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Association of C677T Single Nucleotide Polymorphism of MTHFR with Susceptibility to Autism Spectrum Disorders

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Abstract

In general, people with Autism Spectrum Disorders (ASD) have problems in social, emotional, and communication skills. Genome-Wide Association Studies (GWAS) have suggested a potential association of the C677T polymorphism of Methylenetetrahydrofolate Reductase (*MTHFR*) with autism spectrum disorders. The present study intended to investigate the relationship between this polymorphism of *MTHFR* and the severity of autism symptoms in two groups of children affected by autism and healthy children to elucidate its potential role as a risk factor for ASD.

study included 40 patients with autism and 40 healthy participants with matched age as control. The samples from the participants underwent ARMS-PCR for *MTHFR* genotyping. The CC genotype was reported in 50% (n=20) and 72.50% (n=29) of the children in the study and control groups, respectively, while the CT genotype was observed in 35% (n=14) of the study group and 17.50% (n=7) of the control group. Also, 15% (n=6) of the study group and 10% (n=4) of the control group had the TT genotype.

According to our results, the genotype distribution and allele prevalence were significantly different between the groups.

INTRODUCTION

Disorders (ASD) are a group of developmental disabilities that can lead to significant social, communication, and behavioral challenges for the affected individuals (1). These people show impairments with various degrees in emotional, social, and communication functions (2). Moreover, most of them use different ways from normal individuals for paying attention, learning, or reacting. The manifestations of these disorders start in early childhood and usually last throughout life (3). The diagnosis is challenging since no medical testing is available for a definite diagnosis. Only the behavioral pattern and development of the child can be used for this purpose (4). For most affected individuals, the cause is somehow unknown; however, a genetic predisposition can be found in up to 25% of the cases. According to the studies, 10-20% of the individuals with ASD have genetic impairments, such as chromosomal rearrangements and rare and de novo Copy Number Variants (CNV), while the prevalence of these genetic problems is 1%-2% in the general population or the siblings of the affected individuals (5). Moreover, 5-10% of the individuals with ASD have rare and de novo coding-sequence mutations in neuronal genes. Also, it seems that Single Nucleotide Polymorphisms (SNV) can cause susceptibility to ASD, while these effects seem to be low. An identifiable Mendelian condition or genetic syndrome have been observed in about 10% of the affected individuals (6). A recent review identified more than 103 genes involved in the disease and 44 genomic loci in individuals with ASD or autistic behavior (7). All these genes are typically involved in intellectual disability, indicating that these two neurodevelopmental disorders often share a common genetic basis. There is insufficient evidence for individual common variants that affect ASD risk (8). Three large, independent Genome-Wide Association Studies (GWAS) have reported that the C677T polymorphism of 5,10-Methylenetetrahydrofolate Reductase (MTHFR) is associated with various diseases, including vascular diseases, cancers, neurological diseases, diabetes, psoriasis, etc. The

epidemiology of this polymorphism varies in different geographies and ethnicities (9). The *MTHFR* locus is mapped on 1p36.6 (10). It codes an enzyme that is important for folate metabolism, which is an integral process for cell metabolism in the DNA, RNA, and protein methylation. The *MTHFR* mutation that causes C677T polymorphism is located at exon 4. This mutation results in the conversion of valine to alanine at codon 222, a common polymorphism that reduces the enzyme activity (11). The individuals who are homozygous for this mutation have higher levels of homocysteine, while the heterozygous individuals have mildly raised homocysteine levels than normal, non-mutated controls. The present study intended to investigate the relationship between this polymorphism of MTHFR and the severity of autism symptoms in two groups of children affected by autism and healthy children to elucidate its potential role as a risk factor for ASD.

METHODS AND MATERIALS

The present study was included 40 children affected by autism who were randomly selected and referred by the Clinical Genetics Department. Their age ranged from 5 to 10 years, the mean age was 4.6 years. Apparently and 40 healthy participants with matched ages. All the affected participants met the autism diagnostic criteria in the Diagnostic and Statistical Manual of Autism according to diagnostic and statistical manual of mental disorders.

Peripheral blood samples (5 μL) were obtained from all cases and controls in tubes containing EDTA. Total genomic DNA was extracted from leukocytes using a standard salting-out method. The DNA concentration was firstly measured by a spectrophotometer. Then, ARMS-PCR was performed using three primers for each mutation, including one forward primer and two reverse primers specific for the wild-type and mutant alleles shown in Table 1. PCR was carried out on a 20 μL volume. First denaturation step (96° C, 2 min) was followed by 10 cycles of denaturation (96° C, 15 s) and annealing/extension (65° C, 60 s). Then, there was a final 20 cycles of denaturation (96° C, 10 s), annealing (61° C, 50 s), and extension (72° C, 30 s). The PCR products were electrophoresed on 2% agarose gel and stained using ethidium bromide.

ARMS-PCR primers for sequencing the C677T variant of *MTHFR*.

RESULTS

The study included 80 patients with a mean age of 4.6 years and an age range of 5-10 years old. The genotype distribution of both groups was in a state of Hardy-Weinberg equilibrium ($p < 0.415$). The CC genotype was reported in 50% (n=20) and 72.50% (n=29) of the children in the study and control groups, respectively, while the CT genotype was observed in 35% (n=14) of the study group and 17.50% (n=7) of the control group. Also, 15% (n=6) of the study group and 10% (n=4) of the control group had the TT genotype. According to our results, the genotype distribution and allele prevalence were significantly different between the groups (Table 2).

DISCUSSION

The C677T polymorphism in exon 4 of the *MTHFR* gene causes an amino acid substitution, replacing alanine with valine at codon 222 (A222V). This alteration leads to a thermolabile enzyme with lower activity. Individuals with TT genotype have 30% of the normal enzyme activity level, whereas those with CT genotype have 60% of the normal activity. Therefore, they tend to accumulate 5,10-methylene-THF by displacing the reaction toward the DNA synthesis at the expense of pooling of methyl donors (12). Our data showed that CT, CT, and TT genotypes and T allele had a significantly higher frequency in the study group than the control subjects. There have been several meta-analyses on the correlation between the C677T polymorphism of *MTHFR* and ASD risk. Given the association of reduced *MTHFR* enzyme activity with ASD, Liu et al. selected two common polymorphisms (C677T and a1298C) to investigate the simplex and multiplex ASD families (13).

Primer No	Forward sequences	Reverse sequences	Product
MTHFR C677T	5-TGCTGTTGGAAGGTGCAAGAT-3	RW 5-GCG TGA TGA TGA AAT CGG-3	226
		RM 5-GCG TGA TGA TGA AAT CGA-3	226

*RW= reverse wild-type allele; RM=reverse mutant allele

Table 2. Frequency of C677T allele and related genotypes by group

Frequency Alleles and Genotypes	AutisticStudy group	Control group	sigP-value
CC	20 (50.00%)	29 (72.50%)	-
CT	14 (35.00%)	7 (17.50%)	0.01
TT	6 (15.00%)	4 (10.00%)	0.001
C	54 (67.5%)	65 (81.25%)	
T	26 (32.5%)	15 (18.75%)	0.000

They found that the T allele was more prevalent in children with ASD (42.9%) compared with the controls (32.3%) (P-value: 0.0004). However, the prevalence of heterozygous genotype was not significantly different between the study (47.8%) and control groups (43.2%) (14). The allele and genotype frequencies of the polymorphism in multiplex families were very similar to those in the control group. Finally, they suggested that reduced *MTHFR* activity is a risk factor for autism only in simplex families (15). Moreover, James et al. reported lower methionine, lower S-adenosyl methionine (SAM)/S-adenosyl homocysteine (SAH) ratio, lower cysteine, and lower glutathione levels in the ASD group than in the control group. They also found a significant alteration in homocysteine levels in autism (16). Paşca et al. studied this polymorphism in three groups of children diagnosed with autism (n = 15), Asperger syndrome (n = 5), and PDD-NOS (n = 19) and their age- and sex-matched controls (n = 25). They found a normal distribution of the C677T polymorphism in children with ASDs, but the T allele frequency was slightly higher in autistic patients (17). Therefore, they concluded that one-carbon metabolism alterations had a potential role in the pathophysiology of ASDs. In the present study, we found that 20 (50%), 14 (35%), and 6 (15%) children in the study group had the CC, CT, and TT genotypes, respectively. These results were compatible with the study by dos Santos et al. Thus, we could not find an association between this polymorphism and autism, which is incompatible with some of the studies.

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