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Title: **Apolipoprotein E 4 accelerates dementia and increases cerebrospinal fluid tau levels in Alzheimer's disease**

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Brief running head title: ApoE4 declines dementia and increases CSF tau level

Abstract
To examine the effect of apolipoprotein E (ApoE) 4 on the progression of Alzheimer's disease (AD), the clinical course of 33 AD patients (17 cases with ApoE ε4 and 16 cases without ApoE ε4) was evaluated with the mini-mental state examination (MMSE) and cerebrospinal fluid (CSF) biological markers. The decline of MMSE scores to zero was shortened in the ApoE4 group. During a mean follow-up of 20 months, a significant increase of CSF tau levels was observed in the ApoE4 group. A lower level of CSF Aβ1-42(43) was found in both the ApoE4 and non-ApoE4 groups than in age-matched normal controls. The ApoE ε4 allele accelerates the progression of dementia and increases the levels of CSF tau in AD patients.

Key words: Alzheimer's disease; Apolipoprotein E 4; cerebrospinal fluid; tau; Aβ
Apolipoprotein E (ApoE) is a 299 amino acid plasma protein with a molecular weight of 34 kDa. Three major isoforms of ApoE (ApoE2, ApoE3 and ApoE4) are products of alleles (ε2, ε3 and ε4, respectively) at a single gene locus on chromosome 19q13.2. ApoE4 is a major risk factor for the development of Alzheimer’s disease (AD) [3, 16, 17]. After the initial report of an association between late-onset familial AD and ApoE ε4, numerous studies have established that ApoE ε4 is a common risk factor of AD because of acceleration of the AD onset. Possession of homozygous ε4 alleles increases the risk for AD from 20% to 90% and decreases the mean onset age from 84 to 68 years old [3, 14]. ApoE4 binds tightly to Aβ amyloids [8], and facilitates aggregation of Aβ [2] and hyperphosphorylation of tau [18]. In sporadic and familial AD, the presence of the ApoE ε4 is related to severity of senile plaque deposits [7, 15, 18]. The levels of Aβ1-40 and Aβ1-42 in the AD brains are correlated with the ApoE ε4 gene dosage [13]. Finally, the role of ApoE in accelerating Aβ amyloid deposits was confirmed by a study in which PDAPP+/+ transgenic mice were crossed with ApoE-/- knockout mice [1]. However, it is unclear whether ApoE4 also accelerates the progress of clinical dementia in AD. Although 3 reports found no relationship between ApoE4 and progress of dementia [5, 7, 9], one recent study did find accelerated progression of dementia in ApoE ε4 homozygotes with AD [4]. These reports evaluated the ApoE ε4 effect on clinical progression using only various clinical tests. Here, we examined its effect using the biological diagnostic markers in addition to the mini-mental state examination (MMSE). Definite AD subjects were strictly selected by diagnostic criteria, magnetic resonance imaging (MRI) of the brain and biological CSF markers of AD, that is, tau, Aβ1-40 and Aβ1-42(43) [10]. Additionally, two groups consisting of 17 ApoE4 and 16 non-ApoE4 subjects were matched for age, gender, duration from onset, the initial MMSE score, estimated MMSE score at onset and the value of CSF markers. We followed up the 33 AD patients in the two groups retrospectively for a mean period of 20 months using MMSE and CSF biological markers.

The diagnosis of AD was based on the National Institutes of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria, MRI of the brain and the AD Index (tau x Aβ1-40 / Aβ1-42(43)) of CSF markers [10]. The MMSE was performed to evaluate the clinical severity of dementia.

Of the total 33 AD subjects, 3 AD patients (2 males and 1 female) had homozygous ε4 alleles, and 14 patients (5 males and 9 females) had heterozygous ε4 alleles (ε4 / ε3) (ApoE4 group). Fifteen patients (4 males and 11 females) had homozygous ε3 alleles and one male had ε3 / ε2 alleles (non-ApoE4 group). No patient had a heterozygous ε4 allele of the ε4 / ε2 type. The ApoE4 and non-ApoE4 groups did not differ in gender (p=0.55), onset age (p=0.75), age at initial examination (p=0.64), duration from onset (p=0.64), or length of follow-up period (p=0.94). Initial MMSE scores (17.3 ± 1.6 in the ApoE group and 17.9 ± 1.9 in the non-ApoE4 group; mean ± standard error) and clinical stages, assessed by the Functional Assessment Staging Test (FAST), (stage 5 in both groups) were not significantly different (Table 1). After informed consent was obtained, CSF was taken by lumbar puncture and blood was obtained for ApoE analysis. The assay for CSF tau was performed using a sandwich ELISA (Innogenetics, Zwindrecht, Belgium) [10]. The CSF Aβ1-40 and CSF Aβ1-42(43) levels were measured with hypersensitive ELISA systems [10]. The age-matched normal control values of the respective CSF markers were described previously [10].

DNA was extracted from blood using a standard phenol / chloroform protocol. The region containing the ApoE polymorphic sites in the ApoE gene was amplified by polymerase chain reaction (PCR). An upstream and downstream primer pair was used as previously described [20]. The PCR products were digested with Cfo I (Boehringer, Mannheim, Germany) and then analyzed by 5% agarose gel electrophoresis with ethidium bromide staining.
The data were analyzed by the SPSS. The analyses included the regression analysis by the mean value between initial and second points in every subject, determination of the 95% confidence interval (95%CI), and F-tests in ANCOVA.

During the follow-up period, the MMSE scores were progressively decreased in the ApoE4 group (Y = -1.6X+20.8, r²=0.28, p=0.03, Fig. 1a) and in the non-ApoE4 group (Y = -1.1X+20.0, r²=0.25, p=0.046, Fig. 1b). The estimated value at onset, Y-intercept, of the ApoE4 group was 20.8 (95%CI, 14.6 – 26.9) points and that of the non-ApoE4 group was 20.0 (95%CI, 14.6 – 25.4) points. There was no significant difference in the Y-intercept between the two groups (p>0.05). The rate of decline in MMSE score was 1.6 (95%CI, -3.1 to -0.2) points per year in the ApoE4 group, and 1.1 (95%CI, -2.2 to -0.1) points per year in the non-ApoE4 group. The mean duration from onset to the zero point of the MMSE score was 12.6 years (Fig. 1a, arrow) in the ApoE4 group and 18.3 years (Fig. 1b, arrow) in the non-ApoE4 group.

The initial levels of tau, Aβ1-40, Aβ1-42(43) and the AD Index are shown in Table 1. The mean values of the tau level and the AD Index were significantly higher than those of age-matched normal control (aNC) subjects (tau: 217 ± 11 pg/ml; AD Index: 1405 ± 32; p<0.01) [10]. There were no significant differences in the levels of tau, Aβ1-40, or Aβ1-42(43) between the ApoE4 and non-ApoE4 groups. After a mean follow-up of 20 months, no significant differences were found in any marker between the two groups.

During the follow-up period, a linear regression curve fitted to the first order revealed a significant increase of the mean tau level in the ApoE4 group (Y=94X+159; r²=0.28, p=0.03, Fig. 1c) but not in the non-ApoE4 group (Y=19X+368; r²=0.05, p=0.42, Fig. 1d). The slope of the regression line of the tau level in the ApoE4 group (95%CI, 11 - 177) was significantly higher than that in the non-ApoE4 group (95%CI, -30 to 68) (F=4.5, p=0.04). No change of the Aβ1-40 level was observed in the ApoE4 group (Y=65X+1351; r²=0.02, p=0.61) or in the non-ApoE4 group (Y=59X+1544; r²=0.04, p=0.48). The mean Aβ1-42(43) levels of the ApoE4 and non-ApoE4 groups were significantly lower both at the initial and second examinations compared to the levels obtained for the aNC group (242 ± 13 fmol/ml; p<0.01) [10]. In linear regression analysis of the Aβ1-42(43) levels, the slope of the ApoE4 group was –10.4 fmol/ml/year (95%CI, -48.8 to 27.9), which was slightly steeper than that of the non-ApoE4 group (-2.7 fmol/ml/year; 95%CI, -22.6 to 17.2). The AD Index was increased in both the ApoE4 group (Y=1850X+1900; r²=0.28, p=0.03, Fig. 1e) and the non-ApoE4 group (Y=1398X+4636; r²=0.22, p=0.04, Fig. 1f), and the rates of increase rate were not significantly different between the two groups (F=0.1, p>0.05).

Our study showed that the MMSE scores progressively deteriorated in both the ApoE4 and non-ApoE4 groups. However, the mean duration of illness from onset until the zero point of MMSE was significantly shorter in the ApoE4 group (12.6 years) than in the non-ApoE4 group (18.3 years; p<0.05). Since an MMSE score of zero indicates the clinical end-point [11], this finding indicates that AD patients with ApoE ε4 reach the clinical end-point about 6 years faster than AD patients without ApoE ε4. It is important to note that the accelerated progression of dementia was observed even in ApoE ε4 heterozygotes. Recently, Craft et al. [4] has reported the progressive decline of Dementia Rating Scale values in ApoE ε4 homozygote AD patients, in agreement with our results. Three other reports which found no facilitating effect of ApoE4 on clinical progression included older subjects and more female subjects than the present study [5, 7, 9]. Since the CSF tau levels are increased with aging and in female AD patients [10], these factors might make it difficult to show a direct effect of ApoE4 on progression of dementia. Our data indicate that ApoE4 is a risk factor that not only accelerate the disease onset, but also accelerates the progression of dementia.

There have been conflicting reports as to whether CSF tau levels are altered according to the ApoE genotype [6, 19]. In order to examine whether there were differences in the CSF concentration of tau between
carriers and non-carriers of the ε4 allele, CSF tau levels of AD subjects divided into ε4 carrier and non-carrier groups were analyzed using ELISA. Our longitudinal data revealed a significantly higher rate of increase of the CSF tau level in the ApoE4 group. The discrepancy between the findings of several conflicting reports and the present study appears to be due to differences in the methodology used, i.e., the reports concluding that ApoE4 status had no effect were based on one-time evaluation of the CSF tau level at initial entry. In fact, a similar ApoE isoform-specific difference was found in the brain; significantly greater amounts of NFTs were accumulated in the brains of AD patients with the ε4 allele [15]. Quantitative neuropathological assessment of the AD brains revealed that neurofibrillary tangles (NFT) are strongly associated with the clinical severity and duration of dementia [7], which is corroborated by our previously published findings that the levels of CSF tau are correlated with clinical stages [10].

Our findings suggest that the level of CSF tau may mirror the ApoE4-related NFT accumulation in the AD brains. One way by which ApoE4 might modulate the appearance of NFTs is through an inability of ApoE4 to interact with tau, and a resultant effect on NFT formation, leading to tau phosphorylation and self-assembly into PHF.

To our knowledge, this is the first report showing that ApoE4 accelerates the progression of the decline in the clinical dementia scale (MMSE), based on the evidence of biological markers. ApoE ε4 thus plays an important role in the progression of dementia in addition to exerting an accelerating effect on disease onset in AD.

This longitudinal study of 33 AD patients revealed an acceleration of the dementia and an elevation of the CSF tau levels in the ApoE4 group. Thus, ApoE ε4 accelerated not only the onset but also the clinical deterioration of AD, and the levels of CSF tau were inversely correlated with this decline.

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References


ApoE4 declines dementia and increases CSF tau level


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Figure legends

Fig. 1. Chronological changes of MMSE (a and b), the levels of CSF tau (c and d) and the AD Index (e and f). The 3 left-hand figures (a, c and e) show data for the ApoE4 group, and the open circles represent ApoE ε4 homozygotes while the closed circles represent ApoE ε4 heterozygotes. The 3 right-hand figures (b, d and f) show data for the non-ApoE4 group, and the closed circles represent ApoE ε3 homozygotes while the open circles signify ApoE ε3 / ε2 heterozygotes. The X-axes show the duration of illness in years and zero represents the onset of AD symptoms. Bold, solid lines indicate a significant correlation between the mean values of marker and the duration, while a broken line indicates a non-significant relationship.

MMSE scores deteriorated progressively in the ApoE4 group (Y=−1.6X+20.8; r²=0.28, p=0.03; a) and in the non-ApoE4 group (Y=−1.1X+20.0; r²=0.25, p=0.046; b). The arrows indicate the periods to reach the zero point in the MMSE. The ApoE4 group (c) showed a significantly faster rate of increase of the CSF tau level than the non-ApoE4 group (d) (F=4.5, p=0.04). Both the ApoE4 group (Y=1850X+1900; r²=0.28, p=0.03; e) and the non-ApoE4 group (Y=1398X+4636; r²=0.22, p=0.04; f) showed significant increases of the AD index during the follow-up.

Table 1. Summary of the characteristics and marker values of the subjects

<table>
<thead>
<tr>
<th></th>
<th>ApoE4 group</th>
<th>non-ApoE4 group</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients (Male / Female)</td>
<td>17 (7/10)</td>
<td>16 (5/11)</td>
<td>33 (12/21)</td>
</tr>
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Initial Examination, mean (SE)

<table>
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<th>ApoE4 group</th>
<th>non-ApoE4 group</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age [Range]</td>
<td>64 [46~86]</td>
<td>66 [55~82]</td>
<td>65 [46~86]</td>
</tr>
<tr>
<td>Duration from Onset, months</td>
<td>36.4 (4.6)</td>
<td>40.6 (7.9)</td>
<td>38.4 (4.4)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>17.3 (1.6)</td>
<td>17.9 (1.9)</td>
<td>17.6 (1.2)</td>
</tr>
<tr>
<td>FAST score</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CSF tau, pg/ml</td>
<td>442 (73)</td>
<td>404 (68)</td>
<td>424 (49)</td>
</tr>
<tr>
<td>CSF Aβ1-40, fmol/ml</td>
<td>1610 (280)</td>
<td>1700 (225)</td>
<td>1654 (178)</td>
</tr>
<tr>
<td>CSF Aβ1-42(43), fmol/ml</td>
<td>131 (39)</td>
<td>119 (27)</td>
<td>125 (23)</td>
</tr>
<tr>
<td>The AD Index</td>
<td>6969 (1041)</td>
<td>8237 (2000)</td>
<td>7583 (1096)</td>
</tr>
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</table>

Second Examination, mean (SE)

<table>
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<th>ApoE4 group</th>
<th>non-ApoE4 group</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration from 1st Exam., months</td>
<td>19.4 (4.3)</td>
<td>19.8 (2.8)</td>
<td>19.6 (2.6)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>11.6 (1.7)</td>
<td>12.9 (1.4)</td>
<td>12.2 (1.1)</td>
</tr>
<tr>
<td>CSF tau, pg/ml</td>
<td>598 (98)</td>
<td>489 (66)</td>
<td>545 (59)</td>
</tr>
<tr>
<td>CSF Aβ1-40, fmol/ml</td>
<td>1591 (229)</td>
<td>1888 (275)</td>
<td>1735 (177)</td>
</tr>
<tr>
<td>CSF Aβ1-42(43), fmol/ml</td>
<td>135 (31)</td>
<td>124 (27)</td>
<td>130 (20)</td>
</tr>
<tr>
<td>The AD Index</td>
<td>11021 (2922)</td>
<td>12794 (4039)</td>
<td>11881 (2435)</td>
</tr>
</tbody>
</table>
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(a) 0 5 10 15 years
(b) 0 5 10 15 years
(c) pg/ml
(d) pg/ml

(e) 0 5 10 years
(f) 0 5 10 years

(g) 0 5 10 years
(h) 0 5 10 years