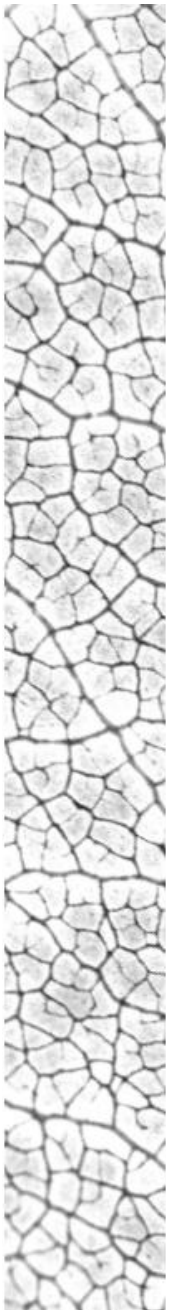

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 1471-1475 (2020)

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

Reshetnikov EA, Altukhova OB, Orlova VS, Batlutskaya IV, Elykova AV, Kulikovsky VF (2020) The relationship between placental gene polymorphisms and preeclampsia risk in Russian women. Eurasia J Biosci 14: 1471-1475.

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.

Received: July 2019
Accepted: April 2020
Printed: June 2020

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.

REFERENCES

Abalos E, Cuesta C, Carroli G, Qureshi Z, Widmer M, Vogel JP, Souza JP (2014) Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG,121(Suppl 1): 14-24. https://doi.org/10.1111/1471-0528.12629

Bilano VL, Ota E, Ganchimeg T, Mori R, Souza JP (2014) Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low- and middle-income countries: a WHO secondary analysis. PLoS One, 9(3): e91198.

Chen B, Nelson DM, Sadovsky Y (2006) N-Myc downregulated gene 1 (NDRG1) modulates the response of term human trophoblasts to hypoxic injury. J Biol Chem, 281(5), 2764-2772.Duley L., 2009. The global impact of pre-eclampsia and eclampsia. Semin Perinatol, 33(3): 130-137.

Choi SJ, Oh SY, Kim JH, Sadovsky Y, Roh CR (2007) Increased expression of N-myc downstream-regulated gene 1 (NDRG1) in placentas from pregnancies complicated by intrauterine growth restriction or preeclampsia. Am J Obstet Gynecol, 2007 Jan;196(1): 45.e1-e7. https://doi.org/10.1016/j.ajog.2006.08.029

Fu Y, Wei J, Dai X, Ye Y (2017) Increased NDRG1 expression attenuate trophoblast invasion through ERK/MMP-9 pathway in preeclampsia. Placenta, 51: 76-81.

Golovchenko OV (2019) Molecular genetic determinants of pre-eclampsia. Research Results in Biomedicine, l5(4): 139-149. (In Russian) https://doi.org/10.18413/2658-6533-2019-5-4-0-11

Hansson SR, Nääv A, Erlandsson L (2014) Oxidative stress in preeclampsia and the role of free fetal hemoglobin. Front Physiol, 5: 516.

Johnson MP, Brennecke SP, East CE, Göring HH, et al. (2012) Genome-wide association scan identifies a risk locus for preeclampia on 2q14, near the inhibin, beta B gene. PLoS One, 7(3): e33666.

Loset M, Mundal SB, Johnson MP, Fenstad MH, et al. (2011) A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 204(1): 84 e1-e27. https://doi.org/10.1016/j.ajog.2010.08.043

Louwen F, Muschol-Steinmetz C, Reinhard J, Reitter A, Yuan J (2012) A lesson for cancer research: placental microarray gene analysis in preeclampsia. Oncotarget 3(8): 759-773.

Polonikov A, Bykanova M, Ponomarenko I, Sirotina S, et al. (2017b) The contribution of CYP2C gene subfamily involved in epoxygenase pathway of arachidonic acids metabolism to hypertension susceptibility in Russian population. Clin Exp Hypertens 39(4): 306-311. https://doi.org/10.1080/10641963.2016.1246562

Polonikov A, Kharchenko A, Bykanova M, Sirotina S, et al. (2017a) Polymorphisms of CYP2C8, CYP2C9 and CYP2C19 and risk of coronary heart disease in Russian population. Gene, 627: 451-459. https://doi.org/10.1016/j.gene.2017.07.004

Polonikov AV, Ushachev DV, Ivanov VP, Churnosov MI, et al. (2015) Altered erythrocyte membrane protein composition mirrors pleiotropic effects of hypertension susceptibility genes and disease pathogenesis. J Hypertens, 33(11): 2265-77. https://doi.org/10.1097/HJH.0000000000000699

Ponomarenko I, Reshetnikov E, Altuchova O, Polonikov A, et al. (2019) Association of genetic polymorphisms with age at menarche in Russian women. Gene, 20;686: 228-236. https://doi.org/10.1016/j.gene.2018.11.042

Reshetnikov E, Ponomarenko I, Golovchenko O, Sorokina I, Batlutskaya I, Yakunchenko T, Dvornyk V, Polonikov A, Churnosov M (2019) The VNTR polymorphism of the endothelial nitric oxide synthase gene and blood pressure in women at the end of pregnancy. Taiwan J Obstet Gynecol, 58(3): 390-395. https://doi.org/10.1016/j.tjog.2018.11.035

Reshetnikov E, Zarudskaya O, Polonikov A, Bushueva O, Orlova V, Krikun E, Dvornyk V, Churnosov M (2017) Genetic markers for inherited thrombophilia are associated with fetal growth retardation in the population of Central Russia. J Obstet Gynaecol Res, 43(7): 1139-1144. https://doi.org/10.1111/jog.13329

Reshetnikov EA, Akulova LY, Dobrodomova IS, Dvornyk VY, Polonikov AV, Churnosov MI (2015) The insertion-deletion polymorphism of the ACE gene is associated with increased blood pressure in women at the end of pregnancy. J Renin Angiotensin Aldosterone Syst, 16(3): 623-632. https://doi.org/10.1177/1470320313501217

Serebrova VN, Trifonova EA, Gabidulina TV, Bukharina IY, Agarkova TA, Evtushenko ID, Maksimova NR, Stepanov VA (2016) Detection of novel genetic markers of susceptibility to preeclampsia based on an analysis of the regulatory genes in the placental tissue. Mol Biol (Mosk), 50(5): 870-879. [Article in Russian].

Sirotina S, Ponomarenko I, Kharchenko A, Bykanova M, Bocharova A, et al. (2018) A Novel Polymorphism in the Promoter of the CYP4A11 Gene Is Associated with Susceptibility to Coronary Artery Disease. Dis Markers, 2018: 5812802. https://doi.org/10.1155/2018/5812802

Suparman E, Mose JC, Handono B (2018) Comparison of LC3 and Caspase 3 level in normal pregnancy, early onset preeclampsia, and late onset preeclampsia. Electronic Journal of General Medicine, 15(4), em56. https://doi.org/10.29333/ejgm/89509

Trifonova EA, Gabidulina TV, Ershov NI, Serebrova VN, Vorozhishcheva AY, Stepanov VA (2014) Analysis of the placental tissue transcriptome of normal and preeclampsia complicated pregnancies. Acta Naturae, 6(2): 71-83.

Vogel JP, Souza JP, Mori R, Morisaki N, et al. (2014) Maternal complications and perinatal mortality: findings of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG, 121 (Suppl 1): 76-88. https://doi.org/10.1111/1471-0528.12633

Williams PJ, Broughton Pipkin F (2011) The genetics of pre-eclampsia and other hypertensive disorders of pregnancy. Best Pract Res Clin Obstet Gynaecol, 25(4): 405-417.

Williams PJ, Morgan L (2012) The role of genetics in pre-eclampsia and potential pharmacogenomic interventions. Pharmgenomics Pers Med, 5: 37-51.

Yarosh SL, Kokhtenko EV, Churnosov MI, Solodilova MA, Polonikov AV (2015) Joint effect of glutathione S-transferase genotypes and cigarette smoking on idiopathic male infertility. Andrologia 47(9): 980-6. https://doi.org/10.1111/and.12367

Zhao L, Bracken MB, DeWan AT (2013) Genome-wide association study of pre-eclampsia detects novel maternal single nucleotide polymorphisms and copy-number variants in subsets of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study cohort. Ann Hum Genet, 77(4): 277-287. https://doi.org/10.1111/ahg.12021