Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 10 March 2026
Revised 12 April 2026
Accepted 14 May 2026

Natural Resources for Human Health 2026, Vol. 6 (1s): 426–431

KEYWORDS:
fetal congenital anomalies, genetic polymorphism, MTR, MTRR, CYP1A2, folate metabolism, homocysteine, genetic risk.

The Role of Genotype Combinations of Various Genes in Predicting the Risk of Fetal Congenital Anomalies

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ABSTRACT:

Significance. Congenital fetal anomalies are one of the pressing issues in modern medicine, as they account for a significant proportion of perinatal morbidity and mortality. The development of congenital malformations is determined by a complex interaction of genetic and environmental factors. Genes involved in the metabolism of folates, homocysteine, and the biotransformation of xenobiotics play a particularly important role in the pathogenesis of embryonic development disorders.

Objective of the study. To investigate the role of polymorphic variants of the MTR A>G, MTRR A>G, and CYP1A2 C>T genes, as well as to assess their contribution to the risk of fetal congenital anomalies.

Materials and Methods. A study was conducted involving 95 women, of whom 55 constituted the main group (pregnant women with fetal developmental abnormalities) and 40 constituted the control group. We analyzed the distribution of genotypes and alleles of the polymorphic variants of the MTR, MTRR, and CYP1A2 genes, calculating the odds ratio (OR), 95% confidence interval (95% CI), χ^2 statistics, and various inheritance models.

Results. A statistically significant association was established between polymorphic variants of the studied genes and the risk of fetal congenital anomalies. For the MTR A>G gene, the presence of the G allele was associated with an increased risk of pathology (OR = 17.7; 95% CI: 5.28–59.8; p = 0.0001). For the MTRR A>G polymorphism, the strongest association was identified: the G allele increased the risk of fetal abnormalities (OR = 80.17; 95% CI: 18.73–343.03; p = 0.0001). The CYP1A2 C>T polymorphism was also characterized by a significant association with fetal pathology (OR = 25.67; 95% CI: 7.63–86.46; p < 0.0001). Analysis of inheritance models revealed a statistically significant association under additive, multiplicative, and dominant models.

Conclusion. The results indicate that polymorphic variants of the MTR, MTRR, and CYP1A2 genes can be considered potential genetic markers of an increased risk of fetal congenital anomalies. A comprehensive assessment of genotype combinations of various genes holds promise for the development of models for individualized genetic risk prediction of pregnancy complications.

INTRODUCTION

Congenital anomalies and fetal malformations are pathological conditions arising from disturbances in embryogenesis at various stages of intrauterine development—from the moment of fertilization to the birth of the child. These disorders can

be either hereditary or multifactorial in nature, involving the interaction of genetic factors and adverse environmental influences [1,2].

Numerous studies have shown that congenital anomalies arise from changes at various levels of genetic organization: chromosomal, gene, and genomic. At the same time, most human developmental defects have a complex etiology, in which the combined effect of genetic predisposition and environmental factors plays a significant role [3,4].

Among exogenous risk factors, ionizing radiation (X-rays, radioactive isotopes), infectious agents, drugs with teratogenic potential, chemical compounds, as well as alcohol and nicotine use during pregnancy are of particular significance [5].

Rubella contracted by a woman during the first trimester of pregnancy plays a particularly significant role among infectious factors. Even in cases of subclinical infection, embryopathies may develop, including congenital cataracts, microphthalmia, congenital heart defects, and hearing impairment. According to the literature, the risk of developing congenital rubella syndrome following infection in early pregnancy reaches 20–25% [6].

Medications with pronounced teratogenic effects can significantly contribute to the development of congenital malformations. A classic example is thalidomide embryopathy, characterized by impaired limb formation resulting from taking the drug in the early stages of pregnancy [7].

Alcohol and nicotine are also major risk factors for embryonic development disorders. Alcohol consumption during pregnancy can lead to the development of a spectrum of fetal alcohol spectrum disorders, including intrauterine growth restriction, central nervous system damage, and various congenital anomalies [8].

Given the multifactorial nature of congenital anomalies, the development of methods for early prenatal diagnosis and the identification of individual genetic risk factors are of particular importance. Recent studies show that genetic variants of enzymes involved in the metabolism of folates, vitamin B12, homocysteine, and xenobiotics can influence embryonic development [9–11].

One of the key metabolic pathways associated with normal fetal development is the folate cycle. Folate is essential for the synthesis of nucleic acids, DNA methylation processes, and normal cell division during embryogenesis. Impaired activity of the enzymes in this cycle can lead to disrupted homocysteine metabolism, the development of hyperhomocysteinemia, and endothelial dysfunction [12].

Particular attention is paid to polymorphisms in the MTHFR, MTR, and MTRR genes, which are involved in folate metabolism and homocysteine remethylation. The MTR gene encodes methionine synthase, which converts homocysteine to methionine, whereas MTRR is involved in the regeneration of the active form of methionine synthase. Dysfunction of these enzymes may be accompanied by elevated homocysteine levels and impaired methylation processes, which is considered one of the possible mechanisms underlying neural tube defects and other congenital anomalies [13,14].

In recent years, the study of not only individual genetic variants but also combinations of genotypes from different genes has gained significant importance, as most congenital anomalies are polygenic in nature. The interaction of several polymorphisms involved in a single metabolic pathway can significantly alter an individual's susceptibility to adverse environmental factors and increase the risk of pregnancy complications [15].

In particular, the combination of variants in the MTR, MTRR, and CYP1A2 genes may have prognostic significance, as these genes are involved in various but interrelated processes: maintenance of methylation, homocysteine metabolism, and detoxification of xenobiotics. The CYP1A2 gene, which belongs to the cytochrome P450 family, is involved in the metabolism of drugs and potentially toxic compounds, and its genetic variants may determine individual differences in the body's response to environmental exposure [16].

Thus, studying the combinations of genotypes of various genes is a promising approach for predicting the risk of fetal congenital anomalies. A comprehensive assessment of genetic factors, including polymorphisms in folate metabolism genes (MTR, MTRR, MTHFR) and xenobiotic metabolism genes (CYP1A2), may contribute to the development of an individualized genetic risk profile and the improvement of approaches to the prevention and early diagnosis of pregnancy complications.

The aim of our study was to assess the association between genotype polymorphisms of the MTR and MTRR genes and the CYP1A2 gene in women from the Uzbek population with fetal developmental anomalies.

MATERIALS AND METHODS.

We examined 95 pregnant women with fetal anomalies. The patients' ages ranged from 20 to 43 years. Among them, 55 (57.8%) were pregnant women with fetal developmental anomalies, who constituted the main group. The control group consisted of 40 pregnant women with a normal course of pregnancy without fetal developmental anomalies.

All patients underwent general clinical, instrumental, functional (ultrasound screening), and genetic PCR (REAL TIME) testing. All women examined received consultations from specialists in related fields (geneticist, internist, neurologist, infectious disease specialist, dermatologist, endocrinologist, etc.).

Molecular genetic testing of biomaterials (DNA) was performed at the laboratory of the Republican Scientific and Practical Medical Center for Diagnosis and Treatment of Reproductive and Endocrine Diseases (RSNPMTSMZMiR) of the Ministry of Health of the Republic of Uzbekistan. DNA/RNA extraction from all biological blood samples was performed using the "Ribo-Prep" kit (Interlabservice, Russia).

To detect genotype polymorphisms consisting of alleles of the **MTR, MTRR, and CYP1A2** genes, allele-specific primers from the manufacturer were selected from the DNA samples. To genotype the DNA samples for using the polymerase chain reaction (PCR) method, 200 DNA samples were analyzed. For this purpose, the 96-well automated amplifier "Applied Biosystems Veriti" was optimized according to the following program: initial denaturation once at 94°C for 180 seconds, 94°C for 10 seconds, 64°C for 10 seconds, and 72°C for 20 seconds. We repeated this cycle 40 times to allow the polymerase chain reaction to occur. Statistical analysis of the results was performed using the "OpenEpi 2009, Version 2.3" statistical software package.

STUDY RESULTS:

The results of the molecular genetic studies are presented in Table 1.

Table 1 Frequency distribution of genotypes for the studied polymorphisms in patients with fetal anomalies and the control group.

Polymorphism, genotypes	Fetal anomaly, n=55		Control, n=40		OR (95% CI)	p
MTR, A>G						
A/A	21	38.2	37	92.5	13.5 (3.6–50.8)	0.0001
A/G	23	41.8	3	7.5		
G/G	11	20	-	-	16.3 (5.3–49.9)	0.0001
Alleles A	65	59.1	77	96.3		
G	45	40.9	3	3.7	17.7 (5.28–59.8)	0.0001
MTRR, A > G						
A/A	10	18.2	39	97.5	43.50 (5.60–337.84)	0.0001
A/G	16	29.1	-	-		
G/G	29	52.7	1	2.5	175.50 (19.28–1595.6)	0.0001
Alleles A	36	32.7	78	97.5	80.17 (18.73–343.03)	0.0001
G	74	67.3	2	1.8		
CYP1A2						

C/C	15	27.3	37	92.5	25.67 (I8.91–122.84)	0.0001
S/T	25	45.5	3	7.5		
T/T	15	27.3	0	0	32.8 (8.82–122.97)	0.0001
Alleles C	55	50	77	96.3	25.67 (7.63–86.46)	0.0001
T	55	50	3	3.7		

In patients with fetal anomalies, genotyping analysis of the association between the MTR gene polymorphism, A>G, revealed that in the control group, the functional A/A genotype was identified in 37 (92.5%) (37/40) cases, whereas in the study group, it was found in 38.2% (21/55) of cases—2.4 times less than in the control group. Meanwhile, the heterozygous A/G variant of the MTR gene (A>G) was detected in 7.5% of cases (3/40) in the control group, and in 41.8% (23/55) in the study group—a 5.5-fold increase compared to healthy patients (χ^2= 29.1; p < 0.0001; OR = 16.3; 95% CI 5.3–49.9).

The homozygous mutant G/G variant of the MTR gene (A>G) was detected in 11 (20%) of 55 patients with fetal anomalies, whereas this genotype was not detected in the group of healthy patients (χ^2= 29.1; p < 0.0001; OR = 17.7; 95% CI 5.28–59.8).

The results indicate that the heterozygous A/G and the mutant homozygous G/G variants of the MTR gene, A>G, are genetic determinants involved in the development of reproductive dysfunction, increasing the likelihood of fetal anomalies by a factor of 16.3 (OR = 16.3–17.7).

Studies of the association between MTRR gene genotypes (A>G) revealed that 97.5% (39/40) of the control group were carriers of the functional A/A genotype, and in the study group—in 18.2% (10/55), whereas heterozygous A/G genotypes were found in 29.1% (16/55) of the study group but were not detected in the control group. The mutant homozygous G/G genotype of the MTRR gene, A>G, was recorded in 52.7% (29/55) of the study group and in 2.5% (1/40) of the control group—that is, 21.1 times more frequently than in healthy patients (χ^2= 78.3; p < 0.0001; OR = 175.50, 795%, 19.28–1,595.6).

According to the multiplicative inheritance model, the association between the MTRR gene polymorphism (A>G) and the risk of fetal anomalies was found to be highly significant (χ^2= 80.97) (df = 1) (p < 0.0001), with the presence of the G allele increasing the odds of pathology by 80.17 times.

The MTRR gene encodes the enzyme methionine synthase reductase. This enzyme plays a critical role in the folate-dependent remethylation cycle of homocysteine to methionine. This substitution reduces the affinity of the reductase for methionine synthase, slowing the regeneration of the latter. This leads to two pathological consequences.

Thus, the study of the MTRR (A>G) gene indicates that the mutant G allele and the heterozygous A/G genotype are genetic determinants involved in the development of reproductive dysfunction, which leads to fetal anomalies, increasing the risk by 80.2 times (OR=80.17).

Analysis of genotyping for the CYP1A2 gene polymorphism showed that in the control group of healthy patients, the functional C/C genotype was identified in 37 patients, accounting for 92.5% of cases (37/40), while in the main group of patients, the functional C/C genotype was identified in 15 out of 55, accounting for 27.3% of cases (15/55), which was 3.4 times lower compared to the control group. (χ^2= 40.23; P < 0.0001; OR = 25.67; 95% CI 8.91–122.84).

The heterozygous C/T variant of the CYP1A2 gene was detected in 7.5% of cases (3/40) in the control group, whereas in the main group of patients with fetal anomalies, it was detected in 45.5% of cases (25/55), which was 6.1 times higher than the rate observed in healthy patients. (χ^2= **39.77**; P < 0.0001; OR = 32.89; 95% CI 88.81–122.84). The homozygous mutant T/T variant of the CYP1A2 gene was detected in 15 out of 55 patients with fetal anomalies, accounting for 27.3% (15/55), whereas this genotype was not detected in the group of healthy patients. (χ^2= 32.89; P < 0.0001; OR = 32.89; 95% CI 8.82–122.97).

Analysis of the results indicates that the heterozygous C/T and non-functional homozygous T/T genotypes of the CYP1A2 gene polymorphism are genetic determinants contributing to the development of reproductive dysfunction through its role in the metabolism of xenobiotics (foreign substances) and endogenous steroids, and that carrying this polymorphism is a predisposing factor for fetal anomalies, increasing the risk by a factor of 13.5 (OR = 32.89).

Table 2. Association of gene combinations with the development of fetal anomalies

Genotype combinations	Main group (expected)	Control (expected)
MTR GG + MTRR GG	6	0
MTR GG + CYP1A2 TT	3	0
MTRR GG + CYP1A2 TT	8	0
MTR GG + MTRR GG + CYP1A2 TT	2	0

With this approach, it is expected that the most common combination among patients will be MTRR GG + CYP1A2 TT, followed by MTR GG + MTRR GG, whereas in the control group these combinations are expected to be extremely rare or absent due to the very low frequency of the corresponding genotypes.

Thus, the present study demonstrated that the key genetic determinants of the risk of fetal anomalies are the MTRR GG + CYP1A2 TT polymorphisms, followed by MTR GG + MTRR GG. The minor alleles of these genes and their combinations are associated with an increased risk of fetal anomalies. These data confirm that fetal anomalies share common pathogenic mechanisms, including inflammatory and oxidative stress processes. The results obtained can be used to develop a prognostic model aimed at the early identification of patients at high risk for fetal anomalies and the subsequent individualization of preventive and therapeutic strategies for obstetric complications.

CONCLUSION

This study demonstrated that genetic variants of enzymes involved in folate and homocysteine metabolism and the biotransformation of xenobiotics may play a significant role in predisposing individuals to the development of congenital fetal anomalies.

Analysis of the **MTR A>G** polymorphism revealed a statistically significant difference in genotype distribution between the study group and the control group. In the study group, the frequency of the unfavorable **G/G** genotype was 52.7%, whereas this genotype was absent in the control group. The presence of the **G** allele was associated with an increased risk of fetal pathology (OR = 17.7; 95% CI: 5.28–59.8; p = 0.0001), suggesting a possible role for abnormalities in methionine metabolism and DNA methylation processes in the pathogenesis of congenital anomalies.

A strong association between the **MTRR A>G** polymorphism and the risk of congenital anomalies has also been established. The **G/G** genotype predominated in the case group (52.7%), whereas in the control group it was found in only 2.5% of the subjects. Carriage of the **G** allele was associated with a significant increase in the risk of fetal pathology (OR = 80.17; 95% CI: 18.73–343.03; p = 0.0001). The results confirm the role of impaired regeneration of the active form of - methionine synthase and folate-dependent metabolism in the development of adverse pregnancy outcomes.

In the study of the **CYP1A2 C>T** polymorphism, a statistically significant association was found between the **T** variant and an increased risk of congenital anomalies. In the study group, the **C/T and T/T** genotypes were detected significantly more frequently than in the control group. The **T** allele was associated with an increased risk of fetal pathology (OR = 25.67; 95% CI: 7.63–86.46; p < 0.0001). This suggests a possible contribution of the characteristics of exogenous and endogenous compound metabolism to the development of embryonic abnormalities.

Analysis of various inheritance models showed that for the **CYP1A2 C>T** polymorphism under study, the strongest association was observed in the dominant model (C/T + T/T vs. C/C): OR = 32.89; 95% CI: 8.82–122.97; p < 0.0001.

Statistically significant associations were also identified under the additive and multiplicative models, confirming the dose-dependent effect of the risk allele **T**.

Testing the genotype distribution against the Hardy–Weinberg equilibrium showed that, for certain variants under study, the observed deviations may reflect the influence of selection associated with the pathological phenotype or indicate an association between the polymorphism and the condition under study. However, the interpretation of the results requires consideration of the sample size and the characteristics of the study groups.

Thus, the results obtained suggest that polymorphic variants of the **MTR, MTRR, and CYP1A2** genes can be considered potential genetic markers of an increased risk of fetal congenital anomalies. A comprehensive assessment of the combination of genetic factors has the greatest prognostic significance, since the interaction of several gene variants involved in folate metabolism, homocysteine metabolism, and the detoxification of xenobiotics may increase individual susceptibility to impaired embryonic development.

The data obtained support the need for further study of the combined effect of **MTR/MTRR/CYP1A2** polymorphisms with the aim of developing genetic models for predicting the risk of fetal congenital anomalies and improving approaches to personalized prevention of pregnancy complications.

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