



## MTHFR rs1801133 Gene Polymorphism and Autism Susceptibility

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### ARTICLE INFO

**Article type:**  
Original Article

### Article history:

Received: 9 November 2016  
 Accepted: 11 February 2016  
 Available online: 18 February 2017  
 CJNS 2017; 3 (8): 39-45

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### ABSTRACT

**Background:** It is believed that environmental and genetic factors may be responsible for autism. Methylenetetrahydrofolate reductase (MTHFR) and its gene polymorphisms have been shown to be implicated as risk factors in autism. **Objectives:** To analyze MTHFR C677T polymorphism (rs1801133) in autistic patients.

**Materials and Methods:** This study was carried out in 2014 and 2015 in northern Iran. One hundred and seventy-one male autistic patients and 198 healthy males were included in this study. Autism was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria. Each autism spectrum disorder (ASD) patient was also evaluated by the Childhood Autism Rating Scale (CARS). All participants were tested for C677T polymorphism by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Statistical analyzes were performed using MedCalc version 12.1 by the  $\chi^2$  test and logistic regression model. A value of  $p < 0.05$  was considered statistically significant.

**Results:** The mean age $\pm$ SD of case and control groups was 13.5 $\pm$ 2.7 and 15.6 $\pm$ 3.7 years. The mean $\pm$ SD of CARS score was 36.2 $\pm$ 1.7.

The genotype frequencies of CC, TC, and TT in children with autism were 50.9%, 45.6%, and 3.5%, respectively, and in control group were 54.5%, 44.0%, and 1.5%, respectively ( $p > 0.05$ ). The allele frequencies of C and T in children with autism were 73.0% and 27.0%, and in control group were 76.0% and 24.0%, respectively ( $p > 0.05$ ).

**Conclusion:** MTHFR C677T polymorphism is not associated with autism in a population in the north of Iran.

**Keywords:** Methylenetetrahydrofolate Reductase; Autism Spectrum Disorders; Polymorphism, Genetic

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### ➤ Please cite this paper as:

Delshadpour M, Mashayekhi F, Bidabadi E, Shahangian Sh, Salehi Z. MTHFR rs1801133 Gene Polymorphism and Autism Susceptibility. Caspian J Neurol Sci 2017; 3(8): 39-45.

## Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder. ASD is specified by repetitive behavior, early-onset difficulties in

social interaction, and verbal and non-verbal communication. These disorders are estimated to affect almost 1% of the population [1]. Autism affects males more

frequently than females, with a ratio of 4:1 (male: female) [2]. Autism has been propounded as a rare childhood disorder [3]. It has been shown that many genetic and environmental factors play a role in autism [4]. It has been reported that vitamin B12, B6, and folic acid play important roles in the development, maintenance, and function of the brain, and there has been intensive research on elucidating the role of vitamin B deficiency in psychiatric and neurologic diseases [5]. Several environmental factors have been proposed in the etiology of ASD including environmental conditions and immune imbalance [6,7]. ASDs have been accepted as multifactorial inheritance with 90% genetic background [8]. As a complex neurodevelopmental disorder, phenotype, and intensity of autism are extremely heterogeneous with differences from one patient to another [9]. This incongruity involves both locus and allelic heterogeneity in ASD cases [10]. It has been shown that some polymorphisms of the genes involved in the folate/homocysteine pathway are risk factor for autism [11].

MTHFR is one of the significant enzymes in the folate metabolism pathway. It transforms 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate and adjusts the intracellular flow of folate. C677T polymorphism in the MTHFR gene (A222V, rs1801133) is associated with a reduction in enzymatic activity to 35%–70% in homozygotes [12].

The MTHFR gene consists of 11 exons on chromosome 1 at 1p36.6 [13]. Two common polymorphisms for MTHFR gene at nucleotide 677 are C to T replacement (MTHFR C677T) and A1298C polymorphism at base pair 1298, leading to a glutamate to alanine substitution; this variation leads to reduced activity of this enzyme [14,15]. The MTHFR C677T polymorphism changes the amino acid from alanine to valine in the 222 codon [16]. It has been shown that DNA methylation defects are associated with ASDs, and the role of MTHFR gene in folate metabolism may help

the epigenetic mechanisms that modify complex gene expression, thus causing autism [12].

The objective of this study was to analyze MTHFR C677T polymorphism (rs1801133) in autism patients in northern Iran.

## Material and Methods

### Participants

The study group was collected between December 2014 and May 2015. One hundred and seventy-one male ASD patients (mean age $\pm$ SD, 13.5 $\pm$ 2.7 years) and 196 healthy male individuals (control group) (15.6 $\pm$ 3.7 years), were included in this study. ASD patients were diagnosed with ASD by a well-trained psychiatrist and a child and adolescent neurologist, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria, using available historical information from interviews and clinical records. Each ASD patient was also evaluated by the Childhood Autism Rating Scale (CARS), one of the most widely-used instruments to evaluate the developmental degree of autism, applying cutoff score of 30. The mean $\pm$ SD of CARS score was 36.2  $\pm$  1.7. The healthy controls were recruited from subjects who visited Iran Laboratory, Rasht, Guilan, Iran for routine health checkups. Through personal interviews, controls were investigated to determine whether they or their first-degree relatives had psychiatric disturbances or had previous psychiatric treatment. Only unaffected subjects with no psychiatric disorder or family history were included in this study as controls. Informed consent was taken from parents of the autistic patients. This project was approved by the University of Guilan Ethics Committee and was carried out in accordance with the Code

of Ethics of the World Medical Association (Declaration of Helsinki).

#### **DNA Extraction and Polymorphism analysis**

Two milliliter blood samples were obtained from patients with autism and from control group. Genomic DNA was extracted from whole-blood samples using a DNA Extractor Gpp Solution Kit (Gen Pajooan, Iran), according to the manufacturer's instructions. MTHFR C677T polymorphism (rs1801133) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

The C677T polymorphism was identified using 1 µl each of the following primers to amplify a 497-bp region from genomic DNA: 5'-TGGGGTCAGAAGCATATCAGTCA-3' (forward) and 5'-CTGGGAAGAAGCTCAGCGAAC-3' (reverse). PCR reactions were performed in 25 µl reaction volume containing 1 µl of each primer (100 pmol/µl), 2.5 µl of 10x reaction buffer (100 mM Tris-HCL pH 8.3 at 25 °C, 500 mM KCl, 15 mM MgCl<sub>2</sub>), 0.5 µl of dNTPs (2.5 mM), 0.75 µl of MgCl<sub>2</sub>, 0.3 µl of *Taq* DNA polymerase (Cinnagen, Iran), 4 µl of genomic DNA (80 ng/µl) and 14.95 µl H<sub>2</sub>O. Primer sequences are summarized in Table 1.

**Table 1.** Primer sequences and genotype differences after *Taq*I enzymatic digestion of MTHFR C677T polymorphism

Primer sequences	CC genotype	TC genotype	TT genotype
F: 5'- TGGGGTCAGAAGCATATCAGTCA-3'	497bp	497bp	
R: 5'-CTGGGAAGAAGCTCAGCGAAC-3'		271bp	271bp
		226bp	226 bp

PCR cycle conditions consisted of an initial denaturation step of 94 °C for 5 min, and 35 cycles at 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 45 s, followed by a final extension step at 72 °C for 5 min. PCR products were then digested for 2 h at 65 °C with *Taq* I restriction enzyme and checked with 2% agarose gel electrophoresis.

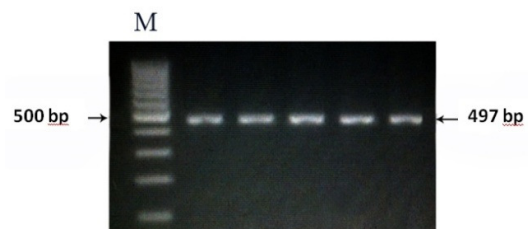
#### **Statistical analysis**

Statistical analysis was performed using MedCalc (version 12.1, Mariakerke, Belgium). Analysis compared the differences in allele and genotype frequencies between cases and controls with the  $\chi^2$  test. To estimate the association between the MTHFR C677T polymorphism (rs1801133) variant and the risk of ASD, odds ratios (ORs) with 95% confidence intervals (95% CI) were evaluated by logistic regression. A value of

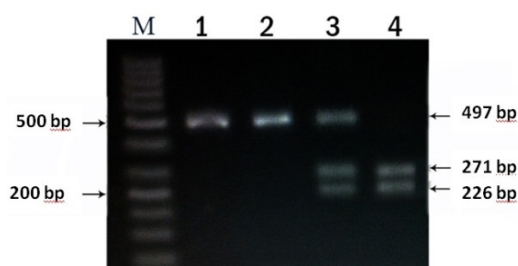
$p < 0.05$  was considered statistically significant.

## **Results**

In the present study 369 subjects, consisting of 171 patients with autism and 198 healthy subjects as control group, were evaluated. Genotyping of rs1801133 was done by PCR-RFLP method (Figs. 1 and 2).



**Figure 1.** Agarose gel electrophoresis of the MTHFR gene PCR amplification products. Fragments of 497 bp indicate the MTHFR gene



**Figure 2.** Agarose gel electrophoresis of the MTHFR gene PCR-RFLP amplification products. CC homozygote had a single band (1, 2) of 497 bp . TC heterozygote had three bands of 497, 271 and 226 bp (3) and TT homozygote had a two fragment of 271 and 226 bp (4).

The MTHFR genotype frequencies among the cases were CC=50.9%, TC=45.6% and TT=3.5%; the C and T allele frequencies

were 73.0% and 27.0%, respectively. The MTHFR genotype frequencies among the controls were CC=54.5%, TC=44.0% and TT=1.5%; the C and T allele frequencies were 76.0% and 24.0%, respectively. Statistical analysis showed that there was no significant difference between two groups ( $p=0.4$ ). All information about allele and genotype frequencies and associated ORs (95% CI) for autism cases and controls are summarized in Table 2. The results of this study indicate that MTHFR C677T polymorphism (rs1801133) was not associated with autism in this population.

**Table 2.** Genotype and allelic distribution of MTHFR C677T polymorphism in patients with autism and control groups

	Controls (n = 198)		patients (n = 171)		
	n (%)	n (%)	OR (95% CI)	P <sup>a</sup>	P <sup>b</sup>
<b>Alleles</b>					
C	303 (76.0)	252 (73.0)	1.00 (reference)	0.42	-
T	93 (24.0)	90 (27.0)	1.16 (0.83- 1.62)		0.374
<b>Genotypes</b>					
CC	108 (54.5)	87 (50.9)	1.00 (reference)	0.40	-
TC	87 (44.0)	78 (45.6)	1.11 (0.73- 1.68)		0.614
TT	3 (1.5)	6 (3.5)	2.48 (0.60- 10.21)		0.207

<sup>a</sup> allele and genotype frequencies in cases and controls were compared using  $\chi^2$  test.

<sup>b</sup> significance level for allele and genotype frequencies in cases and controls

## Discussion

The results of this research show that the relationship between MTHFR 677T gene polymorphism and autism is not significant.

ASDs are neurodevelopmental disorders that affect over 1% of children in the United States [1]. Children with ASD have impairments in large motor skills, motor planning, praxis and motor coordination [17]. Many genes have been shown to be involved in autism, and include MTHFR. MTHFR is a critical enzyme that regulates the metabolism of folate and methionine, which are important factors in DNA methylation and nucleotide synthesis [18]. The MTHFR gene has been reported to increase the risk of birth defects

such as neural tube defects and Down Syndrome [19,20]. Also, MTHFR polymorphism has been demonstrated to be associated with neurodevelopmental disorders such as schizophrenia and autism [21,22]. It has been shown that the MTHFR 677T allele might have an effect on the risk of schizophrenia in the Chinese Han population [23]. It has also been shown that MTHFR gene maybe a potential risk factor for neural tube defects (NTD) in the Chinese population [24]. Studies in Asian populations have shown that there is a significant association between the MTHFR C677T

polymorphism and the risk of breast cancer [25].

Two polymorphisms in the MTHFR gene C677T in N-terminal catalytic domain exon 4 and A1298C in C-terminal regulatory domain exon 7 were shown to be associated with reduced enzyme activity [12]. DNA methylation defects are associated with ASDs, and MTHFR and its role in folate metabolism may contribute to epigenetic mechanisms that modify complex gene expression causing autism [26]. The association of SHANK3, methionine synthase (rs1805087) and contactin-associated-like 2 (CNTNAP2) gene polymorphism in a population in northern Iran has been demonstrated [27-29].

The association between MTHFR C677T polymorphisms and autism is still controversial unclear [30]. Many studies have investigated the association between genetic polymorphisms and the risk of autism [31]. The C677T polymorphism in different subtypes of ASD has also been investigated. The results of the MTHFR gene analysis show a normal distribution of C677T polymorphism in children with ASDs, but the frequency of the 677T allele was slightly more prevalent in autistic disorder (AD) patients. This study indicates a possible role for the alterations in carbon metabolism in the pathophysiology of ASDs, and provides preliminary evidence for metabolic and genetic differences between clinical subtypes of ASDs [32]. The T allele has been shown to be more prevalent in children with ASD (42.9%) compared with controls (32.3%). Szatmari and colleagues suggested that reduced MTHFR activity is a risk factor for autism only in simplex families [33]. A similar study with the Chinese Han population also supported the notion that

MTHFR C677T polymorphism is associated with increased risk of ASD [34]. However, a study performed in south Brazil showed that MTHFR C677T alone is not a risk factor for ASD [35].

## Conclusion

The results show that this gene variation is not associated with the disease. Some limitations should be considered in interpreting our results. One limitation of this study was the small sample size of autism families. For generalizing the results in Iran it is also necessary to study the polymorphism in other populations. Further studies in larger populations including using other genetic and environmental factors, are required for clarifying our results.

## Conflict of Interest

The authors have no conflict of interest.

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