CSF BIOMARKERS IN ALZHEIMER’S DISEASE AND CONTROLS: ASSOCIATIONS WITH APOE GENOTYPE ARE MODIFIED BY AGE
CHAPTER 2.1

ABSTRACT

The object of this study was to elucidate the effect of age in the relationship between APOE genotype and CSF biomarkers amyloid beta 1-42 (Aβ42), total tau (tau) and tau phosphorylated at threonine 181 (ptau-181) in AD and controls. Three-hundred-and-two AD patients and 174 controls were categorized into APOE ε4 carriers and non-carriers, and into younger and older (≥65years).

In controls, older age and APOE ε4 were independently associated with lower Aβ42 and higher tau and ptau-181 levels (p<0.05). For tau and ptau-181 there were also interactions (p<0.10): older carriers had higher levels than older non-carriers, without effect for younger controls. In AD, APOE ε4 genotype had a main effect on Aβ42, but there was also an interaction: older carriers had lower Aβ42 than older non-carriers, without effect for younger AD patients (p<0.05). For tau and ptau-181 there were only interactions: older carriers had higher levels than older non-carriers, while younger AD patients showed the opposite (p≤0.05).

Association between CSF biomarkers and APOE genotype were modified by age in both controls and AD patients. This suggests that cognitively healthy APOE ε4 carriers are more prone to develop AD pathology with aging. For AD patients this provides support for the existence of subtypes within the disease.

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INTRODUCTION
Alzheimer's disease (AD) is the most common form of dementia. The neuropathological hallmarks of AD are progressive accumulation of plaques with amyloid beta and neurofibrillar tangles containing (phosphorylated) tau.\(^1\) Cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain and biochemical changes in the brain are thought to be reflected in CSF.\(^2\) Amyloid beta 1-42 (A\(\beta\)42), total tau (tau) and tau phosphorylated at threonine 181 (p-tau-181) have been proven useful CSF biomarkers in the diagnosis of AD, with good sensitivity and specificity of 80-90%.\(^2\)

The apolipoprotein E (APOE) \(\varepsilon\)4 genotype is an important risk factor for AD.\(^3\) The exact mechanism through which APOE influences the development of AD is yet unknown, but the APOE genotype seems to play a major role in the pathological pathway. APOE \(\varepsilon\)4 genotype has been associated with an increased rate and extent of amyloid beta deposition and neurofibrillary tau pathology in post-mortem studies.\(^4,6\) Furthermore, non-demented subjects carrying the APOE \(\varepsilon\)4 allele were more likely to have amyloid plaques and neurofibrillary tangles than non-carriers.\(^3\) Age is the most important known risk factor for the development of AD. Neurofibrillary tangles and amyloid beta plaques are commonly found in brains of elderly without the clinical syndrome of AD.\(^7\) In controls and AD a clear effect of age on CSF biomarker levels has recently been demonstrated.\(^8,9\) Additionally, the increase of plaques and tangles was much higher with aging for the APOE \(\varepsilon\)4 carriers than for those who lack the APOE \(\varepsilon\)4 allele.\(^3\)

Several articles have been published about the relations between APOE genotype and CSF biomarkers in AD patients and controls,\(^9,17\) but the results of these studies were often conflicting and came from small patient groups. Few studies focused on the combined associations of APOE genotype and age on CSF biomarkers, mostly in controls.\(^9,11,13\)

In this study we aim to investigate the associations of APOE genotype with CSF biomarker levels of A\(\beta\)42, tau and p-tau-181 in a large cohort of AD patients and controls and to explore the effect of age on these associations.

MATERIALS AND METHODS
Subjects
In this ongoing study, 302 patients with sporadic AD and 174 controls (24 volunteers and 150 persons with subjective complaints), with available CSF results and APOE genotyping were included from our memory clinic.\(^8\) All patients underwent a standard dementia screening including physical and neurological examination as well as laboratory tests, EEG and brain MRI. Cognitive screening included a Mini Mental State Examination (MMSE), but usually involved comprehensive neuropsychological testing. The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria\(^1\) by consensus of a multidisciplinary team, without knowledge of CSF results and the APOE genotype. When the results of all examinations were normal, patients were considered to have subjective complaints (i.e. criteria for MCI not fulfilled). In addition we included 24 volunteers without cognitive complaints. The study was approved by the local ethical review board and all subjects gave written informed consent.
CHAPTER 2.1

CSF analysis
CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 10 mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 1800 g for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml and stored at -80°C until further analysis. CSF Aβ42, tau and ptau-181 were measured with Innotest sandwich ELISA as described previously. The inter-assay coefficient of variation for Aβ42 was 12.6%, 12.6% for tau and 10.3% for ptau-181. The team involved in the CSF analysis was not aware of the clinical diagnoses.

APOE genotyping
For APOE genotyping, DNA was isolated from 10 ml EDTA blood by the QIAamp DNA blood isolation kit from Qiagen. The genotype was determined with the Light Cycler APOE mutation detection kit (Roche Diagnostics GmbH, Mannheim, Germany). Subjects were classified as APOE ε4 carriers if they had one or two ε4 alleles, otherwise they were APOE ε4 non-carriers.

Statistical analysis
For statistical analysis, SPSS version 13.0 (for Windows) was used. CSF biomarker levels were log transformed to obtain normal distribution. Frequency distributions for sex were compared with chi-squared tests. Results are expressed as means (SD) unless indicated otherwise. Student’s T-tests were used to compare age and MMSE between groups. Associations between APOE genotype and the CSF biomarkers were assessed for controls and AD patients separately. We first used Analyses of Variance (ANOVA) with APOE genotype as independent factor, biomarker levels as dependent variables and gender and age as covariates. Subsequently we examined if the associations between CSF biomarker levels and APOE genotype were modified by age using two-way ANOVAs, with age (younger: <65 versus older: ≥65 years) and APOE genotype as independent factors and the CSF biomarker levels as dependent variables. In general, statistical significance was set at p<0.05. Interactions were considered significant if p-values were lower than 0.10.

RESULTS
There was no difference in gender distribution between groups. Controls were younger than AD patients (61±10 years vs 67±8, p<0.001). Mean MMSE for controls was 29±1 and 21±5 for AD. Adjusted for gender and age, CSF levels of Aβ42 were lower in AD patients compared to controls (459±170 vs 845±222 pg/ml, p<0.001), while levels of tau (728±432 vs 288±160 pg/ml, p<0.001) and ptau-181 (90±39 vs 48±20 pg/ml, p<0.001) were both higher in AD.

CSF biomarker levels by diagnosis and APOE genotype are presented in Table 1. For controls Aβ42 levels were lower in APOE ε4 carriers than in APOE ε4 non-carriers (p<0.001), while tau (p=0.01) was higher in APOE ε4 carrier controls than in the
non-carriers. For ptau-181 there was a trend for higher levels in APOE ε4 carriers in comparison to non-carriers (p=0.10). In AD patients the levels of Aβ42 were lower in APOE ε4 carriers (p=0.003), but there was no difference in the levels of tau or ptau-181 between carriers and non-carriers.

Subsequently, we assessed if the associations between APOE genotype and CSF biomarkers were modified by age. Results are shown in Figure 1. For controls, two-way ANOVAs with APOE genotype and age as independent variables and CSF biomarker levels as dependent variables revealed main effects of APOE genotype and age for Aβ42 (p<0.001), without interaction. Older age and APOE ε4 genotype were independently associated with lower levels of Aβ42. For tau and ptau-181, there were main effects for APOE genotype (both p≤0.01) and age (both p<0.001) as well. In addition, there were interactions between APOE genotype and age for tau (p=0.06) and ptau-181 (p=0.02), as the effect of APOE genotype on CSF levels of tau and ptau-181 was specific for older controls. Older controls with the APOE ε4 genotype had higher tau and ptau-181 than older controls lacking the APOE ε4 allele, without an effect of APOE genotype in younger controls.

In the group of AD patients we observed a different pattern; there was a main effect of APOE genotype (p=0.007) for Aβ42, but not for age (p=0.08). In addition there was an interaction as Aβ42 was lower in older APOE ε4 carriers compared to older APOE ε4 non-carriers, but there was no effect for younger AD patients (p for interaction p=0.03). For tau and ptau-181 there were no main effects, but there were interactions. Tau and ptau-181 were higher in older APOE ε4 carriers than older APOE ε4 non-carriers. For younger AD patients, we observed the opposite effect; APOE ε4 non-carriers had higher, and thus more abnormal, CSF levels than APOE ε4 carriers (p for interaction: tau p=0.04; ptau-181 p=0.05).

### Table 1. Characteristics and CSF biomarker levels by diagnostic group and APOE genotype

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=174)</th>
<th>AD (n=302)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APOE ε4 -</td>
<td>APOE ε4 +</td>
</tr>
<tr>
<td>N</td>
<td>111</td>
<td>63</td>
</tr>
<tr>
<td>Gender= female, n (%)</td>
<td>50 (45%)</td>
<td>34 (54%)</td>
</tr>
<tr>
<td>Age</td>
<td>61 (10)</td>
<td>60 (10)</td>
</tr>
<tr>
<td>Age &lt;65 years, n (%)</td>
<td>72 (65%)</td>
<td>45 (71%)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29 (2)</td>
<td>29 (1)</td>
</tr>
<tr>
<td>Aβ42</td>
<td>896 (211)</td>
<td>756 (214)*</td>
</tr>
<tr>
<td>Tau</td>
<td>263 (110)</td>
<td>331 (218)*</td>
</tr>
<tr>
<td>Ptau-181</td>
<td>46 (15)</td>
<td>52 (26)</td>
</tr>
</tbody>
</table>

Data are represented as mean (SD) unless indicated otherwise. AD = Alzheimer’s disease, APOE ε4 - = APOE ε4 non-carriers, APOE ε4 + = APOE ε4 carriers, MMSE = mini mental state exam, Aβ42 = amyloid beta 1-42, tau = total tau and ptau-181 = tau phosphorylated at threonine 181 (levels pg/ml). Please note that raw data are presented, while statistical analyses were done after log transformation. * p<0.05 vs APOE ε4 non-carriers.
We found associations between APOE genotype and the most commonly used CSF biomarkers in controls and AD patients. In addition, these associations were modified by age, as older APOE ε4 carrier controls had more abnormal biomarker levels than older APOE ε4 non-carriers, while such an effect was not observed in younger controls. In AD patients we observed a slightly different pattern when age was taken into account. Older APOE ε4 carriers had more abnormal biomarkers levels than older APOE ε4 non-carriers. Conversely, for the younger AD patients CSF levels of tau and ptau-181 were more abnormal in non-carriers than in APOE ε4 carriers.

DISCUSSION

We found associations between APOE genotype and the most commonly used CSF biomarkers in controls and AD patients. In addition, these associations were modified by age, as older APOE ε4 carrier controls had more abnormal biomarker levels than older APOE ε4 non-carriers, while such an effect was not observed in younger controls. In AD patients we observed a slightly different pattern when age was taken into account. Older APOE ε4 carriers had more abnormal biomarkers levels than older APOE ε4 non-carriers. Conversely, for the younger AD patients CSF levels of tau and ptau-181 were more abnormal in non-carriers than in APOE ε4 carriers.
For controls previous studies on the relation between APOE genotype and CSF biomarkers have reported different results. Most studies found that levels of Aβ42 were lower and levels of tau and ptau-181 were higher in APOE ε4 carriers. Few studies described the effects of APOE genotype in combination with aging on the CSF biomarker levels. One group assessed controls only and found lower Aβ42 and higher tau to be independently associated with older age and the APOE ε4 genotype. For ptau-231 an interaction was found as the increase of CSF levels with aging was higher in the APOE ε4 carrier group. Two other studies also reported that Aβ42 was decreased in older controls and even more so if they were APOE ε4 positive. Our findings in controls confirm these former studies as older controls with the APOE ε4 genotype had more abnormal biomarkers, than those who lack the APOE ε4 allele. It is conceivable that these older APOE ε4 carrier controls already have some Alzheimer pathology. This would be in line with former post-mortem studies that showed aging and APOE ε4 genotype to be correlated to a larger burden of plaques and tangles, even in the absence of clinical AD. Furthermore, reduced Aβ42 and an increased ratio of CSF tau/Aβ42 in controls have been described as predictor for future cognitive decline. Moreover, it has been shown that elderly APOE ε4 controls with abnormal CSF tau/Aβ42 ratio are at an increased risk for developing MCI in the first years of follow-up. Future studies in large groups are needed to substantiate the predictive value of abnormal CSF biomarkers for cognitive decline in healthy subjects.

In AD earlier studies examining the relation between APOE genotype and CSF biomarkers reported conflicting results: some studies found more abnormal biomarker levels in APOE ε4 carriers, while others found no relation in relatively large groups. Our results suggest that the modifying effect of age could partially explain these conflicting results. We observed, contrary to our findings in controls, an opposite effect of age by APOE genotype for tau and ptau-181 levels in AD patients, as both older APOE ε4 carriers and younger APOE ε4 non-carriers had higher values. Although at first this may appear counterintuitive, we feel that this may illustrate the existence of subtypes in AD that may be related to both age at onset and genetic make up. This is supported in the literature where clinically different phenotypes of AD have been described. Typically, AD patients are elderly presenting with memory problems, often positive for the APOE ε4 genotype. It has been suggested that APOE ε4 non-carrier AD patients with age at onset before 65 years have a distinct clinical profile with prominent parietal dysfunction. We do not yet have an explanation why younger patients without the APOE ε4 allele have more abnormal CSF biomarker levels. Possibly, the higher tau and ptau-181 levels reflect a more progressive type of the disease.

Among the limitations of our study is the fact that we used patients with subjective complaints as controls. Former research has shown that patients with subjective complaints have a higher risk of developing AD in the future. Although we have not included patients with subjective complaints whom we knew to show clinical progression over time, it is still possible that our results were influenced by the fact that there was a higher percentage of preclinical AD in our group of controls than in the general community. The main strength of our study is the relatively large patient sample. Lumbar puncture and APOE genotyping are performed on a routine basis in the majority of the patients presenting in our memory clinic, resulting in a representative
group of patients for this study. The fact that we have a fairly large proportion of young patients, enabled us to focus on the modifying effect of age. There seems to be a clear distinction between younger and older AD patients, where we have found evidence for the existence of a subgroup of young APOE ε4 non-carrier AD patients with very abnormal levels of CSF biomarkers. Further research in this group is necessary to clarify more thoroughly their clinical presentation and genetic make-up.

REFERENCES


