Brazilian Journal of Medical and Biological Research (2003) 36: 919-923
ISSN 0100-879X Short Communication

Association of apolipoprotein E polymorphism in late-onset Alzheimer's disease and vascular dementia in Brazilians

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Abstract

The genetic basis for dementias is complex. A common polymorphism in the apolipoprotein E (*APOE*) gene is considered to be the major risk factor in families with sporadic and late-onset Alzheimer's disease as well as in the general population. The distribution of alleles and genotypes of the *APOE* gene in late-onset Alzheimer's disease (N = 68), other late-life dementias (N = 39), and in cognitively normal controls (N = 58) was determined, as also was the risk for Alzheimer's disease associated with the ε4 allele. Peripheral blood samples were obtained from a total of 165 individuals living in Brazil aged 65-82 years. Genomic DNA was amplified by the polymerase chain reaction and the products were digested with *Hha*I restriction enzyme. *APOE* ε2 frequency was considerably lower in the Alzheimer's disease group (1%), and the ε3 allele and ε3/ε3 genotype frequencies were higher in the controls (84 and 72%, respectively) as were the ε4 allele and ε3/ε4 genotype frequencies in Alzheimer's disease (25 and 41%, respectively). The higher frequency of the ε4 allele in Alzheimer's disease confirmed its role as a risk factor, while ε2 provided a weak protection against development of the disease. However, in view of the unexpectedly low frequency of the ε4 allele, additional analyses in a more varied Brazilian sample are needed to clarify the real contribution of apolipoprotein E to the development of Alzheimer's disease in this population.

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Research supported by FAPESP and CNPq.

Received July 3, 2002
Accepted February 28, 2003

Key words
- Alzheimer's disease
- Vascular dementia
- Dementia
- Apolipoprotein E
- Aging
- Genetic polymorphisms

In North America and Europe the most common dementia that affects the elderly is Alzheimer's disease (AD), corresponding to 55.6 to 72% of all dementia cases (1,2). In a community-dwelling Brazilian population the prevalence of AD and vascular dementia (VD) was 55.1 and 9.3%, respectively (3).

The genetic basis for dementias is complex. For familial early-onset AD, there is some evidence for mutations in the ß-amy-

loid precursor protein (APP, chromosome 21) and presenilin 1 and 2 genes (chromosomes 14 and 1, respectively). Considering the development of sporadic and late-onset AD (LOAD), a common polymorphism in the apolipoprotein E (*APOE*) gene (chromosome 19) is the major risk factor in families with the disease, as well as in the general population (4). More recently, a new LOAD locus on chromosome 10 independent of the *APOE* genotype was discovered (5).

There are three common *APOE* alleles (ε2, ε3 and ε4), accounting for more than 99% of the isoforms of the product of apo E. The ε3 allele is the most frequent, representing 74 to 86% of all alleles in European and American Caucasian populations. The ε4 allele frequency is approximately 7 to 16% and the ε2 allele frequency is 6.3 to 12% (6-10). Of these three, ε4 is recognized as a risk factor for LOAD, whereas the ε2 and ε3 alleles appear to enhance tolerance to the brain degenerative process, extending the survival of affected neurons. The effect of the *APOE* ε4 allele has been associated with the pattern of regional brain atrophy in AD (11). In addition, the combination of low head circumference and ε4 predicts early-onset AD (12). Furthermore, the apo E polymorphism in a community sample of middle-aged adults was associated with memory performance (13).

Several studies have confirmed the association of apo E and the accumulation of amyloid ß-protein, a characteristic of AD. The early description of this peptide was reported to be associated with the presence of the ε4 allele in autopsy cases even without signs of dementia (14). In addition, individuals with the ε4 allele and mutations in the *APP* gene have earlier-onset disease compared to those with the ε2 or ε3 allele and mutations in *APP* (15).

In fact, many case-control and post-mortem studies of LOAD patients have shown ε4 allele frequencies ranging from 17 to 57% in both sporadic and familial cases (6-9,16,17). The *APOE* genotype, also investigated in other types of dementias, has shown a slight association with VD, Pick's disease and Lewy's body disease but no association with Parkinson's disease or Creutzfeldt-Jakob disease (10,18).

The literature, in general, suggests that there is a relationship between the ε4 allele and AD. In the present study we analyzed the distribution of *APOE* alleles and genotypes in Brazilian patients with clinical signs of LOAD or other types of late-life dementias, mainly VD. We also evaluated the risk for AD associated with the ε4 allele.

A total of 165 Caucasian individuals aged 65-82 were studied. Cognitively impaired patients were divided into two groups: a LOAD group (39 men and 29 women) and a group with other late-life dementias (23 men and 16 women) including VD (N = 35), Parkinson's disease (N = 3) and hypothyroidism dementia (N = 1). Control subjects (28 men and 30 women) were members of an elderly healthy group attended in a neurogeriatric unit of a teaching hospital (Faculty of Medicine of São José do Rio Preto, São José do Rio Preto, SP, Brazil). The median ages of the participants at the beginning of this study were 71.5, 72.0 and 70.0 years for patients with AD, other dementias and controls, respectively, without significant difference among them (P = 0.14). A trained physician interviewed each subject or person responsible and obtained informed consent and blood samples. The subjects were diagnosed and classified by historical and physical examination as well as by neuropsychological (NINCDS-ADRDA) criteria and laboratory tests according to standard protocols and guidelines. The study was approved by the Hospital Ethics Committee.

Genomic DNA extraction and *APOE* genotyping were conducted according to standard procedures (19,20). Intragroup frequencies of *APOE* ε2, ε3 and ε4 alleles were evaluated by adjusting the chi-square test with equiprobability. Allele and genotype

frequencies were compared among groups by the test of proportion by normal approximation. The association between the ε4 allele and AD and between the ε2 allele and a protective effect was evaluated by the odds ratio (OR). The level of significance was set at $\alpha = 0.05$ for all analyses.

Table 1 shows the distribution of the *APOE* alleles and genotypes in patients and controls. *APOE* ε2 was less frequent in AD than in controls and the ε4 frequency was significantly higher in AD than in controls (P = 0.004) but no significant difference was observed for other dementias versus controls (P = 0.46).

Population data have shown a wide variation in the frequency of the ε4 allele, probably reflecting different methodologies, ethnic diversity, and sample sizes for AD (17 to 57%) versus control groups (7 to 16%) (6-9,16,17). As reported by other investigators, patients also showed a slightly increased frequency of ε4 in both VD and Lewy's body disease (10,18).

In the present study, although the ε4 frequency was twice as high in AD (0.25) compared to controls (0.12), it was still lower than in many other studies. However, it was consistent with the limited Brazilian data ranging from 0.21 to 0.39 (7-10,16), without a significant difference between patients with presenile or senile dementia (8).

Furthermore, there are the possible confounding effects of age and gender (11). This suggests that each group is a different subset in the general population. Studies of patients with AD and their relatives can permit a reliable expansion of experiments to measure the prevalence of the *APOE* alleles in familial or sporadic AD, due to the hereditary nature of the apo E polymorphism. Cação J, De Godoy MR, Pinhel MA, Scudeler D, Fernandez MR, Ruiz V, Romero AMM, Tognola W, Hotta J, Dos Santos JE and Souza DRS (2002, personal communication) showed a significant increase of the ε4 allele in relatives of AD patients (0.24) compared with relatives of controls in a Brazilian population (0.04).

The ε3/ε3 genotype frequencies were higher than other apo E genotype frequencies (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε4, ε4/ε4), specifically in controls when compared to AD in this study (Table 1). Contrasting results were observed for the ε3/ε4 genotype in AD (41%) and in controls (16%; P = 0.0008). This prevalence is in agreement with other Brazilian series only for controls but not for patients, with the values reported in the literature being 27.3 and 35% for Caucasian patients versus 17.9 (P = 0.234) and 16% for Caucasian controls, respectively (8,9), or black controls (27%) (9). On the other hand, the ε4/ε4 genotype was rare mainly in series from the southeast, with values around 5% (8), including this study, while in a southern Brazilian population this prevalence was 17%, but in a small series (9).

The OR for ε4 in AD was >1 (Table 2) with a significant effect, again suggesting its influence on the development of the disease. In addition, the estimated OR for ε3/ε4 confirms the higher risk for AD, as reported in

Table 1. Apolipoprotein E (*APOE*) allele and genotype frequencies in patients with Alzheimer's disease (AD), other late-onset dementias (OD) and controls (C).

APOE allele	Number of individuals (absolute frequency)		
	AD (N = 68)	OD (N = 39)	C (N = 58)
ε2	1 (0.01)	3 (0.04)	5 (0.04)
ε3	101 (0.74)*	62 (0.79)	97 (0.84)
ε4	34 (0.25)*	13 (0.17)	14 (0.12)
APOE genotype	Number of individuals (%)		
	AD	OD	C
ε2ε2	0	0	0
ε2ε3	1 (1)	1 (3)	4 (7)
ε2ε4	0	2 (5)	1 (2)
ε3ε3	36 (53)*	26 (67)	42 (72)
ε3ε4	28 (41)*	9 (23)	9 (16)
ε4ε4	3 (5)	1 (3)	2 (3)
Total	68 (100)	39 (100)	58 (100)

*P<0.05 compared to AD and controls (test for two independent proportions based on normal approximation).

other populations (18). However, no association was found with other late-life dementias, represented in this study mainly by VD, although a slight increase in frequencies of the ε4 allele or ε3/ε4 genotypes was observed in such cases. As many patients with clinical diagnoses of VD prove to have neuropathologic signs of AD, higher values for ε4 are expected in this group (6). Similar results were observed for ε2 and ε3 in other dementias. Furthermore, the presence of the ε3/ε4 genotype increased the risk for AD by a factor of 3.6 (95% CI = 1.5-8.7) using ε3/ε3 homozygotes as the baseline. However, no significant effect of the ε3/ε4 genotypes was found in other dementias.

The reduced frequency of genotypes with at least one ε2 allele in AD substantiates the role of this allele as a protective element. Very low ε2 allele frequencies were observed in case-control studies (0.03 to 0.08) and even lower ones in AD (0.01 to 0.08) (7-10,15-18). However, a nonsignificant OR for ε2 in AD was observed in the present study, suggesting that the ε2 allele has a very little or no effect in terms of presence or absence of AD, while Bahia et al. (16) observed a significant difference between patients with presenile or senile AD (0.05) and controls (0.15; P = 0.009).

The higher frequency of the ε4 allele confirmed its role as a risk factor associated with AD, while the ε2 and ε3 alleles showed a weak protection, if any, against AD development. Considering the unexpected reduced frequency of the ε4 allele and the almost complete lack of studies in the Brazilian population, additional analysis with larger and more varied samples is needed to clarify the real contribution of apo E to the development of AD in this population.

Table 2. Odds ratio for Alzheimer's disease (AD) and other late-life dementias (OD) according to apolipoprotein E allele frequencies and ε3/ε4 genotype compared to ε3/ε3.

Allele	Odds ratio (95% confidence interval)	
	AD (N = 68)	OD (N = 39)
ε2	0.16 (0.02-1.43)	0.89 (0.20-3.83)
ε3	0.57 (0.30-1.06)	0.76 (0.36-1.59)
ε4	2.43 (1.23-4.79)	1.46 (0.64-3.30)
Genotype ε3/ε4	3.63 (1.5-8.7)	1.6 (0.5-4.5)

Acknowledgments

The authors wish to thank Marcia R.F. Ferraz, José Antonio Cordeiro, Márcio Colombo, Flávio A.V. Seixas, Marcos R.H. Estécio, Luis Carlos Mattos, Rosa Kawasaki Oyama, Peter James Harris, Livia C. Burdmann, Carlos Alexandre A. Torres, Mariléia Scartezini and David A. Hewitt for technical assistance, discussions and comments.

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