

Protective effect of the *APOE*-*e3* allele in Alzheimer's disease

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Abstract

Although several alleles of susceptibility to Alzheimer's disease (AD) have been studied in the last decades, few polymorphisms have been considered as risk factors for the disease. Among them, the *APOE*-*e4* allele appears to be the major genetic risk factor for the onset of the disease. However, it is important to confirm the potential susceptibility of these genetic variants in different populations in order to establish a genetic profile for the disease in specific communities. This study analyzed the *APOE* polymorphisms regarding susceptibility to AD in a sample of 264 individuals (primarily Caucasians; 82 cases and 182 controls) in the population from Vitória, ES, Brazil, by PCR restriction fragment length polymorphism (PCR-RFLP) methods. The patients were selected according to clinical criteria for probable AD. Whereas the *e4* allele showed statistically significant positive association with susceptibility to AD (OR = 3.01, 95%CI = 1.96-4.61; $P < 0.0001$), the *e2* allele did not. The results of the *e4* allele confirm the role of this polymorphism as a risk factor for AD in the sample studied as observed in other populations. Although the *e3* allele has been considered neutral in several studies, our results suggest that it acts as a protective factor against AD in the population studied (OR = 0.46, 95%CI = 0.30-0.67; $P < 0.0001$). This study may provide a new insight into the role of the *APOE*-*e3* allele in the etiology of AD and might help to establish a profile of risk for AD in the population from Vitória, ES.

Key words: Alzheimer's disease; *APOE*-*e3* allele; *APOE*-*e4* allele; Brazilian population; Case-control study

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive deficit of cognitive function, with greater emphasis on memory loss and interference with occupational and social activities (1). Sporadic and late-onset AD (LOAD) shows a multifactorial heredity pattern caused by genetic and complex environmental interactions associated with several predisposing factors and age. The rate of cognitive deterioration during the development of AD varies among individuals (2,3) and seems to be guided by a combination of genetic and environmental factors (4). Some genes, such as *CLU*, *PICALM*, and *CR1*, have been shown to be related to AD as indicated by genome-wide association studies (GWAS) (5,6). However, only apolipoprotein E (*APOE*) polymorphisms have been established as consistent genetic susceptibility factors for LOAD in all populations studied in the world (7).

In humans, the *APOE* gene is found in the 19q13.2 region and presents three alleles (*e2*, *e3*, and *e4*). Stud-

ies of association throughout the world have shown that the *e4* allele acts as a strong dose-dependent risk factor for LOAD (8-10). Individuals with two copies of this allele are at a higher risk than those with only one copy (11). The *e2* allele seems to represent a protective factor both for familial AD and for LOAD, although this protection is not observed in every population (12,13). The *e3* allele, considered neutral, is the most frequent in all populations studied (10,14). Due to their clinical significance, it is very important to know the distribution of the alleles of the *APOE* gene in different populations in order to determine whether the polymorphisms of this gene has a universal effect on the risk for the development of AD.

Previous studies carried out on samples from Southeast [São Paulo, SP (15-18) and São José do Rio Preto, SP (19)], Northeast [Recife, PE (15,16)] and South [Porto Alegre, RS (20,21)] of Brazil investigated the polymorphisms of the *APOE* gene, demonstrating a positive association of the *e4*

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allele with susceptibility to AD. Since the Brazilian population has a high degree of miscegenation, with prevalence of Afro-descendants in the Northeast and of Caucasian-descendants in the South, it is important to determine the role of *APOE* gene polymorphisms in the susceptibility to AD in distinct communities. Thus, this study aimed to investigate the influence of *APOE* gene polymorphisms among patients with LOAD and non-affected controls in a case-control study on the population from Vitória, ES, a State in the Southeast of Brazil, where the population is primarily composed of Caucasians, Afro-descendants and Native Brazilians with higher miscegenation.

Material and Methods

Volunteers

A total of 264 non-consanguineous individuals from Vitória, ES, a city of the Southeast of Brazil, were studied during the years 2007 and 2008. Among them, 82 participants were patients with a diagnosis of LOAD and the other 182 individuals were controls paired for gender and age at a ratio of ~1:2. All participants were selected from the same community in order not to change the ethnic profile among the groups. The participants were primarily Caucasians (approximately 80% in both cases and controls) and the remaining were Afro-Brazilians.

The volunteers, patients and controls, were recruited by invitation to participate in this study while awaiting care in two public geriatric units in the city of Vitória, ES, Brazil: the Geriatric Unity of the Hospital Santa Casa de Misericórdia de Vitória (HSCMV) and the Centro de Referência de Atendimento ao Idoso (CRAI). Almost 90% of the invited subjects or their legal representatives agreed to participate. The participants were diagnosed at the Neurogeriatric Unity of the HSCMV or at the CRAI. All patients fulfilled the clinical criteria for probable AD (22) and had a complete diagnostic evaluation for dementia, including CT scan, standard laboratory tests performed at the time of diagnosis and repeated after 2 years (complete blood count, serum electrolytes, serum glucose, blood urea nitrogen, vitamin B12, folate, thyroid function, and syphilis serology), Mini-Mental State Examination (MMSE) (23), and Clinical Dementia Rating Scale (CDR) (24). All patients received treatment with cholinesterase inhibitors at the time of enrollment in the study. The control sample consisted of volunteers who presented a score >28 on the MMSE and who did not have any cognitive deficit or any known relatives with AD. The study was approved by the Ethics Committee of Human Research of Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória and written informed consent was obtained from all participants or their representatives if the participant could not give consent.

Polymorphisms of the *APOE* gene

DNA was extracted according to the methodology de-

scribed by Miller et al. (25) from 5 mL peripheral blood collected into tubes containing 5% EDTA. The polymorphisms of the *APOE* gene were investigated by PCR restriction fragment length polymorphism (PCR-RFLP) using the primers described by Hixson and Vernier (26) and the following conditions for amplification: 94°C for 5 min for denaturation, followed by 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and an extra extension phase at 72°C for 7 min. The PCR products were subjected to digestion with the enzyme *HhaI* (New England Biolabs, USA) at 37°C overnight and visualized on 10% polyacrylamide gel stained with silver nitrate.

Statistical analysis

The comparison of allelic and genotypic frequencies between patients and controls was performed by the chi-square test (X^2) and the Fisher exact test was used to estimate the risk of developing AD, with a 95% confidence interval (95%CI), using the GraphPad Instat 3.06 software for Windows (27).

Results

The assessment was performed on 82 LOAD patients (26 males and 56 females, average age of 82.2 ± 7.5 years) and 182 non-demented controls (51 males and 131 females, average age of 78.3 ± 8.3 years), with no significant differences in age or gender between the two groups.

Table 1 shows the distribution of each genotype and of the allele frequencies and the relative risk of developing AD for each genotype and allele. The distributions of the allele frequencies in LOAD patients and controls were in

Table 1. Genotypic and allelic distribution of *APOE* polymorphisms in subjects from Vitória, ES, Brazil.

	LOAD group (N = 82)	Control group (N = 182)	OR (95%CI)
Genotype			-
e2e2	1 (1%)	4 (2%)	0.55 (0.06-4.99)
e2e3	4 (5%)	12 (7%)	0.73 (0.22-2.32)
e2e4	1 (1%)	8 (4%)	0.27 (0.03-2.18)
e3e3	30 (37%)*	112 (62%)	0.36 (0.21-0.61)
e3e4	35 (43%)*	44 (24%)	2.33 (1.34-4.06)
e4e4	11 (13%)*	2 (1%)	13.94 (3.14-64.50)
Allele			
e2	7 (4%)	28 (8%)	0.54 (0.22-1.25)
e3	99 (60%)*	280 (77%)	0.46 (0.30-0.67)
e4	58 (36%)*	56 (15%)	3.01 (1.96-4.61)

Data are reported as number with percent in parentheses. LOAD = late-onset Alzheimer disease; OR = odds ratio; CI = confidence interval. *P < 0.004 compared to control group (Fisher exact test).

Hardy-Weinberg equilibrium ($P = 0.0015$).

When comparing the *APOE* genotype frequencies between patients and controls, we observed a significant difference for the genotypes *e3e3* ($P = 0.0003$), *e3e4* ($P = 0.0038$), and *e4e4* ($P < 0.0001$). A significantly increased risk of AD was observed in carriers of the *APOE-e4* allele. The odds ratio (OR) for the association of LOAD patients with the genotypes *e3e4* and *e4e4* were 2.33 (1.34-4.06; $P = 0.0038$) and 13.94 (3.14-64.50, $P < 0.0001$), respectively. Interestingly, the *e3e3* genotype showed a positive association with AD protection (OR = 0.36, 95%CI = 0.21-0.61; $P = 0.0003$).

The over-represented allele was *e3* (0.60), followed by *e4* (0.36) and *e2* (0.04), in LOAD patients, and *e3* (0.77), *e4* (0.15), and *e2* (0.08) in the control group. We found a significant difference in the frequencies of the *e4* and *e3* alleles between the LOAD group and controls ($P < 0.0001$), whereas the *e2* allele did not reach statistical significance ($P = 0.2020$).

Discussion

The frequency of the *e4* allele was significantly higher in the LOAD patient group (36%) than in the control group (15%), indicating that this allele acts as a risk factor for AD in the population of Vitória, ES (OR = 3.01, 95%CI = 1.96-4.61; $P < 0.0001$) as demonstrated for other populations (28). Since this is a dose-dependent risk, individuals with the *e3e4* and *e4e4* genotypes are 2.3- and 13.94-fold more likely to develop AD, respectively. However, the protective factor attributed to the *e2* allele was not observed in the sample analyzed, possibly because it is a rare allele in the population studied.

On the other hand, we obtained unusual results for the analysis of the *e3* allele, suggesting that carriers of the *e3* allele have a 46% protection against AD in this population (OR = 0.46, 95%CI = 0.30-0.67; $P < 0.0001$). This protection is maintained when we compare the frequency of the *e3e3* genotype between patients with LOAD and controls (OR = 0.36, 95%CI = 0.21-0.61; $P = 0.0003$).

A recent study, using meta-analysis strategy to determine the prevalence of *APOE-e4* among AD patients across the global population, suggested that *e4e4* genotype frequency varies among AD patients in regional patterns similarly to those of the general population (29). Whereas European and North American populations were often used in this meta-analysis, the South American continent was analyzed grouped with other communities, showing that there are few studies related to the genetic aspects of AD in South American. Thus, it is important to improve our knowledge about the genetic aspects of AD through studies of different Brazilian cities. Compared to other studies, our sample size was one of the largest analyzed thus far in Brazil for *APOE* polymorphisms (15,17,21,30).

The frequency of *APOE* polymorphisms is highly heterogeneous throughout the world (31-33). For example, the risk attributable to the *e4* allele varies by region and by race and ethnicity (29). European researchers observed a gradient with a higher frequency of the *e4* allele in the North European continent, and a low frequency in the South. In contrast, the *e3* allele goes in an opposite direction (34). The *e3* allele is the most frequent in all human groups (10). High frequencies of the *e4* allele (37%) have also been detected in the African population (35). A high frequency of the *e3* allele (51-98%) has been observed in the Brazilian Indian population, followed by *e4* (0-47%) and *e2* (0-4%) (14,36). Our results of allele and genotype frequencies are in agreement with the aforementioned data, possibly due to the high miscegenation of the Brazilian population, which displays genetic influences from all of these populations (15,37).

A recent study performed on the Tunisian population, also using the PCR-RFLP method, reported high frequencies of the *e4* allele in AD patients, whereas the *e2* allele was under-represented (38), as also observed in the present study. Contrary to other studies (12,13,39), an association of the *e2* allele with protection against AD was not observed in the present study, probably due to the low frequency of this polymorphism in the sample. Despite the fact that the *e3* allele is considered to be the wild type, in our study this allele was associated with a significant protective factor against AD, which was only observed in a study from Sicily, Southern Italy (10).

We found higher frequencies of the *e3* allele in the control group than in the LOAD patient group. We believe that this allele represents a protective factor against AD in the population from Vitória, ES. Additionally, we found that subjects of this population who carry the *e4* allele have an increased risk of developing the disease. These peculiarities might be explained by the ethnic background, in which the frequencies of other alleles of risk to AD can vary among populations. Moreover, distinct environmental factors like oxidative stress, antioxidant intake and aluminum exposition may contribute to the development of the disease (40). These data may provide a new insight into the research on Alzheimer's disease.

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