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+10 T/C polymorphisms in the gene of transforming growth factor-β1 are associated with neurodegeneration and its clinical evolution

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Abstract

Transforming growth factor-β1 (TGF-β1) acts as an immunosuppressant by inhibiting the expression of several pro-inflammatory cytokines. Its gene contains single nucleotide polymorphisms (SNPs) at codon +10 (T → C) and +25 (G → C) that appear to influence the level of expression of TGF-β1. We investigated these SNPs in 198 healthy controls (HC), 193 patients with Alzheimer's disease (AD) and 48 patients with mild cognitive impairment (MCI). Among the latter, after a 4-year follow-up, 21 were diagnosed as AD (MCI → AD) while 18 did not progress (stable MCI).

We observed that both the +10 C allele and the CC genotype were over-represented in AD when compared to HC. These variants significantly raised the risk of disease independently of the status of apolipoprotein E4. The CC genotype was also over-expressed in MCI, especially in MCI → AD.

These results suggest that TGF-β1 may be one of the early markers involved in the inflammatory mechanisms underlying the pathogenesis of AD.

Keywords: Alzheimer's disease; Inflammation; Cytokines; TGF-β1; Gene polymorphisms

1. Introduction

Transforming growth factor-β1 (TGF-β1) is a member of a super-family of multifunctional peptides whose strong immunosuppressive effects are brought about by inhibiting the expression of both pro-inflammatory cytokines such as TNF-α and IL-1 (Suzumura et al., 1993; Benveniste et al., 1994) and adhesion molecules such as ICAM-1 and VCAM-1 (Shrikant et al., 1996; Winkler and Benveniste, 1998). TGF-β1 also regulates growth, function and differentiation of various cell types (Letterio and Roberts, 1998), and is involved in tissue repair after injury.

There is evidence that TGF-β1 besides protecting the brain from neuronal degeneration (Lindholm et al., 1992; Logan et al., 1992; Prehn et al., 1993) could also be involved in the pathogenesis of AD. This hypothesis is supported by experimental data showing that in vitro TGF-β1 regulates the synthesis and processing of amyloid precursor protein (APP) (Lahiri et al., 2005), the generation of amyloid-β (Aβ) fibrills (Cacquevel et al., 2004) and the formation of stable deposition of amyloid-β peptide on ApoE (Mosseau et al., 2003). Thus TGF-β1 could take part in the ''cytokine cycle'' of AD pathology, in which the anti-inflammatory cytokines (IL-4, IL-10, IL-13) regulate Aβ-induced microglial/macrophagic inflammatory responses (Szczepanik et al., 2001) and the presence of single nucleotide polymorphisms (SNPs) in genes of pro- and anti-inflammatory cytokines seems to enhance the risk of AD (Arosio et al., 2004). This is particular relevant since

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such cytokine polymorphisms are common in the general population and the chance that any single individual has of inheriting one or more of the high-risk alleles is substantial. A growing body of evidence support the hypothesis the overall risk of developing AD can be profoundly affected by a ''susceptibility profile'' reflecting the combined influence of inherited multiple high-risk alleles (Candore et al., 2007).

TGF-β1 gene, located on chromosome 19q13.1–3, contains several polymorphisms upstream and in the transcript region (Fujii et al., 1986; Awad et al., 1998; Hutchinson et al., 1998; Perrey et al., 1999). In literature conflicting results have been reported on the association between such polymorphisms and AD (Luedecking et al., 2000; Araria-Goumidi et al., 2002; Dickson et al., 2005; Hamaguchi et al., 2005; Van Oijen et al., 2006). A number of studies have also attempted to establish a relationship between polymorphisms in the TGF-β1 gene and serum or in vitro levels of TGF-β1, but rather ambiguous results have been obtained (Hutchinson et al., 1998; Yamada et al., 1998). An increased expression of TGF-β1 has been described in AD patients: in plasma and in CSF (Tarkowski et al., 2002) as well as in the intrathecal compartment and in the brain parenchyma (Luterman et al., 2000). However, a reduction of both its active (25 kDa) and inactive (50 kDa) forms has been also reported in AD plasma (Mocali et al., 2004).

In order to cast some light on the role of this cytokine in the pathophisiology of AD, we investigated the genetic variability of TGF-β1 in patients with mild cognitive impairment (MCI) and in Alzheimer's disease (AD). We focused our attention on point mutations of those alleles that are supposed to be involved in the expression of TGF-β1: namely alleles T (leucine) and C (proline) at codon 10 and alleles G (arginine) and C (proline) at codon 25.

2. Materials and methods

2.1. Patients and controls

We enrolled 48 (33F/15M, mean age 78 years ± S.D. 5.16) subjects with mild cognitive impairment (MCI). At a 4-year follow-up, 21 subjects were diagnosed with AD, 9 with vascular dementia (VD) and 18 had not progressed (stable MCI).

Our study also included 193 AD patients (129F/64M, mean age 80 years ± S.D. 5.9, range 63–92 years) and 198 healthy controls (HC), matched for sex and age (130 F/68 M, mean age 82 years ± S.D. 6, range 67–99 years).

Probable AD was diagnosed according to standard clinical procedures and on the basis of DMS IV and NINCDS-ADRDA criteria (McKhan et al., 1984). Functional status was assessed by means of the scales for the activities of daily living (ADL) and the instrumental activities of daily living (IADL). Cognitive performance was assessed by means of the mini-mental state evaluation (MMSE) and an extensive neuropsychological evaluation. All patients underwent computed tomography (CT) or magnetic resonance imaging (MRI) brain scan. For the diagnosis of a normal cognitive status the following criteria had to be met: (1) no active neurological or psychiatric disorders, (2) a normal neurological exam, (3) no psychotropic drugs, (4) no ongoing medical problems interfering with cognitive function and (5) living and functioning independently in the community.

The criteria used for MCI were those defined by Petersen and colleagues (Petersen et al., 1999; Petersen, 2004) and included: a memory-complaint, preferably corroborated by an informant; an objective memory impairment adjusted for age and education; a preserved general cognitive function; activities of daily living normal or only slightly impaired, no dementia according to DSM-IV criteria. Patients with MCI underwent a four-year follow-up. Patients who were diagnosed with AD during follow-up were required to meet the DMS IV and NINCDS-ADRDA criteria (McKhan et al., 1984). All patients were Caucasian and lived in northern Italy. They were selected from a larger population of outpatients visiting the Geriatric Unit of the Ospedale Maggiore Policlinico in Milan. There were no significant differences among the three groups in age or years of education.

In order to minimize the risk of an inflammatory process being present, for patients to be selected there had to be no evidence of inflammation—neither clinically (i.e. normal body temperature, no history of inflammatory conditions), nor on blood tests (normal red blood cell sedimentation rate, normal plasma concentrations of albumin, transferrin and C reactive protein).

Informed consent was obtained from the patients or their relatives. The study protocol was approved by the Ethics Committee of the University Hospital.

2.2. Blood sampling

Whole blood was collected by venipuncture in Vacutainer tubes containing EDTA (Becton Dickinson Co., Rutherford, NJ).

2.3. Genotyping

Genomic DNA was extracted by the salting-out method as described (Miller et al., 1988). The concentration and purity of DNA were determined by spectrophotometric analysis. In order to establish TGF-β1 genotypes we employed a polymerase chain reaction using sequence-specific primers (PCR-SSP). The sequence in the coding region of the TGF-β1 genes (polymorphic positions +10 and +25 in exon 1) was amplified using the cytokine genotyping tray method (One Lambda, Canoga Park, CA, USA). The human β-globin gene was amplified as an internal control for the genomic DNA preparation. PCR conditions were indicated by the One Lambda PCR program (OLI-1) and the PCR products were visualised by electrophoresis in 2.5% agarose gel.

ApoE genotypes were determined by PCR amplification of a 234 base-pair fragment of exon 4 of the ApoE gene, followed by digestion with Cfo1. The restriction patterns were obtained by gel electrophoresis.

Il-10 and IL-6 alleles were detected using the PCR-SSP assay previously described (Arosio et al., 2004).

2.4. Statistical analysis

Statistical analysis was carried out using the SPSS statistical package (SPSS version 10, Chicago, IL). Genotype frequencies in the study groups were compared by the χ^2-test. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated as estimates of the risk of AD in carriers of TGF-β1, IL-10 and IL-6 polymorphisms, compared to non-carriers. Adjusted estimation for ApoE ε4 carrier status was done by logistic regression analysis; $p < 0.05$ was taken as the cut-off for statistical significance.

3. Results

3.1. Distribution of TGF-β1 genotype and haplotype in AD and MCI subjects

AD patients had a significantly higher frequency of the +10 C (proline) allele (50.5% vs. 41%; $p = 0.009$), which skews the genotype distribution in AD compared to HC with an increase of the +10 CC genotype (24.9% vs. 16.2%; $p = 0.025$) (Table 1).

MCI subjects as a whole had an intermediate pattern between AD and HC subjects, the percentages of +10 C allele and CC genotype being 44.8% and 20.8%, respectively (Table 1).

Table 1
Frequency of the different +10 SNP genotypes and alleles observed in Alzheimer's disease (AD), in mild cognitive impairment (MCI) and in healthy sex- and age-matched controls (HC)

	Genotype			Allele	
	TT	TC	CC	T	C
AD ($n = 193$)	46 (23.8%)	99 (51.3%)	48 (24.9%)	191 (49.5%)	195 (50.5%)
HC ($n = 198$)	68 (34.3%)	98 (49.5%)	32 (16.2%)	234 (59%)	162 (41%)
MCI ($n = 48$)	15 (31.3%)	23 (47.9%)	10 (20.8%)	53 (55.2%)	43 (44.8%)

Genotype AD vs. HC – $\chi^2 = 7.388$; GF: $2p = 0.025$; allele – $\chi^2 = 6.892$; GF: $1p = 0.009$.

Table 2
Frequency of the different +25 SNP genotypes and alleles observed in Alzheimer's disease (AD), in mild cognitive impairment (MCI) and in healthy sex- and age-matched controls (HC)

	Genotype			Allele	
	GG	GC	CC	G	C
AD ($n = 193$)	165 (85.5%)	26 (13.5%)	2 (1%)	356 (92.2%)	30 (7.8%)
HC ($n = 198$)	172 (86.9%)	25 (12.6%)	1 (0.5%)	369 (93.2%)	27 (6.8%)
MCI ($n = 48$)	39 (81.2%)	8 (16.7%)	1 (2.1%)	86 (89.6%)	10 (10.4%)

Genotype AD vs. HC – $\chi^2 = 0.434$; GF: $2p = 0.805$; allele – $\chi^2 = 0.141$; GF: $1p = 0.707$.

The genotype and allele frequencies of +25 C → G SNP were similarly distributed in AD subjects, MCI subjects and controls (Table 2).

3.2. Follow-up

After a 4-year follow-up 21 MCI subjects progressed to AD (Hansson et al., 2006).

Table 3 shows +10 SNP distributions in MCI progressing (MCI → AD) and not progressing to AD (stable MCI).

In MCI → AD the percentages of both +10 C allele and CC genotype are higher than in stable MCI (genotype: $p = 0.001$, allele: $p = 0.008$).

Table 3
Frequency of the different +10 genotypes and alleles observed in stable MCI (MCI stable) and progressing to AD (MCI → AD)

	Genotype			Allele	
	TT	TC	CC	T	C
MCI stable	9 (50%)	7 (38.9%)	2 (11.1%)	25 (69.4%)	11 (30.6%)
MCI → AD	6 (28.6%)	9 (42.8%)	6 (28.6%)	21 (50%)	21 (50%)

Genotype – $\chi^2 = 13.727$; GF: $2p = 0.001$; allele – $\chi^2 = 7.036$ GF: $1p = 0.008$.

Table 4
TGF-β1 genotype and risk for Alzheimer's disease

+10 SNP	Cases	Controls	OR (CI)	Adj. OR (CI)
TT	46	69	1[a]	1[a]
TC	97	95	1.53 (0.96–2.45)	1.36 (0.79–2.34)
CC	46	34	2.03 (1.14–3.62)[*]	2.47 (1.21–5.08)[**]

The odds ratio indicates the risk for Alzheimer's disease in individuals carrying the CC genotype. OR: crude odds ratio; Adj. OR: odds ratio adjusted for sex, IL-6, IL-10 and apolipoprotein E ε4.
[a] Reference category.
[*] $p = 0.017$.
[**] $p = 0.014$.

None of the control subjects developed AD or MCI.

3.3. CC genotype and relative risk of developing AD

The presence of CC genotype significantly raised the risk of disease, independently of the ApoE4 status (OR 2.475, CI 1.206–5.079, $p = 0.014$) (Table 4).

The genotyping of our samples revealed the presence of ApoE4 in 42% and 44% of AD and MCI cases, respectively; as expected these values were higher than the value for HC (28%) and the value for the general population of the same region of Italy (10%), as was recently reported in literature (Singh et al., 2006).

When the data were stratified according to the presence or absence of the ApoE4 allele, the distribution of the polymorphisms in the TGF-β1 gene was similar in both cases and controls, in both ApoE4 carriers and non-carriers ($p = 0.708$ and 0.504, in AD and HC, respectively).

We also adjusted the OR for the presence of both IL-10 A and IL-6 C alleles, previously described as risk factors in our AD population, and we found that the risk was not dependent of these interleukin polymorphism (Table 4).

4. Discussion

TGF-β1 is though to play an important role in cellular response to brain injury and to protect the brain against neuronal degeneration (Prehn et al., 1993). Inconclusive data have been reported on the involvement of TGF-β1in the pathogenesis of AD (Van der Wal et al., 1993; Wyss-Coray et al., 1997).

We found the highest percentage of TGF-β1 +10 C allele and CC genotype carriers among AD and MCI subjects. The homozygous state for the C allele was associated with an increased risk of AD (more than twofold) in a fashion unrelated to the IL-10 and IL-6 genotypes (Arosio et al., 2004).

The risk of disease in CC carriers was independent of ApoE4 status, indicating that such an association cannot be the expression of a linkage disequilibrium between TGF-β1 and ApoE genes, which are neighbours on chromosome 19.

Since the TGF-β1 and ApoE genes are neighbours on chromosome 19, this association might be the expression of a linkage disequilibrium with the ApoE4 allele. However, stratification by ApoE4 status actually shows that the risk is independent.

These findings disagree with those obtained by the Rotterdam Study (Van Oijen et al., 2006), possibly because of differences in study design.

It is interesting to remark that polymorphism in the TGF-β1 gene seems to influence the levels of expression of such inflammatory cytokine (Awad et al., 1998). Although several other factors may influence protein expression in the periphery, in our MCI → AD patients the CC genotype was associated with reduced serum level of TGF-β1 (unpublished data) suggesting that low levels of this cytokine could favour neurodegeneration in the early stage of AD (Tarkowski et al., 2003). However, because of the small size of the sample, our results will need to be confirmed in a larger number of patients.

In conclusion, our results suggest that the TGF-β1 gene polymorphism may contribute to the AD ''susceptibility profile'' characterized by the presence of high-responder alleles of pro-inflammatory cytokines and low-responder alleles of anti-inflammatory cytokines (Licastro et al., 2000). These finding are in line with the hypothesis that a genetic background leading to an excessive inflammation response may be involved in the onset of AD.

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