

Genetic markers for inherited thrombophilia are associated with fetal growth retardation in the population of Central Russia

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Abstract

Aim: The aim of this study was to examine the role of hereditary thrombophilia in the development of fetal growth retardation (FGR) in the population of Central Russia.

Methods: The case-control study sample included 497 women in the third trimester of pregnancy recruited during 2009–2013. The participants were enrolled into two groups: patients with FGR ($n = 250$) and controls without FGR ($n = 247$). The participants were genotyped for four genetic markers of hereditary thrombophilia: factor V Leiden ($G > A$ *FV*, rs6025), prothrombin ($G > A$ *FII*, rs1799963), factor VII ($G > A$ *FVII*, rs6046), and fibrinogen ($G > A$ *FI*, rs1800790).

Results: The genetic factors for an increased risk of FGR were allele *G* of rs6046 (odds ratio [OR] = 2.34) and genotype *GG* of rs6046 (OR = 2.64), whereas genotype *GA* of rs6046 had the protective value (OR = 0.42). A combination of alleles *G* of rs1799963, *A* of rs6046, and *G* of rs1800790 (OR = 0.31) reduces the risk of FGR.

Conclusion: Polymorphism rs6046 of the *FVII* gene is associated with the development of FGR.

Key words: factor VII coagulant, fetal growth retardation, pregnancy, single nucleotide polymorphism.

Introduction

Thrombophilia is the pathological condition of the body characterized by an increased risk of intravascular thrombosis due to inherited or acquired disorders of hemostasis.^{1,2} Women with acquired or inherited thrombophilia are at increased risk for thrombotic and gestational complications.^{3–10} At least 50% of thrombotic complications of pregnancy are associated with thrombophilia.^{6,11} Thrombophilia is the most important risk factor for several obstetric complications, for example, fetal loss syndrome (39.1%), severe pre-eclampsia (18.2%), chorionic detachment (11.5%),

thrombosis during pregnancy and the post-partum period, and placental abruption (13.63%).¹² The most significant markers for the development of gestational complications of congenital thrombophilia are mutation Leiden 1691*G > A* *FV*, prothrombin 20210*G > A* *FII*, plasminogen activator inhibitor 4*G > 5G* *PAI-1*, 677*S > T* *MTHFR*, deficiency of protein S, protein C, antithrombin III, and others.^{9,10,12} Over 52% of women with obstetric complications have thrombophilic gene defects.⁷

Factor V Leiden thrombophilia is caused by a substitution of *G* by *A* at position 1691 of the gene and, respectively, replacement of arginine by glutamine at

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position 506 of the protein.^{13,14} This results in resistance of the factor V Leiden variant to inactivation by the activated protein C and thus promotes clotting.^{14,15} The polymorphism inheritance follows the autosomal dominant principle. About 4–7% of European Caucasians are heterozygous for this mutation.¹⁶

The 20210G > A *FII* variant of prothrombin is located in the 3'-untranslated region of the gene. Although this substitution does not result in an amino acid change, it was associated with the up to 1.5–2 times elevated level of the normal prothrombin.¹⁷ The elevated level of prothrombin results in the higher level of thrombin that activates clotting through the positive feedback mechanism. Such an activation of the clotting mechanism confers an increased risk of thrombosis.¹⁷ Inheritance of the polymorphism follows the autosomal dominant principle. The frequency of heterozygotes in Caucasians is about 2–5%.^{1,18,19}

The –455G > A *FI* polymorphism is located in the promoter of the fibrinogen β -chain. It has been associated with the increased risk of thrombosis.²⁰ The prevalence of this polymorphism in European populations is 5–10%.¹

Mutation 10976G > A *FVII* results in a replacement of Arg by Gln in position 353 of the respective protein. This mutation was associated with the low concentration of the FVII protein in blood plasma.²¹ Heterozygotes 10976G > A *FVII* have 25% lower concentration of the protein, homozygotes 10976AA – 50% lower as compared to the most common genotype 10976GG. In addition, homozygotes 10976GG are at higher risk for cardiovascular health problems and complications at pregnancy.^{21,22} The frequency in the European population is 10–20%.^{23,24}

The available data about the role of the genetic markers for inherited thrombophilia in the fetal growth retardation are inconsistent. Several studies have reported a significant association of the 1691G > A *FV* with FGR in Caucasian females from: Canada (odds ratio [OR] = 2.7; 95% confidence interval [CI], 1.3–5.5),⁵ the USA (OR = 1.23; 95%CI, 1.04–1.44),²⁵ and France (OR = 2.58; 95%CI, 0.83–8.04).²⁶ The significant association of the 20210G > A *FII* polymorphism with FGR was determined in Caucasian women from Canada (OR = 2.5; 95%CI, 1.3–5.0)⁵ and France (OR = 2.03; 95%CI, 0.51–8.01).²⁶ The contradictory data were reported by Franchi *et al.*,⁴ who did not find significant associations of the 1691G > A *FV*, 20210G > A *FII*, and 677C/T *MTHFR* polymorphisms with FGR. We have not found any data about the association of the –455G > A *FI* and 10976G > A *FVII* polymorphisms with FGR.

Methods

Study population

In total, 497 unrelated pregnant women in the third trimester of pregnancy were recruited for the study during 2009–2013. All participants signed an informed consent before entering the study. The clinical and laboratory examination of the participants was conducted in the perinatal center of the Saint Joasaph Belgorod Regional Clinical Hospital. The following inclusion criteria were used to check eligibility of the participants: singleton pregnancy and Russian ethnicity. Patients having congenital malformations of internal genitals, uterine fibroids, anomalies of placental location, isosensitization of Rh factor or ABO, or genetic diseases Q5 were excluded from the study.

Two hundred and fifty participants were diagnosed with the syndrome of intrauterine growth retardation of varying severity. The diagnosis of the syndrome was based on clinical data, ultrasound fetometry (TOSHIBA XARIO SSA-660A) and parameters of growth and weight after the birth. Based on the differences between the fetometric data and nomograms, three degrees of FGR were classified: first degree corresponded to the reduction of the estimated gestational ages from the standard ones by 2 weeks; second degree – by 3–4 weeks; and third degree – by more than 4 weeks.²⁷

The controls were 247 women without the syndrome of intrauterine growth retardation.

DNA isolation and genotyping assay

Each participant donated 6 mL of blood drawn from a cubital vein. Genomic DNA was isolated according to Miller *et al.*²⁸ The participants were genotyped for four candidate genetic markers for hereditary thrombophilia: Leiden mutation of coagulation factor V (G > A *FV*, rs6025), prothrombin (G > A *FII*, rs1799963), factor VII (G > A *FVII*, rs6046), and fibrinogen (G/A *FI*, rs1800790).

The polymorphisms were genotyped using the TaqMan real-time PCR assays with the standard primers and probes (Syntol, Russia).^{29,30} The data were processed using BioRad CFX Manager 3.1 (Bio-Rad Laboratories, Inc.).

Statistical analysis

The obtained genotypes were checked for their correspondence to the Hardy–Weinberg distribution (HWE) using the χ^2 -test. Association of the polymorphisms with FGR was estimated by analyzing 2 × 2 contingency tables with the χ^2 -test and Yates' correction for continuity. The risk was estimated using

OR with 95%CI. Association of the polymorphisms with FGR was analyzed using logistic regression with adjustment for cofactors (somatic and gynecological pathologies). The analyses were performed using STATISTICA 6.0 (StatSoft).

Possible allele combinations of the studied polymorphisms and their significance for FGR were analyzed using the Markov chain Monte Carlo and Bayes nonparametric statistics as implemented in APSampler (<http://sources.redhat.com/cygwin/>).³¹ Bonferroni correction was applied to adjust for multiple comparisons.

Results

The summary characteristics of the case and control groups are given in Table 1.

First-degree FGR was diagnosed in 133 patients (53.20%), second-degree in 96 patients (38.40%), and third-degree in 21 patients (8.40%).

The FGR patients had a 1.8-fold higher rate of spontaneous abortion predominantly in the first trimester of pregnancy than the controls.

All genotypes but one showed no deviation from the HWE. The only case of the deviation was observed in the case group at the *G/A FV rs6025* locus ($\chi^2 = 6.58$; P

< 0.05). This can be explained by the presence of a single homozygote *AA FV rs6025*. After this genotype was removed, no deviation was observed ($\chi^2 = 0.09$; $P > 0.05$).

FGR patients had higher frequency of allele *G FVII rs6046* (88.72%) and genotype *GG FVII rs6046* (77.87%), and lower frequency of genotype *GA FVII rs6046* (21.70%) as compared to the controls (77.07%, 57.07%, and 40.00%, respectively; Table 2).

The frequency of a combination of alleles *G FII rs1799963*, *A FVII rs6046*, and *G FI rs1800790* was approximately half (19.8%) that in the case group as compared to the controls (44.5%) (OR = 0.31; 95%CI, 0.20–0.48; P Fischer's < 0.0001 , P Bonferroni < 0.0001).

The results of the logistic regression analysis showed that polymorphism *G > A FVII rs6046* was significantly associated with a risk for FGR both individually and after adjustment for the cofactors, which were present in the patients and absent in the controls (i.e., miscarriage, essential hypertension; Table 3).

Discussion

The present study shows the association of genetic markers of hereditary thrombophilia *G > A FVII rs6046* with the development of FGR in the population of

Table 1 Physical characteristics and various medical pathologies in the study participants

	FRG patients, <i>n</i> (%)	Controls, <i>n</i> (%)	<i>P</i>
<i>N</i>	250	247	—
Age, years (min–max)	26.78 ± 4.81 (16.0–45.0)	26.20 ± 5.01 (19.0–41.0)	>0.05
BMI, kg/m ²	23.37 ± 4.31	23.90 ± 3.96	>0.05
Somatic pathologies			
Essential hypertension	50 (20.00)	29 (11.74)	0.02
Chronic pyelonephritis	63 (25.20)	55 (22.27)	0.51
Obesity	23 (9.20)	22 (8.91)	1.00
Varicose veins	13 (5.20)	10 (4.05)	0.69
Venous thromboembolism at pregnancy	4 (1.60)	1 (0.41)	0.38
Chronic gastroduodenitis	26 (10.40)	22 (8.89)	0.68
History of cerebrovascular disease	2 (0.80)	0	0.50
Gynecological pathologies			
History of medical abortion	80 (32.00)	65 (26.32)	0.20
History of infertility	5 (2.00)	8 (3.24)	0.56
History of miscarriage (total)	46 (18.40)	25 (10.12)	0.01
Miscarriage in first trimester	36 (14.40)	20 (8.10)	0.04
Pregnancy loss in first trimester	12 (4.80)	12 (4.86)	1.00
Ectopic pregnancy	10 (4.00)	15 (6.07)	0.39
History of disorders of the menstrual cycle	20 (8.00)	14 (5.67)	0.39
History of pelvic inflammatory disease	58 (23.20)	59 (23.89)	0.94
Intrauterine infection during pregnancy	83 (33.20)	80 (32.39)	0.68
Pre-eclampsia	55 (22.00)	68 (27.53)	0.19
Antenatal intrauterine fetal death	4 (1.60)	0	0.14

Table 2 Frequencies of the studied SNP and respective genotypes in case and control samples

Polymorphism	Studied groups	Alleles, genotypes	Frequency, %	OR (95%CI)	χ^2	<i>P</i>
<i>G > A FII rs1799963</i>	Case	G	98.40	0.44 (0.09–1.83)	0.87	0.35
	Control	G	99.29			
	Case	GG	96.80	0.44 (0.09–1.83)	0.88	0.35
	Control	GG	98.58			
	Case	GA	3.20	2.29 (0.55–11.04)	0.88	0.35
	Control	GA	1.42			
	Case	AA	0	—	—	—
	Control	AA	0	—	—	—
<i>G > A FI rs1800790</i>	Case	G	79.20	1.18 (0.87–1.61)	1.04	0.31
	Control	G	76.32			
	Case	GG	64.40	1.27 (0.87–1.86)	1.47	0.23
	Control	GG	58.70			
	Case	GA	29.60	0.77 (0.52–1.15)	1.55	0.21
	Control	GA	35.22			
	Case	AA	6.00	0.99 (0.46–2.19)	0.001	1.00
	Control	AA	6.07			
<i>G > A FV rs6025</i>	Case	G	97.77	0.59 (0.19–1.74)	0.63	0.43
	Control	G	98.67			
	Case	GG	95.95	0.65 (0.21–1.97)	0.34	0.56
	Control	GG	97.35			
	Case	GA	3.64	1.32 (0.44–4.46)	0.12	0.73
	Control	GA	2.65			
	Case	AA	0.40	—	—	—
	Control	AA	0	—	—	—
<i>G > A FVII rs6046</i>	Case	G	88.72	2.34 (1.60–3.44)	20.53	0.0005
	Control.	G	77.07			
	Case	GG	77.87	2.64 (1.71–4.09)	20.88	0.0005
	Control	GG	57.07			
	Case	GA	21.70	0.42 (0.27–0.64)	16.52	0.0006
	Control	GA	40.00			
	Case	AA	0.43	0.21 (0.01–2.03)	1.13	0.29
	Control	AA	2.93			

Table 3 Results of the univariate regression analysis (*P*-values) of association of the *G > A FVII rs6046* polymorphism with FGR

Parameters	Genotype <i>G > A FVII rs6046</i>	Genotype adjusted for cofactors
History of miscarriage (total)	0.0003	0.04
Miscarriage in first trimester	0.004	0.04
Essential hypertension	0.003	0.04

Central Russia. Genetic factors of an increased risk for FGR are allele *G FVII rs6046* and genotype *GG FVII rs6046*, whereas allele *A FVII rs6046* has a protective value. The combination of alleles *G FII*, *A FVII*, and *G FI* reduces the risk of FGR (OR = 0.31; 95%CI, 0.20–0.48).

The association of the *rs6046* polymorphism of the *FVII* gene with FGR remained significant after

adjustment for cofactors (miscarriage and essential hypertension). However, the significance level was lower (*P* = 0.04 vs *P* = 0.003–0.004 without the adjustment). This may be due to the opposite effect of this polymorphism and the cofactors: Allele A was protective for FGR (OR = 0.43 for allele A [95%CI, 0.29–0.63] and 0.42 for genotype GA [95%CI, 0.27–0.64]), while essential hypertension and miscarriage were 1.7–1.8-fold more common in the FGR patients than in the controls, which was implicated in a higher risk for FGR.^{32,33}

The observed association of the *G > A FVII rs6046* polymorphism with the development of FGR is consistent with the available data on the physiological role of this factor in the hemostatic system. According to modern concepts, coagulation factor VII (proconvertin) is one of the key factors in the coagulation cascade and participates in the external way of the activation of blood coagulation.^{22,34} The complex consisting of tissue factor, the active form of the

Table 4 Literature data about the association of the hereditary thrombophilia and FGR in various Caucasian populations

Country	Results/conclusions	Reference
Canada	Association of Leiden 1691G/A FV with FGR (OR = 2.7; 95%CI, 1.3–5.5)	Howley <i>et al.</i> ⁵
USA	Association of Leiden 1691G/A FV with FGR (OR = 1.2; 95%CI, 1.04–1.44)	Facco <i>et al.</i> ²⁵
France	Association of Leiden 1691G/A FV with FGR (OR = 2.6; 95%CI, 0.83–8.04)	Verspyck <i>et al.</i> ²⁶
Canada	Association of prothrombin 20210G/A FII with FGR (OR = 2.5; 95%CI, 1.3–5.0)	Howley <i>et al.</i> ⁵
France	Association of prothrombin 20210G/A FII with FGR (OR = 2.0; 95%CI, 0.51–8.01)	Verspyck <i>et al.</i> ²⁶
Italy	No association of Leiden 1691G/A FV and prothrombin 20210G/A FII with FGR	Franchi <i>et al.</i> ⁴

FGR, fetal growth retardation.

coagulation factor VIIa, and calcium ions promotes formation of the active forms of factor X (Xa), which is a common way of initiating the coagulation cascade. In addition, the complex is able to activate factor IX (IXa) of the intrinsic pathway of blood coagulation that further strengthens the process of blood coagulation.^{22,34}

The *Factor VII* gene is located on the long arm of chromosome 13 (13q34). The GA heterozygotes and AA homozygotes of *FVII rs6046* have, respectively, approximately 25% and 50% lower blood concentration of Factor VII as compared with the wild-type GG heterozygotes.^{21,35} The elevated levels of this factor can result in a hypercoagulable state and thrombotic complications,^{21,22,33} causing the development of placental insufficiency with the formation of FGR.

We have not found any significant associations of markers $G > A$ *FI rs1800790*, $G > A$ *FII rs1799963*, or $G > A$ *FV rs6025* with a risk for FGR. This is in concordance with some previous studies on American and European Caucasians,^{1,4} whereas it contradicts other studies^{5,25,26} (Table 4).

The inconsistency in the results may be related to the respective differences in the ethnic and genetic backgrounds of the studied populations. Population genetic stratification was shown to have a significant effect on the prevalence and expression of quantitative traits in various populations.³⁶

The results of the study show that the polymorphism $G > A$ *FVII rs6046* alone, as well as in combination with other markers of hereditary thrombophilia, $G > A$ *FI rs1800790* and $G > A$ *FII rs1799963*, is associated with the development of FGR. These results extend the existing ideas about the role of hereditary thrombophilia in the pathogenesis of placental insufficiency syndrome and fetal growth retardation.

Disclosure

No author has any potential conflict of interest.

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