SHORT REPORT

Association study of three polymorphisms of TGF-β1 gene with Alzheimer's disease

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Background: There is evidence that inflammatory processes may contribute to the development of Alzheimer's disease through production of cytokines and free radicals that damage neurones. A recent study has shown that transforming growth factor β1 (TGF-β1) signalling in astrocytes promotes Aβ production and could play a critical role in the formation of amyloid plaques in the brain.
Objectives: To explore the impact of the –800 and –509 TGF-β1 promoter polymorphisms and the +25 polymorphism on the risk of occurrence of Alzheimer's disease in a large population of sporadic cases and controls, and on the amyloid β (Aβ) load in the brains of Alzheimer patients.
Methods: The TGF-β1 genotypes of the three polymorphisms were determined in 678 sporadic Alzheimer's disease patients and 667 controls. They were also characterised, along with Aβ load, in the brains of 81 necropsy confirmed Alzheimer patients.
Results: No significant variations in the distribution of the genotypes and haplotypes were observed between Alzheimer patients and controls, or in the amount of Aβ deposition.
Conclusions: These results do not suggest an influence of genetic variability at the TGF-β1 gene locus on the occurrence of Alzheimer's disease.

Inflammatory processes may play a critical role in the pathogenesis of degenerative changes in Alzheimer's disease. The observation that activated microglial cells are concentrated in and around senile plaques suggests that this accumulation may contribute to neurodegeneration through the production of cytokines and free radicals that can damage and kill neurones. There are several lines of evidence that cytokines produced during inflammatory process—such as transforming growth factor β1 (TGF-β1)—may contribute to the development of Alzheimer's disease by initiating or exacerbating amyloid β (Aβ) deposition.[1–4] TGF-β1 is present in senile plaques and is overexpressed in Alzheimer's disease brain compared with controls.[5] TGF-β1 has also been reported to accelerate amyloid deposition in transgenic mice coexpressing human TGF-β1 and mutated amyloid precursor protein, an animal model for Alzheimer-like pathology.[1 6] Recent studies have shown that TGF-β1, upregulated in Alzheimer patients, drives astrocytic overexpression of the mRNA encoding for the amyloid precursor protein.[7]

These observations led us to propose TGF-β1 as a possible candidate gene for susceptibility to Alzheimer's disease. TGF-β1 contains seven polymorphisms: three upstream and four in the transcript region of the gene.[8] Three polymorphisms located in the coding region may be associated with different effects of TGF-β1. Among these, a polymorphism at codon 25 (+25 polymorphism) is associated with an increased risk of myocardial infarction and a reduced risk of hypertension.[8] Two polymorphisms located in the promoter region at position –800 and –509 from the transcription start site may also contribute to the regulation of the expression of this gene.

Our aim in the present study was therefore to explore the impact on Alzheimer's disease of these two TGF-β1 promoter polymorphisms, together with the +25 polymorphism, in a population of sporadic Alzheimer's disease patients and controls. The impact of these polymorphisms was also studied in a collection of Alzheimer brains in which we have determined the cerebral load of the $A\beta_{n-40}$ and $A\beta_{n-42(43)}$ peptides.

METHODS

Case–control population

The Alzheimer's disease patients and the controls were of European ethnic origin and all came from France. There were 678 Alzheimer's disease patients (mean (SD) age 72.9 (8.6) years; age at onset 69.6 (8.5) years; 63% female, 37% male), and 667 controls (age 73.1 (8.5) years; 63% female, 37% male). A diagnosis of probable Alzheimer's disease was established according to DSM-III-R and NINDCS-ADRDA criteria. The controls were defined as subjects without DMS-III-R dementia criteria and with intact cognitive function. Each individual or next of kin gave informed consent.

Brain samples

Brains from 81 pathologically confirmed Alzheimer's disease patients (age of onset 64.8 (10.2) years; age at death 73.1 (9.4) years; 53% female, 47% male) were collected from the Manchester region in the United Kingdom. DNA was extracted from the frozen brain tissues of these cases by standard methods. The proportion of tissue area occupied by $A\beta_{40}$, $A\beta_{42(43)}$, and total Aβ was quantified in immunohistochemically stained sections from Brodmann areas 8/9 of the frontal cortex, as previously reported.[9]

Genotypes

DNA amplification was performed by polymerase chain reaction (PCR) as described previously.[8] The –509 and +25 PCR products were, respectively, digested by *Dde*I and *Sau*96 and separated by agarose and polyacrylamide gel electrophoresis. The –800 genotype was determined by allele specific oligonucleotides, as described by Cambien *et al*.[8]

Statistical analysis

SAS software, release 6.11, was used (SAS institute, Cary, North Carolina, USA). Univariate analyses were performed with Pearson's χ^2 test. In multivariate analyses, we coded the genotypes according to tested hypotheses (at least one TGF-β1 –800 allele: AA+GA/GG; at least one TGF-β1 –509 allele: TT+CT/CC; at least one TGF-β1 +25 allele: CC+GC/GG). The

Table 1 Genotype distribution of –800, –509, and +25 polymorphisms in a case–control population, and in relation to amyloid peptide (Aβ40, Abβ2, and total Abβ) in brains of patients with Alzheimer's disease

	–800			–509			+25		
	GG	GA	AA	CC	CT	TT	GG	GC	CC
AD patients	528 (83.7)	98 (15.5)	5 (0.8)	298 (41.4)	335 (46.5)	87 (12.1)	602 (87.8)	97 (13.8)	3 (0.4)
Controls	550 (86.1)	86 (13.4)	3 (0.5)	285 (42.0)	307 (45.2)	87 (12.8)	577 (85.5)	96 (14.2)	2 (0.3)
AD brains (n)	60	7	1	38	35	5	68	13	–
$A\beta_{40}$	4.5 (4.2)	2.0 (1.8)	3.9	3.2 (3.1)	4.8 (4.8)	5.3 (3.9)	4.2 (4.1)	2.9 (3.4)	–
$A\beta_{42(43)}$	10.7 (4.7)	12.5 (5.5)	4.2	9.7 (4.2)	11.0 (5.0)	12.2 (5.3)	10.8 (4.7)	9.0 (4.7)	–
Total Aβ	15.2 (7.5)	14.5 (6.5)	8.1	12.9 (5.9)	15.8 (8.2)	17.5 (8.3)	15.0 (7.3)	11.9 (6.3)	–

Values are n (%) (patients) or mean (SD) (brains).
AD, Alzheimer's disease; Aβ, amyloid β peptide.

effects of these variables on the disease were estimated using multiple logistic regression models adjusted for age and sex. The amyloid load for different genotypes was compared using Wilcoxon non-parametric tests. Extended haplotype frequencies of the three markers were estimated on collapsed data using the myriad haplotype algorithm described by MacLean *et al*.[10] Pairwise linkage disequilibrium coefficients were estimated in the control samples.[8]

RESULTS

For all three polymorphisms tested, the genotype distributions in control subjects were in Hardy–Weinberg equilibrium (table 1). The allele frequencies and genotype distributions of the three TGF-β1 polymorphisms in controls were similar to those previously reported.[8 11] We did not observe any differences in genotypic or allelic distributions between Alzheimer's disease and control groups for any of the polymorphisms.

We also tested the impact of these three polymorphisms on the Aβ load in Alzheimer's disease brains. No effect was detected for –800, –509, or +25 polymorphisms (table 1). No effect of these polymorphisms on age at onset was found in either case–control or Alzheimer brain cohorts. No difference in the distribution of estimated haplotype frequencies between patients and controls was observed. Furthermore, no particular haplotype correlated with Aβ load in Alzheimer brains (data not shown).

The linkage disequilibrium coefficients between pairs of polymorphisms were calculated. A negative sign in front of the coefficient indicates that the less frequent allele at one site is associated with the most frequent allele at the other site. As previously reported by Cambien *et al*,[8] there was a strong negative linkage between –800 and –509 (–0.97), and between –509 and +25 (–0.94). Complete linkage disequilibrium was observed between –800 and +25 in our population.

DISCUSSION

Despite some previous evidence to the contrary, we found that genetic variability at the TGF-β1 gene locus did not appear to be associated with Alzheimer's disease risk.

TGF-β1 appears to be a potential candidate susceptibility gene for Alzheimer's disease for the following reasons:

- In Alzheimer patients, TGF-β1 is present in senile plaques and in neurofibrillary tangles[4];
- TGF-β1 protein is overexpressed in Alzheimer brain tissues compared with control brain[5];
- Recent data on transgenic mice support the involvement of TGF-β1 in Alzheimer pathology—for example, overproduction of TGF-β1 in transgenic mice induces an Alzheimer-like cerebrovascular degeneration[12]; and immunoreactive astrocytes for TGF-β are present in early Aβ deposits in mice containing the Swedish double mutation of human amyloid precursor protein 695 as transgene.[13]
- Recent studies have shown that the TGF-β1 signalling in astrocytes promotes Aβ production and might play a critical role in the formation of amyloid plaques in the brain.[7]

These observations suggest that an increased level of TGF-β1 could play a significant role in neurodegeneration. These increased levels of TGF-β1 might be constitutionally associated with genetic variability at the TGF-β1 gene locus. Thus the aim of our study was to assess whether genetic variability at the TGF β1 gene locus could be a potential risk factor for Alzheimer's disease.

We first investigated the impact of the –509 polymorphism of the TGF-β1 gene in a large case–control population. The –509 T allele had previously been associated with higher serum concentration of TGF-β1 in a twin study, suggesting a functional role for this polymorphism in the regulation of TGF-β1 levels.[14] Moreover, a weak association of the –509 T allele with Alzheimer's disease was recently reported in an American population.[11] However, while the distribution of this polymorphism was similar in our control subjects to that reported by Luedecking *et al*,[11] we did not observe any impact of this polymorphism on the disease. Despite linkage disequilibrium reported at the TGF-β1 locus, we also tested the –800 and +25 polymorphisms in this population. The –800 and +25 polymorphisms were in strong linkage disequilibrium with –509, and not surprisingly no effect of these polymorphisms could be found in our case–control study. Moreover none of the polymorphisms tested showed an impact on Aβ load, and no haplotype appeared to influence the occurrence of Alzheimer's disease, the age at disease onset, or the Aβ load.

In conclusion, the three polymorphisms tested in this gene do not seemed to be implicated in the development of Alzheimer's disease.

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