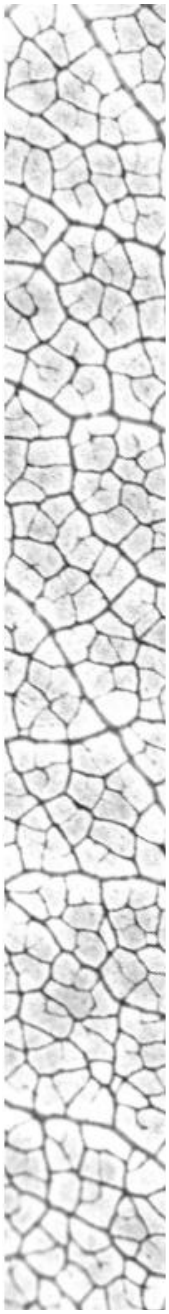

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The relationship between placental gene polymorphisms and preeclampsia risk in Russian women

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Abstract

To study the associations of polymorphisms of the NDRG1 gene, which is differentially expressed in the placenta, with the risk of preeclampsia (PE) development. The study group included 997 women: 366 pregnant women with preeclampsia and 631 women with physiological pregnancy. Clinical and laboratory examination of pregnant women was carried out in the Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph. DNA was isolated from peripheral venous blood lymphocytes by phenol-chloroform extraction. All women underwent typing of four single nucleotide polymorphisms of the N-myc downstream regulated gene 1 (NDRG1). The analysis of SNPs associations with the development of preeclampsia was performed using logistic regression analysis within the framework of additive, dominant and recessive genetic models. The average age in the group with preeclampsia was 0.75 years higher compared to the control group (p = 0.01). Among pregnant women with PE, body weight and body mass index exceeded those of the control group (p = 0.0001). Also, in this group, the percentage of women with obesity is 2.26 times higher (p = 0.001). The analysis of risk factors for PE revealed a higher incidence of arterial hypertension before pregnancy and a history of preeclampsia in the patient group as compared with the control (p = 0.0001). Compared with the control group, women with PE have a greater number of pregnancies (1.32 times, p = 0.03) due to a greater number of stillbirths (p = 0.02), miscarriages (0.01), and artifactual abortions (p = 0.0005). The rs12678229 NDRG1 allele A was found to be associated with the development of preeclampsia in the framework of the recessive model (OR = 1.46, 95% CI 1.01-2.12, p = 0.046). The associations of other studied polymorphic markers with the development of preeclampsia were statistically insignificant. Thus, the rs12678229 NDRG1 allele is a risk factor for the development of preeclampsia among the women of the Central Black Earth Region of Russia.

Keywords: pregnancy, preeclampsia, gene, single nucleotide polymorphism, NDRG1

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INTRODUCTION

Preeclampsia (PE) is a multisystem pathological condition that occurs in the second half of pregnancy (after the 20th week), characterized by arterial hypertension in combination with proteinuria (≥3 g/l in daily urine), often with edema and manifestations of multiple organ / multisystem dysfunction / failure (Abalos et al. 2014; Suparman et al., 2018).

According to world literature and WHO, the incidence of preeclampsia is 2-8% (Duley, 2009; Abalos et al., 2014). PE remains an important cause of maternal, perinatal, and neonatal morbidity and mortality (Abalos et al., 2014; Voge et al., 2014).

With the development of severe preeclampsia and eclampsia, the risk of such complications as hemorrhage and cerebral edema, placental abruption, Disseminated Intravascular Coagulation (DIC), massive obstetric hemorrhage, HELLP syndrome, hemorrhage and rupture of the liver capsule, pulmonary edema, respiratory distress syndrome among adults, acute renal and hepatic insufficiency increases significantly (Abalos et al., 2014).

Children born after preeclampsia have a low weight and an increased risk of stroke, coronary heart disease, and metabolic syndrome in adulthood (Abalos et al., 2014; Bilano et al., 2014).

Numerous studies indicate the multifactorial nature of preeclampsia, which suggests an assessment of the genetic component in the development of this pregnancy complication. Currently, research on the molecular genetic study of preeclampsia is being actively conducted around the world.

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Table 1. Biomedical characteristics of the studied groups of pregnant women

Indicators	Pregnant women with preeclampsia (n =366) $\bar{X}$ ± SD/%(n)	Control (n =631) $\bar{X}$ ± SD/%(n)	P
Age, years (min - max)	27.32±5.17	26.57±4.94	**0.01**
Height, m	1.65±0.05	1.65±0.06	0.52
Weight, kg	69.46±15.76	63.50±11.22	**0.0001**
BMI, kg/m2	25.65±5.71	23.40±3.47	**0.0001**
The distribution of individuals by BMI, % (n):			
lack of weight (<18.50)	4.10 (15)	5.55 (35)	
normal body weight (18.50-24.99)	51.91 (190)	66.72 (421)	**0.001**
overweight (25.00-29.99)	22.40 (82)	22.19 (140)	
obesity (>30)	21.58 (79)	5.55 (35)	

Note: BMI – body mass index; P – the significance level of differences between the compared groups according to the Kruskal-Wallis test

One of these types of studies is genome-wide association studies (GWAS) of single nucleotide polymorphisms with the development of preeclampsia (Johnson et al., 2012; Zhao et al., 2013). Another field of the molecular genetic study of PE is the study of candidate genes differentially expressed in the placenta (Loset et al., 2011; Louwen et al., 2013; Trifonova et al., 2014; Serebrova et al., 2016). A more common approach in the molecular genetic study of preeclampsia and its clinical manifestations (arterial hypertension, proteinuria, etc.) is related with associative studies (Williams et al., 2011; Williams, Morgan, 2012; Polonikov et al., 2015; Yarosh et al., 2015; Reshetnikov et al., 2015; Polonikov et al., 2017a; Polonikov et al., 2017 b; Reshetnikov et al., 2017; Sirotina et al., 2018; Golovchenko, 2019; Reshetnikov et al., 2019; Sami, et al, 2018). Dynamic Simulation and Modeling of a Novel Combined Hybrid Photovoltaic-Thermal Panel Hybrid System. *International Journal of Sustainable Energy and Environmental Research*, *7*(1), 1-23).

It should be noted that the results of the studies on the search for candidate gene associations with the risk of PE development are contradictory, which is associated with the genetic heterogeneity of different populations.

MATERIALS AND METHODS

The study group included 997 women: 366 pregnant women with preeclampsia and 631 women with normal pregnancy (control group).

The studied samples included the women of Russian nationality who were born in the Central Black Earth Region of Russia, who had no kinship among themselves, who lived in the Belgorod Region and voluntarily agreed to conduct the study. Clinical and laboratory examination of pregnant women was carried out on the basis of the Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph.

The study was approved by the ethics committee of the Belgorod National Research University. All participants signed informed consent to participate in this study.

The diagnosis of preeclampsia was made on the basis of generalized edema, arterial hypertension and proteinuria. DNA was isolated from peripheral venous blood lymphocytes by phenol-chloroform extraction.

Genotyping of DNA samples was performed by the method of matrix-activated laser desorption/ionization (MALDI) on the iPLEX platform of the MassARRAY Analyzer 4 mass spectrometer (“Seqeunom”) at the Scientific Research Institute of Medical Genetics of the Tomsk National Research Medical Center of the Russian Academy of Sciences. All women underwent typing of four single nucleotide polymorphisms of N-myc downstream regulated 1 gene (NDRG1): A / G NDRG1 (rs2977559), T / C NDRG1 (rs2227262), A / G NDRG1 (rs12678229), T / C NDRG1 (rs3802252). Differences in the studied traits between the compared groups were evaluated using the Kruskal – Wallis method. The analysis of SNP associations with the development of preeclampsia was performed using logistic regression analysis in the framework of additive, dominant and recessive genetic models (Ponomarenko et al., 2019). The study was carried out taking into account correction for covariates: age, body mass index, the history of artifactual abortions, the history of stillbirths, the presence of hypertension before pregnancy, and the history of preeclampsia. The odds ratio (OR) indicators and their 95% confidence interval (95% CI) were calculated.

They estimated the observed distribution of genotypes by 4 SNPs included in the analysis, and its correspondence to the expected distribution, according to Hardy – Weinberg equilibrium, the observed (Ho) and expected (He) heterozygosity were calculated.

RESULTS

The main biomedical and clinical-anamnestic characteristics of the studied groups of pregnant women are presented in **Table 1**.

The average age in the group with preeclampsia was 0.75 years higher as compared with the control group (p = 0.01). Among the pregnant women with PE, body weight and body mass index exceeded those of the control group (p = 0.0001). Also, the percentage of women with obesity in this group is 2.26 times higher (p = 0.001).

During the analysis of PE risk factors, they revealed a higher incidence of arterial hypertension before

Table 2. Results of logistic regression analysis of SNPs associations of the NDRG1 gene with the development of preeclampsia

Chr	SNP	Additive model				Dominant model				Recessive model			
		OR	95%CI		P	OR	95%CI		P	OR	95%CI		P
			L95	U95			L95	U95			L95	U95	
8	rs2977559	0.97	0.78	1.21	0.795	1.00	0.73	1.37	0.997	0.91	0.60	1.36	0.635
	rs2227262	0.92	0.70	1.21	0.553	0.94	0.69	1.28	0.714	0.67	0.27	1.66	0.385
	rs12678229	1.10	0.89	1.37	0.367	0.95	0.69	1.30	0.743	**1.46**	**1.01**	**2.12**	**0.046**
	rs3802252	0.87	0.70	1.08	0.212	0.86	0.63	1.17	0.323	0.81	0.54	1.21	0.296

Note: - The results are obtained taking into account the corrections for covariates.
- OR - odds ratio, 95% CI - 95% confidence interval, L95 - lower limit of the 95% confidence interval, U95 - upper limit of the 95% confidence interval, P - significance level.

pregnancy and a history of preeclampsia in the patient group as compared with the control (p = 0.0001).

As compared with the control group, the women with PE differ by a large number of pregnancies (1.32 times, p = 0.03) due to a greater number of stillbirths (p = 0.02), miscarriages (0.01), and artifactual abortions (p = 0.0005).

They studied the distribution of 4 polymorphic loci among the studied groups of pregnant women. For all SNPs studied, both in the group of pregnant women with PE and in the control group, the frequencies of minor alleles (MAF) were 5% higher. The analysis of the observed distribution of genotypes did not reveal deviations from the expected distribution in accordance with Hardy-Weinberg equilibrium (HWE) (p > 0.05).

At the next stage, using the logistic regression analysis in the framework of additive, dominant and recessive genetic models, they performed the analysis of NDRG1 gene polymorphism associations with a risk of preeclampsia. At the same time, biomedical and clinical-anamnestic indicators, according to which significant differences were found between the studied groups of pregnant women, were used as covariates. The results are presented in **Table 2**.

The rs12678229 NDRG1 allele A was found to be associated with the development of preeclampsia in the framework of the recessive model (OR = 1.46, 95% CI 1.01-2.12, p = 0.046).

The associations of other polymorphic markers studied with the development of preeclampsia were statistically insignificant.

DISCUSSION

The results of this study indicate a connection between rs12678229 NDRG1 polymorphism and the risk of preeclampsia development among pregnant women of the Central Black Earth Region of Russia.

Other studies have also examined the role of the NDRG1 gene in the formation of preeclampsia. Thus, in the study on the Siberian population, including Russians, Yakuts and Buryats, they determined the associations of polymorphisms rs12678229, rs2227262 and rs2227262 and rs2227262 NDRG1 with an increased risk of preeclampsia (Serebrova et al., 2016).

A number of other studies have evaluated the effect of NDRG1 expression in the placenta on preeclampsia development (Choi et al., 2007; Fu et al., 2017). So, Choi et al. (2007) revealed an increased level of NDRG1 expression in placentas of women with PE. In the work by Fu et al. (2017) an increased expression of NDRG1 was also established, and in placentas with early PE, the expression level was higher as compared to the placentas with late PE.

According to published data, the isoform of the NDRG1 protein, which is a member of the NDRG family, is most actively expressed in the placenta during the second and the third trimester of pregnancy, mainly in syncytiotrophoblast. Activation of NDRG1 gene expression in trophoblast cells is carried out under conditions of hypoxia (Chen et al., 2006; Choi et al., 2007).

Violation of cytotrophoblast invasion under hypoxia leads to placental hypoperfusion, the development of oxidative stress and inflammation, and the appearance of systemic endothelial dysfunction, which is responsible for the characteristic clinical manifestations of preeclampsia (Hansson S.R. et al., 2014).

Thus, the results of the study indicate that the rs12678229 NDRG1 allele is a risk factor for the development of preeclampsia among the women of the Central Black Earth region of Russia. The data obtained expand the existing understanding of genetic factor role in the development of preeclampsia and allow the future use of these data to predict or detect this pregnancy complication.

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