Polymorphisms of *APOE* and *LRP* Genes in Brazilian Individuals With Alzheimer Disease

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Abstract: Alzheimer disease (AD) is the most frequent cause of dementia in Western countries. Putative genetic risk factors for AD are polymorphisms in the apolipoprotein E (*APOE*) gene and in the low-density lipoprotein receptor-related protein (*LRP*) gene. Our objective was to investigate the role of the *APOE* coding region polymorphisms ε2, ε3, and ε4 and *APOE* promoter variants A/T at position -491 and G/T at -219, as well as *LRP* polymorphism C/T, as risk factors for AD in Brazilian individuals. One hundred and twenty patients with probable AD, along with 120 controls were analyzed. A significant difference between patients and controls for ε4 alleles was observed: frequency of this allele in AD was 0.31, and 0.10 in controls. Individuals with 2 ε4 alleles had a higher risk for AD than subjects with only 1 such allele; presence of 1 ε2 allele proved protective. The presence of the T allele of the -219 polymorphism was also associated with an increased risk of AD, but this polymorphism is in linkage disequilibrium with *APOE* ε polymorphisms. No significant differences between patients and controls were observed for -491 *APOE* or *LRP* polymorphisms. In this Brazilian population, both the ε4 allele and T -219 polymorphism were associated with an increased risk for AD.

Key Words: dementia, Alzheimer disease, apolipoprotein E, low-density lipoprotein receptor-related protein, genetic risk factor

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Alzheimer disease (AD) is the major cause of dementia in Western countries.[1,2] AD is characterized by progressive memory deterioration, cognitive decline, and behavior disturbances. The disease is denominated presenile when the onset of symptoms occurs before 65 years of age, and senile when manifestations beyond this age.[3]

AD is a multifactorial condition in which genetic and environmental factors play a role. Several genetic risk factors for sporadic AD have been studied and the only factor recognized as a major risk factor is the presence of the ε4 allele on the apolipoprotein E (*APOE*) gene,[4–6] whereas the ε2 allele is considered a protective factor for the condition.[7,8] Furthermore, the presence of the ε4 allele is linked to earlier and faster cognitive decline.[9] *APOE* is located on chromosome 19 and its coding region is polymorphic, with 3 commonly recognized alleles: ε2, ε3, and ε4. The most common allele is the ε3, whereas ε2 and ε4 are considered variants.[10,11]

ApoE is a protein comprising 299 amino acids with a molecular weight of 34 kd.[10] In the nervous system, ApoE is involved in cholesterol mobilization and redistribution as well as myelin maintenance and neuronal membrane formation and repair.[12] It is also important for membrane remodeling, and is associated with synaptic plasticity mechanisms.[11,13]

There are several hypotheses regarding the role of ApoE in AD pathophysiology. Presence of the ApoE4 might interfere with β-amyloid (βA) deposition in brain tissue,[14,15] have a regulatory role in neuronal τ protein metabolism,[16] influence dendritic outgrowth and branching,[17] act in a negative way on the cholinergic system,[18] produce a less efficient antioxidant effect,[19] and be associated with increased low-density lipoprotein cholesterol levels.[20] ApoE2 is believed to have the opposite effect. It is also possible that other polymorphisms that cosegregate with APOE ε polymorphisms are ultimately responsible for the increased risk for AD.

In 1998, Bullido et al[21] studied the association of AD with the A/T polymorphism at position -491 of the *APOE* promoter region, with the presumption that a single base substitution in this region of the gene could produce a significant change in the level of RNA transcription and, consequently, protein expression.[22] This finding would have represented an important breakthrough, because the simple presence of the ε4 allele is known to be neither sufficient nor necessary to cause AD. This initial study found an association between AD and genotype A/A at position -491 and AD, independently of the ε4 allele. These results were confirmed by some investigators,[23,24] but not by others.[25,26]

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Also in 1998, Lambert et al[27] reported that polymorphism G/T at position -219 of the *APOE* promoter, also known as T_H1E47cs, was a risk factor for AD. They demonstrated that individuals with at least 1 T allele had a higher risk for AD, independently of the *APOE* genotype. This same group of researchers demonstrated an increased expression of *APOE* mRNA in heterozygotes -219 G/T with the *APOE* ε3/ε4 genotype.[28]

Supporting the view that the T -219 genotype might be a risk factor for AD, a study involving 1732 patients with probable AD and 1926 matched controls, drawn from 6 different populations, demonstrated a significant effect of the -219 G/T polymorphism in older age groups where both results were independent of the ε4 allele of the *APOE*.[29] The same authors in a recent publication[30] have demonstrated, in the brains of 114 patients with late and early onset sporadic AD, that the level of βA total and βA_{40} was increased in those individuals bearing the -219 TT genotype, compared with GT and GG. In older subgroups, βA total, βA_{40}, and βA_{42} were significantly increased independent of the coding region *APOE* variants, whereas the level of *APOE* mRNA was also lowered by 65%. In the same study, no effect of the -491 polymorphism on βA deposition was observed.

Low-density lipoprotein receptor-related protein (*LRP*) is the main ApoE receptor in the brain, being selectively located in neurons and activated astrocytes.[31] *LRP* is also found in senile plaques, one of the pathologic hallmarks of AD.

In 1997, Kang et al[32] investigated the polymorphism C/T at position 766 of the *LRP* gene, and found that the C/C genotype was associated with increased risk for senile AD. This finding was subsequently confirmed by some investigators[33,34] but not by others.[35]

It is speculated that the complex βA-ApoE linked to *LRP* might represent the initial intracellular amyloid precursor protein (*APP*) processing pathway, and that *LRP* effects on *APP* processing are mediated through its cytoplasmatic domains. When *LRP* is absent, levels of βA are much reduced, secondary to a specific reduction in *APP* internalization.[36,37] Nevertheless, it has been suggested that this genetic marker is in linkage disequilibrium with a putative nearby AD susceptibility locus.[34] In a recent meta-analysis,[38] including 4668 AD patients and 4473 matched controls, investigators found no association between the C/T766 *LRP* gene polymorphism in the total sample or in senile or presenile subgroups. In addition, no association between this genotype and brain levels of βA or τ proteins was detected.

The aim of this investigation was to evaluate the association of AD with certain polymorphisms of the promoter and coding region of the *APOE*, and of the coding region of *LRP* in a Brazilian population.

METHODS

One hundred and twenty controls and 120 patients who fulfilled both Diagnostic and Statistical Manual of Mental Disorders-4th edition criteria[39] for dementia and National Institute for Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria[3] for probable AD were studied. Patients and controls were recruited at the Behavioral and Cognitive Neurology Unit of Hospital das Clínicas, and all were evaluated by neurologists and neuropsychologists. All individuals or their caregivers signed an informed consent previously approved by Hospital das Clínicas Institutional Ethics Commission. Controls were selected from spouses or nonrelated individuals who displayed no evidence of neurologic or psychiatric impairment, or systemic decompensated disease. To screen for dementia among controls, the Mini-Mental State Examination and a verbal fluency test were used. Race was determined according to phenotypic appearance: color of skin, together with hair, nose, and mouth characteristics.

Genomic DNA was prepared from peripheral blood using standard procedures.[40] Genotyping for *APOE*[21,41,42] and *LRP*[43] polymorphisms was performed according to published protocols. Some modifications employed to increase efficiency of *APOE* -219 genotyping can be supplied upon request.

Statistical studies included analysis of variance, followed by the Tukey multiple comparison test, whenever a statistically significant difference was detected between groups. Whenever nonhomogeneous variances were found, Kruskal-Wallis test was used. Associations among categorical variables were tested by the χ^2 test. In cases where at least 1 of the expected frequencies was below 5, Fisher exact test was applied. The 95% confidence intervals (95%CI) of the percentages were calculated by assuming a binomial distribution.

Logistic regression analysis was performed to control for age, sex, and race effects and also to test for interactions between promoter genotypes and APOE status. We used both the stepwise and the ad hoc methods to verify which variables were associated with AD.

We used the SPSS 11.0 software for the statistical analysis, and the R-program 2.4.1 (http://www.r-project.org/) for the assessment of the linkage disequilibrium. For all tests, $P < 0.05$ was considered as statistically significant.

RESULTS

In the patient group, 68.3% were females with 63.3% females in the control group ($P = 0.41$). Age at ascertainment was also similar in cases and controls: for patients, the mean age was 75.2 years (SD = 9.2) and for controls, 72.5 years (SD = 8.6) ($P = 0.26$). Mean age of AD onset was 71.1 years (SD = 9.5). In this sample, 89.2% of patients and 94.2% of controls were whites ($P = 0.16$). The distribution of genotypes within patients group was in Hardy-Weinberg equilibrium.

Sample characteristics are described in Table 1 and genotypes results are shown in Table 2.

We observed that the frequency of the ε4 *APOE* allele was approximately 3 times more common in

TABLE 1. Characteristics of the Sample

Category	AD	Controls	*P*
Total	120	120	
No. individuals			
Senile	90	93	> 0.05
Presenile	30	27	
Sex (%)			
Male	31.7	36.7	0.41
Female	68.3	63.3	
Race (%)			
Whites	89.2	94.2	0.16
Non-whites	10.8	5.8	
Age (y) (mean ± SD)	75.2 ± 9.2	72.5 ± 8.6	0.26*
Age of onset (y) (mean ± SD)	71.1 ± 9.5		

Levene test *P* value < 0.05.
*Assuming equality of the variances.

patients (0.31) than in controls (0.10) ($P < 0.001$) and the presence of the ε2 allele was 3 times more frequent in controls (0.09) than in patients (0.03) ($P = 0.005$). Presence of the ε4 allele was strongly associated with risk for AD [odds ratio (OR) = 3.83; 95%CI = 2.27 to 6.50, $P < 0.001$]. The presence of the ε2 allele showed a protective effect (OR = 0.33; 95%CI = 0.13 to 0.78, $P = 0.005$).

Presence of T at position -219 of the *APOE* promoter region was associated with an increased risk for AD (OR = 1.52; 95%CI = 1.04 to 2.22, $P = 0.02$), whereas the presence of G at this same position showed a protective effect (OR = 0.66; 95%CI = 0.45 to 0.96, $P = 0.02$).

We were able to demonstrate that alleles -219 T and ε4 are in linkage disequilibrium, with $P < 0.001$ ($D' = 0.50$ and $r^2 = 0.05$), whereas the same occurred with -219 G and ε2 *APOE* alleles, with $P < 0.013$ ($D' = 0.21$ and $r^2 = 0.11$).

Using logistic regression analysis, we were able to demonstrate that the presence of the ε4 APOE allele was the only risk factor for AD in this sample (OR = 4.35; 95%CI = 2.45 to 7.78, $P < 0.001$). No association was seen between risk for AD and polymorphism A/T at position -491 of the *APOE* promoter region. In addition, polymorphism C/T 766 of the *LRP* coding region was not recognized as a risk factor for AD.

DISCUSSION

Our study has confirmed previous investigations regarding the association between AD and the presence of the ε4 allele in a dose-dependent manner.[4,5,7,44]

Frequency of ε2 and ε4 alleles is variable among different populations, and to date, very few studies on this issue have been performed in Latin America. In our sample, frequency of the ε4 allele among AD patients and controls (0.31 and 0.10, respectively) were closer to levels observed in the United States[45] and Northern Europe[46] yet higher than those observed in other Latin-American studies conducted in Brazil,[47,48] Argentina,[49] Venezuela,[50] and Colombia[51] (Table 3). These studies included patients with both probable and possible AD, except in Argentina whose study included only patients with probable AD, and also involved much smaller samples than the present study, with the exception of Venezuela. Furthermore, our sample might have included more individuals with European ethnic background than other Latin-American studies. To our knowledge, no other studies on *LRP* or *APOE* promoter polymorphisms have been conducted in Latin America. Nevertheless, even though our sample size is one of the largest ever published in Latin America, it is still not sufficiently large to provide a definitive result regarding ε2, ε3, and ε4 allele frequency.

Although the majority of our sample is constituted by whites, it should be noted that the Brazilian population is the result of extensive mixing of whites, Africans, and Amerindians. Mitochondrial DNA analysis in Brazilians with white phenotype[52] has shown Amerindian (33%) and African (28%) contributions to be high. On the other hand, a study of the Y chromosome unique-event polymorphisms in 200 Brazilian white males demonstrated that most of the Y chromosomes were of European origin.[53] These data suggest a strong European

TABLE 2. *APOE* and *LRP* Gene Polymorphisms in AD Patients and Controls

Polymorphism	Genotype	AD, n (%)	Controls, n (%)	*P*	OR 95%CI
APOE ε	2/2	0	4 (3.3)	—	—
	2/3	6 (5.0)	15 (12.5)	0.303	0.59 (0.21; 1.61)
	2/4	2 (1.7)	0	—	—
	3/3	53 (44.2)	78 (65.0)	Reference	—
	3/4	46 (38.3)	21 (17.5)	< 0.001	3.22 (1.73; 6.01)
	4/4	13 (10.8)	2 (1.7)	0.004	9.57 (2.07; 44.13)
	A/A	79 (65.8)	82 (68.3)	0.74	0.91 (0.51; 1.61)
APOE -491	A/T	34 (28.3)	32 (26.7)	Reference	—
	T/T	7 (5.8)	6 (5.0)	0.88	1.09 (0.33; 3.62)
	T/T	37 (30.8)	24 (20.0)	0.15	1.56 (0.85; 2.88)
APOE -219	T/G	69 (57.5)	70 (58.3)	Reference	—
	G/G	14 (11.7)	26 (21.7)	0.10	0.55 (0.26; 1.13)
	C/C	87 (72.5)	86 (71.7)	0.79	1.08 (0.59; 1.96)
LRP	C/T	28 (23.3)	30 (25)	Reference	—
	T/T	5 (4.2)	4 (3.3)	0.68	1.34 (0.33; 5.49)

TABLE 3. Frequency of *APOE* ε4 in AD and Controls Among Different Populations

Publications	Country	AD (n)	ε4 Frequency	Controls (n)	ε4 Frequency
Souza et al[47]	Brazil	68	0.25	58	0.12
Almeida et al[48]	Brazil	43	0.22	56	0.09
Bahia et al, current study	Brazil	120	0.31	120	0.10
Morelli et al[49]	Argentina	45	0.22	45	0.08
Jacquier et al[50]	Colombia	83	0.23	44	0.07
Molero et al[51]	Venezuela	121	0.17	1665	0.11
Wang et al[45]	USA	235	0.30	274	0.11
Adroer et al[46]	Spain	160	0.29	207	0.06
Lambert et al[27]	France	292	0.33	308	0.11

male contribution and a more diverse European, African and Amerindian matrilineal heritage in the formation of the Brazilian population.

In line with other studies,[7,8] the presence of the ε2 allele proved a protective factor for AD.

The presence of at least 1 T at position -219 of the *APOE* promoter region was associated with increased risk for AD, whereas the presence of the G allele showed a protective effect. Our results are in agreement with those of Lambert et al,[29,30] which showed an association of increased risk for AD and polymorphism − 291 T, but we were unable to demonstrate that this risk was independent of *APOE*ε4. Indeed, our results suggested that − 291 T is in linkage disequilibrium with *APOE*ε4, and − 291G with *APOE*ε2.

We did not find any relationship between -491 *APOE* polymorphism and risk for AD. This result is in agreement with several other published studies, despite slight effect of the -491 A/T polymorphism on risk for AD, independent of the ε4 allele, having been demonstrated in a meta-analysis.[45]

The C/T766 *LRP* polymorphism is a silent nucleotide substitution, without RNA processing or amino acid sequence changes. Akin to several other investigators,[35,38,54] we were unable to detect a relationship between AD and the polymorphism C/T 766 of the *LRP* gene in our sample.

In conclusion, we were able to demonstrate that in the Brazilian population, frequency of the ε4 allele among AD patients is similar to that observed in Northern European countries and the United States. We also demonstrated that ε4 is in linkage disequilibrium with T -219 *APOE* promoter polymorphism, but we could not distinguish the isolated effect of ε4 and T -219 as risk factors for AD.

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