The −48 C/T polymorphism in the presenilin 1 promoter is associated with an increased risk of developing Alzheimer’s disease and an increased Aβ load in brain

Jean-Charles Lambert, Alistair Cumming, John Coates, Helen Lemmon, David StClair, Takeshi Iwatsubo, Corinne Lendon

Abstract

Mutations in the presenilin 1 gene (PS1) account for the majority of early onset, familial, autosomal dominant forms of Alzheimer’s disease (AD), whereas its role in other late onset forms of AD remains unclear. A −48 C/T polymorphism in the PS1 promoter has been associated with an increased genetic risk in early onset complex AD and moreover has been shown to influence the expression of the PS1 gene. This raises the possibility that previous conflicting findings from association studies with homozygosity for the PS1 intron 8 polymorphism might be the result of linkage disequilibrium with the −48 CC genotype. Here we provide further evidence of increased risk of AD associated with homozygosity for the −48 CC genotype (odds ratio=1.6). We also report a phenotypic correlation with AD cases and controls and have hypothesised that the −48 C/T polymorphism in the PS1 promoter may increase the risk of AD, perhaps by altering PS1 gene expression and thereby influencing Aβ load.

Epidemiological and molecular studies suggest that multiple genes and environmental factors underlie the aetiology of AD. To date, four genes have been shown to play a role in AD. The apolipoprotein E (APOE) gene is recognised as a major risk factor for complex forms of AD (that is, non mendelian patterns of inheritance), while pathogenic mutations in the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) genes are responsible for some rare, early onset, autosomal dominant forms, with 18-50% of cases being caused by mutations in the PS1 gene. In vitro and in vivo studies show that pathogenic mutations in PS1 favour Aβ peptide production, particularly Aβ42/40, the species suspected to initiate the formation of amyloid plaques. This observation is of particular relevance given the greater Aβ42/40 deposition in the brains of patients bearing PS1 mutations, greater than found in complex forms of AD.

The role of genetic variations of PS1 in complex forms of AD is unclear. Despite our original observation of an association between a single nucleotide polymorphism (SNP) in intron 8 of PS1 and AD, these data have not always been replicated and no functional role has been ascribed to this polymorphism. It has been suggested that the association with the intron 8 polymorphism might be spurious or that the disease associated allele might be in linkage disequilibrium with a functional variant elsewhere in the gene. The −48 C/T polymorphism in the PS1 promoter is a possible candidate and has recently been associated with an increased risk of early onset AD (EOAD). We therefore tested the impact of this polymorphism in our UK white AD cases and controls and have hypothesised that because the PS1 pathogenic mutations affect APP metabolism, this polymorphism might also modulate Aβ load in human AD brains.

Methods

STUDY POPULATION

All AD cases were white (n=287, mean age=67.1 (SD 13.0) years, mean age at onset=63.1 (SD 10.4) years, 49% males), ascertained from two UK centres, the central belt of Scotland (n=164, 25% had been
Table 2: Allele and genotype distribution of the −48 C/T polymorphism in early and late onset populations

<table>
<thead>
<tr>
<th></th>
<th>Allele distribution (%)</th>
<th>Genotype distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>C</td>
</tr>
<tr>
<td>Early onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD cases</td>
<td>176</td>
<td>334 (0.93)</td>
</tr>
<tr>
<td>Control</td>
<td>254</td>
<td>454 (0.89)</td>
</tr>
<tr>
<td>Late onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD cases</td>
<td>105</td>
<td>196 (0.93)</td>
</tr>
<tr>
<td>Control</td>
<td>209</td>
<td>374 (0.90)</td>
</tr>
</tbody>
</table>

Number of subjects (frequency).

Values are % area occupied in Brodmann area 8/9 of the frontal cortex, as previously reported.4

confirmed as definite AD; in this definite AD population, only one case had early onset AD) and Greater Manchester (n=123, all of which were probable AD cases). Diagnoses of definite or probable AD were established according to DSM-III-R and NINDCS-ADRDA criteria. Early (EOAD) and late onset AD (LOAD) were defined as cases with onset before 65 years of age or ≥65 years of age (EOAD n=177, LOAD n=110). The proportion of AD cases with a family history was 20%. The white controls were collected from the same geographical areas as the AD patients and were defined as subjects without DSM-III-R dementia criteria and with full integrity of their cognitive functions (n=482, mean age=62.5 (SD 14.4) years, 42% males). Ethical approval for the study and informed consent was obtained from all participants and their relatives and data were anonymised to ensure subject confidentiality.

Table 3: Aβ42, Aβ42/40, and total Aβ loads according to the −48 CT genotype in AD brains

<table>
<thead>
<tr>
<th></th>
<th>CC n=81</th>
<th>CT n=17</th>
<th>TT n=1</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42</td>
<td>4.2 (4.0)</td>
<td>1.9 (1.9)</td>
<td>0.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Aβ42/40</td>
<td>10.5 (4.2)</td>
<td>8.3 (5.5)</td>
<td>0.6</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Total Aβ</td>
<td>14.7 (6.7)</td>
<td>10.2 (6.8)</td>
<td>8.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are % area occupied in regions 8 and 9 (mean (SD)).

confirmed as definite AD; in this definite AD population, only one case had early onset AD) and Greater Manchester (n=123, all of which were probable AD cases). Diagnoses of definite or probable AD were established according to DSM-III-R and NINDCS-ADRDA criteria. Early (EOAD) and late onset AD (LOAD) were defined as cases with onset before 65 years of age or ≥65 years of age (EOAD n=177, LOAD n=110). The proportion of AD cases with a family history was 20%. The white controls were collected from the same geographical areas as the AD patients and were defined as subjects without DSM-III-R dementia criteria and with full integrity of their cognitive functions (n=482, mean age=62.5 (SD 14.4) years, 42% males). Ethical approval for the study and informed consent was obtained from all participants and their relatives and data were anonymised to ensure subject confidentiality.

Table 4: Aβ40, Aβ42/40, and total Aβ loads according to the −48 CT polymorphism and APOE genotypes in AD brains

<table>
<thead>
<tr>
<th></th>
<th>Non ε4 bearers</th>
<th>ε4 bearers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC n=26</td>
<td>CT+TT n=5</td>
</tr>
<tr>
<td>Aβ40</td>
<td>2.5 (2.4)</td>
<td>1.0 (0.9)</td>
</tr>
<tr>
<td>Aβ42/40</td>
<td>10.7 (6.4)</td>
<td>6.9 (1.2)</td>
</tr>
<tr>
<td>Total Aβ</td>
<td>13.1 (6.2)</td>
<td>7.9 (1.6)</td>
</tr>
</tbody>
</table>

Values are % area occupied in regions 8 and 9 (mean (SD)).

confirmed as definite AD; in this definite AD population, only one case had early onset AD) and Greater Manchester (n=123, all of which were probable AD cases). Diagnoses of definite or probable AD were established according to DSM-III-R and NINDCS-ADRDA criteria. Early (EOAD) and late onset AD (LOAD) were defined as cases with onset before 65 years of age or ≥65 years of age (EOAD n=177, LOAD n=110). The proportion of AD cases with a family history was 20%. The white controls were collected from the same geographical areas as the AD patients and were defined as subjects without DSM-III-R dementia criteria and with full integrity of their cognitive functions (n=482, mean age=62.5 (SD 14.4) years, 42% males). Ethical approval for the study and informed consent was obtained from all participants and their relatives and data were anonymised to ensure subject confidentiality.

STATISTICAL ANALYSIS

Univariate analyses were performed by Pearson’s χ² test. In the multivariate analysis, we tested the hypothesis that possession of the −48 CC genotype increases the risk of AD (that is, −48 CC versus −48 CT + TT genotypes).8 9 The effect of the CC variant on risk for AD was assessed using a multiple logistic regression model adjusted for age and gender. The amyloid load for −48 CC bearers was compared with −48 CT + TT bearers using the Wilcoxon non-parametric test.

Results

The distributions of the −48 CT alleles and genotypes for AD and control subjects are shown in table 1. The frequency of the −48 T allele in the control population was 11%, similar to that previously reported in a Dutch population.9 We observed a significant difference for both allele and genotype distributions between the AD and control populations (p=0.01 and p=0.03, respectively), the −48 CC genotype being associated with an increased risk of developing AD (OR=1.55, 95% CI 1.03-2.35, p=0.04). Similar trends were observed in the Scottish and Manchester populations (respectively, OR=1.31, 95% CI 0.81-2.13, p=0.27 and OR=1.75, 95% CI 0.82-3.69, p=0.14) (table 1). The effect of this polymorphism was similar whether familial or sporadic disease (in the population without a family history, OR=1.63, 95% CI 1.04-2.57, p=0.034) or whether definite or probable AD cases were analysed separately. This effect appears to be independent of the APOE ε4 allele (OR=1.68, 95% CI 1.09-2.59, p=0.02, adjusted for the presence of at least one ε4 allele). No significant interaction with PS1 was detected with age or sex. We also observed a stronger effect of −48 CC genotype in EOAD cases compared to LOAD and age matched control cases; however, they were not significantly different (OR=1.56, 95% CI 0.91-2.75, p=0.11 and 1.27, 95% CI 0.72-2.82, p=0.31, respectively) (table 2).

We next tested the hypothesis that the −48 C/T polymorphism may be associated with Aβ peptide load in brains from AD patients. We observed that all three measures of Aβ load (Aβ40, Aβ42/40, and total Aβ) were significantly increased in −48 CC bearers (table 3). Subjects bearing the −48 CC genotype presented a 100% and 57% increase in Aβ40 (p=0.007) and Aβ42/40 (p=0.01) load, respectively, leading to a 47% (p=0.003) increase in total Aβ load. This increased load appears to be independent of the APOE ε4 allele (table 4). We found no relationship between −48 CC genotype and the age of onset of disease in the Manchester cohort of brains, but we did detect a trend towards a shorter duration of illness (CC=7.8 (SD 3.7) years, CT and TT=9.5 (SD 3.1) years, p=0.067 with Wilcoxon non-parametric test).

Discussion

In this study we provide further evidence of an association in our UK population between AD and the −48 C/T polymorphism in the PS1 gene promoter. We detected an overall in-
increased frequency of the −48 CC genotype in AD cases (OR=1.6) whereas a slightly stronger effect had been reported in a Dutch EOAD sample (OR=2.6). We also found a trend towards a stronger effect in EOAD cases in our population. Interestingly, in a second Dutch LOAD cohort, no association with the −48 C/T polymorphism was detected.10 Thus, we could speculate that the −48 C/T polymorphism may have a greater impact in earlier onset forms of the disease, as do the dominant AD mutations in the PS1 gene.

Considerable research effort has been directed towards understanding the function of PS1 protein. Evidence suggests that it plays a role in cell trafficking14 and apoptosis15 and chromosomal segregation.16 PS1 has been implicated in the γ-secretase activity that generates the carboxy-terminus of Aβ peptides.14 Because mutations in PS1 in familial AD are directly implicated in APP metabolism and production of Aβ, we hypothesised that variations of PS1 expression because of polymorphisms in the promoter may similarly influence the production of Aβ. Indeed, Aβ peptide production can be reduced after inhibition of PS1 expression in cultured cells.17 In this present study, we have reported that the −48 CC genotype correlates with increased Aβ deposition. Furthermore, the view that genetic variants in the PS1 promoter increase the risk of developing AD by modulating PS1 expression and consequently APP metabolism. Mutations in the PS1 gene increase the total amount of Aβ secreted and deposited through selectively influencing the activity of γ-secretase in favour of the production of Aβ42.18,19 We have shown here that the −48 C/T polymorphism is associated with brain increases in both Aβ40 and Aβ42,21 implying that its modulatory effect on PS1 activity is unselective, at least as far as the composition of the C-terminal Aβ peptides that are produced is concerned. Hence, while the −48 C/T polymorphism leads to increased deposition of Aβ, this might be achieved by driving more APP per se through the catablic cascade rather than, as is the case with the PS1 mutations, by facilitating the preferential production of the more highly aggregable species Aβ42.21

Our epidemiological studies and those of others are supported by genotype-phenotype correlations that suggest that the −48 C/T polymorphism might exert a functional role by influencing PS1 gene expression20 and Aβ load, as described here. Other polymorphisms have been reported in the PS1 promoter, which appear to be in linkage disequilibrium with the −48 C/T polymorphism.5 Further epidemiological and functional studies are required to determine which of these modifies the risk of AD. These data emphasise the potential importance of control of gene expression in the pathogenesis of AD. Genetic variability in the APP promoter has been suggested to increase the risk of late onset AD, and we have recently reported that polymorphisms in the APOE promoter modulate risk for AD.

In conclusion, our findings are consistent with the established effects of PS1 mutations on APP metabolism, suggesting that variations in the level of PS1 expression per se may have an impact upon AD pathology.
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