Cytokine Gene Polymorphisms and Alzheimer’s Disease in Brazil

Clayton F. Moraes a, d · Andrea L. Benedet a · Vinícias C. Souza b · Túlio C. Lins c · Einstein F. Camargos a, e · Janeth O. S. Naves b · Ciro J. Brito f · Cláudio Córdova g · Rinaldo W. Pereira c, g, h · Otávio T. Nóbrega a, b

a Programa de Pós-Graduação em Ciências Médicas, b Programa de Pós-Graduação em Ciências da Saúde e, c Programa de Pós-Graduação em Patologia Molecular, Universidade de Brasília, d Hospital da Universidade Católica de Brasília, e Hospital Universitário de Brasília, f Programa de Pós-Graduação em Educação Física, Universidade Federal de Sergipe, g Programa de Pós Graduação em Educação Física e h Programa de Pós-Graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, Brasília, Brasil

Introduction

Alzheimer’s disease (AD) is the most common progressive neurodegenerative disorder worldwide, affecting nearly 27.7 million people presently and with over 4.5 million new cases diagnosed every year, with major health care and socioeconomic impacts [1]. This disease is characterized by injury to the central nervous system with memory loss along with a decline in any other cognitive function [2, 3], and may exhibit early- or late-onset forms depending on intrinsic pathophysiological mechanisms [4]. The ultimate cause of dementia is the loss of neurons and of the synaptic connections between them. However, the insults that produce this loss remain to be determined. Immune response and the resulting neuroinflammation may be important in mediating neuronal damage in AD. A typical immune response at the central nervous system includes activation of microglia cells and

Key Words
Alzheimer’s disease · Cytokine · Inflammation · Genetic ancestry · Admixture

Abstract

Background: Single-nucleotide polymorphisms in genes encoding immunological mediators can affect the biological activity of these molecules by regulating transcription, translation, or secretion, modulating the genetic risk of inflammatory damage in Alzheimer’s disease (AD). Nonetheless, the Brazilian contingent is highly admixed, and few association trials performed herein with AD patients have considered genetic ancestry estimates as co-variables when investigating markers for this complex trait. Methods: We analyzed polymorphisms in 10 inflammatory genes and compared the genotype distribution across outpatients with late-onset AD and noncognitively impaired subjects from Midwest Brazil under a strict criterion, and controlling for ancestry heritage and ApoE genotype. Results: Our findings show an almost 40% lower chance of AD (p = 0.004) among homozygotes of the IL10 -1082A allele (rs1800896). Dichotomization to ApoE and mean ancestry levels did not affect protection, except among those with greater Europe-an or minor African heritage. Conclusion: The IL10 locus seems to affect the onset of AD in a context sensitive to the genetic ancestry of Brazilian older adults.

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astrocytes along with increased cytokine and/or acute-phase protein expression [5]. The hazardous effect of a pro-inflammatory profile in AD is supported by epide-
microbiological evidence of a potential protective effect of an-
anti-inflammatory responses [6, 7]. In addition, evidence
suggests that the risk of AD is affected by genetic varia-
tion in inflammatory modulators, such as interleukin-
1alpha (IL1-α), IL1-β, IL6, tumor necrosis factor (TNF), and
others [8, 9]. A recent genome-wide association study
has described an association between AD risk and the
polymorphic site (rs3818361) at the complement recep-
tor 1 (CR1) gene, placing an immunological marker in the
framework of the etiology of AD by indicating unsuit-
able immune pathways for Aβ clearance as a risk-modi-
fying factor to the progress of the late-onset forms of the
disease [10, 11]. Single-nucleotide polymorphisms (SNPs)
in genes encoding these molecules could affect biological
activity by regulating transcription, translation, and se-
cretion. Therefore, factors modulating the degree of in-
flammatory damage might influence the risk of AD.

Considered a complex phenotype, the etiology of late-
onset AD is largely affected by modifiable (e.g. literacy
level) and nonmodifiable (e.g. genetic architecture) fac-
tors [2, 12]. Regardless of the technology employed (from
direct gene to genome-wide centered), most of the AD-
related genetic risk factors were identified by the ‘associa-
tion study’ approach, in which the frequency of a specific
genotype is compared among affected and nonaffected
people. Though useful, this approach has methodologi-
ical biases mostly in stratified populations, when spurio-
us associations arise from a nonperceived genetic struc-
ture within an admixed population [13]. For traits that
bear evidence of differential risk among populations with
distinct continental backgrounds (e.g. Europeans and Af-
ricans), estimating ancestry proportions is a strategy that
can help obtain knowledge about the structure of com-
plex traits [14–17]. The Brazilian contingent is highly ad-
mixed, with roughly 60–75% of its ancestry derived from
Iberian whites, 10–30% from West Africans, and 5–20%
from Native Americans [18, 19]. Studies worldwide have
linked AD to ethnic differences between populations [20,
21], including recent work suggesting that migrant popu-
lations attain rates between those of their homelands and
adopted countries [22]. However, few association trials
performed with Brazilian AD patients have considered
genetic ancestry estimates as main or accessory variables
when investigating markers for this complex phenotype.

Our purpose was to investigate whether the genotypes
of 10 important immunological mediators could be asso-
ciated with the development of the late-onset form of AD
in the Brazilian contingent. Ancestry informative mark-
ers were used to ascertain the structure of the sample, thus
placing ancestry estimates as a correction factor in our
analyses.

Materials and Methods

Subjects
The present study represents an analysis of data from an ongo-
ing initiative for cognitive assessments in Brasília, Brazil [23],
whose methods are reproduced below. This city (∼2.6 million in-
habits) has the important feature of having been planned and
constructed to bring the administrative capital from the coast to
the midwest of Brazil, giving rise to a migration process over the
last 50 years. For that reason, the capital’s elderly population
(∼200,000 inhabitants) is considered an expression of the genetic
diversity of all Brazilian regions.

Subjects with or without a previous diagnosis of AD were con-
secutively enrolled and clinically followed for a minimum time
span of 2 years, either at the Medical Center for the Aged Outpa-
tient at the Universidade de Brasília or at the Ambulatory for
Elderly Care at the Universidade Católica de Brasília. Both institu-
tions are located in the metropolitan area of the Brazilian Federal
District and employ skilled staff for the management of cognitive
disorders. To participate in this research, each volunteer or re-
sponsible caregiver provided written consent approved by the In-
stitutional Review Board. Project enrollment eligibility criteria
were: age 60 years or older and fulfillment of clinical assessments.

Data Collection
All clinical assessments were conducted by two experienced
geriatricians. Clinical evaluation of each patient consisted of his/
her follow-up for a time span of at least 2 years to confirm or rule
out the diagnosis of AD or any other form of dementia. All patients
assigned to the control group were followed prospectively and
considered cognitively preserved whether a decline was observed
during follow-up or not. The neuropsychological assessments ad-
ministered to these participants in each encounter encompassed
everal cognitive domains including short- and long-term episod-
ic memory, processing speed, and attention and executive func-
tions, as recommended by the Diagnostic and Statistical Manual
of Mental Disorders (DSM-IV) criteria [24]. Demented individu-
als could be followed either retrospectively when enrolled with a
prior diagnosis of AD or by prospective means whenever newly
admitted, newly diagnosed among the nondemented, or followed
at the Centers for less than 2 years at the onset of the study. The
diagnosis or confirmation of probable AD was made according to
NINCDS/ADRDA criteria and determined by the clinicians on the
basis of consultation with the patient, interviews with a knowl-
edgeable informant, review of medical records, neuropsychologi-
cal assessments, and/or laboratory tests, whenever applicable.

At admission, all patients were assessed with a validated Bra-
Zilian Portuguese version of the Mini-Mental State Examination
(MMSE), whereas the Clinical Dementia Rating (CDR) scale was
obtained for demented patients only. Also at admission, data
such as age (years), waist circumference (WC; cm), systolic (SBP;
mmHg) and diastolic blood pressure (DBP; mm Hg), and literacy
level were collected. This latter variable was assessed in terms

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of having either a complete basic cycle of educational or higher (≥4 years) or an incomplete basic cycle or no formal education at all (<4 years). For patients enrolled with a prior diagnosis of AD throughout our assessments, baseline characteristics consisted of data collected in the year of onset of the disease.

**Marker Selection and Genotyping**

For laboratory experiments, peripheral venous blood samples were collected into EDTA-containing tubes and DNA extraction was performed using standard extraction kits (QIAamp DNA Mini Kit; Qiagen, Brazil). For genetic analyses of 10 important inflammatory mediators (IL-1α, IL-1β, IL-6, IL-8, IL10, IL12-β, IL18, transforming growth factor (TGF)-β1, toll-like receptor (TLR)-4, and TNF), one polymorphic site in each was selected based on prior evidence of a functional role for the mediator’s expression [25–33]. Characteristics of selected markers are shown in table 1. Allelic frequencies from the parental populations were retrieved from a public genomic database [34]. From those, 5 (rs1800587, rs1143627, rs1800795, rs4073, and rs1800896) displayed at least a modest difference (δ > 0.2) between European and African frequencies, 2 between European and Native American frequencies (rs1143627, rs1800795, rs4073, and rs1800896), and 1 between African and Native American frequencies (rs1800587).

Since all selected markers were SNPs, genotyping was conducted by a single base extension procedure followed by capillary electrophoresis. Briefly, DNA fragments encompassing the polymorphic site and sized 100–200 pb were amplified by standard polymerase chain reaction (PCR) using a Qiagen Multiplex PCR Kit. Multiplex reactions were optimized with primers segregated into two sets (set 1: rs1143627, rs4073, rs3212227, rs1800469, rs1800629; set 2: rs1800587, rs1800795, rs1800896, rs1946518, rs4986790), according to the size of the single-base sequencing primer, and co-amplified in a Veriti™ Dx Thermal Cycler (Applied Biosystems, USA) using the following cycles: denaturation at 95°C for 15 min, followed by 39 cycles of 30 s at 94°C, 90 s at 57°C, and 60 s at 72°C, with a final extension step at 72°C for 10 min. Following amplification, products were purified to eliminate nonincorporated dNTPs and primers by adding 1 U of Exo I and 1 U of CIAP to 3 μl of each PCR product corrected to have 1× the CIAP reaction buffer. This mixture was incubated for 60 min at 37°C followed by denaturation at 75°C for 15 min.

For minisequencing, each multiplex was genotyped by single-base extension reaction using a SNAPshot Multiplex System Kit (Applied Biosystems) following the manufacturer’s recommendations. A second purification step was carried out by mixing 1 U of CIAP with 5 μl of the single-base extension reaction corrected to 1× the CIAP reaction buffer, which was incubated for 1 min at 37°C and for 15 s at 85°C. Following addition of 1 μl of each purified product to 8.83 μl Hi-Di formamide and 0.17 μl GS120 LIZ internal size standard, capillary electrophoresis was carried out on an ABI Prism 3130XL genetic analyzer (Applied Biosystems) using ABI 3700 POP 7 polymer. The obtained data were analyzed using GeneMapper software (Applied Biosystems). Electropherograms were rendered successful when only mild noise was observed and allowed coincident identification of the polymorphic site by two independent readers after visual inspection. Samples with less than 90% call rate were repeated, after which blanks were filled with unitary (singleplex) reactions until the entire panel of SNPs was fulfilled.

To genotype the apolipoprotein E gene (ApoE) and determine the classic e2, e3, and e4 alleles for statistic control, a PCR method was adapted from a multiplex refractory mutation system [35]. Quality control for the ApoE polymorphism consisted of systematic repetition of all e2 and e4 carriers. All reassessments were done by an independent technician, and the genotyping success rate and concordance scored at 100%. The concentrations used and the sequence of all primers are available upon request. Genetic ancestry estimates were determined for each subject exactly as described previously [23].

**Statistical Analysis**

Cases were labeled as positive (affected) or negative (nonaffected) regarding the clinical diagnosis of AD. The Kolmogorov-Smirnov test was performed to check for normal distribution of the

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>SNP</th>
<th>Allele</th>
<th>Frequencies</th>
<th>AFR</th>
<th>AMR</th>
<th>EUR</th>
<th>sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1-α</td>
<td>2q14</td>
<td>rs1800587</td>
<td>A</td>
<td>0.460</td>
<td>0.250</td>
<td>0.252</td>
<td>0.296</td>
<td></td>
</tr>
<tr>
<td>IL1-β</td>
<td>2q14</td>
<td>rs1143627</td>
<td>C</td>
<td>0.646</td>
<td>0.500</td>
<td>0.363</td>
<td>0.442</td>
<td></td>
</tr>
<tr>
<td>IL6</td>
<td>7p15</td>
<td>rs1800795</td>
<td>C</td>
<td>0.000</td>
<td>0.160</td>
<td>0.535</td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td>IL8</td>
<td>4q21</td>
<td>rs4073</td>
<td>T</td>
<td>0.175</td>
<td>na</td>
<td>0.600</td>
<td>0.458</td>
<td></td>
</tr>
<tr>
<td>IL10</td>
<td>1q32</td>
<td>rs1800896</td>
<td>G</td>
<td>0.274</td>
<td>0.320</td>
<td>0.531</td>
<td>0.329</td>
<td></td>
</tr>
<tr>
<td>IL12-β</td>
<td>5q33</td>
<td>rs3212227</td>
<td>C</td>
<td>0.327</td>
<td>0.370</td>
<td>0.190</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>IL18</td>
<td>11q23</td>
<td>rs1946518</td>
<td>T</td>
<td>0.345</td>
<td>0.460</td>
<td>0.392</td>
<td>0.448</td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td>19q13.1</td>
<td>rs1800469</td>
<td>T</td>
<td>0.208</td>
<td>0.370</td>
<td>0.288</td>
<td>0.376</td>
<td></td>
</tr>
<tr>
<td>TLR-4</td>
<td>9q33</td>
<td>rs4986790</td>
<td>G</td>
<td>0.040</td>
<td>0.031</td>
<td>0.033</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>6p21.3</td>
<td>rs1800629</td>
<td>A</td>
<td>0.088</td>
<td>0.060</td>
<td>0.173</td>
<td>0.234</td>
<td></td>
</tr>
</tbody>
</table>

na = Not available; AFR = African; AMR = Amerindian; EUR = European.
Results

From 2009 to 2011, data from 630 participants (~0.32% of the whole elderly segment in Brasília; aged from 60 to 96 years) was gathered according to the inclusion criteria. The number of clinical interviews with each patient ranged from 2 to 11 different consultations throughout up to 9 years of follow-up, with the group of AD patients displaying significantly more encounters with the clinical staff (5.8 ± 2.7 consultations) and more years of follow-up (5.2 ± 3.4 years) compared to the nondemented group (5.8 ± 2.7 consultations) and more years of follow-up (5.2 ± 3.4 years) compared to the nondemented group (5.8 ± 2.7 consultations and 2.5 ± 0.5 years, respectively). Of all participants, 120 individuals were diagnosed with AD (3.3 ± 1.2 consultations and 2.5 ± 0.5 years, respectively). The latter analysis was confirmed with the χ² test. Individual genetic ancestry was estimated with an algorithm based on maximum likelihood estimation [36] and determined exactly as described previously [23]. p values were rendered significant following the Bonferroni correction for multiple genotypic tests (α = 0.005). Single gene analyses within subsets were considered significant at the 0.05 level. SPSS software, version 19 (SPSS Inc., Chicago, Ill., USA), and Epi-Info, version 3.5.1 (Centers for Disease Control, Atlanta, Ga., USA) were used for statistical calculations.

| Variable | AD (n = 120) | Control (n = 412) | p
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>72.3 ± 7.4</td>
<td>70.8 ± 7.2</td>
<td>0.38</td>
</tr>
<tr>
<td>WC, cm</td>
<td>93.5 ± 10.4</td>
<td>92.1 ± 10.0</td>
<td>0.53</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>134.1 ± 20.3</td>
<td>137.5 ± 22.0</td>
<td>0.80</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.3 ± 10.6</td>
<td>82.1 ± 13.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MMSE (points)</td>
<td>13.7 ± 7.1</td>
<td>24.5 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

| Male | 41 | 34.2 | 48 | 11.6 | <0.001 |
| e4 carriers | 57 | 47.5 | 81 | 19.7 | 0.001 |
| <4 years of schooling | 73 | 60.8 | 231 | 56.1 | 0.68 |
| CDR ≤2 | 52 | 43.3 | – | – | – |

a Significance verified by Student’s t test. b Significance verified by the χ² test.

Discussion

Patients with AD display a pro-inflammatory phenotype characterized by local overproduction of cytokines in the plaques as well an increased elevation of cytokines

Table 2. Comparison of continuous (mean and SD) and categorical [absolute number (n) and proportion (%)] variables across AD patients and control, nondemented subjects at baseline

| Variable | AD (n = 120) | Control (n = 412) | p
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>70.8 ± 7.2</td>
<td>0.38</td>
</tr>
<tr>
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<tr>
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<td>82.1 ± 13.1</td>
<td>&lt;0.01</td>
</tr>
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</tr>
</tbody>
</table>

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| <4 years of schooling | 73 | 60.8 | 231 | 56.1 | 0.68 |
| CDR ≤2 | 52 | 43.3 | – | – | – |

a Significance verified by Student’s t test. b Significance verified by the χ² test.
Table 3. Frequency analysis of the genotypes investigated according to a recessive (left) or dominant model (right) across AD patients and control, nondemented subjects

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotypes</th>
<th>Condition (%)</th>
<th>RR (95% CI)</th>
<th>Genotypes</th>
<th>Condition (%)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>control</td>
<td></td>
<td>AD</td>
<td>control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[χ²; p]</td>
<td>[χ²; p]</td>
<td></td>
<td>[χ²; p]</td>
<td>[χ²; p]</td>
</tr>
<tr>
<td>IL1-α</td>
<td>GG</td>
<td>273</td>
<td>53.3</td>
<td>50.7</td>
<td>1.08 (0.79–1.49)</td>
<td>G_</td>
</tr>
<tr>
<td></td>
<td>A_</td>
<td>259</td>
<td>46.7</td>
<td>49.2</td>
<td>[0.25; 0.678]</td>
<td>AA</td>
</tr>
<tr>
<td>IL1-β</td>
<td>TT</td>
<td>162</td>
<td>28.3</td>
<td>31.1</td>
<td>0.90 (0.64–1.28)</td>
<td>T_</td>
</tr>
<tr>
<td></td>
<td>C_</td>
<td>370</td>
<td>71.7</td>
<td>68.9</td>
<td>[0.33; 0.652]</td>
<td>CC</td>
</tr>
<tr>
<td>IL6</td>
<td>GG</td>
<td>331</td>
<td>59.2</td>
<td>63.1</td>
<td>0.88 (0.64–1.21)</td>
<td>G_</td>
</tr>
<tr>
<td></td>
<td>C_</td>
<td>201</td>
<td>40.8</td>
<td>36.9</td>
<td>[0.61; 0.455]</td>
<td>CC</td>
</tr>
<tr>
<td>IL8</td>
<td>AA</td>
<td>154</td>
<td>31.7</td>
<td>28.2</td>
<td>1.14 (0.81–1.59)</td>
<td>A_</td>
</tr>
<tr>
<td></td>
<td>T_</td>
<td>378</td>
<td>68.3</td>
<td>71.8</td>
<td>[0.56; 0.492]</td>
<td>TT</td>
</tr>
<tr>
<td>IL10</td>
<td>AA</td>
<td>225</td>
<td>30.8</td>
<td>45.6</td>
<td>0.61 (0.43–0.86)</td>
<td>A_</td>
</tr>
<tr>
<td></td>
<td>G_</td>
<td>307</td>
<td>69.2</td>
<td>54.4</td>
<td>[8.34; 0.004]</td>
<td>GG</td>
</tr>
<tr>
<td>IL12-β</td>
<td>AA</td>
<td>279</td>
<td>54.2</td>
<td>51.9</td>
<td>1.07 (0.78–1.47)</td>
<td>A_</td>
</tr>
<tr>
<td></td>
<td>C_</td>
<td>253</td>
<td>45.8</td>
<td>48.1</td>
<td>[0.18; 0.679]</td>
<td>CC</td>
</tr>
<tr>
<td>IL18</td>
<td>GG</td>
<td>160</td>
<td>32.5</td>
<td>29.4</td>
<td>1.16 (0.80–1.56)</td>
<td>G_</td>
</tr>
<tr>
<td></td>
<td>T_</td>
<td>372</td>
<td>67.5</td>
<td>70.6</td>
<td>[0.43; 0.500]</td>
<td>TT</td>
</tr>
<tr>
<td>TGF-β</td>
<td>CC</td>
<td>207</td>
<td>40.0</td>
<td>38.6</td>
<td>1.05 (0.76–1.44)</td>
<td>C_</td>
</tr>
<tr>
<td></td>
<td>T_</td>
<td>325</td>
<td>60.0</td>
<td>61.4</td>
<td>[0.08; 0.831]</td>
<td>TT</td>
</tr>
<tr>
<td>TLR4</td>
<td>AA</td>
<td>434</td>
<td>83.3</td>
<td>81.1</td>
<td>1.13 (0.74–1.73)</td>
<td>A_</td>
</tr>
<tr>
<td></td>
<td>G_</td>
<td>98</td>
<td>16.6</td>
<td>18.9</td>
<td>[0.32; 0.688]</td>
<td>GG</td>
</tr>
<tr>
<td>TNF</td>
<td>GG</td>
<td>313</td>
<td>60.0</td>
<td>58.5</td>
<td>1.05 (0.76–1.45)</td>
<td>G_</td>
</tr>
<tr>
<td></td>
<td>A_</td>
<td>219</td>
<td>40.0</td>
<td>41.5</td>
<td>[0.08; 0.833]</td>
<td>AA</td>
</tr>
</tbody>
</table>

Data are expressed as frequencies (%) within the diagnostic group.

Table 4. Comparison of the frequency of IL6 and IL10 genotypes across AD patients and control, nondemented subjects in subsets according to the genotype of ApoE and the degree of genetic ancestry from parental populations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n</th>
<th>IL6</th>
<th>IL10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GG vs. C_</td>
<td>G_ vs. CC</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>ε4 carriers</td>
<td>138</td>
<td>0.28; 0.721</td>
<td>1.55; 0.318</td>
</tr>
<tr>
<td></td>
<td>ε4 noncarriers</td>
<td>394</td>
<td>2.15; 0.157</td>
<td>2.38; 0.166</td>
</tr>
<tr>
<td>European ancestry</td>
<td>&lt;59%</td>
<td>257</td>
<td>0.06; 0.859</td>
<td>0.24; 0.448</td>
</tr>
<tr>
<td></td>
<td>≥59%</td>
<td>275</td>
<td>0.57; 0.499</td>
<td>4.24; 0.061</td>
</tr>
<tr>
<td>African ancestry</td>
<td>&lt;30%</td>
<td>258</td>
<td>0.43; 0.522</td>
<td>1.85; 0.185</td>
</tr>
<tr>
<td></td>
<td>≥30%</td>
<td>274</td>
<td>0.20; 0.670</td>
<td>4.10; 0.080</td>
</tr>
<tr>
<td>Amerindian ancestry</td>
<td>&lt;6%</td>
<td>265</td>
<td>1.85; 0.192</td>
<td>3.29; 0.092</td>
</tr>
<tr>
<td></td>
<td>≥6%</td>
<td>267</td>
<td>3.64; 0.062</td>
<td>0.75; 1.000</td>
</tr>
</tbody>
</table>

Data are expressed as χ² and p values.
in the cerebrospinal fluid [38, 39]. In this environment, microglial cells account for most of the mediators produced, and it is possible that their well-acknowledged responsiveness to amyloid-β (Aβ) deposits triggers reactions that are proportional to transcriptional and/or translational properties of immune genes. Therefore, we analyzed whether polymorphisms in 10 inflammatory genes may increase the risk of AD.

The present study shows a significant difference in the genotypic distribution of the IL10 -1082 SNP (rs1800896) between AD patients and noncognitively impaired subjects. Analyses according to the Cohen convention [40] confirmed differences that could be categorized as an elevated magnitude of the effect size (ES) of IL10 genotypes among subjects with and without dementia (AA vs. G_, ES = 1.2), whereas a rather low magnitude of ES was found for IL6 genotypes compared likewise (G_ vs. CC, ES = 0.4). In addition, the IL10 SNP almost deviated from Hardy-Weinberg equilibrium (P = 0.054) when the whole group was considered. Nonetheless, the genotype distributions for all investigated genes (including IL10) were in Hardy-Weinberg equilibrium within each AD and control subset. This may be further evidence towards an association of the locus with the disease. Physiologically, our finding is in accordance with results published elsewhere which attribute the status of ‘high-producer’ of circulating IL10 to the -1082A allele [41, 42], contributing to the rationale that suppression or attenuation of neuroinflammation in susceptible subjects may protect against the onset of AD. The -1082 SNP is in complete linkage disequilibrium, with variances at other positions also allegedly functional as the -819 T/C (rs1800871) and the -592 C/A (rs1800872) SNPs, yielding promoter haplotypes already implicated in the risk for AD [43, 44] and others that deserve proper investigation by subsequent genomic approaches.

Apart from the well-characterized contribution of the classic susceptibility genes to the etiology of AD, a body of recent evidence accounts for the overall genetic heritage as an additional genetic risk factor, as observed in the highly admixed Brazilian population [23, 45]. However, our stratified analyses did not detect an interaction between either the ApoE ε4 allele or the degree of American ancestry and the genotypes of IL10, rendering the apolipoprotein alleles and Native American heritage as potential confounders rather than effect-modifying variables. Still regarding ethnicity, it is noteworthy that carriers of the -1082A allele exhibited protection against AD only among those with greater African or minor European heritage. It is likely that coevolution and/or linkage disequilibrium between genes encoding functionally interconnected proteins can explain, at least in part, these results. In this particular, immune mediators bear remarkable evidence of co-selection in the same human population. Even though the setting for selection may not depend on the AD phenotype itself, a genetic background enriched in elements prone to a pro-inflammatory response may act synergistically in the context of immune cells and underlie the AD pathophysiology. Altogether, our results suggest that allelic architectures inherent to these parental populations may interact with the IL10 gene and act as risk modifiers of the AD phenotype, which may in part justify a number of reports that do not replicate this finding among white European individuals [46, 47]. Given the possibility that the Bonferroni correction overcorrects for the inflated false-positive rate and thereby throws away valid information, similar analyses with subjects dichotomized to ApoE and ancestry were performed for genotypes of IL6, but no associations were rendered significant after these corrections. Therefore, a statistical association of AD with IL6 genotypes should be regarded as borderline at this time and subjected to further investigations in different scenarios. It was established that for a sample comprising 120 AD cases and 412 control individuals, a significance level set at α = 0.005, and an ES between 0.4 and 1.2, the test power exceeded 80% for all of investigated variables using G*Power 3 software [48].

To the authors’ best knowledge, no previous report has sought to investigate the relationship between a number of immunogenomic markers and the AD phenotype in the context of (and correcting for) the high admixture and social heterogeneity of the Brazilian contingent. In our conditions, differences in gender distribution between affected and nonaffected individuals probably derived from cultural aspects since older women tend to be greater consumers of outpatient services, and older men of inpatient healthcare [50]. Lower DBP may be attributed to the frequent dysautonomia of AD patients. Despite these discrepancies, both aspects might be considered of minor interest to clinically distinguish subjects according to the main phenotype under study (AD). Regarding social profile, the high proportions of individuals with less than 4 years of formal education among demented and nondemented subjects is consistent with the general low literacy level of older adults in Brazil, and comparable frequencies in both groups attenuate disparities related to socioeconomic status.

Despite this study tending to make a contribution by considering sociodemographic and biological character-
istics, there are important limitations to acknowledge. Remarkable heterogeneity produced by differences in immune-modulating pharmacotherapy (past or present) and by the sampling method not being based on the pattern of clinical evolution and severity, among other factors not considered herein, may add complexity to this scenario. Another bias akin to all associative studies relies on the fact that association does not imply causation. We recall that the SNPs studied herein were selected based on prior evidence of functional properties, but our results should be interpreted with caution since actual genetic causation may rely on element(s) fairly adjacent to and/or away from the indicated SNP. In this matter, one should remember that the IL10 and CR1 genes share the same chromosomal localization (1q32), and the results found here for IL10 may in part be attributable to linkage disequilibrium with this relevant susceptibility gene to AD.

In summary, our findings suggest that the IL10 locus affects the development of late-onset AD in a context that may be sensitive to the overall genetic heritage of Brazilian older adults. Mapping by admixture linkage disequilibrium, for instance, may be important to refine the search for elements in the genetic architecture of a major continental population that can confer protection against or predispose to this form of dementia.

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Disclosure Statement

The authors declare minor self-plagiarism by reusing elements from published work of our own to help describe the sample. Appropriate references are provided.

References


