Tumor necrosis factor-α−308A/G polymorphism is associated with age at onset of Alzheimer’s disease

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Abstract

Pro-inflammatory cytokines and acute-phase proteins play an important role in Alzheimer’s disease (AD) neurodegeneration, and common polymorphisms of genes controlling their production have been shown to be associated with AD. Tumor necrosis factor (TNF)-α is an inflammatory cytokine involved in the local immune response occurring in the central nervous system of AD patients. Genetic variation could contribute to the risk of developing AD or influence the age at the onset of the disease. We genotyped 222 patients (152 women, 70 men; age range 60–87) and 240 non-demented age-matched healthy controls for TNF-α −308 G/A single nucleotide polymorphism (SNP). No significant differences were observed in genotyped frequencies between patients and controls, whereas carriers of −308A showed a significantly lower mean age at onset than non-carriers of this allele. This difference was more evident taking into account ApolipoproteinE (ApoE) status since the lowest age at onset was observed in patients carrying the −308ATNF+/APOE4+ genotypes. In conclusion, our data support previous suggestions that, at least in Caucasians, the TNF gene is a disease modifier gene in patients in which AD is rising, bringing to light the importance of genetic variation at the pro-inflammatory components in the progression of AD.

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Keywords: Alzheimer’s disease; Cytokine; Inflammation; SNP; TNF-α

1. Introduction

Alzheimer’s disease (AD) is a complex disease with multifactorial aetiology. Genetic factors have been found to be associated with the sporadic form of the disease, and the ApolipoproteinE (APOE) ε4 allele significantly increases the risk of AD (Myers and Goate, 2001; Nussbaum and Ellis, 2003). The brain lesions associated with AD, which are referred to as neurofibrillary tangles and senile plaques, are characterized by the presence of a broad spectrum of inflammatory mediators, produced by resident brain cells, including neurons (Akiyama et al., 2000). Although secondary to the fundamental pathology caused by the presence of tangles and plaques, there is strong evidence that inflammation exacerbates the neuronal loss (McGeer and McGeer, 2001a,b). In fact, local inflammatory processes can exert a direct neurotoxicity, interfere with β-amyloid expression and metabolism, and maintain a chronic intracerebral acute phase protein secretion, in turn favouring formation of β-amyloid fibrils (Licastro and Chiappelli, 2003). Accordingly, several reports have appeared indicating that the risk of AD is substantially influenced by polymorphisms in the promoter region, and other untranslated regions, of genes encoding inflammatory mediators. Alleles that favour increased expression of the inflammatory mediators or alleles that favour decreased expression of anti-inflammatory mediators are more frequent in patients with AD than in controls. However, the association of allelic variants of a few molecules cannot account for all sporadic AD
cases and other largely unknown genetic and environmental factors are implicated in the disease (Arosio et al., 2004; Candore et al., 2004a; Caruso et al., 2003; Licastro and Chiappelli, 2003; Licastro et al., 2003, 2005; Lio et al., 2003, 2005; McGeer and McGeer, 2001a, b).

The pleiotropic pro-inflammatory cytokine tumor necrosis factor (TNF-α), which maps within the class III region of human leukocyte antigen (HLA), is an important mediator of the inflammatory responses with multiple biologic activities (Candore et al., 2002, 2004a, b; Cipriano et al., 2005). Several polymorphic areas are documented within the TNF gene cluster. Notably, the −308A single nucleotide polymorphism (SNP) located in the promoter region of the TNF-α gene is reported to have an increased frequency in autoimmune and inflammatory diseases and is associated with stronger transcriptional activation than the G allele, although it may be due to linkage with other genes in the HLA region (Candore et al., 2002, 2004a, b; Perry et al., 2001; Price et al., 1999). TNF-α might be involved in AD, based on observations that patients with AD display significantly higher intrathecal levels of TNF-α compared to the controls. However, when TNF polymorphisms were related to intrathecal levels of the cytokine, its levels did not differ significantly in patients displaying different alleles of the TNF gene, suggesting that increased intrathecal production of TNF-α in AD is preferentially controlled by environmental stimuli rather than genetic makeup (Tarkowski et al., 2000).

Moreover, opposite effects of TNF-α on AD development have been claimed, being the effect protective or enhancing neuro-inflammation depending on the time of action (Akiyama et al., 2000; Wick et al., 2003). Accordingly, contrasting results have been obtained in the studies performed on the role of TNF polymorphisms in AD (Candore et al., 2004a).

In the light of these considerations, we planned to evaluate whether the −308 TNF-α SNP was related to the development of AD, in a homogeneous population, from Northern Italy of sporadic late-onset AD patients.

2. Methods

2.1. Patients and controls

Two hundred twenty two AD patients (152 women, 70 men; age range 60–87) and 240 non-demented age-matched healthy controls (132 women, 108 men) were enrolled. Subjects affected by cancer or cardiovascular diseases (both in AD and control groups) were not included in the study. The patients were followed at the Geriatric Department of the Ospedale Maggiore IRCCS, University of Milan, Italy. We applied the DMS IV and NINCDS-ADRDA criteria to obtain the clinical diagnosis of AD (McKhann et al., 1984); every subject had a recent brain magnetic resonance imaging/computed tomography scan available. Cognitive performances of all enrolled subjects were assessed according to the Mini-Mental State Evaluation (MMSE) (Cockrell and Folstein, 1988). AD patients and healthy controls were living at home and a careful physical examination was done again on the day of blood collection, and their clinical records were consulted. The study protocol was approved by the Ethics Committee of the University Hospital. Written informed consent for enrolling in the study and for personal data management was obtained from controls and relatives of AD patients according to Italian laws.

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele</th>
<th>AD</th>
<th>Women</th>
<th>Men</th>
<th>HC</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>G/A</td>
<td>A/A</td>
<td>G</td>
<td>A</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>163 (75.4)</td>
<td>54 (24.3)</td>
<td>5 (2.3)</td>
<td>380 (85.6)</td>
<td>64 (14.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110 (72.4)</td>
<td>38 (25.0)</td>
<td>4 (2.6)</td>
<td>258 (84.9)</td>
<td>46 (15.1)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>53 (75.7)</td>
<td>16 (22.9)</td>
<td>1 (1.4)</td>
<td>122 (87.1)</td>
<td>18 (12.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>164 (68.3)</td>
<td>65 (27.1)</td>
<td>11 (4.6)</td>
<td>393 (81.9)</td>
<td>87 (18.1)</td>
<td></td>
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</tr>
<tr>
<td>95 (71.6)</td>
<td>34 (25.4)</td>
<td>5 (3.7)</td>
<td>224 (83.6)</td>
<td>44 (16.4)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>69 (65.1)</td>
<td>31 (29.2)</td>
<td>6 (5.7)</td>
<td>169 (79.7)</td>
<td>43 (20.3)</td>
<td></td>
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</tbody>
</table>

The distribution of genotypes was in HW equilibrium in all the groups. No significant differences were observed between the AD patients and the controls.

2.2. Genotyping

Genomic DNA was obtained from blood samples. DNA extraction was carried out and typing of G/A −308 (Gene Bank Accession number: CR941560) SNP in the TNF-α promoter region, was performed as described (Lio et al., 2001). The allelic ApoE polymorphisms were assessed by PCR-based method (Arosio et al., 2004).

2.3. Statistics

Genotypic and allele frequencies were evaluated by gene count and contingency tables ($\chi^2$-test) constructed to determine statistical significance of differences in frequency for the alleles under study between the groups. The data were tested for the goodness of fit between the observed and expected genotype values and their fit to Hardy–Weinberg equilibrium (HWE) by $\chi^2$-test. Differences in the mean age of onset, fitting in Gaussian distribution (assessed by Kolmogorov–Smirnov test) between the patients carrying the different alleles were calculated by Student t-test or by ANOVA and Tukey–Kramer post-test, when multiple comparisons were made.

3. Results

Table 1 shows the frequency of genotypes and alleles for 308A/G TNF-α gene SNP in our sample of 222 patients with AD and 240 healthy Italians. No significant differences were observed in frequency genotypes and alleles between controls and patients both when data were analyzed on the whole and according to gender.

Table 2 shows the mean age at onset in AD patients carrying the −308ATNF-α SNP (subjects bearing −308AA homozygous

<table>
<thead>
<tr>
<th>−308A negative patients</th>
<th>−308A positive patients</th>
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<tbody>
<tr>
<td>No</td>
<td>Age at onset</td>
</tr>
<tr>
<td>AD</td>
<td>163</td>
</tr>
<tr>
<td>Women</td>
<td>110</td>
</tr>
<tr>
<td>Men</td>
<td>53</td>
</tr>
</tbody>
</table>

Data fit in Gaussian distribution for all groups (Kolmogorov–Smirnov test). Differences in the mean age of onset between the patients carrying the different alleles were calculated by Student t-test. A significant difference in mean age at onset was observed between −308A positive patients and the negative ones ($p = 0.0023$). By analysing data according to gender, the significance was attained both in women ($p = 0.0084$) and in men ($p = 0.0405$).
and −308A heterozygous genotypes) and those homozygous for the −308G TNF-α SNP. It can be observed that −308A-positive patients showed a significant lower mean age at onset than negative ones (p = 0.0023). By analysing data according to gender, the significance was attained both in women (p = 0.0084) and in men (p = 0.0405).

As expected and already reported (Locke et al., 1995), the frequency of the well known genetic risk factor APOE4 allele was increased in AD patient group (23.2% versus 9.5%; p = 1 × 10⁻⁷). In some studies the association between AD and cytokine polymorphisms has been shown evident (or more evident) in APOE4 allele non-carrier patients, suggesting a complex interaction between these genetic factors (Licastro and ChiapPELLI, 2003; Lio et al., 2005; Myers and Goate, 2001). However, no significant differences in −308 TNF-α allele frequencies were observed between AD APOE4 positive and negative subjects, also by analysing data according to gender (data not shown).

Table 3 shows the effect of combined TNF-α and APOE genotypes on the age at onset of AD patients under study. AD patients were classified according to APOE4 negative (APOE2/2; APOE2/3; APOE3/3) or positive (APOE2/4; APOE3/4; APOE4/4) genotypes and TNF −308A negative (−308GG) or positive (−308AA; −308GA) genotypes. The distribution of age at onset was found significantly different by ANOVA analysis among the different subject groups classified according to APOE and TNF genotypes (p = 0.0141). Tukey–Kramer multiple comparison post-test showed that APOE4 and −308A positive subjects have the lowest significant onset age (p < 0.05) when compared to APOE4 positive and −308A negative group of subjects as well as to APOE4 and −308A negative ones.

<table>
<thead>
<tr>
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<th>−308A TNF positive</th>
<th>−308A TNF negative</th>
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<tbody>
<tr>
<td>APOE4 positive</td>
<td>70.2 ± 6.8 (28)</td>
<td>74.2 ± 6.0 (64)</td>
</tr>
<tr>
<td>APOE4 negative (31)</td>
<td>71.4 ± 5.9 (31)</td>
<td>73.9 ± 7.4 (99)</td>
</tr>
</tbody>
</table>

Age of onset distribution was found significantly different by ANOVA analysis among the different subject groups classified according to ApoE and TNF genotypes, that ApoE4 and −308A genotype and TNF −308A negative (−308GG) or positive (−308AA; −308GA) genotypes. Numbers of subjects bearing the combined genotypes are reported in brackets.

4. Discussion

TNF is one of the main pro-inflammatory cytokine that plays a central role in initiating and regulating the cytokine cascade during an inflammatory response. However, the pathophysiological actions of TNF-α in the nervous system are surprisingly controversial, since depending on the experimental model it has been claimed to be trophic or killer for neurons, by respectively inducing either the expression of protective molecules or of the transcription factor that elevates expression of pro-inflammatory factors. This dichotomy could be also explained by the fact that TNF-α elicits its biological effects through the activation of two distinct receptors, the p55 TNF receptor (type I TNFR) and the p75 TNF receptor (type II TNFR). The type I TNFR contains an intracellular death domain and contributes to cell death when activated. By contrast, type II TNFR is likely to play a trophic or protective role in neuronal survival (Li et al., 2004; Uberti et al., 2004; Wick et al., 2003).

TNF gene cluster is highly polymorphic and reporter gene constructs containing the A allele of the −308 SNP appear to have higher transcriptional activity than with the −308G allele and some in vitro studies have demonstrated higher levels of TNF are released from cells with the −308A allele of TNF (Candore et al., 2002, 2004a,b; Lio et al., 2001; Price et al., 1999).

Thus, the candidate gene under study may be involved in the AD phenotype and −308 SNP has functional effects on the protein. Therefore, the association is biologically plausible and it is rationale to perform such a case control study (Tabor et al., 2002). In spite of that, we were unable to demonstrate the association between −308A genotype and AD. Accordingly, negative results were obtained by the few case-control studies investigating the relationship between the TNF-α −308G/A SNP and AD (Alvarez et al., 2002; Culpan et al., 2003; Lehmann et al., 2001; Perry et al., 2001; Zhang et al., 2004). Concerning age at onset in these case control studies, two studies performed this analysis but contrasting results were obtained. In a study, which included 111 African-Americans with AD, Perry et al. (2001), showed a significant increase in age of onset for patients carrying the TNF −308 A SNP when compared to patients with G (mean age 73.9 versus 70.6, P = 0.02). On the other hand, in 315 Spanish patients, Alvarez et al. (2002), were able to show that carriers of −308A showed a mean age at onset 3 years younger than noncarriers of this allele (mean age 72.2 versus 75.1, P = 0.019). So, our results and Alvarez’ ones were contradictory with that by Perry et al. (2001). Some of these discrepancies might be due to genetic heterogeneity of the populations studied (Tabor et al., 2002). In particular, TNF gene may show different effects upon different ethnic groups, depending on its interaction with other unknown different susceptibility genes and/or risk factors in another nearby locus which may vary in different populations. In fact, TNF gene is an HLA gene and in HLA complex many other genes encode for proteins involved in immune-inflammatory responses (Candore et al., 2002, 2004b; Price et al., 1999). In this view, the common genetic soil shared between Spanish and Italian populations allows to hypothesise that the HLA 8.1 ancestral haplotype, involving −308A allele (Candore et al., 2002, 2004b) might be a disease modifier gene in patients in which AD is rising, bringing to light the importance of genetic variation at the pro-inflammatory components in the progression of AD pathogenesis.

APOE4 variant is a major risk factor for sporadic AD. Several hypotheses have been proposed to explain the association of the APOE4 allele with AD being involved in

Table 3

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<thead>
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<th></th>
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deposition or clearance of β-amyloid (Carter, 2005). On the other hand, data on the effect of APOE4 allele on mean age of onset of Alzheimer’s disease are not univocal (Reiman et al., 2005; Tiraboschi et al., 2004; Khachaturian et al., 2004; Rogaea, 2002; Locke et al., 1995). Our data show that the ApoE4 allele has only a marginal, if any, influence on the age of AD onset, being the lowest significant onset mean age observed in ~308A TNF and ApoE4 positive subject group. Intriguingly, our data and some of other studies on sporadic AD patients of Italian ancestry, in spite of a constant observation of the association of an increased risk for AD, demonstrate only a partial influence on the age of onset of the disease of the ApoE4 allele (Bosco et al., 2005; Marra et al., 2004; Panza et al., 2000). As well known, the frequency of ApoE4 allele follows a geographic North-South decreasing trend in Western Europe (Panza et al., 1999), so the effect of this allele on age of onset might be more easily detected in North- than in South-Europe (Bosco et al., 2005).

In conclusion, our data add another piece of evidence on the hypothesis that a pro-inflammatory genetic profile affects the pathogenetic mechanisms and/or the progression of AD (Lio et al., 2003), suggesting that definition of the genetic background of pro- or anti-inflammatory relevant molecules might be useful in the identification of new therapeutic strategies for the prevention of the Alzheimer’s disease.

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