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The Genetics of Alzheimer's Disease in Brazil: 10 Years of Analysis in a Unique Population

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Abstract Alzheimer's Disease (AD) is the most common type of dementia among the elderly, with devastating consequences for the patient, their relatives, and caregivers. More than 300 genetic polymorphisms have been involved with AD, demonstrating that this condition is polygenic and with a complex pattern of inheritance. This paper aims to report and compare the results of AD genetics studies in case-control and familial analysis performed in Brazil since our first publication, 10 years ago. They include the following genes/markers: Apolipoprotein E (APOE), 5-hidroxytryptamine transporter length polymorphic region (5-HTTLPR), brain-derived neurotrophin factor (BDNF), monoamine oxidase A (MAO-A), and two simple-sequence tandem repeat polymorphisms (DXS1047 and D10S1423). Previously unpublished data of the interleukin-1α (IL-1α) and interleukin-1 β (IL-1β) genes are reported here briefly. Results from others Brazilian studies with AD patients are also reported at this short review. Four local families studied with various markers at the chromosome 21, 19, 14, and 1 are briefly reported for the first time. The importance of studying DNA samples from Brazil is highlighted because of the uniqueness of its population, which presents both intense ethnical miscegenation, mainly at the east coast, but also clusters with high inbreeding rates in rural areas at the countryside. We discuss the current stage of extending these studies using high-throughput methods of large-scale genotyping, such as single nucleotide polymorphism microarrays, associated with bioinformatics tools that allow the analysis of such extensive number of genetics variables, with different levels of penetrance. There is still a long way between the huge amount of data gathered so far and the actual application toward the full understanding of AD, but the final goal is to develop precise tools for diagnosis and prognosis, creating new strategies for better treatments based on genetic profile.

Keywords Alzheimer's disease · Polymorphisms · Genetic risk factors · Brazil

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Introduction

Recently, the plethora of data produced at the field of genetic risk factors for Alzheimer's Disease (AD) was compiled and summarized in a new public database entitled ALZGENE. This website centralizes and curates most of the genotyping data generated so far from AD case-control analysis and might be used especially for meta-analysis studies (http://www.alzforum.org).

This was a key initiative, considering the amount of articles published about this issue, especially during the last 15 years.

More than 300 genetic polymorphisms have been involved with AD, but only a few were independently confirmed, demonstrating that this condition is polygenic and with a complex pattern of genetic inheritance (Bertram et al. 2007).

When our group started studying this issue, the foundations of molecular studies in AD were based on specific studies of families with early-onset Alzheimer's disease (EOAD) and late-onset Alzheimer's disease (LOAD). Mutations at the amyloid precursor protein (APP) and at the presenilin genes (PS1 and PS2) were already reported and the apolipoprotein E ε4 allele (ApoE ε4) was considered the most important polymorphism involved mainly in LOAD (Eastwood et al. 1996).

Around a decade ago, the first case-control studies and family analysis were performed in Brazilian patients with some genes previously linked to AD, such as APOE, 5HTTLPR, DXS1047, D10S1423, BDNF, and MAO-A (Oliveira et al. 1997, 1998, 1999a, b; Nishimura et al. 2000, 2001, 2004, 2005). Previously unpublished data of the interleukin-1α (IL-1α) and interleukin-1 β (IL-1β) genes are also reported here together with a brief report of markers at the chromosomes 21, 19, 14, and 1 in a few families with AD.

The Brazilian population has various interesting genetic idiosyncrasies, initially characterized 50 years ago. The groups living at the coast are highly mixed, with low inbreeding rates and presenting a major genetic contribution from Native Indians, Europeans, and African Americans. On the other hand, the population living in rural areas presents some of the highest inbreeding rates reported so far (Freire-Maia 1957).

We focused our recruitment at the coast population in two major cities: Recife and São Paulo. Performing case-control studies in these groups with low inbreeding rates is crucial due to the possibility of finding risk factors that are widely relevant in various ethnicities and not only limited to a specific population because the overrepresentation of rare variants in specific ethnic groups may confuse interpretation of association analyses (Keen-Kim et al. 2006).

After the publication from various other research groups studying the same mutations and also other polymorphisms, it is clear that the genetic background for AD is polygenic and complex.

A more detailed analysis of our findings will follow along this article.

Case-control Studies

In general, the genetic background of the population recruited in our case-control studies presented a distribution of 3:1 for Caucasoids and African/Brazilians, respectively. This disproportioned distribution decreased the number of subject available to correlation studies between the ethnical backgrounds and specific genetic entity.

The sample sizes were progressively increased since 1997 and the estimated allele frequencies were consistently checked for the Hardy–Weinberg equilibrium.

Studying AD patients and another group with memory deficit, but not demented (pre-clinical subjects), we found significant differences at the APOE ε4 allele distribution between AD patients and controls. This study also verified a significant association between the ε4 allele and the pre-clinical subjects with positive family history for dementia, confirming this polymorphism as a susceptibility factor and suggesting that it might be useful for screening risk groups (Oliveira et al. 1997). This result coincides with the majority of studies that bestowed to the APOE ε4 allele the status of the most important risk factor for AD (Bertram et al. 2007).

The polymorphism at the promoter region of the serotonin transporter gene (5-HTTLRP), an insertion/deletion of 44 base pairs generating two alleles with the short version (*s*) decreasing the gene transcriptional activity was widely studied in various neuropsychiatric conditions and personality traits (Canli and Lesch 2007). The short version was also considered a risk factor to develop AD in a Brazilian group of AD patients (Oliveira et al. 1998). Curiously, we did not find significant association for risk increase when studying Brazilian patients with both *s* variant and ε4 allele (Oliveira et al. 1999a).

This study confirmed for the first time the findings of Li et al. (1997), but later other studies in Japanese population found no statistically significant association between AD and the short allele (Kunugi et al. 2000).

Nevertheless, studies with polymorphisms related with serotonin, involving receptors, transporters, and enzymatic machinery, enhanced our knowledge about specific pathological mechanisms involved at this neuropsychiatric condition (Oliveira and Zatz 1999b).

Simultaneously, an important Genome wide scan found that the 202 bp allele in locus DXS1047 and 234 bp in locus D10S1423 were associated with AD. These researchers had observed that the patients with these two alleles presented reduced levels of dopamine in six cortical regions and high concentrations of cortical norepinephrine, suggesting a dosage effect of the alleles in question (Zubenko et al. 1998).

Both locus DXS1047 and D10S1423 were analyzed in Brazilian patients, but no statistically significant association was found (Nishimura et al. 2000, 2001).

The C-270T polymorphism, located at the 5′ non-coding region of brain-derived neurotrophin gene (BDNF) was also associated with AD and the patients with the T variant present an increase of the susceptibility to develop the illness. In accordance with these authors, the polymorphism can modify the expression of protein BDNF, being capable of relating to the pathogenesis of AD (Kunugi et al. 2001).

However, we found no significant association between these polymorphisms and the AD (Nishimura et al. 2004).

Monoamine oxidase A (MAOA) is a mitochondrial enzyme that catalyzes the degradation of different amines including neurotransmitters like dopamine, norepinephrine, and serotonin. Several studies have shown that MAOA plays important role in human behavior and physiology, being associated with mild mental retardation and impulsive aggressive behavior, mood disorders, and alcoholism with antisocial personality (Sabol et al. 1998).

We found no association between the locus of MAOA and LOAD, but curiously, a combination of the MAOA allele 1, the 5HTTLPR short variant and APOE ε4 was more frequent in patients, reinforcing the hypothesis that different genes might act together to modulate the disease (Nishimura et al. 2005).

Other studies have attempted to find a relationship between inflammatory pathways and psychiatric disorders. Polymorphisms of the interleukin-1 (IL-1) gene complex such as IL-1α and IL-1β have been associated to different neuropsychiatric conditions, including AD (Katila et al. 1999; Du et al. 2000; Mattila et al. 2002; Fertuzinhos et al. 2004)

To test the association of these polymorphisms and AD, a total of 195 LOAD Brazilian patients with mean age of 68.7±8 years were selected in accordance with the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria. The 188 age-matched controls with mean age of 72.3±9.75 were selected according to the Mine Mental State Examination (MMSE) and/or Blessed Scale, socio-cultural and comparable ethnic background. All patients and healthy subjects gave informed consent for participation in this study with the approval of the ethical committee of the Institute of Biosciences, University of São Paulo.

In order to evaluate the distribution of IL-1 polymorphisms in the Brazilian population, genomic DNA was isolated from peripheral blood according to standard procedures and the genotyping was based on previous study (Katila et al. 1999).

No significant association was observed when we compared either allele or genotype distribution for IL-1α and IL-1β polymorphisms (P=0.813 and P=0.607) for allelic and for genotype distribution (P=0.825 and P=0.427), respectively, suggesting they might not confer an increased risk for LOAD in the Brazilian population.

A few other Brazilian groups also performed similar studies, examining the association of the methylenetetrahydrofolate reductase enzyme (MTHFR) with AD. MTHFR is a central enzyme of the folate metabolism forming the substrate needed for the transferring reaction, an important co-factor to homocysteine (Hcy) methylation. Epidemiological studies have reported an association between increased plasma Hcy level and AD (Fernandez and Scheibe 2005; Religa et al. 2003).

Da Silva et al. (2006) found no association between MTHFR polymorphisms and AD patients from Brazil, but found a difference at the level of expression at the blood serum.

Recently, meta-analyses aimed for this polymorphism did not confirm it as a major risk factor for the AD (Bertram et al. 2007).

All these Brazilian case-control studies are summarized in Table 1.

Family Studies

The study of families with LOAD is extremely difficult due to the fact that potential affected subjects will decease from

Table 1 A summarized analysis of the Brazilian case-control studies

References	Polymorphisms	P value	Sample size
Oliveira et al. (1997)	ApoE-ε4	$P<0.05$	57
Oliveira et al. (1998)	5-HTTLPR	$P<0.05$	81
Nishimura et al. (2000)	DXS1047	$P>0.05$	130
Nishimura et al. (2001)	D10S1423	$P>0.05$	130
Nishimura et al. (2004)	BDNF (C-270T)	$P>0.05$	188
	BDNF (C-270T) + ApoE-ε4	$P<0.05$	
Nishimura et al. (2005)	MAOA	$P>0.05$	128
	MAOA + ApoE-ε4 + 5-HTTLPR	$P<0.05$	
Unpublished data	IL-1α and IL-1β	$P>0.05$	195
	ApoE-ε4	$P<0.05$	195
Fernandez and Scheibe (2005)	MTHFR (C-677T)	$P>0.05$	30
	ApoE-ε4	$P<0.05$	
da Silva et al. (2006)	MTHFR (C-677T)/(A-1298C)	$P>0.05$	43

various age-related causes before developing the dementia symptoms, so the number of affected subjects available for Linkage analysis and association studies is often scarce. This is why the most elucidative family studies came from the analysis of EOAD (Shen and Raymond 2007).

We were able to recruit four small kindred with LOAD and tested the pattern of segregation with markers for the chromosomes already involved with familial AD, such as D1S158, D1S479, F13B, D1S53, D1S179, D1S217, D1S249, D1S175, D1S178, D14S52, D14S53, D14S42, N141I, D21S120, D21S13E, APP, APOCII (MFD5), D19S278, D19S178, D19S224, D19S47, and D19S75. None of these markers showed a distinct and significant segregation pattern across the families based on Linkage analysis. The APOE and 5-HTTLPR were also genotyped at these patients to check possible association. Curiously, these were the only markers associated with the affectation status (unpublished data).

Other Genetic Studies at the Brazilian Population

Cytogenetic studies were also performed at the Brazilian population in order to find the major differences between AD patients and controls. Kormann-Bortolotto et al. (1992) examined chromosomal fragility in both AD and elderly control groups and found a higher frequency of fragile sites in 6p21, but not in the young controls. These caryotype bands overlap with the position of the MTBT2 and MTBT1 genes, which are microtubule (beta)-associated protein tau-like and tau 1, respectively.

The maturation rates of the 28S/18S subunits of rRNA were analyzed in order to establish an association of this event with the AD. Payão et al. (1998) and da Silva et al. (2000) found reduced maturation and transcription rate, suggesting a possible change of these processes or preference degradation of the 28S subunit.

By analyzing the maturation rate the subunits 28S/18S and the effect of the allele APOE e4, no association was found with AD, suggesting that the two different events are involved in the etiology of the AD, but traveling through different paths (Tavares et al. 2004).

Another genetic study checked for association at the Werner helicase polymorphism and AD, but the result was negative (Payão et al. 2004).

Possible Reasons to Explain the Results Disparity in Brazilian Studies

Over the 10 years of research with the Brazilian population, eight polymorphic markers were addressed by our group; however, only two showed statistically significant differences of distribution in case-control studies.

Some possible reason to explain our results when comparing with positive studies are the relative reduced sample size and clinical diagnostic criteria.

Our patients were diagnosed with probable/possible AD, and we did not have access to brain tissue. This procedure increases the chances of including patients with other types of dementia. However, the positive findings with the APOE analysis confirm that we were working with a population well selected enough to detect the significance similar to most of the studies performed worldwide.

Another possible explanation for this result would be the great ethnical variability in the Brazilian population that could mask the relevance of some markers in a few specific groups or that other susceptibility genes would be acting as risk factors at the Brazilian population (NCI-NHGRI Working Group on Replication in Association Studies 2007).

For various reasons, case-control studies testing allelic association might produce positive and negative results in different populations also due to different ethnical background, and this is a good reason to compare data from different groups of patients (Keen-Kim et al. 2006).

Controversial results are found when different analyses are performed in complex disorders from various populations worldwide (Freimer and Sabatti 2007).

New Perspectives Using High-throughput Methods of Genotyping Genetic Variations, Such as Single-nucleotide Polymorphism (SNPs) Microarrays

The availability of straightforward techniques to perform high-throughput genotyping, by using SNPs microarrays, multiplex and real-time polymerase chain reaction (PCR) will provide faster and more precise methods to extend the studies genetic risk factor for AD at this same population (Pearson et al. 2007).

Given the large number of candidate genes that have already been identified for AD, (http://www.alzforum.org), the most recent studies focus on understanding how these genes interact with each other to trigger the disease. Analysis of microarray suits this purpose, pointing differences in the levels of gene expression on a large scale or, more recently, identifying variations SNPs in LOAD. The different approaches with the same technique specify groups of genes involved in the pathological process (Colangelo et al. 2002; Hamshere et al. 2007).

The bioinformatics has a fundamental importance in speeding up that process, allowing the analysis of multiple data simultaneously, using complex algorithms to help the better understanding of various complex disorders, such as AD (Perez-Iratxeta et al. 2002).

There is still a long way between the huge amount of data gathered so far and the actual application toward the

full understanding of AD, but the final goal is to develop precise tools for diagnosis and prognosis, creating new strategies for better treatments based on genetic profile.

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