CHAPTER 5

APOE genotype modifies the predictive value of CSF biomarkers in preclinical Alzheimer’s Disease

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*Alzheimers Dement.* Under review.
ABSTRACT

Background. We investigated whether Apolipoprotein E (APOE) genotype modifies the predictive value of preclinical Alzheimer’s disease (AD) for clinical progression in patients with subjective complaints.

Methods. We included 224 patients with subjective complaints (61±9 years old; 92 (41%) female; MMSE 28±2) from our Amsterdam Dementia Cohort, with a follow-up of 3±2 years. Cerebrospinal fluid (CSF) biomarkers amyloid-beta1-42 (Aβ42), total Tau (Tau) and hyperphosphorylated tau-181 (pTau) were used to define National Institute on Aging-Alzheimer’s Association (NIA-AA) preclinical AD stages. APOE genotype and preclinical AD were used to predict clinical progression to Mild Cognitive Impairment (MCI) or dementia due to AD in individual and combined models using Cox proportional hazard models. Analyses were corrected for age, sex and baseline MMSE.

Results. Twenty-eight patients (13%) showed clinical progression during follow-up. In univariate models, both APOE4 positivity [HR (95% CI) 2.2 (1.0-4.9)] and preclinical AD increased risk of clinical progression [HR (95% CI) stage 1: 8.5 (2.2-33.1), stage 2: 23.2 (7.2-88.5)]. The combined model showed an interaction between APOE genotype and preclinical AD. Stratified analyses indicated predictive value of preclinical AD in APOE4 negative patients increased dose-dependently [stage 1: 29.8 (4.7-190.8), stage 2: 45.1 (6.9-293.8)]. In APOE4 positive patients only stage 2 increased risk of clinical progression [stage 1: 2.1 (0.2-19.9), stage 2: 12.3 (3.0-50.0)].

Conclusions. The predictive value of preclinical AD is especially strong in APOE4 non-carriers. In future, this may have implications for trial design in preclinical intervention studies. Our results indicate that preclinical AD can have a distinct APOE4 independent pathway.
1. Introduction

Intervention studies in Alzheimer’s disease (AD) have started to focus on cognitively normal subjects with an increased risk of developing dementia due to AD.\(^1\) For the selection of these subjects it is important to know which clinical variables, genes and biomarkers predispose for dementia due to AD. Having subjective complaints has been suggested to be a clinical factor that predicts dementia due to AD,\(^2,4\) but heterogeneity within this entity makes it necessary to identify further predictors of clinical progression within this group.

Prior evidence suggested cerebrospinal fluid (CSF) evidence of preclinical AD predicts incidence of Mild Cognitive Impairment (MCI) or dementia due to AD and cognitive decline in patients with subjective complaints.\(^7\) A factor that could modify the predictive value of preclinical AD is Apolipoprotein E (APOE) \(\varepsilon4\) genotype. APOE4 is the strongest known genetic risk factor for AD. Two prior studies indicated it may predict clinical progression in cognitively normal subjects as a single predictor,\(^8,9\) while in two others its predictive value did not reach significance.\(^10,11\) APOE4 allele frequency is increased in subjective complaints, compared to cognitively normal individuals without complaints.\(^12\) Cross-sectionally, APOE4 was associated with worse memory performance in patients with subjective complaints.\(^13\) Also, healthy APOE4 carriers have lower CSF amyloid-beta\(_{1-42}\) (A\(\beta\)\(_{42}\)) values and increased Pittsburgh compound B (PiB) binding.\(^14,15\) The combination of preclinical AD and APOE genotype may therefore improve predictions made by any marker individually.

We investigated whether APOE genotype modifies the predictive value of preclinical AD for clinical progression in patients with subjective complaints.

2. Materials and methods

2.1 Subjects

Two hundred and thirty patients with subjective complaints from our Amsterdam Dementia Cohort were eligible for this study, because they underwent APOE genotyping and CSF analysis at baseline and had at least one year of follow-up. Six patients were excluded from the current study, because they progressed to a different type of dementia than dementia due to AD, resulting in inclusion of 224 patients. The study population partly overlapped with an earlier publication.\(^7\) We used the same methodology as the one employed in that study. All patients underwent a standardized dementia screening, including neuropsychological, physical and neurologic examination as well as laboratory tests, electroencephalography (EEG) and brain magnetic resonance imaging (MRI) at baseline. Diagnoses were made in a multidisciplinary meeting, without knowledge of APOE genotype or CSF results. Patients were labelled as having subjective complaints when they presented with cognitive complaints, but ancillary investigations were normal and criteria for MCI, dementia or any other neurological
or psychiatric disorders known to cause cognitive complaints were not met (i.e. cognitively normal elderly). Follow-up took place by annual routine visits to our memory clinic, in which patient history, cognitive tests and a general physical and neurologic examination were repeated. In 2010, patients who had not undergone at least two years of routine follow-up were contacted by telephone. Their cognitive status was evaluated by a standardized interview, complemented by the Telephone Interview for Cognitive Status (TICS). When the telephone interview gave rise to a suspicion of potential clinical progression, patients were invited for a more thorough investigation at the outpatient clinic. The primary outcome measure was clinical progression, defined as progression to MCI or dementia due to AD. Petersen’s criteria were used for MCI and NINCDS-ADRDA criteria for probable AD. In addition, patients with dementia due to AD fulfilled National institute on Aging-Alzheimer’s Association (NIA-AA) core clinical criteria for AD and patients with MCI fulfilled NIA-AA criteria for MCI.

2.2 Standard protocol Approvals, Registrations and Patient Consents

The medical ethics committee of the VU University Medical Center approved the study. All patients provided written informed consent.

2.3 CSF analysis

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space by a 25-gauge needle and collected in polypropylene tubes. CSF analysis took place at the Neurochemistry Laboratory of the VU University Medical Center in Amsterdam. Amyloid-beta1-42 (Aβ42), Tau, and hyperphosphorylated Tau (pTau) were measured using sandwich ELISAs (Innotest, beta-amyloid1-42, Innotest hTAU-Ag and Innotest PhosphoTAU-181p, Innogenetics, Belgium) as previously described. The intra-assay coefficient of variation (mean±SD) was 2.0±0.5% for Aβ42, 3.2±1.3% for tau and 2.9±0.8% for pTau as calculated from averaging the CV of duplicates from 5 runs randomly selected over 2 years. The inter-assay coefficient of variation (mean±SD) was 10.9±1.8% for Aβ42, 9.9±2.1% for tau and 9.1±1.8% for ptau-181, as analyzed in a high and low pool from 13 consecutive pool preparations used in total in 189-231 runs during this period.

Based on previously published CSF biomarker cut-points from our own laboratory, we defined preclinical AD stages according to NIA-AA criteria. These criteria define 3 preclinical stages. As it is not completely clear how to define subtle cognitive dysfunction based on NIA-AA criteria, we categorized preclinical AD stages based on CSF biomarker values only. We defined stage 0 (reference category), “no evidence of underlying AD pathology,” as normal values of all CSF biomarkers; stage 1, “evidence of amyloid pathology only,” as abnormal CSF Aβ42 only, and stage 2, “evidence of amyloid pathology and neurodegeneration,” as abnormal CSF Aβ42 and abnormal CSF tau and/or pTau concentrations. Patients who had
abnormal CSF Tau and/or pTau values with normal CSF Aβ42 were defined as Suspected Non-Alzheimer Pathology (SNAP) patients.\textsuperscript{23,24}

2.4 APOE genotyping

APOE genotyping was performed after automated genomic DNA isolation from 7-10 mL EDTA blood. It was subjected to PCR, checked for size and quantity using a QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) and sequenced using Sanger sequencing on an ABI130XL. Subjects were classified as APOE4 negative or positive.

2.5 Statistical analysis

Data were analyzed using SPSS for Macintosh, version 20. Baseline characteristics were compared using t-tests, chi-square tests or Mann-Whitney U tests as appropriate. Cox proportional hazard models were used to assess the predictive value of APOE genotype and NIA-AA preclinical AD stages for clinical progression to MCI or dementia due to AD. First, we assessed the predictive value of APOE genotype and CSF biomarker stage in separate models. Secondly, the combination of NIA-AA preclinical stage and APOE genotype was evaluated, including the interaction term APOE*NIA-AA preclinical stage. As a significant interaction was found, we estimated the effects of NIA-AA preclinical stages for APOE4 negative and positive patients separately. Progression to MCI or dementia due to AD was taken as outcome measure. If a patient first converted to MCI and then to dementia due to AD, the moment of conversion to MCI was taken as time of clinical progression. All analyses were first performed without correction, then adjusted for age and sex and lastly adjusted for age, sex and baseline MMSE. For main effects, a p-value below p<0.05 was considered significant and for interaction effects a p-value below p<0.10. Results are presented as hazard ratio (HR) with 95% confidence intervals (CI).

3. Results

Patients were on average 61±9 years old. Their average MMSE was 28.3±1.6. Average follow-up duration was 3±2 years. Ninety (40%) patients were APOE4 positive. Seventy two (32%) of these patients were heterozygous and 18 (8%) homozygous. At baseline, 132 (59%) patients had no CSF evidence of underlying AD pathology (NIA-AA stage 0). Eighteen (8%) patients had CSF evidence of amyloid pathology only (NIA-AA stage 1) and another 18 (8%) had CSF evidence of amyloid pathology and neuronal injury (NIA-AA stage 2). Fifty-six patients (25%) fulfilled criteria for SNAP. Preclinical AD (stage 1 or 2) was more often present in APOE4 carriers than in APOE4 non-carriers (27% vs. 9%, p<0.001).

During follow-up 28 patients showed clinical progression (13%): 19 to MCI, 4 first to MCI and then to dementia due to AD, and 9 to dementia due to AD without being diagnosed with MCI.
first. Patients who showed clinical progression during follow-up were older at baseline and tended to have a lower baseline MMSE (Table 1). Also, they were more often APOE4 positive and their CSF biomarker concentrations were more often abnormal.

### Table 1. Baseline characteristics according to follow-up diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Stable (n=196)</th>
<th>Clinical progression (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>60±9</td>
<td>66±7**</td>
</tr>
<tr>
<td><strong>Female sex, n (%)</strong></td>
<td>84 (42%)</td>
<td>8 (33%)</td>
</tr>
<tr>
<td><strong>GDS (n=197)</strong></td>
<td>2.5±2.3</td>
<td>1.9±1.9</td>
</tr>
<tr>
<td><strong>Baseline MMSE</strong></td>
<td>28.4±1.6</td>
<td>27.7±1.8§</td>
</tr>
<tr>
<td><strong>Follow-up duration, years</strong></td>
<td>2.7±1.7</td>
<td>2.4±2.1</td>
</tr>
<tr>
<td><strong>APOE4 positive</strong></td>
<td>75 (38%)</td>
<td>15 (63%)*</td>
</tr>
<tr>
<td><strong>Aβ42, ng/microL</strong></td>
<td>887 (293-1363)</td>
<td>547 (232-1370)**</td>
</tr>
<tr>
<td><strong>tau, ng/microL</strong></td>
<td>243 (68-2088)</td>
<td>390 (116-1092)**</td>
</tr>
<tr>
<td><strong>ptau, ng/microL</strong></td>
<td>44 (12-190)</td>
<td>58 (20-136)**</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, no. (%) or median (range). *p<0.05; ** p<0.01; ***p<0.001.

Univariate Cox proportional hazards models showed that APOE4 positivity was associated with a modestly increased risk of clinical progression (adjusted HR 2.2(1.0-4.9); Table 2, Figure 1). Evidence of preclinical AD was strongly associated with an increased risk of clinical progression in a dose dependent manner (Table 2). The predictive value for clinical progression of SNAP only became significant after adjustment for age, gender and baseline MMSE.

### Table 2. Predictive value of APOE and NIA-AA preclinical stages in univariate models

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Clinical progression</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APOE genotype</strong></td>
<td></td>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 3</td>
</tr>
<tr>
<td>APOE4 neg.</td>
<td>134</td>
<td>11 (8%)</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>APOE4 pos.</td>
<td>90</td>
<td>17 (18%)</td>
<td>2.4 (1.1-5.2)</td>
<td>2.3 (1.1-5.1)</td>
<td>2.2 (1.0-4.9)</td>
</tr>
<tr>
<td><strong>Preclinical AD</strong></td>
<td></td>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 3</td>
</tr>
<tr>
<td>Stage 0</td>
<td>132</td>
<td>6 (5%)</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Stage 1</td>
<td>18</td>
<td>4 (22%)</td>
<td>5.8 (1.6-20.7)</td>
<td>6.9 (1.9-25.1)</td>
<td>9.6 (2.5-36.6)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>18</td>
<td>11 (61%)</td>
<td>19.1 (6.9-52.3)</td>
<td>22.3 (7.2-69.4)</td>
<td>23.2 (7.2-75.3)</td>
</tr>
<tr>
<td>SNAP</td>
<td>56</td>
<td>7 (13%)</td>
<td>2.4 (0.8-7.5)</td>
<td>2.5 (0.8-7.9)</td>
<td>3.4 (1.0-10.9)</td>
</tr>
</tbody>
</table>

Data are presented as HR (95%CI). APOE genotype and NIA-AA preclinical AD stages (including SNAP) were evaluated in separate models. In Model 1 APOE genotype or NIA-AA preclinical stage were evaluated as categorical variables. Model 2 was adjusted for age and sex. Model 3 was adjusted for age, sex and baseline MMSE.
When APOE genotype and NIA-AA preclinical AD stages were entered into a combined model, we found an interaction between APOE genotype and preclinical AD stage 1 \( (p<0.10) \). Therefore, we estimated predictive value of NIA-AA preclinical AD stages for APOE4 carriers and non-carriers separately (Table 3; figure 2). In APOE4 negative patients, NIA-AA preclinical AD stages were strongly associated with an increased risk of clinical progression in a dose-dependent manner. SNAP patients also tended to have an increased rate of clinical progression. In APOE4 positive patients, we found no added risk of clinical progression in patients with CSF evidence of NIA-AA stage 1 or SNAP, but patients in NIA-AA stage 2 had a twelve times increased risk of clinical progression compared to the baseline risk of APOE4 positive patients.
### Table 3. Predictive value of NIA-AA preclinical AD stages according to APOE genotype

<table>
<thead>
<tr>
<th>APOE4 non-carriers</th>
<th>APOE4 carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td><strong>Stage 0</strong></td>
<td>89</td>
</tr>
<tr>
<td><strong>Stage 1</strong></td>
<td>7</td>
</tr>
<tr>
<td><strong>Stage 2</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>SNAP</strong></td>
<td>33</td>
</tr>
</tbody>
</table>

Data are presented as n (%) or HR (95% CI). Clinical progression was defined as incidence of progression to MCI or dementia due to AD during follow-up. The Cox proportional hazard model included terms for APOE genotype (dichotomous), NIA-AA preclinical stages including SNAP and the interaction between NIA-AA preclinical stage and APOE genotype. Presented values are adjusted for age, sex and baseline MMSE. The hazard ratio associated with APOE4 positivity in patients in preclinical stage 0 was 4.6 (0.9-26.2).

### Figure 2. Kaplan-Meier curve for clinical progression based on NIA-AA preclinical stage for APOE4 carriers and non-carriers separately

Kaplan-Meier curves for clinical progression based on CSF evidence for NIA-AA preclinical AD. Black line: NIA-AA stage 0; Blue line: NIA-AA stage 1; Red line: NIA-AA stage 2; Grey line: SNAP patients. Vertical tics represent censored cases. (A) for APOE4 negative patients. Log rank test: p<0.001. (B) for APOE4 positive patients. Log rank test: p<0.001.
4. Discussion

The most important finding of this study is that APOE genotype modifies the predictive value of CSF evidence of preclinical AD in patients with subjective complaints. In APOE4 negative patients, each advancing preclinical AD stage increased the risk of clinical progression, while in APOE4 positive patients only NIA-AA stage 2 further increased baseline risk of clinical progression. Additionally, we found that APOE4 positivity on its own predicted clinical progression and we confirmed that preclinical AD predicted clinical progression in patients with subjective complaints.

Similar to previous research, the prevalence of abnormal CSF Aβ42 was higher in APOE4 positive than in APOE4 negative cognitively normal subjects.\textsuperscript{14,15} Counter-intuitively however, preclinical AD stage 1 did not increase risk of clinical progression in APOE4 carriers, while it did in APOE4 non-carriers. Baseline risk of clinical progression in APOE4 carriers was only increased when there was evidence of both amyloidosis and neuronal injury, which may imply that amyloid deposition on its own is an insufficient trigger for clinical progression in cognitively normal APOE4 carriers. This finding does not stand alone: we previously found similar results in a group of MCI patients.\textsuperscript{25} Increased prevalence of abnormal CSF Aβ42 concentrations lends support to the thought that APOE4 genotype increases risk of dementia due to AD by causing amyloid deposition in the brain (for a review see Liu et al., 2013\textsuperscript{26} and Holtzman et al., 2012\textsuperscript{27}). Based on our findings, one might hypothesize that amyloid pathology in APOE4 carriers may – in spite of its high prevalence – be relatively controllable until it is combined with evidence of neuronal injury. This is in line with results from a previous study, where Aβ42 in itself was reported not to be associated with volume loss, while the combination of CSF Aβ42 and pTau was.\textsuperscript{28} Aside from influencing amyloid deposition, APOE4 genotype is related to a number of other aspects that may be more closely related to clinical progression. Possible mechanisms for such an amyloid independent effect include vascular risk factors such as hypertension, diabetes mellitus and cholesterol metabolism,\textsuperscript{29,30} but also effects on synaptic plasticity and neuronal repair.\textsuperscript{26,27} It is conceivable that evidence of neuronal injury – in this study operationalized as increased levels of CSF tau – is an indicator of these amyloid independent effects. Hypothetically, in APOE4 carriers amyloid dependent and amyloid independent effects cause an increased risk of clinical progression when they are combined, while they are largely insufficient to add to the baseline risk of APOE4 positivity when only one of them is present.

In APOE4 non-carriers we found NIA-AA preclinical AD stages strongly increased the risk of clinical progression in a dose-dependent manner. This finding underlines that Alzheimer’s pathology can also follow a course leading to MCI or dementia due to AD that is unrelated to APOE4 positivity. When patients developed preclinical AD in spite of being APOE4 negative, they had a more aggressive disease course, which was especially evident in preclinical stage
1. This finding is in line with several previous studies: absence of the APOE4 allele in patients with dementia due to AD was associated with a higher whole-brain atrophy rate, and with shorter survival. In APOE4 non-carriers with MCI, biomarkers indicative of Alzheimer pathophysiology were associated with a strongly increased risk of clinical progression. The current findings provide support for the existence of this alternate pathway as early as the preclinical AD stage. It remains to be determined what triggers the deposition of amyloid in these APOE4 negative patients. Nevertheless, it seems that when amyloid deposition has occurred despite the absence of APOE4 genotype, the cascade of events leading to dementia will undoubtedly unfurl.

Our study has several strengths: it evaluated the predictive value of several important risk factors for AD in a large number of patients with subjective complaints, who may already be at increased risk of developing dementia due to AD. Also, the longitudinal character of this study allows inferences about causality. Follow-up extended up to nine years for individual patients, but was three years on average. This is substantial, but the study could be improved by longer follow-up as the preclinical phase of AD may take 15-20 years. Another reason for caution when interpreting our results is the small number of patients in each preclinical AD stage. Another possible limitation is the use of MCI as an outcome measure. Like subjective complaints, MCI is a heterogeneous disorder that could have multiple underlying causes. Still, four out of 24 patients who developed MCI subsequently developed dementia due to AD and none progressed to a different dementia diagnosis, which indicates AD is likely to be the predominant underlying disease process of patients who progressed to MCI.

Twenty-five percent of patients with subjective complaints fulfilled criteria for SNAP. Purely based on this prevalence it would seem unlikely that all these patients are on their way to a neurodegenerative disorder, which would mean SNAP could be an aspecific finding, at least in some patients with subjective complaints. However, in contrast to two population-based studies, SNAP did increase risk of clinical progression in our study in univariate models and at trend level in APOE4 non-carriers. As we also took MCI as outcome measure these patients could indeed be on their way to another form of dementia than AD. Alternatively, it may be argued that the predominant disease process in these patients is AD in spite of normal CSF Aβ42 values. Aβ42 values may be borderline normal and therefore counted as indicating no evidence of amyloidosis, while in fact there would be some amyloid deposition already. Alternatively, these could be patients where CSF tau concentrations change first, while Aβ42 changes later. This would mean the order of pathological events leading to dementia due to AD in these patients differs from the original hypothetical biomarker model as postulated by Jack et al., because tau pathology precedes amyloid pathology in SNAP patients. It would be more in line with the thought that CSF Aβ42 and tau represent independent processes that lead to AD only when they are combined. Finally, a number of these patients may suffer
from a form of AD pathology in which tangles remain histopathologically overrepresented until the dementia stage of the disease.\textsuperscript{38}

It has been supposed subjective complaints are a clinical stage along the Alzheimer continuum;\textsuperscript{2,3,6} but also that they are mostly indicative of depressive symptoms or certain personality traits.\textsuperscript{39,40} Our results indicate they are neither by definition benign, nor necessarily indicative of future dementia due to AD. In these patients, the predictive value of CSF biomarkers indicative of preclinical AD can be improved by additionally determining APOE genotype. This may, in future, have implications for the selection of patients for intervention studies.

**Acknowledgments**

Research of the VUmc Alzheimer center is part of the neurodegeneration research program of the Neuroscience Campus Amsterdam. The VUmc Alzheimer center is supported by Alzheimer Nederland and Stichting VUmc fonds. Wiesje M. van der Flier is recipient of a project grant for the Subjective Cognitive Impairment Cohort (SCIENCe) by the Gieskes-Strijbis fonds. The clinical database structure was developed with funding from Stichting Dioraphte.

**Disclosures**

The Alzheimer Center receives unrestricted funding from various sources through the VUmc Fonds. This study received no specific funding. A.C van Harten reports no disclosures. F. Duits reports no disclosures. Dr. Pijnenburg reports no disclosures. Dr. Teunissen served as a consultant for the international advisory board of Innogenetics and Roche. Dr. Oudejans reports no disclosures. Dr. Scheltens serves/has served on the advisory boards of: Novartis, Pfizer, Roche, Danone, Jansen Al, Baxter and Lundbeck. He has been a speaker at symposia organised by Lundbeck, Lilly, Merz, Pfizer, Jansen Al, Danone, and Roche. He is co-editor-in-chief of Alzheimer’s Research & Therapy. He is a member of the scientific advisory board of the EU Joint Programme on Neurodegenerative Disease Research (JPND) and the French National Plan Alzheimer. He acts as vice-chair of the Dutch Deltaplan Dementia. Dr. Scheltens receives no personal compensation for the activities mentioned above. Dr. van der Flier reports no disclosures.
Reference List


38 Nelson PT, Kukull WA, Frosch MP. Thinking outside the box: Alzheimer-type neuropathology that does not map directly onto current consensus recommendations. *J Neuropathol Exp Neurol* 2010;69:449-54.
