PROGRESSION FROM MCI TO AD: PREDICTIVE VALUE OF CSF Aβ42 IS MODIFIED BY APOE GENOTYPE

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ABSTRACT

Objective: To study CSF biomarkers amyloid-beta 1-42 (Aβ42) and total tau (tau) in relation to APOE genotype in their ability to predict progression from mild cognitive impairment (MCI) to Alzheimer’s disease (AD).

Methods: In 100 MCI patients CSF Aβ42, tau and APOE genotype were determined. At follow-up of 18(13-24) months 58 patients remained non-progressive and 42 progressed to AD.

Results: Cox proportional hazards models showed an interaction between Aβ42 and APOE genotype (p<0.05). Stratification for APOE revealed HR(95% CI) for abnormal Aβ42 of 8.2(2.1-31.9) for ε4 non-carriers, 3.9(0.8-18.5) for heterozygotes and 0.3(0.0-1.7) for homozygotes. Inversely, stratification for Aβ42 revealed that in patients with normal levels of Aβ42, ε4 homozygotes had a strongly increased risk of progression to AD with HR(95%CI) 20.8(2.4-182.8). Tau and APOE independently predicted progression to AD.

Conclusions: Aβ42 was a stronger predictor of progression to AD in APOE ε4 non-carriers than in carriers. Furthermore, the risk of progression for ε4 homozygotes was very high, also in patients with normal levels of Aβ42.

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INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia. Patients with mild cognitive impairment (MCI) do not have overt dementia and are not cognitively normal for age either. A substantial proportion of MCI patients will progress to dementia, mostly AD, during follow-up. Adequate identification of MCI patients prone to develop AD is essential to patients and their caregivers, and will become even more important when new drug candidates prove to have disease arresting effects.

The neuropathological hallmarks of AD are progressive accumulation of plaques with amyloid-beta and neurofibrillary tangles containing (phosphorylated) tau. In AD, levels of CSF amyloid-beta 1-42 (Aβ42) are decreased, whereas levels of total tau (tau) are increased. CSF Aβ42 and tau have been proven sensitive and specific in the diagnosis of AD, and are thought to reflect pathology. Pathological changes like plaques and tangle formations are present before the onset of clinical dementia and CSF changes can already be detected in patients with MCI who will progress to AD.

Besides age, the APOE ε4 genotype is an important risk factor for AD. APOE ε4 genotype has been associated with an increased rate and extent of amyloid-beta deposition in post-mortem studies. This seems a possible pathological pathway for the APOE ε4 genotype as risk factor. Furthermore, this genotype has been related to cardiovascular problems, increased inflammation and decreased plasticity of the brain.

APOE ε4 genotype and abnormal CSF biomarkers have both been described as predictors of cognitive decline in MCI patients. In the current study, we aimed to examine the combined value of the CSF levels of Aβ42 and tau in relation to APOE ε4 genotype, as predictors for progression to AD in a group of MCI patients.

METHODS

Study population

In the period between January 2001 and May 2008, 153 patients with available CSF results and APOE ε4 genotyping were diagnosed with MCI in our memory clinic. Of 107 of these MCI patients we had follow-up data. Patients underwent a standardized clinical assessment, including medical history, physical, neurological and neuropsychological examination including mini-mental state examination (MMSE), laboratory tests, EEG and brain MRI as described earlier. Of all patients we had information about education defined in the Verhage scale. At least one follow-up investigation was performed in all MCI patients. Follow-up time was defined as the time until date of dementia diagnosis (in case of progression to dementia) or until the last visit to the memory clinic (in case of non-progressive MCI). The median follow-up period was 18 (IQR 13-24) months. The initial and follow-up diagnoses were made by consensus in a multidisciplinary team. Criteria of Petersen and co-workers were used for MCI. At follow-up, NINCDS-ADRDA criteria were used for AD. Of the 107 MCI patients with clinical follow-up, 58 remained non-progressive and 42 progressed to AD. Three patients progressed to frontotemporal lobar degeneration, 2 to vascular dementia, 1 to dementia with Lewy bodies, and
1 developed dementia due to normal pressure hydrocephalus. These patients were excluded from this study, resulting in a total number of 100 patients that were included in the analysis. The baseline characteristics of the 100 patients included in our analysis did not differ from the baseline characteristics of the MCI patients without clinical follow-up. The study was approved by the local ethical review board and all subjects gave written informed consent.

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 x g for 10 minutes in 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 and tau were measured with Innotest sandwich ELISA as described previously. As the manufacturer does not supply controls, the performance of the assays was monitored with pools of surplus CSF specimens. In the study period multiple specimens with various concentrations, which were included in 7 to 18 runs, have been used for this purpose. The inter-assay coefficient of variation (mean±SD) was 11.3±4.9% for Aβ42 and 9.3±1.5% for tau. The team involved in the CSF analysis was not aware of the clinical diagnoses. Levels of CSF Aβ42 <495 pg/ml and CSF tau >356 pg/ml were considered abnormal.

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 non-carriers, APOE ε4 heterozygotes or APOE ε4 homozygotes.

Statistical analysis
For statistical analysis, SPSS version 15.0 (for Windows) was used. Results are expressed as means (SD) unless indicated otherwise. Frequency distributions for categorical variables were compared with chi-squared tests. Student’s T-tests or Analyses of Variance were used to compare age and MMSE. Mann-Whitney-U or Kruskal-Wallis tests were used to compare duration of follow-up, education level and CSF biomarker levels between groups. Cox proportional hazards models were used to assess associations between CSF biomarkers and APOE genotype (independent variables) and time to diagnosis of AD (dependent variable). In the first model we assessed the predictive value of Aβ42, tau (both dichotomized as normal or abnormal) and APOE genotype (non-carriers, ε4 heterozygotes, ε4 homozygotes) separately. In a second model, the combination of CSF biomarkers and APOE genotype was evaluated (separate models for Aβ42 and tau): the CSF biomarker and APOE genotype were entered simultaneously. If there was a significant interaction between the CSF biomarker and APOE genotype, the interaction term (CSF biomarker*APOE genotype) was also included in the model. To provide further insight in how the predictive value
of the CSF biomarker was modified by APOE genotype and vice versa, the analysis was subsequently stratified (i.e. in case the interaction term CSF biomarker*APOE genotype was significant). We first stratified by APOE genotype, and then by CSF biomarker. All Cox proportional hazards models were adjusted for age, sex and education level. Data are presented as hazard ratios (HR's) with accompanying 95% confidence interval (CI). A p-value <0.05 was considered significant.

RESULTS

Baseline characteristics of non-progressive and progressive MCI patients are presented in Table 1. Non-progressive MCI patients and MCI patients who progressed to AD did not differ with respect to sex, age, MMSE or duration of follow up. MCI patients who progressed to AD were more often APOE ε4 homozygous carriers and less often ε4 non-carriers than the non-progressors (p<0.005). The group of MCI patients who progressed to AD had lower levels of CSF Aβ42 and higher levels of CSF tau than the group of non-progressive MCI patients (p<0.001). In Table 2 baseline characteristics by APOE genotype are shown. The proportion of MCI patients progressing to AD increased with

### Table 1. Baseline characteristics by follow-up diagnosis group

<table>
<thead>
<tr>
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<th>Non-progressive MCI (n=58)</th>
<th>Progressive MCI (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender = female, n (%)</strong></td>
<td>20 (35%)</td>
<td>21 (50%)</td>
</tr>
<tr>
<td><strong>Age, mean (SD)</strong></td>
<td>67 (9)</td>
<td>69 (7)</td>
</tr>
<tr>
<td><strong>MMSE, mean (SD)</strong></td>
<td>27 (2)</td>
<td>26 (3)</td>
</tr>
<tr>
<td><strong>Education level, (SD)</strong></td>
<td>5 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td><strong>Follow up period, months, median (IQR)</strong></td>
<td>18 (12-25)</td>
<td>17 (13-24)</td>
</tr>
<tr>
<td><strong>APOE ε4 non-carrier, n (%)</strong></td>
<td>31 (53%)</td>
<td>12 (29%)</td>
</tr>
<tr>
<td><strong>APOE ε4 heterozygote, n (%)</strong></td>
<td>22 (38%)</td>
<td>17 (41%)</td>
</tr>
<tr>
<td><strong>APOE ε4 homozygote, n (%)</strong></td>
<td>5 (9%)</td>
<td>13 (31%)**</td>
</tr>
<tr>
<td><strong>Aβ42, median (IQR)</strong></td>
<td>608 (471-914)</td>
<td>405 (353-495)**</td>
</tr>
<tr>
<td><strong>Abnormal Aβ42, n (%)</strong></td>
<td>16 (28%)</td>
<td>32 (76%)**</td>
</tr>
<tr>
<td><strong>Tau, median (IQR)</strong></td>
<td>368 (216-523)</td>
<td>698 (462-908)**</td>
</tr>
<tr>
<td><strong>Abnormal tau, n (%)</strong></td>
<td>29 (50%)</td>
<td>35 (83%)**</td>
</tr>
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</table>

MCI= Mild Cognitive Impairment, MMSE= mini-mental state examination, education defined in the Verhage scale, Aβ42= amyloid-beta 1-42, IQR= interquartile range, CSF Aβ42 <495 pg/ml and CSF tau >356 pg/ml were considered abnormal. For each patient group (non-progressive or progressive) the percentage per APOE genotype is given.

* Significant difference for distribution of APOE genotypes between non-progressive and progressive patients with linear-by-linear association (p<0.005)

** Significant difference for comparisons between non-progressive and progressive patients (p<0.001)
the number of APOE ε4 alleles (p<0.005). In addition, CSF levels of Aβ42 were lower and tau levels were higher with an increasing number of ε4 alleles (p<0.001).

Cox proportional hazards models showed that, after adjustment for age, sex and education, abnormal Aβ42 was associated with a more than threefold increased risk of progression, while abnormal tau was associated with a five times increased risk of progression to AD (Table 3, model 1). APOE ε4 genotype was a moderate predictor, with a 40% albeit non-significantly increased risk for the ε4 heterozygous and a threefold increased risk for the homozygous ε4 carriers.

Subsequently, we combined each CSF biomarker with APOE genotype in model 2. Abnormal CSF tau and carriership of APOE ε4 independently predicted progression, without interaction. Risk estimates remained in the same order of magnitude. Combining Aβ42 and APOE genotype, we found an interaction (p<0.05). When the interaction term (Aβ42*APOE genotype) was included in the model, the risk of progression of both abnormal Aβ42 and APOE genotype increased (Table 3). This implies that the predictive value of Aβ42 is strongest in the absence of APOE ε4, while the predictive value of APOE ε4 homozygosity is strongest in patients with normal Aβ42 values.

To further examine the interaction between APOE genotype and CSF level of Aβ42, we then stratified the analysis by APOE genotype. As an illustration for these data we show Aβ42 levels by APOE genotype for MCI non-progressors and MCI progressors in Figure 1. The predictive effect of abnormal Aβ42 was strongest for APOE ε4 non-carriers HR (95%CI) = 8.2 (2.1-31.9), and was lower for heterozygous 3.9 (0.8-18.5) and homozygous 0.3 (0.0-1.7) ε4 carriers. Subsequently, the analysis was stratified for Aβ42 level. For MCI patients with normal levels of Aβ42, homozygous ε4 carriers had a strongly increased risk of HR (95%CI) = 20.8 (2.4-182.8) compared to ε4 non-carriers. Heterozygotes had a risk estimate of 1.6 (0.3-9.7), in comparison with non-carriers. For patients with abnormal levels of Aβ42 there was no added predictive value of APOE ε4 genotype (homozygotes: HR (95%CI) = 0.7 (0.2-1.8); heterozygotes:

<table>
<thead>
<tr>
<th>Table 2. Baseline characteristics by APOE genotype</th>
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<tr>
<td>APOE ε4 non-carriers (n=43)</td>
</tr>
<tr>
<td>Gender = female, n (%)</td>
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<tr>
<td>Age, mean (SD)</td>
</tr>
<tr>
<td>MMSE, mean (SD)</td>
</tr>
<tr>
<td>Education level, (SD)</td>
</tr>
<tr>
<td>Follow up period, months, median (IQR)</td>
</tr>
<tr>
<td>Progression rate, n (%)</td>
</tr>
<tr>
<td>Aβ42, median (IQR)</td>
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<tr>
<td>Tau, median (IQR)</td>
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</tbody>
</table>

* Significant difference of progression rates between the APOE genotypes with linear-by-linear association (p<0.005);
** Significant difference for comparisons between the APOE genotypes (p<0.001)
Table 3. Risk estimates for abnormal biomarker and progression to Alzheimer's disease in relation to APOE ε4 genotype

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Model 1 Separate</th>
<th>Model 2 Combined</th>
<th>Model 2* Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal Aβ42</td>
<td>3.2 (1.6-6.7)</td>
<td>-</td>
<td>7.9 (2.4-26.3)</td>
</tr>
<tr>
<td>Abnormal tau</td>
<td>5.2 (2.0-13.9)</td>
<td>4.9 (1.8-13.5)</td>
<td>-</td>
</tr>
<tr>
<td>APOE ε4 heterozygote</td>
<td>1.4 (0.6-2.9)</td>
<td>0.9 (0.4-2.0)</td>
<td>1.1 (0.2-5.7)</td>
</tr>
<tr>
<td>APOE ε4 homozygote</td>
<td>3.1 (1.4-6.9)</td>
<td>2.1 (0.9-4.7)</td>
<td>10.1 (2.2-46.8)</td>
</tr>
</tbody>
</table>

Cox proportional hazard models with duration of follow-up as time variable and progression to AD as dependent variable. Data are presented as hazard ratios (HR) with 95% confidence interval (95%CI). CSF Aβ42 <495 pg/ml and CSF tau >356 pg/ml were considered abnormal. APOE was considered a categorical variable, with ε4 non-carriers as reference category. In model 1, separate analyses were performed for each biomarker, with adjustment for age and sex. In model 2, APOE was entered simultaneously with a CSF biomarker, corrected for sex, age and education (separate columns for tau and Aβ42). APOE and tau were independently related to progression to AD, without interaction. In the model containing APOE and Aβ42, there was a significant interaction between these two markers, and the interaction term was therefore added to the model.

* Interaction term Aβ42 and APOE genotype p<0.05.

Figure 1. Box and whisker plots for Aβ42 levels in pg/ml per group of APOE genotype, with separate boxes for MCI non-progressors and MCI who progressed to AD. Light grey boxes represent MCI non-progressors and dark grey boxes represent MCI-progressors.
HR (95%CI) = 0.4 (0.1-1.1) in comparison to non-carriers). In summary, these data show that the majority of APOE ε4 homozygous MCI patients progressed to AD, even when they had normal levels of Aβ42. Conversely, the majority of patients with abnormal Aβ42 levels progressed to AD, even when they were APOE ε4 non-carrier. The added predictive value of Aβ42 levels was largest in the MCI patients that did not carry the APOE ε4 allele.

DISCUSSION

The main finding of this study was that the predictive value of CSF Aβ42 for progression to AD was modified by APOE genotype. Besides, we confirm previous studies in which both Aβ42 and tau were good predictors for progression of MCI to AD.6,8,15,16 APOE genotype appeared a moderate predictor, with a threefold increased risk for the ε4 homozygotes and a 40% albeit not significantly increased risk for the ε4 heterozygotes.

In this study we found that the added predictive value of abnormal Aβ42 levels for progression to AD was higher in APOE ε4 non-carriers than in APOE ε4 carriers. The risk of AD associated with abnormal Aβ42 was over eight times higher in the non-carriers, it was fourfold (although not significantly) higher in heterozygotes, whereas there was no additional risk of AD associated with abnormal Aβ42 in the homozygote MCI patients. Conversely, the added value of APOE ε4 carriership was especially evident in patients with normal levels of Aβ42, with an over twenty times increased risk for progression of APOE ε4 homozygotes compared to non-carriers, while there was no added effect of the APOE genotype in patients with abnormal Aβ42 levels.

The results from this study may have implications for the use of CSF biomarkers in the diagnostic work-up of dementia. Tau and APOE genotype can be interpreted independently for the prediction of progression from MCI to AD. However, our data lend support to the interpretation of CSF Aβ42 levels in relation to APOE genotype. In APOE ε4 non-carriers, abnormal Aβ42 should make a clinician alert for pre-Alzheimer’s disease, whereas normal CSF biomarkers could be reassuring. In APOE ε4 carriers, especially homozygotes, the risk of developing AD is in general high, reducing the added value of CSF biomarker levels.

CSF tau has been suggested to reflect the degree of neuronal damage.23-25 It is also elevated after a cerebrovascular accident or in Creutzfeldt-Jakob disease. In our study the predictive value of tau was not modified by APOE genotype. This could be considered in agreement with the hypothesis that tau is a more general marker for brain damage.

Although it has been repeatedly shown that patients with APOE ε4 develop AD at a younger age,9 we did not find an age difference according to APOE ε4 genotype in our sample of MCI patients. For MCI patients, it has not been established that APOE ε4 carriers are younger than APOE ε4 non-carriers. MCI is not the same as pre-AD: not all MCI patients will progress to AD. Conversely, not all AD patients pass through a phase of MCI. Our findings are in agreement with a former study reporting age per APOE genotype in a memory clinic cohort of MCI patients which also showed no age difference between the APOE genotypes.26 A possible explanation for the lack of age difference is that older APOE ε4 non-carriers do not visit a memory clinic in an early
phase of the disease, due to the fact that they more often have non-memory cognitive problems.\textsuperscript{27} It could be considered as a limitation, that it is not completely clear to what extent CSF biomarkers reflect cerebral pathology. A post-mortem study revealed very loose correlations between Braak stages and the CSF biomarkers.\textsuperscript{28} However, in vivo amyloid imaging studies using PET showed that increased amyloid deposition was reflected by reduced levels of CSF Aβ42.\textsuperscript{29,30} In this study we used Cox proportional hazard models to predict progression of MCI to AD. The advantage of this model is that it takes into account variability in time to event, correcting for differences in progression times. However, the process of progression is in reality a continuum, rendering any account of exact date of transition arbitrary to some extent, which could also be seen as a limitation. We have used the date of clinical AD diagnosis as the best possible approximation. These data most likely give information on how near patients with MCI are to developing clinical AD.

The exact mechanism of APOE genotype, by which it acts as a risk factor for the development of AD, is unknown. APOE ε4 carriers usually have lower CSF levels of Aβ42 than those lacking the ε4 allele.\textsuperscript{31} In our current study of MCI patients, we also found that overall the levels of Aβ42 were lower in APOE ε4 carriers, especially among the homozygotes. This finding seems associated with an increased burden of amyloid plaques in the parenchyma of APOE ε4 carrying healthy individuals compared to those who lack the ε4 allele, as has also been shown in amyloid imaging studies using PIB PET.\textsuperscript{32} Furthermore, the APOE ε4 genotype has been associated with an increased rate and extent of amyloid-beta deposition and neurofibrillary tau pathology in post-mortem studies.\textsuperscript{9,10} This effect could be caused by a direct up-regulatory effect of APOE ε4 genotype on amyloid plaque formation.\textsuperscript{9,10,31} Following this line of reasoning, we would have expected that the APOE ε4 genotype would be a confounder of the predictive effect of abnormal levels of CSF Aβ42. However, in our data the APOE ε4 genotype acts as an effect modifier, rather than as a confounder. The predictive effect of Aβ42 levels and APOE genotype are strongest when the effect of the other predictor is not there. Hence, the predictive effect of low levels of Aβ42 is best in APOE ε4 non-carriers, and there is hardly any added predictive effect in APOE ε4 homozygotes. Conversely, the predictive effect of APOE ε4 genotype is best in patients with normal levels of Aβ42, and there is hardly any added predictive effect of the APOE genotype in patients with abnormal Aβ42 levels.

There are several other hypotheses regarding the mechanism of APOE ε4 genotype as risk factor for clinical AD. First, APOE ε4 genotype has been associated with an increased risk for cardiovascular problems, which in turn have been shown to be related to clinical AD.\textsuperscript{12,34} Secondly, inflammation is seen as a contributor to AD pathology and has been shown to be dependent on APOE ε4 genotype.\textsuperscript{14,35,36} Thirdly, recent studies showed that APOE ε4 mice models had an impaired synaptic plasticity in the cortex and hippocampus following environmental stimulation, in comparison to APOE ε3 mice, indicating that the APOE ε4 genotype seems less able to adjust its cognition to a more challenging environment.\textsuperscript{13} All of the above mentioned mechanisms may play a role for APOE ε4 carriers in the progression of MCI to AD. An explanation for the more loose relation between progression and Aβ42 (and thus amyloid) in APOE ε4 homozygosity could be that other damage processes (vascular, inflammation and decreased plasticity) associated with APOE ε4 genotype also play
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a role. The AD pathology could in a larger extent be multifactorial in the APOE ε4 homozygous patients.

Our results may provide a glimpse into a future of more individually tailored therapy. The evolutionary ‘newer’ alleles ε2 and ε3 may be associated with improvements for humans compared to the ‘older’ ε4 allele. The ε2 and ε3 alleles are related to less amyloid pathology, but also with less cardiovascular problems, inflammation and increased neuroplasticity. It is conceivable that for APOE ε4 carriers (especially for homozygotes) therapeutic strategies should not solely focus on amyloid-beta plaque reduction, but also on managing other processes that are involved in AD as well. As an example, a recent publication describing risk reducing effect of NSAID for the development of AD, found that this effect was there only for those with the APOE ε4 genotype.37 Furthermore, it could imply that anti-amyloid therapy in humans is more effective in APOE ε4 non-carriers than in ε4 carriers.

REFERENCES

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