Original Article

Reproductive and Developmental Medicine®

Role of intergenic interactions among folate cycle genes in the development of fetal growth retardation

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Abstract

Objective: Metabolic disturbances in the folate cycle in mothers can lead to fetal growth retardation (FGR). This study was to analyze the role of intergenic interactions among maternal folate cycle genes in the development of FGR.

Methods: This case-control study recruited 365 women in the third trimester of pregnancy, including 122 FGR patients and 243 controls. The women were genotyped for 5 polymorphisms of the 4 folate cycle genes: *MTR* (rs1805087), *MTRR* (rs1801394), serine hydroxymethyl transferase (*SHMT1*; rs1979277), and *TYMS* (rs699517 and rs2790). The SNP × SNP interactions in the two-, three-, and four-locus models were analyzed using the multifactor dimensionality reduction method and a modification of it (the model-based multifactor dimensionality reduction method).

Results: Four loci of maternal folate cycle genes (rs1805087 *MTR*, rs2790 *TYMS*, rs1801394 *MTRR*, and rs1979277 *SHMT1*) were associated with FGR in 3 significant models of single nucleotide polymorphism (SNP) × SNP interactions (two-, three-, and four-locus models) (P <0.05). The highest contribution to FGR was made by polymorphic loci rs1979277 *SHMT1* (1.70% of entropy), rs1805087 *MTR* (0.96%), and interactions between rs1979277 *SHMT1* × rs1805087 *MTR* (-1.11%) and rs1801394 *MTRR* × rs1979277 *SHMT1* (–0.64%). The four-locus maternal genotype combination AG rs1801394 *MTRR* × AA rs1805087 *MTR* × CT rs1979277 *SHMT1* × AG rs2790 *TYMS* was associated with an increased risk of FGR (β = 2.69, P = 0.012). FGR-associated SNPs were correlated with the expression of 16 genes (*MTR, MTRR, SHMT1, ALKBH5, CTD-2303H24.2, ENOSF1, FAM106A, FOXO3B, LGALS9C, LLGL1, MIEF2, NOS2P2, RP11-806L2.6, SMCR8, TOP3A,* and *USP32P2*) in various tissues and organs related to FGR pathophysiology.

Conclusion: SNP × SNP interactions of maternal folate cycle genes (*MTR*, *MTRR*, *SHMT1*, and *TYMS*) are associated with the development of FGR.

Keywords: Polymorphism, Associations, Fetal growth retardation, Folate, SNP × SNP interactions

Introduction

Fetal growth retardation (FGR) is a condition when the rate of fetal growth is lower than expected (considering the race and gender)[1,2]. FGR affects approximately 5% of newborns globally and poses a high risk of perinatal morbidity and mortality[3]. Restriction of intra-uterine progress of the fetus can cause a number of post-natal diseases of the body and metabolic disorders due to impaired growth and development of the embryo during pregnancy[2,4]. Long-term complications are observed in children with FGR; these include an increased risk of developing metabolic syndrome, cardiovascular disease, and type 2 diabetes in adulthood[5,6]. Due to the growing prevalence and lack of effective treatments, FGR has substantial medical, economic, and social implications on families and societies[1,2,5].

Genetic and environmental factors play a role in the development of FGR[1,3,6–9]. Studies have found that 40% of birth weight is due to hereditary factors and 60% is due to environmental factors[5]. Folate plays a key role in normal fetal and placental development and growth[10,11]. Maternal folate deficiency (correlated with higher homocysteine concentrations) is associated with poor pregnancy outcomes, such as FGR[12–14], pre-eclampsia, and pre-term birth[11,15]. Folate is a necessary component for the synthesis of purine/thymidine nucleotides, which are important for deoxyribonuclei acid (DNA) replication, repair, and methylation[10,13]. Folate levels in organisms are genetically determined[16]. Specific gene mutations/polymorphisms can be linked to reduced levels and activity of key folate cycle enzymes, leading to disorders in folate metabolism[17,18]. Genes (genetic polymorphisms) may interact with other genes/environmental factors to modify disease risk[19]. Despite numerous studies on the association between folate cycle genes and FGR/SGA (infants born small for gestational age)[8,19–23], the role of folate cycle-related gene-gene interactions in FGR development has been poorly analyzed.

The purpose of this study was to analyze the role of intergenic interactions between maternal folate cycle genes in the development of FGR.

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Reproductive and Developmental Medicine (2023) 7:1

***Received:** 6 July 2021 **Accepted:** 18 February 2022*

http://dx.doi.org/10.1097/RD9.0000000000000013

Materials and methods

Design and participants

This study was approved by the Ethical Committee of the Medical Institute of Belgorod State University (reference number: ID. 54). The study details were explained to the women before they participated in the study, and informed consent was obtained from all participants. A total of 365 women in the third trimester of pregnancy, including 122 patients with FGR and 243 controls, were recruited for this case-control study. All participants (both cases and controls) were clinically examined at the Perinatal Center of the Belgorod Regional Clinical Hospital between 2015 and 2018. Women who were born in Russia, were of Russian ethnicity[24], and were in the third trimester of spontaneous singleton pregnancy were included in the study. The diagnosis of FGR was based on clinical data, parameters of growth and weight after birth, and ultrasound fetometry (TOSHIBA XARIO SSA-660A) (as described elsewhere[7,8]). The control group consisted of pregnant women with normally developed fetuses. Exclusion criteria included multiple pregnancies, treatment with insulin therapy for gestational diabetes mellitus, severe uncompensated somatic diseases, fetal malformation diseases, and congenital thrombophilia.

Genetic measurements

The material used in this study was a sample of maternal DNA isolated from whole venous blood[25]. DNA was extracted from whole blood using the phenol–chloroform method and then checked for quality (as described earlier[26]). Five SNPs in *MTR* (rs1805087), *MTRR* (rs1801394), serine hydroxymethyl transferase (*SHMT1*; rs1979277), and *TYMS* (rs699517, rs2790) were selected for the analysis according to the significant regulatory potential[27,28]. DNA was genotyped by real-time polymerase chain reaction using TaqMan© probes[29].

Statistical analysis

The correspondence of the observed allele/genotype frequencies to the Hardy–Weinberg equilibrium (HWE) was estimated using the Chi-square test[30]. The interactions between genes were analyzed assuming two-, three-, and four-locus models and using the model-based multifactor dimensionality reduction (MB-MDR) method[31,32] with adjustment for covariates [age of pregnant women as well as their weight and body mass index (BMI) before pregnancy as quantitative variables (Table 1)] and validation by the permutation test (1000 permutations)[33]. The significance level was set at $P \leq 0.05$[34]. Calculations were performed in the *mbmdr* package (version 2.6) using the R software [version 3.4.0 (April 4, 2017) http://www.r-project.org/]. The identified interactions and the proportion of their contribution to the total variance of the trait were visualized using the MDR v. 3.0.2 software (http://sourceforge.net/projects/mdr).

In silico *identification of expression quantitative trait loci*

FGR-associated polymorphisms were analyzed *in silico* for expression quantitative trait locus (eQTL) significance using the GTEx Consortium atlas (http://www.gtexportal.org/)[35]. The eQTL data provided in this study had a significance level of FDR ≤0.05. The relationship between polymorphisms and the level of gene expression was characterized by a linear regression coefficient (β) (showing a change in gene expression per single allele).

Results

Phenotypic data of the study participants are presented in Table 1. Patients with FGR had a lower weight (P = 0.0001) and BMI (P = 0.002) before pregnancy than those in the control group. These parameters and the age of the participants were used as covariates in the MB-MDR association analyses. All 5 SNPs had minor allele frequencies >5%, which corresponded with the HWE (P >0.05) (Table 2).

Using the MB-MDR method, we identified that 3 models of the SNP × SNP interactions of folate cycle genes were significantly ($P_{perm} \leq 0.05$) associated with the development of FGR: 1 two-locus interaction model, 1 three-locus model, and 1 four-locus model (Table 3). The 3 models of intergenic interactions associated with FGR included 4 polymorphic loci from 5 SNPs: rs1805087, rs2790, rs1801394, and rs1979277 *SHMT1*. The polymorphic locus rs1805087 *MTR* was involved in the formation of all 3 of the most significant gene–gene interaction models. Other SNPs were included in the 2 models. It was noted that the paired SNP × SNP interaction rs1805087 *MTR* × rs2790 *TYMS* was the basis of 2 significant models of intergenic interactions – the two-locus and four-locus models. In addition, the triple SNP × SNP interaction rs1801394 *MTRR* × rs1805087 *MTR* × rs1979277 *SHMT1* was the basis of the three-locus and four-locus models.

The associations between individual genotype combinations and FGR within the 3 significant models of SNP × SNP

Table 1.

Baseline characteristics of the study participants.

Parameters	FGR (n =122) M ± SD	Control (n = 243) M ± SD	P
Age (years)	25.48 ± 5.34	26.47 ± 5.63	0.092
Height (m)	1.68 ± 5.29	1.63 ± 6.82	0.151
Weight before pregnancy (kg)	61.43 ± 10.59	67.22 ± 11.54	0.0001
BMI before pregnancy (kg/m^2)	22.13 ± 4.2	24.25 ± 3.56	0.002
Newborn weight (g)	2,647.26 ± 621.15	3,463.26 ± 438.26	0.00002
Newborn growth (cm)	41.27 ± 2.41	54.51 ± 2.26	0.00001

BMI: body mass index; FGR: fetal growth retardation; SD: standard deviation.

Table 2.

Distribution of 5 polymorphic loci of folate cycle genes in the group of pregnant women with FGR and in the control group.

Polymorphic locus	Parameters	FGR (n = 122)	Control (n = 243)
rs1805087 *MTR*	Genotypes*	9/40/70	6/90/131
	Ho/He	0.34/0.37	0.40/0.35
	P_{HWE}	0.325	0.055
rs1801394 *MTRR*	Genotypes*	27/63/29	44/121/78
	Ho/He	0.53/0.50	0.50/0.49
	P_{HWE}	0.585	0.896
rs1979277 *SHMT1*	Genotypes*	18/55/42	17/96/118
	Ho/He	0.48/0.48	0.41/0.40
	P_{HWE}	1.000	0.747
rs699517 *TYMS*	Genotypes*	9/51/58	18/102/115
	Ho/He	0.43/0.41	0.43/0.41
	P_{HWE}	0.824	0.532
rs2790 *TYMS*	Genotypes*	6/38/75	7/67/168
	Ho/He	0.32/0.33	0.28/0.28
	P_{HWE}	0.781	0.821

*Total number of each genotype (minor allele homozygotes/heterozygotes/major allele homozygotes). FGR: fetal growth retardation; He: expected heterozygosity; Ho: observed heterozygosity; HWE: Hardy–Weinberg equilibrium.

Table 3.

Significant models of the SNP × SNP folate cycle gene interactions associated with FGR

N	Models SNP × SNP interactions	NH	βH	WH	NL	βL	WL	P_{perm}
Two-locus models, ($P<2.7\times10^{-3}$)								
1	rs1805087 MTR × rs2790 TYMS	2	0.480	10.30	0	NA	NA	0.036
Three-locus models ($P<1.4\times10^{-5}$)								
2	rs1801394 MTRR × rs1805087 MTR × rs1979277 SHMT1	5	0.325	20.52	1	–0.166	3.765	0.018
Four-locus models ($P<1.4\times10^{-5}$)								
3	rs1801394 MTRR × rs1805087 MTR × rs1979277 SHMT1 × rs2790 TYMS	4	0.505	24.11	0	NA	NA	0.048

Results were obtained using the MB-MDR method with an adjustment for covariates.βH: linear regression coefficient for combinations of genotypes associated with an increased risk of developing FGR; FGR: fetal growth retardation; L: the linear regression coefficient for combinations of genotypes associated with a reduced risk of developing FGR; MB-MDR: model-based multifactor dimensionality reduction; NH: number of significant combinations of genotypes associated with an increased risk of developing FGR; NL: the number of significant combinations of genotypes that are associated with a reduced risk of developing FGR; P_{perm}: significance level of the models after the permutation test (1000 permutations were performed); WH: Wald statistics for combinations of genotypes associated with an increased risk of developing FGR; WL: Wald statistics for combinations of genotypes associated with a reduced risk of developing FGR.

interactions are presented in Table 4. The following four-locus maternal genotype combination was most significantly associated with an increased risk of FGR: AG rs1801394 *MTRR* × AA rs1805087 *MTR* × CT rs1979277 *SHMT1* × AG rs2790 *TYMS* (β = 2.69, P = 0.012).

The dendrogram and graph of the most significant SNP × SNP interactions associated with FGR (Fig. 1) suggest that the highest contribution to the entropy is made by polymorphic loci rs1979277 *SHMT1* (1.70%) and rs1805087 *MTR* (0.96%) and interactions rs1979277 *SHMT1* × rs1805087 *MTR* (–1.11%) and rs1801394 *MTRR* × rs1979277 *SHMT1* (–0.64%).

FGR-associated SNP eQTL effects

Analysis of the GTE × consortium data showed that all FGR-associated SNPs were involved in the expression of quantitative traits (Table S1, http://links.lww.com/RDM/A2). rs1979277 *SHMT1* was associated with the expression of 12 genes (*SHMT1*, *ALKBH5*, *CTD-2303H24.2*, *FAM106A*, *FOXO3B*, *LGALS9C*, *LLGL1*, *MIEF2*, *NOS2P2*, *SMCR8*, *TOP3A*, and *USP32P2*) that had the most significant eQTL effects. Loci associated with FGR demonstrated eQTL influence in multiple organs and tissues involved in the pathogenesis of FGR: different parts of the brain (cortex, basal ganglia, substantia nigra, hypothalamus, pituitary *etc*.) (*MTR*, *MTRR*, *ALKBH5*, *SHMT1*, *SMCR8*, and *CTD-2303H24.2*), ovary (*MTRR* and *SHMT1*), thyroid (*SHMT1*, *ALKBH5*, *USP32P2*, *FAM106A*, *CTD-2303H24.2*, *TOP3A*, and *RP11-806L2.6*), adrenal gland (*SHMT1*, *ENOSF1*, and *RP11-806L2.6*), adipose tissue (sub-cutaneous and visceral) (*MTRR*, *ALKBH5*, *CTD-2303H24.2*, *USP32P2*, *FAM106A*, *SHMT1*, *TOP3A*, *ENOSF1*, and *RP11-806L2.6*), skeletal muscle (*MTR*, *SHMT1*, *ALKBH5*, *CTD-2303H24.2*, *USP32P2*, *FAM106A*, *LLGL1*, *TOP3A*, *FOXO3B*, *MIEF2*, and *ENOSF1*), artery (*SHMT1*, *TOP3A*, *ALKBH5*, *CTD-2303H24.2*, *USP32P2*, and *FAM106A*), and whole blood (*SHMT1*, *SMCR8*, *CTD-2303H24.2*, *USP32P2*, *LGALS9C*, *TOP3A*, and *FAM106A*).

In summary, 4 FGR-associated SNPs were correlated with the expression of 16 genes (MTR, MTRR, SHMT1, ALKBH5, CTD-2303H24.2, ENOSF1, FAM106A, FOXO3B, LGALS9C, LLGL1, MIEF2, NOS2P2, RP11-806L2.6, SMCR8, TOP3A, and USP32P2) in various tissues and organs related to FGR pathophysiology.

Discussion

The present study demonstrates the important role of SNP × SNP interactions in maternal folate cycle genes (rs1805087, rs2790, rs1801394, and rs1979277 *SHMT1*) in the development of FGR. FGR-related polymorphisms are implicated in the regulation of 16 genes (*MTR*, *MTRR*, and *SHMT1*) in various organs and tissues (different parts of the brain, ovary, adrenal gland, adipose tissue (sub-cutaneous and visceral), whole blood, *etc*.) and are involved in the pathogenesis of poor pregnancy outcomes.

Previous studies have investigated the association of folate SNPs with poor pregnancy outcomes, such as spontaneous

Table 4.

Associations of genotype combinations with FGR within the significant models of SNP × SNP interactions

N Models	N Combi nations	Genotype combinations	FGR (n = 122) n (%)	Control (n = 243) n (%)	β	P	Risk
Two-locus model							
1	1	rs1805087 × GGrs2790 AG	4 (3.28)	1 (0.41)	2.11	0.060	H
	2	rs1805087 × AGrs2790 GG	4 (3.28)	1 (0.41)	2.11	0.060	H
Three-locus model							
2	3	rs1801394 AA × rs1805087 AA × rs1979277 CC	6 (4.92)	27 (11.11)	–8.78	0.059	L
	4	rs1801394 AG × rs1805087 AA × rs1979277 CT	12 (9.84)	10 (4.11)	9.37	0.034	H
	5	rs1801394 GG × rs1805087 AG × rs1979277 TT	6 (4.92)	3 (1.23)	1.42	0.047	H
Four-locus model							
3	6	rs1801394 GG × rs1805087 AG × rs1979277 TT × rs2790 AA	6 (4.92)	3 (1.23)	1.42	0.047	H
	7	rs1801394 AG × rs1805087 AA × rs1979277 CT × rs2790 AG	7 (5.74)	1 (0.41)	2.69	0.012	H

Results were obtained using the MB-MDR method with adjustment for covariates. B: logistic regression coefficients for combinations of genotypes; FGR: fetal growth retardation; H/L: high/low risk; MB-MDR: model-based multifactor dimensionality reduction; *P*: significance level.

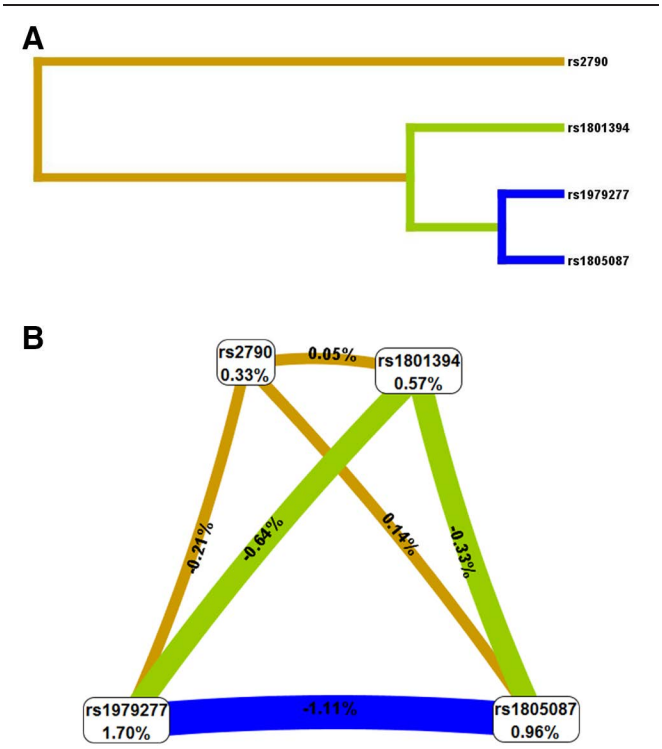

Fig. 1 Dendrogram (A) and graph (B) of the most significant SNP × SNP interactions associated with FGR (obtained by the MDR method). The positive values of entropy indicate synergistic interactions while the negative values indicate redundancy. The brown color denotes an independent effect; green and blue colors denote moderate and strong antagonism, respectively. FGR: fetal growth retardation; MDR: multifactor dimensionality reduction.

pre-term birth (rs1801394 *MTRR* and rs1979277 *SHMT1*)[21], neural tube defects (rs1801394 *MTRR*)[36,37], recurrent spontaneous abortion (rs1801394 *MTRR*)[38–40], and uteroplacental insufficiency (rs1805087 *MTR*)[41]. An important role for the interaction between dietary folate intake and rs1979277 *SHMT1* in the development of spontaneous pre-term birth and small for gestational age (SGA) has been revealed[21]. On the other hand, in some studies, significant associations were found between the analyzed SNPs and several poor pregnancy outcomes, such as uteroplacental insufficiency and FGR (rs1801394 MTRR)[41], pre-eclampsia (rs1805087 MTR, rs1801394 MTRR)[20,22], SGA (rs1805087 MTR, rs1801394 MTRR)[19,20,22], spontaneous pre-term birth (rs1805087 MTR and rs1801394 MTRR)[20], and neural tube defects (rs1979277 SHMT1)[42].

Barbosa *et al*.[43] revealed an association between *MTR* polymorphisms (rs1805087) and total homocysteine levels in pregnant women. In addition, the data presented in this paper indicate that the interaction between Cbl status and polymorphisms of *MTRR* (rs1801394) is associated with total homocysteine levels. Lin *et al*.[40] showed the detrimental effects of rs1801394 *MTRR* on serum homocysteine and lipid levels in patients with recurrent spontaneous abortion. Interestingly, maternal homocysteine levels and uteroplacental insufficiency may correlate with fetal rs1805087 *MTR*[41]. Another study demonstrated the independent association between rs1805087 *MTR* and a higher risk of folate deficiency and hyperhomocysteinemia[44,45]. Low folate status and folate cycle gene polymorphisms (rs1801394 *MTRR* and rs1979277 *SHMT1*) may have synergistic effects and are defined as abnormal lipid metabolism[46].

A previous study revealed a significant interaction between rs1801394 *MTRR* and rs1801133 *MTHFR* on serum homocysteine levels[47,48]. The interactions among homocysteine metabolism gene polymorphisms (*MTHFR* and *MTR*) leading to dramatic elevations in folate deficiency and hyperhomocysteinemia risk were discussed by Li *et al*.[44,45]. The present study suggests that gene-gene interactions of maternal folate cycle genes (*MTRR, MTR*, *TYMS*, and *SHMT1*) are associated with FGR, and all 4 FGR-related polymorphisms are implicated in the regulation of the expression of 16 genes (*MTR, MTRR, SHMT1, etc*.). Interestingly, serum folate and plasma vitamin B-12 levels are strongly inversely linked to plasma homocysteine levels[48]. Previous studies have reported that low folate and high homocysteine levels (determined genetically and by environmental factors) were associated with numerous pregnancy complications, including FGR, SGA, recurrent pregnancy loss, pre-eclampsia, pre-term delivery, and placental abruption[19,20,41,49]. Maternal homocysteine levels are considered important regulators of amino acid transfer in the placenta, and maternal hyperhomocysteinemia can induce FGR by inhibiting this transfer[49].

The folate cycle includes the complex effects of many gene products and other important trace elements obtained through diet, such as vitamin B12, vitamin B6, choline, and methionine. All of these elements are directly or indirectly necessary for the conversion of homocysteine to methionine, which is a direct precursor of S-adenosylmethionine (SAM), the main donor of intra-cellular methyl, to support the methylation of DNA, proteins, and lipids. Subsequently, SAM is first converted to S-adenosyl homocysteine and then to homocysteine. The presence of genetic polymorphisms that alter the functionality of key transport molecules and enzymes necessary for the folate/homocysteine cycle may pre-dispose individuals to genome instability, altered recombination, and abnormal segregation[50,51].

Folate-cycle metabolic enzymes play an important role in maintaining normal fetal development. Therefore, *SHMT1*, a pyridoxal phosphate-dependent enzyme that catalyzes the interconversion of serine and glycine, provides the folate-dependent single-carbon metabolism required for the synthesis of purines and thymidylate as well as for the conversion of homocysteine to methionine. Methionine is subsequently adenylated to SAM, a cofactor that methylates DNA, ribonucleic acid, proteins, and several metabolites[52].

Methionine synthase (*METH*, *i.e.*, *MTR*) is a key enzyme in the folate pathway that plays a critical role in the synthesis, repair, and methylation of DNA. Methionine synthase is a B12-dependent enzyme, and vitamin B12 deficiency can disrupt homocysteine methylation, leading to its accumulation. The demethylation of methionine during metabolism leads to the formation of homocysteine, and folate and cobalamin are required for its methylation. Low FA concentrations of folic acid are associated with vascular complications during pregnancy[20].

Conclusion

Four loci of the maternal folate cycle genes (rs1805087, rs2790, rs1801394, and rs1979277 *SHMT1*) were associated with FGR in 3 significant models of SNP × SNP interactions (two-, three-, and four-locus models) (P_{perm} ≤0.05). FGR-associated SNPs were correlated with the expression of 16 genes related to FGR pathophysiology in various tissues and organs.

Acknowledgments

None.

Author contributions

M.C. conceived of the presented idea. O.E. performed the computations and data alalys. I.P. verified the analytical methods. M.C. and I.P. supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Funding(s)

This research received no external funding.

Conflicts of interest

All authors declare no conflict of interest.

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Edited By: Yong-Qing Zhu

How to cite this article: Efremova O, Ponomarenko I, Churnosov M. Role of intergenic interactions among folate cycle genes in the development of fetal growth retardation. Reprod Dev Med 2023;7(1):32–37. doi: 10.1097/RD9.0000000000000013