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Original article

DNA (cytosine-5)-methyltransferase 3B (*DNMT 3B*) polymorphism and risk of Down syndrome offspring

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ABSTRACT

Down syndrome (DS) is the most common form of human genetic mental retardation. Several polymorphisms in genes coding folic acid cycle enzymes have been associated to the risk of bearing a DS child; however, the results are controversial. S-adenosyl-L-methionine (SAM) is an important intermediate of folic acid pathway and acts as methyl donor and substrate for DNA (cytosine-5)-methyltransferase 3B (*DNMT3B* – EC 2.1.1.37) *de novo* methylation processes during embryogenesis. Recent studies suggest that a functional polymorphism of *DNMT 3B* in maternal genotype may be associated with a decreased risk of having a DS child. We herein investigate the association of this polymorphism with the occurrence of DS in a Brazilian population. We have genotyped 111 mothers of DS infants (MDS) and 212 control mothers (CM) through PCR-RFLP. The observed genotypic frequencies were CC = 0.22; CT = 0.49 and TT = 0.29 in CM, and CC = 0.30; CT = 0.52 and TT = 0.18 in MDS. Allelic frequencies were C = 0.47 and T = 0.53 in CM and C = 0.56 and T = 0.44 in MDS. No deviation of HWE was observed, and both *DNMT 3B* rs2424913 genotype ($\chi 2 = 4.53$; DF = 1; P = 0.03) and allelic ($\chi 2 = 4.90$; DF = 1; P = 0.03) frequencies show significant differences between MDS and CM. The presence of the mutant *DNMT 3B T* allele decreases 30% the risk of bearing a DS child (OR = 0.69; 95% CI: 0.50–0.96; P = 0.03), and the risk is diminished up to 45% in association with the homozygous genotype (OR = 0.54; 95% CI: 0.31–0.96; *P* = 0.04). Our results suggest that women harboring the single nucleotide polymorphism *DNMT 3B* rs2424913 have a decreased risk of a DS pregnancy, and further studies are necessary to confirm this protective effect.

1. Introduction

Down syndrome is the most frequent cause of mental retardation of genetic etiology in humans and occurs in 1/700 stillbirths (Sherman et al., 2007). Failure of segregation machinery during maternal gametogenesis is considered the main cause chromosome 21 missegregation, generating a trisomy 21 zygote. Other causes of DS include chromosomal rearrangements and somatic mosaicism (Hassold and Sherman, 2000). The only validated risk factor associated with DS is advanced maternal age at conception (Allen et al., 2009).

James et al. (1999) published the first report associating a folate pathway gene polymorphism with an increased risk of bearing a DS child. From their original paper to date, several reports have suggested an association between polymorphisms within folate metabolism genes and an increased risk of DS. However, the results are controversial: some reports found an association between DS and folate-related gene polymorphisms, while others failed to find such association. Thus, the association between a given polymorphism and DS varies according to several factors, including nutritional status and genetic background of the studied population (Guéant et al., 2003).

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Cellular processes of nucleotide synthesis and methylation are associated with folic acid cycle, which provides the chemical groups necessary for these reactions. In methylation reactions, DNA (cytosine-5)-methyltransferase 3B (*DNMT3B* – EC 2.1.1.37) uses S-adenosyl-L-methionine (SAM) as methyl donor and substrate for *de novo* methylation during embryogenesis (Bestor, 2000). SAM arises from the methionine generated during homocysteine remethylation and is essential for transmethylation, transsulfuration, and polyamine synthesis (Lu, 2000).

Human DNMT 3B present 23 exons and has been mapped to chromosome 20q11.2 (Xie et al., 1999). Although −149C > T polymorphism has been described as a promoter mutation, it is located in an intronic region according to the SNP databases (NM_006892.3 at NCBI, as well as, ENST00000328111.6 at ENSEMBL). Considering that all the previous publications refer to this polymorphism as a promoter variant 149 base pairs upstream the transcription start site, we adopted dbSNP nomenclature rs2424913. Shen et al. (2002) has described an increased promoter activity *in vitro* in association with this polymorphism.

Most association studies involving *DNMT 3B* polymorphism are related to cancer development; DNA methylation of promoter regions is one of the major regulatory mechanisms of gene expression (Shen et al. 2002) and aberrant DNA methylation may contribute to carcinogenesis through deamination of 5-methyl cytosine to thymine, increasing mutation rates (Lee et al., 2005). Also, an abnormal supply of folic acid cycle intermediates could unbalance the availability of SAM, the main donor of methyl groups for methylation reactions, leading to centromeric hypomethylation and chromosomal nondisjunction (James et al., 1999).

To determine the association of DNA (cytosine-5)-methyltransferase 3 beta rs2424913 polymorphisms over DS pregnancy risk we have performed a case-control study in a population from Rio de Janeiro, a state located in the Southeast region of Brazil.

2. Materials and methods

2.1. Subjects

Mothers of Down syndrome children (MDS) were selected from Genetics Service of IPPMG (Portuguese acronym for Martagão Gesteira Childcare and Pediatrics Institute) at Federal University of Rio de Janeiro and control mothers (CM) were enrolled through Pediatric Clinic from the same Institution. A total of 111 MDS and 212 CM were included in this study and maternal age varied from 14 to 49 years old in MSD and 14 to 43 years old in CM. The inclusion criterion for MDS was give birth to a karyotipically confirmed trisomy 21 child. Preset inclusion criteria for CM were a medical history free of chronic disorders associated to *DNMT 3B rs2424913*, no previous miscarriage and give birth to healthy children without congenital defects. Both case and control subjects were inhabitants of the Rio de Janeiro. MDS and CM were asked to answer a socioeconomic questionnaire and donate a buccal epithelial cell sample. The study protocol was approved by the Ethics Committee of Federal University of Rio de Janeiro (UFRJ) and written informed consent was obtained from all subjects. A total of 111 MDS and 212 CM agreed to participate and were included in this study.

2.2. DNA extraction and DNMT 3B rs2424913 genotyping

Genomic DNA was isolated from buccal epithelial cells by standard procedure (Aidar and Line, 2007) and quantified by spectrophotometry. Genotypes were determined by PCR amplification followed by digestion with restriction endonuclease (PCR-RFLP) as described by Xiao et al. (2008) with minor modifications. Conditions used to amplify a 380 bp fragment containing the polymorphism are shown in Table 1. Reactions were carried out in a final volume of 15 μL containing 50ηg genomic DNA, 1X Green GoTaq™ Reaction Buffer (with 1.5 mM $MgCl_2$), 9 pmol each primer, and 1 U GoTaq™ Polymerase (Promega). PCR products were digested with XmaJI endonuclease (Thermo Fischer Scientific) according to supplier recommendations, and the digested products were resolved in 2% agarose gel, ethidium bromide stained and visualized under UV light. Genotypes are identified according to the band pattern as shown in Table 1.

2.3. Statistical analysis

Deviation from the Hardy–Weinberg equilibrium was determined by Chi-square and Fischer's exact test was used to compare allelic and genotypic distributions. The possible association of *DNMT 3B* rs2424913 and DS pregnancy risk was determined as odds ratios (OR) estimates with 95% confidence intervals (95% CI). The effect associated with the presence of the mutant allele was assessed in co-dominant (TT vs. CC and CT vs. CC), dominant (TT or CT vs. CC) and recessive (TT vs. CT or CC) models. Significance was achieved at $P < 0.05$. All statistical analyses were performed using GraphPad InStat Version 3.06 (GraphPad Software, San Diego, CA).

3. Results and discussion

Due to its high frequency, Down syndrome has great importance for public health worldwide. Although the genetic cause of DS has been discovered decades ago, the molecular mechanism underlying chromosome missegregation remains unknown. Both genetic and environmental factors are related to trisomy 21 and advanced maternal age is the only currently validated risk factor for DS. It has been shown that nutritional components can modulate epigenetic status of mammalian cells (Yen et al., 1994), and maternal polymorphisms in genes coding enzymes involved with folic acid cycle may act as potential risk factors for DS by centromeric hypomethylation and chromosomal nondisjunction secondary to the alteration of maternal folic acid metabolism (James et al., 1999).

DNA methylation is a chemical modification involved in regulation of gene expression that is important for several cellular processes, and DNA methyltransferase family is involved in both the maintenance of imprinted patterns and *de novo* methylation (Okano et al., 1999; Bestor, 2000). Disturbance in epigenetic mechanisms have been implicated in cancer development and several syndromes.

DNMT 3B is a nuclear protein involved in *de novo* methylation processes and it has been proposed that this protein interacts with

Table 1
PCR-RFLP conditions and genotypes observed for DNMT3B −149C > T polymorphism.

Primers (5′-3′)*	PCR conditions	Band pattern (bp)
F: 5′-TGCTGTGACAGGCAGAGCAG-3′ R: 5′-GGTAGCCGGGAACTCCACGG-3′	94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 60 °C for 50 s, and 72 °C for 1 min, and a final elongation step at 72 °C for 4 min	CC: 380 CT: 380, 207 and 173 TT: 207 and 173

* As described by Xiao et al. (2008).

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