Association of a polymorphism of the transforming growth factor-β1 gene with cerebral amyloid angiopathy

T Hamaguchi, S Okino, N Sodeyama, Y Itoh, A Takahashi, E Otomo, M Matsushita, H Mizusawa, M Yamada

Cerebral amyloid angiopathy (CAA) is a cerebrovascular amyloid deposition related to intracerebral haemorrhage and other cerebrovascular disorders.1,2 CAA is commonly found in the elderly as well as in Alzheimer’s disease (AD),1,3 and some genetic risk factors for AD have been reported to be associated with sporadic CAA.3–8 The e4 allele of the apolipoprotein E (apoE) gene (APOE), an established risk factor of AD, has been suggested to be a risk factor of CAA,4 although this was not evident in some populations5,6 and the APOE ε2 allele may be associated with CAA-related haemorrhage.7 We previously reported that the polymorphisms in the presenilin-1, α2-antichymotrypsin, and neprilysin genes may be associated with sporadic CAA.8–10 AD and CAA share risk factors in the common pathogenetic process of amyloid β protein (Aβ) deposition.

The multifunctional cytokine transforming growth factor-β1 (TGF-β1) is a potent regulator of injury and inflammatory responses in the central nervous system11 and has been implicated in cerebral amyloid deposition12–14 and AD pathogenesis.15–16 Cerebral TGF-β1 mRNA levels are correlated positively with the extent of amyloid deposition in cerebral blood vessels in AD cases.12 Astroglial overproduction of TGF-β1 in aged transgenic mice expressing the human β-amyloid precursor protein (hAPP) promotes the deposition of human Aβ in cerebral vessels.13 In hAPP/TGF-β1 bigenic mice, in spite of its amyloidogenic effects on the cerebral vasculature, TGF-β1 strongly reduces the overall cerebral Aβ load by inhibiting the formation of neuritic plaques in the brain parenchyma.14

The levels of TGF-β1 in the central nervous system are reportedly increased greatly in response to ischaemic, excitotoxic, and traumatic brain injury.17–21 Eight polymorphisms of the TGF-β1 gene (TGF-β1) located to 19q13.1–13.322 have been detected.23,24 Although the details of TGF-β1 expression are still unclear, polymorphisms of the TGF-β1 may play a role in the control of the TGF-β1 level in plasma.24–26 Recently, one of these polymorphisms, a T/C transition at nucleotide 29 in the region encoding the signal sequence, which results in a Leu/Pro substitution at amino acid 10, has been reported to be associated with the serum concentration or production of TGF-β124,25 and with diseases such as osteoporosis,26 myocardial infarction,27 rheumatoid arthritis,28 and invasive breast cancer.29

In the present study, we investigated whether the T/C polymorphism at codon 10 in exon 1 of the TGF-β1 is associated with CAA in elderly individuals.

SUBJECT AND METHODS

Patients
We studied 167 Japanese patients (age 62–104 years; mean ± SD, 86.0 ± 7.8 years). They were consecutive autopsy cases in a large geriatric hospital, excluding cases in which brain samples could not be obtained for study and cases of neurodegenerative diseases other than AD. Consent was obtained from all families of participants at autopsy. This study project was approved by the ethics committee of each institution. The 167 patients included 73 patients with sporadic AD, in which the neuropathological findings satisfied the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease,29 and 94 subjects without AD or other neurodegenerative disorders. All the AD patients clinically showed dementia on the basis of the criteria of American Psychiatric Association.30 There was no significant difference in the age at death between AD (86.1 ± 8.0) and non-AD (86.0 ± 7.7) groups. No familial cases of AD or CAA were included in this series.

Abbreviations:
Aβ, amyloid β protein; AD, Alzheimer’s disease; APOE, apolipoprotein E; CAA, cerebral amyloid angiopathy; hAPP, human β-amyloid precursor protein; PCR, polymerase chain reaction; TGF-β1, transforming growth factor-β1
Neuropathological evaluation of CAA

Congophilic deposits with green birefringence under polarised light were identified as amyloid deposits. The cerebrovascular amyloid deposits were immunohistochemically confirmed to be Aβ. Using a large section of the occipital lobe (about 4×4 cm in size), the numbers of meningeal and cortical vessels (small arteries and arterioles) with and without amyloid deposits were counted in the whole section, and the percentage of amyloid-laden vessels was calculated (= CAA count). We examined the occipital lobe because it was the most frequently affected by CAA in our previous study in both AD and non-AD cases and seemed suitable for detecting CAA of very slight degree. The quantification was performed without knowledge of the TGF-β1 or APOE genotypes. Severe vascular wall involvement was commonly found in patients with high CAA counts, and some of them presented with secondary degenerative changes — that is, CAA associated vasculopathies. However, we did not use data of such morphological changes because it was difficult to evaluate totally and quantitatively the extent of amyloid involvement of each blood vessel, and only a small number of patients with CAA associated vasculopathies were included in this series. We used only the CAA counts for our statistical analysis to represent the severity of CAA.

Identification of the TGF-β1 polymorphism

Genomic DNA was isolated from the frozen brain tissue of all patients using a standard phenolchloroform extraction procedure. TGF-β1 genotype was determined by a direct sequencing method as previously described. Briefly, the first exon of the TGF-β1 was amplified by polymerase chain reaction (PCR) with sense (5'-TCTTACCTTTTGCGGGAGAC-3') and antisense (5'-GTGTGGGTTTCCACCATAG-3') primers. The PCR products were sequenced with a fluorescence-based automated DNA sequencer (Prism 310; Applied Biosystems, CA, USA). The APOE genotype was also determined as previously described.

Statistical analysis

Comparison of the distributions of the AD and non-AD patients over the TGF-β1 genotype categories (TT, TC, and CC) were performed using a χ² test.

CAA counts were compared between the TGF-β1 genotypes (TT, TC, and CC) in AD, non-AD, and all patients. Because the counts did not follow a normal distribution in any group, we used the Kruskal-Wallis test for the comparison as a non-parametric test. Similar analyses were performed according to the subgroups of APOE ε4 status. We also used the Mann-Whitney U test to compare CAA counts between AD and non-AD patients. The correlation between the number of T allele and CAA counts was examined with Spearman’s rank correlation analysis.

Statistical significance was defined as p<0.05. The statistical analyses were performed using StatView J-7.5 (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Among the 167 patients examined, TT, TC, and CC genotypes of the TGF-β1 polymorphism were found in 47, 77, and 43 individuals, respectively. Additionally, 0.51 in T-type allele frequency and 0.49 in C-type allele frequency. Age did not differ significantly between the genotypes. The distribution of the TGF-β1 genotype in AD and non-AD is shown in Table 1. The TGF-β1 genotype was not significantly different between AD and non-AD patients. There was a strong association between AD and the APOE ε4 allele in this population (p<0.0001) as we previously reported in a smaller number of samples.

Sixty two (84.9%) of 73 AD patients and 29 (30.9%) of 94 non-AD patients were affected by CAA. Average values of CAA counts and numbers of patients with CAA in the TGF-β1 genotypes are shown in Table 2. Average values of CAA counts were significantly different between TT, TC, and CC genotypes in all patients (p = 0.0026) and in the non-AD patients (p = 0.011), but not in the AD patients. When the patients were categorised by APOE ε4 status, the average CAA count was significantly different between the genotypes in the non-ε4 carriers (p = 0.0099) but not in the ε4 carriers (Table 2). In this population, the CAA count was significantly higher in the AD patients than in the non-AD patients (p<0.0001), and was higher in the APOE ε4 carriers than in the APOE non-ε4 carriers (p = 0.002) (Table 2).

The number of T alleles in the TGF-β1 polymorphism showed a significant positive correlation with the CAA count in all patients (ρ = 0.255, p = 0.0011), non-AD patients (ρ = 0.316, p = 0.0033), and APOE non-ε4 carriers (ρ = 0.272, p = 0.0028), but not in the AD patients or ε4 carriers.

Table 1 Distribution of the TGF-β1 genotypes in Alzheimer’s disease (AD) and non-AD

<table>
<thead>
<tr>
<th>Genotype, n (%)</th>
<th>AD (n = 73)</th>
<th>Non-AD (n = 94)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>23 (31.5%)</td>
<td>24 (25.5%)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>36 (49.3%)</td>
<td>41 (43.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>CC</td>
<td>14 (19.2%)</td>
<td>29 (30.9%)</td>
<td></td>
</tr>
</tbody>
</table>

*Statistical difference in the TGF-β1 genotypes between AD and non-AD by χ² test.

Table 2 Average cerebral amyloid angiopathy (CAA) counts in the TGF-β1 genotype

<table>
<thead>
<tr>
<th>TGF-β1 genotype</th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
<th>Total</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total AD or non-AD</td>
<td>22.6 ± 4.3 (33/47)</td>
<td>21.5 ± 3.4 (44/77)</td>
<td>11.8 ± 4.2 (14/43)</td>
<td>19.3 ± 2.3 (91/167)</td>
<td>0.0026</td>
</tr>
<tr>
<td>AD</td>
<td>21.7 ± 5.7 (21/23)</td>
<td>32.2 ± 5.1 (31/36)</td>
<td>28.9 ± 9.7 (10/14)</td>
<td>25.9 ± 3.6 (62/73)</td>
<td>NS</td>
</tr>
<tr>
<td>Non-AD</td>
<td>18.3 ± 6.3 (12/24)</td>
<td>17.7 ± 4.0 (13/41)</td>
<td>3.6 ± 3.3 (4/29)</td>
<td>10.9 ± 2.6 (29/94)</td>
<td>0.011</td>
</tr>
<tr>
<td>Status of APOE ε4 (+)</td>
<td>23.1 ± 5.9 (12/14)</td>
<td>27.1 ± 6.1 (18/22)</td>
<td>21.1 ± 14.3 (5/7)</td>
<td>24.8 ± 4.3 (35/43)</td>
<td>NS</td>
</tr>
<tr>
<td>ε4 (-)</td>
<td>22.4 ± 5.6 (21/33)</td>
<td>19.2 ± 4.1 (26/55)</td>
<td>10.0 ± 4.3 (9/36)</td>
<td>17.4 ± 2.7 (56/124)</td>
<td>0.0099</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values in parentheses are numbers of CAA patients/total patients. Average CAA counts are calculated by using the data from total patients.

*Statistical difference in CAA counts between the TGF-β1 genotypes by Kruskal-Wallis test; p<0.0001 (AD v non-AD) by the Mann-Whitney U test; p = 0.002 (ε4 (+) v ε4 (-)) by the Mann-Whitney U test. AD: Alzheimer’s disease; APOE: apolipoprotein E; TGF-β1: transforming growth factor-β1.
reduced levels of TGF-β polymorphism has been reported to be associated with TGF-β in exon 5, and polymorphism with CAA may develop cerebral haemorrhages following antithrombotic or anticoagulation treatment against thromboembolic disease. Although TGF-β is present in senile plaques and is overexpressed in AD, it has been shown in recent studies that increased production of TGF-β promotes deposition of Aβ in cerebral blood vessels, but reduces plaque formation in the brain parenchyma in AD patients or AD mice models. By contrast, TGF-β1 has been reported to potentiate Aβ generation in astrocytes and transgenic mice promoting Aβ deposition in the brain. TGF-β1 may have complex effects on the expression and processing of APP, Aβ clearance from the brain, and cerebrovascular Aβ deposition, probably through activation of astrocytes and microglia. Association of TGF-β1 polymorphisms with AD has been studied, including the polymorphisms at –800, –509, codon 263 in exon 5, and +25 in the TGF-β1. A weak association of the –509 T allele with AD was reported in an American population; however, there was no association between the –509 polymorphism and AD in another study. The other polymorphisms of TGF-β1 were not associated with AD. In this study, we investigated the association of the T/G polymorphism at codon 10 in exon 1 of the TGF-β1 with AD for the first time, and failed to find any significant association. Therefore, the association of the T/G polymorphism with CAA would be independent of AD, although there is a close relation between CAA and AD. The T/C polymorphism of the TGF-β1 results in the Leu/Pro substitution of TGF-β1, which is located in the 29-residue signal peptide sequence. TGF-β1 polymorphism may affect the function of the signal peptide, perhaps influencing intracellular trafficking or export efficiency of the preproprotein, and the T/G1 polymorph may be associated with the production of this protein. The T allele of the TGF-β1 polymorphism has been reported to be associated with reduced levels of TGF-β1 proteins in serum. In transfection experiments with HeLa cells, the signal peptide with Pro at residue 10 encoded by the T allele causes an increase in secretion of TGF-β1 compared with the Leu form encoded by the T allele. If the T allele is associated with reduced levels of TGF-β1 in the brain as well as in serum, our data suggest that reduced TGF-β1 levels may be associated with increased severity of CAA. This is inconsistent with the reports that TGF-β1 would promote CAA in AD patients and AD mice models. This discrepancy may be related to the difference in the role of TGF-β1 for cerebrovascular amyloid deposition between AD and non-AD patients, or the difference in the role of this polymorphism in expression of TGF-β1 between systemic circulation and brain. We do not have any definite explanation for this discrepancy because we have no data on the TGF-β1 concentration in the central nervous system from these patients. Our results indicate an important role of TGF-β1 in CAA, requiring further study to elucidate the pathomechanism.

CAA-related cerebrovascular disorders include lobar cerebral haemorrhage, leucoencephalopathy, and cortical small haemorrhage. Patients with asymptomatic CAA may develop cerebral haemorrhages following antithrombotic or anticoagulation treatment against thromboembolic disorders or Aβ immunotherapy against AD. Because there are no definitive biological markers or imaging technologies for definitive diagnosis of the CAA, except for pathological examination of the brain, further development of tests to evaluate risk of CAA is necessary. AD and APOE e4 have been reported to be independent risk factors of CAA, but CAA is also observed in non-AD or APOE non-e4 carriers. Interestingly, the polymorphism of TGF-β1 is a risk factor of CAA especially in non-AD or APOE non-e4 carriers, which may contribute to prediction of CAA in such cases.

In conclusion, our results suggest that the T/C polymorphism at codon 10 in exon 1 of the TGF-β1 would be associated with the severity of CAA, especially in non-AD or APOE non-e4 carriers.

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