Analysis of methionine synthase (rs1805087) gene polymorphism in autism patients in Northern Iran

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Autism is characterized by impairment in reciprocal communication and speech, repetitive behaviors, and social communication. The genetic and environmental factors play roles in the pathogenesis of autism. It was recently shown that the genes involved in the folate/homocysteine pathway may be risk factors for autistic children. One of the genes that may be the risk factor for autism is Methionine synthase (MTR). MTR is responsible for the regeneration of methionine from homocysteine. The aim of this study was to analyze the association of MTR A2756G gene polymorphism (rs1805087) and the risk of autism in a population in northern Iran. The prevalence of MTR A2756G polymorphism was determined in 108 children with autism and 130 controls in northern Iran. Genotypes and allele frequencies were determined in patients and controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The prevalence of genotype frequencies of AA, AG and GG in autistic children were 57.41%, 22.22% and 20.37%, respectively, while in controls were 61.54%, 32.31% and 6.15%, respectively. There was significant difference between the MTR polymorphism distribution in control and patient groups. The prevalence of allele frequencies of A and G in autistic children were 0.69 and 0.31, respectively and in controls were 0.78 and 0.22, respectively (P=0.03). The MTR G allele conferred a 1.6-fold increased risk to autism relative to the A allele (95% CI=1.06–2.41, P=0.02). The present study suggests that the G allele of MTR A2756G polymorphism is associated with an increased risk of autism.

Key words: autism, MTR A2756G, gene polymorphism, PCR-RFLP

INTRODUCTION

Autism spectrum disorders (ASDs) are a collection of neurodevelopmental conditions that are usually of prenatal origin and can be diagnosed in early childhood, when it is severe (Gillberg 2010). The prevalence of ASDs in the United States is currently 1 in 68 children (Centers for Disease Control and Prevention 2014). ASDs affect mainly males, with an estimated 4:1 ratio between males and females, which might be partly related to hormonal involvement in the development of the disease (Lombardo et al. 2012). Although the etiology of ASDs is unknown, many theories support an interaction of environmental and genetic factors (Smalley et al. 1988). The genetic variants participated in ASDs and inherited from parents to affected individuals have been estimated to explain ~40% of ASDs risk. De novo mutations in the patients are thought to contribute to 15–20% of cases (Hallmayer et al. 2011, Devlin and Scherer 2012). Despite the un-success in identifying the candidate genes that are responsible for the most of ASDs cases, epigenetic dys-regulation of genes necessary for normal brain development and growth and cognitive function and behavior are associated with the etiology of ASDs (Liu et al. 2011). Autism is the most severe symbol of a group of neurodevelopmental disabilities known as ASDs and was first described by Leo Kanner (Baird et al. 2006, Kanner 1968). Autism is a heterogeneous neurological disorder defined by three core behavior impairments – for example, fractions in verbal and nonverbal communication, deficits in social interaction, and severe stereotyped behaviors that appear after a period of relatively normal development (American Psychiatric Association 2000). Individuals with Idiopathic autism (IA) have major deficits in temporal information processing (TIP) (Szegi et al. 2004). It has been shown that the genes participated in the folate/homocysteine pathway may be the risk factors for autistic children. Methionine synthase (MTR), methylenetetrahydrofolate reductase (MTHFR), and methionine synthase reductase (MTRR) are key enzymes participated in the folate-mediated one-carbon metabolism, and involves in DNA synthesis, methylation, and repair (Xu et al. 2004). MTR is consisted of five important regions, including homocysteine (HCY)-binding,
5-methyltetrahydrofolate (5-methylTHF) -binding, cap, cobalamin-binding and SAM-binding domains (Evans et al. 2004, Leclerc et al. 1998). MTR gene is located on chromosome 1q43. MTR, a vitamin B12-dependent enzyme involved in the folate-mediated one-carbon metabolism. It catalyzes the methylation of homocysteine to methionine with simultaneous conversion of 5-methyl-tetrahydrofolate (5-methyl-THF) to tetrahydrofolate (THF). THF is essential for nucleotide synthesis. Methionine is essential for S-adenosyl-methionine (SAM) synthesis and DNA methyltransferases (DNMTs) transfer the methyl group from SAM to the DNA (James et al. 1999). It is reported that a polymorphism in MTR A2756G (rs1805087) leads to a change from aspartic acid to glycine at codon 919 (D919G) and it was initially thought to be associated with the lower enzyme activity followed by homocysteine elevation and DNA hypomethylation (Chen et al. 1997, 1996). However, some other studies revealed a modest inverse association between GG genotype (A2756G MTR) and HCY levels, indicating an increased enzymatic activity of the variant genotype (Goode et al. 2004). The polymorphism in many genes including Forkhead Box P3, SHANK and Vitamin D receptor were shown to be associated with the susceptibility of autism (Safari et al. 2016, Mashayekhi et al. 2016, Schmidt et al. 2015). The aim of this study was to investigate the impact of MTR A2756G gene polymorphism on the risk of autism in Iran.

MATERIAlS AND METHODS

Subjects

All participants have been given their informed consent in this study. The current study included a total of 108 patients with autism disorder and 130 disease-free control subjects. Controls and patients were selected from the same population that was recruited in 2014. Data on patient characteristics at the study entry for each subject were collected from the Iran Medical diagnostic Center in Rasht, Iran. The diagnosis of ASD was made according to DSM-5 criteria for ASD. Children were investigated in terms of developing to certain genetic diseases in close relatives, neurological disorders and allergy in infancy and intestinal bacterial infections. Children with fragile x syndrome, tuberous sclerosis, a previously identified chromosomal abnormality, dysmorphic features, or any other neurological condition suspected to be associated with autism were excluded. Each subject donated 2 ml blood and drawn into EDTA-Coated tubes (Venoject, Belgium), which was used for genomic DNA extraction. This study has been approved by the local ethical committee (Protocol number: 1392-4, Date: 2013).

Genomic DNA extraction

Subjects were genotyped for the MTR 2756 SNP using genomic DNA extracted from peripheral blood leukocytes. Genomic DNA was extracted from peripheral blood samples using the Gpp solution kit (Gen Pajoohan, Iran). Extracted DNA was observed and confirmed by electrophoresis on 0.1% agarose gel containing ethidium bromide.

Analysis of genetic polymorphism

For genotyping of the MTR A2756G polymorphism (rs1805087), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used. The PCR primers were synthesized by Shanghai Genewy Biotech. China. The forward and reverse primers of MTR A2756G were 5’-CAGCTTTGCTCATCTATGGCTATC-3’ and 5’-TCTAGCACAGCCCCTAACACCT-3’, respectively. The primers were designed by Oligo7 software (version 7.54, USA). The amplification procedure was carried out in a total reaction volume of 20 μl, containing 10 μl 2X PCR Master mix (CinnaGen, Iran), 1 μl forward primer, 1 μl Reverse primer, 3 μl sterile deionized water and 5 μl Template DNA. The amplification was performed as follows: initial denaturation at 94°C for 5 min, amplification for 35 cycles at 94°C for 45 s, 58°C for 45 s and 72°C for 45 s, followed by a final elongation step at 72°C for 5 min. The resultant PCR product was visualized on a 2% agarose-ethidium bromide gel under UV illumination. To confirm the accuracy of genotyping results, randomly in 10% of subjects, genotyping was repeated to obtain concordance by minimizing genotyping errors. Then the PCR products were digested for 1 hour at 37°C with 2 unit of AvaII (Thermo Scientific Eco47I), and the amplified fragment of 395 bp was cut into fragments of 280 and 115 bp and visualized on a 2% agarose-ethidium bromide gel under UV illumination. This method is able to detect all three possible genotypes for the polymorphism including homozygous wild type (AA: 280 and 115 bp), heterozygous variant type (AG: 395, 280 and 115 bp) and homozygous variant type (GG: 395 bp).

Statistical analysis

Genotype frequencies of MTR polymorphism in patient and control groups were analyzed by χ² test. The Hardy-Weinberg equilibrium assumption was assessed by comparing the genotype frequencies with those expected on the basis of the observed frequencies. Logistic regression approach was used to obtain adjusted odds ratio (OR) and 95% confidence interval (CI) for genetic
RESULTS

This study included 108 children with autism (20 females and 88 males) and 130 controls (34 female and 96 males) in Iran. The undigested PCR product size was 395 bp for MTR A2756G (Fig. 1A). Restriction digestion for the GG genotype generated 395 bp fragment; whereas the AG genotype generated 115, 280 and 395 bp fragments. Moreover, there were two bands (280 and 115 bp) in the presence of homozygous AA (Fig. 1B). The frequency of the A2756G polymorphism of MTR was also analyzed. All information about allele and genotype frequencies and associated ORs (95% CI) for patients and controls is presented in Table I.

There was significant association in MTR 2756 gene polymorphism was seen between patients and control groups (P=0.002). Moreover, GG genotype (A2756G MTR) seems to be the risk factor in our population (P=0.004, OR 3.54, 95% CI 1.47–8.50). The A and G allele frequencies of this polymorphism were 68.52%, 31.48% in the patient group and 77.69%, 22.31% in the control group, respectively, which was statistically significant (P=0.03). Moreover G allele was shown to be associated with the increased risk of autism (P=0.02).

DISCUSSION

MTR catalyzes the remethylation of homocysteine to form methionine using the methyl group bound to cobalamin (Födinger et al. 1999). So Cbl(I) state of cobalamin is a very high reactive “supernucleophile”, and acts as an
indicator of the cellular redox environment until it is remethylated (Jensen 2005). However, the cap domain assumes a position above Cbl(I) and partially protecting it from oxidation (Bandarian et al. 2002). Cbl oxidation stops enzyme activity and diverts HCY to transulfuration pathway, which in turn, increases glutathione (GSH) synthesis until SAM-dependent reductive methylation of Cbl restores MTR activity (Jarrett et al. 1998). Folate and methionine metabolism are required for DNA synthesis and DNA methylation, also their metabolic pathways may play roles in disease susceptibility (Heijmans et al. 2003). Rare genetic defects of MTR, known as the CblG complementation group of cobalamin disorders, which cause in hyperhomocysteinemia, homocystinuria, and megaloblastic anemia without methylmalonic aciduria (Watkins and Rosenblatt 1988). It also indicates that the low activity of MTR results in the hypomethylation of DNA. The AG heterozygotes of the MTR A2756G polymorphism is likely associated with augmented levels of Hcy in Alzheimer’s disease and Parkinson’s disease patients (Dorszewska et al. 2007). This increase in Hcy is likely due to low MTR activity, caused by excessive oxidation of cobalamin (McCaddon et al. 2002) related to oxidative stress, which is observed in aging and degenerative disorders (Rozycka et al. 2013). Increased oxidative stress has been diagnosed in autistic patients (James et al. 2006). It was demonstrated that MTR status might change during aging and in neurological disorders related with oxidative stress and the level of MTR mRNA revealed a considerable age-dependent decrease. Although MTR mRNA levels were lower in autistic subjects, protein levels of MTR were similar to control. These findings suggested that the prematurely low levels of MTR mRNA in the cerebral cortex were associated with autism (Muratore et al. 2013). Mohammad and others (2009) examined the associations between five gene polymorphisms involved in folate pathway including MTR A2756G, MTHFR C677T, MTHFR A1298C, SHMT1 C1420T, MTRR A66G, and the risk of autism in a cohort of autistic children and nonautistic children from the South India. Their studies show that MTR A2756G polymorphism was not associated with an increased risk of autism (Mohammad et al. 2009). Some studies showed that polymorphisms are important in different cancers. For example, it was found that the MTHFR C677T and MTR A2756G polymorphisms are related with breast cancer susceptibility in a Chinese population in their case-control study, and that folate, vitamin B6, and vitamin B12 intakes influence these associations (Jiang-Hua et al. 2014). The data of Zhu and others (2013) supported the hypothesis that the MTHFR 677TT polymorphism is associated with an increased risk of cervical cancer in Asian females, while reverse association applies to Caucasian females. However, their meta-analysis did not support an association of the A2756G polymorphism of MTR and MTHFR A1298C polymorphism with cervical cancer risk (Zhu et al. 2013). It was shown that MTR A2756G polymorphism is a candidate gene polymorphism for cancer susceptibility (Yu et al. 2010). It was demonstrated that the MTHFR A1298C, the MTHFR C677T, the MTR A2756G, the MTRR A66G, and the thymidylate synthase (TS 2R/3R) polymorphisms have consistent roles in the increased risk of sporadic colorectal adenocarcinoma (SCA) susceptibility among the south and southeastern Brazilian population (Guimarães et al. 2011). However, large sample studies are required to confirm these associations.

Some potential limitations should be considered in this study. First, it was conducted in Iran, and may not be representative of other populations. Second, the numbers of cases and controls is rather small, which may limit the statistical power to detect differences between groups. Third, alterations in laboratory procedures such as methods of data collection and genotyping, could also clarify the inconsistent results.

CONCLUSIONS

The MTR G allele conferred a 1.6-fold increased risk to autism relative to the A allele (95% CI=1.06–2.41, \( P=0.024 \)). The present study suggests that the G allele of MTR A2756G polymorphism is associated with an increased risk of autism. Larger studies with more patients and controls are needed to confirm the results.

<table>
<thead>
<tr>
<th>alleles (A2756G)</th>
<th>controls (n= 65)</th>
<th>patients (n= 54)</th>
<th>( p^a )</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>202 (77.69)</td>
<td>148 (68.52)</td>
<td>1.00 (reference)</td>
<td>0.03*</td>
</tr>
<tr>
<td>G</td>
<td>58 (22.31)</td>
<td>68 (31.48)</td>
<td>1.60 (1.06–2.41)</td>
<td>0.02*</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>genotypes (A2756G)</th>
<th>n (%)</th>
<th>OR (95 % CI)</th>
<th>( p^a )</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>80 (61.54)</td>
<td>62 (57.41)</td>
<td>1.00 (reference)</td>
<td>0.002*</td>
</tr>
<tr>
<td>AG</td>
<td>42 (32.31)</td>
<td>24 (22.22)</td>
<td>0.73 (0.40–1.34)</td>
<td>0.32</td>
</tr>
<tr>
<td>GG</td>
<td>8 (6.15)</td>
<td>22 (20.37)</td>
<td>3.54 (1.47–8.50)</td>
<td>-</td>
</tr>
</tbody>
</table>

\* = significant at 5% level of significance (\( P<0.05 \)); ** = allele and genotype frequencies in patients and controls were compared using \( \chi^2 \) test; ^ = significance level for allele and genotype frequencies in patients and controls; n = number of subjects.
ACKNOWLEDGEMENTS

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REFERENCES


