

# Association of a polymorphism of the transforming growth factor- $\beta$ 1 gene with cerebral amyloid angiopathy

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**Background:** A recent study showed that transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) induces amyloid- $\beta$  deposition in cerebral blood vessels and meninges of a transgenic mouse model of Alzheimer's disease (AD), and that TGF- $\beta$ 1 mRNA levels are correlated with cerebral amyloid angiopathy (CAA) in human AD brains. A T/C polymorphism at codon 10 in exon 1 of the TGF- $\beta$ 1 gene has been reported to be associated with the serum TGF- $\beta$ 1 concentration. We investigated whether the TGF- $\beta$ 1 polymorphism is associated with the risk of CAA.

**Methods:** The association between the severity of CAA and the T/C polymorphism at codon 10 in exon 1 of the TGF- $\beta$ 1 was investigated in 167 elderly Japanese autopsy cases, including 73 patients with AD. The apolipoprotein E (APOE) genotype was also determined.

**Results:** The genotypes (TT/TC/CC) were associated with the severity of CAA significantly in all patients ( $p = 0.0026$ ), in non-AD patients ( $p = 0.011$ ), and APOE non- $\epsilon$ 4 carriers ( $p = 0.0099$ ), but not in AD patients or APOE  $\epsilon$ 4 carriers. The number of the T alleles positively correlated with the severity of CAA in all patients ( $p = 0.0011$ ), non-AD patients ( $p = 0.0026$ ), and APOE non- $\epsilon$ 4 carriers ( $p = 0.0028$ ), but not in AD patients or APOE  $\epsilon$ 4 carriers. The polymorphism was not significantly associated with AD.

**Conclusions:** Our results suggest that the polymorphism in TGF- $\beta$ 1 is associated with the severity of CAA, especially in non-AD patients and APOE non- $\epsilon$ 4 carriers.

Cerebral amyloid angiopathy (CAA) is a cerebrovascular amyloid deposition related to intracerebral haemorrhage and other cerebrovascular disorders.<sup>1,2</sup> CAA is commonly found in the elderly as well as in Alzheimer's disease (AD),<sup>1,2</sup> and some genetic risk factors for AD have been reported to be associated with sporadic CAA.<sup>3–10</sup> The  $\epsilon$ 4 allele of the apolipoprotein E (apoE) gene (APOE), an established risk factor of AD, has been suggested to be a risk factor of CAA,<sup>3,4</sup> although this was not evident in some populations<sup>5,6</sup> and the APOE  $\epsilon$ 2 allele may be associated with CAA-related haemorrhage.<sup>7</sup> We previously reported that the polymorphisms in the presenilin-1,  $\alpha$ <sub>1</sub>-antichymotrypsin, and neprilysin genes may be associated with sporadic CAA.<sup>8–10</sup> AD and CAA share risk factors in the common pathogenetic process of amyloid  $\beta$  protein (A $\beta$ ) deposition.

The multifunctional cytokine transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a potent regulator of injury and inflammatory responses in the central nervous system<sup>11</sup> and has been implicated in cerebral amyloid deposition<sup>12–14</sup> and AD pathogenesis.<sup>11,15,16</sup> Cerebral TGF- $\beta$ 1 mRNA levels are correlated positively with the extent of amyloid deposition in cerebral blood vessels in AD cases.<sup>12</sup> Astroglial overproduction of TGF- $\beta$ 1 in aged transgenic mice expressing the human  $\beta$ -amyloid precursor protein (hAPP) promotes the deposition of human A $\beta$  in cerebral vessels.<sup>12</sup> In hAPP/TGF- $\beta$ 1 bigenic mice, in spite of its amyloidogenic effects on the cerebral vasculature, TGF- $\beta$ 1 strongly reduces the overall cerebral A $\beta$  load by inhibiting the formation of neuritic plaques in the brain parenchyma.<sup>14</sup>

The levels of TGF- $\beta$ 1 in the central nervous system are reportedly increased greatly in response to ischaemic, excitotoxic, and traumatic brain injury.<sup>17–21</sup> Eight polymorphisms of the TGF- $\beta$ 1 gene (TGF- $\beta$ 1) located to 19q13.1–13.3<sup>22</sup> have been detected.<sup>23,24</sup> Although the details of TGF- $\beta$ 1 expression are still unclear, polymorphisms of the TGF- $\beta$ 1 may play a role in the control of the TGF- $\beta$ 1 level in plasma.<sup>24–26</sup>

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- $\beta$ 1<sup>24,25,27</sup> and with diseases such as osteoporosis,<sup>25</sup> myocardial infarction,<sup>24</sup> rheumatoid arthritis,<sup>28</sup> and invasive breast cancer.<sup>27</sup>

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

## SUBJECT AND METHODS

### Patients

We studied 167 Japanese patients (age 62–104 years; mean  $\pm$  SD, 86.0  $\pm$  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,<sup>29</sup> 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.<sup>30</sup> There was no significant difference in the age at death between AD (86.1  $\pm$  8.0) and non-AD (86.0  $\pm$  7.7) groups. No familial cases of AD or CAA were included in this series.

**Abbreviations:** A $\beta$ , amyloid  $\beta$  protein; AD, Alzheimer's disease; APOE, apolipoprotein E; CAA, cerebral amyloid angiopathy; hAPP, human  $\beta$ -amyloid precursor protein; PCR, polymerase chain reaction; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1

**Table 1** Distribution of the *TGF- $\beta$ 1* genotypes in Alzheimer's disease (AD) and non-AD

	AD (n = 73)	Non-AD (n = 94)	p
Genotype, n (%)			
TT	23 (31.5%)	24 (25.5%)	NS
TC	36 (49.3%)	41 (43.6%)	
CC	14 (19.2%)	29 (30.9%)	

\*Statistical difference in the *TGF- $\beta$ 1* genotypes between AD and non-AD by  $\chi^2$  test.

### 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 A $\beta$ . 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<sup>31</sup> and seemed suitable for detecting CAA of very slight degree. The quantification was performed without knowledge of the *TGF- $\beta$ 1* 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- $\beta$ 1* polymorphism

Genomic DNA was isolated from the frozen brain tissue of all patients using a standard phenolchloroform extraction procedure. *TGF- $\beta$ 1* genotype was determined by a direct sequence method as previously described.<sup>23</sup> Briefly, the first exon of the *TGF- $\beta$ 1* was amplified by polymerase chain reaction (PCR) with sense (5'-TCCTACCTTTGCCGGGAGAC-3') and antisense (5'-GTTGTGGGTTCCACCATTAG-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.<sup>5</sup>

### Statistical analysis

Comparison of the distributions of the AD and non-AD patients over the *TGF- $\beta$ 1* genotype categories (TT, TC, and CC) were performed using a  $\chi^2$  test.

CAA counts were compared between the *TGF- $\beta$ 1* 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*  $\epsilon$ 4 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- $\beta$ 1* polymorphism were found in 47, 77, and 43 individuals, respectively (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- $\beta$ 1* genotype in AD and non-AD is shown in Table 1. The *TGF- $\beta$ 1* genotype was not significantly different between AD and non-AD subjects. There was a strong association between AD and the *APOE*  $\epsilon$ 4 allele in this population ( $p < 0.0001$ ) as we previously reported in a smaller number of samples.<sup>32</sup>

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- $\beta$ 1* 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*  $\epsilon$ 4 status, the average CAA count was significantly different between the genotypes in the non- $\epsilon$ 4 carriers ( $p = 0.0099$ ) but not in the  $\epsilon$ 4 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*  $\epsilon$ 4 carriers than in the *APOE* non- $\epsilon$ 4 carriers ( $p = 0.002$ ) (Table 2).

The number of T alleles in the *TGF- $\beta$ 1* polymorphism showed a significant positive correlation with the CAA count in all patients ( $\rho = 0.255$ ,  $p = 0.0011$ ), non-AD patients ( $\rho = 0.316$ ,  $p = 0.0033$ ), and *APOE* non- $\epsilon$ 4 carriers ( $\rho = 0.272$ ,  $p = 0.0028$ ), but not in the AD patients or  $\epsilon$ 4 carriers.

**Table 2** Average cerebral amyloid angiopathy (CAA) counts in the *TGF- $\beta$ 1* genotype

	<i>TGF-<math>\beta</math>1</i> genotype			Total	p*
	TT	TC	CC		
Total	22.6 $\pm$ 4.3 (33/47)	21.5 $\pm$ 3.4 (44/77)	11.8 $\pm$ 4.2 (14/43)	19.3 $\pm$ 2.3 (91/167)	0.0026
AD or non-AD					
AD	27.1 $\pm$ 5.7 (21/23)	32.2 $\pm$ 5.1 (31/36)	28.9 $\pm$ 9.7 (10/14)	29.9 $\pm$ 3.6† (62/73)	NS
Non-AD	18.3 $\pm$ 6.3 (12/24)	11.7 $\pm$ 4.0 (13/41)	3.6 $\pm$ 3.3 (4/29)	10.9 $\pm$ 2.6 (29/94)	0.011
Status of <i>APOE</i>					
$\epsilon$ 4 (+)	23.1 $\pm$ 5.9 (12/14)	27.1 $\pm$ 6.1 (18/22)	21.1 $\pm$ 14.3 (5/7)	24.8 $\pm$ 4.3‡ (35/43)	NS
$\epsilon$ 4 (-)	22.4 $\pm$ 5.6 (21/33)	19.2 $\pm$ 4.1 (26/55)	10.0 $\pm$ 4.3 (9/36)	17.4 $\pm$ 2.7 (56/124)	0.0099

Values are means  $\pm$  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- $\beta$ 1* genotypes by Kruskal-Wallis test; † $p < 0.0001$  (AD v non-AD) by the Mann-Whitney U test; ‡ $p = 0.002$  ( $\epsilon$ 4 (+) v  $\epsilon$ 4 (-)) by the Mann-Whitney U test. AD, Alzheimer's disease; *APOE*, apolipoprotein E; *TGF- $\beta$ 1*, transforming growth factor- $\beta$ 1.

## DISCUSSION

Our results revealed that the genotypes and number of T alleles of the T/C polymorphism at codon 10 in exon 1 of the *TGF-β1* were associated with the severity of CAA. An increase in the number of T alleles in the *TGF-β1* polymorphism may be a risk factor of CAA in the elderly, especially for non-AD patients and *APOE* non-ε4 carriers.

*TGF-β1* may be related to the pathogenesis of AD and CAA.<sup>11 12 14–16</sup> It has been reported that cerebral *TGF-β1* levels are higher in AD patients than in non-demented controls,<sup>15 16</sup> and that *TGF-β1* is present in senile plaques and is overexpressed in AD.<sup>11</sup> It has been shown in recent studies<sup>12 14</sup> that increased production of *TGF-β1* 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.<sup>33</sup> *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.<sup>14 33</sup>

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*.<sup>34 35</sup> A weak association of the –509 T allele with AD was reported in an American population;<sup>34</sup> however, there was no association between the –509 polymorphism and AD in another study.<sup>35</sup> The other polymorphisms of *TGF-β1* were not associated with AD.<sup>34 35</sup> In this study, we investigated the association of the T/C 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 *TGF-β1* polymorphism with CAA would be independent of AD, although there is a close relation between CAA and AD.<sup>36</sup>

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.<sup>24 25</sup> The *TGF-β1* polymorphism may affect the function of the signal peptide, perhaps influencing intracellular trafficking or export efficiency of the preproprotein, and the *TGF-β1* genotypes 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.<sup>24 25</sup> In transfection experiments with HeLa cells, the signal peptide with Pro at residue 10 encoded by the C allele causes an increase in secretion of *TGF-β1* compared with the Leu form encoded by the T allele.<sup>27</sup>

If the T allele is associated with reduced levels of *TGF-β1* in the brain as well as in serum,<sup>24 25</sup> 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.<sup>12 14</sup> 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 and cerebellar haemorrhages, leucoencephalopathy, and cortical small haemorrhage.<sup>2</sup> Patients with asymptomatic CAA may develop cerebral haemorrhages following antithrombotic or anticoagulation treatment against thromboembolic disorders<sup>37 38</sup> or Aβ immunotherapy against AD.<sup>39</sup> Because there are no definitive biological markers or imaging

technologies for definite 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* ε4 have been reported to be independent risk factors of CAA, but CAA is also observed in non-AD or *APOE* non-ε4 carriers.<sup>1–10 31 35</sup> Interestingly, the polymorphism of *TGF-β1* is a risk factor of CAA especially in non-AD or *APOE* non-ε4 carriers, which may contribute to prediction of CAA in such cases.

The current study has limitations; our sample population was relatively small and obtained from an autopsy series in a geriatric hospital. Our results warrant further study with a larger sample size from populations with various ethnic backgrounds.

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-ε4 carriers.

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