Longitudinal study of cerebrospinal fluid amyloid proteins and apolipoprotein E in patients with probable Alzheimer’s disease

Tuula Pirttiläa,b,∗, Keijo Koivistoa,c, Pankaj D. Mehtad, Kari Reinikainenb, Kwang S. Kimd, Olavi Kilkke, Esa Heinonenb, Hilkka Soininenb, Paavo Riekkinen Sr, Henryk M. Wisniewskid

a Department of Neurology, Kuopio University Hospital and University of Kuopio, PO Box 1777, FIN-70211, Kuopio, Finland
b Department of Neurology, Tampere University Hospital and University of Tampere, PO Box 2000, FIN-33101 Tampere, Finland
c Department of Neurology, Seinäjoki Central Hospital, Staten Island, NY, USA
d Institute for Basic Research in Developmental Disabilities, Forest Hill Road 1050, Staten Island, NY, USA
e Orion Corporation, ORION PHARMA, PO Box 425, FIN-20101 Turku, Finland

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Abstract

Levels of soluble amyloid β protein (sAβ), amyloid β precursor protein (APP) and apolipoprotein E (apoE) were examined in cerebrospinal fluid (CSF) obtained twice, at baseline and after 3-year follow-up, from 25 patients with probable Alzheimer’s disease (AD). Levels of sAβ and apoE from patients with the apoE4 allele decreased with time, whereas the levels were similar in patients without apoE4 allele. Changes of sAβ and apoE concentrations correlated significantly with those of mini-mental state examination (MMSE) scores. Levels of sAβ did not change with time in patients with mild dementia, whereas they decreased significantly in patients with moderate dementia. ApoE concentrations decreased in both groups whereas APP levels were similar. We conclude that measurements of CSF sAβ and apoE levels may be helpful in monitoring progression of the disease. © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved

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Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by accumulation of fibrillar amyloid-β (Aβ) protein in plaques in brain parenchyma and in the walls of leptomeningeal and parenchymal vessels. Aβ is a 4-kDa peptide that is proteolytically derived from a larger, transmembrane protein, beta-amyloid precursor protein (APP). Soluble APPs and Aβ are normally present in blood and cerebrospinal fluid (CSF) [14]. Apolipoprotein-E (apoE), on the other hand, has been of special interest in research of AD since the presence of the apoE4 allele has been suggested to be the strongest risk factor for the development of late-onset AD [12]. ApoE polymorphism influences levels of amyloid deposits in brains from AD patients and elderly non-demented controls [13].

Comparisons of soluble APP, Aβ and apoE in CSF from AD patients and controls have shown conflicting results and measurements of these proteins are not useful in the diagnosis of AD [5,7–9,15,16,20]. There are two published studies of sAβ or apoE measurements in serial CSF from AD patients. One study reported that CSF apoE levels increased with time in AD patients [5]. The other study showed that levels of APP decreased with progression of disease [18]. However, a combination of markers in serial CSF were not measured in relation to apoE phenotype and mini-mental state examination (MMSE). The aim of the present study was to examine if CSF sAβ, APP and apoE in CSF collected at intervals of 3 years are useful in monitoring the progression of AD.

We collected CSF samples from 25 patients with probable AD [6] at the Department of Neurology, Kuopio University Hospital, Kuopio, Finland. The severity of the dementia was assessed by MMSE during each visit. CSF was collected at baseline and after a period of 3 years. CSF
APP was measured by competitive inhibition assay using an antibody raised against 45–62 N-terminal amino acids of APP [11]. Soluble A\(\beta\) and apoE were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) as previously described [10,11]. ApoE genotyping was done from DNA extracted from venous blood as previously described [19]. Comparison between and within patients groups were accomplished using Student’s two-sample and one-sample \(t\)-tests. Changes from baseline up to 3 years were used as parameters in the analyses. Normality of variables used in the statistical analyses were tested using Shapiro–Wilks test. There were only minor violations in the assumption of normal distribution. In the case of possible violation in the normality also non-parametric Wilcoxon signed-rank and rank-sum tests were used. However, the results of non-parametric tests did not differ from the corresponding parametric \(t\)-test. Therefore the results are presented with parametric tests. Correlation between CSF amyloid proteins or apoE and MMSE were analyzed using the Pearson correlation method.

Levels of APP, s\(\text{A}\beta\) and apoE in CSF collected at the baseline and after 3 years varied greatly. Soluble A\(\beta\) concentrations in CSF decreased with time (Table 1). There was a significant decrease of the ratio of s\(\text{A}\beta/\text{APP}\) with time (0.33 ± 0.18 vs. 0.25 ± 0.12, \(P = 0.019\)). There was no association between the apolipoprotein genotype and levels of APP, s\(\text{A}\beta\) and apoE at baseline. Mean levels of s\(\text{A}\beta\) and apoE in CSF from AD patients with the apoE4 allele decreased over time, whereas there were no significant changes of the levels in CSF from patients without the apoE4 allele (Table 1). CSF APP levels did not change significantly over time.

There was no significant correlation between MMSE scores and the levels of CSF amyloid proteins and apoE at baseline (Table 1). However, the decrease in CSF s\(\text{A}\beta\) levels with time correlated significantly with the decrease in MMSE scores (\(r = 0.77, P < 0.001\)). Previous clinical and neuropathological studies suggested that progression of AD may not be linear [4,17]. Cognitive decline was slower in patients with mild dementia than those with moderate dementia [17]. Here we compared serial CSF measurements between the patients with mild (MMSE 21–25 points) and moderate (MMSE 11–20 points) dementia at baseline. Levels of s\(\text{A}\beta\) were similar in patients with mild dementia, whereas they decreased significantly in patients with moderate dementia (Table 1). There were no significant differences of CSF apoE and APP levels between the groups.

Our study is the first to report the measurements of a combination of three amyloid-associated markers in serial CSF from patients with probable AD. Our results suggest that longitudinal measurements of CSF s\(\text{A}\beta\) and apoE may be helpful in monitoring progression of the disease. Due to lack of control samples we can not exclude the possibility that the decrease of CSF s\(\text{A}\beta\) was an age-related phenomenon. However, previous cross-sectional studies showed no age-related changes of CSF APP and s\(\text{A}\beta\) concentrations [3,10]. In addition, the observation that the changes were not equal in different patient groups, suggests that the changes were related to disease process.

One weakness of the study is that our ELISA measures total s\(\text{A}\beta\) levels instead of different forms of A\(\beta\). However, our previous studies, in which we used the same method, showed a significant difference of s\(\text{A}\beta\) levels between patients with probable AD and controls [10]. We postulated that low CSF concentrations of s\(\text{A}\beta\) may reflect accumulation of A\(\beta\) in the brain. Investigators reported that amyloid load is greater in brains from AD patients with the apoE4 allele compared to that in patients without the apoE4 allele [13]. Our present findings are consistent with the hypothesis that amyloid formation may occur more rapidly in the presence of the apoE4 allele than without the apoE4 allele.

### Table 1

Demographic data, and s\(\text{A}\beta\), APP and apoE levels in CSF from patients with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Age, mean (SD)</th>
<th>ApoE4 + (n = 16)</th>
<th>ApoE4minus (n = 9)</th>
<th>Mild (n = 16)</th>
<th>Moderate (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n/f)</td>
<td>71/8</td>
<td>5/11</td>
<td>2/7</td>
<td>2/7</td>
</tr>
<tr>
<td>MMSE, mean (SD)</td>
<td>20.8 (3.0)</td>
<td>20.1 (3.4)</td>
<td>22.1 (1.8)</td>
<td>22.5 (1.5)*</td>
</tr>
<tr>
<td>s(\text{A}\beta) ng/ml, mean (SD)</td>
<td>3.99 (0.85)</td>
<td>3.78 (0.77)</td>
<td>4.36 (0.90)</td>
<td>4.22 (0.82)</td>
</tr>
<tr>
<td>Follow-up (3 years)</td>
<td>3.44 (1.24)**</td>
<td>3.01 (1.10)**</td>
<td>4.22 (1.14)***</td>
<td>3.91 (1.16)***</td>
</tr>
<tr>
<td>APP ng/ml, mean (SD)</td>
<td>14.96 (6.69)</td>
<td>15.19 (5.74)</td>
<td>14.62 (8.51)</td>
<td>13.91 (7.11)</td>
</tr>
<tr>
<td>Follow-up (3 years)</td>
<td>15.98 (8.58)</td>
<td>13.85 (5.14)</td>
<td>19.78 (12.08)</td>
<td>16.86 (10.25)</td>
</tr>
<tr>
<td>ApoE mg/L, mean (SD)</td>
<td>4.22 (1.01)</td>
<td>4.57 (0.88)</td>
<td>3.59 (0.96)</td>
<td>4.21 (1.12)</td>
</tr>
<tr>
<td>Follow-up (3 years)</td>
<td>3.94 (1.00)</td>
<td>4.01 (0.82)**</td>
<td>3.74 (1.23)***</td>
<td>4.02 (1.10)</td>
</tr>
</tbody>
</table>

*Significant difference between the groups, \(P < 0.01\). **Significant difference between baseline and follow-up samples, \(P = 0.0023\), one sample \(t\)-test. ***Changes with time differed between the groups, \(P = 0.057\) for s\(\text{A}\beta\) and \(P = 0.020\) for apoE, two-sample \(t\)-test. **Changes with time differed between the groups, \(P = 0.048\), two-sample \(t\)-test.
However, our results suggest that the relationship between CSF amyloid proteins and progression of disease may not be linear, and depends on the stage of disease. It is possible that in AD patients with mild dementia CSF sAβ levels reflect the deposition of Aβ that precedes clinical symptoms. However, in the advanced stages of the disease, low CSF levels may be due decreased production of βAPP, sAβ and apoE, or inefficient clearance into CSF due to enhanced binding into the amyloid plaques.

Urakami et al. [18] measured APP levels in CSF from eight AD patients decreased with progression of disease. APP exists in various soluble isoforms in CSF. Previous studies suggest that processing of APP changes with age and in patients with AD [9]. Since we used an antibody raised against N-terminal 45–62 amino acids of APP, our method measured various different forms of APP, whereas Urakami et al. [18] examined APP isoforms that contain Kunitz-type trypsin inhibitor domains (APPI).

Our data and previous studies suggest that secretion of apoE may be compromised in chronic neurodegenerative diseases [1,2,15]. However, other studies reported an increase of CSF apoE levels in AD patients with time [5]. Consistent with these results, CSF apoE levels were stable or increased in 40% of our patients at follow-up and in turn, one fifth of their AD patients showed decreasing apoE levels in CSF over time. AD is genetically and pathologically heterogeneous disorder. Although in some patients, CSF apoE levels may increase, our results indicate that in most patients the levels decrease with time. The longer follow-up time in our study (3 years compared to mean 14 months in a previous study) may also contribute to the different results.

The effect of long-term storage of CSF is not well examined. Southwick et al. [16] reported that Aβ immunoreactivity decreases under certain storage conditions of CSF such as in certain tubes with multiple freeze/thaw cycles. We divided CSF samples into aliquots in polypropylene tubes immediately after obtaining the samples and stored them at −70°C until the measurements.

We conclude that decreases of CSF sAβ and apoE are associated with the progression of AD. However, the changes varied individually, depending on apoE genotype and the stage of disease. Recent studies suggested that measurement of longer forms of Aβ, Aβ1–42/43, in CSF may be more useful as an aid in the diagnosis of AD than examination of shorter form, Aβ1–40 or total sAβ [7]. Additional studies are needed to determine the relationship between longitudinal changes of different forms of sAβ in CSF in relation to apoE phenotype and progression of disease.

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