Interleukin-8-251T > A, Interleukin-1α-889C > T and Apolipoprotein E polymorphisms in Alzheimer’s disease

Alex Augusto Vendramini¹, Roger Willian de Lábio², Lucas Trevizani Rasmussen²,³, Nathali Mattiuzzo dos Reis²,³, Thais Minett⁴, Paulo Henrique Ferreira Bertolucci⁴, Marcela Augusta de Souza Pinhel⁵, Dorotéia Rossi Silva Souza⁵, Diego Robles Mazzotti³, Marília de Arruda Cardoso Smith³ and Spencer Luiz Marques Payão¹,²

¹Pós-Graduação em Biologia Oral, Universidade do Sagrado Coração, Bauru, SP, Brazil.
²Disciplina de Genética, Hemocentro, Faculdade de Medicina de Marília, Marília, SP, Brazil.
³Departamento de Morfologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil.
⁴Disciplina de Neurologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil.
⁵Núcleo de Pesquisa em Bioquímica e Biologia Molecular, Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, SP, Brazil.

Abstract

An inflammatory process has been involved in numerous neurodegenerative disorders such as Parkinson’s disease, stroke and Alzheimer’s disease (AD). In AD, the inflammatory response is mainly located in the vicinity of amyloid plaques. Cytokines, such as interleukin-8 (IL-8) and interleukin-1α (IL-1α), have been clearly involved in this inflammatory process. Polymorphisms of several interleukin genes have been correlated to the risk of developing AD. The present study investigated the association of AD with polymorphisms IL-8 -251T > A (rs4073) and IL-1α-889C > T (rs1800587) and the interactive effect of both, adjusted by the Apolipoprotein E genotype. 199 blood samples from patients with AD, 146 healthy elderly controls and 95 healthy young controls were obtained. DNA samples were isolated from blood cells, and the PCR-RFLP method was used for genotyping. The genotype distributions of polymorphisms IL-8, IL-1α and APOE were as expected under Hardy-Weinberg equilibrium. The allele frequencies did not differ significantly among the three groups tested. As expected, the APOE4 allele was strongly associated with AD (p < 0.001). No association of AD with either the IL-1α or the IL-8 polymorphism was observed, nor was any interactive effect between both polymorphisms. These results confirm previous studies in other populations, in which polymorphisms IL-8 -251T > A and IL-1α-889C > T were not found to be risk factors for AD.

Key words: IL-8, IL-1α, Alzheimer’s Disease, APOE, inflammatory response.

Received: January 20, 2010; Accepted: June 17, 2010.

Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder that causes loss of memory, mental confusion and several cognitive disturbances. It frequently occurs at around 60 years of age, but may also have an early onset at 40 years (Khachaturian, 1985). Dementia is an increasingly common diagnosis in the aging population, and the numbers are expected to rise exponentially in coming years. AD alone affects 5 million people in the US, while millions more are currently affected by vascular dementia, Lewy body disease and frontotemporal dementia (Grossman et al., 2006).

Two neuropathological features characterize the AD brain: amyloid plaques and neurofibrillary tangles. Plaques are mostly characterized by extracellular deposits of amyloid-β peptide (Aβ), which is derived from the processing of the amyloid precursor protein (APP). Neurofibrillary tangles correspond to intracellular accumulation of fibrils called paired helical filaments (Cacquevel et al., 2004). In this sense, the neurological implications of AD come from the coexistence of two degenerative processes, tau protein aggregation and Aβ deposition, that affect polymodal association brain areas, a feature never observed in non-human primates and difficult to model (Delacourte, 2006).
Familial early-onset AD is associated with mutations in the APP and presenilin genes, whereas only a polymorphic variation at the apolipoprotein E (APOE) locus, the e4 allelic form has so far been firmly established as a genetic risk factor for late-onset familial and sporadic AD. However, these genetic risk factors only account for about 30-50 percent of all cases of AD. Thus, an interaction between genetic, biological and environmental factors might account for most AD cases, by promoting inflammatory reactions, particularly those mediated by the release of interleukin-1α (IL-1α) from microglial cells of the brain (Hayes et al., 2004). Infection or injury of the body results in inflammation. A hallmark of this response is the recruitment of neutrophils from the blood to the injured tissue. This process could be directed by chemotactic polypeptides, the so-called chemokines (Zlotnik and Yoshie, 2000). Chemokines are low-molecular-weight chemotactic cytokines that have been shown to play an important role in early inflammatory events (Baggiolini et al., 1994). So far, microglial cells were shown to display an increased migratory response to β chemokines, including monocyte chemotactic protein 1 (MCP-1), suggesting that these molecules may play an important role in the trafficking of mononuclear phagocytes within the brain (Peterson et al., 1997). It is widely accepted that chronic inflammatory reaction plays an important role in the pathogenesis of AD, and a variety of inflammatory factors, including cytokines and chemokines, have been detected in and around plaques and tangles (Galimberti et al., 2006; Pomponi et al., 2008). Moreover, elevated levels of chemokines are demonstrable in the brain in neurodegenerative diseases, such as AD, whereas in healthy brains they are detected at low levels (Galimberti et al., 2006). Thirumangalakudi et al. (2008) made an immunohistochemical analysis of activated microglia and astrocytes as neuroinflammation markers, cytokines expression and cognitive alteration in C57/BL6 and low-density lipoprotein receptor (LDLR) (-/-)-deficient mice fed a fat/cholesterol-rich diet. Their findings link hypercholesterolemia with cognitive dysfunction potentially mediated by increased neuroinflammation and APP processing in a non-transgenic mouse model.

IL-8 enhances the survival of hippocampal neurons in vitro and increases the proliferation of glial cells (Araujo and Cotman, 1993). Strong immunoreactivity for CXCR2 (chemokine (C-X-C motif) receptor 2), the IL-8-related receptor, has been demonstrated in both AD and age-matched subjects with non-inflammatory affections of the nervous system. In particular, CXCR2 expression in AD is close to neuritic plaques, surrounding Aβ deposits (Galimberti et al., 2003, 2006). Increased IL-1 expression, in the form of higher tissue concentrations of the IL-1 protein and increased numbers of IL-1 immunoreactive astrocytes, has been demonstrated in the brains of patients with AD and in those elderly individuals with Down syndrome who show AD-type pathology in their brains (Griffin et al., 1989). Polymorphisms located in the promoter regions of the IL-1α and IL-8 genes have been widely studied as risk factors for AD. For instance, the IL-1α-889C > T polymorphism was strongly associated with late-onset AD in samples from two different centers: Indianapolis, IN, USA, and Munich, Germany (Du et al., 2000). However, other studies conducted in different populations did not show this association (Kuo et al., 2003; Tang et al., 2004; Zhou et al., 2006; Dursun et al., 2009; Hu et al., 2009). In addition, Infante et al. (2004) reported that neither the presence of the IL-1α-889 T allele nor the presence of the IL-8 -251T > A polymorphism TT genotype was associated with AD in Caucasians originating from a homogeneous population in a limited geographical area in Northern Spain. However, subjects carrying both the IL-1A allele T and the IL-8 TT genotype had about twice the risk of developing AD than subjects without these genotypes.

In the present study, we characterized the IL-8 -251T > A and IL-1α-889C > T polymorphisms in AD patients, healthy young and elderly control groups. We also investigated a possible interactive effect between these polymorphisms in the developing of AD, as proposed by Infante et al. (2004)

Materials and Methods

Patients and controls

Peripheral blood samples were obtained from 440 Brazilian individuals: 199 AD patients, 146 healthy elderly controls (EC) and 95 healthy young controls (YC).

The sample of AD patients was composed of 87.44% subjects of European origin, 6.80% of Japanese origin and 5.76% of African origin; the sample of elderly controls was composed of 89.78% subjects of European origin, 5.84% of Japanese origin and 4.38% of African origin; and the sample of young controls was composed of 87.65% subjects of European origin, 6.80% of Japanese origin and 4.75% of African origin. Thus, there was no difference in the ethnic distribution of the three groups. The mean age and standard deviation of the samples was: 73.81 ± 7.95 years in the AD group, composed of 68 men and 131 women; 71.25 ± 9.02 years in the EC group, composed of 53 men and 93 women; and 20.56 ± 1.64 years in the YC group, composed of 31 men and 64 women. The AD patients were selected according to the NINCDS-ADRDA criteria for probable AD (McKhann et al., 1984). Vascular dementia was excluded by a Hachinski score of 5 or higher and by neuro-imaging (Hachinski et al., 1975). Patients as well as controls were from São Paulo City, and all subjects gave informed consent for participation in this study, which was approved by the USC (Universidade do Sagrado Coração de Bauru) ethics committee (Protocol number 0110/2004). The control groups were composed of relatives (spouse or children) or
friends of the patients. For these groups, exclusion criteria were a history and examination findings suggestive of neurological (seizure, brain trauma with loss of consciousness longer than 15 min, stroke, Parkinson’s disease) or psychiatric disease (depression and substance abuse, including alcohol), and evidence of functional decline as shown by a structured questionnaire. All experiments were conducted in accordance with the Declaration of Helsinki.

**Laboratory analysis**

Total genomic DNA was extracted from blood samples using a Qiagen extraction kit, according to the manufacturer’s instructions.

IL-8 -251T > A genotyping: IL-8 -251T > A genotypes were determined using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. A 349 bp fragment was amplified from genomic DNA using the forward and reverse oligonucleotides IL-8F1 5’-CAT AGC ATC TGT AAT TAA CTG-3’ and IL-8R2 5’-CTC ATC TTC TCA TTA TGT CAG AG-3’, respectively, as described previously (Hamajima et al., 2003). PCR conditions included an initial denaturation step of 94 °C/5 min, followed by 30 cycles of 94 °C for 45 s, 52 °C for 45 s, 72 °C for 1 min, and a final extension step of 72 °C for 7 min. The amplification products were digested with MnuI restriction enzyme (Fermentas, Ottawa, ON, Canada), subjected to electrophoresis on a 3% agarose gel, stained with ethidium bromide and analyzed on an Alpha Imager 2200 (Alpha Innotech Corporation, San Leandro, CA, USA). After digestion, three different band combinations were found, viz. a 349 bp fragment (TT genotype), 202 and 147 bp fragments (AA genotype); and 349, 202 and 147 bp fragments (TA genotype).

IL-1α-889C > T genotyping: IL-1α-889C > T genotypes were also determined using PCR-RFLP. A 194 bp fragment was amplified from genomic DNA using the forward and reverse oligonucleotides IL-1AF1 5’-GCA TGC CAT CAC ACC TAG TT-3’ and IL-1AR1 5’-TTA CAT AGC TTG TAG CAG AG-3’, respectively, as described previously (Infante et al., 2004). The PCR conditions included an initial denaturation step of 94 °C/5 min, followed by 30 cycles of 94 °C for 45 s, 52 °C for 45 s, 72 °C for 1 min, and a final extension step of 72 °C for 7 min. The amplification products were digested with Mun1 restriction enzyme (Fermentas, Ottawa, ON, Canada), subjected to electrophoresis on a 3% agarose gel, stained with ethidium bromide and analyzed on an Alpha Imager 2200 (Alpha Innotech Corporation, San Leandro, CA, USA). After digestion, three different band combinations were found, viz. a 349 bp fragment (TT genotype), 202 and 147 bp fragments (AA genotype); and 349, 202 and 147 bp fragments (TA genotype).

APOE genotyping: The APOE genotypes were determined by PCR-RFLP using the oligonucleotides and reaction conditions described by Hixson and Vernier (1990). The amplification products were digested with HhaI (Fermentas, Ottawa, ON, Canada), subjected to electrophoresis on a 4% NuSieve® GTG® agarose gel (Cambrex, Rockland, ME, USA), stained with ethidium bromide and analyzed on an Alpha Imager 2000 (Alpha Innotech Corporation, San Leandro, CA, USA).

**Statistical analysis**

The chi-square test was used to compare categorical variables and to test the deviation from the Hardy-Weinberg equilibrium for each polymorphism. A value of \( p < 0.05 \) was considered statistically significant, and all tests were two-tailed. Logistic regression was used to investigate the individual relationships and interactive effect between independent variables (carriers versus non-carriers of the IL-1α T allele, and carriers versus non-carriers of the IL-8 TT genotype). This approach was based on the findings of Infante et al. (2004), who suggested an interactive effect of the IL-1A allele and the IL-8 TT genotype. For this analysis, we considered both polymorphisms as dependent variables, and gender, age and APOE genotype groups as co-variables in the model. The crude odds ratio (OR) was calculated considering the AD group genotypes in relation to the genotypes of the elderly and of the young control groups, using gender and age as co-variables. The adjusted odds ratio (OR) by APOE genotype considered three groups of APOE genotypes: 1) E2E2 with E2E3; 2) E3E3; and 3) E3E4 with E4E4, as well as gender and age as co-variables. Ninety-five percent confidence intervals were calculated. The statistical analyses were performed using the SPSS 16.0 package.

**Results**

Table 1 presents the IL-8 -251T > A and IL-1α-889C > T genotype distributions in the AD, EC and YC subject groups. The APOE genotype distributions among the three subject groups are also shown in Table 1. There were no statistically significant differences concerning gender among the subject groups (\( p = 0.22; \) data not shown).

The genotype distributions concerning the IL-8 -251T > A and the IL-1α-889C > T polymorphism were in Hardy-Weinberg equilibrium in all three subject groups. The APOE genotype distribution was also in Hardy-Weinberg equilibrium in the three subject groups. The comparison of the genotype frequency distribution in the AD patients and the EC group did not show any significant difference for either IL-8 -251T > A (\( p = 0.05 \)) or IL-1α-889C > T (\( p = 0.23 \)). Similarly, no significant difference was found in the comparison between AD patients and the YC group (Table 1).

Logistic regression analysis did not detect an association of AD with any of the polymorphisms individually. An interactive effect of both polymorphisms in our tri-hybrid Brazilian population was not detected (Tables 1 and 2). As expected, logistic regression analysis revealed a strong as-
association of the E4 allele in AD patients in relation to the EC (OR = 8.137, 95% CI = 4.569-14.489, p < 0.001) and YC (OR = 3.174, 95% CI = 1.850-5.446, p < 0.001) groups (data not shown).

Discussion

This study was the first one to investigate a potential association of polymorphisms *IL-8*-251T > A and *IL-1α*-889C > T with AD. The findings concerning *IL-8*-251T > A confirmed our previous study with a smaller sample (Vendramini et al., 2007), which did not detect an association of this polymorphism with AD either. Infante et al. (2004) also observed a lack of association of polymorphisms *IL-1α*-889C > T and *IL-8*-251T > A with AD. No association of the IL1-A polymorphism with AD was also reported in Chinese samples from Taiwan (Kuo et al., 2003), from the Chengdu area (Tang et al., 2004), and in a Han population (Hu et al., 2009). Similarly, the *IL-1α*-889C > T polymorphism was not associated with AD either in individuals from the Canary Islands, Spain (Deniz-Naranjo et al., 2008) or in a homogeneous Caucasoid population from northern Spain (Infante et al., 2004).

No interactive effect of the *IL-1α*-889T allele and the *IL-8* TT genotype concerning the AD group in relation to EC and YC was detected (Tables 1 and 2). However, our findings differed from those of Infante et al. (2004). This discrepancy may be due to the distinct ethnic composition of both populations. As it is well known, the Brazilian population is mainly composed of European, African and Amerindian descendants. Other variables, such as the AD age of onset or another undetectable stratification bias may also be involved.

Our findings concerning the lack of association of *IL8* and *IL1* with AD and of an interactive effect between them were consistent with the great majority of reports from different population samples. Hence, taking together our results with others from the literature, it appears that *IL-8*-251T > A and *IL-1α*-889C > T do not play a major role in the pathogenesis of late-onset AD.

Acknowledgments

This research was supported by Fundação de Amparo à Pesquisa de São Paulo (FAPESP, Brazil), Grant number -

### Table 1 - Absolute and relative genotype frequencies of the *IL-8* and *IL-1α* polymorphisms and *APOE* genotypes in an Alzheimer’s disease patient group (AD) and in an elderly (EC) and a young (YC) healthy control groups.

| IL-8 -251T > A | AD  | EC  | YC  |
|---------------|-----|-----|-----|
| A/A           | 47  | 28  | 19  |
| T/A           | 101 | 63  | 49  |
| T/T           | 51  | 55  | 27  |

| IL-1α-889C > T | C/C   | T/C   | T/T   |
|---------------|-------|-------|-------|
|               | 96    | 84    | 19    |

APOE genotype

| E2/E2          | 0 (0%) | 1 (0.68%) | 1 (1.05%) |
| E2/E3          | 11 (5.53%) | 11 (7.53%) | 15 (15.79%) |
| E3/E3          | 85 (42.71%) | 117 (80.14%) | 55 (57.89%) |
| E2/E4          | 7 (3.52%) | 2 (1.38%) | 1 (1.05%) |
| E3/E4          | 78 (39.20%) | 14 (9.59%) | 20 (21.05%) |
| E4/E4          | 18 (9.04%) | 1 (0.68%) | 3 (3.17%) |
| Total          | 199 (100%) | 146 (100%) | 95 (100%) |

*Chi-square test p value for AD x EC = 0.056; for AD x YC = 0.751.

**Chi-square test p value for AD x EC = 0.226; for AD x YC = 0.120.

### Table 2 - Odds ratio (OR) - crude and adjusted by *APOE* genotype – and 95% confidence interval (CI) for interaction between *IL-1α* T allele and *IL-8* TT genotype obtained from logistic regression analysis concerning Alzheimer’s disease (AD) and elderly controls (EC), and Alzheimer’s disease (AD) and young controls (YC).

| IL-1α T allele | IL-8 TT genotype | AD (%) | EC (%) | OR(95%CI) | p* | OR(95%CI) | p** |
|---------------|------------------|-------|--------|----------|----|----------|-----|
| -             | -                | 35.70 | 33.60  | 1 (reference) |    | 1 (reference) |    |
| -             | +                | 12.60 | 19.90  | 0.595 (0.312-1.136) | 0.12 | 0.542 (0.277-1.060) | 0.07 |
| +             | -                | 38.70 | 28.80  | 1.265 (0.750-2.135) | 0.38 | 1.110 (0.639-1.926) | 0.71 |
| +             | +                | 13.10 | 17.80  | 0.690 (0.359-1.328) | 0.27 | 0.553 (0.278-1.097) | 0.09 |

| IL-1α T allele | IL-8 TT genotype | AD (%) | YC (%) | OR(95%CI) | p* | OR(95%CI) | p** |
|---------------|------------------|-------|--------|----------|----|----------|-----|
| -             | -                | 35.70 | 42.10  | 1 (reference) |    | 1 (reference) |    |
| -             | +                | 12.60 | 18.90  | 0.782 (0.381-1.606) | 0.50 | 0.666 (0.317-1.401) | 0.28 |
| +             | -                | 38.70 | 29.50  | 1.549 (0.867-2.769) | 0.14 | 1.345 (0.737-2.455) | 0.33 |
| +             | +                | 13.10 | 9.05   | 1.628 (0.695-3.813) | 0.26 | 1.174 (0.486-2.838) | 0.72 |

*Crude OR. **Adjusted by APOE genotypes.
Hayes A, Green EK, Pritchard A, Harris JM, Zhang Y, Lambert HC, Chartier-Harlin MC, Pickering-Brown SM, Lendon CL and Mann DM (2004) A polymorphic variation in the interleukin 1A gene increases brain microglial cell activity in Alzheimer’s disease. J Neurol Neurosurg Psychiatry 75:1475-1477.

Hixson JE and Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 31:545-548.

Hu JL, Li G, Zhou DX, Zou YX, Zhu ZS, Xu RX, Jiang XD and Zeng YJ (2009) Genetic analysis of interleukin-1A C(-889)T polymorphism with Alzheimer disease. Cell Mol Neurobiol 29:81-85.

Infante J, Sanz C, Fernandez-Luna JL, Llorca J, Berciano J and Combarros O (2004) Gene-gene interaction between interleukin-1A and interleukin-8 increases Alzheimer’s disease risk. J Neurol 251:482-483.

Khachaturian ZS (1985) Diagnosis of Alzheimer’s disease. Arch Neurol 42:1097-1105.

Kuo YM, Liao PC, Lin C, Wu CW, Huang HM, Lin CC and Chuo LJ (2003) Lack of association between interleukin-1alpha polymorphism and Alzheimer disease or vascular dementia. Alzheimer Dis Assoc Disord 17:94-97.

McKinnon G, Drachman D, Folstein M, Katzman R, Price D and Stadlan EM (1984) Clinical diagnosis of Alzheimer’s disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 34:939-944.

Peterson PK, Hu S, Salit-Johnson J, Molitor TW and Chao CC (1997) Differential production of and migratory response to beta chemokines by human microglia and astrocytes. J Infect Dis 175:478-481.

Pomponi M, Bria P and Pomponi M (2008) Is Alzheimer’s disease a synaptic disorder? J Alzheimer’s Dis 13:39-47.

Tang MN, Zhang ZX, Han HY, Liu XH and Shen Y (2004) Analysis on association between the polymorphisms in apolipoprotein E, interleukin-1 alpha genes and Alzheimer’s disease in Chengdu area. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 21:176-178.

Tamirivi T, Uzan M, Sanus GZ, Baykara O, Is M, Ozkara C and Buyar N (2006) Lack of association between the IL1A gene (-889) polymorphism and outcome after head injury. Surg Neurol 65:7-10.

Thirumangalakudi L, Prakasam A, Zhang R, Bimonte-Nelson H, Sambamurti K, Kindy MS and Bhat NR (2008) High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. J Neurochem 106:475-485.

Vendramini AA, de Labio RW, Rasmussen LT, Minett T, Bertolucci PH, Smith MAC and Payao SLM (2007) Interleukin-8 gene polymorphism -251T > A and Alzheimer’s disease. J Alzheimers Dis 12:221-222.

Zhou YT, Zhang ZX, Zhang JW, He XM and Xu T (2006) Association between interleukin-1 alpha-889 C/T polymorphism and Alzheimer’s disease in Chinese Han population. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 28:186-190.

Zlotnik A and Yoshie O (2000) Chemokines: A new classification system and their role in immunity. Immunity 12:121-127.

Associate Editor: Francisco Mauro Salzano

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.