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Association of maternal genetic polymorphisms with fetal growth restriction syndrome in Russian pregnant women from Rostov region

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Abstract

Background Fetal growth restriction (FGR) is one of the main syndromes causing fetal morbidity and mortality. It was known to be associated with different factors including maternal, fetal, and environmental. However, the effect of genetic factors in FGR is not totally understood. Recently, researchers have focused on investigating genetic variants as possible markers of FGR. This especially concerns maternal genetic polymorphisms since they could serve as prenatal prognostic biomarkers. Accordingly, we aimed to study the association of several polymorphisms affecting vital processes of pregnancy with FGR in pregnant women. Targeted polymorphisms include methylenetetrahydrofolate reductase (*MTHFR*) 677C > T; methionine synthase reductase *(MTRR)* 66A > G; methionine synthase *(MTR)* 2756A > G; angiotensinogen *(AGT)* 704 T > C; and vascular endothelial growth factor A *(VEGFA)* 634C > G. In addition, this study examined SNP–SNP interactions, linkage disequilibrium (LD), and haplotypes association for these polymorphisms in the studied population.

Results According to our data, *MTRR* 66(GG) carriers had increased FGR risk (OR = 3.18, 95% CI 1.31–7.72) while (AG) genotype was associated with lower FGR risk (OR = 0.37, 95% CI 0.17–0.84). *AGT* 704T > C also showed significant association with FGR with allele (T) as a risk factor. SNP–SNP interactions analysis revealed antagonistic relationship between these two polymorphisms and haplotypes association confirmed this finding. High LD possibility was shown between *MTHFR* 677C > T and *MTR* 2756A > G (D' = 0.999) located on chromosome 1.

Conclusion We suggest *MTRR* 66A > G and *AGT* 704T > C as associated with FGR susceptibility with antagonistic interaction. Result will help to expand our understanding of FGR as a multifactorial syndrome and improve prenatal prognosis using maternal genetic biomarkers, but further studies in different populations are needed to confirm findings.

Keywords Fetal growth restriction, Maternal genetic polymorphism, Methionine synthase reductase, Angiotensinogen

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Background

Fetal growth restriction (FGR) is a fetal syndrome in which fetus growth parameters are not consistent with normal ranges of the same gestational age [17]. This syndrome can be defined as a prenatal period accompanied with slowdown, arrest, or negative dynamics of fetal size which clinically includes pregnancies with fetal body

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weight < 10th percentile (or ≤ 2 or more standard deviations) of normal weight detected for gestational age and sex [26]. FGR affects approximately 7–15% of pregnancies all over the world and six times higher in underdeveloped countries. It is known to be associated with prenatal and postnatal outcomes with a mortality of 12% in fetal and 8% in neonatal period [14, 18]. FGR short-term complications include respiratory distress, perinatal asphyxia, hypoglycemia, and others. Moreover, later in life, patients showed increased risk of cognitive and neurodevelopmental abnormalities, obesity, cardiovascular diseases, and even metabolic syndromes [3]. Hence, developing our understanding about this syndrome is very important to improve pediatric outcomes.

Three main factors were known to contribute in FGR etiology: placental, maternal, and fetal (intrauterine) factors, but many studies used to call them risk factors since their effects in FGR are not fully understood [26]. Though etiology is not fully understood, placental insufficiency was proved as the direct causal factor in most FGR cases. Moreover, it is known that genetic expression of genes regulating placental angiogenesis and nutrients transition from mother to fetus leads to abnormal placental functions and thereby abnormal fetal growth [9]. Recently, researchers have focused on the genetic aspects of FGR syndrome, especially maternal candidate genetic variants associated with its risk. So far, possible effects of several polymorphisms on FGR pathophysiology were widely studied in different populations, but results are contradictory [16]. Finding new maternal biomarkers will improve prenatal diagnosis and prognosis and thus treatment or even prevention. Therefore, more studies are needed to investigate maternal polymorphisms of genes involved in FGR development.

This study aimed to investigate the association of five candidate genetic variants with FGR risk in pregnant women from Rostov region. In addition, research includes haplotypes and gene–gene interactions analysis to identify the possible prenatal biomarkers of this syndrome whether it is a single polymorphism, haplotype, or a set of genetic variants.

Materials and methods

Research subjects

From March 2018 to April 2021, pregnant women (mean age ± SD = 30.18 ± 5.28 years) were recruited in this case–control study to investigate targeted genetic polymorphisms. Samples were collected in "Perinatal Center" State institution of Rostov oblast, Russia. Then, multiple pregnancies, fetal chromosomal aberrations, twins' pregnancy, and induced pregnancy (in vitro fertilization) were excluded from the study. Study subjects ($n = 103$) were classified according to diagnosis into two groups: FGR pregnancy ($n = 46$) and controls corresponding normal pregnancy without any complications ($n = 57$). The diagnosis was made according to ultrasound and Doppler ultrasound.

FGR was defined as estimated fetal weight (EFW) or abdominal circumference (AC) < 3rd centile, or EFW or AC < 10th centile and umbilical artery (UA) pulsatility index (PI) > 95th centile or cerebroplacental ratio (CPR) < 5th centile, diagnosed after 32 weeks [10, 21].

All participants provided willingly written informed consent for inclusion before they participated in the study. The study was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and approved by the Southern Federal University Bioethics Committee.

DNA extraction

Blood samples were collected at an average of 29 weeks of pregnancy into purple top EDTA tubes and then stored at −80 °C. Genomic DNA was extracted from peripheral blood leukocytes by standard procedures using commercially available kits: NK-sorbent «Base»; (Lytech Co. Ltd., Russia) and «DNA-EXTRAN»; (Syntol Co. Ltd., Russia). The isolated DNA was quantitatively assessed using NanoDrop and then stored at −20 °C.

Targeted polymorphisms

In this study, five polymorphisms were targeted: *MTHFR* 677C > T; *MTRR* 66A > G; *MTR* 2756A > G; *AGT* 704 T > C; and *VEGFA* 634C > G. Table 1 illustrates the following information about every targeted polymorphism: the affected gene and amino acid substitution; chromosomal location; function of encoded protein under the normal level of genetic expression; and the effect of mutant allele existence on genetic and protein function.

Genotyping

Real-time allele-specific polymerase chain reaction (AS-PCR) assay was used to determine: *MTHFR* 677C > T (rs1801133); *MTRR* 66A > G (rs1801394); *MTR* 2756A > G (rs1805087); and *AGT* 704 T > C (rs699) with SYBR-Green fluorescence label. This was done using commercially available genotyping kits for those polymorphisms: SNP-Express RT kit (Lytech Co. Ltd., Russia). Results automatically were registered on QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA) in accordance with the manufacturer's instructions. For PCR amplification, the standard program used was as follows: one initial denaturation step of 1 min at 93 °C; followed by 35 cycles of 10 s at 93 °C (denaturation), 10 s at 64 °C (annealing), and 20 s at 72 °C (extension). Melt curves and amplification plots for each genetic variation were

Table 1 Amino acids substitution, normal genetic function, chromosomal location, and effects on genetic expression of the five studied polymorphisms

Gene/polymorphism/amino acid substitution	Chr	Function of encoded protein *	Effect of polymorphism
MTHFR (methylenetetrahydrofolate reductase)/rs1801133 (C677T)/Ala > Val	1	MTHFR is an enzyme involved in folate metabolism. It reduces 5, 10-MTHF to 5-MTHF, which donates a methyl group for the conversion of homocysteine to methionine	The C677T mutation leads to enzyme dysfunction. This contributes to hyperhomocysteinemia and reduces folate levels (Raghubeer and Matsha, 2021)
MTRR (methionine synthase reductase)/rs1801394 (A66G)/Ile > Met	5	MTRR is another enzymatic member of folate metabolism by regenerating methionine synthase to a functional state.	A66G substitution adversely influences the enzyme activity, and thus, it is considered as a genetic risk factor for hyperhomocysteinemia [24]
MTR (methionine synthase)/rs1805087 (A2756G)/Asp > Gly	1	MTR enzyme, also known as cobalamin-dependent methionine synthase, catalyzes the final step in methionine biosynthesis from homocysteine in folate metabolism cycle	2756A > G reduces enzymatic activity and causes higher homocysteine levels and DNA hypomethylation [20]
AGT (angiotensinogen)/rs699 (T704C)/Met > Thr	1	Angiotensinogen is part of renin–angiotensin system. It is cleaved by renin in response to lowered blood pressure. It is involved in maintaining blood pressure, body fluid, and electrolyte homeostasis	ThrThr235 genotype is known to be associated with higher genetic expression and thus increased AGT concentration [1]
VEGFA (vascular endothelial growth factor A)/rs2010963 (634C > G)/polymorphism located in the 5′-untranslated region of the gene	6	Heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells and is essential for both physiological and pathological angiogenesis	Mutant allele (G) is associated with lower level of VEFGA genetic expression. Several studies have shown that this polymorphism could affect angiogenesis, which leads to placental insufficiency and endothelial dysfunction [23]

* The functions of proteins encoded by the corresponding gene according to Genecards database

checked to confirm specificity and accuracy of the used kit.

For *VEGFA* 634C > G (rs2010963), allele-specific PCR was conducted with SNP- Express EF kit (Lytech Co. Ltd., Russia) using the same amplificatory system (QuantStudio™ 5) with an initial denaturation temperature of 93 °C for 1 min followed by 35 cycles of 93 °C for 10 s (denaturation), 64 °C for 10 s (annealing), and 72 °C for 20 s (extension) with a final extension step of 72 °C for 1 min. PCR products were then analyzed with gel electrophoresis using a 1.5% agarose gel stained with ethidium bromide on a UV transilluminator.

Statistical analysis

Statistical analysis was performed using free portal «medical statistics» (Kazan, Russia) (https://www.medstatistic.ru/). Comparisons in qualitative parameters between FGR pregnancies and controls were made by using independent t-test, and results were presented as mean ± SD. The difference in those parameters between studied groups was evaluated by calculating *P* value (< 0.05 was considered as statistically significant). Genotypes were checked for Hardy–Weinberg equilibrium using Chi-square test in an available online calculator [22]. Chi-squared tests were performed to examine the differences in alleles frequency and genotypes distribution of every studied polymorphism in FGR and control pregnancies. Odds ratios (OR) [with 95% confidence interval (CI)] were calculated to measure the association with FGR risk in both groups. OR greater than 1 was considered a positive association (risk effect) whereas OR values less than 1 indicated negative association (protective effect). Statistical significance was assumed for *P* values < 0.05.

To investigate the interactions between targeted polymorphisms, multifactor dimensionality reduction (MDR) algorithm was used. MDR is model-free and non-parametric that uses delineate multiple factors to identify high-dimensional interactions, with the assumption that the level of risk is mainly explained by genetic factors; it is, therefore, ideal to study gene–gene interactions [22]. The following parameters were chosen for MDR analysis: attributes number: 5; cross-validation count: 10; and track top models: 500. In addition, as a correction for small sample size, Fisher's exact test was selected to be used in MDR.

Linkage disequilibrium and haplotypes association with FGR

Linkage disequilibrium (LD) was evaluated for polymorphisms located on the same chromosome using SNPStats software (https://www.snpstats.net/). This included: *AGT* 704T > C, *MTR* 2756A > G, and *MTHFR* 677C > T. For those three polymorphisms, LD coefficient (D′) was calculated to evaluate the possibility of recombination. Generally, D′ = 1 indicates complete LD and thus no evidence of recombination between the two sites, while D′ = 0 indicates no LD. In this study, we considered that D′ > 0.80 indicates strong LD.

To study haplotypes association with FGR risk, the most common haplotype was selected as the reference. P-values, odds ratios, and 95% CI were calculated to estimate the degree of association between haplotypes and the FGR risk in SNPStats. *P* values < 0.05 were considered as an indicator of significant association.

Results

Characteristics of study groups

Maternal blood samples were collected from FGR-diagnosed (aged 20–38 years) and healthy pregnant women (aged 18–43 years). There was no significant difference in age between the two studied groups (*P* > 0.05). Samples were collected after confirming pregnancy state. FGR was diagnosed at the following pregnancy weeks: 24w (one case), 27w (one case), 29w (three cases), 30w (two cases), 31w (three cases), 32w (five cases), 33w (eight cases), 34w (two cases), 35w (six cases), and 36w (five cases) with an average of 33.8 pregnancy weeks. It should be mentioned that 10 cases were with late delivery, and FGR was diagnosed at 37w (seven cases) and even 38w (three cases). Gestational and maternal age of the study groups are shown in Table 2.

Genotypes and alleles frequencies in both studied groups

Genotype and allele frequencies for *MTHFR* 677C > T (rs1801133); *MTRR* 66A > G (rs1801394); *MTR* 2756A > G (rs1805087); *AGT* 704T > C (rs699); and *VEGFA* 634C > G (rs2010963) polymorphisms in healthy controls and FGR pregnancies are shown in Table 3. Genotypes frequencies

Table 2 The maternal and gestational age at sampling point of FGR and normal pregnancy groups

Variable	All (*n* = 103)	FGR (*n* = 46)	Normal (*n* = 57)	P value
Age (years)	30.18 ± 5.28	30.5 ± 5.75	30 ± 4.87	0.68
Gestational age at sampling point (weeks)	29.01 ± 6.64	33.8 ± 3.1	23.86 ± 5.44	0

Table 3 Genotype and allele frequencies of the five studied polymorphisms in FGR and controls

Polymorphism	FGR n (%)	HWE* χ^2 (*P* value)	Control n (%)	HWE* χ^2 (*P* value)
MTHFR 677C > T	*n* = 46		*n* = 55	
CC	33 (71.7)	0.71 (0.7)	29 (52.7)	0.003 (0.998)
CT	11 (23.9)		22 (40)	
TT	2 (4.4)		4(7.3)	
C	77 (83.7)		80 (72.7)	
T	15 (16.3)		30 (27.3)	
MTRR 66A > G	*n* = 44		*n* = 56	
AA	7 (15.9)	0.59 (0.74)	9 (16.1)	4.62 (0.1)
AG	18 (40.9)		36 (64.3)	
GG	19 (43.2)		11 (19.6)	
A	32 (36.4)		54 (48.2)	
G	56 (63.6)		58 (51.8)	
MTR 2756A > G	*n* = 43		*n* = 56	
AA	29 (67.4)	0.27 (0.87)	30 (53.6)	2.71 (0.26)
AG	12 (27.9)		25 (44.6)	
GG	2 (4.7)		1 (1.8)	
A	70 (81.4)		85 (75.9)	
G	16 (18.6)		27 (24.1)	
AGT 704T > C	*n* = 42		*n* = 55	
TT	11 (26.2)	0.11 (0.95)	7 (12.7)	0.34 (0.84)
TC	22 (52.4)		28 (50.9)	
CC	9 (21.4)		20 (36.4)	
T	44 (52.4)		42 (38.2)	
C	40 (47.6)		68 (61.8)	
VEGFA 634C > G	*n* = 45		*n* = 51	
CC	9 (20)	1.25 (0.54)	8 (15.7)	0.3 (0.86)
CG	18 (40)		22 (43.1)	
GG	18 (40)		21 (41.2)	
C	36 (40)		38 (37.3)	
G	54 (60)		64 (62.7)	

*HWE: Hardy–Weinberg equilibrium; *P* value was calculated in an online available calculator to assess genotypes distribution accordance with HWE

were all in accordance with Hardy–Weinberg equilibrium (HWE) for both groups (Table 3).

Association of alleles and genotypes frequency with FGR

Among the five studied polymorphisms, *MTRR* 66A > G (rs1801394) and *AGT* 704T > C (rs699) showed a significant association with FGR risk (*P* value = 0.025 and 0.049, respectively). For *MTRR* 66A > G, (GG) carriers had increased FGR risk (OR = 3.18, 95% CI 1.31–7.72). Moreover, (AG) genotype was associated with lower FGR risk (OR = 0.37, 95% CI 0.17–0.84). According to our data, *AGT* 704T > C major and minor alleles have significant effect on FGR development, with (T) as a risk factor (OR = 1.78, 95% CI 1.002–3.17) and (C) as a protective one (OR = 0.58, 95% CI 0.32–1). In the study population, *MTHFR* 677C > T (rs1801133); *MTR* 2756A > G (rs1805087); and *VEGFA* 634C > G showed no differences in alleles or genotypes frequency between FGR and controls (Table 4).

SNP–SNP interaction of targeted polymorphisms

MDR analysis was conducted to evaluate possible interactions of the five targeted polymorphisms. Results showed two significant models ($P < 0.05$): *VEGFA* 634C > G, *MTRR* 66A > G, and *AGT* 704T > C three-locus model and four-locus model *VEGFA* 634C > G; *MTRR* 66A > G; *AGT* 704T > C; and *MTR* 2756A > G. Table 5 shows balance accuracy and cross-validation consistency (CVC) of each model.

According to the Fruchterman–Rheingold scheme, out of the five analyzed polymorphisms, *MTRR* 66A > G has the highest predictive potential, which supports the previous results of polymorphisms association shown in Table 4. The most effective intergenic interaction is

Table 4 Association of five studied polymorphisms with FGR susceptibility

Polymorphism	OR (95% CI)	χ^2	*P* value
MTHFR 677C > T			
CC	2.28 (1–5.23)	3.82	0.149
CT	0.47 (0.2–1.12)		
TT	0.58 (0.1–3.32)		
C	1.925 (0.96–3.85)	0.195	0.659
T	0.519 (0.26–1.04)		
MTRR 66A > G			
AA	1.01 (0.34–2.96)	7.396	**0.025***
AG	**0.37 (0.17–0.84)**		
GG	**3.18 (1.31–7.72)**		
A	0.61 (0.35–1.1)	2.824	0.093
G	1.63 (0.92–2.88)		
MTR 2756A > G			
AA	1.8 (0.79–4.10)	3.267	0.196
AG	0.48 (0.21–1.12)		
GG	2.68 (0.24–30.61)		
A	1.39 (0.69–2.78)	0.866	0.352
G	0.72 (0.36–1.44)		
AGT 704T > C			
TT	2.43 (0.85–6.95)	4.113	0.128
TC	1.06 (0.48–2.37)		
CC	0.48 (0.19–1.2)		
T	**1.78 (1.002–3.17)**	3.891	**0.049***
C	**0.58 (0.32–0.998)**		
VEGFA 634C > G			
CC	1.34 (0.47–3.84)	0.316	0.854
CG	0.88 (0.39–1.98)		
GG	0.95 (0.42–2.16)		
C	1.12 (0.63–2.01)	0.152	0.697
G	0.89 (0.5–1.6)		

*P value < 0.05; χ^2: chi-square; significant statistical values are shown in bold

VEGFA 634C > G with *AGT* 704T > C (the highest phenotypic entropy), representing a synergistic effect of these two polymorphisms in FGR pathogenesis. In addition, synergism was observed between *VEGFA* 634C > G and *MTRR* 66A > G, which support the significant three-locus model (Table 5). As shown in Fig. 1, a weak synergism was also found between *AGT* 704T > C and *MTHFR* 677C > T. Beside synergism, *MTR* 2756A > G antagonistic interactions were suggested with *VEGFA* 634C > G and *AGT* 704T > C. Moreover, *MTRR* 66A > G revealed antagonistic relationship with *AGT* 704T > C. Dendrogram (Fig. 1a) and Fruchterman–Rheingold scheme (Fig. 1b) represent all types of interactions according to MDR.

Linkage disequilibrium (LD) analysis

Because of their chromosomal location (on chromosome 1), pairwise LD was analyzed between *AGT* 704T > C, *MTR* 2756A > G, and *MTHFR* 677C > T. According to SNPStats analysis, D′ of *AGT* 704T > C and *MTR* 2756A > G was 0.023, D′ of *AGT* 704T > C and *MTHFR* 677C > T was 0.4 which indicates no LD. On the other hand, D′ between *MTR* 2756A > G and *MTHFR* 677C > T was 0.999 indicating a strong LD possibility and no evidence of recombination between these two sites.

Haplotypes association with FGR risk

Using SNPStats software, a pairwise haplotype association analysis was conducted for all the five studied polymorphisms. Results showed four haplotypes significantly associated with FGR risk represented in Table 6. Haplotypes containing *MTHFR* 677T * *MTRR* 66A and *MTR* 2756G * *VEGFA* 634C were associated with a statistically increased FGR risk while those with *MTRR* 66G * *AGT* 704T and *AGT* 704T * *VEGFA* 634G were associated with a statistically decreased risk.

Discussion

FGR is considered as a major cause of fetal mortality and morbidity. It is a multifactorial syndrome in which several maternal mechanisms were known to play a critical role, such as folate metabolism, angiogenesis, and many others [4]. After a detailed scan of scientific literature concerning genetic and psychology aspects of FGR, we aimed to target the genetic variations modulating expression of main proteins in vital process that is known to affect fetal growth during pregnancy. In this study, the most common targeted genetic variants affecting folate metabolism (methionine cycle

Table 5 Best predictive gene–gene interaction models identified by MDR analysis

Locus model	Genes	Training balance accuracy	Testing balance accuracy	CVC consistency	Sensitivity	Specificity	χ^2 (*P* value)
Three	*VEGFA* 634C > G *MTRR* 66A > G *AGT* 704T > C	0.7073	0.6513	9/10	0.8571	1.0	17.6786 (0.0001)
Four	*VEGFA* 634C > G *MTRR* 66A > G *AGT* 704T > C *MTR* 2756A > G	0.6546	-	7/10	1	1	11.0 (0.0009)

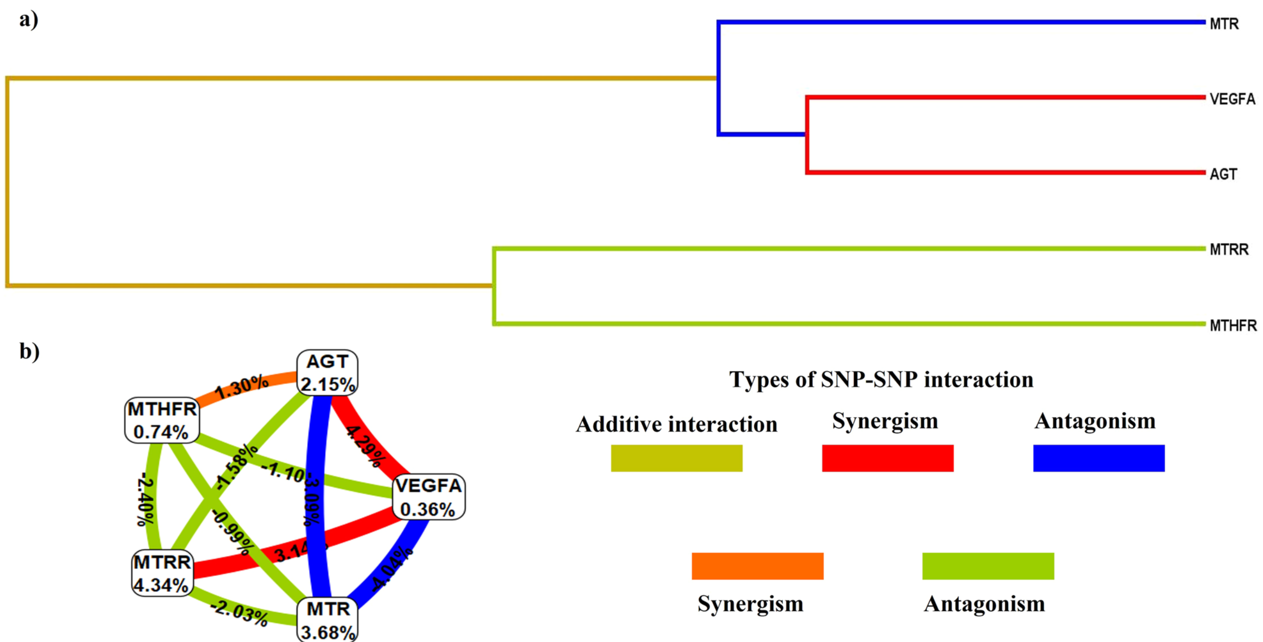

Fig. 1 High-order gene–gene interaction analysis for *MTHFR* 677C > T; *MTRR* 66A > G; *MTR* 2756A > G; *AGT* 235Met > Thr; and *VEGFA* 634C > G (data obtained by multifactor dimensionality reduction): **a** interaction dendrogram and **b** Fruchterman–Rheingold scheme

Table 6 Haplotype analysis on association of *MTHFR* 677C > T; *MTRR* 66A > G; *MTR* 2756A > G; *AGT* 704T > C; and *VEGFA* 634C > G with FGR risk

Haplotype (Alleles)	Frequencies			OR (95%CI)	*P* values
	Total	FGR	Controls		
MTHFR 677T * *MTRR* 66A	0.1	0.05	0.14	4.66 (1.21–17.87)	0.027**
MTRR 66G * *AGT* 704T	0.21	0.31	0.12	0.27 (0.08–0.89)	0.034*
MTR 2756G * *VEGFA* 634C	0.05	0	0.1	> 20	< 0.0001**
AGT 704T * *VEGFA* 634G	0.24	0.32	0.18	0.31 (0.12–0.84)	0.023*

*Decreased FGR risk

**Increased FGR risk

particularly) were investigated in association with FGR risk. Two other candidate polymorphisms were also studied including *AGT* 704T > C and *VEGFA* 634C > G since they affect placental perfusion and angiogenesis, respectively.

Folate is one of the most important micronutrients during pregnancy since it provides the one-carbon units for homocysteine (Hcy) remethylation to methionine. High levels of homocysteine are known to be associated with several pregnancy complications due to different mechanisms [11]. Accordingly, the previous researches have studied genetic variations of folate metabolism enzymes in many fetal and pregnancy syndromes, but studies concerning their association particularly with FGR are limited and inconsistent [27].

According to our data, among the three studied polymorphisms affecting those enzymes (*MTHFR* 677C > T; *MTRR* 66A > G; and *MTR* 2756A > G), only *MTRR* 66A > G showed a significant association with FGR risk. We figured that *MTRR* 66(GG) has a significant association with increased FGR risk since it was more than two times frequent among FGR cases (43.2%) compared to controls (19.6%). MTRR is responsible for converting methionine synthase into the active status (as shown in Table 1). Its gene is located at 5p15.2–p15.3 with the most frequent polymorphism at position 66 causing a substitution of methionine to isoleucine at codon 22 [7]. The figured role of *MTRR* 66GG as a possible FGR risk factor can be explained by the fact that this genotype was known to be associated with decreased enzymatic activity

leading to higher homocysteine concentration and DNA methylation [15]. Increased maternal homocysteine levels are associated with various vascular-related complications of pregnancy including FGR (D'Souza and Glazier 6). It is also known that mid-pregnancy elevated maternal homocysteine has independent effect on placenta-mediated pregnancy complications including FGR [2].

To our knowledge, this is the first study to show *MTRR* 66(AG) genotype as a protective factor against FGR risk. It could be suggested that the presence of the allele (A) in heterozygotes somehow mitigates the effect of allele (G) and provides the optimal level of *MTRR* genetic expression for fetal growth. The previous results are consistent with the fact that *MTRR* 66GG is associated with pregnancy complications, such as recurrent spontaneous abortion [13, 24]. However, less studies concerned its association particularly with FGR and instead focused mainly on *MTHFR* 677C > T. Most of these studies confirmed *MTHFR* 677TT as associated with a higher FGR risk which our data have not shown probably due to population and ethnicity differences. Moreover, in contrast with our results, Vaughn et al. [25] found that existence of *MTRR* 66GG can aggravate the effects of *MTHFR* 677TT (see [15]).

Another polymorphism showed a significant association with FGR risk is *AGT* 235Met > Thr. This gene encodes angiotensinogen, the angiotensin precursor, and renin substrate in renin–angiotensin system (RAS) responsible for blood pressure maintaining. The main polymorphism of *AGT*, located on chromosome 1 (lq42–43), represents a single-base substitution of thymine (T) at the second exon with cytosine (C) leading to methionine to threonine substitution at position 235 and thus higher level of genetic expression [1]. Because of its effect increasing AGT levels and blood pressure, this polymorphism was studied during pregnancy mainly in association with preeclampsia and hypertension and rarely in association with FGR. This study showed *AGT* 704T > C mutant allele as associated with lower FGR risk. In contrast with our results, Zhang et al. [28] have proved mutant allele *AGT* 704C as associated with higher FGR risk and more frequent in idiopathic FGR pregnancies in China. They suggested placental abruption as AGT mechanism of action in FGR (see [5]).

According to our data, *AGT* 704T allele was associated with higher risk of FGR while the mutant allele (C) was significantly more frequent in normal pregnancies compared to those with FGR diagnosis. MDR analysis of gene–gene interaction supports this result since dendrogram, and Fruchterman–Rheingold scheme revealed antagonistic relationship of *MTRR* 66A > G with *AGT* 704T > C. Our finding is supported by the fact that physiologic pregnancy is characterized by an overall upregulation of RAS, increased angiotensinogen production and angiotensin II levels which stimulates aldosterone secretion, causes sodium retention and fluid overload, essential to obtain adequate placental perfusion and thus adequate fetal growth [8]. *AGT* 235Thr carriers, according to many studies, showed an increase in serum AGT levels by 13–20%. Elevated AGT levels are associated with increased angiotensin II concentrations causing higher RAS activation and thus could be suggested as contributing to appropriate fetal growth minimizing FGR risk [12, 29]. The inconsistency of our results with Zhang et al. [28], mentioned above, could be explained by population heterogeneity since all participants in this study are Caucasians. Future clinical trials on different ethnicities are needed to figure the exact role of *AGT* polymorphism in FGR.

We have also analyzed the LD between the three targeted genes *AGT* 704T > C, *MTR* 2756A > G, and *MTHFR* 677C > T since they are all located on chromosome 1. *MTR* 2756A > G and *MTHFR* showed a high possibility of LD in our population ($D' = 0.999$). It could be suggested that women with *MTHFR* 677C > T mutant allele are with high possibility of being *MTR* 2756 (G) allele carriers which will negatively affect the function of two folate metabolism enzymes. Thus, we recommend future investigations to ensure this finding.

It should be mentioned that sample size is a limitation of this study. Small sample size resulted from patient inclusion criteria since all patients were diagnosed with FGR as a separated syndrome. Any case of FGR associated with another complication such as preterm birth, preeclampsia, or diabetes was excluded. Further studies with larger sample size are recommended to confirm all our findings.

Conclusion

In conclusion, this study suggests *MTRR* 66(GG) and *AGT* 704(T) allele as risk factors of FGR in Rostov region population. On the other hand, *MTRR* 66(AG) and *AGT* 704(C) were associated with lower risk of this syndrome and can be considered as protective factors. We have also shown the antagonistic interaction of these two polymorphisms in FGR patients. These findings will help to increase our understanding about genetic aspects of FGR and support the tendency of finding new prenatal prognostic markers and thus improve diagnosis, prognosis, and prevention.

Abbreviations

FGR Fetal growth restriction
MTHFR Methylenetetrahydrofolate reductase
MTRR Methionine synthase reductase
MTR Methionine synthase
AGT Angiotensinogen

VEGFA Vascular endothelial growth factor A
LD Linkage disequilibrium

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Author contributions
D.A. performed data input, methodology, statistical analysis, and writing—review and editing; E.V.B. and I.O.P. contributed methodology and review and editing; and E.A.Z. performed samples collection and FGR diagnosis. T.P.S. and N.B.K. were supervisors.

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Availability of data and materials
Detailed genotyping data are available on request from the corresponding author. The raw data are not publicly available due to privacy or ethical restrictions.

Declarations

Ethics approval and consent to participate
Study design and methods were approved by the Ethics Committee of Southern Federal University. All participants provided willingly written informed consent for inclusion before they participated in the study.

Consent for publication
Not applicable.

Competing interests
The authors have no competing interests to declare that are relevant to the content of this article.

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