ISSN 1022-7954, Russian Journal of Genetics, 2021, Vol. 57, No. 9, pp. 1100–1105. © Pleiades Publishing, Inc., 2021.
Russian Text © The Author(s), 2021, published in Genetika, 2021, Vol. 57, No. 9, pp. 1082–1088.

HUMAN GENETICS

Locus rs833061 of the *VEGF* Gene in Pregnant Women with Preeclampsia Is Associated with Newborn Weight

O. V. Golovchenko[a], M. Y. Abramova[a], I. V. Ponomarenko[a], and M. I. Churnosov[a, *]

[a] *Belgorod State University, Belgorod, 308015 Russia*

**e-mail: churnosov@bsu.edu.ru*

Received October 14, 2020; revised January 27, 2021; accepted February 1, 2021

Abstract—We studied the associations of newborn weight with polymorphic loci of growth factor genes in pregnant women with preeclampsia (PE) and considered their regulatory potential. In the group of pregnant women with PE ($n = 190$), a molecular genetic study of five polymorphic loci of growth factor genes was performed: rs4444903 *EGF*, rs833061 *VEGFA*, rs2981582 *FGFR2*, rs6214 *IGF1*, rs1800469 *TGFβ1*. Newborn somatometry was performed using standard methods. Associations of the studied polymorphic loci with newborn weight were studied using log-linear regression analysis. It was found that the genetic risk factor for the birth of small children in pregnant women with PE is the allele C of the rs833061 polymorphism of the *VEGFA* gene ($p_{perm} = 0.002$): women with the T/T genotype have the highest newborn weight (on average 3524 g), while women with the C/C genotype have the minimum newborn weight (on average 3415 g). It is shown that the allele C of polymorphic locus rs833061 is associated with low transcription of the *VEGFA* gene in the thyroid gland and a higher level of alternative splicing of the *VEGFA* gene transcript in skeletal muscle, increases the affinity of DNA for transcription factors BCL, Pax-5, and Znf143, and affects the interaction of DNA with more than 20 different regulatory proteins (CTCF, RAD21, ZNF263, MAX, etc.). It is revealed that polymorphism rs833061of the *VEGF* gene in pregnant women with PE is associated with weight of the newborn and has considerable regulatory potential.

Keywords: newborn weight, preeclampsia, single-nucleotide polymorphism (SNP), *VEGFA*, associations

DOI: 10.1134/S1022795421090039

INTRODUCTION

Anthropometric parameters (weight, height) are some of the key indicators of the state of the newborn, which, firstly, characterize the course of the past pregnancy—embryonic growth, as we know, is an important indicator of the outcome of pregnancy and reflects the interaction of physiological and pathological factors affecting the fetus during intrauterine development [1]. Often such complications of pregnancy as placental insufficiency and preeclampsia lead to the birth of children with low weight and in some cases to the development of fetal growth retardation [1, 2]. Secondly, low birth weight in newborns is a known risk factor for perinatal morbidity (development of distress syndrome, meconium aspiration, necrotizing enterocolitis, etc.) and mortality [2, 3]. Thirdly, children with low birth weight have an increased risk of developing various diseases (cardiovascular, dyslipidemia, metabolic syndrome, etc.) in adulthood [4, 5].

According to the literature, maternal factors (vascular and metabolic diseases, thrombophilic conditions, nutritional disorders, drugs, etc.) are of great importance in the formation of the anthropometric characteristics of the newborn (weight, height) [3, 4, 6, 7], including the genetic determinants of the maternal organism [8–10]. In recent studies (including those carried out at the whole-genome level), the relationship between the polymorphism of a number of candidate genes of the "maternal" genome and the height and weight of the newborn has been shown [8, 9, 11]. It should be noted that such studies are few in number in the Russian Federation, whereas the establishment of the genetic factors of the maternal organism associated with the height and weight indicators of newborns will make it possible already at the preconception stage to form risk groups for the birth of low-weight children and already in these groups to implement measures to prevent them.

The aim of the study was to study the associations of newborn weight with polymorphic loci of growth factor genes in pregnant women with preeclampsia and consider their regulatory potential.

MATERIALS AND METHODS

This study included 190 pregnant women with preeclampsia (PE) who were under observation at the Perinatal Center of the Regional Clinical Hospital of St. Joseph in Belgorod (average age 26.88 ± 5.37 years). The diagnosis of PE was based on the presence of gen-

eralized edema, arterial hypertension, and proteinuria. The samples included unrelated Russian women born in the Central Black Earth Region of the Russian Federation [12]. Individuals with uterine diseases (fibroids of the uterus, anomalies in the development of internal genital organs), other abnormalities of pregnancy (abnormalities of attachment and location of the placenta, fetal growth retardation, Rh conflict), fetal pathology (CDF), and multiple pregnancy were excluded from the studied samples. Clinical and clinical laboratory examination of pregnant women was carried out at the time of delivery. Newborn somatometry was performed using standard methods. This study was conducted under the supervision of the Ethics Committee of the Faculty of Medicine of Belgorod State University (informed consent was obtained from each woman included in the study).

All pregnant women with PE underwent a molecular genetic study of five polymorphic loci of growth factor genes: rs4444903 *EGF*, rs833061 *VEGFA*, rs2981582 *FGFR2*, rs6214 *IGF1*, rs1800469 *TGFβ1*. The polymorphic variants included in the study are regulatory (rSNP) [13]—according to the HaploReg database (v.4.1) (http://compbio.mit.edu/HaploReg), they have significant regulatory potential. DNA isolation and genotyping of polymorphic loci were carried out according to the method presented earlier [14].

To study the association of the polymorphic loci under discussion with the newborn's weight, we used log-linear regression analysis (allelic, additive, recessive, and dominant genetic models were considered). The calculations were performed using PLINK v. 2.050 (http://zzz.bwh.harvard.edu/plink/). The program calculated the regression coefficients (β) and their errors (SE) characterizing the direction of the change in the studied quantitative indicator (newborn weight) to one polymorphic genetic variant (minor allele). It should be noted that, firstly, owing to the fact that the distribution of the newborn's weight in the study sample assessed using the Shapiro–Wilk test differed from the normal one, for linear regression analysis, we used the transformed values of the newborn's weight. Secondly, the age and body mass index (before pregnancy) of mothers (quantitative variables) were included in the genetic analysis as covariates. Thirdly, during the calculations, correction for multiple comparisons was performed using the adaptive permutation test (p_{perm}). The level $p_{perm} < 0.05$ was considered statistically significant.

For polymorphic loci of growth factor genes that showed significant associations with newborn weight, their relationship to gene expression (eQTL) and alternative splicing (sQTL) was considered (associations of allelic variants of polymorphic loci with the level of gene transcription and alternative splicing were studied using data from the GTExportal project (http://www.gtexportal.org/) according to the previously presented method [15]), as well as epigenetic effects (using the online program HaploReg (v4.1) (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) according to the method described earlier [16]).

RESULTS AND DISCUSSION

The conducted population genetic analysis of the observed distribution of genotypes for the studied polymorphic loci of the genes of growth factors (rs4444903 *EGF*, rs833061 *VEGFA*, rs2981582 *FGFR2*, rs6214 *IGF1*, rs1800469 *TGFβ1*) showed its correspondence to the expected distribution according to the Hardy–Weinberg equilibrium ($p > 0.05$).

The association of the locus of the rs833061 polymorphism of *VEGFA* has been established with the weight of newborns in accordance with all considered genetic models (allelic, additive, dominant and recessive) (Table 1). According to the data obtained, the minor T allele of the rs833061 polymorphism of *VEGFA* is reliably associated with a higher birth weight (for the allelic model, $\beta = 0.171$, $p = 0.003$, $p_{perm} = 0.005$; for the additive model, $\beta = 0.176$, $p = 0.002$, $p_{perm} = 0.002$; for the dominant model, $\beta = 0.212$, $p = 0.02$, $p_{perm} = 0.02$; for the recessive model $\beta = 0.248$, $p = 0.008$, $p_{perm} = 0.009$). Accordingly, the reference allele C of this polymorphic variant of gene *VEGFA* is associated with low birth weight. In women with the T/T genotype (locus rs833061), the weight of the newborn is maximal and averages 3524 g, while in women with the C/C genotype, the weight of the newborn is minimal and averages 3415 g ($p = 0.003$, $p_{perm} = 0.005$).

Thus, we have established that the genetic risk factor for the birth of low-weight children in pregnant women with PE is the C allele of the rs833061 variant of the gene *VEGFA*.

Analysis of the regulatory effects of the rs833061 locus of the gene *VEGFA* (with the help of modern world databases on functional genomics) indicates its extremely important functional significance in the body.

First, according to the online program HaploReg (v4.1), the rs833061 locus (located at a distance of 434 bp from the 5' end of the *VEGFA* gene) is localized in the region of "open" chromatin, as well as in functionally active regions of the genome (promoters and enhancers). rs833061 exhibits significant functional effects in various cell cultures and fetal and adult organs, which are "key" in the formation of anthropometric characteristics of the embryo and fetus (trophoblast and germ layer cell lines; provisional organs—placenta and amnion; etc.).

Second, according to HaploReg (v4.1), the rs833061 polymorphic variant is localized in the region of DNA interaction sites with more than 20 different regulatory proteins (CMYC, E2F6, HDAC2,

Table 1. Association of newborn weight with polymorphic loci of growth factor genes in pregnant women with preeclampsia

Polymorphic variant	Genotypes (genetic models)	*n* (abs.)	%	Newborn weight $\bar{X} \pm SD$, g
rs4444903 *EGF*	A/A	64	35.96	3485 ± 357
	A/G	78	43.82	3456 ± 436
	G/G	36	20.22	3464 ± 323
	Minor allele G (allelic model)	β = –0.014 ± 0.053, $p = 0.79$		
	A/A vs. A/G vs. G/G (additive model)	β = –0.002 ± 0.052, $p = 0.96$		
	A/A vs. A/G + G/G (dominant model)	β = 0.038 ± 0.080, $p = 0.63$		
	A/A + A/G vs. G/G (recessive model)	β = –0.063 ± 0.097, $p = 0.51$		
rs833061 *VEGFA*	C/C	44	23.91	3415 ± 280
	C/T	96	52.18	3492 ± 385
	T/T	44	23.91	3524 ± 520
	Minor allele T (allelic model)	**β = 0.171 ± 0.058, $p = 0.003$**		
	C/C vs. C/T vs. T/T (additive model)	**β = 0.176 ± 0.056, $p = 0.002$**		
	C/C vs. C/T + T/T (dominant model)	**β = 0.212 ± 0.092, $p = 0.02$**		
	C/C + C/T vs. T/T (recessive model)	**β = 0.249 ± 0.091, $p = 0.008$**		
rs2981582 *FGFR2*	C/C	64	35.36	3499 ± 391
	C/T	93	51.38	3485 ± 393
	T/T	24	13.26	3438 ± 463
	Minor allele T (allelic model)	β = 0.001 ± 0.062, $p = 0.99$		
	C/C vs. C/T vs. T/T (additive model)	β = –0.022 ± 0.061, $p = 0.72$		
	C/C vs. C/T + T/T (dominant model)	β = –0.039 ± 0.084, $p = 0.64$		
	C/C + C/T vs. T/T (recessive model)	β = –0.006 ± 0.119, $p = 0.95$		
rs6214 *IGF1*	G/G	68	38.20	3466 ± 399
	G/A	84	47.19	3507 ± 431
	A/A	26	14.61	3405 ± 296
	Minor allele A (allelic model)	β = –0.033 ± 0.061, $p = 0.58$		
	G/G vs. G/A vs. A/A (additive model)	β = –0.019 ± 0.059, $p = 0.74$		
	G/G vs. G/A + A/A (dominant model)	β = 0.021 ± 0.084, $p = 0.79$		
	G/G + G/A vs. A/A (recessive model)	β = –0.113 ± 0.114, $p = 0.32$		
rs1800469 *TGFβ1*	C/C	70	38.04	3406 ± 364
	C/T	90	48.91	3539 ± 447
	T/T	24	13.04	3484 ± 269
	Minor allele T (allelic model)	β = 0.030 ± 0.061, $p = 0.62$		
	C/C vs. C/T vs. T/T (additive model)	β = 0.035 ± 0.059, $p = 0.56$		
	C/C vs. C/T + T/T (dominant model)	β = 0.113 ± 0.081, $p = 0.17$		
	C/C + C/T vs. T/T (recessive model)	β = –0.098 ± 0.119, $p = 0.41$		

β ± SE is the linear regression coefficient (change in the transformed value of the newborn's weight by the minor allele) and its error, obtained using linear regression taking into account the correction for covariates (the age of the pregnant woman and her body mass index before pregnancy); *p* is the level of significance; significant differences are highlighted in bold.

HEY1, YY1, TAF1, CTCF, RAD21, ZNF263, MAX, and others), including proteins that have a "key" role in the regulation of the transcriptional activity of genes in the body (CTCF, etc.) [17].

Third, according to the materials of the HaploReg database (v4.1), the rs833061 variant of gene *VEGFA* is located in the region of DNA regulatory motifs, which are binding sites for three transcription factors (BCL, Pax-5, and Znf143). At the same time, the C allele, associated according to our data with low birth weight, increases the affinity for all three considered transcription factors BCL (the difference between the LOD scores of the C and T alleles is 11.1), Pax-5 (the difference between the LOD scores of the C and T alleles is 2.2), and Znf143 (the difference between the LOD scores of the C and T alleles is 0.8).

Fourth, according to the Genotype-Tissue Expression (GTEx) project, the rs833061 variant is associated with the level of expression of gene *VEGFA* in the thyroid gland (the linear regression coefficient for the T allele is $\beta = 0.11$, $p = 0.00014$, $p_{FDR} \le 0.05$).

Fifth, the rs833061 polymorphic locus, in accordance with the materials of the GTExportal database, is associated with the level of alternative splicing of the transcript of gene *VEGFA* in skeletal muscle (intron ID—43772958:43774341:clu_32412; the normalized intron cutout ratio for the T allele is $\beta = -0.29$, $p =$ 7.1e-9, $p_{FDR} \le 0.05$).

Thus, according to our data, the rs833061 polymorphic locus of the gene *VEGFA* in pregnant women with PE is associated with the weight of newborns, and the medical and biological basis of this association may be the pronounced functional effects of the rs833061 locus in the body: it is associated with the expression of gene *VEGFA* in the thyroid gland, is associated with the level of alternative splicing of the transcript of gene *VEGFA* in skeletal muscles, determines the affinity of DNA for three transcription factors (BCL, Pax-5, and Znf143), determines protein-DNA interactions with a large number (more than 20) of various regulatory proteins (transcription factors CTCF, ZNF263, RAD21, etc.), and is localized in the zone of functionally active "open" chromatin (promoters, enhancers, etc.). At the same time, the C allele of this locus, which is a risk factor for the birth of low-weight children in pregnant women with PE, is associated with low transcription of gene *VEGFA* in the thyroid gland and with a higher level of alternative splicing of the transcript of gene *VEGFA* in skeletal muscles and also increases the affinity of DNA for the transcription factors BCL, Pax-5, and Znf143.

According to the information resource GeneCards: The Human Gene Database (http://www.genecards.org/), gene *VEGFA* is a member of the VEGF growth factor gene family—it encodes vascular endothelial growth factor A. This protein exists as a disulfide-linked homodimer and is heparin-binding. Vascular endothelial growth factor A induces proliferation and migration of vascular endothelial cells and is important for both physiological and pathological angiogenesis [18]. It should be noted that the VEGFA protein plays an important role in vasculogenesis—the growth of vessels in the embryo with the differentiation of angioblasts into endothelial cells. The results of a number of studies indicate the "key" role of VEGFA in the development of the organism, in both the embryonic and early postnatal periods. "Turning off" the *VEGFA* gene can lead to the death of the embryo in the early stages of development [19]. Experimental studies associated with the administration of VEGF inhibitors to mice at the early stages of embryonic development (days 1–8) demonstrated growth arrest and death in laboratory animals [20].

Polymorphism of the gene of vascular endothelial growth factor A is of great medical and biological importance in the body. In the catalog of genome-wide association studies (GWAS) of the National Human Genome Research Institute (http://www.genome.gov/gwastudies/), there is information on more than 90 different GWAS studies of associations of polymorphic loci of gene *VEGFA* with the formation of various signs and diseases of a person (anthropometric indicators, type 2 diabetes mellitus, kidney disease, etc.), as a result of which more than 140 significant associations were established at the whole-genome level.

It should be noted that the involvement of the rs833061 polymorphic locus of the *VEGFA* gene (according to our data, it is associated with the weight of newborns in pregnant women with PE) in the development of various human diseases is being actively studied by various scientific teams—in the NCBI database (https://www.ncbi.nlm.nih.gov/pmc/?term=rs833061) as of February 2020, there are more than 150 works devoted to biomedical studies of rs833061 of gene *VEGFA*. The study by Ben Ali Gannoun et al. [21] demonstrated the association of this polymorphism (within the haplotype) with the development of pre-eclampsia. S.A. Yalcintepe et al. [22] established significant differences in the frequencies of rs833061 genotypes between mothers and their spontaneous abortions. The authors note that the rs833061 locus is a risk factor for spontaneous abortion. The significant role of rs833061 in recurrent miscarriage is noted in [23]. Associations of this polymorphic locus with the development of congenital heart defects [24], polycystic ovary disease [25], various cancers (ovarian cancer [26], lung cancer [27]), and metabolic diseases (diabetic retinopathy in patients with type 2 diabetes mellitus [28]) are shown.

It can be assumed that one of the important mechanisms for the implementation of the biomedical effects of rs833061 of gene *VEGFA* in the body determining its involvement in the formation of various diseases (including the underlying, established in our work, and its association with the weight of newborns)

is its influence on the processes of DNA interaction with more than 20 different regulatory proteins, including those playing the central role in the regulation of gene activity in the body. For example, the transcription factor CTCF, according to the literature, is one of the main participants in various networks of gene regulation, including activation and repression of transcription, formation of independently functioning chromatin domains, regulation of imprinting, organization of insulators, and regulation of alternative splicing [17].

Thus, the locus rs833061 of the gene *VEGFA* in pregnant women with PE is associated with the weight of newborns, and the medical and biological basis of this association may be the pronounced functional effects of rs833061 in the body: it is associated with the expression of gene *VEGFA* in the thyroid gland, is associated with the level of alternative splicing of the transcript of gene *VEGFA* in skeletal muscles, determines the affinity of DNA for three transcription factors (BCL, Pax-5, and Znf143), and affects the interaction of DNA with more than 20 different regulatory proteins (CMYC, E2F6, HDAC2, HEY1, YY1, TAF1, CTCF, RAD21, ZNF263, MAX, etc.). This locus is located in the region of hypersensitivity to DNase-1 and modified histones that mark enhancers and promoters (including "active" enhancers and promoters) in more than 100 different cell cultures, tissues, and organs, both in an adult body and in a fetus.

FUNDING

This work was supported by a grant from the President of the Russian Federation for the leading scientific schools of the Russian Federation (project NSh-2609.2020.7).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in a study involving people comply with the ethical standards of the institutional and/or national committee for research ethics and the 1964 Helsinki Declaration and its subsequent changes or comparable ethical standards.

Informed voluntary consent was obtained from each of the participants.

REFERENCES

1. Strizhakov, A.M., Lipatov, I.S., and Tezikov, Yu.V., *Platsentarnaya nedostatochnost': patogenez, prognozirovanie, diagnostika, profilaktika, akusherskaya taktika* (Placental Insufficiency: Pathogenesis, Forecasting, Diagnostics, Prevention, Obstetric Tactics), Samara: OFORT, 2014.
2. Sharma, D., Shastri, S., and Sharma, P., Intrauterine growth restriction: antenatal and postnatal aspects, *Clin. Med. Insights: Pediatr.*, 2016, vol. 10, pp. 67—83. https://doi.org/10.4137/CMPed.S40070
3. Malhotra, A., Allison, B.J., Castillo-Melendez, M., et al., Neonatal morbidities of fetal growth restriction: pathophysiology and impact, *Front. Endocrinol.*, 2019, vol. 10, p. 55. https://doi.org/10.3389/fendo.2019.00055
4. Devaskar, S.U. and Chu, A., Intrauterine growth restriction: hungry for an answer, *Physiology* (Bethesda), 2016, vol. 31, no. 2, pp. 131—146. https://doi.org/10.1152/physiol.00033.2015
5. Melkozerova, O.A., Bashmakova, N.V., Tret'yakova, T.B., and Shchedrina, I.D., Molecular-genetic and epigenetic aspects of impaired endometrial receptivity in women with low birth weight, *Vopr. Ginekol., Akush. Perinatol.*, 2019, vol. 18, no. 4, pp. 35—43. https://doi.org/10.20953/1726-1678-2019-4-35-43
6. Nardozza, L.M.M., Caetano, A.C.R., Zamarian, A.C.P., et al., Fetal growth restriction: current knowledge, *Arch. Gynecol. Obstet.*, 2017, vol. 295, pp. 1061—1077. https://doi.org/10.1007/s00404-017-4341-9
7. Hwang, L.D., Lawlor, D.A., Freathy, R.M., et al., Using a two-sample Mendelian randomization design to investigate a possible causal effect of maternal lipid concentrations on offspring birth weight, *Int. J. Epidemiol.*, 2019, vol. 48, no. 5, pp. 1457—1467. https://doi.org/10.1093/ije/dyz160
8. Reshetnikov, E., Zarudskaya, O., Polonikov, A., et al., Genetic markers for inherited thrombophilia are associated with fetal growth retardation in the population of Central Russia, *J. Obstet. Gynaecol. Res.*, 2017, vol. 43, no. 7, pp. 1139—1144. https://doi.org/10.1111/jog.13329
9. Beaumont, R.N., Warrington, N.M., Cavadino, A., et al., Genome-wide association study of offspring birth weight in 86 577 women identifies five novel loci and highlights maternal genetic effects that are independent of fetal genetics, *Hum. Mol. Genet.*, 2018, vol. 27, no. 4, pp. 742—756.
10. Golovchenko, O., Abramova, M., Ponomarenko, I., et al., Functionally significant polymorphisms of ESR1and PGR and risk of intrauterine growth restriction in population of Central Russia, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2020, vol. 253, pp. 52—57. https://doi.org/10.1016/j.ejogrb.2020.07.045
11. Horikoshi, M., Beaumont, R.N., Day, F.R., et al., Genome-wide associations for birth weight and correlations with adult disease, *Nature*, 2016, vol. 538, no. 7624, pp. 248—252. https://doi.org/10.1038/nature19806
12. Litovkina, O., Nekipelova, E., Dvornyk, V., et al., Genes involved in the regulation of vascular homeostasis determine renal survival rate in patients with chronic glomerulonephritis, *Gene*, 2014, vol. 546, no. 1, pp. 112—116. https://doi.org/10.1016/j.gene.2014.04.020
13. Reshetnikov, E.A., Akulova, L.Y., Dobrodomova, I.S., et al., 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.,* 2015, vol. 16, no. 3, pp. 623—632. https://doi.org/10.1177/1470320313501217

14. Reshetnikov, E.A., Search for associations of candidate genes differentially expressed in the placenta with the risk of developing placental insufficiency with fetal growth retardation syndrome, *Res. Result. Biomed.*, 2020, vol. 6, no. 3, pp. 338—349. https://doi.org/10.18413/2658- 6533-2020-6-3-0-5
15. Ponomarenko, I., Reshetnikov, E., Polonikov, A., et al., Candidate genes for age at menarche are associated with endometrial hyperplasia, *Gene*, 2020, vol. 757, p. 144933. https://doi.org/10.1016/j.gene.2020.144933
16. Ponomarenko, I., Reshetnikov, E., Polonikov, A., et al., Candidate genes for age at menarche are associated with endometriosis, *Reprod. Biomed. Online*, 2020, vol. 41, no. 5, pp. 943—956. https://doi.org/10.1016/j.rbmo.2020.04.016
17. Starikova, D., Ponomarenko, I., Reshetnikov, E., et al., Novel data about association of the functionally significant polymorphisms of the MMP-9 gene with exfoliation glaucoma in the Caucasian population of Central Russia, *Ophthalmic Res.*, 2021, vol. 64, no. 3, pp. 458—464. https://doi.org/10.1159/000512507
18. Ponomarenko, I., Reshetnikov, E., Altuchova, O., et al., Association of genetic polymorphisms with age at menarche in Russian women, *Gene*, 2019, vol. 686, pp. 228—236. https://doi.org/10.1016/j.gene.2018.11.042
19. Moskalenko, M., Ponomarenko, I., Reshetnikov, E., et al., Polymorphisms of the matrix metalloproteinase genes are associated with essential hypertension in a Caucasian population of Central Russia, *Sci. Rep.,* 2021, vol. 11, no. 1, p. 5224. https://doi.org/10.1038/s41598-021-84645-4
20. Ponomarenko, I., Reshetnikov, E., Polonikov, A., et al., Candidate genes for age at menarche are associated with uterine leiomyoma, *Front. Genet.*, 2021, vol. 11, p. 512940. https://doi.org/10.3389/fgene.2020.512940
21. Zafar, M.I., Zheng, J., Kong, W., et al., The role of vascular endothelial growth factor-B in metabolic homoeostasis: current evidence, *Biosci. Rep.*, 2017, vol. 37, no. 4. BSR20171089. https://doi.org/10.1042/BSR20171089
22. Ferrara, N., Carver-Moore, K., Chen, H., et al., Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene, *Nature*, 1996, vol. 380, no. 6573, pp. 439—442.
23. Jung, Y.W., Ahn, E.H., Kim, J.O., et al., Association of genetic polymorphisms in VEGF-460, -7 and -583 and hematocrit level with the development of idiopathic recurrent pregnancy loss and a meta-analysis, *J. Gene Med.*, 2018, vol. 20, no. 9. e3048. https://doi.org/10.1002/jgm.3048
24. Wang, W., Xu, A., and Xu, H., The roles of vascular endothelial growth factor gene polymorphisms in congenital heart diseases: a meta-analysis, *Growth Factors*, 2018, vol. 36, nos. 5—6, pp. 232—238. https://doi.org/10.1080/08977194.2018.1513505
25. Huang, L. and Wang, L.L., Association between VEGF gene polymorphisms (11 sites) and polycystic ovary syndrome risk, *Biosci. Rep.*, 2020, vol. 40, no. 3. BSR20191691. https://doi.org/10.1042/BSR20191691
26. Xu, C.H., He, Z.H., and Xu, H., Association of four genetic polymorphisms in the vascular endothelial growth factor-A gene and development of ovarian cancer: a meta-analysis, *Oncotarget*, 2017, vol. 8, no. 42, pp. 73063—73078. https://doi.org/10.18632/oncotarget.20379
27. Yang, F., Qin, Z., Shao, C., et al., Association between VEGF gene polymorphisms and the susceptibility to lung cancer: an updated meta-analysis, *Biomed. Res. Int.*, 2018, vol. 2018, p. 9271215. https://doi.org/10.1155/2018/9271215
28. Han, L., Zhang, L., Xing, W., et al., The associations between VEGF gene polymorphisms and diabetic retinopathy susceptibility: a meta-analysis of 11 case-control studies, *J. Diabetes Res.*, 2014, vol. 2014, p. 805801. https://doi.org/10.1155/2014/805801