RESULTS OF MOLECULAR GENETIC ANALYSIS OF MTR AND MTRR GENES IN CHILDREN WITH AUTISM

Majidova Yakutkhan, Farangisbonu Doniyorova, Nargiza Ergasheva
Tashkent Pediatric Medical Institute, Republic of Uzbekistan

Abstract: Statistically significant association of allele G and hetero A/G, homozygous genotype G/G polymorphism rs1801394 MTRR gene with risk of autism development was revealed. The presence of allele A and genotype A/A polymorphism of the rs1801394 MTRR gene reduces the risk of autism. Identification of allele G increased the risk of autism by 1.4 times compared to the presence of allele A (95% CI = 0.68-2.93, df=1). The presence of the allele G of MTR A2756G polymorphism correlates with an increased risk of autism.

Keywords: autism disorder, etiology, pathogenesis, genetic counselling, folate cycle disorders

Introduction. Autism is characterized by disturbance of mutual communication and speech, repeated behavior and social communication. The etiology of autism can be established in 40% of cases, the cause of the remaining 60% is unknown [1, 2]. According to many authors, the risk of autism in a child increases with the age of the father at conception [3]. Exogenous and endogenous factors of autism are distinguished. Exogenous factors include teratogenic effects (viruses, radiation, trauma, acute asphyxia, intoxication) on the fetus during intrauterine development [4]. Endogenous factors include hereditary causes and the influence of the immediate environment (for example, the nature of upbringing of the child, the degree of his deprivation).

It is known that genetic and environmental factors play a role in the pathogenesis of autism. Genetic diagnosis of autism is difficult in practice, despite the fact that the genes that cause it are already known, and methods of genetic diagnosis are widely available. The development of an autistic phenotype depends on many genes expressed in the central nervous system. It is very difficult to determine the endophenotypes of autism, because this requires a clear classification of behavior and thinking, as well as consideration of external factors, such as a model of education. So far, no qualitative biological markers have been developed that can reliably classify genetically homogenous types of autism.

Thus, the genetic nature of autism is a proven fact. However, further research is needed to combine our extensive theoretical knowledge on the genetics of autism with clinical practice and optimize diagnosis.

It has recently been shown that the genes involved in the folate/homocysteine pathway may be risk factors for children with autism. Two polymorphic variants associated with folate cycle disorders have been genetically studied: B12 dependent methioninsintaza (MTR Asp919Gly, A2756G, rs1805087) and methioninsintazareductase (MTRR Ile22Met, A66G, rs1801394) [5, 6].

The MTR gene encodes the amino acid sequence of the enzyme methionine synthase (MS) - one of the key enzymes of methionine metabolism that catalyzes methionine formation from homocysteine by its remethylation [7, 8]. Vitamin B12 (cobalamin) takes part in this reaction as a cofactor. The polymorphism of MTR gene is related to amino acid replacement of Asp919Gly (A2756G- rs1805087) (asparagine acid to glycine) in the enzyme
MS molecule. As a result of this replacement, the functional activity of the enzyme changes, resulting in an increased risk of autism. The influence of polymorphism is aggravated by increased homocysteine levels [9, 10, 11].

The MTRR gene localization on the chromosome is 5p15.31 and encodes the cytoplasmic enzyme methionine synthase reductase (MSR), which plays an important role in protein synthesis and participates in a large number of biochemical reactions associated with the transfer of methyl group. One of the functions of MCR is the inverse transformation of homocysteine into methionine [12, 13]. The genetic marker A66G of the DNA of the MTRR gene, where adenine (A) in position 66 is replaced by guanine (G), is designated as the genetic marker A66G. Consequently, the biochemical properties of the enzyme, in which the amino acid isoleucine is replaced by methionine, also change. A66G is the replacement of adenine nucleotide (A) in position 66 by guanine (G). Ile22Met is a substitution of isoleucine amino acid for methionine [14].

The aim of the study is to analyze the genetic study of two polymorphic variants associated with disorders of the folate cycle: B12-dependent methioninsintaza (MTR Asp919Gly, A2756G, rs1805087) and methioninsintaza-reductase (MTRR Ile22Met, A66G, rs1801394).

Materials and methods of investigation: molecular genetic studies were performed on the basis of the Laboratory of Medical Genetics of NIIG and PC of the Ministry of Health of the Republic of Uzbekistan. The study enrolled 50 children with clinically diagnosed autism and 58 typically developed children (control group).

Method of DNA isolation from peripheral blood. DNA extraction was carried out using the AmplimPrime Ribo-prep kit (Text Bio Ltd., Russia). The principle of action is based on cell lysis and denaturation of cell proteins with a solution for lysis containing guanidine thiocyanate, followed by nucleic acid deposition with isopropanol and further extraction into the solution. The obtained DNA was used for PCR. Concentration and purity of isolated DNA was measured on spectrophotometer NanoDrop 2000 (USA).

The standard polymerase chain reaction was performed on a thermal cycler “Applied Biosystems” 2720 (USA) using the kits “Liteh” (Moscow), according to the manufacturer's instructions. To register PCR products, electrophoresis in 3% agarose gel in the presence of ethidium bromide was used, followed by visualization in ultraviolet light “UV-T-1” (“Biocom”, Russia).

Statistical processing of the study results was performed: using online calculator openepi [8]. Correspondence of observed frequencies distribution of genotypes of the studied genes in the control group, theoretically expected by Hardy-Weinberg equilibrium, was assessed by the criterion of χ². The calculation was performed using an online calculator: http://www.oeg.org/software/hwe-mr-calc.shtml [10].

The frequency of alleles and genotypes (f) was calculated by formula:

\[ f = \frac{n}{2N} \] and \[ f = \frac{n}{N} \], where \( n \) is the occurrence of the variant (allele or genotype), \( N \) is the sample volume. The estimation of the allele frequency was calculated by the formula: \( p = \frac{(2N_1+N_2)/2N} \), \( q = \frac{(2N_3+N_2)/2N} \), where \( p \) is the allele frequency A, \( q \) is the allele frequency a, \( N \) is the total sample volume.

Research results: the obtained results of allele frequency distribution in the study and control groups are presented in tables 1 and 2.
Table 1: Distribution frequency of alleles and genotypes of A2756G polymorphism MTR genes in patient and control groups

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Allelic frequency</th>
<th>Genotypes distribution frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Autism</td>
<td>51</td>
<td>80.4</td>
</tr>
<tr>
<td>Control Group</td>
<td>54</td>
<td>85.2</td>
</tr>
</tbody>
</table>

Analysis of genotype frequencies by A2756G polymorphism of the MTR gene showed that the distribution of the analyzed genotypes in our population corresponds to the distribution of Hardy-Weinberg (HW) ($\chi^2=0.84; p=0.04$) (Table 2).

The prevalence of A2756G polymorphism of the MTR gene was determined in 50 children with autism and in 54 control children. The frequency prevalence of AA, AG, and GG genotypes in children with autism was 64.70%, 31.37%, and 3.92% respectively, while in the control group it was 72.22%, 25.93%, and 1.85% respectively.

Table 2.: Distribution of alleles and genotypes of A2756G polymorphism of MTR gene in investigated groups

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Allelic frequencies</th>
<th>HWE</th>
<th>Genotype Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>G</td>
<td>Observed by</td>
</tr>
<tr>
<td>Control Group</td>
<td>0.852</td>
<td>0.148</td>
<td>0.722</td>
</tr>
<tr>
<td>(n=54)</td>
<td></td>
<td></td>
<td>0.726</td>
</tr>
<tr>
<td>Autism (n=51)</td>
<td>0.804</td>
<td>0.196</td>
<td>0.647</td>
</tr>
</tbody>
</table>

A study of the distribution of alleles and genotypes of the polymorphic marker A2756G of the MTR gene among children diagnosed with autism and in the control group among conditioned children revealed no statistically significant differences. Heterozygote A/G genotype distribution frequencies were 31.4% (16/51) and 25.9% (14/54) in the control group respectively ($\chi^2 = 0.47; p=0.24; OR=1.35; 95% CI 0.57-3.17; df=1$). The allele frequency prevalence of A and G in children with autism was 80.39 and 19.61 respectively, while in the control group it was 85.2 and 14.8 respectively. Detecting allele G increased the risk of autism by 1.4 times compared to having allele A (95% CI = 0.68-2.93, df=1).

The MTRR gene encodes a member of the family of electronic ferredoxine-NADF (+) reductase (FNR) transfers. This protein participates in the synthesis of methionine by regenerating methionine synthase to its functional state. Since methionine synthesis requires...
the transfer of methyl group by a folate donor, the activity of encoded enzyme is important for folate metabolism and methylation of cells.

Methioninsintazareductase (MTRR) is a key enzyme that plays an important role in the metabolism of homocysteine/folate and has been shown to be involved in neurological disorders, including autism. A number of polymorphisms have been found in the MTRR gene, one of which, namely A66G, is shown to be associated with autism [5, 9].

The frequency of AA, AG and GG genotypes in children with autism was 13.7%, 66.7% and 19.6% respectively, while in the control group it was 63.0%, 24.1% and 12.9% respectively (Fig. 1).

![Fig. 1. Distribution (%) of frequencies of A66G polymorphism genotypes of MTRR gene in the examined groups](image)

The allele A, G frequencies in children with autism were 47.1%, 52.9% respectively, and in the control group 75.0%, 25.0% respectively (Figures 2, 3).

Statistically significant deviation from Hardy-Weinberg equilibrium was found for A66G polymorphism of MTRR gene in the group (between expected and observed distribution frequencies) ($\chi^2 = 6.92; p=0.006$).

![Fig. 2. Distribution (%) of allele frequencies of A66G polymorphism of the MTRR gene in the examined groups](image)
Results interpretation: 1. (+/-) genotype A/G; 2. ( - A/A genotype; 6,7,15,16 - homozygous genotype A/A (normal genotype); 3,5,8,9,11,13,14,17,18 - genotype A/G (heterozygous genotype); 4,10,12 - homozygous genotype G/G (mutant genotype).

Fig. 3. Electrophoregram of detection of A66G polymorphism of Methionine Sintazareductase gene (MTRR).

Expected frequency of distribution of genotypes by PCB in autism: A/A = 22.1; A/G = 49.8; G/G = 28.0. Observed frequency of genotype distribution by PCB in the group of patients: A/A = 13.7; A/G = 66.7; G/G = 19.6. Deviation from PCV can be explained by insufficient sample size of the analyzed group.

Statistically significant deviation from Hardy-Weinberg equilibrium was found for polymorphism of A66G of MTRR gene in the group (between expected and observed distribution frequencies) ($\chi^2 = 6.92; p=0.006$). Expected frequency of distribution of genotypes by PCB in autism: A/A = 22.1; A/G = 49.8; G/G = 28.0. Observed frequency of genotype distribution by PCB in the group of patients: A/A = 13.7; A/G = 66.7; G/G = 19.6. Deviation from PCV can be explained by insufficient sample size of the analyzed group (Table 3).

**Table 3: Distribution of alleles and genotypes of A66G polymorphism of MTRR gene in investigated groups**

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Allelic frequencies</th>
<th>HWE</th>
<th>Genotype Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>Control Group (n=54)</td>
<td>0.750</td>
<td>0.250</td>
<td>Observed by</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\chi^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Autism (n=51)</td>
<td>0.471</td>
<td>0.529</td>
<td>Observed by</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\chi^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p</td>
</tr>
</tbody>
</table>
When analyzing the frequency distribution of alleles and genotypes of the polymorphic marker A66G of the MTRR gene, statistically reliable differences were found. Carriers of allele G and genotype G/G were shown to have an increased risk of autism (allele G: OR=3.38; 95%CI: 1.88-6.05; genotype G/G: OR=6.94; 95%CI: 1.96-24.53, respectively), while carriers of allele A and genotype A/A had a decreased risk (allele G: OR=0.3; 95%CI: 0.17-0.53).

Discussion

The problem of studying the relationship between folate cycle disorders, including at the gene level, and autism disorders has been studied sufficiently [2, 5, 6, 7 8]. The most frequent causes of folate cycle abnormalities are enzyme gene mutations (MTHFR, MTR and MTRR) [3, 13, 14]. According to literature data, associations between the distribution of polymorphic loci of the key enzyme gene of methylenfolatreductase (MTHFR) and autism changes have been studied to a greater extent, in the course of which certain relationships have been established by different authors [10, 11]. The association of polymorphisms of the methioninsyntase gene (MTR) has been studied to a lesser extent [3].

The data obtained in the study of rs1805087 (A2756G) polymorphism of the MTR gene, although no statistically significant differences in the distribution of alleles and genotypes were found, all revealed certain correlations.

The analysis of polymorphism genotype distribution results revealed a weak correlation link between the heterozygous genotype A/G ($\chi^2 = 0.47; 95\%\ CI 0.57-3.17$) with a prevalence of 1.2 times among patients with autism compared to the population sample, which generally does not contradict the researchers' data [16, 17].

However, a statistically insignificant correlation was found to be associated with a 1.4-fold increase in the detection rate of allele G in children diagnosed with autism compared to a control sample (95% CI = 0.68-2.93, df=1). Overall, the data obtained do not contradict and are consistent with the researchers' data (12, 15, 16).

Conclusion:

1. Identification of the allele G MTR A2756G 1.4 times increased the risk of autism, compared to the presence of the allele A (95% CI = 0.68-2.93, df=1).
2. The frequency of polymorphism rs1801394 of the MTRR gene of the minor G allele in patients with autism was 52.9%.
3. Statistically significant association of allele G and hetero A/G, homozygous genotype G/G of rs1801394 MTRR gene polymorphism with risk of autism development was revealed. The presence of allele A and genotype A/A polymorphism of the rs1801394 MTRR gene reduces the risk of autism.

References


