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INFLUENCE OF FOLATE CYCLE MTHFR GENE POLYMORPHISM ON THE PROCESS OF FETUS DEVELOPMENT IN RESIDENTS OF THE REPUBLIC OF UZBEKISTAN

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Abstract:

According to recent studies, throughout the world congenital malformations occur in 5% of newborns, and it is estimated that 303,000 children dying from malformations during the first 4 weeks of life (WHO, 2016). Today, women's reproductive health of women is one of the most important medical and social problems. For the birth of healthy offspring, it is worth taking care of a medical genetic examination even before the conception of a child and before his birth. The most common cause of miscarriage in the early stages are genetic abnormalities (up to 70% of non-developing pregnancies before 6 weeks, 50% before 10 weeks and 5% after 12 weeks of pregnancy), all three causes (hormonal disorders, infections (30%) may be present simultaneously (11).

Keywords:

According to recent studies, throughout the world congenital malformations occur in 5% of newborns, and it is estimated that 303,000 children dying from malformations during the first 4 weeks of life (WHO, 2016). Today, women's reproductive health of women is one of the most important medical and social problems. For the birth of healthy offspring, it is worth taking care of a medical genetic examination even before the conception of a child and before his birth. The most common cause of miscarriage in the early stages are genetic abnormalities (up to 70% of non-developing pregnancies before 6 weeks, 50% before 10 weeks and 5% after 12 weeks of pregnancy), all three causes (hormonal disorders, infections (30%) may be present simultaneously (11).

The miscarriage gene network includes polymorphic variants of more than 40 genes that can be used as molecular genetic predictors of the development of reproductive losses in the first trimester of pregnancy. In world populations, SNPs of 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) genes are the most studied, causing pathology of pregnancy, genetic predisposition to chromosomal rearrangements and abnormalities in fetal development [1,3,4, 6].

In recent studies, the role of polymorphisms of other genes of the folate cycle (FC) has been noted: A2756G (A→G) rs1805087, an allelic variant of the B12-dependent methionine synthase (MTR) gene and A66G (66 A→G) rs1801394SNP of the methionine synthase reductase gene (MTRR). According to few data, the substitution of adenine for guanine (A → G) at position 2756 of the MTR gene and, accordingly, 919 Asp → Gly in the amino acid sequence of the MTR protein may be associated with a genetic predisposition to miscarriage and obstetric pathology. They are: neural tube development disorder (spina bifida), chromosomal abnormalities (Down syndrome), isolated cleft lip and palate in the fetus. At A66G MTRR (66 A→G) SNP, the substitution of the amino acid isoleucine for methionine (Ile22Met) changes the biochemical properties of the enzyme, which ultimately leads to similar pathologies [1,3].

Polymorphisms of the MTHFR C677T and MTRR A66G genes have been studied in RPL, Down syndrome, a large number of works are devoted to spina bifida, the role of two other polymorphisms (MTHFR A1298C, MTR A2756G) has been studied to a lesser extent and their involvement in reproductive disorders is not yet completely clear [9,10].

Nair R.R. et al. in 2013 (India), based on a meta-analysis and the results of their own studies using abortive material, showed that A/C and C/C genotypes at the 1298 locus of the MTHFR gene significantly increase the risk of spontaneous abortion [6].

The role of homocysteine in the occurrence of congenital malformations of the fetus has been actively discussed in the press in the past few decades. Increasing the concentration of homocysteine in itself can lead to damage to the nervous tissue. For a number of years, worldwide, the relationship between low dietary folate intake, hyperhomocysteinemia in pregnant women and the risk of obstetric and perinatal complications has been actively studied (2,4,8). Among all known genetic causes of hyperhomocysteinemia, the most common mutations are in the MTHFR gene, which encodes the amino acid sequence of the enzyme methylenetetrahydrofolate reductase, which plays a key

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role in folic acid metabolism. At the moment, two genetic markers are known that lead to a decrease in enzyme activity and, as a result, to a change in the level of homocysteine: MTHFR (C677T) and MTHFR (A1298C) (5,7).

The aim of this stage of our research was to study the distribution of the Glu429Ala allelic polymorphism of the methylenetetrahydrofolate reductase (MTHFR) enzyme gene in women with an undeveloped pregnancy and a history of congenital malformations of the fetus.

This section presents the results of the analysis of the distribution of alleles and genotypes of the MTHFR gene in the groups of patients and controls. The study on a voluntary basis included 155 women of the Uzbek population with congenital malformations of the fetus and a history of frozen pregnancy (main group), observed in the obstetric-gynecological complex TMA. The age of the examined patients ranged from 20 to 40 years (median 29.0 years). As a control group, the DNA of 75 conditionally healthy patients with no history of frozen pregnancy and congenital anomalies of the fetus, selected from the DNA bank of the Department of Molecular Medicine and Cell Technology of the Research Institute of Growth and PC of the Ministry of Health of the Republic of Uzbekistan, was studied. All of them had no family ties and corresponded to the study group by age.

80 women with congenital fetal malformations and a history of frozen pregnancy took part in molecular genetic studies on a voluntary basis (main group). All pregnant women underwent general clinical, laboratory and functional studies according to the standard for diagnosis and therapy (2019) (11).

The paper analyzes the linkage disequilibrium of the studied polymorphism, as well as the prevalence of the polymorphic variant in women with non-developing pregnancy and congenital malformations of the fetus in history and healthy women (Table 1.).

When studying the Glu429Ala polymorphism in the MTHFR gene in the main group of patients and controls, there was no significant decrease in the frequency of the wild A allele compared to healthy women (71.88% versus 79.33%, respectively) and an increase in the frequency of the functionally unfavorable C allele compared to the healthy group. women (28.13% versus 20.67%, respectively).

However, as a result of the study, statistically significant differences were established in the distribution of genotypes and allele frequencies between the group of women with fetal CM and women in the control group for the allelic variant of the Glu429Ala polymorphism in the MTHFR gene (62.5% - A, compared with 79.33 % in control). And an increase in the frequency of the functionally unfavorable allele C compared with the control group (37.5%, 20.67%, respectively).

When studying the Glu429Ala polymorphism in the MTHFR gene in the group of patients with non-developing pregnancy and control, as well as in the main group, there was no significant decrease in the frequency of the wild A allele compared to healthy women (81.25% vs. 79.33%, respectively). The frequency of functionally unfavorable allele C compared with a group of healthy women (28.13% versus 20.67%, respectively).

Table 1.

The distribution frequency of alleles and genotypes of the Glu429Ala polymorphism in the MTHFR gene in the groups of patients and controls

№	Group	Frequency of alleles				Frequency distribution of genotypes					
		A		C		A /A		A /C		C /C	
		n	%	n	%	n	%	n	%	n	%
1	Congenital fetal malformations (n = 40)	50	62,5	30	37,5	14	35,0	22	55,0	4	10,0
2	Non-developing pregnancy (n = 40)	65	81,25	15	18,75	27	67,5	11	27,5	2	5,0
3	Control group (n = 75)	119	79,33	31	20,67	47	62,67	25	33,33	3	4,0

The most common genotype for the wild A alleles in the control group, in women with non-developing pregnancy, is the homozygous A/A genotype, respectively 62.67% and 67.5%. Homozygous mutant C/C genotype in the group with non-developing pregnancy and control groups met with a relatively low frequency of 10% in the group with congenital malformations, 5.0% in the group with non-developing pregnancy and 4.0% in the control group. However, in the group of women with fetal CM, the mutant

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homozygous genotype was 2.5 times more common (10.0% compared to 4.0%) than in the control group and 1.25 times in the group of women with non-developing pregnancy. The highest frequency of the heterozygous A/C genotype was found in the group of women with fetal CM (55.0%). The prevalence of the normal A/A genotype in the group of healthy women confirms the protective function of this genotype.

Table 2.

Expected and observed frequencies of distribution of locus genotypes by RHV (Glu429Ala polymorphism in the MTHFR gene)

Main group					
Alleles	Frequency of alleles				
A	0,72				
C	0,28				
Genotypes	Frequency of genotypes		χ2	p	df
	Observable	Expected			
A /A	0,51	0,52	0		
A /C	0,41	0,4	0,01		
C /C	0,08	0,08	0,02		
Total	1	1	0,03	0,821	1

Control group					
Alleles	Frequency of alleles				
A	0,79				
C	0,21				
Genotypes	Frequency of genotypes		χ2	p	df
	Observable	Expected			
A /A	0,63	0,63	0		
A /C	0,33	0,33	0,01		
C /C	0,04	0,04	0,01		
Total	1	1	0,02	0,846	1

Groups	Ho	He	D*
Main group	0,41	0,4	0,02
Control group	0,33	0,33	0,02

Note: D = (Ho - He)/He

Table 2 presents the results of the analysis of the frequency of alleles and genotypes of polymorphism in women of the main group and healthy women of Uzbek nationality. It was found that the distribution of frequencies of genotypes and alleles of the Glu429Ala polymorphism in the MTHFR gene in both groups corresponded to the expected Hardy-Weinberg equilibrium law ($p>0.05$).

The distribution of genotypes in the analyzed group of patients corresponded to the Hardy-Weiberg equilibrium (HWE), which indicates the representativeness of the sample of the main group and the correctness of the determination of the Glu429Ala polymorphism. The identified slight deviation from RCM is possibly due to a decrease in heterozygosity, i.e. a lack of heterozygotes in the analyzed group due to an increase in the number of representatives with a wild variant of the genotype (selective effect).

This may indicate that heterozygous and, especially, homozygous genotypes have a fairly pronounced statistically significant associative relationship with the development of fetal CM. These data may indicate a good independent effect of the Glu429Ala

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polymorphism in the MTHFR gene on the risk of fetal CM in the Uzbek population.

In the control group, the actual frequency of MTHFR gene genotypes did not change statistically significantly compared to the theoretical distribution of genotype frequencies (P>0.05). These data indicate the homogeneity of the studied population sample.

Table 3

Prognostic efficiency of the studied genetic markers (Glu429Ala polymorphism in the MTHFR gene)

Factor	Groups	SE	SP	AUC	OR	95%CI	p
A	Congenital fetal malformations // Control group	0,6	0,2	0,42	0,4	0,24 - 0,78	0,49
	Non-developing pregnancy // Control group	0,8	0,2	0,51	1,1	0,57 - 2,26	0,33
	Congenital fetal malformations // Non-developing pregnancy	0,6	0,2	0,41	0,4	0,19 - 0,78	0,67

Factors	Groups	SE	SP	AUC	OR	95%CI	p
C	Congenital fetal malformations // Control group	0,8	0,4	0,59	2,3	1,27 - 4,16	0,51
	Non-developing pregnancy // Control group	0,8	0,2	0,49	0,9	0,46 - 1,72	0,67
	Congenital fetal malformations // Non-developing pregnancy	0,8	0,4	0,6	2,6	1,28 - 5,29	0,33

Factors	Groups	SE	SP	AUC	OR	95%CI	p
A /A	Congenital fetal malformations // Control group	0,4	0,4	0,36	0,3	0,15 - 0,7	0,48
	Non-developing pregnancy // Control group	0,7	0,4	0,53	1,2	0,55 - 2,81	0,32
	Congenital fetal malformations // Non-developing pregnancy	0,4	0,3	0,34	0,3	0,1 - 0,64	0,67

Factors	Groups	SE	SP	AUC	OR	95%CI	p
A /C	Main group // Control group	0,4	0,7	0,54	1,4	0,73 - 2,68	0,48

Note: SE - sensitivity; SP, specificity; AUC - predictive efficiency, *p - Fisher's exact test.

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When studying the prognostic efficiency of the Glu429Ala polymorphism in the MTHFR gene, we found a significant increase in the AUC index (P<0.05). The predictive efficiency of the Glu429Ala polymorphism in the main group of women, according to the AUC values, was 0.59 (P>0.05), respectively (Table 3.).

Thus, for the main group, this indicates a low predictive value as an independent marker. However, for women from the group with fetal CM, the prognostic value of Glu429Ala polymorphism in the MTHFR gene as an independent marker is high.

Table 4
Relative chance (OR polymorphism Glu429Ala in the MTHFR gene)

Factors	Congenital fetal malformations	Non-developing pregnancy	Total	Chances
C (existed) =	65	50	115	1,3
C (not existed) =	15	30	45	0,5
Total	80	80	160	

Factors	Congenital fetal malformations	Контрольная группа	Total	Chances
C (existed) =	119	50	169	2,38
C (not existed) =	31	30	61	1,03
Total	150	80	230	

Factors	Non-developing pregnancy	Control group	Total	Chances
C (existed) =	119	65	184	1,83
C (not existed) =	31	15	46	2,07
Total	150	80	230	

Factors	Congenital fetal malformations	Non-developing pregnancy	Total	Chances
A /C (existed) =	22	11	33	2
A /C (not existed) =	18	29	47	0,62
Total	40	40	80	

Factors	Congenital fetal malformations	Non-developing pregnancy	Total	Chances
C /C (existed) =	4	2	6	2
C /C (not existed) =	36	38	74	0,95
Total	40	40	80	

Factors	Congenital fetal malformations	Контрольная группа	Total	Chances
C /C (existed) =	4	3	7	1,33
C /C (not existed) =	36	72	108	0,5
Total	40	75	115	

The risk structure suggests a dominant effect, and the risks of developing pathology compared with the control group were increased when cases were divided according to the options (CMD and non-developing

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pregnancy), a significant difference in the frequency of genotypes was observed only between CM of the fetus and control (OR 1.33). In this group of patients, the risk was increased for both heterozygotes and homozygotes with the mutant allele. However, the risk of heterozygotes was insignificant (0.88) (Table 4.).

We found a significant association between the Glu429Ala polymorphism in the MTHFR gene and an increased risk of fetal CM.

Thus, the folate cycle is important in the etiology of the development of pathologies in women with non-developing pregnancy and a history of congenital malformations of the fetus. The results of the study showed that a significant association was found between the Glu429Ala polymorphism in the MTHFR gene and an increased risk of fetal CM. The prevalence of the normal A/A genotype in the group of healthy women confirms the protective function of this genotype. This may indicate that heterozygous and, especially, homozygous genotypes have a fairly pronounced statistically significant associative relationship with fetal CM. A significant difference in the frequency of genotypes was observed only between the group of women with fetal CM and control (OR 1.33). In this subgroup of patients, the risk of developing pathology was increased for both heterozygotes and homozygotes with the mutant allele. These data may indicate a good independent effect of the Glu429Ala polymorphism in the MTHFR gene on the risk of fetal CM in the Uzbek population.

Thus, it is advisable to include the determination of MTHFR gene mutations and the level of HC in the preconception preparation of all women with an undeveloped pregnancy and a history of congenital fetal malformations.

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