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DNA methyltransferase haplotype is associated with Alzheimer's disease

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HIGHLIGHTS

- Minor alleles of two SNPs (rs998382, rs2424913) in the *DNMT3B* gene were associated with Alzheimer's disease (AD) when compared to healthy controls.
- The *DNMT3B* TGG haplotype was associated with AD.
- This difference was not observed for *DNMT1* polymorphisms.

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ABSTRACT

Epigenetic mechanisms have been implicated in syndromes associated with neuropsychiatric disorders, but little is known about the role of epigenetics in Alzheimer's disease (AD). DNA methylation, one of the main epigenetic mechanisms, is a complex process carried out by specific enzymes, such as *DNMT1* and *DNMT3B*. This study aimed to investigate the association between *DNMT1* and *DNMT3B* polymorphisms and AD. Two hundred and ten elderly subjects (108 healthy controls and 102 with AD-NINCDS/ARDA, DSM-IV-TR criteria) were assessed. DNA was obtained from whole blood, and genotypes were detected by an allelic discrimination assay using TaqMan® MGB probes on a real-time PCR system. The polymorphisms studied were rs2162560, rs759920 (*DNMT1*) and rs998382, rs2424913, rs2424932 (*DNMT3B*). For both genes, the polymorphisms were in strong linkage disequilibrium. Carriers of the *DNMT3B* TGG haplotype were associated with AD (OR = 3.03, 95% CI 1.63 to 5.63, $P < 0.001$). No significant difference between AD and the control group were observed for *DNMT1* polymorphisms. This study is one of the first describing a significant association between *DNMT3B* polymorphisms and AD. This enzyme, which is responsible for methylation in a general way, may be involved in AD.

1. Introduction

Epigenetic mechanisms have been implicated in syndromes associated with neuropsychiatric disorders, but little is known about the role of epigenetics in sporadic Alzheimer's disease (AD), a complex multifactorial neurodegenerative disorder and the most common cause of dementia [3]. In this sense, epigenetic processes may have a role in the gene–environment interaction process, the most accepted model linked with neurodegeneration in sporadic AD. DNA methylation is the most stable epigenetic modification, modulating the transcriptional plasticity of mammalian genomes. It is linked to gene expression, with an inverse correlation between the degree of promoter DNA methylation and the level of expression [15,30]. This process is mediated by a family of conserved enzymes, DNA methyltransferases (DNMT), responsible for adding a methyl group to position 5 of the cytosine pyrimidine ring in the CpG dinucleotide [9]. The *DNMTs* (mainly *DNMT1* and *DNMT3B*) are enzymes responsible for establishing and maintaining DNA methylation patterns. *DNMT1* is a maintenance enzyme, which binds methyl groups to hemi-methylated DNA during DNA replication. *DNMT3B* are de novo methyltransferase, which establish methylation patterns during embryonic development [8,29].

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DNA methyltransferases were first investigated in some varieties of cancer [1,4], but interest in the neuropsychiatric field has been increasing [13,14,36], with most studies considering the *DNMT3B* enzyme. A study conducted with psychiatric patients with a history of suicide attempts showed significantly higher levels of global DNA methylation compared with controls, and this finding was associated with *DNMT3B* polymorphisms [29]. A post-mortem brain regions study revealed a marked reduction in DNA methylation in cortical neurons of AD subjects when compared to controls, and it was associated with increased *PSEN1* gene expression [32]. It is still not clear whether *DNMTs* polymorphisms imply hyper or hypo DNA methylation in Alzheimer's disease.

In addition, some genes that participate in amyloid-beta processing (*PSEN1, APOE*) and methylation homeostasis (*MTHFR, DNMT1*) show significant inter-individual epigenetic variability in AD brain samples, showing a notably age-specific epigenetic drift, which supports the potential role of epigenetic effects in developing the disease [35].

The complexity of AD degeneration, as well as the attractive hypothesis of epigenetic mechanisms contributing to AD pathogenesis and the influence of environmental factors on phenotypic constitution, justifies further studies on epigenetics. This study aims to evaluate the association of AD and five known *DNMT* gene polymorphisms (*DNMT1:* rs2162560, rs759920; *DNMT3B:* rs998382, rs2424932 and rs2424913).

2. Methods and materials

2.1. Study design

This is a case-control study comparing a group of Alzheimer's disease patients to healthy control subjects.

The study was approved by the bioethics committees of the participating institutions and was performed in compliance with the Declaration of Helsinki. All participants or their proxies in AD cases provided written informed consent.

2.2. Participants

All participants (AD patients and healthy controls) were Caucasian and were from a similar geographic region, matched for the same low-income economic status.

One hundred and two sporadic AD patients were recruited by convenience from two academic outpatient neuropsychiatric services located in a southern Brazilian city. All of them fulfilled probable NINCDS-ADRDA [25] and DSM-IV-TR [2] AD criteria. This diagnosis was ascertained by a psychiatrist or neurologist from the research team with expertise in the dementia field. Brain tomography or magnetic resonance imaging and complete medical and laboratory evaluations were performed to exclude other causes of dementia. Other exclusion criteria were history of cancer, family history of dementia and any other neurological or psychiatric disorders.

A control group of 108 age and sex-matched cognitively healthy and independent community-dwelling elderly individuals were recruited from the catchment areas of the same academic services. The inclusion criteria were age greater than 65 years, clinical dementia rating (CDR) of 0 [27], mini mental state examination (MMSE) score higher than 26 [12] and independence for activities of daily living (ADL) [17,20]. Controls were excluded if they presented chronic renal disease, history of significant head injury or stroke, history of cancer, family history of dementia, other psychiatric conditions such as major affective disorder or evidence of current depression, uncorrectable vision or hearing loss or other conditions such as substance abuse or use of medications that could impair cognitive function.

2.3. Genotyping

The DNA was extracted from 500 μL of EDTA-treated whole blood using the salting out method [19]. After extraction, the DNA was quantified on a UV visible spectrophotometer (Biospec® Nano). The final concentration of DNA used was from 10 ng/mL. The single nucleotide polymorphism (SNP) selection investigated in this study was performed using the HapMap (HapMap Genome Browser release #24) (Phases 1 and 2—full dataset) using the following settings for the tool "annotate TagSNP Picker": European population (CEU), minimum frequency of the rarer allele of 20% and a coefficient of determination (R2) of 80%. The five polymorphisms were genotyped with the use of TaqMan Genotyping Master Mix and TaqMan SNP Genotyping assays (Applied Biosystems®).

For each reaction plate, genomic control DNA samples and non-template controls (water) were included. A control on the TaqMan SNP genotyping assay was also performed (25% of randomly chosen samples from both groups) to check for genotyping accuracy, and identical genotypes were identified in all repeated samples. The researchers who performed the genotyping were blinded to the patients' diagnostic status.

2.4. Statistical analysis

The results were entered into a database, and statistical package SPSS® version 18.0 was used to perform the analyses.

A non-parametric Mann–Whitney test was used to calculate the differences in age and education between cases and controls. For sex comparisons, a chi-squared test of association was used. The Student *t* test was used to compare economic income between the AD group and the control group.

Frequencies were described as proportions for categorical variables and as mean plus standard deviations for quantitative variables. Allelic frequencies were obtained by direct counting throughout the genotype frequency.

Chi-square testing was carried out to verify whether the genotypic frequencies were in agreement with Hardy-Weinberger equilibrium. The linkage disequilibrium between the polymorphisms in each genomic region was estimated with MLocus 3.0 [22], and haplotypes were imputed with PHASE 2.1 [33,34]. Haplotypes with frequencies less than 3% were pooled.

Univariate analyses to verify the associations between the polymorphisms in the genes encoding the enzymes *DNMT1* and *DNMT3B* and Alzheimer's disease were carried out by chi-square association tests with a dominant model. The Bonferroni test was performed for multiple testing corrections.

Multivariate logistic regression analysis was performed for the outcome AD, with polymorphisms or haplotypes as independent variables. The confounders entered in the model were age and education, based on the literature review [3,7].

A two-tailed $P < 0.05$ was considered significant for all analyses.

3. Results

The sample is depicted in Table 1. The AD and control groups were comparable by age and sex. Education and MMSE scores were significantly lower in the AD group than in the control group. Family income (US$/mouth) was not significantly different between the AD group (M = 587.32; SD = 491.21) and healthy controls (M = 640.13; SD = 501.12) ($t = 0.96$, $P = 0.31$).

The genotypic frequencies of *DNMT1* and *DNMT3B* polymorphisms were consistent with Hardy-Weinberg equilibrium