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Research Article

Assessment of the Role of Polymorphism of the Folate Methionine Cycle MTR Gene in Pregnant Women with Fetal Malformations

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Abstract

The problem of fetal abnormalities in pregnant women, undeveloped pregnancy (NB) continues to be relevant and socially significant in the practice of an obstetrician-gynecologist. In the structure of reproductive losses in the early stages, the frequency of undeveloped pregnancy and/or fetal abnormalities is 27.3-36.6%.

The purpose of the study: To assess the detectability of the association of folate cycle gene polymorphism (rs1805087) of the MTR gene with the development of fetal abnormality in pregnant Uzbek population.

R**esearch Materials and Methods:** 89 pregnant women with fetal abnormalities were examined. The age of the patients ranged from 24 to 41 years. All patients underwent general clinical, instrumental, functional (ultrasound, Doppler), ELISA, and molecular genetic PCR studies. All the women surveyed had consultations with related specialists. (therapist, neurologist, infectious disease specialist, endocrinologist, etc.) The control group consisted of 30 pregnant women with a physiological course of pregnancy.

The results of the Study: The analysis of the association of polymorphism of the folate cycle gene MTR Asp919Gly (rs 1805087) A>G showed that the favorable A/A genotype in the control group of healthy pregnant women was 96.6% (29/30), whereas in the patients of the main group it was 67.4% (60/89), which was 1.4 times lower compared with the control group. (χ^2=10.24; p<0.006; OR=0.07; 95%CI 0.01 -0.55). The heterozygous variant of A/G was detected by 24.7% (22/89), and in the healthy control group -3.3% (1/30), which was 7.5 times higher than in the control group, respectively. (χ^2=10.24; p<0.006; OR=9.52; 95%CI 1.22 -74.03). The mutant G/G genotype was determined in the main group -7.8% (7/89), and it was not detected in the control group. (χ^2=10.24; p<0.006; OR=5.5; 95%CI 0.31 -100.05)

Conclusions: Thus, the G allele and the hetero-A/G and mutant homozygous genotypes of the G/G polymorphism of the MTR gene are significant markers of an increased risk of fetal abnormality in pregnant Uzbek population. (χ^2=11.7; p<0.0006; OR=14.9; 95%CI 2.00 -111.6). Allele A and functionally favorable genotype A/A are reliable protective markers for the development of pathology (χ^2=11.7; p<0.0006; OR=0.07; 95%CI 0.01 -0.50).

The problem of fetal abnormalities in pregnant women, undeveloped pregnancy continues to be relevant and socially significant in the practice of an obstetrician-gynecologist. In the structure of reproductive losses in the early stages, the frequency of undeveloped pregnancy and/or fetal abnormalities is 27.3-36.6% [1-9] The causes of fetal abnormalities are numerous and often appear in a complex. To date, numerous studies have not formed a consensus on the etiology and pathogenesis of fetal abnormalities [3-6,10-12].

Studies have established that chromosomal imbalance plays a special role in the etiology of fetal abnormality. Thus, among abortions of the first trimester of gestation, about 50% have an abnormal karyotype [4,5,13,14] It was noted that in half of the cases, chromosomal abnormalities are the reason for stopping the development of pregnancy in the first trimester, while the cause of death of the other half of the fetuses may be multifactorial in nature.

One of the factors in the development of obstetric complications, including fetal abnormalities, is a deficiency of folic acid and other B vitamins associated with dietary characteristics and defects in folate metabolism genes, which, in turn, leads to excessive accumulation of homocysteine in the blood of the mother and fetus. It is known that homocysteine has an indirect and pronounced direct embryotoxic effect due to the binding of Nitric Oxide (NO), the main vasodilation factor, thereby stimulating the adhesion of lymphocytes to the endothelium in excessive amounts, disrupting the proliferation of endotheliocytes [12]. Hyperhomocysteinemia is a risk factor for embryo implantation defects, chronic fetoplacental insufficiency, the development of severe malformations, growth retardation or fetal death (Nazarenko M.S., 2006; *Kym, et al*., 2011). Folates are necessary for the synthesis and methylation of DNA, RNA, proteins and phospholipids, neurotransmitters; they are involved in the repair of double-stranded DNA breaks, otherwise the mechanisms of cell apoptosis are triggered. Folates are necessary for the regulation of gene expression through DNA methylation.

The next significant factor in obstetric complications is a violation of the implantation process of a fertilized egg, due to a violation of the formation of a full-fledged vascular system of the chorion and placenta. Vascular endothelial growth factor A (VEGF-A, angiogenesis factor), which is the main inducer of angiogenesis, is also an important factor in placentation and trophoblast invasion. VEGF-A factor stimulates proliferation, migration, permeability and homeostasis of vascular endothelial cells, stimulates cytotrophoblast differentiation.

In this aspect, genetic studies of the main mutations in the genes responsible for folic acid metabolism are of particular interest.

The aim of the study was to assess the detectability of the association of folate cycle gene polymorphism (rs1805087) of the MTR gene with the development of fetal abnormality in pregnant Uzbek population.

Keywords: Fetal anomaly, Genetics, Folate cycle genes, (rs1805087) MTR gene

Materials and Methods of the Study

We examined 89 pregnant women with fetal malformations. The age of the patients ranged from 24 to 41 years. All patients underwent general clinical, instrumental, functional (ultrasound, Doppler), ELISA, and molecular genetic PCR studies. All the women surveyed had consultations with related specialists. (therapist, neurologist, infectious disease specialist, endocrinologist, etc.) The control group consisted of 30 pregnant women with a physiological course of pregnancy.

Molecular genetic examination of biomaterials (DNA) was performed on the basis of the clinical laboratory of Genotechnology LLC. DNA/RNA isolation from all biological blood samples was performed using the Ribot-prep kit (Interlabservice, Russia).

To identify the polymorphism of the genotype consisting of alleles of the MTR Asp919Gly (rs1805087) A>G gene, allele-specific primers from the manufacturer were selected from DNA samples. 200 DNA samples were examined for genotyping DNA samples by polymerase chain reaction (PCR). To do this, the 96-cell automated amplifier "Applied Biosystems Veriti" was optimized according to the following program: initial denaturation once at 180 seconds 94°C, 94°C-10 seconds, 64°C-10 seconds, 72°C - 20 seconds in the program, we performed these specified actions 40 times to occur polymerase chain reaction. The statistical analysis of the results was carried out using the statistical software package "OpenEpi 2009, Version 2.3". The results of the study. The average age of pregnant women in the main group was 29.2 ± 2.3 per 91 examined, whereas in the control group it was 29.4±2.5, respectively, which causes the absence of statistically significant differences in the examined groups. (P > 0.005).

By informed consent, molecular genetic studies were performed in patients for the detection of allelic variants and the association of polymorphism of genotypes of the MTR Asp919Gly (rs 1805087) A>G gene by PCR (Table 1).

Table 1: The frequency of distribution of allelic variants and polymorphism of the MTR Asp919Gly (rs1805087) A>G gene in pregnant women with fetal malformations and the control healthy group of pregnant women.

№	Group	Frequency of Alleles				Frequency of Genotype Distribution					
		A		G		A/A		A/G		G /G	
		n	%	n	%	n	%	n	%	n	%
1	The main group n=89 (178)	142	79,7	36	20,2 *	60	67,4 *	22	24,7 *	7	7,8 *
2	The control group n=30 (60)	59	98,3	1	1,6	29	96,6	1	3,3	0	0

Note*: n - the number of patients examined; *n - the number of alleles studied; * - confidence indicator in relation to the control group (P<0,05).

As follows from the table, in the control group of healthy pregnant women, the detection of the functional allele A of the MTR gene was 98.3% of cases (59/60), whereas in the main group of pregnant women with AP pregnancy, the allele A was determined in 79.7% (142/178) of cases, which was 1.2 times lower compared with the control group. (χ^2=11.7; p<0.0006; OR=0.07; 95%CI 0.01-0.50). The data obtained indicate a reliable association of the allelic frequency of the studied polymorphism with the development of

fetal abnormalities in women of the Uzbek population. An analysis of the distribution of genotypic variants of the MTR gene polymorphism showed that the favorable A/A genotype in the control group of healthy pregnant women was 96.6% (29/30), whereas in the patients of the main group it was 67.4% (60/89), which was 1.4 times lower compared with the control group. (χ^2=10.24; p<0.006; OR=0.07; 95%CI 0.01 -0.55). Whereas the heterozygous variant of A/G was detected by 24.7% (22/89), and in the healthy control group -3.3% (1/30), which was 7.5 times higher than in the control group, respectively. (χ^2=10.24; p<0.006; OR=9.52; 95%CI 1.22 -74.03) (Table 1). The mutant G/G genotype was determined in the main group -7.8% (7/89), and it was not detected in the control group. (χ^2=10.24; p<0.006; OR=5.5; 95%CI 0.31 -100.05).

Analysis of the results of molecular genetic studies indicates that the mutant G allele and the heterozygous A/G genotype and the unfavorable homozygous G/G genotype of the MTR gene are the genetic determinants of folate metabolism disorders - the risk of fetal abnormality, and its carrier is a protector of predisposition to the development of this pathology, increasing its risk in 14,9 times (OR=14.9). Thus, the results of molecular genetic studies of the MTRR gene showed a connection between the unfavorable variant allele "G" and the association of polymorphism of the A/G genotypes of the MTR gene, leading to the replacement of adenine (A) at position 119 with guanine (G) of the amino acid sequence, with the development of fetal abnormalities in pregnant women. The results of the study found that the risk of fetal abnormality in the case of the presence of a variant allele of G polymorphism in the genome was increased by 14.9 times (OR=14.9) and in the heterozygous variant -A/G - 9.52 (OR=9.5) and in the homozygous mutant variant of genotypes G/G - 5.5 (OR=5.6), respectively.

The data obtained indicates that when carrying an unfavorable variant of the A/G genotype of the MTRR gene, the risk of fetal abnormality increases by 1.7 times compared with the homozygous variant of the MTRR gene. (OR=9,52). A statistically significant association of the functionally unfavorable G allele and the heterozygous A/G genotype and the mutant homozygous A/A variant of the MTR gene of the polymorphism under study with the pathogenesis of fetal abnormality may indicate a high probability of association of this pathology with both heterozygous and homozygous non-functional variants of the MTR gene genotypes (Table 2). We conducted a study on the relationship of polymorphism of folate cycle genes in pregnant women with fetal malformations, which characterized the presence of an association of the MTR gene with a predisposition to develop fetal abnormalities, which confirms the relationship of the folate cycle with reproductive disorders.

Table 2: Indicators of differences in the frequency of occurrence of alleles and genotypes of the polymorphism of the MTR Asp919Gly (rs 1805087) A>G gene in the main and control groups of pregnant women.

Alleles and Genotypes	The Number of Alleles and Genotypes Examined		Statistical Difference
	The main group	Control group	
Allele A	142	59	χ^2=11.7; p<0.0006; OR=14.9; 95%CI 2.00 - 111.6
The G allele	36	1	
Genotype A/A	60	29	χ^2=10.24; p<0.006; OR=0.07; 95%CI 0.01 - 0.55
Genotype A/G	22	1	χ^2=10.24; p<0.006; OR=9.52; 95%CI 1.22 - 74.03
Genotype G/G	7	0	χ^2=10.24; p<0.006; OR=5.5; 95%CI 0.31 - 100.05

In this regard, an important step in the study of polymorphic genes potentially associated with the development and pathogenesis of diseases is the analysis of the expected and observed frequency of the genotypes of the studied polymorphisms and the correspondence of the frequency distribution to the Hardy-Weinberg equilibrium (HW) (Table 3). As follows from the table, the indicators of the frequency of genotype distribution by RHB of the MTR Asp919Gly polymorphism (rs 1805087) A>G in the main group of pregnant women with fetal abnormalities showed that the observed frequency of favorable A/A genotypes was 67.4%, heterozygous A/G genotypes - 24.7 and mutant homozygous genotypes -G/G -7.8% cases, respectively, while the expected frequency of favorable A/A genotypes decreased by 1.3 times and amounted to 53.01%, and heterozygous A/G genotypes amounted to -39.6 increased, which was 1.6 times higher, and the observed and expected homozygous mutant G/G genotypes amounted to 7.8 and 7.4% of cases, respectively.

Table 3: Expected and observed frequency of genotype distribution by RHW of the MTR Asp919Gly(rs1805087) A>G gene polymorphism in the main group of pregnant women with multiple pregnancies.

Genotypes	Frequency of Genotypes		χ^2	P
	Observed	Expected		
A/A	67,42	53,01	0,636	
A/G	24,72	39,59	0,323	
G/G	7,87	7,4	0,041	
Bcero	100,00	100,00	1,85	0,17

The data obtained indicate that the frequency of expectation of the phenomenon of fetal abnormality in pregnant women with heterozygous variants of the A/G MTR gene increases 1.6 times, respectively. The results obtained are statistically reliable. (P <0.05). The results of the study were analyzed in a group of control patients with a physiological course of pregnancy (Table 4). Whereas in the control group, the observed frequency of functional A/A genotypes was detected in 96.7%, and the expected one was 60.6%, the heterozygous A/G variant of the MTR gene was observed in 3.3% of cases, and the expected one in 34.5% of cases, which was 10.5 times higher compared to indicators with observed genotypes, (P <0.05). While the unfavorable mutant genotype G/G was not found in the observed frequency, however, it was shown in the expected 4.9 cases. The data obtained are important in predicting the risk of obstetric complications, in particular fetal abnormalities (Table 4). A comparative analysis of the expected and observed frequencies of the genotypes of this polymorphism revealed a statistically significant deviation of the indicators (P <0.05) in all the studied groups. This fact indicates that the observed proportion of genotypes in the studied samples corresponds to the Hardy-Weinberg equilibrium.

Table 4: Expected and observed frequency of genotype distribution by RHB of the polymorphism of the MTR Asp919Gly (rs1805087) A>G gene in the control group of patients with the physiological course of pregnancy.

Genotypes	Frequency of Genotypes		χ^2	P
	Observed	Expected		
A/A	96,7	60,6	0,967	
A/G	3,3	34,5	0,033	
G/G	0	4,9	0,0	1
Total	100,00	100,00	0,0	

Thus, the G allele and the hetero-A/G and mutant homozygous genotypes of the G/G polymorphism of the MTR gene are significant markers of an increased risk of fetal abnormality in pregnant Uzbek population. (χ^2=11.7; p<0.0006; OR=14.9; 95%CI 2.00 -111.6). Allele A and functionally favorable genotype A/A are reliable protective markers for the development of pathology (χ^2=11.7; p<0.0006; OR=0.07; 95%CI 0.01 -0.50). The analysis of the obtained results indicates that pregnant women with the carrier of the allele variant G and the heterozygous A/G genotype of the MTR gene have a risk of fetal anomaly formation. Thus, the results of our own studies published in the literature and conducted by us indicate that the folate cycle gene MTR Asp919Gly(rs1805087) A>G plays an important role in the development of fetal abnormalities in pregnant Uzbek population.

The analysis of the nature of the multifactorial pathology of the pedigrees of the examined pregnant patients with fetal malformation revealed the predominance of cardiovascular pathology (myocardial infarction, stroke, varicose veins), the presence of oncological pathology and endocrine pathologies (polycystic disease, diabetes mellitus, endemic goiter). The data obtained correspond to the literature data of foreign authors that the hereditary burden of cardiovascular and oncological diseases of the examined women carriers of polymorphic variants of folate cycle genes corresponds to world data on possible complications associated with SNP of these genes, etc. 2013]. Studies indicate that in the pedigrees of women with a threat of developing reproductive pathology with thyroid disease with a deficiency in an important biogenic element - iodine, the deficiency of which is of a regional nature [15,16].

Thus, the results of our own studies published in the literature and conducted by us indicate that the folate cycle gene MTR Asp919Gly (rs 1805087) A>G plays an important role in the development of reproductive pathologies, including fetal abnormalities.

Conclusion

a) The analysis of the association of polymorphism of the folate cycle gene MTR Asp919Gly(rs 1805087)A>G showed that the favorable A/A genotype in the control group of healthy pregnant women was 96.6% (29/30), whereas in the patients of the main group - 67.4% (60/89), which was 1.4 times lower compared with the control group. (χ^2=10.24; p<0.006; OR=0.07; 95%CI 0.01 -0.55).

b) The heterozygous variant of A/G was detected by 24.7% (22/89), and in the healthy control group -3.3% (1/30), which was 7.5 times higher than the indicators of the control group, respectively. (χ^2=10.24; p<0.006; OR=9.52; 95%CI 1.22 -74.03). The mutant G/G genotype was determined in the main group -7.8% (7/89), and it was not detected in the control group. (χ^2=10.24; p<0.006; OR=5.5; 95%CI 0.31 -100.05)

c) The data obtained indicate that the carriage of the unfavorable allele G (Asp919Gly(rs 1805087)A>G of the MTR gene may be a diagnostic marker of predisposition to the formation of fetal abnormality, increasing its risk by 9.5 times (OR=9.52).

d) Thus, the G allele and the hetero- A/G and mutant homozygous genotypes of the G/G polymorphism of the MTR gene are significant markers of an increased risk of fetal abnormality in pregnant Uzbek population. (χ^2=11.7; p<0.0006; OR=14.9; 95%CI 2.00 -111.6). Allele A and functionally favorable genotype A/A are reliable protective markers for the development of pathology (χ^2=11.7; p<0.0006; OR=0.07; 95%CI 0.01 -0.50).

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Conflict of Interest

None.

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