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Analysis of the disease risk locus DXS1047 polymorphism in Brazilian Alzheimer patients

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Alzheimer's disease (AD) is a disorder characterized by a progressive deterioration in memory and other cognitive functions. Four genes associated with early onset AD have been identified[1–5] but familial AD is rare.[6] The majority of late onset AD (LOAD) is caused by a complex inheritance with several genes interacting with environmental factors. The ϵ4 allele of the apolipoprotein E (APOE) gene has been reported worldwide as a risk factor associated with LOAD.[7–10] The short variant of a polymorphism in the transcriptional region of the serotonin transporter gene (5-HTTLPR) was analyzed in several psychiatric conditions[11–15] and found to be more frequently associated with European[16] and Brazilian LOAD patients.[17,18] Recently, allelic associations with LOAD were reported for five other loci,[19,20] the most significant for one X-linked 202-bp allele, at the DXS1047 locus. We have analyzed this locus in Brazilian LOAD patients and observed that the 202-bp allele was not significantly more frequent among patients. In contrast, two other alleles (200 bp and 208 bp) were less frequent among AD male patients than in controls, confirming the importance of replicating association studies in different populations. *Molecular Psychiatry* (2000) **5,** 563–566.

In a recent study, Zubenko *et al*,[19,20] reported a genome survey for novel Alzheimer disease risk loci. In addition to APOE polymorphisms, allelic associations with AD were identified at five other loci: D1S518, D1S547, D10S1423, D12S1045 and DXS1047. Among them, the most significant association was found for one X-linked 202-bp allele, at the DXS1047 locus which was twice as common among AD cases than controls. In an attempt to replicate these findings in a different population we have analyzed this polymorphic locus in a group of Brazilian patients affected by Alzheimer's disease.

A total of 130 patients (45 males and 85 females) and 130 age-matched controls (45 males and 85 females) were included in the present investigation. The majority of patients (109) and controls (106) were Caucasians (Table 1).

The comparison of genotype distributions between patients and controls (Table 2) showed no statistically significant differences when both sexes were analyzed

Table 1 Demographic characteristics of AD cases and control group

	AD patients (n = 130)	*Control group (n = 130)*
Sex (male/female)	45/85	45/85
Age (years)	68.7 ± 8	72.3 ± 9.75
Race (white, black, other)	109/6/15	106/17/7

Table 2 Allele frequencies and their standard errors of the marker DXS1047 in Alzheimer's disease and control individuals

Alleles (bp)	*AD cases*			*Control group*		
	Males	*Females*	*Total*	*Males*	*Females*	*Total*
212	0.022 (0.022)	0.012 (0.008)	0.014 (0.008)	–	–	–
210	0.022 (0.022)	0.057 (0.018)	0.050 (0.015)	0.067 (0.037)	0.061 (0.019)	0.062 (0.017)
208	0.044 (0.031)	0.052 (0.017)	0.050 (0.015)	0.178 (0.057)	0.030 (0.013)	0.062 (0.017)
206	0.289 (0.068)	0.161 (0.028)	0.187 (0.026)	0.156 (0.054)	0.226 (0.033)	0.211 (0.028)
204	0.111 (0.047)	0.149 (0.027)	0.142 (0.024)	0.022 (0.022)	0.189 (0.031)	0.153 (0.025)
202	0.444 (0.074)	0.374 (0.037)	0.388 (0.033)	0.333 (0.070)	0.280 (0.035)	0.292 (0.031)
200	0.044 (0.031)	0.109 (0.024)	0.096 (0.020)	0.178 (0.057)	0.104 (0.024)	0.120 (0.022)
198	0.022 (0.022)	0.075 (0.020)	0.064 (0.017)	0.067 (0.037)	0.073 (0.020)	0.072 (0.018)
196	–	–	–	–	0.006 (0.006)	0.005 (0.005)
194	–	0.006 (0.006)	0.005 (0.005)	–	0.018 (0.010)	0.014 (0.008)
192	–	0.006 (0.006)	0.005 (0.005)	–	–	–
190	–	–	–	–	0.012 (0.009)	0.010 (0.007)

together (log-likelihood ratio = 45.33; $P > 0.05$). When each gender was analyzed separately, no statistically significant differences were observed in genotype distributions between patients and controls for females (log-likelihood ratio = 38.42; $P > 0.50$). However, for males, the overall genotype frequencies were found to be significantly different (log-likelihood ratio = 16.65; $P = 0.02$).

According to the bootstrap test, the alleles that can be considered most responsible for such differences in males are the 200-bp and 208-bp alleles (381 higher differences out of 10 000 and 362 higher differences, also out of 10 000, respectively). These results cannot be strictly considered as significant, because they correspond to multiple non independent tests. In this case, the critical value corresponding to a probability of less than 5% should be corrected to 6 out of 10 000, according to the corrections of Dunn-Šidák or Bonferroni. These results can be considered as an indication that this locus can be associated with LOAD in Brazilian males, but they show significant linkage disequilibrium with different alleles when compared to the results obtained in independent samples of the North-American population.

The 202-bp allele was the most frequent both in patients and controls. Its relative frequency was 38.22% in patients (44.4% for males and 36.5% for females) and 28.4% for controls (33.3% for males and 27% for females).

Both patient and control groups showed no departures from the genetic equilibrium ($\chi^2 = 454.7$ for the disease group, $\chi^2 = 293.5$ for the controls; $P = 1.000$ for both, as calculated by the bootstrap algorithm). The combined sample was also shown to be in equilibrium ($\chi^2 = 454.7$, $P = 0.996$). The calculated F values were not significantly different from zero (F = 0.029 for the disease group, F = 0.088 for the controls, and F = 0.065 for the overall sample, $P = 1.000$ for all).

It is widely accepted that AD is a genetically complex disorder involving several susceptibility genes in addition to APOE. Therefore, the identification of candidate regions significantly more often associated to AD will be very important in the search for other genes implicated in LOAD. In a genome survey, Zubenko *et al*[19] detected associations of alleles at five novel microsatellite loci, with the higher significance values for the 202-bp allele of marker DXS1047. According to Zubenko *et al*,[21] the inheritance of the DXS1047 202-bp allele was associated with the relative preservation of cortical aminergic neurotransmitter norepinephrine (NE) levels and lower cortical levels of dopamine. However the underlying mechanism to explain these findings is not understood. According to these authors, the DXS1047 202-bp allele may be associated with a subtype of AD that is accompanied by less degeneration of noradregenic neurons and perhaps somewhat greater degeneration of dopaminergic neurons. Since the confirmation of such associations in other population studies is of utmost importance we have analyzed this polymorphism in Brazilian LOAD patients.

In accordance with Zubenko's findings, we also observed that the 202-bp allele is the most frequent allele of marker DXS1047 for both sexes. It was also slightly more frequent in Brazilian AD patients than in controls, although the difference did not reach the level of significance observed by these authors. According to the reported frequency of the 202-bp allele and its standard error by Zubenko *et al*[19] in their study, our values lie between their control and disease group. This result indicates that the population here sampled has less degree of linkage disequilibrium with the 202-bp allele of the DXS1047 locus.

These authors also found that the proportion of women with AD who carried this allele was nearly double that observed for men, in accordance with X-linked inheritance. In our study it was also more frequent in AD women but the difference was only marginally significant ($P = 0.078$ in the present study; $P < 0.0005$ in Zubenko's study).

Interestingly, however, in the present study, when both genders were analyzed separately, the difference was observed only for males. That is, we observed that the 200-bp and 208-bp alleles were found to be four times more frequent among male controls than patients but unexpectedly did not differ significantly in female patients as compared to controls (although the number of females was almost twice and the number of X chromosomes almost four times greater in females than males).

This observation is also surprising since several studies have shown that both the incidence and the prevalence of AD are increased among women,[22,23] although the causes for this gender difference are not completely understood. One possible explanation would be a greater influence of X-linked alleles as recently shown for panic disorder and the X-linked monoamine oxidase A gene.[24] A sex-specific interaction between different genes has also been reported for Huntington disease and the APOE genotype by Kehoe *et al*.[25] These authors observed that the APOE $\epsilon 2\epsilon 3$ genotype is associated with significantly earlier age at onset in males than in females.

In summary, the present study supports the hypothesis that the DXS1047 locus may be genetically linked to a putative important locus for LOAD, but other studies are warranted before assigning this marker with the risk *per se*. On the other hand, we have no explanation for the apparent gender-dependent effect of the DXS1047 200-bp and 208-bp alleles observed in the present study. It will be very important to confirm these results in other population studies before a risk factor associated with these alleles can be used for prognostic purposes for the Brazilian or other populations.

Indeed, it is relevant that more recently Kehoe *et al*[6] performed a full genome scan for LOAD. These authors found two peaks in the X chromosome, which were apparently more associated with the disease. However, these two peaks do not correspond to the DXS1047 marker.

It is also important to point out that in multifactorial disorders such as LOAD an important environmental

effect is also expected to contribute to the results obtained when different populations are analyzed. For example, food habits, climate (including daylight patterns that are known to interfere with serotonin cycles),[26] longevity, cultural level and other environmental factors may modify the effect of alleles and loci. Therefore, apparent contradictions among different reports may be the result of gene × environment interactions, supporting the importance of replication studies in different populations, before genotype-based prognosis can be widely used.

Methods

The diagnosis of probable/possible Alzheimer disease in the patients was based on NINCDS-ADRDA.[27] Their mean age was 68.7 ± 8.0 (ranging from 51 to 85 years old). The control group was selected based on the Mini Mental State Exam and/or Blessed scale[28,29] or depending on their educational level through familial interviews. Their mean age was 72.4 ± 9.75 years (ranging from 53 to 92 years old).

DNA was extracted from blood[30] after informed consent, and the marker DXS1047 was analyzed by polymerase chain reaction (PCR) according to the method reported by Zubenko *et al*.[19]

Statistical analysis included contingency tables and a bootstrap test specially designed for both allele frequencies comparison among AD and control samples and also for genetic equilibrium (detailed below).

Detailed statistical analysis

The samples were first compared with respect to overall genotype frequencies regardless of the sexes, with contingency tables and log-likelihood test. The bootstrap algorithm was used to test for differences in allele frequencies between the disease group and the control group, for each sex separately and together. This algorithm was written in ANSI-C by one of us (SRM, and its source code is available upon request to srmatiol@ib.usp.br), and consisted in making 10 000 samples of genotypes with reposition, where the presence of Alzheimer's disease was randomly assigned, keeping the sample sizes constant. To test for genetic equilibrium, a Chi-square value (with Yates correction) and Wright's F were calculated for each group (disease and control) and also for the combined sample. These values were then compared to 10 000 values calculated in the same fashion from samples that were made for random allele assignment with reposition from the original sample, within the groups that were to be tested. We made this bootstrap resampling for genetic equilibrium because it is well known that too many classes with low observed values (eg genotypes where the allele number is high) cause severe distortions in conclusions drawn through standard Chi-square tests. The values of Chi-square tests for equilibrium would be highly significant if compared to the tables of Chi-square distribution, but were shown to be non significant with the resampling procedure. In all these tests, significant difference was inferred when less than 500 values obtained in the resampled populations were higher than the observed value. This corresponds to a probability that is less than 5% that the results were obtained at random.

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