JOINT EFFECT OF HYPERTENSION AND APOE GENOTYPE ON CSF BIOMARKERS FOR ALZHEIMER DISEASE
ABSTRACT

We examined the impact of hypertension on CSF biomarkers amyloid-beta 1-42 (Aβ42), total tau (tau) and phosphorylated tau at threonine 181 (ptau-181), and assessed the modifying role of APOE genotype in this relation in 546 patients (mean age 65±10, 47% female) from our memory-clinic. Of these patients 150 had subjective complaints, 140 were diagnosed with mild cognitive impairment, and 256 with Alzheimer’s disease. Linear regression analyses adjusted for age, sex, and diagnosis showed that the association of hypertension with tau and ptau-181 was modified by APOE genotype (p-values for interaction <0.05). In APOE ε4 homozygotes (n=74), and to a lesser extent in APOE ε4 heterozygotes, hypertension was associated with higher tau and ptau-181 levels; β (95%CI) were 188 (11; 364) pg/mL and 22 (3; 42) pg/mL for the APOE ε4 homozygotes. Hypertension was not associated with Aβ42 levels, and APOE genotype did not modify this relation. Our findings suggest that hypertension is directly related to tau pathology in APOE ε4 homozygous carriers.

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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-β (Aβ) and neurofibrillary tangles containing (phosphorylated) tau. \(^1\) Aβ_{1-42} (Aβ42), total tau (tau) and tau phosphorylated at threonine 181 (ptau-181) in cerebrospinal fluid (CSF) have been proven useful biomarkers for the pathology of AD.\(^2,3\)

Over the last decades, evidence has accumulated that risk factors for vascular disease and stroke, including hypertension, are associated with an increased risk for AD.\(^4,6\) It is not yet clear which mechanisms underlie the association between hypertension and AD. Hypertension could cause cerebral small vessel pathology observed as white matter hyperintensities and lacunes on MRI.\(^7\) The small vessel pathology in turn may contribute to the increased risk of AD. Alternatively, hypertension may be associated directly to AD by deposition of amyloid plaques and neurofibrillary tangles in the brain. Limited evidence exists to support this latter possibility. Autopsy studies reported that hypertension was associated with greater numbers of amyloid plaques and neurofibrillary tangles in the brain.\(^8,9\)

A possible modifying factor in the association of hypertension with AD and its markers might be the apolipoprotein E (APOE) ε4 genotype. The APOE ε4 genotype is an important risk factor for AD.\(^10\) The exact mechanism through which APOE influences the development of AD is yet unknown. It has been hypothesized that the APOE ε4 allele is associated with an impaired response to cerebral damage due to, for example, hypertension. This hypothesis has been confirmed by previous studies showing that APOE ε4 carriers with hypertension and other vascular risk factors are most severely affected with respect to white matter lesions, cognitive impairment, and dementia.\(^7,11,12\)

In this study we examined whether hypertension was associated with CSF biomarker levels of Aβ42, tau and ptau-181 and whether the APOE genotype modified these associations in a large memory clinic cohort, including patients with subjective complaints, mild cognitive impairment (MCI) and AD.

METHODS

Study population

For this study we included 546 consecutive patients with a diagnosis of AD, MCI, or subjective complaints with available data on CSF measures, blood pressure status, and APOE genotyping. Of these 546 patients, 150 patients had subjective complaints, 140 patients were diagnosed with MCI, and 256 with AD. 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.\(^1\) The diagnosis of MCI was made according to the criteria of Petersen and co-workers.\(^13\) Patients with other types of dementia, e.g. vascular dementia as defined in the NINCDS-AIREN criteria, were not included.\(^14\) Patients with aspecific
CHAPTER 2.2

Signs of cerebrovascular pathology on MRI, and who did not fulfill criteria for vascular dementia, but fulfilled the criteria for MCI or AD were eligible. When the results of all examinations were normal, patients were considered to have subjective complaints. Patients with a psychiatric disorder, like depression, were not included in this group. Diagnoses were made by consensus of a multidisciplinary team, without knowledge of CSF results and the APOE genotype.

Furthermore, patients were asked about education, current use of medication, alcohol use, smoking history and medical history. Diabetes mellitus was defined as use of glucose-lowering agents, a known history of diabetes, a non-fasting plasma glucose level >11.0 mmol/L, or when available, a fasting plasma glucose level >6.9 mmol/L. Hyperlipidemia was defined as total cholesterol >6.5 mmol/L, low-density lipoprotein cholesterol >2.5 mmol/L or use of lipid lowering drugs. The local ethical review board approved the study and all patients gave written informed consent.

Hypertension

During the first visit to the memory clinic, blood pressure was measured manually in a standardized manner using a sphygmomanometer with the patient in sitting position after five minutes of rest. The first and the fourth Korotkoff sounds were used for the systolic and diastolic blood pressure (SBP and DBP). Values were based on a single measurement. Hypertension was defined as presence of a known history of hypertension, a SBP of ≥140 mm Hg, a DBP of ≥90 mm Hg, or the use of blood pressure lowering drugs.15

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 at 4°C. A small amount of CSF was used for routine analysis, including total cells, 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.16 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 to18 runs, have been used for this purpose. The inter-assay coefficient of variation (mean ± SD) was 11.3 ± 4.9% for Aβ42, 9.3 ± 1.5% for tau and 9.4 ± 2.5% 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). Patients were classified as APOE ε4 non-carriers, APOE ε4 heterozygous, or as APOE ε4 homozygous carriers.
Data analysis

First, patient characteristics were calculated for the separate diagnosis categories (patients with subjective complaints, MCI and AD). Differences between diagnosis categories were assessed using ANOVA (with post hoc Bonferroni corrections for baseline characteristics) or Fisher exact test when applicable. Second, linear regression analysis was used to investigate the association of hypertension with CSF biomarker levels of Aβ42, tau and ptau-181 adjusted for age, sex, APOE genotype, and diagnosis category. Next, we added interaction terms between hypertension and APOE genotype (continuous variable) to the linear regression models. Third, if the regression analyses revealed a significant interaction, we investigated the association between hypertension and CSF biomarker levels within strata of APOE genotype (ε4 non-carriers, ε4 heterozygotes and ε4 homozygotes). To assess the independent relation of hypertension and CSF biomarker levels, analyses were additionally adjusted for diabetes mellitus, hypercholesterolemia, smoking, and alcohol use. Subsequently, to further evaluate the role of blood pressure levels, and the use of anti-hypertensive medication, in the relation between hypertension and CSF biomarker levels, we first analyzed continuous measures of SBP and DBP, and secondly divided the hypertensive subjects in groups with or without anti-hypertensive medication. For these analyses we used the same models as described above, but with altered definitions of hypertension. Finally, to assess whether the associations of blood pressure measures, APOE genotype and CSF biomarker levels were different across diagnosis categories, analyses were repeated within the separate diagnosis categories (patients with subjective complaints, MCI, AD). In all analyses the 95% confidence intervals (CI) are given. SPSS version 15.0 (Chicago, Ill, USA) was used to analyze the data.

RESULTS

In the total population of 546 patients mean ± SD age was 65 ± 10 years and 47% was female.

Table 1 presents the patient characteristics according to diagnosis. Patients with AD and MCI were older, had lower education, had a lower score on the MMSE, and had a higher prevalence of the APOE ε4 allele than patients with subjective complaints. Furthermore, patients with MCI and AD more often had hypertension, and had higher SBP than patients with subjective complaints. The differences in SBP between the diagnosis categories were accountable to age (data not shown). CSF biomarker levels were different between the diagnosis categories with the lowest Aβ42 levels and highest tau and ptau-181 levels in the AD group.

In the total population presence of hypertension was not associated with lower levels of Aβ42 and higher levels of tau and ptau-181; β (95% CI) adjusted for age, sex, diagnosis, and APOE genotype were -2 (-39; 34) pg/mL, 54 (-10; 117) pg/mL and 2 (-4; 8) pg/mL. When interaction terms between hypertension and APOE ε4 genotype were added to the models we found that APOE ε4 genotype modified the association of hypertension with tau and ptau-181 (p-interaction: 0.05 and 0.02), indicating that there was an APOE ε4 allele dose-dependent effect of hypertension on CSF tau and
### Table 1. Patient characteristics for the separate diagnosis categories.

<table>
<thead>
<tr>
<th></th>
<th>Subjective complainers (n=150)</th>
<th>MCI (n=140)</th>
<th>AD (n=256)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60 (52-67)</td>
<td>70 (62-74)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>68 (60-74)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex, n (%) female</td>
<td>62 (41%)</td>
<td>65 (46%)</td>
<td>132 (52%)</td>
</tr>
<tr>
<td>Education (range 1-7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.4 ± 1.3</td>
<td>4.9 ± 1.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.7 ± 1.4&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>MMSE (range 0-30)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29 (28-30)</td>
<td>27 (25-29)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>22 (19-25)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>APOE ε4 genotype</td>
<td></td>
<td></td>
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<tr>
<td>ε4 non-carrier, n (%)</td>
<td>93 (62%)</td>
<td>69 (49%)</td>
<td>82 (32%)</td>
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<tr>
<td>ε4 heterozygote, n (%)</td>
<td>47 (31%)</td>
<td>48 (34%)</td>
<td>133 (52%)</td>
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<tr>
<td>ε4 homozygote, n (%)</td>
<td>10 (7%)</td>
<td>23 (16%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>41 (16%)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>71 (47%)</td>
<td>102 (73%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>160 (63%)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>History of hypertension, n (%)</td>
<td>21 (14%)</td>
<td>43 (31%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>60 (24%)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antihypertensive drugs, n (%)</td>
<td>29 (20%)</td>
<td>53 (38%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>71 (28%)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Systolic BP (mmHg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138 ± 21</td>
<td>147 ± 22&lt;sup&gt;*&lt;/sup&gt;</td>
<td>145 ± 21&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85 ± 10</td>
<td>86 ± 10</td>
<td>86 ± 10</td>
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<tr>
<td>Vascular risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>15 (10%)</td>
<td>17 (12%)</td>
<td>24 (9%)</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>89 (60%)</td>
<td>75 (54%)</td>
<td>136 (53%)</td>
</tr>
<tr>
<td>Smoking current, n (%)</td>
<td>22 (16%)</td>
<td>25 (19%)</td>
<td>47 (20%)</td>
</tr>
<tr>
<td>Alcohol use (units/day)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 (0.0-2.0)</td>
<td>1.0 (0.5-2.0)</td>
<td>1.0 (0.0-2.0)</td>
</tr>
<tr>
<td>CSF Biomarkers</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Aβ42 (pg/mL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>842 (678-1029)</td>
<td>518 (407-717)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>446 (369-535)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>tau (pg/mL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>251 (195-320)</td>
<td>423 (272-707)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>607 (418-868)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>ptau-181 (pg/mL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44 (36-54)</td>
<td>64 (44-92)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>79 (60-108)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> mean ± SD, <sup>b</sup> median (interquartile range), <sup>*</sup> p<0.05 versus subjective complainers, <sup>**</sup> p<0.05 versus MCI patients, Aβ42: amyloid-beta 42; AD: Alzheimer’s disease; BP: blood pressure; MCI: mild cognitive impairment; MMSE: mini mental state examination.

### Table 2. Differences in tau and ptau-181 levels (pg/mL) between hypertensive en non-hypertensive patients within strata of APOE genotype.

<table>
<thead>
<tr>
<th>Hypertension</th>
<th>tau (pg/mL)</th>
<th>ptau-181 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>APOE ε4 non-carriers (n=244)</td>
<td>13 (-59; 84)</td>
<td>-2 (-10; 7)</td>
</tr>
<tr>
<td>APOE ε4 heterozygotes (n=228)</td>
<td>63 (-57; 183)</td>
<td>1 (-9; 12)</td>
</tr>
<tr>
<td>APOE ε4 homozygotes (n=74)</td>
<td>188 (11; 364)</td>
<td>22 (3; 42)</td>
</tr>
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</table>

Adjusted for sex, age and diagnosis categories.
ptau-181 levels. APOE ε4 genotype did not modify the association of hypertension with Aβ42 (p-interaction: 0.33).

Table 2 and Figure 1 present the results of the adjusted association of hypertension with tau and ptau-181 levels within strata of APOE genotype. As can be seen, hypertension was associated with higher tau and ptau-181 levels in APOE ε4 homozygotes, but not in APOE ε4 heterozygotes and APOE ε4 non-carriers. Further adjustment for diabetes mellitus, hypercholesterolemia, smoking, and alcohol use did not change the effect estimates (data not shown).

Furthermore, SBP and DBP levels were not related to CSF biomarker levels and this relation was not modified by APOE genotype. In addition, the relations between hypertension and tau and ptau-181 levels were not different for patients with or without anti-hypertensive medication (data not shown).

Finally, the combined effect of hypertension and APOE homozygosity on tau and ptau-181 levels was in the same order of magnitude across diagnosis categories, albeit analyses had insufficient power. Figure 2 presents the relation between hypertension, APOE genotype and ptau-181 levels for the diagnosis categories. Similar results were found for the effect on tau levels (data not shown).

**DISCUSSION**

In this study in a memory-clinic population we showed that the association of hypertension with CSF tau and ptau-181 levels was modified by APOE genotype. In APOE ε4 homozygous patients, and to a lesser extent in APOE ε4 heterozygous patients, hypertension was independently associated with higher levels of CSF tau.
and ptau-181, whereas in the non-carriers it was not. Hypertension was not associated with Aβ42 levels and APOE genotype did not modify this relation.

To our knowledge, this is the first study examining the impact of hypertension on CSF biomarker levels, while at the same time assessing the modifying role of APOE genotype in this relation. Our observation that hypertension in combination with ε4 homozygosity was associated with increased total tau and ptau-181 levels, is in line with previous post-mortem studies. These studies have shown that patients with a history of midlife hypertension more often had AD pathology like neurofibrillary tangles, than those without this risk factor.8,9 Total tau is considered to be a more general marker for neuronal damage,17,18 whereas ptau-181 is considered to be a specific biomarker for AD as it is related to the process of neurofibrillary tangle formation.19,20 These findings and ours could support the hypothesis that hypertension is directly associated to AD by tangle formation in the brain.

In our cross-sectional study we found that blood pressure as a continuous variable was not related to CSF biomarker levels, whereas defined hypertension (including patients with a history of hypertension and patients on antihypertensive medication) was related to increased total tau and ptau-181 levels. This seems in line with previous studies showing that mainly mid-life hypertension was associated with increased risk of AD, and its pathology, at later life.5,8,12
We found that the effect of hypertension on tau and ptau-181 levels was most robust APOE ε4 homozygous carriers which is in line with earlier population-based studies showing that the effect of hypertension on cognitive impairment, subcortical white matter lesions, and medial temporal atrophy was larger in APOE ε4 carriers than in APOE ε4 non-carriers.7,12,21,22 These results and ours suggest that the effect of hypertension on neuronal cell damage and tau tangle formation seems to be aggravated by the ε4 genotype. There are several explanations for the interaction between hypertension and APOE genotype in relation to tau pathology. First, the APOE genotype has a central role in lipid metabolism, and ε4 carriers have an increased risk of dyslipidemia, atherosclerosis, and cardiovascular disease.23 These factors are in turn associated with AD and its pathology.4,24 Our finding that the effect of hypertension on CSF tau and ptau-181 levels is aggravated by APOE ε4 homozygosity could be caused by co-existence of other vascular risk factors and development and progression of atherosclerosis in the ε4 homozygous carriers. However, in our study adjustment for other vascular risk factors did not attenuate the observed effect. Second, the association of the APOE ε4 genotype with AD pathology could be explained by the contribution of inflammatory processes.25,26 Inflammatory response in APOE ε4 mice was prolonged up-regulated in comparison to APOE ε3 mice,25 and APOE ε4 AD patients had more brain inflammation in post-mortem examination.26 The APOE ε4 homozygous carriers could suffer in larger extent from damage caused by hypertension due to an increased extent of inflammatory response aggravating the neuronal cell damage. Third, 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 to a more deficient environment.27 Another mice study suggested that the APOE ε4 genotype delays the astroglial repair process.28 It could therefore be hypothesized that APOE ε4 homozygous carriers are less able to repair the damage caused by hypertension. The combination of increased inflammation and an impaired repair mechanism could be the keystone for aggravated AD pathology in the hypertensive APOE ε4 carriers.

Among the strengths of this study is that we used a relatively large cohort of patients with subjective complaints, MCI, and AD patients with available CSF measures derived from a memory clinic. All patients were assessed in a standardized way and diagnosed according to commonly used criteria. The interpretability of the results may be limited by several factors. The first limitation of our study is its cross-sectional design, which limits conclusions regarding within-person change or direction of causality. Previous studies have shown that mainly midlife blood pressure is a risk factor for late life clinical and subclinical markers of AD.5,6,8,12 Future studies should focus on the longitudinal relation between blood pressure and CSF biomarkers. Second, we examined the association of hypertension and APOE ε4 genotype with CSF biomarker levels within the total memory clinic cohort and adjusted for diagnosis. Although the differences in tau and ptau-181 levels between hypertensive and non-hypertensive APOE ε4 homozygous carriers were found in all diagnosis categories (data not shown), larger sample sizes are needed to more reliably study the joint effect of hypertension and APOE genotype on CSF biomarkers within the separate diagnosis categories. Third, our study consisted of a clinical-based sample consisting of patients with subjective complaints, MCI and AD, which might implicate that our results cannot be extrapolated
to the general population. Finally, although the diagnosis of hypertension was based on commonly used criteria, our data were derived from a clinical database and were therefore dependent on the reporting of individual patients of their medical history and medication use. This could have led to both under and over reporting of hypertension. If we assume that misclassification was non-differential, the true associations would even be larger.

In summary, the combination of hypertension and APOE ε4 homozygosity was associated with increased CSF levels of tau and ptau-181. Our data imply that hypertension is directly related to Alzheimer pathology, mainly in the APOE ε4 homozygous carriers. Possible clinical implications could be that clinicians should be more aware of hypertension in these APOE ε4 homozygous carriers, and that the ε4 homozygous carriers have more benefit of antihypertensive treatment to prevent dementia. Furthermore, treatment trials for hypertension using dementia or cognitive decline as outcome measure should stratify their results for APOE genotype to examine to what extent treatment effects are modified by the APOE ε4 homozygous genotype.

REFERENCES


