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Original Article

The VNTR polymorphism of the endothelial nitric oxide synthase gene and blood pressure in women at the end of pregnancy



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ABSTRACT

Objective: Examine the association of the 4a/4b polymorphism of endothelial nitric oxide synthase (eNOS) with blood pressure in women at late pregnancy.

Materials and methods: Blood pressure before pregnancy and at the end of gestation (37–40-week term) was measured in 588 women of the Russian ancestry. The women were divided into groups according to the body mass index and the presence of preeclampsia at late pregnancy. The 4a/4b polymorphism of the eNOS gene was genotyped using PCR with subsequent screening of amplified fragment length polymorphisms.

Results: The 4a4a eNOS genotype was associated with higher levels of diastolic blood pressure in pregnant women and with more pronounced dynamics of the diastolic and mean arterial pressure in the development of pregnancy ($p = 0.02$ – 0.03). Pregnant women with the 4a4a genotype and increased body mass index had higher systolic, diastolic, and mean arterial pressure ($p = 0.001$ – 0.009). In pregnant women with preeclampsia, the 4a4a genotype was associated with higher level of diastolic blood pressure at the end of pregnancy ($p = 0.04$), whereas in the women without preeclampsia this genotype was associated with more pronounced changes of blood pressure at pregnancy ($p = 0.02$).

Conclusion: The results of our study suggest that the genotype 4a4a of the eNOS gene is associated with higher levels of blood pressure in women at the end of pregnancy.

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Introduction

Pregnancy is one of the physiological conditions, which requires long-term and fundamental adjustments of many functional systems of the organism in order to maintain homeostasis. In the course of pregnancy, the cardiovascular system experiences an increased load due to augmented metabolism, larger volume of circulating blood, development of uteroplacental circulation, gain in body weight, and elevated production of several hormones [1].

Hypertension is one of the common health complications during pregnancy. Its prevalence among pregnant women is 12–22% [2]. Pregnant women with hypertension are predisposed to several

health problems, such as placental abruption, disseminated intravascular coagulation (DIC), brain hemorrhage, acute renal failure, and eclampsia [3–5].

Nitric oxide (NO) is a potent vasodilator synthesized from L-arginine with the aid of endothelial NO-synthase (eNOS) [6].

The eNOS gene is located on chromosome 7q35–36 and has 26 exons. Among several polymorphisms identified in the gene, Glu298Asp (rs1799983) in exon 7, 4a/4b in intron 4, and T2786C (rs2070744) in the promoter region [7] have been commonly studied for their association with functions of the cardiovascular system in healthy and diseased patients [8–10].

Polymorphism 4a/4b is located within the minisatellite in intron 4 of the gene. Its alleles have varying number of 27-bp long tandem repeats (allele 4a has 4 repeats and allele 4b–5 repeats, respectively) [7]. The polymorphism was associated with a decrease of NO level in blood plasma, which was implicated in respective reduction of nitrite and nitrate levels [11,12].

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There is evidence that heterozygotes of the 4a/4b polymorphism have lower concentration and activity of *e*NOS as compared to homozygotes 4b4b [11]. Homozygotes 4a4a manifested lower level of NO in blood plasma as compared to both 4b4b and 4a4b genotypes [12]. Also, a lower level of NO was reported in carriers of allele 4a as compared to carriers of allele 4b [13].

The previous studies estimated the frequencies of the 4a/4b genotypes in a Russian population as 2.0–4.1% for 4a4a, 23.8–29.4% for 4a4b, and 68.6–72.1% for 4b4b [14,15].

The available data on the association of the 4a/4b polymorphism with blood pressure in pregnant females are contradictory [8,16–23]. In this regard, further studies are needed to investigate the role of this locus in the functioning of the cardiovascular system in pregnant women.

While there are quite a few studies on association of the 4a/4b polymorphism in various ethnic populations, no such analysis has been conducted in Russians. The present study is the first, which examined association of the 4a/4b polymorphism of endothelial nitric oxide synthase (*e*NOS) with blood pressure in Russian women at late pregnancy.

Materials and Methods

Study subjects

This study was approved by the Regional Ethics Committee at Belgorod State National Research University. An informed consent was obtained from each participant prior to the enrollment in the study.

The participants were recruited through the Perinatal Centre of St. Joasaph Belgorod Regional Clinical Hospital. The eligible participants were 588 unrelated women of Russian descent (self-declared) born in Central Russia [24]. The age of the women varied from 20 to 43 years (mean age 27.98 ± 4.50 years). Clinical and laboratory examinations of the participants were conducted at the Perinatal Centre (Division of Pathological Pregnancy) of St. Joasaph Belgorod Regional Clinical Hospital. The inclusion criteria were (a) Russian ethnicity and (b) pregnancy at 37–40 weeks of gestation. Pregnant women diagnosed with diabetes mellitus, hepatic or renal failure were excluded from the study. Blood pressure (BP) level was measured three times according to the American Heart Association recommendations [25] by an experienced obstetrician. The measurements were done in the morning (between 07:00–09:00) and evening (between 19:00–21:00). The data on the BP level before pregnancy was obtained from the medical records of each participant. The mean arterial pressure (MAP, mm Hg) was calculated as follows: $MAP = (SBP + 2DBP)/3$, where SBP is systolic blood pressure and DBP is diastolic blood pressure.

All study participants were divided into three groups according to the BP level at the end of pregnancy: normotensive women (SBP from 90 to 140 mm Hg and/or DBP from 60 to 90 mm Hg), hypotensive women (SBP < 90 mm Hg and/or DBP < 60 mm Hg), and hypertensive women (SBP > 140 mm Hg and/or DBP > 90 mm Hg).

All participants were also categorized according to BMI (kg/m^2): underweight (BMI < 18.5), normal weight (BMI = 18.5–24.9), overweight (BMI = 25–29.9), and obese (BMI \geq 30) [26].

Among 591 participants, 209 were with normal gestation and 382 had preeclampsia. Preeclampsia was defined as the presence of hypertension accompanied by proteinuria (24 h urine protein excretion above 300 mg [1]).

DNA extraction and genotyping

Blood (8–9 ml) was taken from the ulnar vein. Genomic DNA was isolated according to Miller et al. [27]. The 4a/4b VNTR polymorphism (4a/4b *e*NOS) for the analysis were selected based on the

following criteria [28]: previously reported associations with blood pressure, regulatory potential, effect on gene expression. Genotyping of the 4a/4b *e*NOS was performed using a polymerase chain reaction (PCR) assay according to the protocol described elsewhere [29].

Statistical analysis

The correspondence of the allele and genotype frequencies of the 4a/4b *e*NOS gene polymorphism to the Hardy–Weinberg equilibrium (HWE) was estimated by the chi-square test. The *p*-values were adjusted for multiple testing using the Bonferroni correction (p_{cor}). The distribution of the quantitative traits such as SBP, DBP, MAP and pulse pressure (the pressure difference between the systolic and diastolic pressures) before pregnancy and at the end of pregnancy were analyzed by the Shapiro–Wilk's test [30]. Since the values of the quantitative traits did not follow the normal distribution, they were described using median (Me) and interquartile range (Q25–Q75) and compared between the groups using the Mann–Whitney test [31]. The linear multiple regression analysis was used to estimate the effect of age, weight, and *e*NOS genotypes on the BP parameters in the participants. As the dependent variable (BP parameters) did not follow normal distribution, the analysis was performed with the logarithmically transformed values. All statistical analyses were conducted using STATISTICA for Windows v. 6.0 (StatSoft, USA).

Results

The biomedical and clinical characteristics of the study participants are shown in Table 1.

The prevalence of hypertension before pregnancy and at the end of pregnancy among the women was 10.3% and 51.7%, respectively. The prevalence of hypotension dropped from 19.1% prior to pregnancy to 4.1% at the end of pregnancy. Overall, blood pressure tended to increase towards the end of pregnancy as compared to that prior to pregnancy. Obesity was determined in 20.6% of the participants (BMI > 30 kg/m^2); 15.1% of the participants were overweight (BMI = 25–29.9 kg/m^2) and 8.1% were underweight (BMI < 18.5 kg/m^2); 7.8% of the women had somatic diseases (vegetative-vascular dystonia, chronic glomerulonephritis, chronic pyelonephritis, chronic cystitis, polycystic kidney disease, hydro-nephrosis, pyelectasis).

The observed allele and genotype frequencies were in the Hardy–Weinberg equilibrium. The frequency of allele 4b in the entire group ($n = 591$) was 0.801. The frequencies of genotypes 4b4b, 4a4b, and 4a4a were 64.2%, 31.7%, and 4.1%, respectively, which corresponded to the data reported for other European populations [20,29].

No association of the 4a/4b polymorphism with the BP parameters prior to pregnancy was determined ($p > 0.05$) (Table 2).

Pregnant women with genotype 4a4a had higher DBP ($p = 0.02$) and more pronounced changes in DBP and MAP during gestation as compared to the carriers of allele 4b (genotypes 4b4b and 4a4b) (Table 2).

The results of the multiple regression analysis suggested that age, BMI, preeclampsia and the 4a/4b polymorphism are associated with DBP and MAP at the end of gestation (Table 3). Overall, this polymorphism accounts of about 38–65% variation of the BP parameters in pregnant women at the end of gestation.

The results of the univariate analysis suggested that the 4a/4b polymorphism is associated with the BP parameters in women at the end of pregnancy both individually and through the interaction with age, BMI and preeclampsia.

Only in the women with elevated BMI (more 25 kg/m^2), the 4a/4b *e*NOS polymorphism was associated with the blood pressure parameters at the end of pregnancy (Table 4). In this group, the

Table 1

The biomedical and clinical characteristics of the study participants.

Value variables, Me (Q25–Q75)				
Number	591			
Age, year	26.0 (24.0–31.0)			
Height, m	1.65 (1.62–1.68)			
	Before Pregnancy		During pregnancy (37–40 weeks term)	
Weight, kg	70.0 (59.0–81.7)		79.3 (71.6–90.4)	
BMI, kg/m ²	23.0 (21.2–26.9)		29.2 (26.4–33.8)	
			Pregnant women without preeclampsia	Pregnant women with preeclampsia
SBP, mmHg	110.0 (110.0–120.0)	130.0 (115.0–145.0)	110.0 (110.0–120.0)	140.0 (135.0–150.0)
DBP, mmHg	70.0 (70.0–80.0)	80.0 (75.0–90.0)	70.0 (70.0–75.0)	90.0 (85.0–100.0)
PBP, mmHg	40.0 (40.0–45.0)	50.0 (40.0–55.0)	40.0 (40.0–40.0)	50.0 (50.0–60.0)
MAP, mmHg	83.3 (81.7–93.3)	100.0 (88.3–110.0)	85.0 (83.3–90.0)	106.7 (103.3–116.7)
ΔMAP, mmHg		13.3 (3.3–23.3)	5.0 (0.0–10.0)	25.0 (20.0–35.0)
ΔSBP, mmHg		20.0 (10.0–30.0)	0.0 (0.0–10.0)	20.0 (10.0–25.0)
ΔDBP, mmHg		10.0 (0.0–20.0)	3.3 (0.0–8.3)	20.0 (13.3–28.3)
Hypotension	113 (19.1%)		24 (4.1%)	
Normotension	417 (70.6%)		261 (44.2%)	
Hypertension	61 (10.3%)		306 (51.7%)	

Abbreviations: Me, median; Q25–Q75, interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PBP, pulse blood pressure; MAP, mean arterial pressure; Δ MAP, change of mean arterial pressure during gestation; ΔSBP, change of systolic blood pressure; ΔDBP, change of diastolic blood pressure.

Table 2

Association of the 4a/4b polymorphism of the eNOS gene with blood pressure parameters in women prior to pregnancy and women at the end of pregnancy, Me (Q25–Q75).

Parameters of blood pressure	Genotypes		p
	4b4b+4a4b (n = 562)	4a4a (n = 24)	
Women prior to pregnancy			
SBP, mmHg.	110.0 (110.0–120.0)	110.0 (107.5.0–115.0)	0.4
DBP, mmHg	70.0 (70.0–80.0)	70.0 (62.5–70.0)	0.1
PBP, mmHg	40.0 (40.0–45.0)	40.0 (40.0–47.5)	0.6
MAP, mmHg	83.3 (83.3–90.0)	83.3 (76.7–85.0)	0.2
Women at the end of pregnancy			
SBP, mmHg.	130.0 (120.0–145.0)	140.0 (120.0–157.5)	0.1
DBP, mmHg	80.0 (75.0–90.0)	90.0 (80.0–100.0)	0.02
PBP, mmHg	50.0 (40.0–55.0)	50.0 (40.0–57.0)	0.6
MAP, mmHg	100.0 (83.3–110.0)	110.0 (95.0–117.5)	0.05
ΔMAP, mmHg	13.3 (5.0–23.3)	25.0 (5.8–33.3)	0.03
ΔSBP, mmHg	20.0 (5.0–30.0)	30.0 (10.0–40.0)	0.1
ΔDBP, mmHg	10.0 (0.0–20.0)	20.0 (7.5–30.0)	0.02

Abbreviations: Me, median; Q25–Q75, interquartile range; P, p-value for Mann–Whitney test; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PBP, pulse blood pressure; MAP, mean arterial pressure during gestation; ΔMAP, change of mean arterial pressure; ΔSBP, change of systolic blood pressure; ΔDBP, change of diastolic blood pressure.

Table 3

Results of the multiple regression analysis for interaction effect between the age, height, preeclampsia, genotypes of the 4a/4b eNOS polymorphism and blood pressure parameters at the end of pregnancy.

Parameters		Beta	Student's p	R ²	F-test, p
SBP	Age	0.11	<0.0001	0.65	<0.0001
	BMI	0.17	<0.0001		
	preeclampsia	−0.72	<0.0001		
	4a/4b eNOS	0.05	0.037		
DBP	Age	0.09	0.002	0.58	<0.0001
	BMI	0.12	<0.0001		
	preeclampsia	−0.70	<0.0001		
	4a/4b eNOS	0.06	<0.025		
PBP	Age	0.10	0.003	0.38	<0.0001
	BMI	0.17	<0.0001		
	preeclampsia	−0.52	<0.0001		
	4a/4b eNOS	0.02	<0.46		
MAP	Age	0.10	0.0001	0.64	<0.0001
	BMI	0.14	<0.0001		
	preeclampsia	−0.72	<0.0001		
	4a/4b eNOS	0.06	<0.02		

Abbreviations: Beta, standardised regression coefficients; R², correlation coefficient; Student's p, p-value for Student's t-test; P, p-value for F-test; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PBP, pulse blood pressure; MAP, mean arterial pressure during gestation.

4a4a genotypes had the highest values of SBP, DBP and MAP as compared to the 4b4b and 4a4b genotypes.

The results of the univariate regression analysis suggested that the 4a/4b polymorphism is associated with the BP parameters in overweight and obese women (BMI>25) at the end of pregnancy both individually and through the interaction with age, BMI and preeclampsia.

The pregnant women with preeclampsia had a higher frequency of the 4a4a genotype as compared to the women without preeclampsia (4.7% and 2.9%, respectively), but these differences were not statistically significant (OR = 1.63, 95% CI 0.60–4.68, $p = 0.42$).

Changes in DBP were more pronounced in healthy pregnant women with genotype 4a4a than in the women with either genotype 4b4b or 4a4b ($p = 0.02$, Table 5).

Likewise, the 4a4a genotype carriers with preeclampsia had higher DBP at the end of pregnancy as compared to the 4b4b and 4a4b genotype carriers ($p = 0.04$).

Discussion

The results of the present study suggest that genotype 4a4a of the *eNOS* gene is associated with higher levels of diastolic blood pressure in pregnant women and with more pronounced dynamics of the diastolic and mean arterial pressure in the course of

pregnancy ($p = 0.02$ – 0.03). Pregnant women with the 4a4a genotype and increased body mass index had high systolic, diastolic, and mean arterial pressure ($p = 0.001$ – 0.02). In the group of pregnant women with preeclampsia, the 4a4a genotype is associated with a higher level of diastolic blood pressure at the end of pregnancy ($p = 0.04$), whereas in women without preeclampsia this genotype was associated with more pronounced changes in this type of blood pressure during pregnancy ($p = 0.02$).

Endothelial nitric oxide synthase is synthesized predominantly in endothelium of blood vessels and catalyzes synthesis of nitrogen oxide (NO) from L-arginine. NO activates guanylate cyclase, which synthesizes 3'-5'-cyclic guanosine monophosphate. The latter promotes relaxation of smooth muscles of blood vessel walls and vasodilation [6].

Some researchers suggested that the 4a/4b polymorphism may be linked to other functional polymorphisms in the regulatory regions of the *eNOS* gene. For example, haplotype –786C/4b/894G was associated with a reduced risk of arterial hypertension [32]. Earlier Wang et al. [33] reported the highest transcription activity of the –786C/4b haplotype.

The higher level of arterial blood pressure in pregnant women with genotype 4a4a determined in our study may be explained by lower production of NO in these genotype carriers and, respectively, its lower vasodilation effect.

Table 4

Association of the 4a/4b polymorphism of the *eNOS* gene with blood pressure in women with different BMI at the end of pregnancy (Me, Q25–Q75).

BMI	Blood pressure parameters	Genotypes			Differences between the genotypes, p		
		4b4b 1	4a4b 2	4a4a 3	1–2	2–3	1–3
Underweight (BMI <18.5)	N	35	20	0			
	BMI, kg/m ²	18.9 (18.2–19.3)	18.8 (18.2–19.3)	—	0.8	—	—
	SBP, mmHg	130.0 (115.0–135.0)	130.0 (110.0–130.0)	—	0.5	—	—
	DBP, mmHg	80.0 (75.0–90.0)	80.0 (70.0–90.0)	—	0.9	—	—
	PBP, mmHg	40.0 (40.0–50.0)	40.0 (40.0–45.0)	—	0.3	—	—
	MAP, mmHg	96.7 (88.3–103.3)	96.7 (83.3–103.3)	—	0.8	—	—
	ΔMAP, mmHg	13.3 (6.7–20.0)	12.5 (5.8–26.7)	—	0.9	—	—
	ΔSBP, mmHg	20.0 (10.0–30.0)	17.5 (10.0–30.0)	—	0.8	—	—
	ΔDBP, mmHg	10.0 (5.0–20.0)	10.0 (7.5–20.0)	—	0.6	—	—
	N	203	98	12			
Normal weight (BMI 18.5–24.9)	BMI, kg/m ²	22.0 (21.0–23.0)	22.1 (21.0–23.1)	22.6 (21.1–23.9)	0.8	0.3	0.2
	SBP, mmHg	120.0 (110.0–140.0)	130.0 (120.0–140.0)	120.0 (110.0–145.0)	0.2	0.5	0.9
	DBP, mmHg	80.0 (70.0–90.0)	80.0 (70.0–90.0)	90.0 (70.0–90.0)	0.3	1.0	0.8
	PBP, mmHg	45.0 (40.0–50.0)	45.0 (40.0–55.0)	40.0 (40.0–50.0)	0.1	0.2	0.5
	MAP, mmHg	93.3 (83.3–106.7)	98.3 (86.7–106.7)	100.0 (83.3–110.0)	0.3	0.8	1.0
	ΔMAP, mmHg	10.0 (3.3–21.7)	13.3 (5.0–23.3)	23.3 (5.8–33.3)	0.4	0.2	0.1
	ΔSBP, mmHg	15.0 (5.0–30.0)	20.0 (5.0–30.0)	20.0 (5.0–37.5)	0.3	0.6	0.3
	ΔDBP, mmHg	10.0 (0.0–20.0)	10.0 (0.0–20.0)	20.0 (7.5–30.0)	0.4	0.1	0.1
	N	139	65	11			
	BMI, kg/m ²	28.9 (26.4–31.8)	27.7 (25.7–32.2)	30.1 (27.2–33.9)	0.1	0.2	0.5
Overweight (BMI >25.0)	SBP, mmHg	140.0 (130.0–150.0)	140.0 (130.0–150.0)	150.0 (140.0–180.0)	0.7	0.02	0.009
	DBP, mmHg	90.0 (80.0–100.0)	90.0 (80.0–100.0)	100.0 (100.0–110.0)	0.7	0.001	0.002
	PBP, mmHg	50.0 (45.0–60.0)	50.0 (50.0–60.0)	50.0 (50.0–70.0)	0.3	0.4	0.2
	MAP, mmHg	106.7 (100.0–116.7)	106.7 (96.7–116.7)	116.7 (113.3–133.3)	1.0	0.005	0.003
	ΔMAP, mmHg	16.7 (8.3–23.3)	16.7 (6.6–25.0)	23.3 (3.3–38.3)	0.8	0.2	0.1
	ΔSBP, mmHg	25.0 (10.0–30.0)	20.0 (10.0–30.0)	30.0 (10.0–45.0)	0.7	0.2	0.2
	ΔDBP, mmHg	10.0 (10.0–20.0)	10.0 (0.0–25.0)	20.0 (0.0–35.0)	1.0	0.2	0.2
	N	58	22	5			
	BMI, kg/m ²	32.4 (30.9–36.7)	33.7 (32.2–36.8)	33.9 (31.6–34.5)	0.6	0.9	1.0
	SBP, mmHg	150.0 (140.0–160.0)	147.5 (140.0–155.0)	170.0 (150.0–170.0)	1.0	0.1	0.1
Overweight (BMI >29.9)	DBP, mmHg	90.0 (80.0–100.0)	90.0 (90.0–100.0)	100.0 (100.0–110.0)	1.0	0.1	0.1
	PBP, mmHg	55.0 (50.0–60.0)	55.0 (50.0–60.0)	60.0 (50.0–70.0)	1.0	0.4	0.4
	MAP, mmHg	110.0 (103.3–120.0)	109.2 (103.3–118.3)	123.3 (116.7–130.0)	1.0	0.1	0.1
	ΔMAP, mmHg	15.0 (6.7–23.3)	17.5 (10.0–21.7)	10.0 (3.3–23.3)	0.6	0.7	1.0
	ΔSBP, mmHg	20.0 (10.0–30.0)	20.0 (15.0–30.0)	10.0 (10.0–30.0)	0.6	0.6	1.0
	ΔDBP, mmHg	10.0 (5.0–20.0)	10.0 (0.0–20.0)	10.0 (0.0–20.0)	0.6	0.8	0.9

Abbreviations: Me, median; Q25–Q75, interquartile range; P, p-value for Mann–Whitney test; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PBP, pulse blood pressure; MAP, mean arterial pressure during gestation; ΔMAP, change of mean arterial pressure; ΔSBP, change of systolic blood pressure; ΔDBP, change of diastolic blood pressure.

Table 5

Association of the 4a/4b polymorphism with blood pressure in women with/without preeclampsia at the end of pregnancy, Me (Q25–Q75).

Blood pressure parameters	Genotypes		p
	4b4b+4a4b	4a4a	
Pregnant women without preeclampsia (n = 204)			
N	198	6	
SBP, mmHg.	110.0 (110.0–120.0)	112.5 (110.0–120.0)	0.7
DBP, mmHg	70.0 (70.0–75.0)	70.0 (70.0–90.0)	0.5
PBP, mmHg	40.0 (40.0–45.0)	40.0 (30.0–40.0)	0.4
MAP, mmHg	85.0 (83.3–90.0)	84.2 (83.3–100.0)	0.6
ΔMAP, mmHg	3.3 (0.0–6.7)	10.8 (3.3–35.0)	0.1
ΔSBP, mmHg	5.0 (0.0–10.0)	12.5 (0.0–35.0)	0.3
ΔDBP, mmHg	0.0 (0.0–10.0)	10.0 (5.0–35.0)	0.02
Pregnant women with preeclampsia (n = 382)			
N	364	18	
SBP, mmHg.	140.0 (135.0–150.0)	150.0 (140.0–170.0)	0.1
DBP, mmHg	90.0 (85.0–100.0)	100.0 (90.0–100.0)	0.04
PBP, mmHg	50.0 (50.0–60.0)	50.0 (50.0–70.0)	0.6
MAP, mmHg	106.7 (102.5–116.7)	116.7 (106.7–123.3)	0.1
ΔMAP, mmHg	20.0 (13.3–27.5)	28.3 (10.0–33.3)	0.2
ΔSBP, mmHg	25.0 (20.0–35.0)	30.0 (10.0–45.0)	0.4
ΔDBP, mmHg	20.0 (10.0–25.0)	20.0 (10.0–30.0)	0.2

Abbreviations: Me, median; Q25–Q75, interquartile range; P, p-value for Mann–Whitney test; SBP, systolic blood pressure; DBP, diastolic blood pressure; PBP, pulse blood pressure; MAP, mean arterial pressure; Δ MAP, change of mean arterial pressure during gestation; ΔSBP, change of systolic blood pressure; ΔDBP, change of diastolic blood pressure.

Studies of the 4a/4b polymorphism association with arterial blood pressure in pregnant women have reported inconsistent results. Most of these studies were about a possible role of the *eNOS* gene polymorphisms in development of preeclampsia. Hoher et al. [20] did not find any differences in MAP between the 4a/4b genotypes of *eNOS* in a sample of 2186 pregnant Caucasian women. Likewise, no association of the 4a/4b polymorphism was found with either preeclampsia [22] or arterial blood pressure in pregnant women with preeclampsia from different ethnic populations [8,21,23].

Chen et al. [19] reported significantly lower frequency of allele 4a of the 4a/4b polymorphism in pregnant Chinese women with preeclampsia than in the controls. On the contrary, pregnant Mexican women with preeclampsia had higher frequency of this allele as compared to the controls [18]. Also, the carriers of allele 4a with preeclampsia had higher SBP as compared to the 4b4b genotypes [18].

Groten et al. reported an association of allele 4a with the 1.7-fold increased risk of preeclampsia in Caucasian and African women regardless of ethnicity, age, and parity [16].

Four meta-analyses also yielded inconsistent results. Two meta-analyses of 18 [34] and 11 [22] studies reported an association of allele 4a with a risk of preeclampsia, whereas two others of 33 and 41 studies did not [35,36].

The inconsistency in the above results may be attributed to the interethnic differences in allele frequencies of the 4a/4b polymorphism [18,21,22,36], and, respectively, different importance of the polymorphism for the hypertensive disorder at pregnancy in populations of different ethnicity. This assumption is supported by previous studies, which suggested interethnic differences in population genetic structure as an important factor for the observed differences in prevalence of some complex traits [37,38]. Further studies in different ethnicities are needed to resolve this inconsistency.

The results of this study suggest that the 4a/4b polymorphism of *eNOS* is associated with some parameters of arterial blood pressure in pregnant women of Central Russia. In particular, genotype 4a4a

of the polymorphism may confer higher level of arterial blood pressure at pregnancy. In addition, overweight and obesity seem to be important cofactors contributing to the BP parameters in pregnant women. Further studies on larger samples of various ethnicities are needed to validate the results of the present study.

Conflict of interest

The authors declare no conflict of interest.

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References

- [1] ACOG Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002;99(1):159–67.
- [2] Lenfant C. Working group report on high blood pressure in pregnancy. *J Clin Hypertens* 2001;3(2):75–88.
- [3] Roberts JM. Preeclampsia: recent insights. *Hypertension* 2005;46:1243–9.
- [4] Simanov IV. Terms of emergence of the main clinical symptoms of preeclampsia at the present stage. *Res Result Med Pharm* 2017;3(3):51–6 [In Russian, English abstract].
- [5] Gureev VV, Martynova OV, Anciferova OE, Martynov MA, Pokrovskaya TG, Malorodova TN, et al. Correction of adma-induced preeclampsia with the use of phosphodiesterase 5 and selective inhibitor of arginase II ZB49-0010. *Res Result Med Pharm* 2015;4(6):66–8 [In Russian, English abstract].
- [6] Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 2001;357(3):593–615.
- [7] Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem* 1993;268(23):17478–14488.
- [8] Sandrim VC, Palei AC, Cavalli RC, Araújo FM, Ramos ES, Duarte G, et al. *eNOS* haplotypes associated with gestational hypertension or preeclampsia. *Pharmacogenomics* 2008;9(10):1467–73.
- [9] Muniz L, Luizon MR, Palei AC, Lacchini R, Duarte G, Cavalli RC, et al. *eNOS* tag SNP haplotypes in hypertensive disorders of pregnancy. *DNA Cell Biol* 2012;31(12):1665–70.
- [10] Silva BM, Neves FJ, Rocha NG, Sales AR, Medeiros RF, Barbosa TC, et al. Endothelial nitric oxide gene haplotype reduces the effect of a single bout of exercise on the vascular reactivity in healthy subjects. *Transl Res* 2013;161(1):15–25.
- [11] Song J, Yoon Y, Park KU, Park J, Hong YJ, Hong SH, et al. Genotype-specific influence on nitric oxide synthase gene expression, protein concentrations, and enzyme activity in cultured human endothelial cells. *Clin Chem* 2003;49(6 Pt 1):847–52.
- [12] Dosenko VE, Zagoriy VY, Haytovich NV, Gordok OA, Moibenko AA. Allelic polymorphism of endothelial NO-synthase gene and its functional manifestations. *Acta Biochim Pol* 2006;53(2):299–302.
- [13] Tsukada T, Yokoyama K, Arai T, Takemoto F, Hara S, Yamada A, et al. Evidence of association of the *eNOS* gene polymorphism with plasma NO metabolite levels in humans. *Biochem Biophys Res Commun* 1998;45(1):190–3.
- [14] Mustafina OE, Shagisultanova EI, Nasybullin TR, Tuktarova IA, Birkmeeva AM, Polyudova ON. Endothelial nitric oxide synthase gene minisatellite polymorphism in populations of the Volga-Ural region and analysis of its association with myocardial infarction and essential hypertension. *Russ J Genet* 2001;37(5):546–52 [In Russian, English abstract].
- [15] Spiridonova MG, Stepanov VA, Puzirev VP, Karpov RS. Analysis of gene complexes predisposing to coronary atherosclerosis. *Genetika* 2002;38(3):383–92 [In Russian].
- [16] Groten T, Schleussner E, Lehmann T, Reister F, Holzer B, Danso KA, et al. *eNOS*4 and *EPHX1* polymorphisms affect maternal susceptibility to preeclampsia: analysis of five polymorphisms predisposing to cardiovascular disease in 279 Caucasian and 241 African women. *Arch Gynecol Obstet* 2014;289(3):581–93.
- [17] Tempfer CB, Dorman K, Deter RL, O'Brien WE, Gregg AR. An endothelial nitric oxide synthase gene polymorphism is associated with preeclampsia. *Hypertens Pregnancy* 2001;20:107–18.
- [18] Grandone E, Colaizzo D, Martinelli P, Pavone G, Errico M, Vecchione G, et al. Does endothelial nitric oxide synthase gene variation play a role in the occurrence of hypertension in pregnancy? *Hypertens Pregnancy* 2003;22(2):149–55.
- [19] Chen LK, Huang CH, Yeh HM, Lee CN, Shyu MK, Hsieh FJ, et al. Polymorphisms in the endothelial nitric oxide synthase gene may be protective against preeclampsia in a Chinese population. *Reprod Sci* 2007;14(2):175–81.
- [20] Hoher B, Chen YP, Hügle S, Repey J, Krause K, Slowinski T, et al. Impact of maternal endothelial nitric oxide synthase gene polymorphisms on blood

- pressure, protein excretion and fetal outcome in pregnancy. *J Hum Hypertens* 2008;22(9):641–7.
- [21] Salimi S, Naghavi A, Mokhtari M, Noora M, Yaghmaei M. Lack of relationship between endothelial nitric oxide synthase gene 4b/a and T-786C polymorphisms with preeclampsia in southeast of Iran. *Arch Gynecol Obstet* 2012;285(2):405–9.
- [22] Dai B, Liu T, Zhang B, Zhang X, Wang Z. The polymorphism for endothelial nitric oxide synthase gene, the level of nitric oxide and the risk for preeclampsia: a meta-analysis. *Gene* 2013;519(1):187–93.
- [23] Rahimi Z, Aghaei A, Rahimi Z, Vaisi-Raygani A. Endothelial nitric oxide synthase (eNOS) 4a/b and G894T polymorphisms and susceptibility to preeclampsia. *J Reproduction Infertil* 2013;14(4):184–9.
- [24] Rudyh NA, Sirotina SS. Genetic interrelations of Russian and Ukrainian populations of Belgorod region. *Res Result Med Pharm* 2015;1(3):72–9 [In Russian]. [In Russian, English abstract].
- [25] Pickering TG, Hall JE, Appel LJ. Recommendations for blood pressure measurement in humans and experimental animals. Part 1: blood pressure measurement in humans a statement for professionals from the subcommittee of professional and public education of the American Heart Association council on high blood pressure research. *Hypertension* 2005;45:142–61.
- [26] Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Bethesda (MD): National Heart, Lung, and Blood Institute; 1998.
- [27] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- [28] Ponomarenko IV. Selection of polymorphic loci for association analysis in genetic-epidemiological studies. *Res Result Med Pharm* 2018;4(2):38–52 [In Russian, English abstract].
- [29] Aggarwal PK, Jain V, Jha V. Endothelial nitric oxide synthase, angiotensin-converting enzyme and angiotensinogen gene polymorphisms in hypertensive disorders of pregnancy. *Hypertens Res* 2010;33(5):473–7.
- [30] Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965;52:591–611.
- [31] Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Stat* 1947;18:50–60.
- [32] Sandrim VC, Coelho EB, Nobre F, Arado GM, Lanchote VL, Tanus-Santos JE. Susceptible and protective eNOS haplotypes in hypertensive black and white subjects. *Atherosclerosis* 2006;186(2):428–32.
- [33] Wang XL, Mahaney MC, Sim AS, Wang J, Wang J, Blangero J, et al. Genetic contribution of the endothelial constitutive nitric oxide synthase gene to plasma nitric oxide levels. *Arterioscler Thromb Vasc Biol* 1997;17(11):3147–53.
- [34] Chen H, Zhao G, Sun M, Wang H, Liu J, Gao W, et al. Endothelial nitric oxide synthase gene polymorphisms (G894T, 4b/a and T-786C) and preeclampsia: meta-analysis of 18 case-control studies. *DNA Cell Biol* 2012;31(6):1136–45.
- [35] Zeng F, Zhu S, Wong MC, Yang Z, Tang J, Li K, et al. Associations between nitric oxide synthase 3 gene polymorphisms and preeclampsia risk: a meta-analysis. *Sci Rep* 2016;6:23407.
- [36] Qi HP, Fraser WD, Luo ZC, Julien P, Audibert F, Wei SQ. Endothelial nitric oxide synthase gene polymorphisms and risk of preeclampsia. *Am J Perinatol* 2013;30(10):795–804.
- [37] Dvornyk V, Liu Xh, Shen H, Lei SF, Zhao L, Huang QR, et al. Differentiation of Caucasians and Asians at bone mass candidate genes: implication for ethnic difference of bone mass. *Ann Hum Genet* 2003;67(3):216–27.
- [38] Dvornyk V, Liu PY, Long JR, Zhang YY, Lei SF, Recker RR, et al. Contribution of genotype and ethnicity to bone mineral density variation in Caucasians and Chinese: a test for five candidate genes for bone mass. *Chin Med J* 2005;118(15):1235–44.