneficial metabolic and protective effects to different tissues (Letra et al., 2017; Ng & Chan, 2017; Thundyil et al., 2012).

In humans, the expression of AdipoRs was documented in different brain structures such as the hypothalamus, pituitary gland, cortical and subcortical neurons, and also in the hippocampus, the main targeted structure in AD. T-cad is also present in the hippocampus, and studies with T-cad knockout mice show that it plays an important, but still unknown, role in cognitive pathways (Letra et al., 2017; Parimisetty et al., 2016; Thundyil et al., 2012).

Adiponectin has many beneficial metabolic effects. It was shown that it plays a significant role in the regulation of insulin sensitivity, glucose homeostasis, fatty acid catabolism and energy metabolism, as well as has antiinflammatory, antiapoptotic, and antiatherogenic properties (Tables 48.3) (Parimisetty et al., 2016).

Reduction in circulating adiponectin levels has been implicated in the development of insulin resistance syndrome and diabetes, abdominal obesity, and chronic inflammatory diseases such as atherosclerosis, suggesting that adiponectin may have a protective

Table 48.3 Adiponectin functions.

Periphery (adipose tissue, muscle, liver)	Vascular wall	Brain
Fatty acid oxidation ↓ Triglyceride content ↓ Glucose uptake ↑ Glucose production ↓ Insulin pancreatic secretion ↑ Insulin sensitivity ↑	Inflammatory cytokines ↓ Adhesion molecules ↓ Oxidative stress ↓ Antiatherogenic	Energy expenditure ↑ Brain insulin sensitivity ↑ Neurogenesis ↑ Apoptosis ↓ Neuroinflammation ↓ Neuroprotection ↑

Table presents the physiological role of adiponectin in the periphery, in the vessels and in the brain.

role against obesity-associated disorders. Additionally, anticancer and neuroprotective effects of adiponectin have been demonstrated (Ischi & Iadecola, 2016; Letra et al., 2017; Parimisetty et al., 2016).

Adiponectin in the brain – experimental studies

In contrast to considerable information about the effects of adiponectin in peripheral tissues, the influence of ADPN on brain metabolism and function has not been well explained.

The physiological functions of adiponectin in the CNS were first identified in association with body weight control. It acts on the hypothalamus and activates AdipoR1-AMPK signaling to regulate food intake, energy expenditure, and lipid and glucose metabolism during fasting and may thereby promote weight loss (Blüher & Mantzoros, 2015; Ng & Chan, 2017; Thundyil et al., 2012).

Many lines of evidence suggest that adiponectin and adiponectin receptors via activation of AMPK directly modulate brain glucose metabolism and improve insulin sensitivity, thus regulating memory and cognitive dysfunction, and it also regulates severe inflammation observed in mild cognitive impairment and Alzheimer disease (Song et al., 2014; Song & Lee, 2013).

To date, several studies have been concerned with animal and cell line models of dementia. Kurata et al. (2013) showed the reduction of serum ADPN levels in mouse model of AD. Using an established animal model of brain ageing, Pancani et al. (2013) found reduced hippocampal adiponectin levels in ageing rats, suggesting reduced ADPN transport into the brain with ageing. It was demonstrated that ADPN regulates neurogenesis and proliferation of hippocampal neural stem cells (Zhang, Guo, Zhang, & Lu, 2011). Qiu et al. (2011) demonstrated in mice that the AMPK pathway is involved in adiponectin-induced neuroprotection and may mediate the antioxidative and antiapoptotic properties of adiponectin. Recent data have highlighted also the role of sphingolipid metabolism in the pleiotropic effects of adiponectin (Turer & Scherer, 2012). Chan et al. (2012) demonstrated that exogenous adiponectin was

protective against Aβ-induced neurotoxicity under oxidative stress conditions in cell model and raised the hypothesis that NF-κB suppression represents another mechanism underlying the effect of adiponectin against Aβ-induced neuronal cytotoxicity. Insulin-sensitizing action of adiponectin may be an additional mechanism of neuroprotection in Alzheimer disease (Song & Lee, 2013).

The results of the previous in vitro and in vivo research indicate that the brain adiponectin may be important for memory and learning. However, these studies only revealed associations, and showed variation among different study groups, and cannot resolve the problem of causality of adiponectin to AD. Concerning this problem, Ng et al. (2016) recent study reported that chronic adiponectin deficiency inactivated AMPK, causing insulin resistance and eliciting AD-like symptoms in aged mice. Taking into consideration previously demonstrated neuroprotective adiponectin properties (Chan et al., 2012), the authors hypothesized that decrease of ADPN levels or reduction of adiponectin signaling in the brain may lead to Aβ accumulation, which is neurotoxic and may result in neurodegeneration and cognitive impairment (Ng et al., 2017).

Recently, many efforts have been made to understand the mechanisms underlying the pathological process in AD, and several lines of evidence support the involvement of inflammatory mechanisms (Holmes, 2013). Adiponectin has a significant role in immune system in CNS. It is considered as the most abundant antiinflammatory adipokine. Several studies demonstrated that higher levels of adiponectin lead to the decreased expression of proinflammatory cytokines. Therefore, it is possible that lower adiponectin levels may stimulate proinflammatory and inhibiting antiinflammatory cascades, thus favoring the higher proinflammatory state observed in cognitive impairment and AD (Song & Lee, 2013).

It is suggested that antiinflammatory and antiatherosclerotic properties of ADPN exert a protective effect against ischemic brain injury, which can lead to the development of vascular dementia. Clinical reports revealed an association between decreased ADPN levels and ischemic stroke (Ng & Chan, 2017).

Summarizing, adiponectin has been linked to AD-related pathology in the brain by several different mechanisms including insulin-sensitizing, antiinflammatory, antiapoptotic signaling pathway activation, and also by its vascular effects.

Adiponectin and dementia – clinical studies

In contrast to clear association of low adiponectin levels with cerebrovascular disease, the association between ADPN and clinical dementia or cognitive impairment is largely unexplored, despite published epidemiological data supporting associations between obesity and various types of dementia and Alzheimer disease (Ishii & Iadecola, 2016; Parimisetty et al., 2016; Yang et al., 2015).

Table 48.4 presents the current state of epidemiological and clinical research investigating the contribution of ADPN to the risk of cognitive impairment and dementia.

Table 48.4 Epidemiological and clinical studies investigating the association between adiponectin and cognitive impairment and dementia.

Ref.	Study	Study type	Subjects groups (n)	Observation time	Adiponectin levels in dementia	Results
Ban et al. (2007)	Clinical study, Japan	CS	VaD (20) NC (40)	0	No relevance	Total serum ADPN similar in VaD and NC
Roberts et al. (2009)	Rochester Epidemiology Project, USA	CS	MCI (143) NC (747)	0	No relevance	Total plasma ADPN similar in MCI and NC
Kamogawa et al. (2010)	J-SHIPP study, Japan	CS	MCI (120) NC (397)	0	Increased	Total plasma ADPN significantly increased in MCI in men
Gu et al. (2010)	WHICAP II, USA	L	NC (1219)	3.8 ± 1.3 y	No relevance	Total serum ADPN not associated with AD risk
Bigalke et al. (2011)	Clinical study, Germany	CS	AD (41) NC (37)	0	No relevance	Total plasma ADPN similar in early AD and NC
Une et al. (2011)	Clinical study, Japan	CS	AD (27) MCI (18) NC (28)	0	Increased	Total plasma ADPN significantly higher in MCI and AD versus NC CSF ADPN significantly higher in MCI versus NC Positive correlation between plasma and CSF ADPN
Warren et al. (2012)	TARCC, USA	CS	AD (150) NC (197)	0	No relevance	Total plasma ADPN similar in AD and NC

Continued

Table 48.4 Epidemiological and clinical studies investigating the association between adiponectin and cognitive impairment and dementia.—cont'd

Ref.	Study	Study type	Subjects groups (n)	Observation time	Adiponectin levels in dementia	Results
van Himbergen et al. (2012)	Framingham Heart Study, USA	L	NC (826)	13 y	Increased	Total plasma ADPN as independent risk factor for the development of all-cause dementia and AD in women
Teixeira et al. (2013)	Clinical study, Brazil	CS	AD (41) MCI (65) NC (51)	34.6 ± 13.2 m	Decreased	Total serum ADPN significantly lower in MCI and AD versus NC And not associated with progression from NC to MCI and from MCI to AD
Khemka et al. (2014)	Clinical study, India	CS	AD (60) NC (60)	0	Increased	Total plasma ADPN significantly increased in AD versus NC In AD positive correlation ADPN with the severity of dementia
Dukic et al. (2016)	Clinical study, Croatia	CS	AD (70) MCI (48) NC (50)	0	No relevance	Total serum ADPN similar in AD, MCI, and NC

Kitagawa et al. (2016)	OSACA2, Japan	L	NC (466)	6.9 y (median)	No relevance	Serum HMW ADPN not associated with future dementia (AD, VaD, and MD)
Gorska-Ciebiada et al. (2016)	Clinical study, Poland	CS	MCI (62) NC (132)	0	Decreased	Total serum ADPN significantly lower in diabetic MCI versus diabetic NC
Ma et al. (2016)	Clinical study, China	CS	MCI (62) NC (132)	0	Increased	Total serum ADPN significantly increased in AD versus NC In AD positive correlation ADPN with the severity of dementia
Waragai et al. (2016)	Clinical study, Japan	CS	AD (63) MCI (64) NC (62)	0	Serum increased CSF decreased	Total serum ADPN significantly increased in AD and MCI versus NC In AD positive correlation ADPN with the severity of dementia CSF ADPN significantly lover in AD versus MCI and NC

Continued

Table 48.4 Epidemiological and clinical studies investigating the association between adiponectin and cognitive impairment and dementia.—cont'd

Ref.	Study	Study type	Subjects groups (n)	Observation time	Adiponectin levels in dementia	Results
						CSF but not serum ADPN correlated with AD neuroimaging and biochemical biomarkers
Wennberg et al. (2016)	MCSA study, USA	CS	NC (535)	0	Increased	Total plasma adiponectin positively associated with neuroimaging markers of neurodegeneration (brain amyloid content and hippocampal atrophy) and poorer cognitive outcomes in women
Bednarska-Makaruk et al. (2017)	Clinical study, Poland	CS	AD (89) VaD (47) MD (69) MCI (113) NC (107)	0	Increased	Total serum ADPN significantly increased in AD and MD versus NC in nonobese subjects

Table presents a summary of the results of selected epidemiological and clinical studies that explore the association of adiponectin levels with various types of dementia or mild cognitive impairment. *AD*, Alzheimer disease; *ADPN*, adiponectin; *CS*, cross-sectional; *CSF*, cerebrospinal fluid; *HMW*, high molecular weight; *L*, longitudinal; *MCI*, mild cognitive impairment; *MD*, mixed dementia; *NC*, nondemented controls; *VaD*, vascular dementia.

Despite its relevance, few studies have evaluated the circulating levels of adiponectin in dementia, and the results have not been conclusive. Two case-control studies (Khemka et al., 2014; Une et al., 2011) have shown an association of higher circulating or CSF adiponectin levels in AD as compared to controls. Similarly, a large-scale population-based prospective study using the Framingham data revealed that increased plasma ADPN levels were an independent risk factor for the development of both all-cause dementia and AD, particularly in elderly women, not in men (van Himbergen et al., 2012). These findings are in agreement with results of our study in which a significant increase of adiponectin level was observed in all-cause dementia and especially in dementia of neurodegenerative origin (AD and MD) as compared to controls. Interestingly, the difference observed between all-cause dementia and control subjects was significant only in individuals without abdominal obesity (Bednarska-Makaruk et al., 2017).

Une et al. (2011), who showed higher plasma and CSF concentrations of adiponectin in MCI and AD compared with cognitively normal controls, conclude that the high levels of ADPN in MCI could play a role in weight loss and decrease in fat tissue often observed in the early stage of dementia. This finding suggests that this molecule plays a role in the onset of AD.

In a recently published review, Ishii and Iadecola (2016) speculate that the association of increased plasma adiponectin levels with dementia found in several studies may be initially surprising since low adiponectin levels are often associated with obesity-associated disorders and adiponectin is generally considered to have protective properties. However, these studies were conducted predominately in patients with AD, where weight loss is a characteristic feature. This condition could be associated with higher circulating ADPN levels, which may lead to subsequent resistance to adiponectin in a similar fashion to insulin resistance and leptin resistance. Thus in the presence of adiponectin resistance, high circulating adiponectin levels do not improve or decrease the dementia risk (Ischi & Iadecola, 2016).

However, not all of the published results show a relationship between elevated adiponectin levels and dementia. The additional four cross-sectional studies found no significant differences in ADPN level between patients with AD, VaD, MCI, and healthy controls (Ban et al., 2007; Bigalke et al., 2011; Dukic et al., 2016; Roberts et al., 2009; Warren, Hynan, & Weiner, 2012). Another population-based study also did not find a significant association between adiponectin levels and risk of AD in nondemented elderly subjects (Gu, Luchsinger, Stern, & Scarmeas, 2010). Recently, a study of the most biologically active HMW adiponectin level and incident dementia in patients with vascular risk factors showed no association with future dementia (Kitagawa, Miwa, Okazaki, Sakaguchi, & Mochizuki, 2016).

In contrast to previous findings, Teixeira et al. (2013) showed that decreased adiponectin serum concentrations are associated with MCI and AD, but do not predict cognitive decline in elderly individuals. Similarly, the cross-sectional J-SHIPP Study

(Kamogawa et al., 2010) demonstrated the association of reduced amounts of abdominal subcutaneous fat and low levels of plasma adiponectin in male patients with MCI. The lower serum levels of adiponectin were observed in elderly diabetic patients with MCI in comparison to nondemented diabetic controls (Gorska-Ciebiada, Saryusz-Wolska, Borkowska, Ciebiada, & Loba, 2016).

To date, only one meta-analysis assessing the correlation between ADPN and AD has been published. The meta-analysis, which included 5 studies comprising a total of 727 subjects (254 AD and 473 controls), demonstrated higher serum levels of adiponectin in AD patients as compared to controls (Ma et al., 2016).

As discussed by Letra et al. (2017), the observed discrepancy between the results of the presented clinical trials may result from the heterogeneity of the studied cohorts of subjects due to the imperfection of diagnostic methods used to recognize and differentiate cognitive disorders as well as unavailability of some biomarker tests, advanced neuroimaging, and neuropsychological tools in clinical practice. Other potential limitations of these studies were confounding factors, for example, sex differences, body weight changes, and the AD-specific therapy (especially acetylcholinesterase inhibitors) or other medications known from the impact on circulating ADPN levels (Letra et al., 2017). Moreover, most of the studies did not investigate the serum level of specific adiponectin isoforms, mainly due to technical limitations in their detection. These multimers may have distinct biologic effects, for example, the high HMW isoform has higher biological activity than the low-molecular-weight isoform (Swarbrick & Havel, 2008).

Adiponectin and dementia biomarkers

Recently, the relationship between adiponectin and neuroimaging measures of AD pathology and CSF dementia biomarkers, such as β-amyloid, tau, and phospho-tau, have been examined. Wennberg et al. (2016) in the cross-sectional population-based study found a positive association between plasma adiponectin with neuroimaging markers of neurodegeneration (measured as increased brain amyloid content and hippocampal atrophy) and poorer cognitive outcomes in older women. These findings confirm the previously observed sex differences in the risk of AD. Waragai et al. (2016) revealed a reduction of CSF adiponectin levels in AD and its correlations with AD specific CSF biomarkers, including Aβ-42, phospho-tau, and hippocampal atrophy. Moreover, in neurohistochemical studies, adiponectin was colocalized with tau in neurofibrillary tangles in postmortem brains of patients with AD, and immunoblot analysis showed that the functional trimers of ADPN were significantly decreased in AD compared to controls. The authors suggested that an increase of circulating adiponectin levels could be a compensatory effect against neurodegeneration (Waragai et al., 2016).

Adiponectin – therapeutic perspectives

For many years significant efforts have been directed to developing new agents that are based on potential targets associated with the pathological changes seen in AD. But there is still no effective disease-modifying treatment. The results of previous studies indicate that adiponectin plays an important role in brain insulin dysfunction, amyloid β neurotoxicity, and immune system, thus, adiponectin can be considered as a potential target to treat Alzheimer disease and other cognitive deficits.

Besides lifestyle modifications (such as diet and physical exercise), which are able to increase ADPN secretion, there is growing interest in pharmacological strategies that target ADPN, AdipoR, or its downstream signaling pathways.

Recently it was shown that the serum adiponectin levels progressively increased in AD patients who were administered acetylcholinesterase inhibitor (Pákáski et al., 2014). Also, some other medicines often used in the elderly (i.e., statins, metformin, thiazolidinediones, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors) have the ability to increase plasma and probably cerebral ADPN levels (Letra et al., 2017; Nigro et al., 2014).

In humans the most obvious therapeutic strategy seems to be adiponectin replacement therapy. However, because adiponectin is a protein, it cannot be orally administered. Its parenteral peripheral administration also has some limitations; one of them is rather weak ability to cross the BBB.

Currently, the use of AdipoR agonists seems to be the most promising therapeutic approach (Letra et al., 2017; Ng et al., 2017; Yang et al., 2015). Several adiponectin receptor agonists have been discovered. An example is osmotin, a plant homolog of mammalian adiponectin present in fruits and vegetables. Its beneficial effects in AD-like pathology were reported in experimental studies in cell lines and in rodents. Badshah, Ali, & Kim (2016) demonstrated in vivo and in vitro that osmotin prevented neuroinflammation-associated memory impairment and neurodegeneration. The same group had previously shown that osmotin suppresses Aβ-induced memory impairments, tau phosphorylation, and neurodegeneration in mouse hippocampus (Ali, Yoon, Shah, Lee, & Kim, 2015). The authors suggest that AdipoR1 as a therapeutic target for the treatment of neuroinflammation and neurological disorders and its agonist, osmotin, could potentially serve as a novel, promising, and accessible neuroprotective agent against AD.

The inventions of pharmacological agents or changes of lifestyle to elevate endogenous adiponectin expression or activate adiponectin signaling may pave the road for future AD treatment. Recent developments of research on the adiponectin receptor agonists are likely to facilitate the development of future drugs based on adiponectin pathways (Letra et al., 2017).

In sum, the results of previously published clinical and experimental studies indicate that the relationship between adiponectin and dementia has not been completely

elucidated and additional studies are required to clarify these discrepancies. Further investigation is required to explain how the adiponectin peripheral level and its various isoforms can modulate its effects in the brain. Understanding the contribution of ADPN to age-related cognitive decline can lead to the development of prevention and treatment methods.

Key facts on obesity

- Obesity is a condition characterized by an excessive accumulation of fat in adipose tissue in the form of triglycerides.
- Clinically, obesity is defined by measurements of body mass index (BMI) or waist circumference and waist-to-hip ratio.
- The prevalence of obesity has been increasing in the Western countries during recent years. The World Health Organization reported that nearly 40% of adults worldwide are currently overweight.
- Abdominal obesity contributes to chronic inflammation, characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways, and leading to the development of insulin resistance and metabolic disorders.
- Obesity is associated with elevated risk for various diseases including type 2 diabetes, cardiovascular disease, gastrointestinal and respiratory disorders, as well as certain types of cancer.

Summary points

- Obesity is recognized as an important risk factor for Alzheimer disease and dementia, but the exact causal mechanisms are still largely unexplored.
- Adiponectin, the most widespread adipocyte-derived hormone, is inversely correlated with adipose tissue dysfunction.
- Adiponectin regulates the sensitivity of insulin, fatty acid catabolism, and glucose homeostasis and has antiinflammatory, antiatherogenic, and neuroprotective properties.
- Adiponectin has been linked to Alzheimer disease—related pathology in the brain by several mechanisms including insulin-sensitizing, antiinflammatory, antiapoptotic signaling pathway activation and also by its vascular effects.
- Understanding the contribution of ADPN to age-related cognitive decline can lead to the development of prevention and treatment methods.

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CHAPTER 49

The impact of the gut microbiome in Alzheimer's disease: cause or consequence?

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Mini-dictionary of terms

Enteric nervous system Largest and most complex peripheral nervous system; consists of numerous mesh-like arranged neurons and innervates the gastrointestinal tract

Fecal microbiota transfer Fecal bacteria transplantation from healthy donor to recipient, so far applied in patients suffering from, e.g., Crohn's disease or *Clostridium difficile* infection

Gut–brain axis Responsible for bidirectional communication between the central nervous system and enteric nervous system including vagus nerve, immune cells, the hypothalamic–pituitary–adrenal axis, gut microbiota, and its metabolites

Lipopolysaccharide Part of the outer membrane of gram-negative bacteria having protective and integrative function as well as serving as an immune stimulus via TLR4 signaling in the host

Microbiome Sum of all microbial genomes in a defined environment (e.g., gut)

Microbiota Sum of all microbial organisms

Peptidoglycan Macromolecule and part of bacterial cell wall responsible for stability and resistance

Introduction

The gastrointestinal tract and brain are connected via a mutual system of structures and molecules that in sum have been designated as the gut–brain axis (see Fig. 49.1). With the vagus nerve, a hard-wired connection with bidirectional information flow (efferent and afferent fibers) exists. Interestingly, a higher number of afferent fibers are found in comparison to efferent ones, so that delivery of information from the gut to the brain seems at least to have high relevance. The next level of communication might be seen in the immune cells and their secretion products that can transport conditions from either organ to the other. A variety of immune cells are located in the gut such as T cells, mononuclear phagocytes, and innate lymphoid cells (reviewed in Powell, Walker, & Talley, 2017).

One recent example for the effect of immune cell activation in the gut on brain functions is given by the example of excess dietary salt consumption in mice; expansion of TH17 cells in the small intestine by this diet resulted in a marked increase in plasma interleukin-17 that subsequently reduced nitric oxide production in endothelial cells of the brain (Faraco et al., 2018). Besides the host's own signaling molecules, molecules

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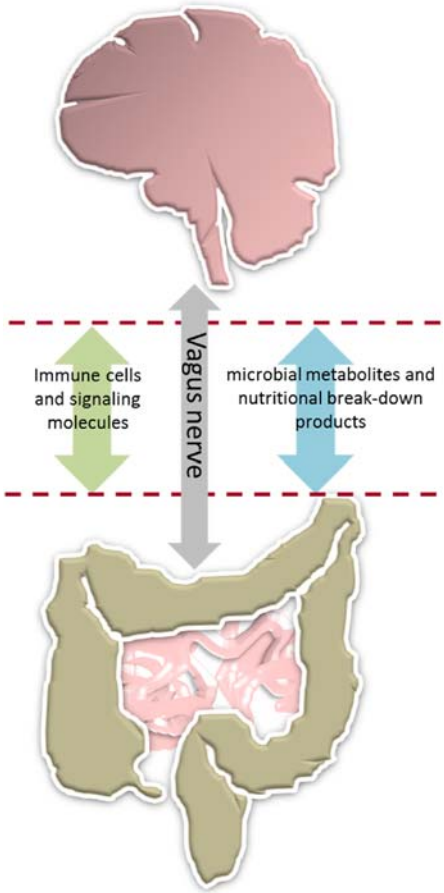

Figure 49.1 ***Scheme of gut–brain axis.*** The central nervous system (CNS) and the gastrointestinal system are mutually connected via different routes: immune cells and their signaling mediators deliver information about potential pathogens and critical situations; microbial metabolites and metabolized food components may enter the bloodstream and be delivered to the CNS; the vagus nerve connects both mutually by efferent and afferent fibers. Intensity and selectivity of this interplay is controlled by two barriers—the blood–brain barrier and the blood–gut barrier (indicated as two *dashed lines*), which may become less impermeable due to aging or pathological condition.

of microorganismic origin also can act as communication mediators; for example, bacterial peptidoglycan (PGN) produced by commensal gut microbiota has been demonstrated to translocate into the brain (Arentsen et al., 2017). Binding of those PGNs to specific pattern-recognition receptors of the innate immune system seems to impact postnatal brain development, as knockout for one of those receptors (PGN-recognition protein 2) resulted, for example, in increased expression of the autism risk gene c-Met in mice. The intensity by which biochemical signatures derived from

host cells in the gut wall or from commensals located within the lumen or attached to the gut surface can affect central nervous functions is determined to a high degree by integrity of two barriers - the blood–gut and blood–brain barriers (for a concise review comparing both barriers, see Spadoni, Fornasa, & Rescigno, 2017). As aging has an impact on all aspects of the aforementioned building blocks of gut–brain communication (immune response, integrity of barriers, composition of microbiota), it is reasonable to speculate about the potential impact of gut properties on the one neurodegenerative disease that has its main risk factor in age - Alzheimer's disease (AD).

In addition, the gut contains an autonomous neuronal network which might serve as an indicator of neurotoxic conditions much earlier than the neurons of the brain. This enteric nervous system (ENS) is the most complex and largest peripheral neuronal assembly and consists of two main structures within the gut wall, the myenteric and the submucous plexuses. The first plexus is located between the circular and longitudinal muscular layer, whereas the second is found underneath the circular muscle layer (Fig. 49.2).

At first glance, the ENS and the brain (central nervous system, or CNS) are apparently different. The brain presents itself as a mass of neuronal and glial cells, while the ENS consists of ganglionated networks interconnected by dense fiber bundles (for a picture of the plexus, see Endres and Schäfer, 2018). However, the neurons of these networks share various similarities with the CNS. For example, subtypes such as catecholaminergic and inhibitory GABAergic neurons are found (e.g., Qu et al., 2008). Additionally, also glial cells can be identified that do not resemble PNS Schwann cells but share similarities with CNS glia such as increased GFAP and GDNF expression due to inflammation (von Boyen et al., 2011).

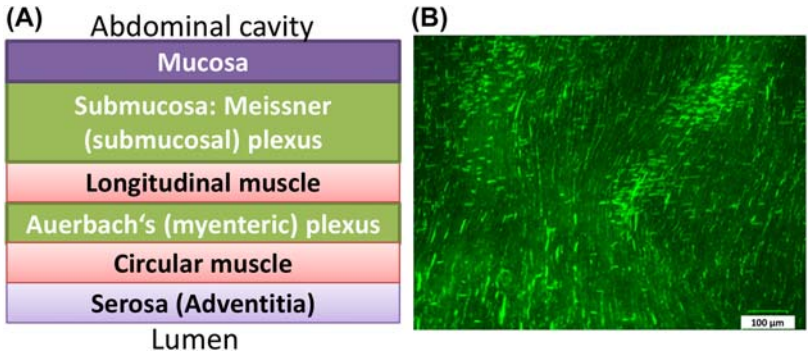

Figure 49.2 ***Localization of nervous plexus in the layering of the wall of the gastrointestinal tract.*** (A): Two networks of enteric neurons are embedded within the submucosa and between the muscular layers of the gut: the Auerbach and the Meissner plexus. (B): By peeling off the longitudinal muscle from tissue samples, the Auerbach plexus can be exposed to microscopic investigation. Here, a staining of nuclei from activated neurons of this plexus from the mouse large intestine is shown.

Neurons of the ENS express, for example, GABA and serotonin receptors (Hyland & Cryan, 2010) as well as toll-like receptors (TLRs) such as TLR2, TLR4, and higher levels of TLR5 (reviewed in Hug et al., 2018). Interestingly, some microbial commensals produce GABA (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012), others influence production of serotonin by enterochromaffin cells (Yano et al., 2015), and a variety of bacterial TLR ligands are known. Therefore, a role for microbiota in physiology and pathophysiology of gut neurons is obvious. That these neurons might serve as predecessors of neurotoxic events of CNS neurons can be demonstrated in the case of Parkinson's disease (PD); characteristic alpha-synuclein deposits have been found in the gut of human PD patients (Shannon, Keshavarzian, Dodiya, Jakate, & Kordower, 2012), sometimes even decades before clinical diagnosis (Hilton et al., 2014). A role for the gut and its microbiota in the transfer of pathology to the CNS can be deduced from the lowered risk for PD in patients with truncal vagotomy (Svensson et al., 2015) and also from PD model mice that have displayed reduced pathophysiological signs upon treatment with antibiotics (Sampson et al., 2016).

Altogether, this hints at a potential influence of the gut's commensals on neurodegenerative diseases of the CNS, and we therefore give an overview within this chapter on what is known about the impact of the gut microbiome on AD and vice versa.

Microbial changes in Alzheimer's disease

The human microbiota is dominated by two bacterial groups, the anaerobic gram-negative Bacteroidetes and the gram-positive Firmicutes. The microbial commensals of the gastrointestinal tract are correlated to age and health status, are probably impacted by the gut–brain axis, and vice versa, specific bacteria play a key role in homeostasis and inflammation. As one of the main risk factors for AD is age, it can be assumed that differences in the microbiome arising along the aging process can also affect AD pathology. In fact, the microbiome of elderly in comparison with the microbiome of children or young adults shows a decrease of Bifidobacteria but an increase of Enterobacteria (Hopkins, Sharp, & Macfarlane, 2001; Woodmansey, McMurdo, Macfarlane, & Macfarlane, 2004). It was also shown that there is a negative correlation of the amount of Bifidobacteria with serum levels of TNF-alpha and IL-10 in the elderly, which delineates that microbiota can also modulate inflammatory responses (Ouwehand, Tiihonen, Saarinen, Putaala, & Rautonen, 2009). Whether AD has a direct influence on gut microbiota or if it is the other way around remains elusive; the research in this field is still quite scarce. To this time, only a few published papers have reported on alterations in gut microbiota in AD mouse models compared with wild-type littermates or in AD patients in comparison with cognitively healthy controls (for a summary: see Table 49.1). If we analyze the results of the different publications, it is obvious that the depth of investigation differs in regard to level of microbial phyla, family, or genus. Therefore, it is hard to

Table 49.1 Microbial changes in the gut associated with Alzheimer's disease.

Reference	Model	Microbial phyla		Microbial family		Microbial genus (microbial species)	
Harach et al. (2017)	AD mouse model	Firmicutes	↓	Erysipelotrichidae		Allobaculum	↓
		Bacteroidetes	↑	Rikenellaceae	↑	n.d.	
				S24-7	↑		
		Verrucomicrobia	↓	Verrucomicrobiales		Akkermansia	↓
		Proteobacteria	↓	n.d.		n.d.	
		Actinobacteria	↓				
		Tenericutes	↑				
Brandscheid et al. (2017)	AD mouse model	Firmicutes	↑	Clostridiaceae		Clostridium (*C. leptum*)	↑
		Bacteroidetes	↓	n.d.		n.d.	
		Verrucomicrobia	–	Verrucomicrobiales		Akkermansia	–
		Proteobacteria	–	Enterobacteriaceae		Escherichia (*E. coli*)	–
		Actinobacteria	–	Bifidobacteriaceae		Bifidobacterium	–
Bauerl, Collado, Diaz Cuevas, Vina, and Perez Martinez (2018)	AD mouse model	Firmicutes		Erysipelotrichaceae	↑	n.d.	
				Ruminococcaceae		Ruminococcus	↓
						Oscillospira	↓
		Proteobacteria	↑	Sutterellaceae		Sutterella	↑
		Bacteroidetes		Rikenellaceae	↓	n.d.	
Shen, Liu, & Ji (2017)	AD mouse model	Proteobacteria		Helicobacteraceae	↑	Helicobacter	↑
				Desulfovibrionaceae	↑	n.d.	
		Bacteroidetes		Porphyromonoadaceae		Oderibacter	↑
				Prevotellaceae		Prevotella	↓
Vogt et al. (2017)	Human	Firmicutes	↓	Ruminococcaceae	↓	n.d.	
				Turicibacteraceae	↓		
				Peptostreptococcaceae	↓		
				Clostridiaceae	↓	Clostridium	↓
				Mogibacteriaceae	↓	n.d.	
				Veillonellaceae		Dialister	↓
				Erysipelotrichaceae		Turicibacter	↓
				Gemellaceae	↑	Gemella	↑
				Lachnospiraceae		Blautia	↑
		Bacteroidetes	↑	Bacteroidaceae	↑	Bacteroides	↑
				Rikenellaceae	↑	Alistipes	↑
		Actinobacteria	↓	Bifidobacteriaceae	↓	Bifidobacterium	↓
				Coriobacteriaceae		Adlercreutzia	↓
		Proteobacteria		Desulfovibrionaceae		Bilophila	↑
Cattaneo et al. (2017)	Human	Firmicutes		Eubacteriaceae		Eubacterium (*E. rectale*)	↓
		Proteobacteria		Enterobactericeae		Escherichia/Shigella	↑

Gut microbes found to be altered in samples of AD mouse models and humans with AD pathology. The alterations have been described on the level of microbial phyla, family and species (increase: ↑; decrease: ↓; no change reported; *n.d.*, not determined).

draw conclusions from those results. Additionally, the time points of pathology in which microbiota were analyzed must be considered—e.g., we reported a decrease in Firmicutes in AD model mice and an increase in Bacteroidetes; however, this was of transient nature and disappeared at 18 weeks of age in the 5xFAD mice (Brandscheid et al., 2017). Therefore, changes in microbiome occurring before clinical diagnosis of AD, in MCI, and in manifested AD also might differ. Generally, we urgently need more data but also a consensus on sampling and analyses to draw an informed picture. Moreover, it can be assumed that not only gastrointestinal microbiota changes but also barrier leakage can directly contribute to pathology. The primary responsibility of the mucosal barrier is to sustain the intestinal milieu and its microbiome. It consists of one single epithelial layer and can disintegrate with age (Man et al., 2015), so the penetrance of bacteria or their metabolites via the gut mucosal barrier can increase. Besides the mucosal barrier, the blood–brain barrier might show an increase of permeability with increasing age, and analyses of AD postmortem tissue samples indicate blood–brain barrier damage (Montagne et al., 2015). As these barriers play a key role in maintenance of endogenous microbiome and integrity of the host's homeostasis, failure or changes in their probabilities easily might affect both, the microbiome as well as the pathological conditions of the human being.

Eating and digestion habits in Alzheimer's disease

Microbiota seems to be changed in AD as described in the preceding section. However, it is rather unclear how and why this happens. One obvious option is that eating habits in AD patients might change with onset of or along pathology. Several reports exist that describe altered feeding behavior in patients; however, to estimate the impact of those is difficult because of incoherent phenotypes. Besides increased food intake, its decrease, or even altered food choice, has been described (Morris, 1989; Cullen, 1997), with a tendency to crave high-fat, high-sugar chow (Mungas et al., 1990). Additionally, changes in eating abilities such as swallowing and transit time have been found (Priefer & Robbins, 1997; Ikeda, Brown, Holland, Fukuhara, & Hodges, 2002). All of those phenomena might explain the weight loss that can be demonstrated in AD patients even before cognitive clinical signs (e.g., Jimenez et al., 2017). Interestingly, AD mouse models also display weight gain changes and altered feeding habits such as increased frequency and duration of feeding bouts (Pugh, Richardson, Bate, Upton, & Sunter, 2007). Speculation has been made that altered eating behavior in patients depends on sensory impairment due to degradation processes in the entorhinal cortex, cognitive decline, behavioral disturbances, comorbidities, or even environmental factors regarding hospitalization (e.g, Slaughter, Eliasziw, Morgan, & Drummond, 2011). For example, a correlation between a high-glycemic diet and amyloid burden in the brain of cognitively normal older adults has been reported (Taylor et al., 2017), and this indicates that feeding behavior might be an important factor for AD development in general.

Impact of symptomatic treatments on the gut microbiome

Besides feeding habits, medication for symptomatic treatment in AD might have impact on microbiota. Single case reports indicated potentially increased higher GI tract bleeding probability in patients using cholinesterase inhibitors (ChEIs) such as donepezil or rivastigmine (e.g., Cholongitas, Pipili, & Dasenaki, 2006). However, a retrospective population-based cohort study with residents from Ontario (Canada) with about 50,000 ChEI users and 50,000 nonusers reported no correlation of upper GI tract bleeding and the respective medication (Thavorn et al., 2014). By analyzing nearly 19,000 reports on adverse drug reactions by ChEIs from 58 countries, Kroger and colleagues found 15.9% of the reactions being of gastrointestinal quality, with 11.6% being serious (Kroger et al., 2015). Adverse GI events due to ChEIs seem to be typically transient and to vanish or decrease with continued use of the respective drug. Nevertheless, this in sum leads to the assumption that AD symptomatic treatment itself or other comedication can have an impact on microbiota of the gut and must also be considered in investigations on microbial commensals.

Pro- and antipathological properties of bacterial endogenous metabolites

There are various putative options by which bacterial products might influence pathomechanisms of AD: toxic effects, activation of immune cells, amyloidogenesis-enhancing products or even molecular mimicry by sharing of host epitopes. A first hint of the impact of bacterial metabolites might be seen in an early publication from 1990: Fabiszewski and colleagues reported that within a cohort of 104 institutionalized patients, those who developed fever, one of the hallmarks of inflammation, had more advanced disease (Fabiszewski, Volicer, & Volicer, 1990). Meanwhile, increasing data exist that describe, for example, elevated levels of lipopolysaccharide, a component of the outer leaflet of gram-negative bacteria, in the brain of AD patients and also report on amyloidogenic peptides from bacteria (see Table 49.2). While several bacterial compounds display devastating properties, microbial proteinases were found that could degrade amyloid beta or prevent its aggregation and therefore might also be of therapeutic value. In addition to endogenous products of bacteria, breakdown of nutritional compounds ingested by the host can be of relevance; one example are urolithins from pomegranate. They have been considered neuroprotective, and it has been shown that their protective properties might be due to their microbial metabolites, the ellagitannins (Yuan et al., 2016). Such polyphenols derived from plant-based alimentation often are ingested as inactive precursors and might mandatorily need metabolization by microbial commensals to be transformed into bioactive substances (e.g., reviewed by Valdes et al., 2015). Validating beneficial or detrimental effects of single nutritional compounds on brain physiology or pathology, therefore, must account for this potential conversion, and single species capable of this might therefore indirectly contribute to disease progression.

Table 49.2 Bacterial products potentially affecting in Alzheimer's disease pathology.

Bacterial product	Model	Observation	Reference
Lipopolysaccharide (LPS)	CD14 (LPS receptor) transfected CHO cells	Direct interaction of CD14 with Abeta42	Liu et al. (2005)
	Brain sections of patients	Spatial correlation of CD14 with AD typical lesions	
	AD mouse model	Exacerbation of tau pathology	Sy et al. (2011)
	AD brain samples	Detection of LPS from *Porphyromonas gingivalis*	Poole, Singhrao, Kesavalu, Curtis, and Crean (2013)
	AD brain samples	*E. coli* K99 pili proteins and LPS elevated	Zhan et al. (2016)
Amyloidogenic peptides		Pore forming toxin of *Klebsiella pneumoniae*	Bieler et al. (2005)
		Curli amyloid fibers (biofilm) from *E. coli*	Wang, Smith, Jones, & Chapman (2007)
		Histidine-rich protein forms amyloid-like oligomers from *Helicobacter pylori*	Ge, Sun, Wang, Zhou, & Sun (2011)
Toxins	Pulmonary microvascular endothelial cells	Exotoxin Y from *Pseudomonas aeruginosa* causes tau phosphorylation, accumulation of insoluble tau, and increases BBB leakage	Ochoa, Alexeyev, Pastukh, Balczon, and Stevens (2012)
	In vitro	*E. coli* endotoxin potentiates Abeta fibrillogenesis	Asti and Gioglio (2014)
Bacterial DNA	Senile plaques of AD cases	*Spirochetes* DNA in senile plaques	Miklossy (2016)

Table 49.2 Bacterial products potentially affecting in Alzheimer's disease pathology.—cont'd

Bacterial product	Model	Observation	Reference
A-beta degrading enzymes	SH-SY5Y cells	Maltose binding protein from *E. coli* inhibits Abeta aggregation and toxicity	Sharoar et al. (2013)
	In vitro, SH-SY5Y cells	SKAP from *Streptomyces* sp. degrades Abeta monomers, oligomers and fibrils	Yoo, Ahn, Park, Kim, and Jo (2010)
	In vitro	Nattokinase from *Bacillus subtilis natto* degrades Abeta40 fibrils	Hsu, Lee, Wang, Lee, and Chen (2009)
	In vitro, SH-SY5Y cells	Proteinases of (B) *pumilus* degrade Abeta42	Danilova et al. (2014)

Metabolites derived by bacteria influencing AD-related proteins (such as Tau) or bacterial products found associated with AD (-like) lesions are summarized. *Abeta*, amyloid beta.

Therapeutic and diagnostic options

Future research definitely will lead to a deeper understanding of the role of the gut microbiome in AD. If it reveals that the microbiome directly influences pathological hallmarks and development or progression of disease, then several therapeutic implications are thinkable; restoration of a "healthy" microbiome via fecal microbiota transfer is already being used successfully in patients with inflammatory bowel disease (Chen et al., 2018). Additionally, shifting the microbiome via pre- or probiotics to a certain beneficial composition might also be helpful. For instance, administration of *Lactobacillus rhamnosus* HN001 during pregnancy was able to lower, postpartum, clinically relevant anxiety (Slykerman et al., 2017), indicating that at least for short-term treatments, probiotics might act on psychological conditions. Moreover, if consistent data on changes in microbiota are found throughout multiple investigations, this might lead to the development of new microbial biomarkers for AD. In this regard, the use of the ENS as a surrogate for brain neurons can be considered; the tissue is easier to access and has the advantage of potentially maintaining age-related hallmarks while iPSC-derived neurons might not. In sum, this gives reason to expect that if microbial changes observed in AD are not merely side effects of the disease, they will open up a whole world of innovative medication strategies. However, much work to demonstrate cause versus consequence still must be done.

Key facts of the gut and its microbiome

- The gut microbiota consists of about 1.5 kg in an adult human.
- The microbiome outnumbers the genome of its host by a factor of 150.
- The gut inherits an autonomous nervous system that has similar neuronal cells and uses the same neurotransmitters as neurons of the brain.
- This enteric nervous system is directly confronted with the microbiota of the gut and their metabolites.
- Therefore, the gut might consist of a body side with the potential to indicate neurological pathological conditions or even contribute to their origin.
- For PD, depositions of alpha-synuclein have been shown in the gut decades before clinical diagnosis.
- For AD, research on the role of gut commensal microbes has just started to arise.

Summary points

- This chapter focuses on the impact of gut microbiota on AD.
- The microbiome of AD patients seems to differ from that of healthy individuals of the same age.
- However, it is hard to decipher whether this is a cause or consequence of the disease.
- AD patients show deviating eating habits and weight loss even before clinical diagnosis, both of which might affect their gut commensals.
- Interestingly, the blood–brain and gut–brain barriers, both of which compromise exchange between the brain and the gut, decrease in integrity along with aging—the most relevant risk factor for sporadic AD.
- Microbial metabolites or nutrients processed by microbes might have both, deleterious or protective effects on brain pathology.
- Therefore, understanding the microbial community in health and disease might offer new avenues for therapeutic intervention or symptomatic treatments.

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CHAPTER 50

(-)-Epigallocatechin-3-gallate and Alzheimer's disease

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List of abbreviations

AChE acetylcholinesterase
AD Alzheimer's disease
ADAM10 A disintegrin and metalloproteinase domain-containing protein 10
AMT abbreviated mental test
APP amyloid precursor protein
Aβ amyloid beta peptide
BACE-1 beta secretase 1
Bcl-2 B-cell lymphoma 2
BDNF brain-derived neurotrophic factor
c-Abl-FE65 Abelson tyrosine kinase—amyloid beta precursor protein binding family B member 1
C99 beta-secretase-derived C-terminal fragment of APP
CamKII calmodulin-dependent protein kinase II
CAT catalase
CDR Clinical Dementia Rating
COX-2 cyclooxygenase 2
CREB cAMP-response element binding protein
CVF categorical verbal fluency
DFT design fluency task
DG dentate gyrus
DNA deoxyribonucleic acid
DSM—III—R *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition, Revised
DST digit span task
EGCG (-)-epigallocatechin-3-gallate
ERK extracellular signal-regulated kinase
FC functional connectivity
G6PDH glucose-6-phosphate dehydrogenase
GDS global degeneration scale
GFAP glial fibrillary acidic protein
Gli1 GLI family zinc finger 1
GPx glutathione peroxidase
GR glutathione reductase
GSH glutathione
GSSG glutathione disulphide

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GTC green tea catechin
GTE green tea extract
ICAM1 intercellular adhesion molecule 1
IL-16 Interleukin 16
IL-1β Interleukin 1 beta
IL-6 Interleukin 6
iNOS inducible nitric oxide synthase
IQCODE informant questionnaire on cognitive decline in the elderly
IRS-1 insulin receptor substrate 1
ITGAM integrin alpha M
KOLT Kendrick object learning test
LTCI long-term care insurance
m-BD modified block design
M-CSF macrophage colony-stimulating factor
m-DST modified digit symbol test
MCI mild cognitive impairment
MDA malondialdehyde
MMSE mini mental state evaluation
MMSE-J MMSE Japanese version
MMSE-K MMSE Korean Version
MWM Morris water maze
NEP neprilysin
NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells
NGF nerve growth factor
NIA-AA National Institute on Aging and Alzheimer's Association
NMADR1 glutamate ionotropic receptor NMDA subunit 1
NPI-Q neuropsychiatric inventory—questionnaire
p-Akt phospho-protein kinase B
p-JNK phospho c-Jun N-terminal kinase
p65 transcription factor p65
$p75^{NTR}$ p75 neurotrophin receptor
PAT passive avoidance test
pc-Raf phospho-RAF proto-oncogene serine/threonine-protein kinase
pGSK3β phopho-glycogen synthase kinase 3 beta
PKA protein kinase A
PKC protein kinase C
PSD95 postsynaptic density protein 95
Ptc patched
RAVLT Rey Auditory Verbal Learning Test
RAWM radial arm water maze
ROS reactive oxygen species
S-task Controlled Oral Word Association test
S100B S100 calcium-binding protein B
SAMP8 senescence accelerated mouse-prone 8
sAPPα soluble alpha-APP
SCD subjective cognitive decline
SDMT symbol digit modality test
Shh sonic hedgehog
SOD superoxide dismutase

SST spatial span task
T-SOD total superoxide dismutase
T2DM type II diabetes mellitus
TBARS thiobarbituric acid reactive substances
TCA tricarboxylic acid cycle
TICS telephone interview for cognitive status
TMT-A trail making test, part A
TNF-α tumor necrosis factor alpha
TrkA tropomyosin receptor kinase A
VRT visual reproduction task
WT wild-type
4-HNE 4-Hydroxynonenal
8-oxodG 8-Oxo-2′-deoxyguanosine
3MS modified mini-mental state

Mini-dictionary of terms

Adult neurogenesis The process of formation of new neurons in the adult brain; in humans, this process is thought to occur exclusively in the subgranular zone of the hippocampus.
Catechins Natural secondary metabolites produced by plants, among them the tea tree; catechins are part of the flavonols family.
Epigallocatechin gallate The most abundant catechin in green tea.
Green tea extract Mix of flavonols and other secondary metabolites obtained from green tea through an extraction process.
Insulin resistance Process by which cells, in our case neurons, have reduced or depleted response to insulin signaling, impairing the uptake of glucose.
Neuroinflammation Inflammatory response specific to the neurological system that includes both chemokines and increased activation of glial cells.
Synaptic plasticity The formation and modulation of synapses connecting neurons between them secondary to neuronal activity; it is the process underlying learning and memory.

Therapeutic potential of (-)-epigallocatechin-3-gallate in the secondary prevention of cognitive decline and Alzheimer's disease development

Alzheimer's disease (AD) is characterized by the accumulation of both insoluble amyloid β-peptide (Aβ) in extracellular plaques and intracellular neurofibrillary tangles of aggregated tau protein. Current knowledge has allowed a shift in the definition of AD from a syndromic to a biological construct based on biomarkers that are proxies of pathology. However, little is known about those mechanisms underlying disease progression at its early stages that would be more sensitive to preventive treatments. Recent research suggests that the shift from "silent" altered neural circuit activity to memory impairments are secondary to abnormal firing and synaptic plasticity in the corticohippocampal circuits. Alterations in synaptic plasticity and functionality would be at the core of mild cognitive impairment (MCI) in early AD caused by the accumulation of nonfibrillar, oligomeric Aβ occurring well in advance of evident widespread synaptic loss and neurodegeneration

(Skaper, Facci, Zusso, & Giusti, 2017). In humans, alterations in functional connectivity (FC) have been observed in two early AD stages, subjective cognitive decline (SCD) and MCI. A hypersynchronized anterior network and a posterior network characterized by a decrease in FC are the spatial features. These disruptions are also seen in AD and indicate that FC alterations appear very early in the course of the disease (Lopez-Sanz et al., 2017).

For this reason, it is important to develop therapeutic interventions that are multifaceted and can affect synaptic plasticity and functional connectivity in the early stages of the disease, including SCD; one such intervention is epigallocatechin-3-gallate (EGCG). We recently reviewed the mechanisms by which EGCG may confer neuroprotection to different AD insults (Xicota, Rodriguez-Morato, Dierssen, & de la Torre, 2017). Among them, the most relevant that will be developed in this chapter are (1) antioxidant activity through the Nrf2-pathway, (2) protection against neuroinflammation, (3) targeting some key partners of AD such as amylogenesis and tau hyperphosphorylation, and (4) modulation of plasticity.

Green tea and (-)-epigallocatechin-3-gallate antineurodegenerative effects: epidemiological and clinical evidence

Green tea consumption in Asian countries is considered a protective factor for the development of neurodegenerative diseases, with its health claims being attributed to its antioxidant nature (Lee et al., 2017; Tomata et al., 2016). This chapter will capitalize on the hypothesis that green tea consumption, in particular its main flavanol EGCG, could have beneficial effects on cognitive decline and AD development and progression as well as summarize the evidence supporting it.

Several observational studies have analyzed the effects of green tea on cognition. Due to the nature of these studies, findings are limited to the usages of the populations analyzed and thus tend to focus on populations with a steady consumption of tea such as the Chinese or Japanese populations. Fewer analyze the consumption of tea in European or American populations and in general take into account the effect of other dietary or lifestyle factors such as coffee consumption. The results of all these studies have been summarized in Table 50.1.

As explained previously, observational studies depend vastly on the origin of the study population. Thus, we will first focus on the evidence gathered from populations that do not traditionally consume green tea but that have other alternate sources of flavonoids. For example, one study focused on the global health benefits of flavonoids, including but not restricted to tea, showing that intake of flavonoids was inversely associated with the risk of developing dementia (Commenges et al., 2000). However, the specific effect of each source of flavonoids was not explored, and we cannot ascertain whether part of the effect was due to the ingestion of tea. Other studies have compared the effects of tea with other flavonoid or caffeine-rich sources such as wine, chocolate, and coffee.

Table 50.1 Summary of the observational studies analyzing the effect of green tea or its catechins on neurodegeneration and cognition.

References	Study type	Population	Age	N	% females	Food	Demented	Cognitive test	Results
Commenges et al. (2000)	Longitudinal (3-year follow-up)	French	Over 75: 50.6% (Dordogne) 55.5% (Gironde)	689 (Dordogne) 1626 (Gironde)	51.2% (Dordogne) 58.5% (Gironde)	Flavonoid rich food	No	DSMIII-R and MMSE	↑flavonoid intake = ↓risk of dementia
Dai et al. (2006)	Longitudinal Mean follow-up: 6.3 ± 2.6 years	Japanese Americans	71.8 years	1589	54.4%	Sake/Wine Tea Fruit or Vegetable juice	No	Cognitive abilities screening instrument	No association between tea drinking and AD risk.
Kuriyama et al. (2006)	Cross-sectional	Japanese	Older than 70.	1003	~57.1%	Green tea	Yes	MMSE	↓ prevalence of cognitive impairment with ↑ consumption of tea. ↑ GT consumption = ↓ cognitive impairment.
Ng et al. (2008)	Cross-sectional and longitudinal (1 −2 years)	Chinese in Singapore	55 or older.	Cross-sectional: 2501 Longitudinal: 1438	63.7%	Tea (black/oolong, English black, green)	Yes, on cross-sectional. Subjects from longitudinal were nondemented at baseline.	MMSE	↑ total tea consumption = ↑ scores in MMSE. ↑ black tea consumption = ↓ cognitive impairment and cognitive decline. ↓GT consumption = ↓ cognitive impairment.
Eskelinen et al. (2009)	Longitudinal. Mean follow-up: 21 ± 4.9 years	Finland	71.3 ± 4.0 at follow-up	1409	62%	Coffee and tea at midlife	Yes.	MMSE, DSM-IV, NINCDS-ADRDA	No associations between tea consumption and dementia.

Continued

Table 50.1 Summary of the observational studies analyzing the effect of green tea or its catechins on neurodegeneration and cognition.—cont'd

References	Study type	Population	Age	N	% females	Food	Demented	Cognitive test	Results
Huang et al. (2009)	Cross-sectional	Chinese	93.48 ± 3.30 (range 90 −108)	681	67.25%	Tea (all)	Yes.	MMSE.	No significant OR for tea consumption and cognitive impairment.
Nurk et al. (2009)	Cross-sectional	Norway	Unreported	2031	Unreported	Wine, chocolate, tea.	Unspecified.	KOLT, TMT-A, m-DST, m-BD, modified MMSE, S-task	Tea consumers had better scores than nonconsumers in TMT-A, m-DST, m-BD, and m-MMSE.
Feng et al. (2010)	Cross-sectional	Chinese in Singapore	64.5 ± 6.8	716	61.5%	Tea (black/oolong, green), coffee.	No. MMSE ≥24.	DST, SST, RAVLT, VRT, CVF, DFT, SDMT, MMSE.	↑ tea consumption = ↑ MMSE score, memory, executive functions, and information processing speed. Green and black/oolong tea consumers: ↑ MMSE, memory, executive function, and information processing speed.
Arab et al. (2011)	Longitudinal. Median follow-up: 7.9	USA (North Carolina, California, Maryland, Pennsylvania)	65 and older.	4809	56.6%	Caffeinated drinks (coffee and tea)	Unspecified.	3MS, TICS or IQCODE.	↑ coffee/tea/caffeine consumption = ↓ rates of decline in women.
Feng et al. (2012)	Longitudinal	Chinese	91.4 (80−115 range)	7139 at baseline, 913 last follow-up.	57%	Tea	No	Verbal fluency test	Daily tea drinkers had higher verbal fluency scores at baseline and follow up than nondrinkers.
Tomata et al. (2012)	Longitudinal. Follow-up: 3 years.	Japanese	65 or older.	13988	55.8%	Green tea.	Unspecified, without a disability.	LTCI test for functional disability.	↑ frequent green tea consumption = ↑ cognitive activity. ↑ GT consumption = ↓ incident functional disability.

Noguchi-Shinohara et al. (2014)	Longitudinal. Follow-up: 4.9 ± 0.9 years	Japanese	60 or older.	490	Unreported.	Tea (green and black), coffee	No	MMSE, CDR, DSM−III−R.	Baseline: GT consumers ↑ MMSE scores. Follow-up: ↑consumption of GT = ↑ MMSE scores, ↓ incidence of dementia.
Shen et al. (2015)	Cross-sectional	Chinese	70.0 ± 7.69 (60−100)	9375	51.5%	Tea (all kinds)	Yes	MMSE	↑ black tea consumption = ↓ cognitive impairment.
Kitamura et al. (2016)	Cross-sectional	Japanese	68.9	1143	44.6%	Modifiable factors (exercise, BMI) Including green tea consumption.	Yes	MMSE	↓ GT consumption = ↑ cognitive impairment.
Yang et al. (2016)	Cross-sectional	Chinese	79.5 ± 7.6	2015	58.8%	Tea	Yes	NIA-AA criteria, MMSE, CDR	Tea drinkers have lower prevalence of dementia, AD, and cognitive impairment
An et al. (2017)	Longitudinal (between 1998 and 2012)	Chinese	80 or older	4749	53%	Diet, including tea intake	Yes, not in baseline	MMSE	No association of tea consumption and cognitive impairment.
Gu et al. (2017)	Cross-sectional	Chinese	60 or older	4579	52%	Tea	Yes	AMT	↑ Frequency of tea drinking = ↑ AMT scores. Tea drinkers: ↓OR for cognitive impairment, Not statistically significant for GT.

This table summarizes the characteristics of all the observational studies focusing on the effect of tea on cognition and/or neurodegeneration. Within the detailed characteristics there are the kind of study (longitudinal or cross-sectional), the origin of the study population, their age, sample size, and sex ratio, the source of flavonoids, the cognitive tests used, and the results.

Two of them found an inverse association between tea consumption and cognitive impairment, in a cross-sectional study (Nurk et al., 2009), or with the rate of cognitive decline in women, in a longitudinal one (Arab et al., 2011). The latter, however, also found a similar association for coffee and general caffeine consumption, and therefore it is not clear whether the beneficial effect of tea consumption is related to the flavonoids present in tea or to the caffeine. The possibility that the effect is due to caffeine consumption is supported by a longitudinal study analyzing the effect of tea or coffee intake in a Finnish population on the midlife development of dementia. In this study, no protective effect for tea consumption was observed, while a decreased risk of dementia in moderate coffee drinkers was found (Eskelinen, Ngandu, Tuomilehto, Soininen, & Kivipelto, 2009).

Studies in Asian populations, and therefore populations that have a steady consumption of tea, can be divided depending on the kind of tea consumed. Chinese populations tend to drink preferably black or oolong tea, while Japanese drink mostly green tea. In those studies focusing on general tea consumption but not on the consumption of a specific kind of tea, several point toward a general direct association between lower tea consumption and cognitive impairment (Feng, Gwee, Kua, & Ng, 2010; Gu et al., 2017; Shen et al., 2015; Yang et al., 2016) or cognitive decline (Feng et al., 2012; Ng, Feng, Niti, Kua, & Yap, 2008). When analyzing the kind of tea consumed, only two studies found an inverse association between green tea consumption and dementia (Feng et al., 2010; Ng et al., 2008), while most pointed to a beneficial effect of black tea (Gu et al., 2017; Ng et al., 2008; Shen et al., 2015). Other similar studies on general tea consumption did not find any beneficial effect independently of the type of tea (An, Liu, Khan, Yan, & Wang, 2017; Dai, Borenstein, Wu, Jackson, & Larson, 2006; Huang, Dong, Zhang, Wu, & Liu, 2009).

In studies performed in Japanese populations, with a long tradition of consuming green tea, inverse associations were found between the amount of green tea consumed and both the prevalence (Kitamura et al., 2016; Kuriyama et al., 2006) and the incidence of dementia or functional disability (Noguchi-Shinohara et al., 2014; Tomata et al., 2012).

Although the results of these observational studies are mixed, we need to bear in mind that they are the result of epidemiological studies where tea flavonoid consumption is self-reported and originates from different sources, making it difficult to establish a proper dose—response relationship. Clinical trials conducted in a more controlled setting should allow the study of not only the effects of green tea but also the comparison of them with an untreated group. However, clinical trials are costly, and for this reason there are only three published studies analyzing the effects of green tea on cognitive decline, with a fourth one declared in ClinicalTrials.gov without available results (NCT00951834). The results from the published clinical trials (Table 50.2) are conflicting, with two

Table 50.2 Summary of the Clinical trials analyzing the effect of green tea or its catechins on neurodegeneration and cognition.

References	Study type	Age	N	% Females	Treatment	Dementia	Cognitive test	Results
Park et al. (2011)	Double-blind Placebo-Controlled	40–75	91 46 LGNC-07 45 Placebo	72.5%	LGNC-07 430 mg twice a day (360 mg GTE, 60 mg L-theanine) 16 weeks	Yes	MMSE-K, GDS, Rey-Kim memory test, Stroop color-word reading test	MMSE-K 21–23: ↑Rey-Kim test at 8 weeks and of immediate and delayed recall after 16 weeks compared to baseline, without treatment effect. Treatment: ↑ Stroop word read at 8 weeks and color reading at 16. Independently of MMSE-K score: ↑ Stroop color reading at 16 weeks compared to baseline.
Ide et al. (2014)	Open-label	88 ± 7.6	12	83.3%	Green tea powder (2 g/day, 227 mg catechins and 42 mg theanine) 3 months	Yes 8 vascular dementia 3 AD 1 dementia with Lewy body	MMSE-J	MMSE-J scores improved after 3 months of green tea consumption. Treatment improved short-term memory.
Ide et al. (2016)	Randomized placebo-controlled	84.8 ± 9.3	33 Placebo: 16 Green tea: 17	87.9%	Green tea powder, 2g a day 12 months	Yes 17 AD 15 vascular dementia 1 dementia with Lewy bodies	MMSE-J, NPI-Q	No differences after 1 year of treatment.

This table summarizes the characteristics of all the clinical trials focused on the effect of tea on cognition and/or neurodegeneration. Within the detailed characteristics are the kind of study, age of the study population, sample size, sex ratio, source of flavonoids, neurodegenerative disease included, cognitive tests used, and results.

reporting improvements in memory and attention (Park et al., 2011) or cognitive impairment (Ide et al., 2014) and the other reporting no differences after treatment (Ide et al., 2016).

Another flaw of the available human studies is that they do not look into the mechanisms that cause improved cognition. For this reason, animal and cellular studies are needed, and we focused on evidence provided by animal models; for an extended assessment that includes cell models, see a previous review (Xicota et al., 2017).

Mechanisms behind the effect: evidence from animal models

When trying to understand the mechanisms of EGCG on neurodegeneration, it is important to look into not only models that mimic the disease but also models that study the effect on a healthy brain. Thus, the results presented in this section include disease models, aging models, and healthy young animals. Likewise, they also include experiments with any of the formulations that contain EGCG—green tea extracts (GTEs) and green tea catechins (GTCs) (Table 50.3).

Before analyzing the possible mechanisms of action behind the procognitive effect observed in humans, it is important to highlight that similar beneficial effects have been observed in animal models for GTEs or GTCs—either as pre- or posttreatment—on learning and memory (Rezai-Zadeh et al., 2008; Schimidt, Garcia, Martins, Mello-Carpes, & Carpes, 2017; Unno et al., 2007, 2008), especially in spatial learning and memory (Biasibetti et al., 2013; Chang et al., 2015; Jia et al., 2013; Lee, Lee et al., 2009; Lee et al., 2013; Lee, Yuk et al., 2009; Li et al., 2009a, 2009b; Lin, Chen, Chiu, Way, & Lin, 2009; Liu et al., 2014; Mi et al., 2017; Walker et al., 2015; Wang et al., 2012). However, a limited number of studies failed to find an effect of GTEs or GTCs on behavior (Flores et al., 2014; Gibbons et al., 2014) and found a beneficial effect for exercise instead.

These beneficial effects on cognition are secondary to the molecular and cellular mechanisms of action that will be subsequently explored (Fig. 50.1).

Reduction of oxidative stress

Oxidative damage is one of the hallmarks of aging and has been tightly related to several neurodegenerative diseases (Mariani, Polidori, Cherubini, & Mecocci, 2005). GTCs, in particular EGCG, have long been studied as antioxidant molecules (Lee et al., 2017; Tomata et al., 2016), although whether the effect is direct or subsequent to the activation of endogenous antioxidant defenses is controversial. Most studies agree on the general effect of EGCG (or green tea/GTEs) in decreasing oxidative status—induced, due to aging, or genetic—as proven by the reduction in DNA (Unno et al., 2007), protein (Li, Zhao, Zhao, Zhang, & Li, 2010; Srividhya et al., 2009), and lipid oxidative damage (Gibbons et al., 2014; Li et al., 2010; Schimidt et al., 2017; Srividhya et al., 2009) as well as the production of ROS (Biasibetti et al., 2013; Flores et al., 2014; Schimidt et al., 2017) and

Table 50.3 Summary of animal model studies analyzing the effect of green tea or its catechins.

References	Model	Alzheimer model	Treatment	Behavioral test	Results	Conclusion
Levites et al. (2003)	Mice C57/BL ♂ 20-22 gr N = 6 per group (2)	No, young	EGCG 2 mg/kg/day oral 3, 7, and 14 days	None	↓ APP levels in hippocampal membrane fraction, ↑ soluble fraction. ↑ membrane-bound PKC.	EGCG modifies the APP processing through PKC.
Rezai-Zadeh et al. (2005)	Mice Tg2576 ♀ 12 months n = 10	Yes, genetic model	EGCG 20 mg/kg Intraperitoneal 60 days	None	↓ $A\beta_{1-40}$ and $A\beta_{1-42}$ ↑ α-secretase activity ↓ Aβ deposits	EGCG decreases Aβ production through an increase of the nonamyloidogenic processing.
	Mice Tg2576 ♀ 7 months n = 10		EGCG 10 μg intraventricular single injection		↓ $A\beta_{1-40}$ and $A\beta_{1-42}$. ↑ α-secretase activity.	
Unno et al. (2007)	Mice SAMP10/TaSlc ♂ 1 month N = 66	No, senescence prone	0.02% Polyphenon 70S 35 mg/kg/day drinking water 1, 5, 8, 11, and 14 months	Passive avoidance test	↓ levels of 8-oxodG in 15-month old mice. = learning time, ↑ memory. ↑ antioxidant activity.	GTC decrease oxidative DNA damage and improve memory in senescent mice.
Unno et al. (2008)	Mice SAMP10/TaSlc ♂ 6–9 months N = 45 in three groups	No, senescence model	Polyphenon 70S 0.02% catechin drinking water 3 or 6 months	Passive avoidance test	↓ learning times for 6 month-treated animals. ↑ synaptophysin	GTC alleviate brain dysfunction in aging.

Continued

Table 50.3 Summary of animal model studies analyzing the effect of green tea or its catechins.—cont'd

References	Model	Alzheimer model	Treatment	Behavioral test	Results	Conclusion
Rezai-Zadeh et al. (2008)	Mice Tg2576 and WT ♀ 8 months N = 10 per group Tg2576 (2) + 10 WT	Yes, genetic	EGCG 50 mg/kg Drinking water 6 months	RAWM	↓ Aβ deposition and plaques ↓ soluble forms of Aβ ↑ ADAM10 maturation and sAPPα ↑ working memory performance to WT level	EGCG activates the nonamyloidogenic pathway, decreasing Aβ production. Decreases tau phosphorylation. Improves working memory.
	Mice Tg2576 ♀ 12 months N = 10 Tg2576 + 5 WT		EGCG (20 mg/kg) Intraperitoneal 60 days		↓ phospho-tau to WT levels ↑ working memory performance to WT level	
Srividhya et al. (2009)	Rats Wistar ♂ 3–4 months Older than 24 months N = 6 per group (4)	No	EGCG 2 mg/kg/day gavage 30 days	None	↑ enzymatic and nonenzymatic antioxidants of aged rats ↓ MDA and protein carbonyls in aged rats ↑ TCA and electron transport chain complexes enzyme activities ↓ 4-HNE from Purkinje cells	EGCG improves antioxidant status in aged rats.

Lee, Lee et al. (2009)	Mice IcrTacSam:ICR ♂ 5 weeks N = 10 per group (4)	Yes, induced LPS i.c.v.	EGCG 1.5 and 3 mg/kg Drinking water 3 weeks before induction	MWM and passive avoidance test	↓ latency in PAT in a dose-dependent manner ↓ escape latency in MWM in a dose dependent manner ↓ $A\beta_{1\text{-}42}$ in cortex and hippocampus ↓ GFAP immunoreactivity ↓ γ-secretase activity in cortex and hippocampus ↓ β-secretase only in cortex. ↓ iNOS and COX-2 ↓ apoptosis	EGCG prevents learning and memory loss, through a decrease in production of $A\beta_{42}$ and activity of secretases, apoptosis and inflammatory response.
Lee, Yuk et al. (2009)	Mice IcrTacSam:ICR ♂ 5-week-old N = 9 per group (4)	Yes, induced $A\beta_{1\text{-}42}$ i.c.v.	EGCG 1.5 and 3 mg/kg drinking water 3 weeks before induction	Passive avoidance test, MWM	↓ MWM escape latency ↓step-through latency in PAT ↓ β and γ-secretases in cortex and hippocampus ↑ α-secretase High dose: ↓ $A\beta_{1\text{-}42}$ in cortex and hippocampus Low dose: ↓$A\beta_{1\text{-}42}$ in cortex. ↓ $A\beta_{1\text{-}42}$ and C99 ↓ cell death ↓ ERK and NF-kB	EGCG prevents the memory and learning problems caused by the injection of $A\beta_{42}$. Due to the decrease of the production of $A\beta_{42}$ through the inhibition of β and γ-secretases and the activation of α-secretase. It also inhibits apoptosis reducing, thus neurodegeneration.

Continued

Table 50.3 Summary of animal model studies analyzing the effect of green tea or its catechins.—cont'd

References	Model	Alzheimer model	Treatment	Behavioral test	Results	Conclusion
	Mice PS2 11−17 months N = 6 per group (2)	Yes, genetic	EGCG 3 mg/kg Drinking water 1 week		↓escape latency in MWM ↓ $A\beta_{1-42}$ ↑ α-secretase in cortex and hippocampus ↓ β-secretase in hippocampus and γ-secretase in cortex	EGCG rescues the learning deficits seen in transgenic mice while affecting secretase activities and decreasing Aβ production.
Lin et al. (2009)	Mice Tg2576xc57BL/6 and WT 8 months N = 35 transgenic (in 2 groups), 17 WT	Yes, genetic	EGCG Oral 20 mg/kg 4 months	MWM	↓ $A\beta_{1-40}$ and $A\beta_{1-42}$ levels. ↓ Aβ plaques ↓ c-Abl-FE65 interaction ↓ escape latency in MWM	EGCG prevents impairments in spatial memory potentially through decrease in Aβ production and decrease in apoptosis.
Li et al. (2009a)	Mice C57BL/6J ♀ 1 month N = 15 14 month N = 60	No	GTC 0.025%, 0.05%, and 0.1% drinking water 6 months	Open field, MWM	Medium and high doses: ↑ performance in MWM ↑ CREB and PKA phosphorylation Medium and high doses: ↑BDNF and Bcl-2 ↑PSD95 and CamKII	GTC prevent deficits in spatial memory. Effects could be due to the maintenance of the phosphorylation of CREB and postsynaptic proteins involved in long term memory formation.

Li et al. (2009b)	Mice SAMP8 and SAMR1 ♂ 4 month N = 48 SAMP8 in 3 groups, 15 SAMR1	No, senescence prone	GTC 0.05% and 0.1% Drinking water 6 months	Open field, MWM	↓ escape latency, ↑ time in target quadrant and platform crossings, similar to age matched SAMR1. ↓ $A\beta_{1-42}$ oligomer levels ↑ pCREB and pPKA ↑ BDNF, PSD95, and CamKII	GTC prevent spatial memory deficit by decreasing $A\beta_{1-42}$ oligomer formation and ameliorating the deficits on the CREB pathway.
Assunção et al. (2010)	Rats Wistar ♂ 12 months N = 15	No	Green tea Drinking fluid 52.32 ± 0.90 mg/kg/day 7 months	None	↑ GSH and ↓ GSSG ↑ SOD activity, ↓CAT ↑ CREB activation ↑ BDNF and Bcl-2	Green tea has neuroprotective and antioxidant effects.
Li et al. (2010)	Mice C57L/6J ♀ 14 months N = 30 in 2 groups 1 month N = 15	No	GTC 0.5 g/L Drinking water 6 months	None	↑ T-SOD and GPx ↓ hippocampal TBARS and protein carbonyls ↓ nuclear p65 expression, higher in cytoplasm in hippocampus. ↓ lipofuscin granules ↑ of postsynaptic proteins PSD95 and NMDAR1	GTC decrease the oxidative stress, prevent the activation of the NF-kB pathway, and neurodegeneration.

Continued

Table 50.3 Summary of animal model studies analyzing the effect of green tea or its catechins.—cont'd

References	Model	Alzheimer model	Treatment	Behavioral test	Results	Conclusion
Wang et al. (2012)	Mice C57BL/6J ♂ 2 months N = 10–12 group	No, disruption of the Shh pathway: 10 mg/kg/day i.p. cyclopamine/2-hydroxypropyl-beta-cyclodextrin 10 days	EGCG 20 mg/kg/day i.p. 60 days	MWM	↑ proliferation of the NPCs in DG ↑ performance in the MWM hidden platform ↑ time in target quadrant ↑ *Ptc* and *Gli1* in the hippocampus (*Ptc* in DG) specific ↑ Ptc and Gli1 ↑ glutamate concentrations	EGCG enhances learning and memory in 4-month-old mice (improvements in object recognition and spatial memory). EGCG stimulates NPCs neurogenesis in adult mice through the Shh pathway.
Biasibetti et al. (2013)	Rats Wistar ♂ 90-day old N = 10–11 per group (4)	Yes, induced STZ 3 mg/kg i.c.v.	EGCG 10 mg/kg Gavage 4 weeks (2 after surgical procedure)	MWM	↑ MWM performance = glucose uptake ↓ S100B in hippocampus ↑ S100B CSF ↓ AChE activity ↑ GPx activity = Glutathione ↓ ROS and nitrite content	EGCG reverses cognitive deficit by modulating oxidative and nitrative stress as well as astroglial alterations.
Jia et al. (2013)	Mice APP/PS1 C57BL/6 ♀ 12 months N = 8 in groups (3)	Yes, genetic	EGCG 2 or 6 mg/kg Gavage 4 weeks	MWM	↓ escape latency ↑ time spent in target quadrant ↓ $A\beta_{42}$ in hippocampus. ↓ IRS-pS636 in hippocampus ↓ TNF-α and p-JNK levels ↑ pAkt and pGSK3β	EGCG improves spatial learning and memory, while decreasing Aβ production, neuroinflammation and restoring of altered insulin signaling.

Lee et al. (2013)	Mice IcrTacSam:ICR ♂ 5 weeks N = 10 per group (4)	Yes, induced with LPS (250 μg/kg ip daily 7 days after EGCG treatment)	EGCG 1.5 and 3 mg/kg drinking water 3 weeks	MWM, passive avoidance test	↓ escape latency ↑ time spent on target quadrant ↑ step-through latency ↓ iNOS positive cells in cortex and hippocampus ↓ iNOS and COX-2 expression in brain ↓ $A\beta_{1-42}$ positive plaques, levels of $A\beta_{1-42}$, activity of β and γ-secretases ↓ BACEs-1 and BACE-1 positive cells, and expression of APP, BACE1 and C99 ↓ GFAP-reactive cells and GFAP expression ↓ cells positive for both GFAP and $A\beta_{42}$ ↓ M-CSF, ICAM-1 and IL-16	EGCG ameliorates memory deficits, decreases inflammation.

Continued

Table 50.3 Summary of animal model studies analyzing the effect of green tea or its catechins.—cont'd

References	Model	Alzheimer model	Treatment	Behavioral test	Results	Conclusion
Liu et al. (2014)	Mice APP/PS1 and WT ♂ and ♀ 9 months N = 20 transgenic and 10 WT	Yes, genetic	EGCG 2 mg/kg/day Intragastric 4 weeks	Passive avoidance test, MWM, locomotion test	↑ performance in PAT ↓ latency and path length in MWM, ↑ time in target quadrant = locomotion ↓ $A\beta_{40}$ plaque formation ↓ APP expression ↓ apoptotic cells ↓ caspase-3 levels ↑ NGF and proNGF levels. ↑ TrkA pathway: ↑ pTrkA, pc-Raf, pERK1/2, and pCREB ↓ $p75^{NTR}$ pathway	EGCG improves learning and memory impairment, reverting spatial acquisition deficits and memory consolidation. It decreases APP expression and $A\beta_{40}$ plaque formation. EGCG also inhibits $p75^{NTR}$ and activates the TrkA pathway leading to an improvement of neurodegeneration, amyloidosis, and cognitive deficits.
Gibbons et al. (2014)	Mice BALB/cByJ ♂ 19 months old	No	EGCG + β-alanine 182 mg/kg/day In diet 4 week Voluntary wheel running	MWM, Contextual Fear Conditioning	Voluntary wheel running: ↑ performance in MWM and contextual fear conditioning. ↑ Bdnf, ↓ IL-1β and Itgam/CD11b EGCG and β-alanine: ↓ 4-HNE	EGCG decreases oxidative stress but does not have an effect in behavior or in gene expression.

Flôres et al. (2014)	Rats Wistar ♂ 9 months N = 73 in 4 groups 2 months N = 10	No	Green tea 13.33 gr/L Drinking water 3 months Exercise	Object recognition, Inhibitory avoidance, Open field, plus maze, tail flick	↑ discrimination index. Exercise: ↑ step-down latency in inhibitory avoidance. ↑ GSH in prefrontal and GPx in hippocampus and striatum. Exercise + GT: ↑ prefrontal GSH and striatum GPx GT: ↑ striatal GPx and cortex GSH ↓ ROS in hippocampus and striatum	Green tea decreases oxidation but no effect on behavior.
Walker et al. (2015)	Mice TgCRND8 and WT CH3/C57BL/6 ♂ and ♀ 2 months N = 42 transgenic and 12 WT	Yes, genetic	EGCG 0.2 mg/mL drinking water 4 months exercise	Open field, light/dark box, Barnes maze, and nest building	EGCG and/or exercise: ↑ nesting behavior EGCG: ↑ hyperactivity in open field Exercise ± EGCG: ↓ locomotor activity EGCG and/or exercise: ↑ performance in Barnes, ↑ learning acquisition. ↓ soluble Aβ in cortex and hippocampus	EGCG and/or exercise improves nesting behavior and spatial memory, decrease in brain Aβ.

Continued

Table 50.3 Summary of animal model studies analyzing the effect of green tea or its catechins.—cont'd

References	Model	Alzheimer model	Treatment	Behavioral test	Results	Conclusion
Chang et al. (2015)	Mice SAMP8 and SAMR1 4 months N = 10 per group SAMP8 (3) and 10 SAMR1	No, senescence model	EGCG 5 and 15 mg/kg Intragastric 60 days	MWM	↓ escape latency and distance traveled ↑ platform crossings with high dose Recovery of neuron arrangement and number of neurons ↓ Aβ levels ↑ NEP expression	EGCG improves spatial learning and memory. It promotes neuronal survival, decreases Aβ accumulation, while promoting its degradation.
Schimidt et al. (2017)	Rats Wistar ♂ 2 months N = 8−12 per group (8)	Yes, induced with 2 μL $A\beta_{25-35}$ 8 weeks after start of treatment	Green, red, or black tea as drinking water 10 weeks	Object recognition memory, social recognition memory test	Green and red tea: ↑ short term recognition in OR ↑ social recognition All teas: ↑ long term recognition in OR Green tea: ↓ROS and TBARS. = antioxidant capacity	Green tea decreases oxidation without affecting the antioxidant capacity, improves short- and long-term memory.

This table summarizes the characteristics of the animal model studies detailed in this chapter. It includes information about the model, whether it is a model of AD and which kind, treatment including dose and administration route, behavioral tests performed, a summary of the results, and a conclusion.

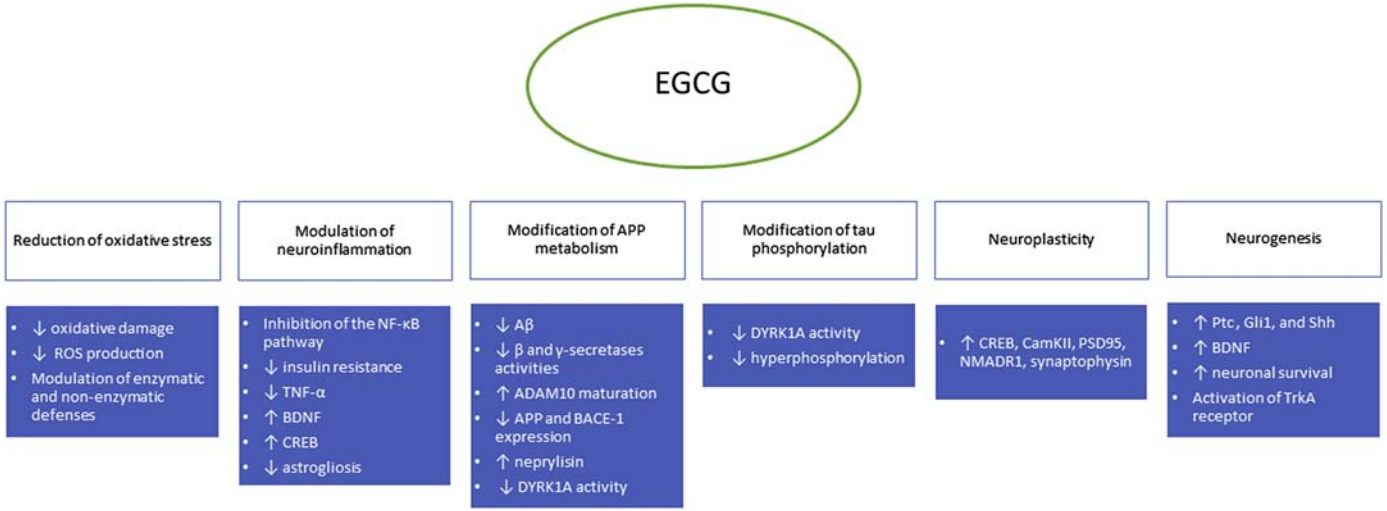

Figure 50.1 Schematic representation of the mechanisms of action of EGCG. Summarizes the EGCG mechanisms of action against AD discussed in this particular chapter (*clear boxes*) with specific evidence for each (*dark boxes*).

nitrite content (Biasibetti et al., 2013). These antioxidant effects could be explained by the modulation of both enzymatic (including SOD, catalase, GPx, GR, and G6PDH) (An et al., 2017; Assuncao, Santos-Marques, Carvalho, & Andrade, 2010; Flores et al., 2014; Li et al., 2010; Srividhya et al., 2009) and nonenzymatic antioxidant defenses (Srividhya et al., 2009) and could be derived from EGCG-dependent Nrf2 activation (Na & Surh, 2008).

Modulation of neuroinflammation

Another prevalent occurrence in AD brains is an elevated inflammatory status deeply related to oxidation (Niranjan, 2013). Indeed, one of the master regulators of inflammation such as NF-κB is activated by oxidation, and EGCG prevents the activation of its pathway (Li et al., 2010) by decreasing its phosphorylation as well as that of p38 (Mi et al., 2017). Interestingly, this latest effect is also linked to a decrease in insulin resistance with a decrease in plasma insulin and of the altered phosphorylation of IRS-1 (Mi et al., 2017); a similar effect on insulin resistance was observed by Jia et al.; however, in this case it was associated to a decrease in the proinflammatory TNF-α (Jia et al., 2013), which was also observed in microglia treated with EGCG (Lai et al., 2018). The effect on the NF-κB pathway can probably be linked to several other decreases in proinflammatory proteins, including but not limited to iNOS (Lai et al., 2018; Lee et al., 2013; Lee, Yuk et al., 2009; Mi et al., 2017), interleukins such as IL-1β and IL-6 (Lai et al., 2018), and the cyclooxygenase COX-2 (Lai et al., 2018; Lee et al., 2013; Lee, Yuk et al., 2009). In addition to inhibition of NF-κB, EGCG could be exerting its effects on these proinflammatory proteins through an increase in brain-derived neurotrophic factor (BDNF) (Lai et al., 2018), which could be secondary to an increase in cAMP-response element binding protein (CREB) protecting against synaptic degeneration (Mi et al., 2017). Furthermore, EGCG has proven to alleviate astrogliosis, as shown by decreases in glial fibrillary acidic protein (Lee et al., 2013) and S100B (Biasibetti et al., 2013).

Modification of amyloid precursor protein metabolism and tau phosphorylation

Amyloid precursor protein (APP) and its amyloidogenic cleavage products have long been identified as possibly causative of AD (Hardy & Higgins, 1992); therefore, it is of interest to understand whether GT or GTC has any effect on its metabolism or expression.

Indeed, several studies have shown that GTC and EGCG have the ability to decrease the production of Aβ (Walker et al., 2015), which translates into a decrease in intra- and extracellular concentrations (Chang et al., 2015), the levels of amyloid oligomers (Li et al., 2009b), and the number of plaques (Lee et al., 2013; Lin et al., 2009; Liu et al., 2014; Rezai-Zadeh et al., 2008). Such effects can be explained by the specific actions

of EGCG on APP metabolism, including a decrease in β and γ-secretase activities (Jia et al., 2013; Lee, Lee et al., 2009; Lee, Yuk et al., 2009; Lin et al., 2009), an increase in the nonamyloidogenic processing (Rezai-Zadeh et al., 2005, 2008) secondary to the increase of A disintegrin and metalloproteinase domain-containing protein 10 maturation—a metalloprotease with α-secretase activity (Fernandez, Rezai-Zadeh, Obregon, & Tan, 2010)—a decrease in the expression of APP and/or beta secretase 1 in the brain (Lee et al., 2013; Liu et al., 2014) or of APP in the membrane (Levites, Amit, Mandel, & Youdim, 2003), and an increase of the expression of the amyloid-degrading enzyme neprilysin (Chang et al., 2015).

Additionally, EGCG has been shown to inhibit dual-specificity tyrosine-phosphorylation regulated kinase in vivo (Guedj et al., 2009) a kinase with increased expression in brains of patients with AD (Ferrer et al., 2005) and that is known to phosphorylate both tau, priming it for hyperphosphorylation (Park & Chung, 2013), and APP, leading to an increased amyloidogenic processing (Ryoo et al., 2008). Interestingly, EGCG has also been proven to decrease tau phosphorylation in transgenic mice to the level of age-matched wild-type, which could potentially reduce the number of neurofibrillary tangles (Rezai-Zadeh et al., 2008), although the exact mechanism of action is not discussed.

Specific effects on plasticity and neurogenesis

Although all the previously described effects have an indirect effect on plasticity, several direct effects of EGCG on this process have been described. Particularly, EGCG has proven to increase plasticity or prevent synaptic degeneration through an increase in CREB (Assuncao et al., 2010), CamKII and PSD95 (Li et al., 2009a), NMADR1 (Li et al., 2010), BDNF (Li et al., 2009b), and synaptophysin (Unno et al., 2008).

One of the less studied effects of EGCG related to neurodegeneration is the effect on adult neurogenesis described by Wang et al., with EGCG increasing Ptc and Gli1 in the hippocampus leading to an increase in neurogenesis in the dentate gyrus (Wang et al., 2012). In vitro studies have observed an increase in Shh expression after EGCG treatment linked to increased BDNF levels (Lai et al., 2018). However, neurogenesis without survival is of little use. Fortunately, EGCG has also proved to increase neuronal survival in SAMP8 mice, including the arrangement of neurons (Chang et al., 2015). This could be related to its effect on the NGF pathway, activating the TrkA receptor while inhibiting the $p75^{NTR}$ and thus favoring cell survival (Liu et al., 2014).

(-)-Epigallocatechin-3-gallate as a possible treatment for Alzheimer's disease? Future perspectives

After examining all the available evidence in both humans and animal models, a question remains: Is it feasible to use EGCG as a treatment for AD? Most research done in animal

models points in that direction, especially considering the nonamyloidogenic, antiinflammatory, antioxidant, and neuroprotective effects that the compound has either alone or in combination with other GTCs. It is worth pointing out, however, that in many induced models, the treatment started before the insult, and in the case of the genetic ones, the treatment was chronic and started before any sign of the disease. This would mean that any treatment would be preventive, although there is a possibility that EGCG could be useful in the first stages of the disease in order to stop further progression.

A large number of modifiable risk factors for AD have been identified in observational studies, many of which do not appear to exert effects through amyloid or tau. This suggests that primary prevention studies focused on risk reduction and lifestyle modification may offer additional benefits (Galvin, 2017).

In this context, clinical trials designed to evaluate the efficacy of EGCG should incorporate a personalized medicine approach that includes a multicomponent intervention (nutritional, physical, cognitive, and medical) looking at improving person-centered outcomes.

One such example is early phase I studies in young adults with Down syndrome, which showed that while subjects were under EGCG, improvements in cognition were observed, but these vanished when treatment was discontinued (De la Torre et al., 2014). Phase II studies combining EGCG with cognitive training showed improvements in cognitive performance and adaptive functionality, but interestingly, had sustained effects after treatment discontinuation (De la Torre et al., 2016). Observations made in humans are in agreement with preclinical studies showing that EGCG combined with environmental enrichment results in an improvement of age-related cognitive decline (Catuara-Solarz et al., 2015; Pons-Espinal, Martinez de Lagran, & Dierssen, 2013). These observations favor the option of combining EGCG with personalized multicomponent intervention, taking into account medical comorbidities (i.e., metabolic syndrome, T2DM), diet (including nutritional status), physical exercise, cognitive training, and behavioral intervention to aid the subject's adherence and empowerment to the intervention proposed. This is in-line with other clinical studies in AD showing the superiority of multicomponent interventions versus a single lifestyle intervention (e.g., single nutrient, physical activity) (McEwen et al., 2018).

Key facts of (-)-epigallocatechin-3-gallate

- Green tea, which is mainly consumed in Asian countries, accounts for 20% of world tea production.
- EGCG is the main polyphenol present in green tea, accounting for 50%–70% of the total polyphenols present in this beverage.
- EGCG is a pleiotropic compound that has been attributed with a number of biological activities in vitro and in animal models, but few of them translate into health benefits in humans.

- In humans, EGCG is safe and well tolerated. Not surpassing a daily dose of 800 mg is recommended.
- EGCG is the first compound able to improve selected cognitive functions and adaptive functionality in persons with intellectual disabilities.

Summary points

- Tea consumption has been linked to a lower dementia risk in specific Asian populations.
- Clinical trials, albeit inconclusive, show a potential beneficial effect of EGCG on AD.
- EGCG has been proven to decrease oxidative stress through the modulation of antioxidant defenses secondary to the activation of Nrf2.
- EGCG can directly modulate neuroinflammation through a reduction of insulin resistance, a decrease in the activation of the NF-κB pathway, and an increase in BDNF.
- EGCG decreases APP expression and its cleavage through the amyloidogenic pathway while at the same time decreasing tau phosphorylation.
- EGCG is able to reduce synaptic degeneration and increase adult neurogenesis.
- EGCG shows its potential as a preventive treatment for AD in conjunction with other lifestyle changes.

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CHAPTER 51

Lead, cadmium and Alzheimer's disease

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List of abbreviations

AChE acetylcholinesterase
AD Alzheimer's disease
ALS amyotrophic lateral sclerosis
APP amyloid precursor protein
Aβ amyloid-beta
BBB blood–brain barrier
CERAD Consortium to Establish a Registry for Alzheimer's Disease
CI confidence interval
CNS central nervous system
CSF cerebrospinal fluid
DMT1 divalent metal transporter-1
IQ intelligence quotient
IQR interquartile range
MAPT microtubule-associated protein tau
MMSE mini-mental status exam
mTOR mammalian target of rapamycin
NAS Veteran's Affairs Normative Aging Study
NHANES National Health and Nutrition Examination Survey
PND postnatal day

Mini-dictionary of terms

- Lead is a bluish-gray metal with symbol Pb and atomic number 82.
- Cadmium is a bluish-white metal with symbol Cd and atomic number 48.
- The blood–brain barrier is a network of closely spaced cells that help keep harmful substances in the blood from reaching the brain.
- The choroid plexus is a plexus of blood vessels or nerves that produces the CSF in the ventricles of the brain and a component of the blood–CSF barrier.
- Metallothionein is a low-molecular-weight sulfhydryl-rich metal-binding protein.
- Oxidative stress is an excess imbalance between free radicals and antioxidants that causes harm in cells.

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Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disease attributable to a combination of genetic and environmental factors. The prevalence of AD has recently increased dramatically, and environmental factors in the etiology of AD have received increasing attention as potentially preventable or modifiable risk factors. Among nongenetic environmental factors, the roles of toxic heavy metals, such as lead and cadmium, are poorly understood despite their known neurotoxic effects. Lead and cadmium are notable for their toxic effects even at low levels of exposure encountered in the general environment. Given their widespread exposure in the general population, it is important to understand the role of these toxicants in the etiology of AD. In this chapter, we review the experimental and epidemiologic literature of the associations between AD and lead and cadmium.

Lead and Alzheimer's disease

Prevalence

Toxicity due to lead exposure was noted as early as 370 BC (Tong et al., 2000). The removal of lead from paint and gasoline is a major public health success, though lead's persistence in soil, dust, old plumbing, and historic house paint make avoidance difficult, and lead is still used in industrial applications, including automobile lead-acid batteries (Dissanayake & Erickson, 2012). The U.S. Centers for Disease Control and Prevention has established a reference level of 5 μg/dL blood lead for children and pregnant women, however, a safe level of blood lead has not been identified (Grandjean & Landrigan, 2014). Approximately 500,000 children ages 1−5 years in the United States have levels exceeding the reference (Raymond & Brown, 2017), particularly concentrated in cities and low socioeconomic areas (Campbell et al., 2016). Globally, high lead levels are associated with electronic waste recycling, lead mining, and smelting (Ericson et al., 2016). Lead exposure remains widespread worldwide and domestically.

Overall lead health effects

Lead is responsible for approximately 1% of the global burden of disease (WHO, 2010), including permanent effects on childhood intelligence quotient (IQ) and behavioral problems (Grandjean & Bellanger, 2017). In U.S. children under 5 years of age, there are annually 22,947,450 IQ points lost due to lead exposure at an estimated cost of $50 billion (Grandjean & Landrigan, 2014). In older adults, lead exposure is associated with amyotrophic lateral sclerosis (ALS) (Kamel et al., 2005), Parkinson's disease (Weisskopf et al., 2010), hearing loss (Choi et al., 2012), age-related cataracts (Schaumberg et al. 2004), glaucoma (Wang et al. 2018), and other chronic conditions. Specific to this review, lead exposure is associated with accelerated cognitive decline and dementia.

General mechanisms linking lead exposure to AD

Exposure sources: ingestion, inhalation, endogenous

Common exposure sources to lead vary by age and geographic location (Fig. 51.1). Housing build prior to 1970 may have paint containing lead, contributing to house dust, which adults and children inhale (Jacobs et al., 2002). Children ingest lead dust due to frequent hand-to-mouth behavior. Older homes may also have leaded pipes or solder in their plumbing, which adults and children ingest through water. Industrial lead smelters and trash incinerators release lead into the local atmosphere as a by-product. Local residents have higher body burden of lead due to contamination of air and soil from deposits (Meyer et al., 2008). Globally, most people are exposed to lead through inhalation or ingestion (Fig. 51.2).

In older adults, the primary source of lead exposure can be endogenous. Excretion of lead is relatively slow, and accumulation is common (Papanikolaou et al., 2005). During early and middle life, lead is sequestered in the bones, where it replaces calcium in the hydroxyapatite structure (Hu et al., 1998). The skeleton contains 70%–95% of the body burden of lead and lead can remain in bones for decades (Hu et al., 1998), a useful feature for exposure assessment research. Adults experiencing loss of bone mass via osteoporosis release lead into the bloodstream. In older adults, 40%–70% of blood lead can be attributed to previous body stores (Hu et al., 1998). Lead that entered the body during previous periods of high exposure can become biologically active decades later.

Absorption into the bloodstream and travel to the brain

Once lead enters the body, it is absorbed into cells and tissues. Inhaled lead particles cause local damage in the lungs and 30%–40% can be absorbed into the bloodstream,

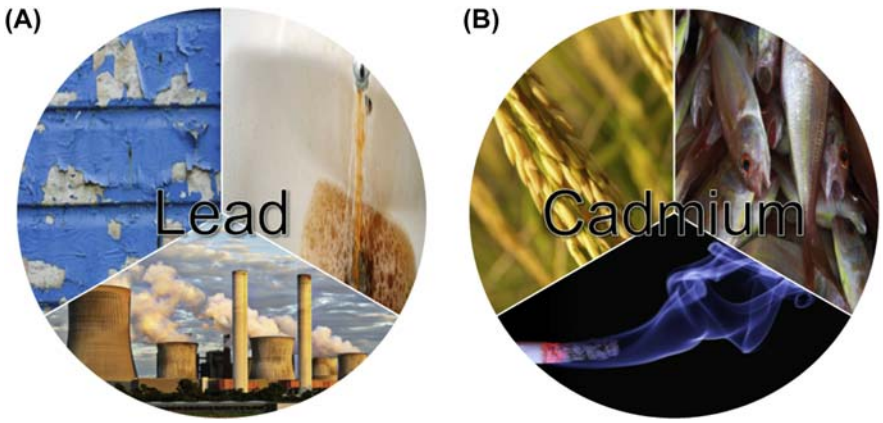

Figure 51.1 Exposure sources for lead (A) and cadmium (B). Primary sources of lead exposure include paint contributing to house dust, water from leaded pipes, and air from industrial processes. Cadmium exposure sources include cigarette smoking and dietary seafood and vegetables.

Figure 51.2 Most human exposure to lead and cadmium occurs through ingestion or inhalation.

depending on particle size (Papanikolaou et al., 2005). The gastrointestinal track of adults absorbs 10%–15% of ingested lead, while pregnant women and children absorb 50% of ingested lead (Papanikolaou et al., 2005). Individual level factors, such as diet (low iron, calcium, phosphorus, or zinc) and genetic polymorphisms, such as in the delta-aminolevulinic acid dehydratase and hemochromatosis genes, influence absorption rate (Onalaja & Claudio, 2000). Organic lead is absorbed by the skin, and this route is most often observed in occupational settings (Papanikolaou et al., 2005).

Absorbed lead circulates in the bloodstream. Lead enters cells by hijacking divalent metal transporters, designed to carry essential metals such as iron and copper (Zhu et al., 2013). Lead crosses the placental barrier and lead can be detected in infant cord blood at similar levels to maternal blood (Al-Saleh et al., 2011). The blood–brain barrier (BBB) physically separates the brain from water-soluble compounds in the bloodstream and transport is tightly regulated. Lead crosses the BBB by substituting for calcium (Sanders et al., 2009) and accumulates in the brain. Lead enters the body through ingestion or inhalation and is distributed in the bloodstream for transport to the brain (Fig. 51.3).

General consequences in the brain

Lead is a known neurotoxicant causing nonspecific brain disruption (Fig. 51.4). First, lead is a redox-inactive metal that causes oxidative stress by depleting thiols and damaging the antioxidant defense system (Ercal et al., 2001). Excessive oxidative stress results in endoplasmic reticulum stress, mitochondrial damage, and ultimately apoptosis of neurons (Sanders et al., 2009). Neurons experience excitotoxic damage from overactivation by

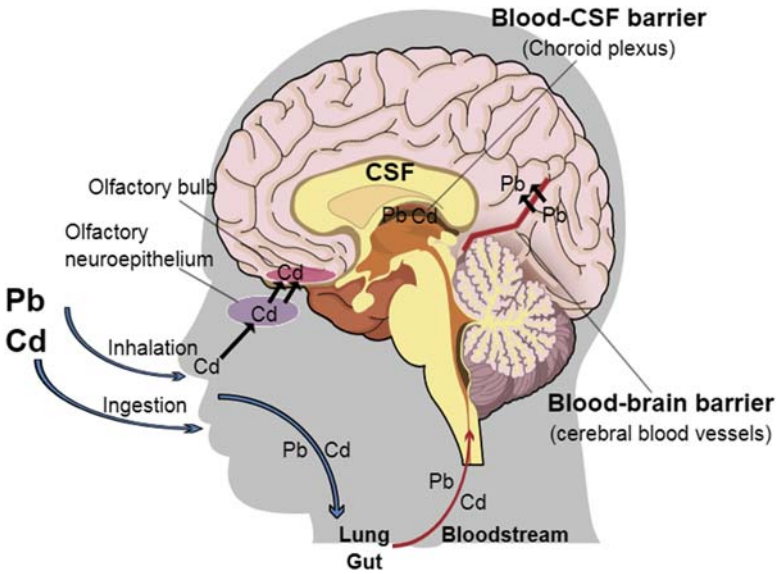

Figure 51.3 Transport of lead (Pb) and cadmium (Cd) to the brain. Lead and cadmium enter the body through the gut and lung, and are distributed in the bloodstream and transported to the brain. Cadmium also reaches the brain through the olfactory nervous system. Lead crosses the blood–brain barrier and accumulates in the brain. Cadmium and lead can accumulate in the choroid plexus, a component of the blood–cerebrospinal fluid barrier. The image was created in the Mind the GRAPH (https://mindthegraph.com/).

calcium associated with lead exposure (Sanders et al., 2009). Lead disrupts homeostatic levels of essential metals and alters normal metal signaling (Zhu et al., 2013). These actions together result in neuroinflammation (Sanders et al., 2009). Similar damage occurs to support cells, such as oligodendrocytes, microglia, astrocytes, and cerebrovascular endothelial cells (Sanders et al., 2009). Lead induces oxidative stress, endoplasmic reticulum stress, neuroinflammation, apoptosis, excitotoxicity, and essential metal disruption in the brain.

Experimental studies linking lead exposure and AD

Alzheimer's mechanisms and symptoms are observed in animal models (mouse, rat, and monkey) with lead treatment. Lead effects vary by species, timing, dose, and duration of exposure, though consistent impairments related to AD are observed.

Mouse model lead treatment: Aβ deposition, tau expression, altered learning

Mice are the most common model organism for studying lead's effects on the brain, due to the availability of transgenic AD susceptibility mice. Amyloid precursor protein (*APP*) transgenic mice treated with 50 mg/kg lead acetate oral gavages for 6 weeks had elevated

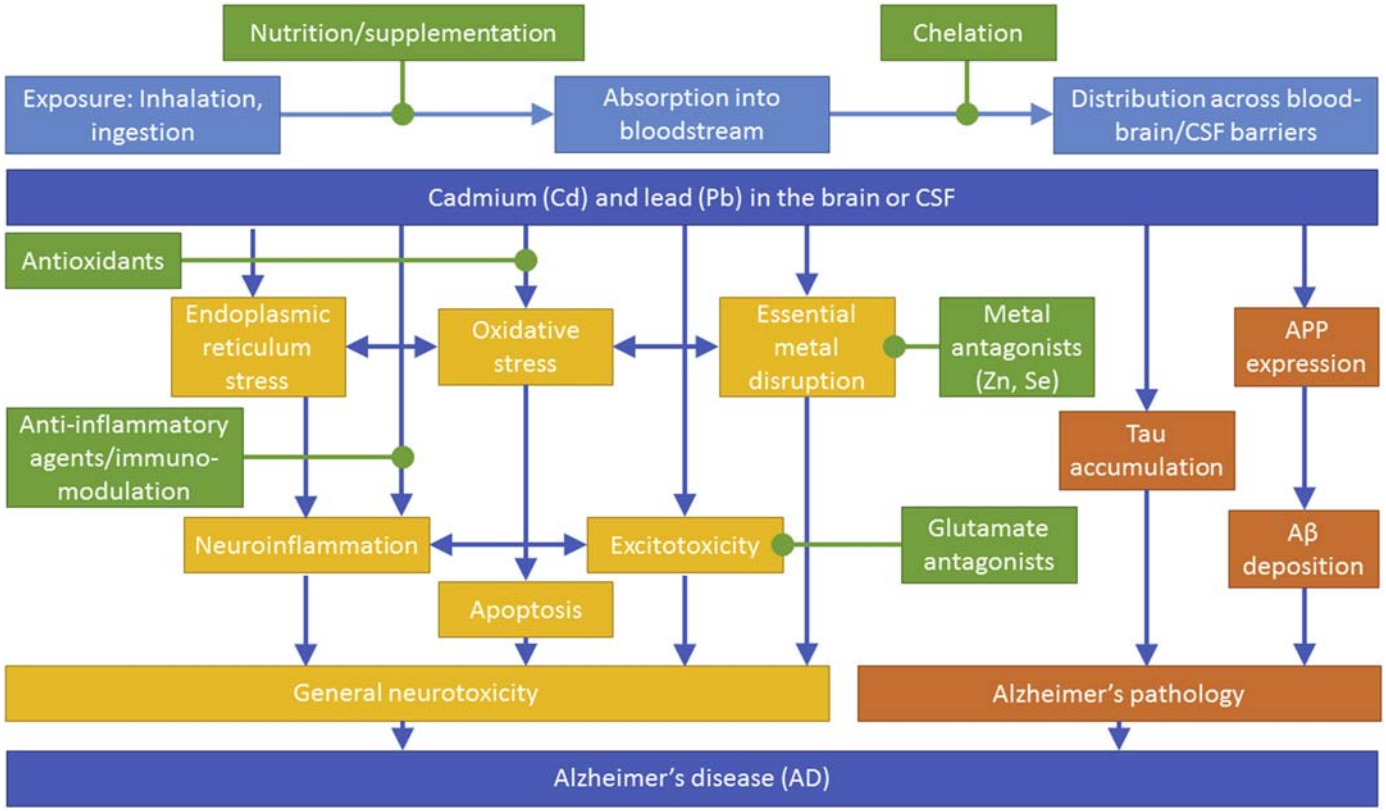

Figure 51.4 Mechanisms of general neurotoxicity action (yellow) and Alzheimer's specific toxicity (orange) of cadmium and lead on Alzheimer's disease. Possible intervention options are in green and exposure routes and body distribution are in light blue.

amyloid-beta $(A\beta)_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in the cerebrospinal fluid (CSF), cortex, and hippocampus, corresponding with impaired spatial learning on the Morris water maze test (Gu et al., 2012). Transgenic microtubule-associated protein tau (*MAPT*) mice exposed to 0.2% lead acetate from postnatal day (PND) 1–20 had altered expression of *MAPT* and miR-34c, an miRNA that targets *MAPT* (Dash et al., 2016). Similarly, lead-treated mice performed poorly on the Morris water maze test at 7 months of age, only when tau gene was knocked out (Wright et al., 2018).

Nontransgenic mouse studies identified timing of susceptibility and molecular targets of lead exposure. C57BL/6J mice exposed to 0.2% lead acetate from PND1-20 had altered miRNA expression that targets epigenetic mediators at PND180 (Masoud et al., 2016) and elevated tau protein and mRNA levels in 7 months age mice (Bihaqi et al., 2014). Similar effects were not observed with adult lead treatment (Bihaqi et al., 2014). Mice exposed to lead acetate had variable results on the Morris water maze based on the developmental timing of exposure (Liu et al., 2014).

Rat model lead treatment: Aβ deposition, tau expression, synaptic density, impaired learning

Male rats treated with 50 mg/kg lead acetate via intraperitoneal injection at 8–9 weeks of age had triple $A\beta_{1\text{-}40}$ fluorescence levels in the choroid plexus and lower RNA and protein levels of low-density lipoprotein receptor-1 (Behl et al., 2009). Rats of both sexes exposed to lead in the maternal drinking water PND1-30 had poorer performance on tests for learning, short-term memory, and long-term memory, which correlated with reduced number of synapses in the hippocampus and higher tau expression (Rahman et al., 2012). Rats of both sexes exposed to very low levels of lead (0.1%) in the maternal drinking water PND1-21 had increased tau protein in the forebrain and cerebellum and tau hyperphosphorylation, which caused cytoskeleton stability impairment and neuronal dysfunction (Gassowska et al., 2016).

Monkey model lead treatment: APP expression, Aβ deposition, tau levels

A unique long-term monkey lead exposure model has provided the strongest evidence for AD-related neurodegeneration. Female *Macaca fascicularis* exposed PND1-400 to 1.5 mg/kg/day lead acetate and then sacrificed at age 23 (Bihaqi et al., 2011). The aging primates exposed to lead exhibited overexpression of *APP*, Aβ, and enhanced pathologic neurodegeneration (Bihaqi et al., 2011). In the same cohort, early life lead exposure was associated with elevated *tau* mRNA, tau protein, its transcriptional regulators (*Sp1* and *Sp3*), and site-specific tau hyperphosphorylation (Bihaqi & Zawia, 2013). Early life lead exposure has a lagged effect on AD-related molecular pathways in older life.

Summary of lead exposure and human dementia epidemiology

Properties of the lead biomarker matrix are important factors for study design and interpretation. Common tissues used for lead measurement and their respective rate of decay in the body are blood (30 day half-life), patella bone (10–15 years half-life), and tibia bone (10–30 years half-life) (Chettle, 2005). Associations may differ based on timing and type of the measurements. In early and midlife, blood lead is expected to reflect exogenous exposure, while in late life, blood lead can be attributed to release of sequestered endogenous bone lead.

Exposure to lead is associated with neurodegeneration in cross-sectional human epidemiology studies (Shih et al., 2007). In a small matched case-control study of clinically confirmed AD, occupational exposure to lead was not associated with odds of AD (odds ratio = 1.12, 95% Cl: 0.63–2.00) (Gun et al., 1997). This suggestive observation inspired population-based studies in larger samples to investigate related outcomes. Among men (mean age 66.6 years) in the Veteran's Affairs Normative Aging Study (NAS), tibia bone lead was associated with poorer cognition, particularly pattern memory and spatial reasoning (Payton et al., 1998). The tibia lead association replicated in a larger NAS sample and similar findings were extended to patella lead and blood lead (Wright et al., 2003). Soon after, in the Baltimore Memory Study of men and women ages 50–70 years, tibia lead was associated with concurrent lower cognition, while blood lead was not associated with cognition (Shih et al., 2006). Lead exposure measured in blood, tibia bone, and patella bone was associated with clinically diagnosed ALS in a matched case-control study (Kamel et al., 2002), as well as Parkinson's disease in a large case-control study (Weisskopf et al., 2010), suggesting that lead exposure may be associated with multiple neurodegenerative processes and may not be specific to AD or dementia.

Epidemiology evidence is strengthened by the use of longitudinal studies to assess temporal relationships between exposure and disease. In the NAS when at least two Mini-Mental Status Exam (MMSE) scores were available, one interquartile range (IQR) (20 μg/g of bone mineral) higher patella bone lead concentration was associated with 0.24 points lower MMSE scores (95% CI: −0.44, −0.05) (Weisskopf et al., 2004). In a follow-up NAS analysis of up to five repeated cognitive measures over 18 years, an IQR higher level of patella lead was associated with 0.13 points lower MMSE score (95% CI: −0.251, −0.004) (Farooqui et al., 2017). Longitudinal studies of lead and cognitive decline require replication across study populations, but they suggest midlife lead exposure is associated with faster rates of cognitive decline.

Current epidemiologic studies of lead exposure are limited in the reach of their exposure measures (Bakulski et al., 2012). Adult bone lead estimates of cumulative lead stretch into midlife. The brain has periods of particular vulnerability to toxicants and exposure during vulnerable periods may increase risk of AD. Newer exposure methods include

tooth lead, where through targeted laser ablation, timing of metal exposure can be pinpointed (Hare et al., 2011). Future clinical studies of AD may incorporate lead exposure measures. AD is the most common form of dementia in late life, representing 70% of dementia cases (Brookmeyer et al., 2011), however, diagnosis requires specific clinical or pathological characteristics. Many lead exposure studies were conducted in population-based samples, and a large study sample would be required to observe enough cases to rigorously test AD's association with lead exposure.

Cadmium and Alzheimer's disease

Cadmium is a bluish-white metal naturally found in the earth's crust, which is environmentally persistent. Anthropogenic sources of cadmium include mining and refining, combustion of fossil fuels, waste incineration and disposal, and the manufacture and application of phosphate fertilizers (ATSDR, 2012). Cadmium has no essential physiologic function in humans and is classified as a Group-I carcinogen by the International Agency for Research on Cancer (Straif et al., 2009). Diet is the primary cadmium exposure source (Satarug et al., 2010) and cigarette smoking is another important source for nonsmokers and smokers (Fig. 51.1). Ingestion of contaminated foods and inhalation of air cadmium are major routes of exposure (Fig. 51.2). Long-term exposure to low-level cadmium increases risks for kidney damage, osteoporosis, hypertension, lower lung function, and diabetes (Satarug et al., 2010). Recently, cadmium has emerged as a neurotoxicant, although evidence in humans is still limited.

How can cadmium reach the brain?

Cadmium enters the body through the gut and lung (Fig. 51.3). The transport systems for divalent essential elements play a role in the cellular uptake of cadmium. Calcium, iron, and zinc transport systems (e.g., divalent metal transporter-1 [DMT1], calcium transporter-1, and calcium channels), transport cadmium (Himeno et al., 2009). Intestinal absorption of cadmium primarily occurs through DMT1 and depends on the body stores of other metals, especially iron. Iron deficiency increases intestinal absorption of cadmium through DMT1 (Park et al., 2002). Under normal conditions, only small amounts of cadmium can cross the BBB in adults (Takeda et al., 1999). DMT1, calcium transporters, and zinc transporters are expressed in neurons and vascular endothelial cells of the brain (Jenkitkasemwong et al., 2012; Siddappa et al., 2002). The choroid plexus, a component of the blood–CSF barrier, restricts blood toxicant access to the CSF and maintains internal central nervous system (CNS) homeostatic environment (Zheng, 2001). The choroid plexus is the main site of cadmium accumulation in the brain (Zheng, 2001).

Cadmium also reaches the brain through the olfactory nervous system. Intranasal instillation of cadmium in mice increased cadmium concentrations in the olfactory mucosa and olfactory bulbs (Bondier et al., 2008). After mouse intranasal cadmium instillation, odorant-evoked neurotransmitter release from the olfactory nerve was reduced, followed by diminished axonal projections from the olfactory epithelium to olfactory bulbs, supporting olfactory neurons as a direct transport pathway of cadmium to the brain (Czarnecki et al., 2011). Cadmium treatment damaged hippocampus-dependent spatial learning and memory and olfactory memory in mice (Wang et al., 2018). Evidence from mice suggests cadmium directly passes into the CNS through the olfactory system, causing persistent, irreversible damage by inhibiting adult neurogenesis in the hippocampus and olfactory bulb.

Experimental studies linking cadmium exposure and AD

Toxicological studies provide potential mechanistic pathways for cadmium's influence on the CNS. Direct effects through oxidative stress, neuroinflammation, and apoptosis in neuronal cells are well defined. Cadmium may also induce neurotoxicity by changing permeability of the BBB and interacting with other neurotoxicants, leading to Aβ aggregation and tau neurofibrillary tangle production. Pathogenic processes following cadmium exposure may result in cognitive impairment and AD (Fig. 51.4).

Oxidative stress, neuroinflammation, and apoptosis in neuronal cells by cadmium

Cadmium is a redox-inactive metal that indirectly induces oxidative stress (Ercal et al., 2001). Cadmium has a high affinity for sulfhydryl group of thiols, such as glutathione (GSH) and metallothionein (Figueiredo-Pereira et al., 1998). Acute high-level exposure or long-term persistent low-level exposure interferes with the antioxidant defense system (Cuypers et al., 2010). Cadmium induces oxidative stress in neuronal cells (Figueiredo-Pereira et al., 1998) and brain endothelial cells (Tobwala et al., 2014). GSH detoxification is activated at low cadmium doses and GSH depletion occurs at higher doses of cadmium with continued oxidative stress. Cadmium causes oxidative stress-dependent neuroinflammation and impaired neurodevelopment in young rats, enhanced with exposure to mixtures of lead, cadmium, and arsenic (Ashok et al., 2015). Rats treated with N-acetyl cysteine, a medication typically used to increase GSH levels following acetaminophen overdose, had toxic effects of cadmium reversed, including memory deficits, increased thiobarbituric acid reactive substances (a marker of lipid peroxidation), and decreased hippocampus, cerebellum, and hypothalamus acetylcholine esterase activity (Goncalves et al., 2010). Cadmium treatment induced brain oxidative stress and treatment with an antioxidant ameliorated cadmium neurotoxicity.

Oxidative stress from cadmium activates neurodegeneration signaling pathways, such as mitogen-activated protein kinase (MAPK), protein kinase B (Akt), mammalian target of rapamycin (mTOR), and CD95/APO-1 (Fas)/Fas Ligand (FasL)-mediated mitochondrial apoptotic pathways, leading to neuronal apoptosis (Chen et al., 2008, 2011; Yuan et al., 2018). These signaling pathways are crucial for growth, proliferation, and survival of neurons and are central in synaptic plasticity and learning and memory formation in the brain (Zhang et al., 2017).

Metallothionein and trace metals are involved in cadmium neurotoxicity via signaling pathways. Metallothionein, a low-molecular-weight sulfhydryl-rich metal-binding protein, can protect against cadmium toxicity by binding free cadmium ions within cells (Carrasco et al., 1999). Metallothionein-III is downregulated in the brain of AD patients (Vasak & Meloni, 2017). Insufficient production of metallothionein-III by prolonged exposure to cadmium causes neuronal apoptosis (Wang & Du, 2013). Cadmium exposure disrupts intracellular calcium homeostasis and increases extracellular calcium influx, triggering neuronal apoptosis via activation of MAPK and mTOR signaling pathways (Xu et al., 2011). Cadmium also impairs the cerebral microvascular endothelium and increases permeability of the BBB, disrupting brain ion balance and nutrient uptake (Shukla et al., 1996).

Increased Aβ production and tau tangles by cadmium

Cadmium exposure increases Aβ aggregation and tau neurofibrillary tangle accumulation, two major pathological phenotypes of AD (Hardy & Selkoe, 2002). APP/presenilin-1 (PS1) transgenic mice, treated with 2.5 mg Cd/kg/day in drinking water, had higher levels of $A\beta_{1\text{-}42}$, reduced α-secretase protein expression and reduced soluble APPα (sAPPα) (Li et al., 2012). Cd-treated mice had poorer learning and memory abilities and higher free zinc ion levels and senile plaque depositions in the brain. Cadmium exposure may exacerbate AD-related learning and memory deficits by inhibiting α-secretase and promoting the amyloidogenic APP processing (APP metabolism through the b-secretase pathway), which in turn leads to $A\beta_{1\text{-}42}$ accumulation and senile plaque deposition. Interactions between zinc and cadmium were important in AD pathways (Li et al., 2012). In replication testing, cadmium treatment was again associated with higher levels of $A\beta_{1\text{-}42}$ and lower levels of α-secretase and sAPPα (Notarachille et al., 2014).

Cadmium treatment in vitro induced aggregation of the third repeat (R3) fragment of the microtubule-binding domain of tau (Jiang et al., 2007). R3 is critical in the nucleation of the tau filament formation process (Tomoo et al., 2005). Cadmium forms Cd-tau dimers by binding to the nitrogen atoms of imidazole groups of histidine residues, affecting the nucleation step on tau aggregation (Jiang et al., 2007). The static electric strike of cadmium ion to the surrounding R3 peptide chains can prompt conformation conversion and enhance interactions with the R3 dimers, leading to enhanced aggregation through the elongation step (Jiang et al., 2007).

Cadmium-induced cholinergic neuron toxicity

Cadmium-induced apoptosis alters acetylcholinesterase (AChE) and degeneration of basal forebrain cholinergic neurons (Del Pino et al., 2016). Loss of cholinergic neurotransmission due to degeneration of cholinergic neurons in the basal forebrain is associated with significant memory deficits seen in AD patients (Francis et al., 1999). In SN56 cells, a cholinergic murine neuroblastoma cell line model of the basal forebrain, cadmium treatment induced apoptosis, mediated by blockade of muscarinic M1 receptors (related to memory loss in rats and humans), overexpression of neurotoxic AChE-S, downregulation of neuroprotective AChE-R, and increased Aβ and tau protein levels.

Human studies of cadmium exposure and AD and cognition

Postmortem cadmium comparison between AD brains and normal brains

Few studies have examined the associations between cadmium exposure and AD in human populations. Cadmium concentrations were significantly higher in AD brain tissues (hippocampus: 0.547 g/g dry weight (d.w); cerebral cortex: 0.518 g/g d.w.) compared with those in age-matched controls (hippocampus: 0.472 g/g d.w; cerebral cortex: 0.496 g/g d.w.) in an Eastern Canada sample but not in the United Kingdom sample (Ward & Mason, 1987). In a recent study, cadmium concentrations in the frontal cortex were lower in AD cases (20 ng/g) than in controls (30 ng/g) (Szabo et al., 2016), however, this finding may be problematic because controls (mean age = 88 years) were older than AD cases (mean age = 78 years). A meta-analysis of eight publications spanning 405 AD patients and 424 control subjects showed circulating concentrations of cadmium (along with aluminum and mercury) were higher in AD (Xu et al., 2018). Interestingly, circulating lead concentrations were lower in AD patients. These findings should be interpreted with caution as many previous studies were subject to confounding by age or other important AD risk factors.

Epidemiologic studies of the association between cadmium exposure and AD

Due to methodologic challenges such as low incident rate, late onset, and lack of exposure data, there are few epidemiologic studies of prevalence or incidence of AD in relation to cadmium exposure, although they consistently show cadmium exposure is associated with impaired cognitive function in adults (Ciesielski et al., 2013; Li et al., 2018). In 2068 adults aged 60 years and older participating in the U.S. National Health and Nutrition Examination Survey (NHANES), blood cadmium (median = 0.35 μg/L) was associated with lower cognitive function as measured by global cognitive function using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning Test, the CERAD Word List Recall Test, the Animal Fluency Test, and the Digit Symbol Substitution Test (Li et al., 2018).

Cadmium exposure is associated with AD mortality. In NHANES 1999–2004 cycles, among 4064 participants aged 60 and over, high blood cadmium (>0.6 μg/L) was associated with 3.83-fold increased AD mortality rate, compared to low blood cadmium (≤0.3 μg/L), even after adjustment for important risk factors for AD (Min & Min, 2016). With urinary cadmium, a longer-term biomarker of cadmium exposure, and extended follow-up time, a 58% higher rate of AD mortality per 0.51 μg/L increase in urinary cadmium was observed in both sexes (Peng et al., 2017). Both studies were underpowered due to low mortality rate (1.1%–1.3% AD risk over mean 7.5 follow-up years (Peng et al., 2017)). AD was assessed by death certificate, which could underestimate AD. Mortality studies are also vulnerable to competing risks, where individuals with high exposure to cadmium could die from other causes before having a chance to die of AD.

Conclusion

Human population evidence linking lead or cadmium to AD is limited. More epidemiologic studies with incident cases of AD and high-quality exposure data are warranted. Globally, 50 million people are currently estimated to have dementia, and this number is expected to reach 152 million in 2050 (ADI, 2018). AD and dementia are related to ageing and as the ageing population grows, the burden of disease, especially in developing countries, is tremendous. Identification of modifiable risk factors is critical to prevention with a significant public health impact. Exposures to lead and cadmium are ubiquitous in our environments and stored in our bodies. Older adults carry historic lead exposure from before policy changes to reduce use of lead in commercial products. They also retain high body burdens of cadmium due to cadmium's long half-life. Older adults are poised to experience lead- and cadmium-related accelerated declines in cognition as they age. Even modest declines in cognition associated with low levels of exposure have a massive public health reach when extended across large exposed populations. If lead and/or cadmium indeed elevate the risk of AD, reduction in exposure to lead and cadmium could have a huge impact on the global burden of AD.

Key facts of lead and cadmium

- Lead and cadmium are heavy metals that occur naturally in the earth's crust and persist in the environment.
- The primary sources of lead in the general population are dust from lead-based paint in older houses and drinking water in houses containing lead pipes.
- In the general population, the primary sources of cadmium are food and cigarette smoking.
- Lead and cadmium have no essential physiologic function in humans.

- Because both lead and cadmium are divalent metals, the transport systems for divalent essential elements, such as calcium and iron, play a role in their cellular uptake.
- Lead and cadmium are ranked as the second and the seventh hazards, respectively, which pose the most important potential threat to human health on the 2017 priority list of the U.S. Agency for Toxic Substances and Disease Registry.

Summary points

- This chapter focuses on the role of two toxic heavy metals, lead and cadmium, in the pathogenesis of AD.
- Lead can cross the BBB and accumulates in the brain.
- Cadmium can reach the brain through the blood–CSF barrier and/or the olfactory nervous system.
- Lead and cadmium treatment induces AD-related memory deficits and molecular features (Aβ and tau tangles), through oxidative stress, neuroinflammation, and apoptosis in neuronal cells, mouse models, rat models, and monkey models.
- Epidemiologic studies have consistently shown that both lead and cadmium are associated with impaired cognitive function in adults.
- No longitudinal human epidemiology study has assessed lead exposure on AD specifically.
- Two human studies using data from the U.S. NHANES reported a possible link between cadmium exposure and AD mortality.
- Evidence linking lead or cadmium to AD is very limited, especially in human populations.
- Given the widespread exposure, reduction in exposure to lead and cadmium could have a significant impact on the global burden of AD.

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PART V

Models and modelling in dementia

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CHAPTER 52

Alzheimer model 5xfad mice and applications to dementia: transgenic mouse models, a focus on neuroinflammation, microglia, and food-derived components

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List of abbreviations

AD Alzheimer's disease
APP amyloid precursor protein
Aβ amyloid-β
BDNF brain-derived neurotrophic factor
FAD familial Alzheimer's disease
GDNF glial cell line−derived neurotrophic factor
IBA-1 ionized calcium binding adapter molecule 1
IL interleukin
MIP-1α macrophage inflammatory protein-1α
ROS reactive oxygen species
TNF-α tumor necrosis factor-α

Mini-dictionary of terms

Familial Alzheimer's disease (FAD) mutations FAD is an early onset, gene-related form of AD. FAD cases are linked to mutations in the APP and presenilin genes. These FAD mutations have been used to produce AD mouse models.

Microglia Innate immune cells that reside in the brain and contribute to maintaining homeostasis. Microglial functions include defending against viral infection and removing damaged neuronal cells by phagocytosis and inflammatory responses.

Neuroinflammation Inflammation in the nervous system, mainly caused by M1 type (proinflammatory) microglia. These microglia produce proinflammatory cytokines, chemokines, and ROS, lead to neuronal cell injury. Neuroinflammation is observed in various central nervous system disorders including AD.

Novel object recognition memory A memory function depending on both the hippocampus and cerebral cortex, which is defined as the recollection of past episodes. It is usually evaluated using the novel object recognition test.

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Peroxisome proliferator-activated receptor (PPAR) PPARs are ligand-activated transcription factors that are involved in the regulation of cellular metabolism, differentiation, and development. PPARs are also expressed in microglia and regulate their phagocytotic activity and inflammatory responses.

Spatial working memory A memory function depending on hippocampal activity that is defined as the retention of spatial information in short term. In rodents, it is usually evaluated using the Y-maze or radial arm water maze tests.

Introduction

With the rapid growth of the elderly population, cognitive decline and dementia have become major public health problems worldwide. Alzheimer's disease (AD) is the most common type of dementia. AD is characterized by extracellular amyloid plaques, deposits of amyloid-β (Aβ) peptides (Glenner & Wong, 2012), and intracellular neurofibrillary tangles of hyperphosphorylated tau protein (Querfurth & LaFerla, 2010). Further, recent pathological and immunological studies have revealed that inflammation in the brain induced by Aβ deposition plays a major role in the neurodegenerative pathologies of AD (Amor, Puentes, Baker, & van der Valk, 2010; Fung, Vizcaychipi, Lloyd, Wan, & Ma, 2012). These lesions are associated with progressive cognitive impairment.

Though marked research efforts have been applied to fundamental and pharmacological studies, the therapies for AD that are currently available only slow the progression of its symptoms and no treatments have been established to be effective after the onset of AD. The preventive approaches such as daily exercise or dietary habits are reported to be effective, but the underlying mechanisms and responsible components are still controversial. These are due to the late onset of the AD pathology and the lack of methods for its early diagnosis. AD pathologies progress over the course of a few decades before patients are diagnosed, and the definitive diagnosis of AD is still only made by the postmortem analysis of pathological lesions in the brain. These characteristics of AD confer severe difficulties in assessing the efficacy of pharmacological agents in clinical trials. To complement the clinical studies on AD, preclinical strategies using animal models that exhibit symptoms of human AD with an earlier onset have been sought. In this chapter, we introduce several AD mouse models, with a particular focus on the 5xFAD mouse model, and discuss the usefulness of these mouse models in evaluating the effectiveness of therapeutic and preventive strategies for AD.

Development of transgenic mouse models for AD

Aβ plays a key role in the pathogenesis of AD. Aβ peptides are produced from amyloid precursor protein (APP) by the sequential actions of β-secretase and γ-secretase. Since the cleavage site of γ-secretase is not precise, several variants of Aβ with amino acid chains of different lengths are produced. $A\beta_{40}$ and $A\beta_{42}$ are the major subtypes of Aβ deposited in the brain, and $A\beta_{42}$ has been reported to exhibit higher fibrillogenicity

and toxicity than $A\beta_{40}$. Molecular genetic studies have revealed strong genetic relationships with the onset of familial forms of AD (FAD). Mutations associated with FAD have been found in the APP genes and presenilin genes 1 and 2, which are involved in the cleavage of APP and generation of Aβ (Pastor & Goate, 2004; St George-Hyslop & Petit, 2005). FAD mutations in the APP gene cluster near the β- and γ-secretase cleavage sites increase total Aβ production, as described for the Swedish mutation (K670N/M671L) (Mullan et al., 1992), or elevate $A\beta_{42}$ production, such as the Florida mutation (I716V) (Eckman et al., 1997) and London mutation (V717I) (Goate et al., 1991). FAD mutations in the presenilin genes also specifically elevate $A\beta_{42}$ production.

In the effort to generate AD model animals, FAD mutations have been used to increase Aβ production and accelerate the onset of AD-like pathologies. The Tg2576 model, which overexpresses APP with Swedish mutations, was first described by Hsiao et al. (1996). Histological analyses of the brains of aged Tg2576 mice have shown a large number of amyloid plaques (Kim et al., 2012; Poirier, Amin, Good, & Aggleton, 2011; Shirvan, Reshef, Yogev-Falach, & Ziv, 2009) and a significant reduction in the number of cholinergic neurons (Apelt, Kumar, & Schliebs, 2002; Lüth, Apelt, Ihunwo, Arendt, & Schliebs, 2003). Arendash et al. (2004) reported that Tg2576 mice exhibit a reduced spatial working memory at 5 months of age. However, most reports have used middle-aged or older mice to detect a significant cognitive decline in spatial memory at 8–9 months of age (Hsiao et al., 1996), in novel object recognition memory at 12–15 months of age (Oules et al., 2012), or in working and contextual memory at 16–18 months of age (Corcoran, Lu, Turner, & Maren, 2002).

The APP + PS1 mouse model was established by Holcomb et al. (1998) by combining two strategies to accelerate Aβ production, i.e., overexpressing the human APP gene with the Swedish mutation and presenilin-1 mutation (M146L) to additionally increase $A\beta_{42}$ cleavage. The induction of these two mutations successively induced the early onset of an amyloid burden, with the presence of cerebral amyloidosis at 6–8 weeks of age (Kurt et al., 2001; Radde et al., 2006). However, the progression of cognitive impairments in this model has been found to be relatively slow and not robust. Deficits in spatial working memory were observed at 6–9 months of age, but impairments in spatial reference memory were not observed at 9 months of age, and these behavioral changes were not associated with the amount of amyloid deposits (Holcomb et al., 1999). Arendash, Gordon et al. (2001), Arendash, King et al. (2001) did not detect any impairment in working memory via the Y-maze test at 16 months of age, but they detected such an impairment in the radial arm water maze test at 15–17 months of age (Arendash, Gordon et al., 2001; Arendash, King et al., 2001). Deficits in object recognition memory were first observed at 12 months of age (Mori, Koyama, Guillot-Sestier, Tan, & Town, 2013). The late onset of the pathologies of AD in the APP + PS1 model is a disadvantage because experiments using this model take a long time to obtain results. To facilitate

research on developing a treatment for AD, another mouse model that expresses AD-like pathologies at an earlier age was needed.

To further accelerate Aβ production and the onset of cognitive impairment, Oakely et al. (2006) established a mouse model coexpressing the five FAD mutations described above (i.e., APP K670N/M671L (Swedish), I716V (Florida), V717I (London), and PS1 M146 + L286V). This mouse model was termed "5xFAD" because of the induction of five FAD mutations. The 5xFAD mouse model first exhibits intracellular Aβ accumulation at 1.5 months of age and develops cerebral amyloid plaques and a massive Aβ burden at 2 months of age. This accumulation of amyloid peptides leads to the loss of noradrenergic (Kalinin et al., 2012) and cholinergic (Devi & Ohno, 2010) neurons. Cognitive impairments in hippocampus-dependent forms of memory such as spatial working memory occur by 3 months of age (Oakley et al., 2006; Ohno et al., 2006). The deficits in cortex-dependent memory occur at 6–8 months of age (Girard et al., 2013; Tohda, Urano, Umezaki, Nemere, & Kuboyama, 2012). These reports suggest that the 5xFAD mice exhibit major features of AD amyloid pathologies at an earlier age than other mutant mouse lines Fig. 52.1.

Neuroinflammation and microglial functions as therapeutic targets for AD

Inflammatory changes in AD are induced throughout the brain, especially around the regions with amyloid deposits, and these changes are associated with the activation of microglia. Microglia are the innate immune cells of the central nervous system and exhibit two phenotypes: M1 (proinflammatory) and M2 (antiinflammatory). Microglia play roles in defending against viral infection, removing damaged neuronal cells, and clearing Aβ peptides (Hanisch & Kettenmann, 2007; Tremblay et al., 2011). However, in the AD

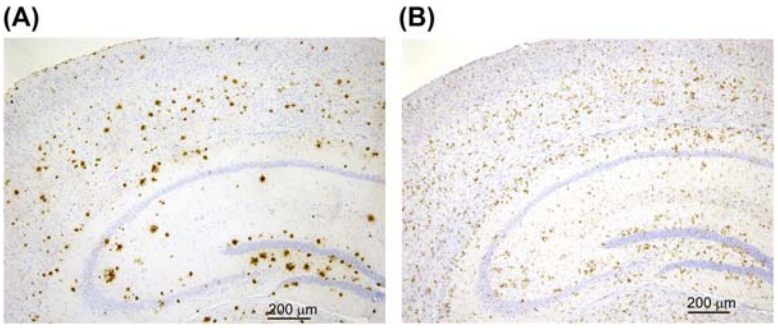

Figure 52.1 ***Amyloid-β deposition and microglial activation in the brain of 5xFAD mice.*** Immunohistochemical detection of amyloid-β_{1-42} (A) and Iba-1-positive microglia (B) in the brain of 5xFAD mice at 5 months of age. 5xFAD mice exhibit severe amyloidosis and microglial activation in early age. Unpublished data of our group.

brain, microglia infiltrate the brain tissue around Aβ plaques and are induced to differentiate to the M1 phenotype, after which they begin to produce proinflammatory cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (Bianca, Dusi, Bianchini, Dal Pra, & Rossi, 1999; Jekabsone, Mander, Tickler, Sharpe, & Brown, 2006). These inflammatory responses induce neuronal cell loss and accelerate the progression of AD.

Neuroinflammation is a significant pathology in the brain of 5xFAD mice. Immunohistological studies have revealed that 5xFAD mice exhibit the activation of microglia (Ou-Yang & Van Nostrand, 2013; Spangenberg et al., 2016; Torika, Asraf, Roasso, Danon, & Fleisher-Berkovich, 2016), and the degree of microglial activation was proportional to the extent of $A\beta_{42}$ and amyloid depositions (Oakley et al., 2006). The sustained activation of microglia in 5xFAD mice results in a chronic neuroinflammatory response and increases the expression and production of proinflammatory cytokines and chemokines such as IL-6, TNF-α, IL-1β, and MIP-1α (Ano et al., 2015; Malm, Mariani, Donovan, Neilson, & Landreth, 2015; Marsh et al., 2016). Spangenberg et al. (2016) demonstrated that eliminating microglia via the pharmacological inhibition of colony-stimulating factor 1 receptor, which is an essential component for the survival of microglia, resulted in the reduction of microglial activation, neuroinflammatory signals, and neuronal loss in 5xFAD mice. These results demonstrate the essential role of microglia in the pathologies of AD Fig. 52.2.

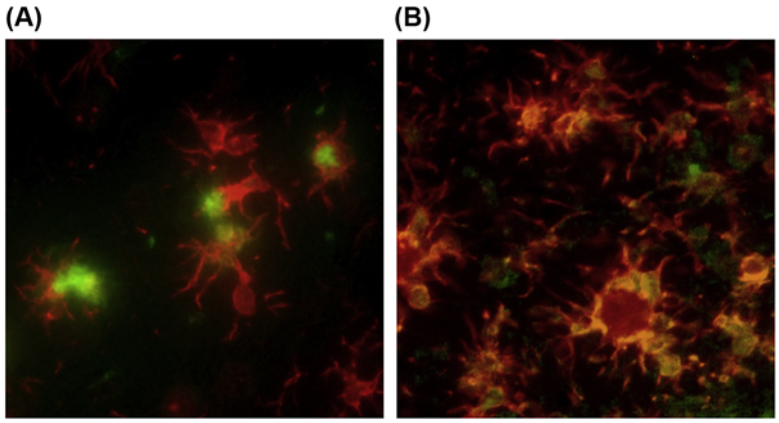

Figure 52.2 ***Inflammatory response in the brain of 5xFAD mice.*** Immunofluorescent detection of Iba-1-positive microglia (red), and either amyloid β_{1-42} (A) or macrophage inflammatory protein-1α (MIP-1α) (B) (green), respectively. Microglia are activated around amyloid plaques and produce inflammatory cytokines in the brain of 5xFAD mice. *(Reproduced from Ano, Y., Ozawa, M., Kutsukake, T., Sugiyama, S., Uchida, K., Yoshida, A., et al. (2015). Preventive effects of a fermented dairy product against Alzheimer's disease and identification of a novel oleamide with enhanced microglial phagocytosis and anti-inflammatory activity.* PLoS One, 10*(3), e0118512. https://doi.org/10.1371/journal.pone.0118512, with permission under the terms of the Creative Commons Attribution License.)*

The effects of several pharmacological agents targeting neuroinflammation or microglial activities were evaluated using 5xFAD mice. Angiotensin-converting enzyme inhibitors have been reported to suppress the inflammation in 5xFAD mice (Torika, Asraf, Cohen, & Fleisher-Berkovich, 2017; Torika et al., 2016). The peripheral administration of a soluble TNF inhibitor decreased amyloid deposits, inhibited the mRNA expression of proinflammatory factors, and rescued neuronal activity (MacPherson et al., 2017). Malm et al. (2015) reported the effect of GW0742, a PPAR-δ agonist, using 5xFAD mice. PPARs are transcription factors that enhance microglial phagocytosis and induce microglia into the antiinflammatory M2 phenotype. In that report, 5xFAD mice aged 4.5 months were given GW0742 by oral gavage for 2 weeks. Immunohistological studies revealed that GW0742 reduced the amount of Aβ deposits and microglial activation in the hippocampus of 5xFAD mice. Furthermore, quantitative PCR revealed a significant elevation of the proinflammatory milieu in the 5xFAD mice. These reports using 5xFAD mice have suggested the efficacy of modulating neuroinflammation or microglial activities as a therapeutic target for AD.

Evaluating the preventive effects of food-derived components using 5xFAD mice

Although several compounds have been reported to suppress neuroinflammation and other AD pathologies in 5xFAD mice, pharmacological agents are usually administered after patients are diagnosed with AD, which means that it is difficult to use pharmacological agents for prevention. Thus, our group focused on exploring whether food-derived bioactive components may have preventive effects against AD. We identified two AD-preventive, food-derived components using 5xFAD mice: oleamide, which is a fermented dairy product–derived component; and iso-α-acids, which are hop-derived bitter components in beer Fig. 52.3.

To identify potential AD-preventive food materials, we focused on epidemiological studies. Several studies have suggested that the consumption of fermented dairy products can reduce the risk of cognitive decline and dementia, including AD (Camfield, Owen, Scholey, Pipingas, & Stough, 2011; Crichton, Murphy, & Bryan, 2010; Ozawa et al., 2013). To reveal the underlying mechanism and identify the responsible components, our group evaluated the effects of a *Penicillium candidum*–fermented dairy product (camembert cheese) on AD-like pathologies using 5xFAD mice (Ano et al., 2015).

5xFAD mice aged 3 months and age-matched wild-type control mice were fed with or without fermented dairy extracts (2% w/w) for 3 months, and the effects were evaluated at the age of 6 months. The amount of soluble $A\beta_{42}$ in the brain of 5xFAD mice significantly decreased by 17% in the group that consumed fermented dairy extract. An examination using pathological staining techniques revealed that the Aβ burden was reduced by 21% in the cerebral cortex of the group tested with the fermented dairy extract. Activated microglia massively infiltrated into brain tissues and produced MIP-1α in the control

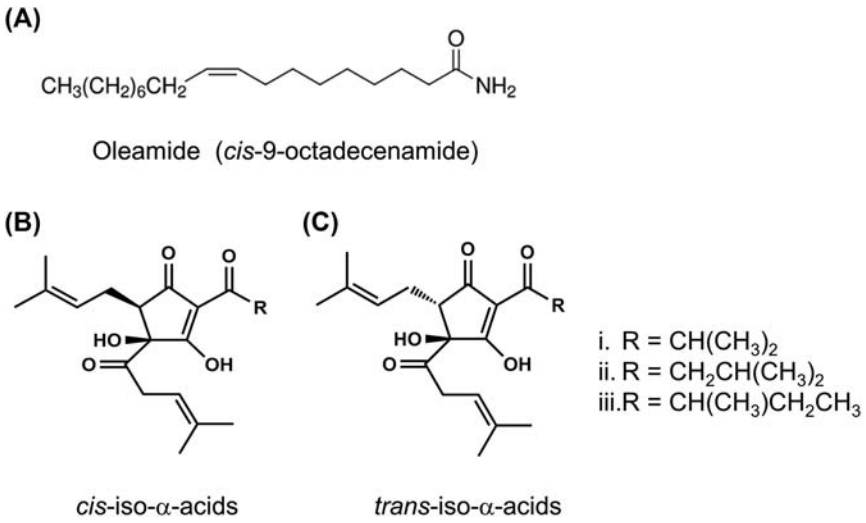

Figure 52.3 ***Chemical structures of oleamide and iso-α-acids.*** (A) Oleamide, (B) *cis*-iso-α-acids, *cis*-isocohumulone (i), *cis*-isohumulone (ii), and *cis*-isoadhumulone (iii). (C) *trans*-iso-α-acids, *trans*-isocohumulone (i), *trans*-isohumulone (ii), and *trans*-isoadhumulone (iii).

5xFAD mice, and this increase in MIP-1α production was significantly suppressed by the administration of the fermented dairy extract. These results suggested that dietary supplementation with fermented dairy extracts prevented the development of high Aβ burden, microglial activation, and inflammation in the brain of 5xFAD mice. To estimate the effects of the fermented dairy extract on cognitive function, the levels of neurotrophic factors were measured. The production of BDNF and GDNF in the hippocampus was significantly lower in the 5xFAD mice as compared with the wild-type control group. The production of these factors was significantly higher in the group treated with the fermented dairy extract as compared with the control 5xFAD mice, suggesting that the intake of fermented dairy extract had neuroprotective effects in the 5xFAD mouse model.

In this report, candidate components responsible for the AD-preventive effect of the fermented dairy extract were screened based on the antiinflammatory activity of microglia. Using gas chromatography-mass spectrometry, oleamide, which is the amide of oleic acid, was identified as a candidate component. Oleamide was not detected in dairy products that had not been fermented with *P. candidum*. We examined the physiological functions of oleamide, and these investigations revealed that oleamide enhances microglial antiinflammatory activity in vitro and in vivo. Although we have not examined the effects of oleamide on the pathologies of AD, we hypothesize that oleamide may improve AD-like pathologies because of its microglial antiinflammatory effect. In summary, using 5xFAD mice as a model of AD, we revealed that fermented dairy products exhibit an AD-preventive effect and identified oleamide as a responsible component Fig. 52.4.

Iso-α-acids, bitter components in beer, prevent the development of AD-like pathologies in 5xFAD mice

Epidemiological studies on the relationship between lifestyle behaviors and health have found that a low-to-moderate consumption of alcoholic beverages such as wine or beer may reduce the risk for dementia (Horvat et al., 2015; Matsui, Yoshimura, Toyama, Matsushita, & Higuchi, 2011; Neafsey & Collins, 2011). Resveratrol, which is a polyphenolic compound in red wine, has been reported to have neuroprotective effects or improve cognitive function (Huang, Lu, Wo, Wu, & Yang, 2011; Witte, Kerti, Margulies, & Floel, 2014). However, until recently, no constituents of beer, which is the most commonly consumed alcoholic beverage worldwide, had been examined for their effect on preventing dementia.

Hops (*Humulus lupulus* L.) are used as a main ingredient of beer to add bitterness and flavor. Iso-α-acids are the main bitter components of beer and are generated from the α-acids contained in hops during the brewing process. Previous studies have demonstrated that iso-α-acids prevented dyslipidemia and type 2 diabetes in a diet-induced obese rodent model (Miura et al., 2007; Yajima et al., 2004, 2005), while they improved glucose metabolism and decreased body fat in a clinical trial (Obara, Mizutani, Hitomi, Yajima, & Kondo, 2009). These physiological functions are thought to be attributable to the potent agonistic activity of iso-α-acids toward PPAR-γ (Yajima et al., 2004). Since PPAR-γ regulates the function of microglia, PPAR-γ is known as a therapeutic target for AD (Agarwal, Yadav, & Chaturvedi, 2017). For example, rosiglitazone, which

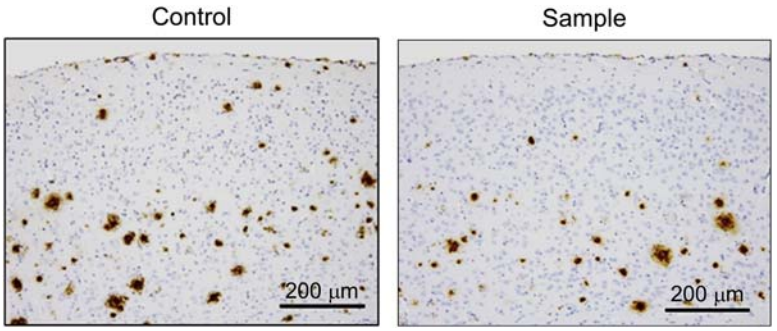

Figure 52.4 ***Amyloid-β depositions in the brain of 5xFAD mice treated with or without fermented dairy sample.*** Immunohistochemical detection of amyloid- β_{1-42} distribution in the cerebral cortex of 5xFAD mice, which were fed with control diet (control) or 2% (w/v) fermented dairy product contained diet (sample). *(Reproduced from Ano, Y., Ozawa, M., Kutsukake, T., Sugiyama, S., Uchida, K., Yoshida, A., et al. (2015). Preventive effects of a fermented dairy product against Alzheimer's disease and identification of a novel oleamide with enhanced microglial phagocytosis and anti-inflammatory activity.* PLoS One, 10*(3), e0118512. https://doi.org/10.1371/journal.pone.0118512, with permission under the terms of the Creative Commons Attribution License.)*

is a potent PPAR-γ agonist, improved cognitive impairment in Tg2576 mice (Denner et al., 2012; Rodriguez-Rivera, Denner, & Dineley, 2011) and in AD patients (Risner et al., 2006; Watson et al., 2005). Thus, we investigated the effect of iso-α-acids on the pathologies of AD, using 5xFAD mice (Ano et al., 2017).

At 2.5 months of age, 5xFAD mice were fed standard diets or pellets containing 0.05% (w/w) iso-α-acids for 3 months, and at 5.5 months of age, the effects of iso-α-acids on the AD pathologies of the 5xFAD were evaluated. An immunohistochemical analysis revealed a significant reduction of the $A\beta_{42}$ burden in the iso-α-acids fed mice, which was reduced to 21% of that in the control group. The level of Aβ in brain homogenates was also reduced in the group fed with iso-α-acids. Microglia were isolated from the brain tissues of the mice to evaluate their phenotypes. The expression of CD36 and phagocytotic activity toward Aβ were significantly lower in the microglia from the 5xFAD mice as compared with those from the age-matched wild-type mice, and these dysfunctions were improved in the 5xFAD mice that were supplemented with iso-α-acids.

Next, the abundance of inflammatory cytokines and chemokines was measured. The concentrations of IL-1β, TNF-α, and MIP-1α were significantly higher in the 5xFAD mice as compared with the age-matched wild-type mice. Meanwhile, the concentrations of IL-1β and MIP-1α were significantly lower in the iso-α-acids-treated 5xFAD mice than in the vehicle-treated mice. These results suggested that the oral administration of iso-a-acids suppressed Aβ deposition and the associated inflammation in the 5xFAD model.

The effects of dietary supplementation of iso-α-acids on cognitive function in 5xFAD mice were examined by the novel object recognition test, which evaluates the function of episodic memory. Novel object recognition memory was significantly impaired in the 5xFAD mice at 5.5 months of age, and this memory deficit was significantly improved by the oral administration of iso-α-acids. Our results in this study indicated that dietary administration of iso-α-acids suppressed AD pathologies such as Aβ burden and neuroinflammation, while this supplementation regime improved cognitive impairment in the 5xFAD model mouse Figs. 52.5 and 52.6.

Conclusion

Animal models for AD have played an essential role in elucidating the pathophysiology of AD and developing therapeutic and preventive strategies. Although no animal models completely mimic the pathologies of human AD, each of them has some characteristic features. The 5xFAD mouse model was developed by accelerating Aβ production as much as possible, resulting in the rapid and severe progress of amyloid burden, neuroinflammation, and cognitive impairments. Evaluating therapeutic and preventive strategies using 5xFAD mice may accelerate the development of solutions for AD and dementia.

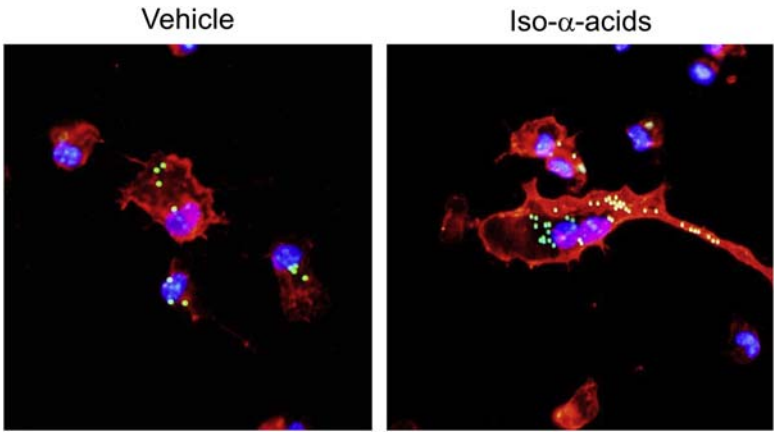

Figure 52.5 ***Iso-α-acids enhance phagocytotic activity of microglia toward amyloid β.*** In vitro phagocytotic activity of microglia (red) toward amyloid-β (green). Compared with vehicle-treated microglia, iso-α-acids-treated microglia increased the amount of amyloid β inside the cell. Unpublished data of our group.

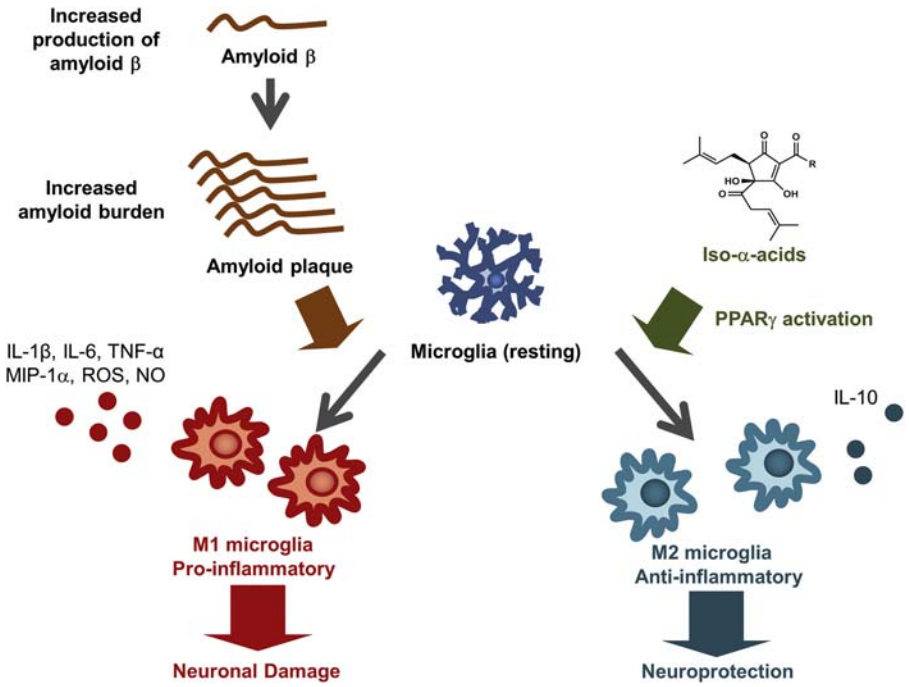

Figure 52.6 ***Induction of inflammatory response in 5xFAD and microglia-modulating effect of iso-α-acids.*** In 5xFAD mice, an increased production of amyloid β enhances the generation of amyloid plaques. The amyloid β burden induces microglia to differentiate into the proinflammatory M1 phenotype and causes neuroinflammation, resulting in neuronal damage. Iso-α-acids enhance the phagocytotic activity of microglia toward Aβ and induce their differentiation into the antiinflammatory M2 phenotype via the activation of microglial PPARγ. *IL*, interleukin; *MIP-1α*, macrophage inflammatory protein-1α; *NO*, nitric oxide; *PPARγ*, peroxisome proliferator-activate receptor γ; *ROS*, reactive oxygen species; *TNF-α*, tumor necrosis factor-α. Original graphical summary.

Key facts of dementia prevention

- Due to the lack of effective therapies after the onset of dementia, preventive strategies have drawn attention.
- Cognitive training, daily exercise, or dietary habits are reported to have dementia-preventive effects.
- Among dietary habits, the Mediterranean diet, which is characterized by high intake of fruits, vegetables, unsaturated fats, and so on, is associated with a lower risk of Alzheimer's disease.
- ω-3 fatty acids such as docosahexaenoic and eicosapentaenoic acids have been suggested to be effective for dementia prevention.
- There is a relationship between lifestyle-related diseases and Alzheimer's disease. Thus, the prevention of lifestyle-related diseases contributes to the prevention of Alzheimer's disease.

Summary points

- This chapter focuses on mouse models of Alzheimer's disease, especially the 5xFAD line.
- 5xFAD mice exhibit severe amyloid β deposition at 2 months of age and cognitive impairment by 3 months of age.
- Microglial activation and neuroinflammation are induced in 5xFAD mice, similar to the human Alzheimer's disease pathologies.
- The effects of pharmacological agents targeting neuroinflammation are evaluated using 5xFAD mice.
- The daily intake of fermented dairy products has been identified to exhibit an Alzheimer's disease-preventive effect in 5xFAD mice.
- Iso-α-acids, which are hop-derived bitter components in beer, improved amyloid β burden, neuroinflammation, and cognitive function by modulating microglial activities in 5xFAD mice.
- Because of the early onset of Alzheimer's disease–like pathologies, the 5xFAD mouse model is valuable for accelerating the development of therapeutic and preventive strategies for Alzheimer's disease.

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CHAPTER 53

Use of 192 IgG-saporin as a model of dementia and its application

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List of abbreviations

AchE acetylcholine esterase
AD Alzheimer's disease
BF basal forebrain
ChAT choline acetyl transferase
DBS deep brain stimulation
DG dentate gyrus
ERPs event-related potentials
HDB horizontal limb of diagonal band of Broca
ICV intracerebroventricular
IgG-SAP 192 IgG-saporin
MS medial septum
NBM nucleus basalis magnocellularis
NGFr nerve growth factor receptor
VDB vertical limb of diagonal band of Broca

Alzheimer's disease (AD) is characterized by histopathological features of extracellular amyloid-β, neurite plaques, and intracellular neurofibrillary tangles that relate to a basic pathomechanism as cholinergic degeneration in the basal forebrain (BF) (Francis, Palmer, Snape, & Wilcock, 1999; Sassin et al., 2000). Dysfunctions of the cholinergic system can be estimated as decreased cholinergic markers in the BF and cholinergic target organs such as choline acetyl transferase (ChAT), acetylcholine esterase (AchE), P75 nerve growth factor receptor (NGFr) positivity (Leanza, Nilsson, Wiley, & Bjorklund, 1995; Perry, Gibson, Blessed, Perry, & Tomlinson, 1977), reduced cholinergic neurons and nicotinic receptors, cortical atrophy, and the presence of amyloid plaques (Bartus, Dean, Beer, & Lippa, 1982). The dysfunction is thought to result from excitotoxicity, growth factor deprivation, oxidative stress, inflammation, mitochondrial dysfunction, and amyloid toxicity (McKinney & Jacksonville, 2005). The severity of cholinergic neuronal degeneration is known to correlate with the severity of AD clinical manifestations (Perry et al., 1978).

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Basal cholinergic system

The cholinergic system is composed of an ascending system and a BF cholinergic system. The BF cholinergic system consists of four principal sectors in rats (Mesulam, Mufson, Wainer, & Levey, 1983) and humans (Zaborszky, Pang, Somogyi, Nadasdy, & Kallo, 1999): the medial septum (MS), the vertical limb of the diagonal band of Broca (VDB), the horizontal limb of the diagonal band of Broca (HDB), and the basal nucleus of Meynert, which corresponds to the nucleus basalis magnocellularis (NBM) in rats. Cholinergic and noncholinergic neurons are intermingled within these nuclei (Kiss, McGovern, & Patel, 1988); therefore, with mechanical lesioning in these areas, we cannot induce selective cholinergic neuronal loss in experimental animals. The MS mainly projects to the hippocampus (septohippocampal system). It is traditionally considered to be involved in the short-term spatial working memory process. The VDB projects to the hippocampus, hypothalamus, occipital cortex, and cingulate cortex. Cholinergic neurons compose 70% or more of the VDB. The HDB sends its outflow principally to the olfactory bulb, piriform cortex, and entorhinal cortex. Between 25% and 75% of cholinergic neurons exist within this sector. Cholinergic projections from the diagonal band to the cingulate influence conditional discrimination. The NBM is a major source of cholinergic neurons to the cortex, including the frontal, occipital, parietal, and temporal cortices as well as amygdala and thalamus. Ninety percent of the NBM comprises cholinergic neurons. The nucleus basalis-cortical projection is involved in visual attention (Everitt & Robbins, 1997), and nucleus basalis-amygdala projections are considered to play some role in the retention of affective conditioning. The cholinergic system with its acetylcholine is considered to play a modulatory role on the excitability of cortical and hippocampal functions (Krnjevic, 1993). The noncholinergic neuronal system in the BF, most importantly GABAergic neurons and the ascending cholinergic system from pontine nuclei, acts with the cholinergic basal system to activate an electroencephalogram, to enhance sensory input processing (Jacobs, Code, & Juliano, 1991), and to modulate behavioral arousal (Buzsaki et al., 1988), cerebral blood flow (Sato, Sato, & Uchida, 2004), the sleep—wake cycle, and cognitive functions (McKinney & Jacksonville, 2005).

192 IgG-saporin—induced rat model of dementia

An adequate animal model of AD should demonstrate pathophysiologic mechanisms, display pathologic hallmarks, implement behavioral abnormalities, and test new therapeutic trials. The antineuronal immunotoxin 192 IgG is a monoclonal antibody having selective affinity for p75, a low-affinity neurotrophin receptor. The receptor is expressed only in BF cholinergic neurons and cerebellar Purkinje cells. Saporin is a member of the ribosome-inactivating protein family derived from the plant *Saponaria*

officinalis. The antibody component is coupled to saporin with a disulfide bond (Wiley, Oeltmann, & Lappi, 1991). Following receptor binding and internalization, saporin enzymatically inactivates the large ribosomal subunit, thereby blocking protein synthesis and ultimately resulting in cell death. 192 IgG-saporin (IgG-SAP) is toxic to cholinergic neurons expressing p75 without damaging noncholinergic neurons. After intracerebroventricular (ICV) injection, the cholinergic degenerative process achieves completion in about 2 weeks (Wrenn & Wiley, 1998), and the selective loss of cholinergic markers in both the cortex and the hippocampus continues for up to 8 months (Leanza et al., 1995). Properly lesioned rats may reveal dose-dependent hyperactivity on an open field and in swimming speed, impaired acquisition, spatial acuity, and working memory in the water maze, diminished response and habituation to acoustic startling, impaired passive avoidance retention, and motoric disturbances with high doses of immunotoxin (Leanza et al., 1995; Waite et al., 1995; Walsh et al., 1995).

The extent of cholinergic loss in the BF measured by p75-positive cell counts and the density of AChE fibers is important to achieving appropriate behavioral deficits and correlated with the administered dose (ICV injection) of immunotoxin. In our study, when different doses of IgG-SAP in rats (0.63 μg/μL; 6, 8, and 10 μL) were injected intraventricularly, the animals with 8 μL showed significant behavioral impairment in the probe test (Jeong, Chang, Hwang, Lee, & Chang, 2011) (Fig. 53.1). Lesion extent was related with developing impairment in spatial working memory (Leanza et al., 1995; Wrenn & Wiley, 1998). That study reported that greater than 85% lesions of the MS/VDB and more than 70% concomitant lesions in the NBM/HDB would be necessary to produce behavioral deficits. Overall, more than 75% of the entire basal cholinergic cell loss was required to induce spatial working memory deficits when checked with the radial arm maze test.

A remarkable point of this study was that just a lesion of the septohippocampal pathway (MS/VDB lesioning) was not enough to induce working memory impairment. Depletion of nucleus basalis-cortical projection (HDB/NBM lesioning) was also required. Leanza et al. noted that in ICV lesioning rats, impairment of spatial learning in a Morris water maze and impairment of passive avoidance retention were paralleled by a reduction of ChAT activity in target organs and depletion growth factor positivity in the BF (Leanza et al., 1995). Waite et al. found that impaired acquisition, impaired spatial working memory in a water maze, and impaired retention of passive avoidance were seen only in rats with 89%–94% reduction in hippocampal ChAT activity and 73%–91% reduction in cortical ChAT activity (Waite et al., 1995). Intraventricular injection of IgG-SAP destroys cholinergic neurons in the BF more diffusely and completely and produces partial loss of cerebellar Purkinje cells. Cholinergic interneurons of the striatum, cholinergic innervation to amygdala, and brain stem cholinergic neurons seem to be spared by IgG-SAP due to its lack of p75 expression. Compared with intraventricular injection, intraparenchymal injections in each nucleus in the BF

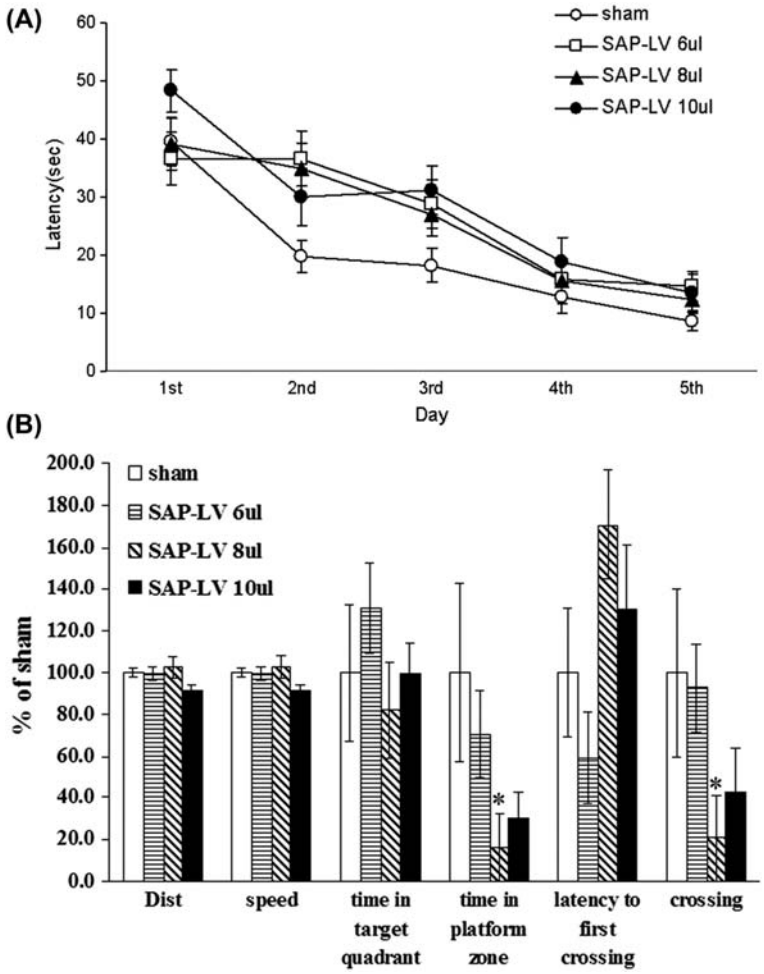

Figure 53.1 Different doses of intraventricular 192 IgG-saporin injections and memory. (A) Memory acquisition. During the acquisition phase, latency until the time of finding the platform was significantly delayed in each group of 6, 8, and 10 μL injections. (B) Effects on spatial memory. Time in the platform zone and number of crossings decreased significantly in the 8 μL injection group. Indices are expressed as a percentage of the values for the sham. *(Reproduced with permission Jeong, D. U., Chang, W. S., Hwang, Y. S., Lee, D., & Chang, J. W. (2011). Decrease of GABAergic markers and arc protein expression in the frontal cortex by intraventricular 192 IgG-saporin.* Dementia and Geriatric Cognitive Disorders*, 32(1), 70–78. https://doi.org/10.1159/000330741.)*

can induce a more selective lesion. Intraparenchymal injections are less diffusible, subsequently producing small, circumscribed lesions in portions of the BF. In view of behavioral consequences, it has been accepted that IgG-SAP injection into a specific nuclei in the BF cannot easily lead to deficits in spatial learning and memory performance (Baxter & Chiba, 1999). Selective lesioning into the MS or NBM produced behavioral

deficits in a delayed radial-arm maze task and in a delayed nonmatch-to-sample task dose-dependently as an ICV injection of immunotoxin (Walsh, Herzog, Gandhi, Stackman, & Wiley, 1996). Bilateral injections of IgG-SAP into the MS, NBM, or MS/NBM induced mild impairments of nocturnal activity, open-field activity, passive avoidance, and delayed nonmatching-to-position but not water maze performance (Dornan et al., 1997; Torres et al., 1994). Following either MS/VDB or NBM lesioning, spatial working memory was mildly impaired when tested in an operant two-choice delayed nonmatching-to-position task (Torres et al., 1994). McMahan et al. hypothesized it would be possible to induce spatial learning impairment when using a more complicated task, such as a delayed nonmatching-to-place task in a water version of the radial arm maze, with selective MS/VDB lesion rats. Cholinergic loss in the MS/VDB was extensive, though they failed to find impairment of the deficits (McMahan, Sobel, & Baxter, 1997). Lamprea et al., with intraseptal injection of IgG-SAP in rats, showed decreased total running distance, less activity in an open-field test, decreased exploratory behavior in an elevated plus-maze, and a deficit in habituation of exploration. They suggested that the septohippocampal cholinergic pathway may relate to the motivation to explore new environments and the acquisition and storage of spatial memory (Lamprea, Cardenas, Silveira, Walsh, & Morato, 2003). In humans, episodic memory is one of the principal functions of the hippocampus. Easton et al. did not find impairment of episodic-like memory in rats with MS/VDB selective lesioning for a task of integrated memory for objects, places, and contexts. With these results, the authors hypothesized that cholinergic hippocampal neurons were not related structurally to episodic memory, but rather the combination of other neurons, like GABAergic, was necessary to build episodic-like memory (Easton, Fitchett, Eacott, & Baxter, 2011). Episodic memory impairment after fornix transection induced by excitotoxins or mechanical lesioning, therefore, indicates that induction of memory deficits through cholinergic and noncholinergic interruption of hippocampal pathways was required. To clarify GABAergic contribution to memory acquisition and consolidation, these authors made a sequential combination model that involved selective cholinergic lesioning with IgG-SAP and/or GABAergic depletion with orexin-saporin into MS/VDB. They observed choline-depleted rats remembering the task after 5 days, though this was impaired at the 25-day delay in the spatial memory test. The rats with selective GABAergic denervation or combined lesions were impaired as early as 5 days postacquisition. These studies suggested sequential involvement of the GABAergic pathway earlier and the cholinergic pathway later for spatial memory consolidation (Lecourtier et al., 2011). Fear conditioning is known to be dependent upon the amygdala and hippocampus, and it is often measured with freezing or a fear-potentiated startle. In animals with cholinergic BF nucleus lesioning in the MS, VDB, HDB, and NBM, 94.5% ChAT depletion was caused in the hippocampus and 76.1% in the neocortex; however, neither group showed impairment from fear conditioning (Frick, Kim, & Baxter, 2004).

The authors suggested that the roles of the hippocampus and cortex on acquisition, consolidation, or retrieval of contextual fear were not necessary. This could be due to insufficient lesion extent in the cholinergic BF; however, many authors have posited that GABAergic and serotonergic modulation in addition to cholinergic innervation may be necessary to impair hippocampus-dependent memory processing (Koenig, Lecourtier, Cosquer, Pereira, & Cassel, 2011). Overall results indicate that the role of the BF cholinergic system in cognition is more limited than was previously believed. According to the aims of studies or behavioral tests, researchers should choose a proper injection route and dose of IgG-SAP. Nevertheless, to induce the impairment of some behaviors, a large proportion of basal cholinergic neuronal loss should be a prerequisite.

Experimental application of 192 IgG-saporin–induced dementia

The dementia model using IgG-SAP has been utilized as a test model for clarification of the physiologic or pathologic roles of the cholinergic system and acetylcholine in cognitive dysfunction and for applying therapeutic strategies for AD. In this section, we briefly introduce examples of the usefulness and pitfalls of this model.

Neurogenesis and the role of the cholinergic signal

There is some evidence that the BF cholinergic system is related to cortical network maturation and neurogenesis in neonates and adults (Cullen et al., 1997). Therefore, the selective cholinergic BF represents a valuable tool. The BF cholinergic system in rodents is known to maturate after birth, after which the cortical and hippocampal afferents from the cholinergic system reach the target areas. Cortical innervation is earlier in the postnatal week (Coyle & Yamamura, 1976), and innervation in the hippocampus comes slightly later (Linke & Frotscher, 1993). Meanwhile, p75 NGFr levels are low during the first week of birth. It increases and reaches its peak during the second postnatal week (Koh & Loy, 1989). The period of cholinergic development and its innervation into the cortex coincides with the time of differentiation of cortical neurons to make their synapses (Berger-Sweeney & Hohmann, 1997).

With neonatal IgG-SAP lesioning in rats occurring on postnatal 0 (P0) and 2 (P2) days, Robertson et al. reported reduced cortical thickness. The degree of reduction was correlated with the loss of AchE-positive fibers. It continued until 3 months of age. They also noted a reduced level of dendritic branching and reduced spines of apical dendrite on layer V pyramidal neurons in the visual cortex (Robertson et al., 1998). Ricceri, Hohmann, & Berger-Sweeney (2002), in their model of P1 injection neonates, found that cholinergic interruption led to slower acquisition of passive avoidance in female rats, and reactivity to spatial changes was impaired in their adulthood in both sexes. Histologically, they found undifferentiated cortical neurons that were densely spaced and smaller than layer V neurons. However, the thickness of the cortical cell layers

was variable and sex-specific. This dimorphism was due predominantly to sex differences in cortical layer VI. Cell layer IV-II was reduced in both sexes but was more prominent in males (Pappas et al., 1996; Ricceri et al., 2002). However, in animals with P7 IgG-SAP lesions through ICV injection, Pappas et al. did not find abnormal cortical morphogenesis or thickness, and naturally no impaired spatial learning/memory was found as assessed by the Morris water maze and delayed spatial alternation. The animals just showed less activity and more timidity. In this study, BF cholinergic loss was near complete, and an 84% loss of hippocampal and 52% loss of ChAT activity were noted. The reason for this discrepancy was hypothesized to be due to lesion size (Pappas et al., 1996). As in adults, for lesioning to show behavioral impairment, the extent of cholinergic loss should be enough to near complete. Alternatively, the age at lesion-making would be a cause for the difference. For example, cholinergic afferent to the cortex and hippocampus was early postnatal, thus as early as P3 or earlier, lesioning is required to induce cortical cytoarchitectural alteration and subsequent behavioral deficits (Ricceri et al., 2002). Berger-Sweeney (1998) made an ICV injection of IgG-SAP in P1 rats. It did not affect passive avoidance acquisition or retention or cortical anatomical development. It was reported that ChAT activity was reduced by 17% in the cortex, and no reduction occurred in the hippocampus. The rat groups lesioned on P4 and P7 also showed no significant impairment in spatial navigation performance when checked in their adulthood (Leanza, Nilsson, Nikkhah, Wiley, & Bjorklund, 1996; Pappas et al., 1996). There was only transient impairment of acquisition of passive avoidance and decreased exploratory behavior in P7-lesioned rats. However, in adulthood, P7-lesioned rats exhibited a subtle spatial deficit, decreased response to spatial rearrangement, and a different habituation profile in spontaneous exploration of objects. These animals showed subtle impairment in detecting spatial arrangements that were measured to assess cortical function. They thus concluded that behavioral impairment in neonatal IgG-SAP lesions was not as severe as in adulthood. Fréchette with Pappas, later in their series using ICV P7 neonatal lesion models, reported that the enriched environment improved behavioral deficits but did not reverse cytoarchitectural abnormalities in CA1 pyramidal cells. They remarked that cholinergic dysfunction was a critical cause for loss of synapses in a young brain (Frechette, Rennie, & Pappas, 2009). Nonspecific lesioning with electrolytes is thought to include noncholinergic fibers. It indicates that noncholinergic neurons contribute to learning and memory in adult models. Neurogenesis in adults has been implicated in cognitive function. IgG-SAP lesions in adult rats reduced the number of cells in the granule cell layers of the dentate gyrus (DG) and olfactory bulb and increased the numbers of apoptotic cells in the subgranular zone, the progenitor region of the dentate gyrus, and within the periglomerular layer of the olfactory bulb (Cooper-Kuhn, Winkler, & Kuhn, 2004). After IgG-SAP injection, the brain-derived neurotrophic factor mRNA has been reported to decrease. This was interpreted to imply that decreased neurotrophic factor could be related to the early death of newly formed cells

(Berchtold, Kesslak, & Cotman, 2002). There was a similar report of decreased adult neurogenesis with cholinergic denervation (Mohapel, Leanza, Kokaia, & Lindvall, 2005). In the DG, neurogenesis decreased, which was measured with AChE positivity in the DG, including an 80% reduction and impairment in spatial learning. It was rescued with administration of a cholinergic agonist.

Therapeutic drug tests

This model can be used for therapeutic drug testing. Selective augmentation through nicotinic receptor or muscarinic receptor has been attempted. Donepezil is one of the therapeutic drugs for AD. Its effectiveness was proved histologically with this dementia model. Using donepezil in an IgG-SAP rat model, a group in Italy showed its effects on neuronal morphology (De Bartolo et al., 2009). They found increased spines of apical and basal dendrites and decreased basal dendritic arborization. Chronic administration of donepezil with an enriched environment in lesioned rats attenuated the compensatory upregulation of dendritic spines, and it enhanced acquisition of procedural competence and localization functions (Cutuli et al., 2009). Pretreated donepezil even elicited beneficial effects on behavior deficits induced by the IgG-SAP model. For enhancing attention performance, a cholinergic depletion model was used (Cutuli et al., 2013). Nicotine receptor agonists and methylphenidates were tried, and the nicotine agonists showed beneficial effects on attention in lesioned animals. In nonlesioned animals, the beneficial effects were even better. In addition, a donepezil steroid sulfatase inhibitor was tried in a selective lesioning model of the septohippocampal cholinergic system (Babalola et al., 2012). It facilitated contextual fear memory in a passive avoidance test and could not facilitate working memory in lesioned animals. Chronic lithium treatment rescued memory performance (Gelfo et al., 2017).

Deep brain stimulation for dementia

It has been established that electrical brain stimulation can modify neurological and psychiatric dysfunction in some movement disorders and psychiatric diseases. Recently, several clinicians and researchers have tried brain stimulation for AD. Plausible targets are hypothalamus-fornix (Hamani et al., 2008), NBM (Freund et al., 2009), and the entorhinal cortex (Suthana et al., 2012). The IgG-SAP model could be a test model for brain stimulation for various targets before human clinical trials. We induced an AD model with ICV IgG-SAP rats that properly showed spatial memory impairment in the Morris water maze test. When applying deep brain stimulation (DBS) into MS with 120 μS, 100 μA, and 60 Hz electrical parameters (Jeong et al., 2014), the stimulation group showed better performance in a spatial memory test. Duration spent in the platform zone was decreased to 26% in the AD model rats when it compared to the normal

control group performance and 98% in the MS stimulation rats. The number of platform crossings was reduced 27% in the AD model group and 98% in the stimulation group compared to the normal control group (Fig. 53.2). We could identify that these behavioral improvements were probably related to enhanced hippocampal neurogenesis. Doublecortin-positive neurons in the hippocampus were significantly increased in the stimulation group (Fig. 53.3). Hippocampal AChE activity was comparable to that of the normal group, whereas in the lesioned group it was significantly decreased. When applying DBS into NBM with 90 μS, 1 V, 120 Hz stimulation parameters (Lee, Jeong, Lee, Chang, & Chang, 2016), the stimulation group showed equivalent performance to that of the normal group in the Morris water maze test. GABA activity in the medial prefrontal cortex and the reduced glutamate acid decarboxylase level after cholinergic lesions were recovered with DBS. We speculate that NBM stimulation could induce an increase GABAergic and glutamatergic activity in the medial prefrontal cortex, which contributes to improvement in memory consolidation and retrieval of visuospatial memory. It is unclear whether these effects resulted from DBS. Further studies are needed to examine electrical stimulation and its relationship with neurotransmitter systems.

Others

To clarify the physiologic efficacy of a cognition-enhancing material, a selective septohippocampal-lesioned model lesioning into the MS and diagonal band is practical. Wu, Shanabrough, Leranth, and Alreja (2000) suggested in septohippocampal-lesioned animals that muscarinic agonists enhancing cognitive function were caused not by acetylcholine increase but by GABAergic disinhibition. GABAergic neurons were measured with parvalbumin positivity as well as spontaneous firing with short-duration spikes, which were characteristics of GABAergic neurons. For a more realistic model of AD, an IgG-SAP-induced cholinergic depletion model can be used with additional manipulation. Antonini et al. (2011) injected the preaggregated $A\beta_{25-35}$ peptide bilaterally into the hippocampus in addition to ICV IgG-SAP. It would be characterized by cholinergic depletion concomitant amyloid peptide accumulation. They tested a sigma-1 receptor agonist in this model and revealed a favorable result in cognition and in the protective effects on neurons. Simultaneous lesions of selective hippocampal serotonergic denervation using a serotonergic toxin, 5, 7-dihydroxytryptamine, into the fimbria fornix and cingulum bundle and IgG-SAP into MS and DB (Lehmann et al., 2002) was also introduced. To validate the suitability of the AD model, electrophysiologic methods can be used. P300 (P3) event-related potentials (ERPs) have been suggested to be an endogenous marker of cognitive function, and auditory oddball paradigms are frequently used to evaluate P3 ERPs in clinical settings. Deficits in P3 amplitude and latency reflect some of the neurological dysfunctions related to several psychiatric and neurological diseases like AD. They used an ICV IgG-SAP model and identified that P3 amplitude

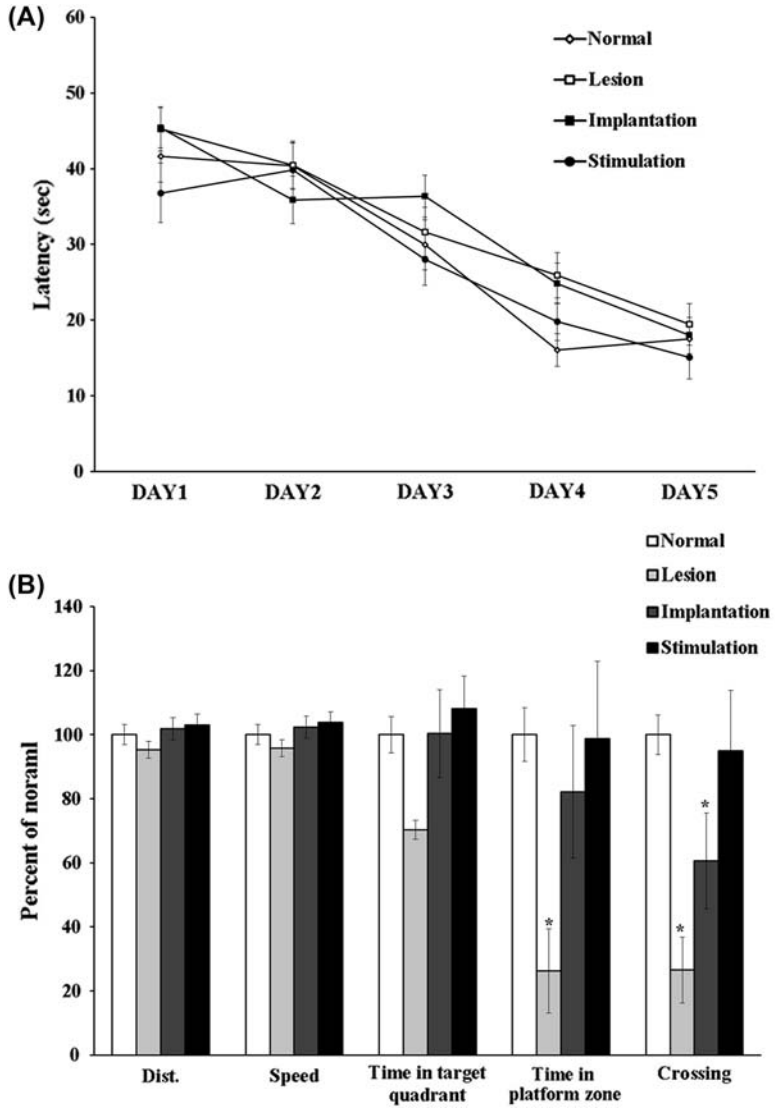

Figure 53.2 Effect of deep brain stimulation (DBS) of medial septum (MS) on spatial memory. During training trials (A), all groups showed decreased latency to find the platform. After a delay of 2 days, spatial memory improved in the stimulation (MS-DBS) group (B). Time spent in the platform zone ($P < .05$) and number of crossings ($P < .005$) were significantly different between lesion and normal groups. However, the stimulation group caught up with the time of the normal group. Data are shown as mean ± SEM (A). Indices are expressed as a percentage of normal group values (B). *(Reproduced with permission Jeong, D. U., Lee, J. E., Lee, S. E., Chang, W. S., Kim, S. J., & Chang, J. W. (2014). Improvements in memory after medial septum stimulation are associated with changes in hippocampal cholinergic activity and neurogenesis.* BioMed Research International*, 568587. https://doi.org/10.1155/2014/568587.)*

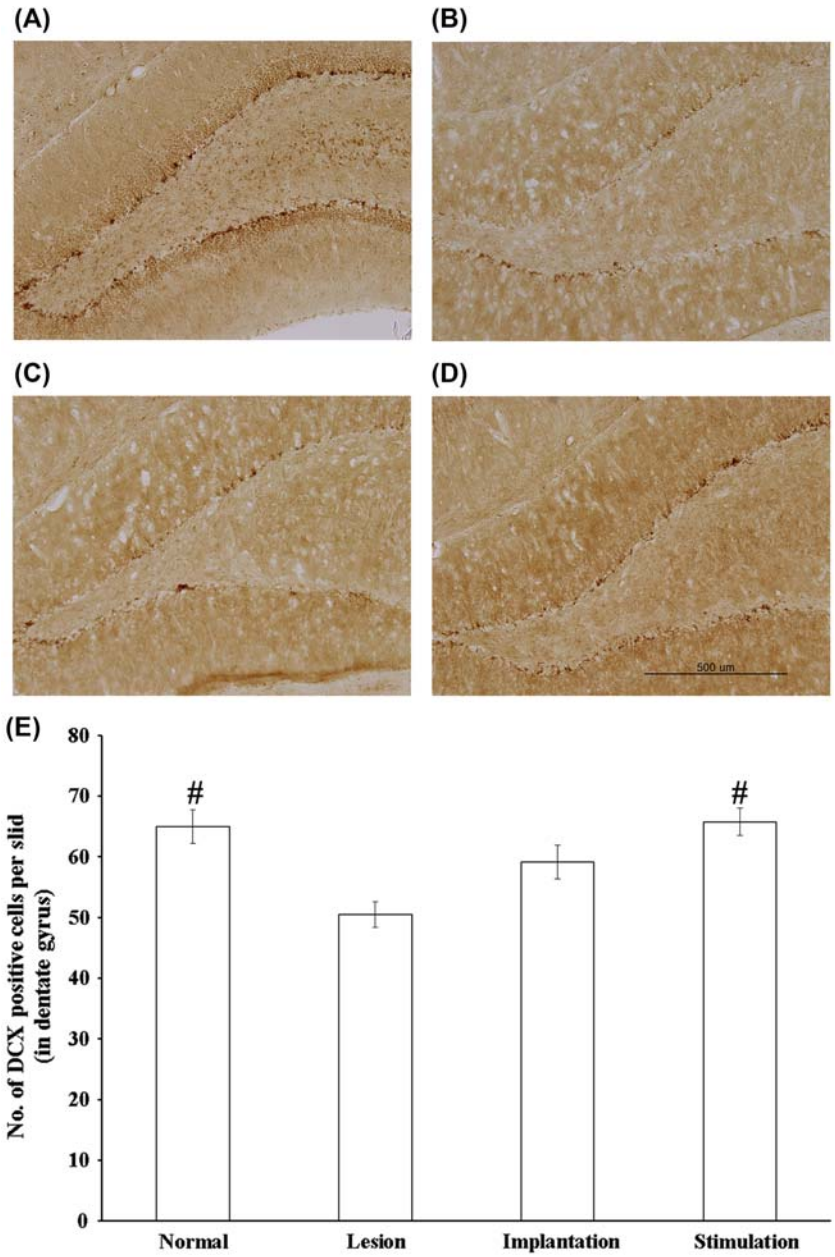

Figure 53.3 Effects of medial septum deep brain stimulation on adult hippocampal neurogenesis (immunohistochemical stain with doublecortin): (A) normal group, (B) lesion group, (C) implantation group, (D) stimulation group, and (E) number of immunopositive cells. The lesion and implantation groups revealed decreased neurogenesis, whereas the stimulation group showed an amount of neurogenesis comparable to that of the normal group ($P < .05$). *(Reproduced with permission Jeong, D. U., Lee, J. E., Lee, S. E., Chang, W. S., Kim, S. J., & Chang, J. W. (2014). Improvements in memory after medial septum stimulation are associated with changes in hippocampal cholinergic activity and neurogenesis.* BioMed Research International*, 568587. https://doi.org/10.1155/2014/568587.)*

was significantly increased in SAP-treated rats. The abnormality was reversed by donepezil. They suggested that P3-like ERPs in IgG-SAP rat models could be electrophysiological measures of cognitive processing (Laursen, Mork, Kristiansen, & Bastlund, 2014). P7 ICV injections of IgG-SAP lesioned rats were used to evaluate susceptibility to seizures (Silveira, Cha, & Holmes, 2002). In this study, choline-depleted neonates had significantly shorter latencies to the onset of myoclonic jerks and tonic-clonic seizures than exhibited by controls. However, no significant differences were found in the duration of seizures or in EEG ictal duration. They deciphered this result as cholinergic denervation on hippocampal inhibitory GABAergic interneurons' decreased inhibitory inputs to pyramidal cells in the CA1 region (Chapman & Lacaille, 1999) that subsequently did not attenuate or abolish spontaneous firing in the hippocampal pyramidal cells following increasing seizure susceptibility (Jouvenceau, Billard, Lamour, & Dutar, 1997).

The rat model using IgG-SAP could be useful for evaluating cholinergic neurons or organs in their role in learning and memory or for testing therapeutic tools in AD. To use this model, appropriate behavioral impairment should be accomplished. Cholinergic loss should be demonstrated with the activity of various cholinergic neuronal markers. Controversial results exist relative to the behavioral consequences of ICV or intraparenchymal IgG-SAP lesioning. Nevertheless, the range of lesion extent should reach near total loss of cholinergic fibers.

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CHAPTER 54

Amyloid beta 1–42-induced animal model of dementia: a review

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List of abbreviations

APP amyloid precursor protein
Aβ peptide - Amyloid-β
AβOs soluble Aβ oligomer
BACE1 beta-site APP cleaving enzyme 1
BDNF brain-derived neurotrophic factor
COX-2 cyclooxygenase-2
CXCR2 C-X-C motif chemokine receptor 2
ERK extracellular signal-regulated kinase
GCL granule cell layer
GFAP glial fibrillary acidic protein
GPx glutathione peroxidase
HEt hydroethidine
IFN-γ interferon γ
IgG immunoglobulin G
IL-1β interleukin-1β
iNOS inducible nitric oxide synthase
JNK Jun N-terminal kinase
MDA malondialdehyde
NF-κB nuclear factor κB
NGF nerve growth factor
NLRP3 nod-like receptor family pyrin domain containing 3
ROS reactive oxygen species
SOD superoxide dismutase
TGF-β transforming growth factor-β
TLR-4 toll-like receptor-4
TNF-α tumor necrosis factor-α
4-HNE 4-hydroxynonenal

Mini-dictionary of terms

Amyloid-β peptide oligomers Small peptides produced by the cleavage of APP.
Amyloid plaques Extraneuronal deposits of amyloid- β peptide.
Neurofibrillary tangles Intraneuronal aggregations of hyperphosphorylated tau protein.

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Monomer A small-molecule single-peptide chain.
Dimer A molecular complex formed by a two-monomer peptide chain.
Aggregation Accumulation of protein in the cell.

Introduction

Alzheimer's disease (AD) is the most common form of dementia among Western countries, corresponding to about 60%–80% of cases (Chinthapalli, 2014). In fact, the number of AD cases has increased since the first description by Alois Alzheimer, more than 100 years ago, to a very high prevalence nowadays (Cornutiu, 2015; Prince et al., 2013). In the coming decades, because of increasing life expectancy, global AD prevalence is expected to reach epidemic levels (Prince et al., 2016). AD is an irreversible, progressive brain disorder that affects a patient's early memory, thinking skills, emotions, behavior, and mood. Over time, this disease impairs all intellectual functions and leads to complete dependence for basic functions of daily life (Tarawneh & Holtzman, 2012).

Neuropathologically, AD is characterized by the presence of plaques consisting of amyloid-β (Aβ) peptide (extracellular senile plaques), intracellular neurofibrillary tangles, and loss of cholinergic neurons (Zhang, Ma, Zhang, & Xu, 2012). It's worth mentioning that many other pathogenic aspects related to the process and development of this neurodegenerative disease remain largely unknown (Dong, Duan, Hu, & Zhao, 2012; Holtzman, John, & Goate, 2011). In this regard, experimental studies, mainly those using animal models, have contributed and still are contributing to increased knowledge about the pathophysiology of AD (Sasaguri et al., 2017). Nonetheless, some challenges in this research field still remain (Medina & Avila, 2014). This chapter aims to explore an important rodent model—Aβ1–42 oligomers intracerebroventricular or intrahippocampal injection—widely used to study the pathogenesis of AD.

Alzheimer's disease pathogenic events

The amyloid cascade was proposed some years ago and remains the most accepted theory to explain the pathological events of AD (Hardy & Selkoe, 2002; Selkoe & Hard, 2016). Fig. 54.1 shows the sequential mechanisms in Aβ-induced AD.

The oligomerization, aggregation, and deposition of Aβ extracellularly causes neurodegeneration in AD (Crews & Masliah, 2010). Aβ peptides are 39–43 amino acid residue peptides (~4 kDa) derived from the sequential enzymatic proteolysis of β-secretase (BACE1) and γ-secretase on transmembrane amyloid precursor protein (APP) (Zhang, Thompson, Zhang, & Xu, 2011). The length of Aβ varies at C-terminal according to the cleavage position of APP by γ-secretase. The Aβ1–40 isoform is the most abundant (approximately 90%), followed by Aβ1–42, which is hydrophobic and aggregates at a faster rate than Aβ1–40 (Xu, 2009). Aβ1–42 is the main constituent of amyloid plaques

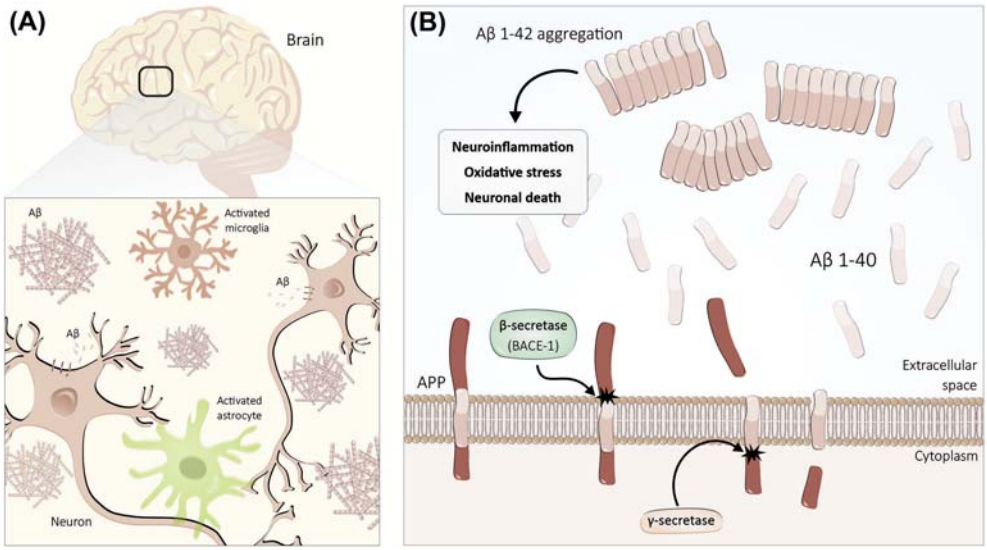

Figure 54.1 ***Sequential mechanisms in amyloid β (Aβ) peptide -induced Alzheimer's disease (AD).*** (A) In the AD brain, the Aβ produced by neurons accumulates and deposits in amyloid plaques. Amyloid plaques are associated with microglial and astrocyte activation and a cascade of events that ultimately result in neuronal loss and cognitive impairment. (B) Aβ peptides are 39−43 amino acid residue peptides derived from the sequential enzymatic proteolysis of β-secretase (BACE-1) and γ-secretase on transmembrane amyloid precursor protein (APP). The Aβ1−40 isoform is the most abundant, followed by Aβ1−42, which is hydrophobic and aggregates at a faster rate than Aβ1−40. Aβ1−42 is the main constituent of amyloid plaques. Aβ1−42 accumulation and aggregation causes neuroinflammation, oxidative stress, synaptic dysfunction, and neuronal death.

and is thought to play a central role in initiating the amyloid cascade (Murphy & LeVine, 2010). It's important to know that besides Aβ1−40 and Aβ1−42, γ-secretase produces a range of Aβ peptides (Xu, 2009). Aβ can exists in different forms: monomers, dimers, oligomers, fibrils, and plaques (Frost & Li, 2017).

Genetic and/or environmental factors alter Aβ production and metabolism, causing increased levels of toxic Aβ 1−42 (Tayeb, Murray, Price, & Tarazi, 2013). Aggregation of Aβ 1−42 is followed by its oligomerization and the formation of fibrils and protofibrils, effectively forming amyloid plaques (Tycko, 2016). Aβ deposition and diffused plaque formation leads to local microglial activation, cytokine release, and reactive astrocytosis. This cascade of events leads progressively to synaptic dysfunction, apoptosis, neuronal loss, and neurotransmitter dysfunction, and consequently to cognitive impairment and dementia (Hardy & Selkoe, 2002; Selkoe & Hard, 2016; Weitz & Town, 2016).

Taking into account this strong participation of Aβ in AD development and progression, the animal models to study this disease present, in the majority, an alteration in the brain's Aβ peptide amounts (LaFerla & Green, 2012).

Experimental models of Alzheimer's disease

Despite years of study, the pathogenesis of AD remains only partially understood, which is reflected in the few available treatment options. In this regard, experimental models of AD are critical for exploring the pathogenesis and testing the potential of novel molecules to treat the disease. There are several different in vitro and in vivo models to study AD. The primary AD experimental models used are conducted in rodents. In order to conduct research on this disease, which is not observed in animals, some tools are used—e.g., genetic modifications and cerebral injection of Aβ species (Hall & Roberson, 2012; LaFerla & Green, 2012; Sasaguri et al., 2017; Van Dam & De Deyn, 2011).

Over the past decades, many experimental models, mainly using mice and rats, have been used to create genetically altered phenocopies of human AD. The transgenic mouse strains that express a mutant form of human APP and/or some of the enzymes implicated in their metabolic processing, and tau, have been developed (Van Dam & De Deyn, 2011). The first model described was human amyloid precursor protein V717F transgenic mice (Games et al., 1995), followed by the transgenic 2576 (Tg2576) (Hsiao et al., 1996) and APP23 (Stürchler-Pierrat et al., 1997) mouse models. These mouse models recapitulate some aspects of the classic AD pathologies—i.e., amyloid plaque formation in the cortex. However, these mutations in the genes of protein related to Aβ generation are associated with early-onset (familial) AD, which accounts for 1% of this pathology cases (Zou, Liu, Che, & Huang, 2014). Though these transgenic models have utility in characterizing how abnormal APP processing may be linked to cognitive impairment, they may present an incomplete perspective of pathology (Kitazawa, Medeiros, & Laferla, 2012).

Rather than attempting to mimic all the characteristics of the AD brain, another approach is to use specific animal models that focus on specific components of AD pathology (La Ferla & Green, 2012). Therefore, another important manner of exploring features of AD in rodents is by intrahippocampal or intracerebroventricular infusion of Aβ peptides in the brain. Aβ species can be administered acutely using a single stereotactic injection (Harkany et al., 1998, 2000) or repetitively using injections through an implanted cannula (Yamada et al., 2005). These rodent models based on intracerebroventricular or intrahippocampal Aβ injection also support the Aβ cascade hypothesis, appointing mechanisms and secondary events of different Aβ species neurotoxicity, such as Aβ1-40 (Takeda et al., 2009; Weldon et al., 1998), Aβ1−42 (Cetin & Dincer, 2007), and Aβ25−35 (Diaz, Limon, Chávez, Zenteno, & Guevara, 2012; Stepanichev, Moiseeva, Lazareva, & Gulyaeva, 2005). More specifically, Aβ injection is considered a useful tool in the study of the inflammation role in AD in the animal brain (McLarnon & Ryu, 2008). In addition, with this type of experimental model it's possible to screen for drugs targeting Aβ (Lawlor & Young, 2010; Van Dam & De Deyn, 2011). Our focus is review of the evidence related to the animal model of AD induced by Aβ 1−42.

Dementia animal model induced by amyloid beta 1−42

Evidence suggests that a variety of neuronal insults, such as the forms of Aβ, can lead to tau hyperphosphorylation, neuronal death, neuroinflammation, and gliosis, culminating in neurodegeneration in the hippocampus and cerebral cortex and ultimately cognitive impairment. Aβ abnormal production and accumulation is an important hallmark of AD. However, the Aβ form or other APP cleavage products specifically related to human neurodegeneration and the clinical dementia of AD have not yet been directly identified (Brody, Jiang, Wildburger, & Esparza, 2017). It has been demonstrated that when injected in vivo, the Aβ 1−42 fibril induces major toxicity because it causes more pathophysiological damage than Aβ 1−40 (Verdurand et al., 2016). Therefore, Aβ 1−42 peptide has been considered a potent activator of neuroinflammation and other pathological characteristics of AD (McLarnon, 2014; McLarnon & Ryu, 2008).

Intrahippocampal or intracerebroventricular injection of Aβ 1−42 peptide in rats or mice is a simple, reliable, and useful paradigm to investigate the mechanisms through which Aβ oligomers interfere in neuronal processes and to test the efficacy of new therapeutic approaches specifically against these species (Fig. 54.2) (McLarnon & Ryu, 2008).

The effectiveness of this animal model involves predictive, face, and construct validity. For predictive validity, it is possible to note that classic drugs revert the behavioral and molecular changes of the animal model. For face validity, the animal model induces cognitive damage, mainly spatial memory impairment. For construct validity, the animal model induced by Aβ 1−42 peptide administration leads to inflammation (similarity of immunohistochemical staining between the model and human tissue), oxidative stress, blood−brain barrier leakage, and increased markers of synaptic damage (Garcez et al., 2019;

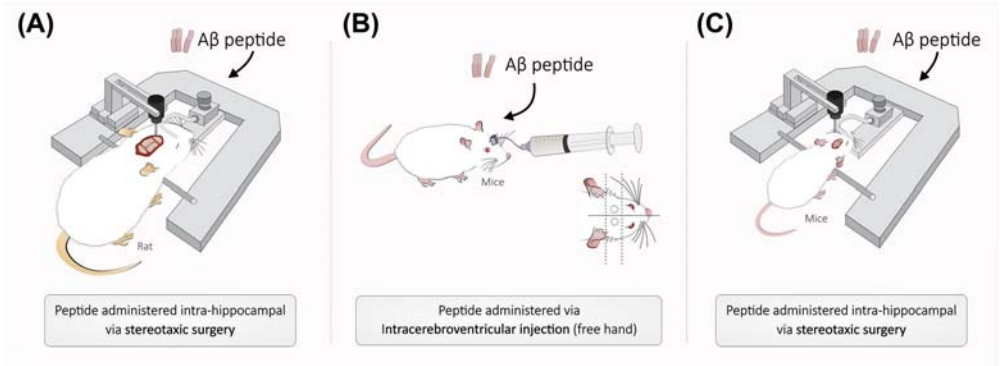

Figure 54.2 ***Rodent models of Alzheimer's disease induced by beta amyloid peptide (Aβ) 1−42 injection in the brain.*** (A) Intrahippocampal injection of aggregated Aβ 1−42 in rats via stereotaxic surgery. (B) Aggregated Aβ 1−42 administered intracerebroventricularly in mice performed via freehand method. (C) Intrahippocampal infusion of aggregated Aβ 1−42 in mice using stereotaxic frame.

Gupta, Sil, Ghosh, Ghosh, & Ghosh, 2018; McLarnon & Ryu, 2008; Ryu & McLarnon, 2009; Tian, Zhai, Zhao, Chen, Zhao, 2017).

We will summarize in Table 54.1 and the text that follows the main findings about Aβ 1−42 peptide administration as an animal model of AD.

First, we will show studies reporting on the mice model of AD induced by intracerebroventricular or intrahippocampal Aβ1−42 peptide administration (Chang et al., 2018; Chen et al., 2016; Fernández, Llacuna, Fernández-Checa, & Colell, 2009; Garcez et al., 2017, 2019; Min et al., 2017; Qi et al., 2018).

A study performed in transgenic mice overexpressing sterol regulatory element binding protein 2 that induces a cholesterol load showed that intracerebroventricular human Aβ increased oxidative stress, neuroinflammation, and neuronal damage. This indicates that mitochondrial cholesterol accumulation is susceptible to Aβ1−42-induced neurotoxicity (Fernández et al., 2009).

Chen et al. (2016) showed that intrahippocampal administration of Aβ1−42 in mice caused cognitive impairment and increased the expression of toll-like receptor 4 (TLR-4), nuclear factor κB (NF-κB), p65, and Bax as well as decreased Bcl-2 expression. The Aβ1−42 peptide induced high levels of tumor necrosis factor-α (TNF-α) and interleukin (IL) 1β. In line with this, Aβ1−42 intracerebroventricular administration in male 10-week-old ddY mice led to cognitive impairment accompanied by increased TNF-α, inducible nitric oxide synthase (iNOS), and $p22^{phox}$ levels in the hippocampus (Min et al., 2017).

A study performed by our group showed that Aβ1−42 peptide induces memory damage. In the hippocampus, the Aβ1−42 peptide caused high levels of IL-1β, TNF-α, and IL-10. In the cortex, it enhanced the levels of IL-1β, TNF-α, and IL-4. In the serum, Aβ1−42 increased the levels of IL-1β and IL-4. Also, exposition to the peptide increased the levels of brain-derived neurotrophic factor in the hippocampus and levels of nerve growth factor in the total cortex (Garcez et al., 2017).

Male ICR mice administered Aβ 1−42 peptide showed learning and memory impairments. An increase of glial fibrillary acidic protein (GFAP) and cluster of differentiation 11b levels in the hippocampus was observed, indicating microglia and astrocyte activation, respectively. iNOS, cyclooxygenase-2 (COX-2), NF-κB, and TLR2 levels were increased by Aβ1−42 peptide in the hippocampus. Also, phosphorylation of extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), and p38 was increased (Qi et al., 2018).

In addition, Chang et al. (2018) showed that C57BL/6 mice subjected to administration of Aβ1−42 oligomers presented high induction of cell death and increased levels of Bax, Bcl-2, caspase 3, and cytochrome C in the hippocampus and cortex. The peptide induced oxidative stress and inflammation verified by immunostaining for GFAP and Iba1 in the hippocampus. The study found increased phosphorylation of p38 and JNK and reduced ERK in the hippocampus and cortex.

Table 54.1 Summary of the main findings about Aβ 1−42 peptide administration as an animal model of AD.

Administration route of Aβ 1−42 form	Animal species	Main results	References
Aβ1 42 injection (5 μg/μL) into the nucleus basalis	Male Wistar rat	Hippocampus: astrogliosis, microgliosis, increase of IL-lβ level, COX 2, and iNOS expression; Cholinergic hypofunction	Giovannini et al. (2002)
Infusion induced by pump released over a period of 28 days at a rate of ∼0.11 μL/h (50 ng/h Aβ; 36 pmol/g brain/h)	B6; SJL Tg(rPEPCKSREBF2) 788R eh/j, BALB/CJ NPC1NIH and B6C3-Tg	Oxidative stress, neuroinflammation and neuronal damage	Fernández et al. (2009)
Unilateral hippocampal Aβ1−42 (2 nmol/μL) injection	Male Sprague−Dawley rats	Hippocampus: microgliosis (lba-1) and astrogliosis (GFAP), increase of fibrinogen and microglial (OX-42) staining; enhance of IgG level	Ryu and McLarnon (2009)
Intrahippocampal Aβl-42 (2 nmol) Injection	Male Sprague−Dawley rats	Hippocampus: Increase of CXCR2 and IL- 8 expression, gliosis, increase of 4-HNE and HEt levels; reduction of neurons in GCL	Ryu et al. (2015)
Intracerebroventricular injection of Aβl-42 (10 mM/10 μL) bilaterally	Male Wistar rats	Cognitive Impairment; Mitochondrial dysfunction, increase of TNF-α, TGF-β and IL-1β levels; increase of NF-κB and caspase-3 activities.	Sachdeva and Chopra (2015)

Continued

Table 54.1 Summary of the main findings about Aβ 1−42 peptide administration as an animal model of AD.—cont'd

Administration route of Aβ 1−42 form	Animal species	Main results	References
Bilateral. hippocampal Aβ1−42 (4 μg/μL) injection	Sprague−Dawley rats	Cognitive impairment; Increase of TNF-α, IL-1β, iNOS, IFN-y, IL-2, IL17 and IL-22 levels	Chen et al. (2015)
Intrahippocampal injection of Aβl-42 (410 pM/mouse) bilaterally	Male ICR mice	Cognitive impairment; increase of TLR-4, NF-κB, p65, and Bax expression; reduction of Bcl-2 expression; increase of TNF-α and IL-1β levels.	Chen et al. (2016)
Intrahippocampal Aβ 1−42 (400 pmol/site)	Male Wistar rat	Cognitive damage; hippocampus and cortex: increase of IL-1β and decrease of IL-4 levels	Budni et al. (2017)
Intracerebroventricular Ap 1−42 (400 pmol/site)	Male BALB/c mice	Cognitive impairment; Hippocampus: increase of IL-1β. TNF-α, IL-10 and BDNF levels; Total cortex: increase of IL-1β. TNF-α, IL-4 and NGF levels; Serum: increase of IL-1β and IL-4 levels.	Garcez et al. (2017)
Intracerebroventricular Aβ 1−42 (200 pmol/3 μL)	Male 10-week-old ddY mice Sprague−Dawley rats	Cognitive impairment; increase of TNF-α, iNOS and $p22^{phox}$ levels in the hippocampus	Min et al. (2017)

Table 54.1 Summary of the main findings about Aβ 1−42 peptide administration as an animal model of AD.—cont'd

Administration route of Aβ 1−42 form	Animal species	Main results	References
5 μg/μL of Aβ1−42 in the hippocampal		Learning and memory impairments; Hippocampus: increase of GFAP expression MDA level and reduction of SOD and GPx activities; Serum; elevation of MDA, ROS, TNF-α, IL-lα, GFAP, p53, BAX, caspase 3 and caspase 9 levels and reduction of Bcl-2 expression, SOD and GPx activities.	Tian et al. (2017)
Intracerebroventricular Aβ 1−42 (1 mg/mL)	Male albino rats (Charles-Foster strain)	Cognitive impairment; Hippocampus and serum: increased levels of ROS, nitrite, TNF-α, and IL-lβ	Gupta et al. (2018)
Intracerebroventricular Aβ 1−42 (400 pmol/site)	Male BALB C mice	Cognitive impairment; increase of TLR2, MyD88, and NLRP3 content in the hippocampus	Garcez et al. (2019)
Intracerebroventricular Aβ1-42 peptide (≈410 pmmol/mouse)	Male ICR mice	Cognitive impairment; Hippocampus: increase of IL-6 level and reduction of 1L 4 level;	Qi et al. (2018)

Continued

Table 54.1 Summary of the main findings about Aβ 1−42 peptide administration as an animal model of AD.—cont'd

Administration route of Aβ 1−42 form	Animal species	Main results	References
		Immunoreactivity for GFAP and CD 11b; Increase of iNOS, COX-2, NF-κB, TIR2 levels and ERK, J N K and p38 activities	
Aβ1−42 oligomers (3 μL, 10 μM, 0.135 μg) unilaterally injected into the right intrahippocampal region	C57BL/6 mice	Cognitive impairment; Hippocampus and cortex: increase of the expression of Bax, caspase-3, and cytochrome *c*; Reduction of Bcl-2 level and ratio of Bd-2/Bax; Increase of SOD, CAT activities and decrease of MDA level; Immunostaining for GFAP and Iba1 in the hippocampus; Increase of JNK and p38 and reduction of ERK expression.	Chang et al. (2018)

4-HNE, 4-hydroxynonenal; *BDNF*, brain-derived neurotrophic factor; *CXCR2*, C-X-C motif chemokine receptor 2; *GCL*, granule cell layer; *GPx*, glutathione peroxidase; *HEt*, hydroethidine; *IFN-γ*, interferon γ; *IgG*, immunoglobulin G; *NGF*, nerve growth factor; *NLRP3*, nod-like receptor family pyrin domain containing 3; *ROS*, reactive oxygen series; *SOD*, superoxide dismutase; *TGF-β*, transforming growth factor-β.

Finally, a study performed by our group showed that intracerebroventricular injection of Aβ1−42 caused cognitive impairment related to microgliosis, elevated level of TLR2, the adapter protein MyD88, and a high level of the protein nod-like receptor family pyrin domain containing 3, which is involved in the assembly of inflammasome (Garcez et al., 2019).

As in mice, rats can also be useful as a model of AD induced by Aβ 1−42 peptide. Several studies have shown that intrahippocampal or intracerebroventricular Aβ1−42 peptide in rats induces neuroinflammation, an important hallmark of AD (Budni et al., 2017; Chen, Ke, Lu, Qiu, & Peng, 2015; Giovannini et al., 2002; Gupta et al., 2018; Ryu, Cho, Choi, Jantaratnotai, & McLarnon, 2015; Ryu & McLarnon, 2009; Sachdeva & Chopra, 2015; Tian et al., 2017) (Fig. 54.2).

A study conducted by Giovanini et al. (2002) revealed that Aβ1−42 produced microgliosis and astrogliosis and a strong inflammatory reaction characterized by IL-1β production, increased COX-2, and iNOS expression in rats. The inflammatory reaction was accompanied by cholinergic hypofunction.

In rats, Ryu & McLarnon (2009) injected Aβ1−42 peptide, which induced microgliosis and astrogliosis. This study showed that microglial response to Aβ1−42 can induce extensive vascular remodeling leading to blood−brain barrier leakage and subsequent plasma protein infiltration. Therefore, microglia initiating an inflammatory response to Aβ 1−42 also can amplify and sustain inflammation in response to fibrinogen extravasation.

A study performed in rats revealed that Aβ1−42 induced memory damage, mitochondrial dysfunction, and neuroinflammation. Increased levels of TNF-α, transforming growth factor, and IL-1β levels were observed as well as elevated NF-κB and caspase-3 activities (Sachdeva & Chopra, 2015).

The intrahippocampal injection of Aβ1−42 in rat induced an increase of C-X-C motif chemokine receptor 2 and IL-8 expression and gliosis. The peptide led to reduction of neurons in granule cell layer and caused oxidative stress by the increase of 4-hydroxynonenal and hydroethidine levels (Ryu et al., 2015).

Another study, performed by Chen et al. (2015), observed that Aβ1−42 induced increases in glia-derived proinflammatory mediators (TNF-α, IL-1β, and iNOS) and T cell-derived proinflammatory cytokines (interferon γ, IL-2, IL-17, and IL-22) in the hypothalamus, serum, and cerebrospinal fluid of rats.

Our group reported that intrahippocampal injection of Aβ1−42 (400 pmol) bilaterally in rats induced spatial memory damage. This damage was accompanied by elevation of IL-1β levels and reduction of IL-4 levels in the hippocampus and cortex (Budni et al., 2017).

The administration of Aβ1−42 oligomers in the hippocampus of rats induces learning and memory impairments and high levels of reactive oxygen species (ROS) and malondialdehyde (MDA) in the serum. Also observed in the hippocampus were high levels of MDA. The activities of superoxide dismutase and glutathione peroxidase were reduced in the serum and hippocampus. Also, the peptide induced high expression of TNF-α, IL-1α, GFAP, p53, BAX, caspase 3, and caspase 9 in the serum. Bcl-2 expression was diminished. Moreover, GFAP was elevated in the hippocampus (Tian et al., 2017).

Gupta et al. (2018) showed hippocampal and serum increased levels of ROS, nitrite, TNF-α, and IL-1β in the AD animals related to increased chromatolysis and cognitive impairments. Also observed was a significant increase in phagocytic activity and cytotoxicity of splenic polymorphonuclear as well as a decrease in the phagocytic activity of white blood cells in rats administered Aβ 1−42 as compared with control group. These results indicate that increased levels of inflammatory markers in the hippocampus may provide signals to the periphery and can alter systemic immune responses.

Therefore, rats and mice can be useful for inducing the animal model of AD by Aβ1−42 peptide administration in the hippocampus or brain lateral ventricle. The main hallmark of these animal models involves cognitive impairment and inflammation.

Taking to mind that some evidence has pointed to the soluble Aβ oligomer (AβOs) as also a toxic Aβ form involved in AD neuropathological processes (Ferreira, Lourenco, Oliveira, & De Felice, 2015; Heinitz, Beck, Schliebs, & Perez-Polo, 2006; Li et al., 2009; Perez et al., 2010; Viola, Velasco, & Klein, 2008), another tool used to explore AD is animal exposition to an intracerebroventricular injection of AβOs. This injection of AβOs in mice causes impairments in signaling pathways (Bomfim et al., 2012; Lourenco et al., 2013), which results in cognitive damage, particularly memory loss, that mimics the clinical characteristics of AD individuals (Ferreira et al., 2015; Figueiredo et al., 2013; Ledo et al., 2013; Lourenco et al., 2013).

These models present some advantages as well as a few limitations. As strengths of cerebral administrations of Aβ, we can point to the relative ease of implementation. In addition, it represents an acute model, as neuropathology and cognitive deficits are detected between 1 day and a few weeks following a single intracerebroventricular or intrahippocampal injection of Aβ, much faster than the course of several months for disease progression in transgenic mice. However, the acute impact of Aβ in rodent brains triggers signaling pathways that might not play significant roles in the chronic nature of human disease. Other disadvantages are (1) the lack of AD multifactorial aspects, (2) the use of adult rodents instead of old ones, (3) normal production of endogenous Aβ from APP, (4) exacerbated inflammatory response, and (5) negligence of other AD hallmarks such as neurofibrillary tangles (McLarnon & Ryu, 2008).

Conclusion

This chapter suggests that Aβ 1−42 peptide injection could serve as a useful experimental animal model mimicking the brain inflammation of AD. The administration of Aβ 1−42 peptide in vivo can considered a simple, nontransgenic, and prodromal animal model of AD. This animal model can exhibit predictive, face, and construct validity, although the pathophysiological entirety of AD is unknown and thus far has no effective therapy. Therefore, this animal model is an important tool to investigate the mechanism of disease and screen for protective therapeutic targets.

Key facts

- BACE1 is a beta-secretase enzyme that initiates the formation of Aβ from APP.
- The Aβ1−42-induced dementia animal model is a pharmacological rodent animal model that leads to inflammation.
- APP is a single-pass transmembrane protein expressed in brain cells including neurons that present a large number of functions. The precise function of APP is unknown. This protein was cloned in 1987.
- Neuroinflammation is defined as an inflammatory response within the brain or spinal cord, and also it is a term to describe the role of inflammatory processes in the pathophysiology of most neurodegenerative diseases.
- Cytokines are proinflammatory mediators that include interferons, the interleukins, the chemokine family, mesenchymal growth factors, the tumor necrosis factor family, and adipokines.

Summary points

- Toxicity of amyloid-β peptide is a hallmark of AD.
- Aβ1−42 is the main constituent of amyloid plaques.
- Experimental models are useful for studying AD.
- Aβ 1−42 injection is a widely used animal model of AD.
- The Aβ 1−42-induced animal model exhibits predictive, face, and construct validity.

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CHAPTER 55

Resources for the neuroscience of dementia

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Introduction

The global prevalence of dementia is at pandemic proportions. In 2015, it was estimated that there were nearly 50 million people living with dementia, worldwide. This was projected to double every 20 years, reaching 74.7 million in 2030 and 131.5 million in 2050 (Ali, Guerchet, Wu, Prince, & Prina, 2015).

Dementia is not a specific disease. It is a group of symptoms affecting memory, thinking, and social abilities severely enough to interfere with daily functioning. This causes significant social, psychological, and financial difficulties to those affected as well as their families.

The concept of dementia has existed since time immemorial. Although dementia is not inevitable, mental decay does occur with aging and the prevalence of dementia has risen as the lifespan of humans has increased. In the late 1800s, with advances in medicine and the ability to examine the brain with microscopes, it was realized that diseases could cause dementia.

While there are several causes of dementia; Alzheimer's disease is the most common cause of progressive dementia in adults. In 1910, it was named after Alois Alzheimer (1864–1915), a German psychiatrist. In 1906, Alzheimer described "an unusual disorder of the cerebral cortex" in Auguste Deter, a 56-year-old woman with dementia. She initially presented with significant memory loss and hallucinations and so was admitted under Alzheimer's psychiatric service in 1901 at 51 years of age. Her dementia deteriorated quickly and she sadly passed away 5 years later.

Over 100 years ago, Alzheimer, who dissected and studies the brains of affected people postmortem, saw microscopic amyloid plaques and neurofibrillary tangles in Deter's brain (Alzheimer, 1907; Müller, Winter, & Graeber, 2013). These neuropathological changes are now known to be characteristic of Alzheimer's disease.

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It is now well recognized that the etiology of all causes of dementia including Alzheimer's is multifactorial. Both genetic variants and environmental factors contribute to disease. However, in a few cases (<2%), Alzheimer's disease is an autosomal dominant trait. The histological slides of Auguste Deter's brain studied by Alzheimer were recently found (Müller et al., 2013; Maurer, Volk, & Gerbaldo, 1997; Graeber, Kösel, Grasbon-Frodl, Möller, & Mehraein, 1998). Reanalysis confirmed the neuropathological diagnosis (Graeber et al., 1998; Enserink, 1998). Astoundingly, analysis of DNA extracted from Auguste Deter's brain identified a *PSEN1* mutation (Müller et al., 2013). Mutations in *PSEN1* are the most common cause of autosomal dominant Alzheimer's disease (Müller et al., 2013).

This revelation demonstrates the explosion in the knowledge and understanding of the neuroscience of dementia since Alzheimer first described amyloid plaques and neurofibrillary tangles. It is now difficult even for experienced scientists to remain up-to-date. For those new to the field, it is difficult to know which of the myriad of available sources are reliable. To assist colleagues who are interested in understanding more about this field, we have therefore produced tables containing reliable, up-to-date resources in this chapter. The experts who assisted with the compilation of these tables of resources are acknowledged below.

Tables 55.1–55.4 list the most up-to-date information on the regulatory bodies and professional societies (Table 55.1), journals on the neuroscience of dementia (Table 55.2), books (Table 55.3), and online resources (Table 55.4) that are relevant to an evidence-based approach to the neuroscience of dementia.

Table 55.1 Regulatory bodies, professional societies, and organizations.

Ageing and Aged Care	www.agedcare.health.gov.au/older-people-their-families-and-carers/dementia
Alzforum	www.alzforum.org
Alzheimer Argentina	alzheimer.org.ar
Alzheimer Europe	www.alzheimer-europe.org
Alzheimer's Association (USA)	www.alz.org
Alzheimer's Association (Journal Alzheimer's and Dementia)	www.alzheimersanddementia.com
Alzheimer's Association Japan	www.alzheimer.or.jp/?p=2978
Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART)	action.alz.org/personifyebusiness/default.aspx?tabid=1516
Alzheimer's Disease and Dementia Caregiver Support	www.alzwell.com
Alzheimer's Disease Association (ADA)	alz.org.sg
Alzheimer's Disease International	www.alz.co.uk
Alzheimer's Drug Discovery Foundation	www.alzdiscovery.org
Alzheimer's Foundation of America	www.alzfdn.org

Table 55.1 Regulatory bodies, professional societies, and organizations.—cont'd

Alzheimer's Disease Neuroimaging Initiative (ADNI)	adni.loni.usc.edu
Alzheimers.net	www.alzheimers.net
Alzheimers New Zealand	www.alzheimers.org.nz
Alzheimer's Research UK	www.alzheimersresearchuk.org
Alzheimer Society of Ireland	alzheimer.ie/Home.aspx
Alzheimer Society Ontario	alzheimer.ca/en/on
Alzheimer's Society	www.alzheimers.org.uk
Alzheimer's Society Canada	alzheimer.ca/en/Home
Alzheimer's Society (UK)	www.alzheimers.org.uk
Alzheimer Society Toronto	www.alz.to
Alzheimer's wa	www.alzheimerswa.org.au
American Academy of Clinical Neuropsychology	www.theaacn.org
American Academy of Neurology	www.aan.com
American Board of Clinical Neuropsychology	www.div40.org
American Delirium Society	www.americandeliriumsociety.org
American Federation for Aging Research	www.afar.org
American Geriatrics Society	www.americangeriatrics.org
American Psychological Association	www.apa.org
Asociación Lewy Body Argentina (ALBA)	www.lewyargentina.org
Association for frontotemporal degeneration	www.theaftd.org
Associazione Gruppo Anchise	www.formalzheimer.it
Associazione Italiana Malattia di Alzheimer	www.alzheimer-aima.it
Associazione Italiana Psicolgeriatria (AIP)	www.psicogeriatria.it/home
Associazione Italiana di Psicologia	www.aipass.org
Associazione Malattia di Alzheimer Sardegna	www.amas-alzheimer.it
Australasian Delirium Association	www.delirium.org.au
Australian Frontotemporal Dementia Association	www.theaftd.org.au
Banner Alzheimer's Institute	www.banneralz.org
BrainHQ	www.brainhq.com
Brain Foundation	www.brainfoundation.org.au/disorders/alzheimers-disease
Brain Injury Association of America	www.biausa.org
Brain Research Center	www.brainresearchcenter.nl
BrightFocus Foundation	www.brightfocus.org
British Neuroscience Association	www.bna.org.uk
Canadian Institute for Health Information	www.cihi.ca
Chinese Dementia Research Association	www.cdra.org.hk
Dementia Action Alliance USA	www.daanow.or
Dementia Alliance International	www.dementiaallianceinternational.org
Dementia Australia	www.dementia.org.au
Dementia Australia Research Foundation	www.dementia.org.au
Dementia Care Central	www.dementiacarecentral.com
Dementia Care International	dementiacareinternational.com

Continued

Table 55.1 Regulatory bodies, professional societies, and organizations.—cont'd

Dementia Friendly Communities-Dementia Australia	www.dementiafriendly.org.au
Dementia Friendly America	www.dfamerica.org
Dementia Friends Canada	www.dementiafriends.ca
Dementia Friends USA	www.dementiafriendsusa.org
Dementia Society	www.dementiahelp.ca
Dementia Society of America	www.dementiasociety.org
Dementia Support Australia	www.dementia.com.au
Dementia Training Australia	www.dementiatrainingaustralia.com.au
Dementia UK	www.dementiauk.org
European Academy of Neurology	www.ean.org
European Alzheimer's Disease Consortium (EADC)	www.eadc.info
Fundacion Alheimer España	www.alzfae.org
Fundación Centro de Investigación Enfermedades Neurológicas (CIEN)	fundacioncien.es
Fundacion Reina Sofía (Spain)	www.fundacionreinasofia.es
Federation of European Neuroscience Societies	www.fens.org
France Alzheimer	www.francealzheimer.org
French Neuroscience Society	www.neurosciences.asso.fr
Global Alzheimer's Association Interactive Network	www.gaain.org
Greek association of Alzheimer's Disease and Related Disorders (Alzheimer Hellas)	www.alzheimer-hellas.gr
Health Quality Ontario	www.hqontario.ca
HealthyWA	healthywa.wa.gov.au/Articles/A_E/Alzheimers-disease
Federazione Alzheimer Italia	www.alzheimer.it
Interdem	www.interdem.org
International Brain Research Organization	www.ibro.org
International Neuropsychological Society	www.the-ins.org
International Psychiogeriatrics Associations	www.ipa-online.org
International Society for Serotonin Research	www.serotoninclub.org
Italian Federation Alzheimer	www.alzheimer.it
Japan Geriatric Society	www.jpn-geriat-soc.or.jp
Japan Neuroscience Society	www.jnss.org
Japanese Psychogeriatric Society	www.rounen.org
Japan Society for Dementia Prevention	ninchishou.jp
Japan Society for Dementia Research	dementia.umin.jp
Knight Alzheimer Disease Research Center	www.knightadrc.wustl.edu
Lewy Body Dementia Association	www.lbda.org
Ministry of Health, Labour, and Welfare-Japan	www.mhlw.go.jp/stf/seisakunitsuite/bunya/hukushi_kaigo/kaigo_koureisha/ninchi/index.html

Table 55.1 Regulatory bodies, professional societies, and organizations.—cont'd

Myagedcare	www.myagedcare.gov.au/getting-started/health-conditions/dementia
NHMRC National Institute for Dementia Research	www.nnidr.gov.au
National Academy of Neuropsychology	www.nanonline.org
National Institute of Aging	www.nia.nih.gov
National Institute on Aging: Alzheimer's Disease Education and Referral Center	www.nia.nih.gov/health/alzheimers
National Institute of Mental Health	www.nimh.nih.gov/index.shtml
National Institute of Neurological Disorder and Stroke	www.ninds.nih.gov
National Institutes of Health	www.nih.gov
National Center for Geriatrics and Gerontology	www.ncgg.go.jp/english/index.html
National Center of Neurology and Psychiatry	www.ncnp.go.jp/english/index.html
PalliAGED	www.palliaged.com.au
Piano Nazionale Demenze	www.iss.it/demenze
Polish Alzheimer's Association (Polskie Towarzystwo Alzheimerowskie)	www.alzheimerpolska.pl
Polish Psychogeriatric Association	www.medicalguidelines.pl
National institutes for Quantum and Radiological Science and Technology	www.qst.go.jp/site/qst-english
Rethink Dementia	www.rethinkdementia.ca
RIKEN Center for Brain Science	cbs.riken.jp/en
Società Italiana di Neurologia (SIN)	www.neuro.it
Società Italiana di Neurologia – Dementia (SINDEM)	www.sindem.org
Society for Neuroscience	www.sfn.org
Sociedad Española de Neurología (Spanish Society of Neurology)	www.sen.es
Sociedad Neurológica Argentina	www.sna.org.ar/web/
Società Italiana di NeuroPsicologia	sinp-web.org
Società Italiana Per gli Studi dell'Invecchiamento (SIPI)	www.sipinvecchiamento.it
Swedish Dementia Centre	www.demenscentrum.se
Wicking Dementia Research & Education Centre	www.utas.edu.au/wicking
World Federation of Music Therapy (WFMT)	www.wfmt.info
World Health Organization	www.who.int

This table lists the regulatory bodies, professional societies, and organizations relevant to dementia. Some of these sites are very comprehensive in that they offer advice, resources, and other information related to dementia. While some sites are country-specific, some information contained within these sites is useful for other countries. Some resources are also identified in Table 55.4. Please note, occasionally, the location of the websites or web address changes. In these cases the use of the "Search" tabs or options should be explored at the parent address or site.

Table 55.2 Journals relevant to the neuroscience of dementia.

Journal of Alzheimer S Disease
Plos One
Neurobiology Of Aging
Scientific Reports
Alzheimer's And Dementia
International Psychogeriatrics
Frontiers In Aging Neuroscience
Neurology
Journal Of The American Geriatrics Society
Current Alzheimer Research
International Journal Of Geriatric Psychiatry
Molecular Neurobiology
Dementia
Lecture Notes In Computer Science Including Subseries Lecture Notes In Artificial Intelligence And Lecture Notes In Bioinformatics
Alzheimer S Research And Therapy
American Journal Of Geriatric Psychiatry
Brain
Journal Of The American Medical Directors Association
American Journal Of Alzheimer S Disease And Other Dementias
International Journal Of Molecular Sciences
Aging And Mental Health
JAMA Neurology
ACS Chemical Neuroscience
Neuroscience Letters
Journal Of The Neurological Sciences
Lancet Neurology
Journal Of Neuroscience
Frontiers In Neuroscience
Geriatrics And Gerontology International
Journal Of Neurochemistry
Alzheimer Disease And Associated Disorders

Journals publishing original research and review articles related to dementia in connection neuroscience and treatments. Included in this list are the top 30 journals which have published the most number of articles over the past 5 years. *Nature Medicine* and *The New England Journal of Medicine* were also recommended by authors (though only Plos One appears in this list). Data derived from Scopus.

Table 55.3 Books relevant to the neuroscience of dementia.

100 Simple Things You Can Do to Prevent Alzheimer's and Age-Related Memory Loss. Carper J. Hachette book group, 2012, USA
Aβ Metabolism and Alzheimer's Disease. Saido TC. Landers Bioscience, 2003, USA
Abeta peptide and Alzheimer's disease. Celebrating a Century of Research. Barrow CJ, Small DH. Springer, 2007, UK
Addiction Biology. Simonnet A, Cador M, Caillé S. Wiley, 2013, UK
Advances in Alzheimer's Research Volume 1 and 2. Lahiri DK. Bentham Science, 2018, Netherlands
Aging, Communication and Health: Linking Research and practice for successful Aging. Hummert ML, Nussbaum JF. Routledge, 2001, USA
Ahead of Dementia: A Real-World, Upfront, Straightforward, Step-by-Step Guide for Family Caregivers. Mitzkun L, Aldenderfer KD. CreateSpace Independent Publishing Platform, 2016, USA
Alzheimer: 100 Years and Beyond. Jucker M, Beyreuther K, Haass C, Nitsch RM, Christen Y. Springer, 2006, Germany
Alzheimer's Action Plan: What You Need to Know-and What You Can Do-about Memory Problems, from Prevention to Early Intervention and Care Doraiswamy PM, Gwyther LP, Adler T. St. Martin's Griffin, 2009, USA
Alzheimer's disease. Waldemar G, Burns AS. Oxford University Press, 2017, UK
Alzheimer's Disease: Advances for a New Century. Perry G, Zhu X, Smith MA, Sorensen A, Avila J. IOS Press, 2013, The Netherlands
Alzheimer's Disease: A Physician's Guide to Practical Management 2nd Edition. Richter RW, Zoeller B. Humana Press, 2014, USA
Alzheimer's Disease: Pathogenic mechanism, and development of novel diagnosis, drug discovery, and therapy. Arai H. NTS Inc, 2018, Japan
Alzheimer's From The Inside Out. Taylor R. Health Professions Press Inc, 2007, USA
Aromatherapy: Basic Mechanisms and Evidence Based Clinical Use. Bagetta G, Cosentino M, Sakurada T. CRC Press, 2016, USA
Astrocyte Physiology and Pathology. Gentile MT. Intech Open Limited, 2018, UK
Behavioral Neurology of Dementia, 2nd edition. Miller BL, Boeve BF. Cambridge University Press, 2017, UK
Calcium Paradox and its Impact on Neurological and Psychiatric Diseases, 2nd edition. Bergantin LB, Caricati-Neto A. Cambridge Scholars Publishing, 2018, UK
Clinical Practice with Caregivers of Dementia Patients. Kaplan M. Taylor and Francis, 1996, USA.
Connecting in the Land of Dementia: Creative Activities to Explore Together. Shouse D. Central Recovery Press, 2016, USA
Continuum, Lifelong Learning in Neurology: Dementia. Finger EC. LWW, 2016, USA
Dancing with dementia. Bryden C. Jessica Kingsley Publishers, 2005, UK
Dealing with Dementia: A guide to Alzheimer's Disease and Other Dementias Brian Draper Allen & Unwin
Dementia. McNamara P. Praeger, 2011, USA
Dementia: A Clinical Approach, 3rd edition. Mendez MF, Cummings JL. Butterworth-Heinemann 2003, USA

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Table 55.3 Books relevant to the neuroscience of dementia.—cont'd

Dementia, Aging, and Intellectual Disabilities: A Handbook. Janicki MP, Dalton AJ. Taylor and Francis, 1999, USA
Dementia: A Global Approach. Krishnamoorthy ESS, Prince MJ, Cummings JL. Cambridge University Press, 2010, New York, America
Dementia: Alzheimer's and Other Dementias: The 'at Your Fingertips' Guide. Cayton H, Graham N, Warner J. Class Publishing (London) Ltd, 2002, UK
Dementia and Memory: A Handbook for Students and Professionals. Thompson SBN. Ashgate Publishing, 2006, USA
Dementia and Normal Aging. Huppert FA, Brayne C, O'Connor DA. Cambridge University Press, 1994, UK
Dementia and Well-Being: Possibilities and Challenges Ailsa Cook Dunedin Academic
Dementia Beyond Disease: Enhancing Well-Being. Power A. Health Professionals Press Inc, 2011, USA
Dementia Beyond Drugs: Changing the Culture of Care. Power A. Health Professionals Press Inc, 2011, USA
Dementia: Challenges and New Directions. Hunter S. Jessica Kingsley Publishers, 1997, USA
Dementia: Comprehensive Principles and Practice. Dickerson B, Atri A. Oxford University Press, 2014, USA
Dementia: From Diagnosis to Management - A Functional Approach. Bourgeois MS, Hickey EM. Taylor and Francis Group (Psychology Press), 2009, USA
Dementia in Close-up. Miesen BML. Routledge, 1999, UK
Dementia: Metamorphosis in Care. Biernacki C. John Wiley and Sons, 2007, UK
Dementia: Mind, Meaning, and the Person. Hughes JC, Louw SJ, Sabat SR. Oxford University Press, 2005, UK
Dementia: New Skills for Social Workers. Chapman A, Marshall M. Jessica Kingsley Publishers, 1993, UK
Dementia: Presentations, Differential Diagnosis, and Nosology, 2nd edition. Emery VOB, Oxman TE. John Hopkins University Press, 2003, USA
Diabetes, Insulin and Alzheimer's Disease. Craft S, Christen Y. Springer, 2010, Germany
Five Stages of Health. Walker R. Transworld Publishers, 2012, Australia
Flourish: A Visionary New Understanding of Happiness and Well-being. Seligman M. Free Press, 2011, USA
Forget Memory. Basting A. John Hopkins University Press, 2009, USA
Handbook of Animal models in Alzheimer's disease. Casadesus G. IOS Press, 2011, USA
Handbook of Dementia: Psychological, Neurological, and Psychiatric Perspectives. Lichtenberg PA, Murman DL, Mellow AM. Wiley, 2005, USA
Handbook of Dementing Illnesses, 2nd Edition. Morris JC, Galvin JE, Holtzman DM. CRC Press, 2006, USA
Handbook of Infection and Alzheimer's Disease. Miklossy J. IOS Press, 2017, The Netherlands
Handbook of neuropsychology. Boller F, Grafman J. Elsevier, 2000, Amsterdam
Hodges' Frontotemporal Dementia. Dickinso BC. Cambridge Medicine, 2016, UK
Human Sleep and Cognition. Van Dongen H, Kerkhof GA. Elsevier Science, 2011, USA

Table 55.3 Books relevant to the neuroscience of dementia.—cont'd

Importance of Food and Mealtimes in Dementia care. Berg G. Jessica Kingsley Publishing, 2006, USA
In Search Of My Father. Popovic H. Bookpal, 2011, Australia
Intermittent Hypoxia and Human Diseases. Xi L, Serebrovskaya TV, Springer Science & Business Media, 2012, USA
Intermittent Hypoxia: From Molecular Mechanisms to Clinical Applications. Xi L, Serebrovskaya TV. Nova Science Publishers Inc, 2009, USA
La neuropsicología en preguntas y respuestas. Alemán C, Berríos W, Bonifacio A, Cal AC et al. Editorial Dunken, 2017, Argentina
Learning Life from Illness Stories. Willis P, Leeson K. Post Pressed, 2012, Australia
Live and Laugh with Dementia. Low LF. Exisle Publishing, 2014, Australia
Living Well with Dementia: The Importance of the Person and the Environment for Wellbeing. Rahman S. CRC Press, 2014, UK
Living Better with Dementia: Good Practice and Innovation for the Future. Rahman S. Jessica Kingsley Publishers, 2015, UK
Love Life Loss A Roller Coaster of Poems. Swaffer K. Kelbane: Graphic Print Group, 2012, Australia
Malattia di Alzheimer e Altre Demenze - Diagnosi e Terapia Integrata. Caltagirone C, Sancesario G. Società Editrice Universo, 2017, Italy
Management of Dementia, 2nd edition. Gauthier S, Ballard C. CRC Press, 2009, USA
Memory loss, Alzheimer's disease, and dementia: A practical guide to clinicians.Buddson AE, Solomon PR. Elsevier, 2015
Microglia in Health and Disease. Tremblay M-È. Springer, 2016, Germany
Microglia: Methods and Protocols (Methods in Molecular Biology) Joseph B. Springer, 2016, Germany
Neurobiology of Brain Disorders. Zigmondm MJ, Rowland LP, Coyle JT. Academic Press, 2015, UK
Neuroimaging Diagnosis for Alzheimer's Disease and Other Dementias. Matsuda H, Asada T, Tokumaru AM. Springer, 2016, Japan
Neuropathology of Dementia. Esiri M, Lee VM-Y, Trojanowski JQ. Cambridge University Press, 2004, UK
Nicotinic Acetylcholine Receptor Signaling in Neuroprotection. Akaike A, Shimohama S, Misu Y. Springer, 2018, Germany.
Nutrition for brain health. Fighting dementia. Town L, Kassel K. Omega press, 2016, USA
Oxford Textbook of Cognitive Neurology and Dementia. Husain M, Schott JM. Oxford University Press, 2016, UK.
Recent Advances in Alzheimer Research. Salehi A, Rafii M, Phillips C. Bentham Science, 2015, USA
Recent Advances in the Biology of Secretases, Key Proteins in Alzheimer's Disease. Araki W. Research Signpost, 2008, India
Research Progress in Alzheimer's Disease and Dementia. Sun M-K. Nova Science Publishers, 2007, USA

Continued

Table 55.3 Books relevant to the neuroscience of dementia.—cont'd

Simplicity of Dementia: A Guide for Family and Carers. Buijssen H. Jessica Kingsley Publishers, 2005, USA
Sleep, Drugs & Alzheimer's Disease. Reid LD, Lavash VA. Hexagon 18 Cherry Lane, LLC, 2018, USA
Soft-Wired: How the New Science of Brain Plasticity Can Change Your Life. Merzenich MM. Parnassus Publishing, 2013, USA
Super Brain: Unleashing the Explosive Power of Your Mind to Maximize Health, Happiness, and Spiritual Well-Being. Tanzi R, Chopra D. Harmony Books, 2012, USA
Synaptic Plasticity and the Mechanism of Alzheimer's Disease. Selkoe DJ, Triller A, Christen Y. Springer, 2008, Germany.
The 36-Hour Day: A Family Guide to Caring for People Who Have Alzheimer Disease, Other Dementias, and Memory Loss (A Johns Hopkins Press Health Book), 6th edition. Mace NL, Rabins PV. Grand Central Life & Style, 2012, USA
The Bad The Brilliant - Lessons from the Journey of Living with Dementia. Ellison P, Sandlant V. Resthaven Incorporated, 2012, Australia
The Biology of Belief: Unleashing the Power of Consciousness, Matter and Miracles. Lipton B. Hay House Inc, 2005, Australia
The Brain That Changes Itself. Doidge N. Scribe Publications, 2012, UK
The Brain Way of Healing. Doidge N. Penguin Books, 2016, UK
Thinking About Dementia Culture, Loss, and the Anthropology of Senility. Leibing A, Cohen L. Rutgers University Press, 2006, USA
Treating Dementia Dependence with Nitrous Oxide/Oxygen (PAN): A manual for Health Professionals. Gillman M. Cerebrum Publishers, 2010, South Africa
Trial Designs and Outcomes in Dementia Therapeutic Research. Rockwood K, Gauthier S. Taylor and Francis, 2006, UK
Two Faces of Evil: Cancer and Neurodegeneration. Curran T, Christen Y. Springer, 2011, Germany
Type 2 Diabetes and Dementia. Srikanth V, Arvanitakis Z. Academic Press, 2018, USA
Vascular Dementia: Cerebrovascular Mechanisms and Clinical Management. Paul RH, Cohen R, Ott BR, Salloway S. Humana Press, 2005, USA
Women and Smoking: A Report of the Surgeon General. Office on Smoking and Health (USA). Centers for Disease Control and Prevention (USA), 2001, USA
World Report on Ageing and Health World Health Organization. Beard J, Officer A, Cassels A. World Health Organization, 2015, Geneva, Switzerland

This table lists recommended books on dementia.

Table 55.4 Relevant online resources and information on emerging techniques.

ACT on Alzheimer's	www.actonalz.org
AFNP MED	www.afnpmed.com
AiMediq	www.aimediq.com
AlzBase gene dys-regulation network	alz.big.ac.cn/alzBase
AlzBetter	www.alzbetter.com
Alzforum Network	www.alzforum.org/about-ad
Alzheimer Disease & Frontotemporal Dementia Mutation Database	www.molgen.ua.ac.be/admutations/default.cfm
Alzheimer's Disease Cooperative Study	www.adcs.org
Alzheimer's Drug Discovery Foundation	www.alzdiscovery.org
Alzheimer's Disease Neuroimaging Initiative	www.adni.loni.usc.edu
Alzheimer Italia	www.alzheimer.it
Alzheimer's Statistics	www.alzheimers.net
Alzheimer Uniti Roma ONLUS	www.alzheimeruniti.it
Amylgen	www.amylgen.fr
Arizona Alzheimer's Disease Center/Banner Alzheimer's Institute	www.azalz.org
Asociación de Ayuda a la Afasia (Help to Aphasia Association)	www.afasia.org
Asociación Lucha contra el Mal de Alzheimer (ALMA)	www.alma-alzheimer.org.ar/es
Associazione Italiana Ricerca Alzheimer Onlus	www.airalzh.it
Biobank (Fundacion CIEN)	https://bt.fundacioncien.es/
Care-givers Voice	www.thecaregiversvoice.com/about-us
Cellular Dynamics	www.fujifilmcdi.com
Centro de Investigación en Salud Mental (Research Center in Mental Health)	bi.cibersam.es
ClinicalTrials.gov	clinicaltrials.gov
Clinical Trials on Alzheimer's disease Conference	www.ctad-alzheimer.com
CRE Alzheimer (Inserso, Spain) Apps for caregivers, patients, and professionals	www.crealzheimer.es
Dementia Action Alliance USA	www.daanow.org
Dementia and Elderly Care News	www.dementianews.wordpress.com

Continued

Table 55.4 Relevant online resources and information on emerging techniques.—cont'd

Dementia Care Central	www.dementiacarecentral.com
Dementia Connections	www.dementiaconnections.ca
Dementia Discovery Fund	www.theddfund.com
Dementia Down Under	www.dementiadownunder.com
Dementia Enabling Environments	www.enablingenvironments.com.au
Dementia Guide	www.dementiaguide.com
Dementia Platform UK	www.dementiasplatform.uk
Dementia Support	www.dementiasupport.ca/web
Department of Neurology. Weill Cornell Medicine	neurology.weill.cornell.edu
Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford	www.dementiasplatform.uk
Eisai Co. (VSRAD)	www.vsrad.info/index2.html
European Delirium Association	www.europeandeliriumassociation.com
First Link Program (Alzheimer Society of Canada)	alzheimer.ca/en/Home/We-can-help/Resources/For-health-care-professionals/first-link
Fisher Center for Alzheimer's Research Foundation	www.alzinfo.org
Fondation Vaincre Alzheimer	www.vaincrealzheimer.org
Frontier Frontotemporal Dementia Research group	sydney.edu.au/brain-mind/our-research/healthy-ageing-and-neurodegeneration/forefront-ageing-and-neurodegeneration-team/frontier-frontotemporal-dementia-research-group.html
Fundación de familiares de enfermos de Alzheimer	www.alzheimers.org
German Center for Neurodegenerative Diseases within the Helmholtz Association	www.dzne.de/en
Global Alzheimer's Platform Foundation	globalalzplatform.org
Harvard Medical School	www.med.harvard.edu/AANLIB/home.html
Human Brain Project	www.humanbrainproject.eu/en/
Icare4someone:	icare4some1.wordpress.com/2015/04/15/64/
iGeriCare	igericare.healthhq.ca
Imaging mass spectrometry society	www.imagingmssociety.org
Innovations in Dementia	www.innovationsindementia.org.uk

Interdem	interdem.org
International Conference on Alzheimer's and Parkinson's Diseases and related neurological disorders (AD/PD Conference)	adpd.kenes.com
International Taurine Meeting	www.taurinesociety.org
International Union of Basic and Clinical Pharmacology (IUPHAR)	www.guidetopharmacology.org
Interval Hypoxic Hyperoxic Treatment	www.reoxy.lu
InterVivo Solutions	intervivo.com
ITALPLANED (Italian Platform for Neurodegenerative Diseases)	www.italplaned.sindem.org
Lewy Body Dementia Association	www.lbda.org
Life-enhancement	www.life-enhancement.com/pages/taurine-stops-memory-loss
LIPID-MAPS	www.lipidmaps.org
Lumocity: Daily Brain Games	itunes.apple.com/us/app/lumosity-mobile/id577232024?mt=8
Magnetic Resonance Imaging-based Gradient Echo Plural Contrast Imaging	www.mir.wustl.edu
Mayo Clinic Alzheimer's Blog	www.mayoclinic.com
MindMate - For a healthy brain	itunes.apple.com/us/app/mindmate-empowering-people/id1030422375?mt=8
Molecular and Clinical Bioinformatics (MCBI)	mcbi.co.jp
Montreal Cognitive Assessment	www.mocatest.org
National Alzheimer's Association (USA)	www.alz.org/index.asp
National Alzheimer's Coordinating Center	www.alz.washington.edu
National Institute of Aging, Alzheimer's Disease and Related Dementias	www.nia.nih.gov/health/alzheimers/dementia-research-and-clinical-trials
neuGRID	www.neugrid4you.eu
Neuroscience Research Australia	www.neura.edu.au
ParentGiving: Home Care Products	www.parentgiving.com
Polish Association for Assisting People with Alzheimer's Disease (Polskie Stowarzyszenie Pomocy Osobom z Choroba Alzheimera)	www.alzheimer-waw.pl
Primary progressive aphasia connection	www.ppaconnection.org

Continued

Table 55.4 Relevant online resources and information on emerging techniques.—cont'd

Quantib	www.quantib.com
Reme	www.remindmecare.com
Reminisence Interactive Therapy Activities (RITA)	www.myimprovementnetwork.com
REPROCELL	www.reprocell.com
Scottish Dementia Informatics Platform	www.sdrc.scot/research/scottish-dementia-informatics-platform
Sexuality and Dementia	fightdementia.org.au/sites/default/files/20101001_Nat_QDC_6DemSexuality.pdf
Spaced Retrieval Therapy	itunes.apple.com/us/app/spaced-retrieval-therapy-memory/id498787795?mt=8
Stanford Health Care	stanfordhealthcare.org/search-results.conditions.html/DEMENTIA
Teepa Snow	www.teepasnow.com
Toronto Dementia Research Alliance	www.tdra.ca
Transpharmation	www.transpharmation.co.uk
University of Greifswald	demenznetzwerke.de
UK Dementia Research Institute	ukdri.ac.uk
Vaiomer	www.vaiomer.com
Validation Training Institute	vfvalidation.org
Wisconsin/Michigan State Brain Collections	www.wisc.edu
World Alzheimer Report 2015: the Global Impact of Dementia.	www.worldalzreport2015.org
Worldwide clinical trials	clinicaltrials.gov
Young Dementia UK	www.youngdementiauk.org
Younger Onset Dementia and ME	youngeronsetdementiaandme.blogspot.com/
Younger onset dementia association Inc	www.youngeronset.net

This table lists some internet resources and other relevant materials in relation to the neuroscience of dementia. Some of these sites are also listed in Table 55.1. Please note, occasionally, the location of the websites or web address changes. In these cases the use of the "Search" tabs should be explored at the parent address or site.

Summary points

- The global prevalence of dementia is at pandemic proportions.
- In 2015, there were nearly 50 million people living with dementia, worldwide.
- The prevalence of dementia is projected to double every 20 years, reaching 132 million in 2050.
- Dementia is not a specific disease.
- The neuronal pathologies responsible for dementia are relatively recent discoveries.
- This chapter lists the resources on the regulatory and professional bodies, journals, books, and websites that are relevant to an evidence-based approach to the neuroscience of dementia.

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Alzheimer, A. (1907). Über eine eigenartige Erkrankung der Hirnrinde. *Allgemeine Zeitschrift fuer Psychiatric, 64*, 146–148, 1907.

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The Neuroscience of Dementia Volume 2

Genetics, Neurology, Behavior, and Diet in Dementia

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Colin R. Martin and Victor R. Preedy

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