# Cerebrospinal Fluid Markers for Alzheimer's Disease over the Lifespan: Effects of Age and the APOEε4 Genotype

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**Abstract.** In Alzheimer's disease (AD), the cerebral pathological changes begin many years before the clinical manifestation of the disease. Biomarkers for AD, such as the cerebrospinal fluid (CSF) concentrations of amyloid-$\beta_{1-42}$ ($A\beta_{1-42}$) and tau phosphorylated at threonine 181 (pTau181), may reflect these cerebral changes relatively early. Accordingly, cognitively healthy subjects at risk for AD often have altered CSF concentrations of $A\beta_{1-42}$ and pTau181. In this study, we assessed the effects and interaction of two strong risk factors for AD, aging and the presence of the APOEε4 allele, on the CSF $A\beta_{1-42}$ and pTau181 concentrations in 280 adults with normal cognition across the lifespan. For comparison, we further included 152 patients with probable AD. We found significant effects of age on the CSF $A\beta_{1-42}$ and pTau181, and of the APOEε4 genotype on the $A\beta_{1-42}$ levels in the cognitively normal participants. Carrying the APOEε4 allele was associated with a significant decrease of the $A\beta_{1-42}$ concentrations in middle-aged and older participants. In the group of participants with AD, the $A\beta_{1-42}$ levels were significantly lower in the APOEε4 carriers compared to the non-carriers. These findings demonstrate significant age effects on the CSF $A\beta_{1-42}$ and pTau181 across lifespan. They also suggest that the decrease of $A\beta_{1-42}$, but not the increase of pTau181 CSF levels is accelerated by the APOEε4 genotype in middle-aged and older adults with normal cognition.

Keywords: Alzheimer's disease, amyloid-$\beta$, APOEε4, cerebrospinal fluid, hyperphosphorylated tau, normal aging

## INTRODUCTION

Sporadic Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Neuropathological changes, such as the progressive deposition of amyloid-$\beta$ protein ($A\beta$) and the formation of neurofibrillary tangles consisting primarily of hyperphosphorylated tau protein (pTau), begin many years previous to the clinical manifestation of the disease. Related alter-

ations of cerebrospinal fluid (CSF) $A\beta_{1-42}$ and pTau concentrations can already be detected in asymptomatic older subjects at risk for AD [1,2], and predict cognitive decline in cognitively healthy older adults [3,4] and in subjects with mild cognitive impairment [5,6].

The presence of the apolipoprotein ε4 (APOEε4) allele is a well-established genetic risk factor for AD. Carrying the APOEε4 allele may accelerate the pathophysiological process [7] and lower the age of clinical onset of the disease [8,9]. Some postmortem studies [10,11], but not all [12], have found associations of the APOEε4 genotype with higher levels of amyloid plaque deposition and neurofibrillary pathology. Studies on the associations of CSF biomarker levels with

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the APOEε4 genotype in patients with AD produced conflicting results [11–15].

Effects of age and the APOEε4 genotype may play an important role for the cerebral amyloid deposition and pTau accumulation early in life, and this may be reflected by changes in the CSF levels of $A\beta_{1-42}$ and pTau. In older cognitively normal subjects, a few studies have investigated the associations between the APOEε4 genotype and the CSF levels of $A\beta_{1-42}$ and total tau [1,14], tau phosphorylated at threonine 231 (pTau231) [16], or tau phosphorylated at threonine 181 (pTau181) [15]. A study including a large number of younger and older participants without cognitive impairment focused on the effects of age and the APOEε4 genotype on $A\beta$ proteins only, and did not address possible effects on pTau CSF concentrations [17]. Some of these studies found lower levels of $A\beta_{1-42}$ and higher levels of total tau or pTau in APOEε4 carriers, others reported no differences between APOEε4 carriers and non-carriers.

To evaluate the effects and interactions of age and the presence of the APOEε4 allele on CSF biomarkers for both amyloid and neurofibrillary pathology across a broad age range, we measured the concentrations of $A\beta_{1-42}$ and pTau181, and determined the APOE genotype in a large cohort of subjects with normal cognition, beginning in the young adulthood. In addition, we investigated the effects of age and the APOEε4 genotype on the pTau181/$A\beta_{1-42}$ ratio, as this ratio may be a more specific indicator of cerebral AD pathology than either CSF concentration alone, and has been shown to predict cognitive decline in nondemented older adults [3]. For comparison, we further addressed these relationships in a cohort of patients with clinically diagnosed sporadic AD.

## METHODS

### Patients

Two hundred and eighty participants without cognitive impairment (118 women and 162 men, aged 16–89 years) and one hundred and fifty two patients with AD (91 women and 61 men, aged 55–92 years) were included into the study. The participants without cognitive impairment were healthy volunteers or subjects with disorders not affecting the central nervous system (CNS) (CON, $n = 139$), or patients with affections associated with pathological conditions of the CNS (ND, $n = 141$) referred to the Department of Neurology, University of Bonn for the evaluation or exclusion of neurological disorders. The CON subgroup included ninety-three participants with disorders not associated with pathological conditions of the CNS, like tension headache, peripheral cranial nerve palsy, peripheral neuropathy, myasthenia, and myalgia. The subgroup of participants with disorders affecting the CNS consisted of patients with spinal stenosis ($n = 67$), idiopathic intracranial hypertension ($n = 16$), encephalitis/myelitis ($n = 14$), seizures ($n = 8$), cerebral ischemia ($n = 8$), motor neuron disease ($n = 7$), lymphoma ($n = 3$), spinal cord infarction ($n = 2$), cerebellar ataxia ($n = 2$), and cerebral lesions, encephalopathy or spinal cord atrophy of unknown etiology ($n = 12$). All participants underwent detailed clinical evaluation that consisted of medical history, physical and neurological examination, laboratory tests including CSF routine analysis, and neuropsychological assessment. The cognitive function of all participants in this group was assessed with the Mini-Mental State Examination (MMSE) [18]. To exclude beginning cognitive decline, the participants aged 50 years or older additionally received extended clinical evaluation and, in most cases, a comprehensive neuropsychological assessment. The included participants had MMSE scores ≥ 26, and no signs or symptoms suggesting cognitive decline. Usually, structural imaging of the brain was performed by magnetic resonance tomography, or, in some cases, by computed tomography.

The 152 study participants with AD were referred to the Memory Clinic, at the Department of Psychiatry, University of Bonn, for investigation of their cognitive complaints. They met clinical diagnostic criteria for probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and Related Disorders Association [19], and DSM-IV criteria for dementia of the Alzheimer type. The diagnosis of AD was based on comprehensive neuropsychological and clinical evaluation and was made by a consensus conference of psychiatrists and neuropsychologists prior to the CSF analysis. In this group, the MMSE score of one patient was 29, and those of two other study participants were 28. However, extended neuropsychological tests revealed distinct impairment in several cognitive domains – including the declarative memory – and information about impaired daily functioning provided by the patients and their relatives confirmed the diagnosis of dementia. The presence of relevant vascular cerebral lesions was excluded for all study participants with AD by computed tomography or magnetic resonance tomography.

The study was approved by the local ethics committee. Written informed consent was obtained from all study participants or their legal representatives.

## CSF collection and analysis

Diagnostic lumbar punctures were performed at the Departments of Neurology or Psychiatry, University of Bonn. A standardized technique with a 22G "atraumatical" spinal needle and a sitting or lying position for the patient was applied. The CSF samples were kept on ice for a maximum of 1 h until being centrifuged for 10 min at 2000 g at 4°C. Samples were aliquoted to 0.25 ml and were stored in polypropylene tubes at −80°C until assay procedures.

Quantitative assessment of serum and CSF albumin was performed during the clinical routine. CSF $A\beta_{1-42}$ and pTau181 concentrations were measured by ELISA using commercially available assays (Innogenetics, Gent, Belgium) and according to the protocols described earlier [20].

## APOE genotyping

Leukocyte genomic DNA was isolated from 10 ml EDTA blood with the Qiagen blood isolation kit (Qiagen, Hilden, Germany) and the APOE genotype was determined.

## Statistical analysis

The distribution of the biomarker data was analyzed using the Kolmogorov-Smirnov test. Not normally distributed variables were log-transformed to approach Gaussian normal distribution.

First, we compared the $A\beta_{1-42}$ and pTau181 concentrations as well as the pTau181/$A\beta_{1-42}$ ratios between the cognitively normal subjects and the AD group with univariate analysis of covariance (ANCOVA) with the diagnostic group, the presence or absence of the APOE ε4 allele and gender as independent factor, and age as covariat.

To evaluate homogeneity of the group of participants with normal cognition, demographic information (age, gender), the presence of APOEε4 allele, MMSE score, CSF/serum albumin ratio, CSF concentrations of $A\beta_{1-42}$ and pTau181, and the pTau181/$A\beta_{1-42}$ ratio were compared between the CON and ND subgroups. In addition, potential subgroup differences between the APOEε4 allele carriers and non-carriers were explored. Pearson's $\chi^2$ test was used for gender and the presence of APOEε4 allele (if appropriate), and t-test for age, MMSE score, albumin ratio, and CSF levels of $A\beta_{1-42}$ and pTau181.

To explore correlations between age, albumin ratio, CSF levels of $A\beta_{1-42}$ and pTau181, and pTau181/$A\beta_{1-42}$ ratio values were separately analyzed in the cognitively healthy and the AD group with Pearson's correlation. The effects of age and the APOEε4 allele on the $A\beta_{1-42}$ and pTau181 concentrations, and on the pTau181/$A\beta_{1-42}$ ratio were analyzed using ANCOVA and including gender as a further factor.

To explore whether age and APOEε4 effects on the CSF markers may differ between CON and ND, ANCOVA with age as covariate was separately used in these subgroups. In an additional step, the group of APOEε4 allele carriers was divided into heterozygous and homozygous carriers to analyze the effects of one vs. two APOEε4 alleles on the CSF $A\beta_{1-42}$ concentrations. Again, ANCOVA with age as covariate was separately used in the subgroups. To further investigate whether the effects of the APOEε4 allele on the CSF $A\beta_{1-42}$ and pTau181 concentrations may be observed in older age only, we separately analyzed these relationships in the cognitively not impaired participants aged 16–45 years ($n = 67$), 46–65 years (132), and 66–89 years ($n = 81$).

All statistical analyzes were performed using the statistical analysis software package SPSS 17.0 for Windows.

## RESULTS

Subject characteristics and biomarker data by diagnostic group and APOE genotype are presented in Table 1.

As expected, the CSF concentrations of $A\beta_{1-42}$ were higher (mean: 849.07 pg/ml, SD: 239.71 vs. mean: 443.29 pg/ml, SD: 198.44; $F_{(1;426)} = 194.367$; $p < 0.001$) and those of pTau181 were lower (mean: 44.03 pg/ml, SD: 18.67 vs. mean: 101.97 pg/ml, SD: 47.66; $F_{(1;420)} = 183.390$; $p < 0.001$) in the participants with normal cognition compared to the AD patients. Furthermore, the pTau181/$A\beta_{1-42}$ ratios were lower in participants with normal cognition compared to subjects with AD (mean: 0.0539, SD: 0.0372 vs. mean: 0.2670, SD: 0.1801; $F_{(1;358)} = 173.512$; $p < 0.001$).

In the group of subjects with normal cognition, there were no significant subgroup differences between CON and ND regarding the presence of the APOEε4 allele ($\chi^2 = 0.12$; $p = 0.777$), age ($F_{(1;278)} = 3.768$; $p = 0.187$), gender ($\chi^2 = 1.82$; $p = 0.185$), MMSE scores ($F_{(1;240)} = 0.027$; $p = 0.589$), albumin ratio ($F_{(1;192)} = 4.56$; $p = 0.942$), the concentrations of
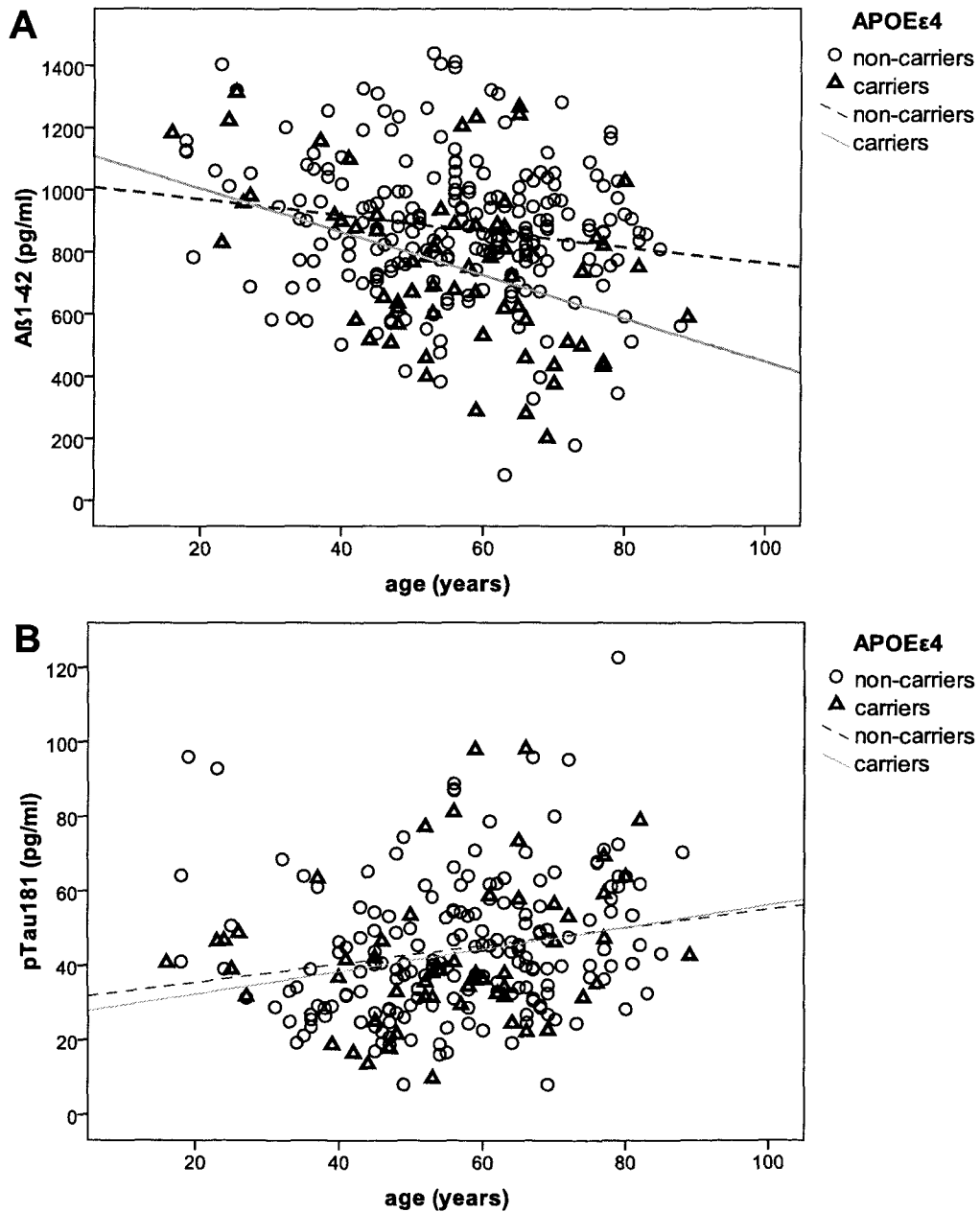
Fig. 1. CSF concentrations of $A\beta_{1-42}$ (A) and pTau181 (B) by APOEε4 allele status in relation to age; triangles represent the values of the APOEε4 carriers, circles those of the APOEε4 non-carriers. There was a significant inverse correlation between age and $A\beta_{1-42}$ concentrations ($r = -0.411$; $p = 0.001$), and no correlation of age and pTau181 ($r = 0.229$; $p = 0.087$) in the group of APOEε4 carriers, whereas in the APOEε4 non-carriers both concentrations of pTau181 ($r = 0.206$; $p = 0.004$) and of $A\beta_{1-42}$ ($r = -0.171$; $p = 0.012$) were weakly correlated with age.

$A\beta_{1-42}$ ($F_{(1;268)} = 0.125$; $p = 0.579$) and pTau181 ($F_{(1;244)} = 0.04$; $p = 0.320$), and the pTau181/$A\beta_{1-42}$ ratios ($F_{(1;228)} = 1.60$; $p = 0.576$). There were no significant age, gender, or albumin ratio differences be-tween the APOEε4 carriers and non-carriers. APOEε4 carriers had significantly lower $A\beta_{1-42}$ concentrations and pTau181/$A\beta_{1-42}$ ratios, whereas pTau181 levels did not differ between the groups (Table 1).
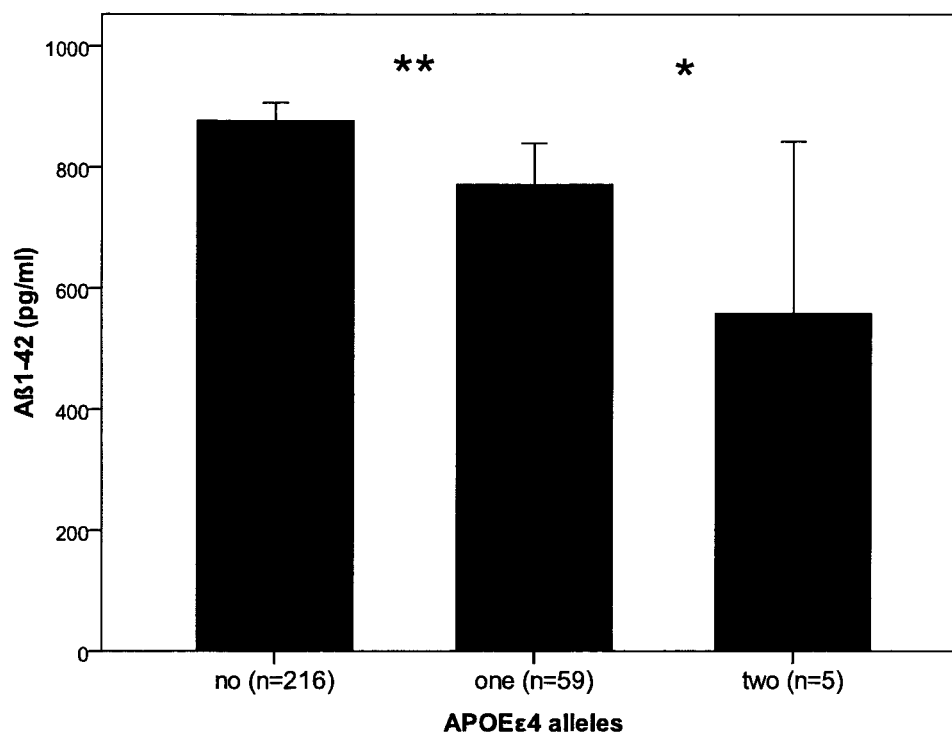
Fig. 2. Mean values of CSF $A\beta_{1-42}$ levels in relation to the number of APOEε4 alleles. **Non-carriers vs. carriers of one APOEε4 allele: $F_{(1;272)} = 10.185; p = 0.002$; * carriers of one vs. carriers of two APOEε4 alleles: $F_{(1;61)} = 4.134; p = 0.046$ (controlled for age).

In the whole group of participants with normal cognition, age was correlated with pTau181 levels ($r = 0.213, p = 0.001$) and the pTau181/$A\beta_{1-42}$ ratios ($r = 319; p < 0.001$), and inversely correlated with $A\beta_{1-42}$ levels ($r = -0.227, p < 0.001$). Albumin ratio increases with age ($r = 0.370, p < 0.001$), whereas there were no correlations of albumin ratio with $A\beta_{1-42}$ or pTau181 levels ($r = -0.117, p = 0.104$, and $r = 0.145, p = 0.056$, respectively).

Using ANCOVA, we found significant effects of age ($F_{(1;275)} = 16.981, p < 0.001$) and of the presence of the APOEε4 allele ($F_{(1;275)} = 16.852, p < 0.001$) on the $A\beta_{1-42}$ concentrations. The effects remained significant when analyses were separately performed in the CON and ND subgroups (for age: $F_{(1;136)} = 5.985, p = 0.016$ and $F_{(1;138)} = 9.980, p = 0.002$, respectively; for the presence of the APOEε4 allele: ($F_{(1;136)} = 6.407, p = 0.013$ and $F_{(1;138)} = 7.440, p = 0.007$, respectively). Furthermore, homozygous carriers of the APOEε4 allele ($n = 5$) had significantly lower $A\beta$1-42 levels compared to heterozygous carriers (Fig. 2).

The levels of pTau181 were associated with age ($F_{(1;241)} = 11.347, p = 0.001$), but not with the presence of the APOEε4 genotype ($F_{(1;241)} = 0.433, p = 0.511$). Furthermore, the pTau181/$A\beta_{1-42}$ ratios were associated with age ($F_{(1;230)} = 30.231, p < 0.001$) and the APOEε4 genotype ($F_{(1;230)} = 11.238, p = 0.001$).

In the younger participants, the $A\beta_{1-42}$ levels did not differ between the APOEε4 carriers and non-carriers whereas in the participants aged 46–65 years and in those aged 66 years or older the $A\beta_{1-42}$ levels were significantly lower in the APOEε4 carriers (Fig. 3A). The CSF levels of pTau181 did not differ significantly between the APOEε4 carriers and non-carriers in the three age groups (Fig. 3B). The interaction of the factors age-group and APOEε4 genotype was significant for the $A\beta_{1-42}$ ($F_{(2;268)} = 5.568, p = 0.004$), but not for the pTau181 concentrations ($F_{(2;234)} = 0.067, p = 0.935$). The effect size of the interaction was medium for the $A\beta_{1-42}$ ($f = 0.204$) and negligible for the pTau181 concentrations ($f = 0.023$). The achieved power to find an effect for pTau181 greater or equal to the effect of $A\beta_{1-42}$ at a given alpha $= 0.05$ was 82%.

In the group of participants with AD, there were no significant correlations between the pTau181 or $A\beta_{1-42}$ levels and age, the MMSE scores, and the albumin ratio, respectively (not shown). The subgroup
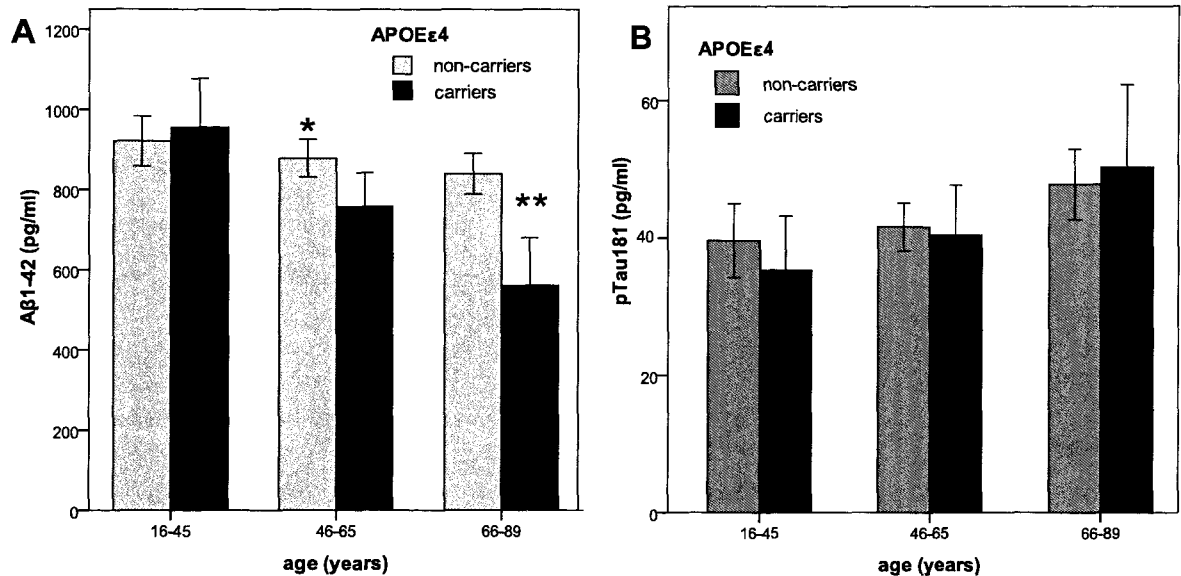
Fig. 3. Mean values of CSF $A\beta_{1-42}$ (3A) and pTau181 levels (3B) in APOEε4 carriers and non-carriers by age group. In the participants aged 46–65 years and in those aged 66 years or older the $A\beta_{1-42}$ levels were significantly lower in the APOEε4 carriers compared to non-carriers (mean: 757.68 pg/ml, SD: 236.49 vs. mean: 878.41 pg/ml, SD: 235.39; $p = 0.014$, and mean: 561.20 pg/ml, SD: 222.46 vs. mean: 839.56 pg/ml, SD: 205.50; $p < 0.001$, respectively). There were no significant differences of the pTau181 concentrations between APOEε4 carriers and non-carriers in the three age groups (data not shown).

of APOEε4 carriers did not differ significantly from the subgroup of APOEε4 non-carriers regarding age, gender, MMSE score, albumin ratio, pTau181 levels, or $pTau181/A\beta_{1-42}$ ratios. The $A\beta_{1-42}$ concentrations were significantly lower in the APOEε4 carriers compared to the non-carriers (Table 1). ANCOVA tests revealed significant effects of the APOEε4 genotype ($F_{(1;148)} = 4.709$, $p = 0.032$), but no age effects ($F_{(1;148)} = 0.498$, $p = 0.482$) on the $A\beta_{1-42}$ levels, and no effects of APOEε4 genotype or age on either the pTau181 levels or the $pTau181/A\beta_{1-42}$ ratios in this group.

## DISCUSSION

In the present study, we found significant age effects on the CSF $A\beta_{1-42}$ and pTau181 levels of subjects without cognitive impairment across a broad age range. Carrying the APOEε4 allele was associated with a more marked decrease of the $A\beta_{1-42}$ concentrations with age, especially in the middle-aged and older participants, whereas there were no effects of the APOEε4 genotype on the pTau181 levels. Moreover, higher $pTau181/A\beta_{1-42}$ ratios were associated with older age and the APOEε4 genotype in this group. Furthermore, in an additional group of patients with clinically diag-

nosed AD, lower CSF $A\beta_{1-42}$ levels were associated with the presence of at least one APOEε4 allele.

Autopsy studies have shown that AD-like cerebral amyloid deposition and tau pathology can be found in a large part of older individuals, which had no clinical relevant cognitive impairment ante mortem [21–23]. Decreased $A\beta_{1-42}$ and increased pTau181 CSF concentrations may reflect these presumably progressive cerebral changes [11]. Previously published studies on the changes of CSF $A\beta_{1-42}$ and tau levels with increasing age in cognitively not impaired subjects have reported controversial results. In a study including a relatively small sample [16], but also in a study considering a large number of participants [24], no relationships were found between age and the $A\beta_{1-42}$ concentrations. Other studies have described associations of decreased $A\beta_{1-42}$ levels with older age [1,15,17,25]. In line with these reports, our findings demonstrate significant age effects on the CSF $A\beta_{1-42}$ levels, suggesting a progressive alteration of the amyloid metabolism in the CNS in subjects with normal cognition.

In a recent study, higher CSF total tau and pTau181 levels were found to be associated with older age [15]. However, most of the subjects included were elderly participants with subjective memory impairment, which may have a higher prevalence of CSF biomarker AD-like profile than cognitively healthy subjects with-

Table 1
Subject characteristics and biomarker data by diagnostic group and the presence of the APOEε4 allele

| | Controls $n = 280$ | | | AD patients $n = 152$ | | |
|---|---|---|---|---|---|---|
| | APOEε4 non-carriers $n = 216$ | APOEε4 carriers $n = 64 (59/5)^a$ | | APOEε4 non-carriers $n = 50$ | APOEε4 carriers $n = 102 (79/23)^a$ | |
| **Age** (years): mean (SD), range | 56.06 (14.94), 18–88 | 55.69 (15.58), 16–89 | $F = 0.25$; $p = 0.866$ | 72.54 (7.15) | 71.58 (7.51) | $F = 0.39$; $p = 0.445$ |
| **Gender** (m/f): n | 130/86 | 32/32 | $\chi^2 = 2.10$; $p = 0.153$ | 23/27 | 38/64 | $\chi^2 = 1.07$; $p = 0.379$ |
| **CNS affection**: yes/no | 110/106 | 31/33 | $\chi^2 = 0.12$; $p = 0.777$ | | | |
| **MMSE**: mean (SD), range | 29.24 (1.07); 26–30 | 29.17 (1.05); 26–30 | $F = 0.012$; $p = 0.657$ | 20.88 (5.55); 5–28 | 20.95 (4.30); 5–29 | $F = 7.50$; $p = 0.932$ |
| **albumin ratio**: mean (SD) | 5.95 (2.22) | 6.30 (4.18) | $F = 14.96$; $p = 0.520$ | 6.15 (2.66) | 5.42 (2.17) | $F = 0.34$; $p = 0.109$ |
| **pTau181** (pg/ml): mean (SD) | 44.38 (1.85) | 42.87 (1.95) | $F = 0.09$; $p = 0.604$ | 97.77 (51.49) | 104.20 (45.68) | $F = 0.01$; $p = 0.479$ |
| $A\beta_{1-42}$ (pg/ml): mean (SD) | 877.00 (224.95) | 754.79 (264.71) | $F = 4.31$; $p < 0.001$ | 493.69 (286.75) | 418.35 (129.94) | $F = 19.00$; $p = 0.028$ |
| **pTau181/** $A\beta_{1-42}$ ratio: mean (SD) | 0.0497 (0.0300) | 0.0653 (0.0658) | $F = 15.54$; $p = 0.039$ | 0.2646 (0.2165) | 0.2682 (0.1589) | $F = 1.01$; $p = 0.913$ |

SD: Standard-Deviation; MMSE: Mini-Mental State Examination.
$^a$(one/two APOEε4 alleles).

out subjective complaints [2], and may be considered a group at high risk for developing cognitive impairment and AD dementia over time [26]. In two further studies that have addressed the age effects on the CSF concentrations of tau proteins in cognitively healthy adults, associations were reported between older age and higher levels of total tau [24] and of pTau231 [16]. While increased total tau levels may reflect neuronal and axonal damage which can be related to many different pathological conditions, changes in pTau231 or pTau181 CSF concentrations are thought to be more specific for neurofibrillary pathology in AD. In our sample, higher pTau181 levels were significantly associated with older age, which is consistent with the presumption that hyperphosphorylated tau protein and neurofibrillary pathology accumulate with increasing age in the absence or before the clinical manifestation of AD.

As the presence of the APOEε4 allele may accelerate the pathophysiological processes in AD [7], one can expect age related changes of the CSF markers reflecting these cerebral processes to be more pronounced in APOEε4 carriers than in non-carriers. Studies in mouse models of AD have demonstrate that apolipoprotein E markedly influences the $A\beta$ metabolism even before plaque formation [27], and that the presence of the APOEε4 allele leads to elevated brain levels of $A\beta$ peptides at a very early point in time, and to a further substantial increase with age [28]. In humans, postmortem studies have reported associations

of the APOEε4 genotype with higher levels of amyloid plaque deposition in patients with AD and in cognitively healthy subjects [10,23,29]. Recent amyloid imaging studies have confirmed these findings *in vivo* in patients with AD [30] and in cognitively normal subjects [31].

Several CSF studies in patients with clinically diagnosed probable or post mortem confirmed AD have described significantly lower CSF $A\beta_{1-42}$ levels in APOEε4 carriers compared to non-carriers [1,13–15, 32]. A few studies in older cognitively normal subjects have found associations between the APOEε4 genotype and the CSF levels of $A\beta_{1-42}$ [1,14,15], but others did not [16]. Investigating this relationship in a large sample of younger and older participants, Peskind et al. reported a substantially greater age-related decrease in CSF $A\beta_{1-42}$ concentration in APOEε4 allele carriers compared with non-carriers [17]. However, the authors did not address possible effects on tau CSF concentrations in this sample. In line with this study, our data show that the presence of the APOEε4 allele is associated with significantly lower $A\beta_{1-42}$ levels in middle-aged and older persons with normal cognition. In addition, in our study carrying two APOEε4 alleles seems to result in a more marked decrease of the $A\beta_{1-42}$ levels than in heterozygous carriers. These findings are consistent with the hypothesis that the presence of the APOEε4 allele contributes early in life to the pathological changes resulting in cerebral amyloid deposition. Moreover, we found similar effects of the APOEε4

genotype on the CSF $A\beta_{1-42}$ levels in the group of participants with AD. This is in accordance with several previous studies in patients with AD dementia [1, 13–15,32] and suggests that these effects persist in later disease stages.

In postmortem studies, both amyloid plaque formation and neurofibillary pathology were found to begin early in adulthood. However, significant associations with the APOEε4 genotype were found only for the levels of cerebral amyloid deposition, but not for the neurofibrillary pathology [23]. In a recent ante mortem study including a large number of older participants with subjective cognitive impairment and patients with AD, there were no associations between the CSF pTau181 levels and the APOEε4 genotype [15]. In line with these observations, we did not find any significant effects of the APOEε4 genotype on the pTau181 concentrations in neither the participants with normal cognition nor the AD group. However, other phosphorylated tau species than pTau181 have been proposed as markers reflecting the cerebral neurofibrillary pathology, and it is controversially discussed which one may be the most appropriate to investigate early CSF changes in AD [16]. In this context, the comparison between different phosphorylated tau species and total tau in the same sample would be particularly interesting, but this was not performed in the present study.

The pTau181/$A\beta_{1-42}$ ratio may be a more specific indicator of cerebral AD pathology than the CSF concentrations of either $A\beta_{1-42}$ or pTau181 alone, and it has been shown to predict cognitive decline in nondemented older adults [3]. In this sample, we found higher pTau181/$A\beta_{1-42}$ ratios to be related to both older age and the presence of the APOEε4 allele. Even though the effects of the APOEε4 genotype on the pTau181/$A\beta_{1-42}$ ratio may essentially reflect the association found for APOEε4 and the $A\beta_{1-42}$ concentrations, these results suggest that the APOEε4 genotype increases the risk for AD with older age and accelerates the development of presumed cerebral pathology related to preclinical AD.

A limitation of our study is related to the fact that an important proportion of the cognitively normal participants underwent lumbar puncture for the evaluation or exclusion of neurological disorders. Therefore, these participants may have altered CSF biomarker levels, in particular the persons presenting affections of the CNS. However, there were no significant subgroup differences regarding age, gender, APOEε4 genotype, MMSE scores, albumin ratio, and the concentrations of $A\beta_{1-42}$ and pTau181 between CON and ND. More-

over, and most important, in both subgroups the effects of age and the APOEε4 genotype on the CSF markers were similar to the effects found for the whole sample. Also, the function of the blood-CSF barrier as assessed by the albumin ratio did not influence the CSF $A\beta_{1-42}$ and pTau181 levels in our study, which confirms previous reports [24]. These findings strongly suggest that disorders affecting the CNS do not substantially interfere with the effects of age and the APOEε4 genotype on the CSF $A\beta_{1-42}$ and pTau181 concentrations.

In conclusion, the results demonstrate significant age effects on the CSF markers for AD $A\beta_{1-42}$ and pTau181, and for the pTau181/$A\beta_{1-42}$ ratio across the lifespan. They also suggest that the decrease of $A\beta_{1-42}$, but not the increase of pTau181 CSF levels is accelerated by the presence of the APOEε4 genotype in middle-aged and older adults with normal cognition. The findings of effects and interaction of age and the presence of the APOEε4 allele on the CSF $A\beta_{1-42}$ concentrations are consistent with the hypothesis that the APOEε4 genotype substantially contributes to the early cerebral amyloid deposition.

## ACKNOWLEDGMENTS

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