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APOE-Dependent Phenotypes in Subjects with Mild Cognitive Impairment Converting to Alzheimer's Disease

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Abstract.
Background: The E4 isoform of the APOE genotype is the most significant genetic risk factor for sporadic Alzheimer's disease (AD) and has recently been found to modulate disease expression in patients with AD.
Objective: To investigate APOE-dependent cognitive and structural phenotypes in subjects with mild cognitive impairment who converted to AD within the following three years.

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Methods: Subjects converting to AD ($n = 63$) were compared to a control group with stable mild cognitive impairment ($n = 131$). Clinical, neuropsychological, and MRI data were obtained by the German Dementia Competence Network. Subgroups of converting and stable APOE E4 carriers and non-carriers were investigated longitudinally with MRI to examine structural correlates of conversion. Voxel-based morphometry was applied to investigate gray matter distribution.

Results: At baseline, executive performance correlated with global and bilateral prefrontal gray matter volume and predicted conversion only among non-carriers. Converting carriers and non-carriers presented distinct patterns of brain atrophy on longitudinal analysis, in line with a dissociation between more pronounced occipital atrophy in carriers and more frontoparietal volume loss in non-carriers at follow-up.

Conclusions: The current findings suggest that in APOE E4 non-carriers with AD, executive dysfunction is closely linked to frontal gray matter atrophy and predictive of progression to dementia. The results are consistent with APOE genotype-dependent profiles of structural damage and cognitive decline in patients with imminent conversion to AD.

Keywords: APOE, Alzheimer's disease, magnetic resonance imaging, mild cognitive impairment, phenotypes, voxel-based morphometry

INTRODUCTION

Patients with Alzheimer's disease (AD) typically present with impairment of episodic memory, accompanied by structural changes of medial temporal regions. But other cognitive deficits, such as executive dysfunction or visuospatial impairment may occur as initial symptoms of the disease and reflect early neocortical pathology. The phenotypic heterogeneity of the disease has increasingly been recognized and is addressed in the new diagnostic guidelines recommended by the National Institute on Aging and the Alzheimer's Association [1, 2].

Though multiple genetic risk factors for AD have been identified, it remains unclear to what extent the phenotype of the disease is genetically determined. Evidence has emerged that the E4 isoform of the apolipoprotein E (APOE) gene, which represents the most significant risk factor for sporadic AD, may also modulate its cognitive and structural phenotype. In two recent studies of patients with early AD, distinct patterns of gray matter (GM) atrophy occurred dependent on the APOE genotype, with carriers of the APOE E4 allele exhibiting medial temporal and occipital foci of atrophy, and non-carriers showing more pronounced frontoparietal volume loss [3, 4]. Moreover, memory deficits in carriers were observed in conjunction with predominant atrophy in medial temporal and other limbic regions, whereas more pronounced executive dysfunction in non-carriers was found to be concomitant with more severe atrophy in superior frontal gyrus [4]. Earlier studies of patients with AD have been less consistent regarding dissociable effects of the APOE-genotype on cognitive and structural phenotype [3, 5–7].

Less is known about the impact of the APOE-genotype on brain atrophy and cognitive deficits in patients with mild cognitive impairment (MCI) and subsequent conversion to AD. Patients with early or prodromal AD and a dysexecutive focus of cognitive impairment were recently found to show reduced frontoparietal cortical thickness compared to patients with memory-predominant deficits; moreover, non-carriers of the E4 allele were significantly overrepresented among patients with dysexecutive-predominant impairment. In contrast, hippocampal volume was not significantly associated with cognitive phenotype [8]. In a longitudinal magnetic resonance imaging (MRI) study using voxel-based morphometry (VBM), carriers of the APOE E4 allele with MCI developed atrophy in hippocampus, insula, temporal, and parietal cortex before converting to AD, while structural changes underlying the conversion to dementia in non-carriers did not become apparent [9].

The purpose of the current longitudinal VBM study was to conduct a combined analysis of cognitive and structural phenotype in AD patients prior to their conversion to dementia. We hypothesized that there would be a closer link between executive dysfunction and prefrontal atrophy in non-carriers of the APOE E4 allele than in carriers and that patterns of atrophy would differ according to APOE E4 carrier status.

MATERIAL AND METHODS

Subjects

One-hundred and thirty-one patients with MCI who remained clinically stable over a follow-up period of 2.1 ± 0.9 years (44 carriers of the APOE E4 allele and

87 non-carriers) and 63 patients with MCI who converted to AD within a follow-up period of 2.1 ± 0.7 years (34 carriers and 29 non-carriers) were included in the study. Data was used from a prospective multicenter trial conducted by the German Dementia Competence network [10]. Patients selected for the current study were recruited in nine German centers and fulfilled the criteria of MCI according to Petersen [11] and the International Working Group on Mild Cognitive Impairment [12]. Further inclusion criteria were clinical follow-up after a minimum of one year and the availability of at least a baseline set of neuropsychological test results as well as a baseline high resolution 3D T1-weighted sequence. Moreover, patients were only included after quality control of the MRI images, which consisted of a test of image homogeneity covariance and noise estimation (VBM8 toolbox: Gaser, http://dbm.neuro.uni-jena.de/author/admin/) as well as visual inspection. Exclusion criteria were stroke, motor symptoms associated with other neurodegenerative diseases such as Lewy body dementia, and cognitive impairment secondary to recognizable diseases such as head injury, multiple sclerosis, or normal pressure hydrocephalus. Subjects with clinically relevant depression, defined as a score of 4 or more on the depressive symptom subscale of the Neuropsychiatric Inventory [13] were also excluded. Eight patients with stable MCI and three patients who converted to AD had to be excluded because of motion or susceptibility artifacts. Characteristics of the remaining 131 stable MCI and 63 converting subjects are listed in Table 1. MCI was classified as amnestic or non-amnestic and the cutoff used to classify memory test performance as impaired was 1.5 SD below age norms [11, 12]. Fifty-eight MCI converters (92.1%) fulfilled the criteria of amnestic MCI (aMCI) and 5 had non-amnestic MCI (naMCI, 7.9%). In the group with stable MCI, there were 81 subjects with aMCI (61.8%) and 50 with naMCI (38.2%). Subgroups of 17 APOE E4 carriers and 17 non-carriers matched for age, gender, education, and clinical status (Clinical Dementia Rating [CDR] Scale [14]) were investigated longitudinally on MRI to examine structural correlates of their conversion to AD. Moreover, matched subgroups of 24 carriers and 24 non-carriers, who remained clinically stable at follow-up were analyzed longitudinally on MRI. Clinical evaluation of patients included a complete neurological and psychiatric evaluation. Cognitive status overall was assessed with the Mini Mental Status Examination (MMSE) [15] and the CDR scale during a semi-structured interview with the patient and caregiver. In addition to the global CDR score, the CDR sum of boxes (CDR SOB) [16] was determined by assigning a severity score in six domains (memory, orientation, judgment and problem solving, community affairs, home, and hobbies). Because global CDR scores in patients with MCI lack variability, we used the CDR SOB for group comparisons.

The study was approved by the Ethics Review Board of the Erlangen medical faculty (coordinating center) and by the Ethics Committees at each individual center. All subjects gave informed consent.

Neuropsychological testing

Psychometric tasks included immediate and delayed recall of word lists, the Boston Naming Test (BNT, test of word retrieval), drawing of increasingly complex figures (constructional praxis), and free recall of drawings from the cognitive battery designed by the Consortium to Establish a Registry for AD [17]. The MMSE [15] was used to assess the overall severity of cognitive impairment. Subjects were also tested with the Trail Making Test (TMT) B, which is sensitive to speed of information processing, mental flexibility and executive function.

Structural image parameters

MRI examinations were performed on 1.5 T whole body units. Siemens scanners (Siemens Magnetom Vision, Symphony or Sonata; Erlangen, Germany) were used at seven centers and Philips Scanners (Gyroscan and Intera; Eindhoven, Netherlands) at the remaining two centers. Scans were performed with a sagittal magnetization prepared rapid gradient echo (MPRAGE) sequence on the Siemens scanners and a 3D fast T1-weighted gradient echo sequence on the Philips scanners. Between the centers, the TR varied between 9.3 and 20 ms and the TE between 3.93 and 4.38 ms. The flip angle was approximately 15°, slice thickness 1–1.2 mm, matrix between 256×256 and 512×512, field of view between 250×250 and 300×300 mm.

Voxel-based morphometry with T1-weighted MRI

High-resolution T1-weighted images were processed according to the unified segmentation model [18] with SPM8 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm/) and Matlab 8b software (The Mathworks, MA, USA). This method involves an iterated scheme of bias correction, segmen-

Table 1
Baseline characteristics of patients with MCI who remained stable or converted to AD (subgroups analyzed longitudinally indicated in parentheses)

	Stable MCI patients		MCI patients converting to AD	
	APOE E4 Carriers $n = 44$ (24) Mean ± SD	APOE E4 Non-carriers $n = 87$ (24) Mean ± SD	APOE E4 Carriers $n = 34$ (17) Mean ± SD	APOE E4 Non-carriers $n = 29$ (17) Mean ± SD
Age (years)[3]	66.7 ± 7.9 (68.4 ± 7.2)	65.1 ± 8.1 (69.9 ± 6.7)	69.4 ± 7.5 (69.8 ± 6.5)	69.5 ± 8.6 (69.2 ± 8.1)
Gender (men/women)	27/17 (13/11)	45/42 (13/11)	15/19 (9/8)	14/15 (9/8)
Education (y)	9.1 ± 1.6 (9.2 ± 1.6)	9.6 ± 2.3 (9.3 ± 2.1)	9.3 ± 2.1 (9.2 ± 2.4)	9.1 ± 1.9 (9.2 ± 2.0)
Time to conversion and follow-up MRI (y)	N/A	N/A	1.3 ± 0.5 (1.6 ± 0.6)	1.5 ± 0.6 (1.4 ± 0.6)
Clinical follow-up period (y)	2.0 ± 0.8 (1.8 ± 0.8)	2.1 ± 0.9 (2.2 ± 0.9)	2.1 ± 0.7 (2.1 ± 0.7)	2.1 ± 0.7 (2.2 ± 0.7)
CDR SOB at baseline[2,3]	1.4 ± 0.9 (1.6 ± 0.9)	1.3 ± 0.9 (1.5 ± 1.0)	2.0 ± 1.0 (2.1 ± 1.1)	2.4 ± 1.1 (2.3 ± 1.0)
MMSE (score)[3]	27.5 ± 2.0 (27.5 ± 2.1)	27.8 ± 1.8 (27.6 ± 1.7)	26.9 ± 1.9 (26.9 ± 2.0)	25.7 ± 2.3 (25.7 ± 2.2)
Delayed word recall (score) [2,3]	5.6 ± 2.2 (5.7 ± 2.2)	5.4 ± 2.1 (5.2 ± 2.0)	3.7 ± 2.0 (3.3 ± 1.7)	2.8 ± 2.1 (2.2 ± 1.6)
Verbal learning[2,3]	17.6 ± 3.5 (18.1 ± 4.1)	17.4 ± 3.4 (16.5 ± 3.4)	14.9 ± 4.2 (15.2 ± 2.9)	13.9 ± 5.2 (14.1 ± 2.0)
Trail Making Test B (s)[3]	157.8 ± 67.8 (160.3 ± 69.7)	140.0 ± 58.0 (154.2 ± 56.5)	168.1 ± 59.2 (154.2 ± 60.5)	**198.3 ± 53.3 (199.9 ± 49.8)**[1]
Constructive Praxia	10.2 ± 1.2 (10.4 ± 1.1)	10.0 ± 1.3 (9.8 ± 1.4)	9.4 ± 2.4 (9.4 ± 1.8)	9.5 ± 1.5 (9.7 ± 1.4)
Boston Naming Test	13.8 ± 1.4 (13.8 ± 1.2)	13.6 ± 1.8 (12.8 ± 2.5)	13.9 ± 2.4 (14.1 ± 2.0)	13.4 ± 1.5 (13.7 ± 1.9)

[1]Significantly lower performance in non-carriers that converted to AD than in carriers indicated in bold print ($p < 0.05$); [2]Predictors of conversion to AD in carriers with MCI; [3]Predictors of conversion to AD in non-carriers with MCI.

tation into white matter (WM), GM, and cerebrospinal fluid and warping of prior images in stereotactic space to the data, which is repeated until no significant change occurs anymore. During normalization, images were interpolated to isotropic $1 \times 1 \times 1$ mm voxels. The VBM8-toolbox extends this model with a partial volume estimation to account for partial volume effects and the application of a spatially adaptive nonlocal Means (SANLM) filter [19] for bias-correction. Normalization to stereotactic space consisted of a linear affine registration and a linear deformation corresponding to a high-dimensional DARTEL normalization [20] implemented in VBM8. The resulting gray matter probability maps were modulated, i.e., intensity-corrected for local volume changes during normalization, to make them more sensitive to the distribution of GM and WM volume. The modulated GM maps were smoothed with a 12-mm FWHM kernel. For the longitudinal analyses, preprocessing of T1-weighted images was conducted as implemented in the longitudinal preprocessing tool of the VBM8 toolbox. First, the follow-up MRI was registered to the baseline image for each subject. Then the realigned follow-up images were corrected for signal inhomogeneities with regard to the baseline image. Subsequently, spatial normalization was estimated using segmentations of the baseline image and applied to the follow-up image.

APOE E4 genotyping

Leukocyte DNA was isolated with the Qiagen blood isolation kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany). The APOE genotype was determined with restriction isotyping by gene amplification and HhaI cleavage as described by Hixson and Vernier [21]. Carriers of at least one APOE E4 allele were compared to non-carriers.

Statistical analysis

Statistical analysis of clinical, neuropsychological, and genetic data

Main effects of conversion and APOE carrier status on structural and neuropsychological measures were examined with a univariate analysis of variance model including age, gender, and education level as covariates of no interest. A partial correlation analysis was conducted to investigate associations between neuropsychological performance and global GM volume; because of potential center-effects on MR measures, centers were included as covariates in addition to age, gender, and education (SPSS for Windows, Version 18.0.1, 2009. Chicago: SPSS Inc.).

Statistical analysis of regional GM volume

We used SPM8 to investigate group differences (with one-way ANOVA) and perform multiple regression analyses (corrected for age, gender, education level, total intracranial volume, and site) on a voxel-by-voxel basis. To evaluate the degree of GM atrophy in APOE E4 allele carriers and non-carriers who converted to AD, we performed a baseline comparison of each APOE-subgroup with the entire group of stable MCI patients; we also compared converting carriers and non-carriers cross-sectionally at baseline and follow-up. To examine the progression of GM atrophy between baseline and follow-up, we performed paired *t*-tests for each individual subgroup (carriers and non-carriers with or without progression to AD). Finally, to investigate the relationship between GM atrophy pattern and executive dysfunction, we correlated performance on the TMT-B with GM volume at baseline. Effects were reported as significant when whole-brain cluster-level family-wise error (FWE)-corrected $p < 0.05$. To adjust for variations in local smoothness of the probability maps, we conducted a correction for non-stationarity with SPM8. Additional voxel-based region-of-interest (ROI) analyses were performed for the hippocampus, which is known to be affected at early stages of AD and to be essential for memory consolidation, as well as for regions previously shown to be differentially affected in APOE E4 carriers and non-carriers converting to AD, i.e., medial occipital cortex, superior frontal, and posterior parietal cortex at $p < 0.05$ voxel-level FWE-corrected [3, 4, 22]. FWE-corrections based on Gaussian random field theory were applied as implemented in SPM8. Masks were created with the Harvard Oxford probabilistic atlas of human cortical and subcortical areas [23]. A medial occipital mask was created by adding individual masks of occipital pole, cuneus and lingual gyrus; masks of superior and inferior parietal lobule were combined to obtain a mask of posterior parietal cortex.

RESULTS

Baseline analyses

Comparison of carriers and non-carriers of the APOE E4 allele regarding clinical status and neuropsychological test results

The TMT-B was the only neuropsychological test that indicated a significant difference between

carriers and non-carriers subsequently converting to AD [F(1, 57) = 4.5, $p < 0.05$]. Tests of verbal learning [F(1, 57) = 0.59, $p = 0.45$], verbal delayed recall [F(1, 57) = 3.4, $p = 0.07$], constructive praxia [F(1, 57) = 0.27, $p = 0.60$], BNT [F(= 1.4, $p = 0.24$], MMSE [F = (1, 57) = 3.9, $p = 0.06$], CDR SOB [F = (1, 57) = 1.6, $p = 0.21$], and age [F(1, 58) = 0.02, $p = 0.88$] did not reveal significant differences. Results were similar for the subgroup comparison of E4 carriers and non-carriers who were scanned longitudinally on MRI [TMT B: F(1, 36) = 5.4, $p = 0.027$; verbal learning: F(1, 36) = 0.86, $p = 0.36$; verbal delayed recall: F(1, 36) = 4.0, $p = 0.06$; constructive praxia: F(1, 36) = 0.56, $p = 0.46$; BNT: F(1, 36) = 0.51, $p = 0.48$; MMSE: F(1, 36) = 2.9, $p = 0.10$; CDR SOB: F(1, 36) = 1.4, $p = 0.71$; age: F(1, 37) = 0.05, $p = 0.82$]. Within the group of stable MCI patients, there were no significant APOE-dependent effects on neuropsychological test performance.

Predictive factors of conversion included age, APOE E4 carrier status, aMCI, CDR-SOB, and neuropsychological test performance and are reported in detail in Wagner et al. (unpublished results). Of note, performance on the TMT B predicted conversion to AD only in non-carriers of the APOE E4 allele, whereas performance on tests of verbal learning and delayed recall predicted conversion in E4 carriers and non-carriers.

Group differences in GM volume

The subgroups of converting carriers ($n = 34$) and non-carriers ($n = 29$) of the APOE E4 allele showed a similar pattern of GM atrophy compared to stable MCI patients ($n = 131$) at baseline. In both groups of converters, reduced volume was measured in bilateral hippocampi and insula as well as superior frontal gyrus, lateral temporal, and lateral occipital cortex. Carriers also showed decreased volume in medial occipital cortex, whereas non-carriers exhibited atrophy in precuneus and bilateral posterior parietal cortex, which was not apparent in the other subgroup, respectively (Table 2, Fig. 1). However, a direct cross-sectional comparison of the converting APOE subgroups revealed no significant differences. We also compared baseline GM volumes of the APOE E4 allele carriers and non-carriers, who were scanned at least twice and were included in a longitudinal MRI analysis ($n = 17$ in each APOE subgroup). The baseline comparison again did not reveal differences. There were no GM volume differences between stable MCI carriers and non-carriers of the E4 allele at baseline or follow-up.

Table 2
Cross-sectional group comparison of gray matter (GM) volume: stable MCI group ($n = 131$) versus converting APOE E4 carriers ($n = 34$) and non-carriers ($n = 29$) at baseline

Location	MNI coordinates			Z-value	p corrected cluster-level
	x	y	z		FWE-corrected
GM volume in stable MCI patients > GM volume in converting APOE E4 carriers					
Left superior frontal gyrus	−16	48	28	4.07	0.02
Right superior frontal gyrus	3	54	21	4.14	0.02
Left insula	−38	−18	−8	3.86	0.001
Right insula	39	−22	−8	4.79	0.001
Left hippocampus	−26	−18	−8	4.63	0.001
Right hippocampus	36	−22	−6	4.88	0.001
Left lateral temporal cortex	−36	−84	16	4.30	0.001
Right lateral temporal cortex	58	−16	−15	3.84	0.001
Left median occipital cortex	−16	−94	−3	4.63	0.001
Left lateral occipital cortex	−33	−88	−13	4.53	0.001
GM volume in stable MCI patients > GM volume in converting APOE E4 non-carriers					
Right superior frontal gyrus	8	52	4	3.78	0.04
Left insula	−40	−22	−8	3.46	0.001
Right insula	40	−22	−8	4.17	0.001
Left hippocampus	−34	−13	−21	4.19	0.001
Right hippocampus	38	−12	−18	4.64	0.001
Left lateral temporal cortex	−46	−37	−6	3.85	0.001
Right lateral temporal cortex	45	−60	10	4.69	0.001
Left inferior parietal lobule	−39	−51	42	3.62	0.01
Right supramarginal gyrus	54	−33	24	3.74	0.01
Left precuneus	−10	−60	27	4.47	0.001
Right precuneus	4	−60	25	4.28	0.001
Right lateral occipital cortex	45	−60	10	4.69	0.001

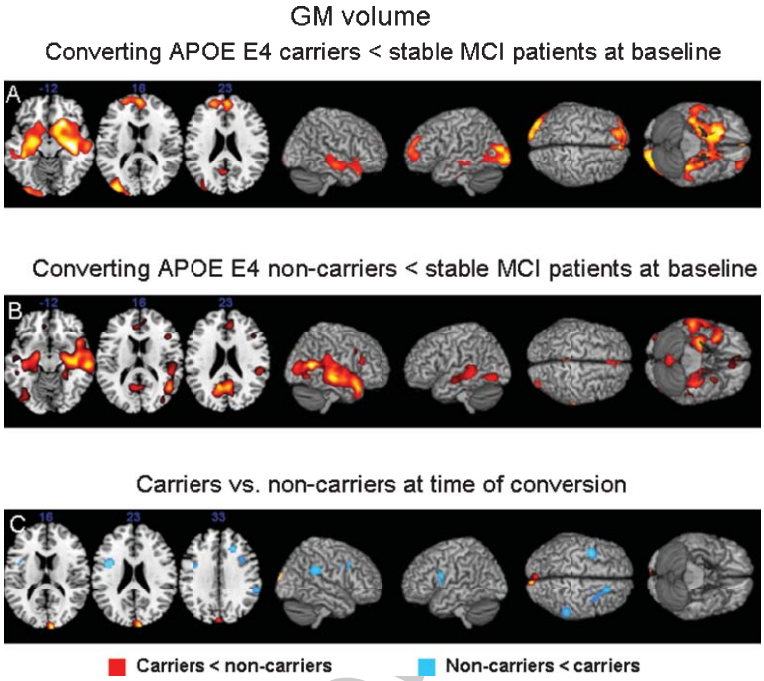

Fig. 1. Cross-sectional analysis at baseline (A and B) and follow-up (time of conversion [C]): converting APOE E4 carriers (A) and non-carriers (B) showed reduced GM volume compared to stable MCI patients in medial temporal and neocortical regions. There were no significant differences in GM volume between converting carriers and non-carriers at baseline; however, at time of conversion, carriers exhibited more occipital atrophy (red), while non-carriers showed more frontoparietal volume loss (blue, C). Results are presented at $p < 0.001$ whole-brain uncorrected.

Correlations between GM volume and cognitive performance

In non-carriers of the APOE E4 allele who converted to AD, TMT-B performance correlated with global GM volume ($r = -0.60$, $p < 0.01$). The voxel-based analysis indicated that performance on the TMT-B correlated with GM volume in bilateral superior frontal gyrus and left middle frontal gyrus. There were no correlations between GM volume and results on the TMT-B in carriers converting to AD or in the subgroups of stable carriers and non-carriers. To investigate whether the correlation was driven by patients with low MMSE scores, we performed a separate *post-hoc* analysis of a subgroup of converting non-carriers with a MMSE score of ≥ 26 ($n = 19$). The mean MMSE score of this subgroup (27.0 ± 1.1) matched the mean MMSE score in the group of carriers of the APOE E4 allele (26.9 ± 1.9). A correlation between TMT-B performance and global GM volume also occurred in this subgroup with high MMSE performance ($r = -0.63$, $p = 0.028$). A voxel-based analysis revealed significant correlations with prefrontal cortex volume in this subgroup comparable to the group results for converting non-carriers overall (Table 5, Fig. 3).

Analysis at follow-up (cross-sectional group differences in GM volume)

A cross-sectional comparison of APOE E4 allele carriers and non-carriers converting to AD at follow-up ($n = 17$ in each subgroup) indicated more atrophy in medial occipital cortex in carriers and more pronounced frontoparietal atrophy in non-carriers (Table 3, Fig. 1).

Longitudinal analysis (progression of atrophy)

Between baseline and follow-up, carriers of the APOE E4 allele converting to AD showed a progression in GM atrophy in left hippocampus, right parahippocampal gyrus, bilateral anterior cingulate cortex, left midcingulate cortex, bilateral posterior cingulate cortex, left lateral temporal and posterior parietal cortex, and right superior occipital cortex, whereas non-carriers showed a loss in GM volume in right hippocampus, bilateral superior frontal gyrus, left inferior frontal gyrus, left insula, left inferior temporal gyrus, and left posterior parietal cortex. Within the group of stable MCI patients, carriers of the APOE E4 allele developed GM atrophy in bilateral

Table 3
Cross-sectional comparison of gray matter (GM) volume in APOE E4 carriers and non-carriers converting to dementia at follow-up*

Location	MNI coordinates			Z-value	*p* corrected voxel-level FWE-corrected (ROI analysis)
	x	y	z		
Subgroup analysis of patients with follow-up MRI					
GM volume in carriers (n = 17) < GM volume in non-carriers (n = 17)					
Right medial occipital cortex	9	−97	19	3.63	0.02
GM volume in non-carriers (n = 17) < GM volume in carriers (n = 17)					
Right superior frontal gyrus	20	30	20	3.87	0.03
Right posterior parietal cortex	58	−39	30	3.61	0.05

*No significant baseline differences in GM volume between APOE E4 carriers ($n = 34$) and non-carriers ($n = 29$) overall or between subgroups followed longitudinally (17 carriers and 17 non-carriers).

Table 4
Progression of gray matter atrophy between baseline and follow-up

Location	MNI coordinates			Z-value	*p* corrected cluster-level FWE-corrected*
	x	y	z		
Subgroup analysis of patients converting to dementia with follow-up MRI					
Carriers (n = 17)					
Left hippocampus	−32	−21	−11	3.52	0.01*
Right parahippocampal gyrus	24	0	−35	3.88	0.05
Left anterior cingulate cortex	−9	32	18	3.91	0.01
Right anterior cingulate cortex	6	39	16	3.74	0.01
Left middle cingulate cortex	−8	−9	42	4.42	0.01
Left posterior cingulate cortex	−4	−49	30	3.89	0.01
Right posterior cingulate cortex	3	−36	40	4.14	0.01
Left middle temporal gyrus	−57	−39	1	3.88	0.03
Left inferior parietal lobule	−46	−55	42	4.09	0.04
Right superior occipital gyrus	33	−73	43	3.89	0.01
Non-carriers (n = 17)					
Left superior frontal gyrus	−4	44	22	3.70	0.03*
Right superior frontal gyrus	3	35	31	3.51	0.03*
Left inferior frontal gyrus	−36	11	16	3.79	0.01
Left insula	−24	8	9	3.65	0.01
Right hippocampus	28	−9	−18	3.41	0.02*
Left inferior temporal gyrus	−60	−48	−15	3.91	0.01
Left supramarginal gyrus	−57	−37	15	3.84	0.01
Subgroup analysis of stable MCI-patients with follow-up MRI					
Carriers (n = 24)					
Left hippocampus	−32	−28	−8	3.40	0.02*
Right hippocampus	34	−25	−8	3.37	0.02*
Left parahippocampal gyrus	−26	2	−33	4.05	0.01
Left middle temporal gyrus	−42	−60	10	4.19	0.01
Right middle temporal gyrus	40	−63	4	3.90	0.04
Left medial occipital cortex	−9	−94	−3	4.33	0.02
Right medial occipital cortex	3	−87	4	3.88	0.02
Non-carriers (n = 24)					
Right hippocampus	33	−31	−12	3.30	0.03*
Right temporal pole	38	6	−27	4.13	0.01
Right insula	33	9	−13	4.12	0.01
Right inferior frontal gyrus	39	36	−8	3.87	0.01

*Significant at voxel-level FWE-corrected in ROI.

hippocampus, left parahippocampal gyrus, bilateral middle temporal gyrus, and bilateral medial occipital cortex. Non-carriers showed a loss of GM volume in right hippocampus, insula, inferior frontal gyrus and lateral temporal cortex (Table 4, Fig. 2).

DISCUSSION

The current study provides evidence for APOE genotype-dependent patterns of GM atrophy during conversion from MCI to AD, consistent with

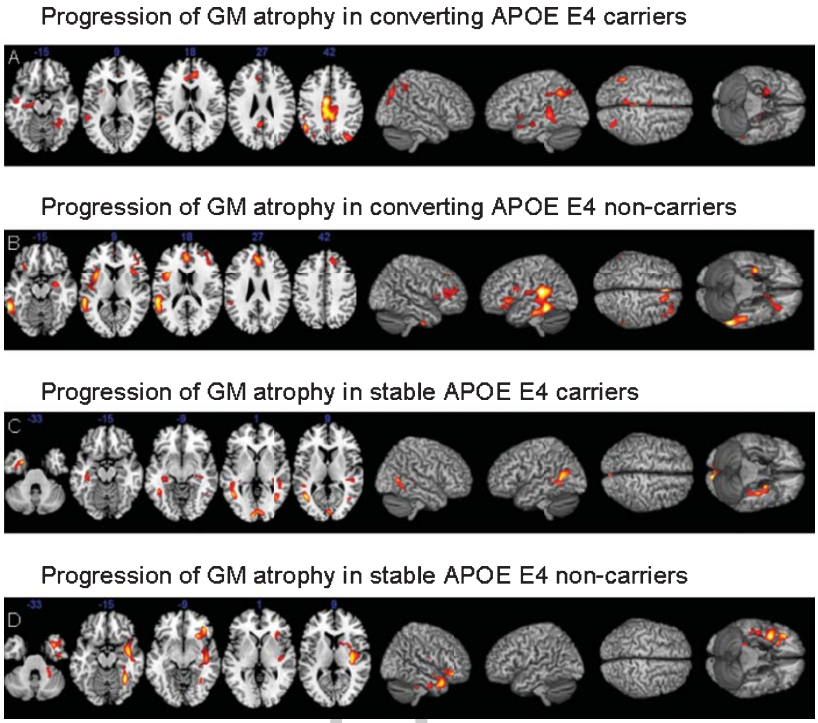

Fig. 2. Longitudinal analysis: converting APOE E4 carriers (A) showed significant progression of GM atrophy in left hippocampus, bilateral cingulate cortex, left lateral temporoparietal and right occipital cortex, while non-carriers developed GM volume loss in superior frontal gyrus, left inferior frontal gyrus and insula, right hippocampus and left temporoparietal cortex during conversion to AD (B). Progression in stable MCI carriers (C) partially occurred in the same regions as in converting MCI patients, specifically in medial and lateral temporal cortex; medial occipital cortex also showed volume loss. Of note, changes in volume did not occur in frontal and parietal cortex. Stable non-carriers developed atrophy in medial and lateral temporal cortex as well as in right insula/inferior frontal gyrus (D). Results are presented at $p < 0.001$ whole-brain uncorrected.

Table 5
Baseline correlations between gray matter volume and performance on the Trail Making Test (TMT)-B*

Location	MNI coordinates			Z-value	p corrected cluster-level
	x	y	z		FWE-corrected
Non-carriers converting to dementia (n = 29)					
Left superior frontal gyrus	−14	51	19	4.24	0.01
Right superior frontal gyrus	28	50	10	3.80	0.04
Left middle frontal gyrus	−28	23	36	4.13	0.04
Subgroup of non-carriers converting to dementia with high MMSE ≥26 (n = 19)					
Left superior frontal gyrus	−24	29	27	4.33	0.01
Right superior frontal gyrus	24	45	28	4.09	0.01

*No correlations between GM volume and TMT-B performance in carriers or non-carriers with stable MCI; no correlations between GM volume and TMT-B performance in carriers converting to dementia.

findings in more advanced AD patients. While APOE-dependent differences in GM volume were not evident at baseline, a dissociation became apparent at the time of conversion between more pronounced occipital atrophy in carriers and more accentuated frontoparietal atrophy in non-carriers. These differences are in line with recent cross-sectional MR findings in carriers and non-carriers of the APOE E4 allele with established AD and with histopathological evidence for occipital tissue changes in carriers of the E4 allele with AD [3, 4, 22].

Between baseline and the time of conversion, carriers of the APOE E4 allele showed cingulate and occipital atrophy, whereas progression in non-carriers was marked by atrophy in prefrontal cortex and insula; temporal and parietal atrophy occurred in both groups.

The significance of prefrontal atrophy for cognitive deterioration in converting non-carriers was under-

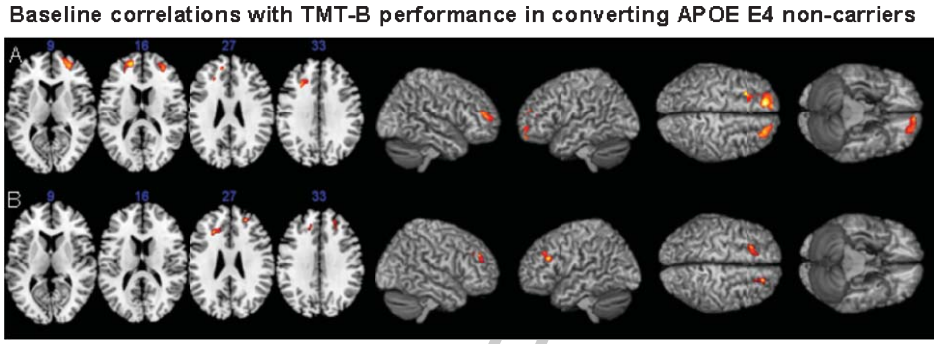

Fig. 3. Baseline analysis: correlations between TMT B performance and bilateral prefrontal volume occurred overall in the group of converting APOE E4 non-carriers (A), as well as in the subgroup of converting non-carriers with high MMSE scores (B). Results are presented at $p < 0.001$ whole-brain uncorrected.

scored by the baseline correlation between executive function and prefrontal GM volume in this subgroup. Executive function, in turn, predicted conversion only among non-carriers of the APOE E4 allele. The association between executive dysfunction and prefrontal damage is in line with a previously reported preponderance of APOE E4 non-carriers among AD patients with a dysexecutive-predominant subtype of cognitive impairment and a frontoparietal focus of atrophy [8].

Impact of APOE on AD phenotype

The APOE-genotype related patterns of GM atrophy in AD patients during conversion to dementia, including the association between executive dysfunction and prefrontal atrophy in APOE E4 non-carriers, are compatible with patterns of cognitive impairment and brain atrophy found in patients with established AD [4]. The mechanisms underlying these patterns remain unclear, however. The E4 isoform of the APOE gene constitutes the most important genetic risk factor in sporadic AD. While APOE functions as a carrier protein for lipids and contributes to the maintenance and repair of cell membranes, the E4 isoform promotes the deposition of amyloid-β (Aβ) and neurofibrillary tangles [24]. Specifically, the E4 isoform has been shown to be less efficient in clearing Aβ than E2 and E3 [25]. Other evidence suggests that APOE function contributes to the selective vulnerability of brain regions to AD pathology by modulating neuronal activity, which in turn has been found to correlate with local Aβ aggregation [26]. Brain regions with a high resting state metabolism, known as the default network, show elevated levels of amyloid deposition [27]. In young cognitively normal carriers of the APOE E4 allele, abnormally high default network neuronal activity has been detected and may constitute a harbinger of AD, decades prior to its clinical expression [28].

Additional modulating factors likely to determine the APOE-dependent regional specificity of brain atrophy in AD, are the detrimental impact of the E4 isoform on synaptic plasticity and APOE-related developmental effects on fiber tract integrity [29–31]. Moreover, several genetic risk factors besides the APOE E4 isoform have been identified in genome-wide association studies; their effects on AD structural phenotypes have recently begun to be investigated [32].

Progression of atrophy in MCI converters

MCI converters overall showed reduced GM volume in medial temporal and neocortical regions known to be affected in AD compared to stable MCI patients. A pattern of significantly greater global and medial temporal lobe-specific atrophy in MCI converters than in stable MCI patients has also been described in the ADNI cohort as well as in several previously investigated samples [33–41].

The widespread progression of atrophy in medial temporal structures and neocortical regions observed during the relatively short follow-up period in the current group of converters is in accord with an acceleration of GM atrophy recently described within the last year before conversion [42]. Consistent with previous evidence for a strong predictive value of hippocampal volume loss regarding the time of AD diagnosis, the conversion to dementia was accompanied by a progression in hippocampal atrophy in both APOE-groups [9, 43]. Contrary to previous findings on patients with AD [4], converters with the APOE E4 allele did not show more pronounced hippocampal atrophy than non-carriers in the present study. Memory impairment associated with hippocampal damage may have been

crucial to the diagnosis of AD in the current group of patients. Alternatively, a divergence in the progression of hippocampal atrophy may not become apparent until more advanced stages of AD. Despite the absence of APOE-dependent regional differences in GM volume among MCI converters at baseline, and CDR-SOB scores that were comparable between converting APOE-subgroups in the present study as well as with scores of MCI converters in similar studies [9, 36, 42], stage-related effects cannot entirely be excluded. Of note, MMSE scores in the subgroup of converting non-carriers were relatively low. To investigate whether the correlation between executive function and prefrontal volume among non-carriers converting to AD was driven by patients with potentially more advanced pathology, we conducted a *post-hoc* correlation analysis of non-carriers with a minimum MMSE score of 26 points, generally considered within the pre-clinical range with a high degree of confidence [44]. The mean MMSE in this subgroup of non-carriers matched the mean MMSE in the group of converting carriers. Baseline correlations of executive performance with global and prefrontal GM volume were similarly strong as among non-carriers overall, consistent with an effect specific to APOE-status rather than to stage.

Cognitive decline and GM atrophy

In carriers of the APOE E4 allele, the conversion to AD was characterized by a pattern of cingulate as well as temporal, parietal, and occipital atrophy. In addition to hippocampal tissue changes, atrophy of the cingulate cortex has been identified as predictor of rapid conversion [35]. Moreover, cognitive decline in early AD patients has been found to be most strongly linked to concomitant atrophy in left cingulate, temporal, and parietal cortex [45]. In contrast, atrophy underlying conversion in non-carriers occurred in temporoparietal cortex as well as in prefrontal cortex and insula, in accord with earlier findings of a frontoparietal focus of atrophy in APOE E4 non-carriers with AD [3, 4]. The fact that prefrontal atrophy correlated with performance on the TMT-B, a test of executive function, in this subgroup, is in accord with the involvement of medial and dorsolateral prefrontal cortex demanded by this task [46].

GM atrophy in stable MCI patients

Loss of GM volume occurred in all subgroups over the relatively short follow-up period, i.e., in converting as well as clinically stable E4 carriers and non-carriers. On the one hand, participants in the stable MCI group may actually have had AD and converted after the follow-up phase. This would explain similarities in the progression pattern between converting and stable APOE E4 carriers, such as the involvement of medial occipital cortex, which has previously been identified in E4 carriers with AD and is not typical of age-related volume loss [3, 4, 47].

On the other hand, significant and typically distributed GM volume loss, in part attributable to cell shrinkage and changes in perfusion, fat, and water content [45, 48], is known to occur with aging over periods as short as one year [47, 49]. Areas vulnerable to extensive aging-related changes are medial temporal structures, other subcortical regions, lateral temporal and prefrontal cortex [47]. Age was included as covariate in statistical analyses. Because age constitutes a risk factor for AD, its effects are, however, not easily differentiated from AD-associated changes.

Limitations

Because MR images were collected at different centers, it is possible that heterogeneity of MR hardware and protocols reduced the sensitivity for volume effects. To control for center effects, center affiliations were included as covariates. Though multicenter pooling of MR data obtained with different scanner types and protocols for VBM analyses has been found to produce an additive average gain in power to detect group differences, some brain regions, specifically along the midsagittal plane, may be more vulnerable to differences in scanning parameters [50]. Another limitation is that the sample analyzed longitudinally was relatively small; thus the longitudinal findings should be confirmed in a larger investigation, which may indicate additional small volume effects. Furthermore, the follow-up period was comparatively short; thus the stable MCI group presumably included subjects who converted to AD later as well as subjects with cognitive impairment unrelated to AD.

CONCLUSIONS

The current findings suggest that in APOE E4 non-carriers with MCI, executive dysfunction is closely linked to frontal GM atrophy and predictive of conversion to AD. Moreover, the APOE-dependent dissociation found between more pronounced occipital atrophy in carriers and more frontoparietal atrophy in non-carriers converting to dementia is in accord with previous findings in patients with more advanced AD.

An earlier detection of distinct subtypes of disease may ultimately facilitate more specific diagnostic procedures and treatment. Future investigations including measures of vascular risk and WM damage as well as other genetic risk factors associated with AD will further contribute to an understanding of the heterogeneity of the disease.

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