ISSN 1022-7954, Russian Journal of Genetics, 2022, Vol. 58, No. 12, pp. 1543–1550. © Pleiades Publishing, Inc., 2022.
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HUMAN GENETICS

The Polymorphic Locus rs167479 of the *RGL3* Gene Is Associated with the Risk of Severe Preeclampsia

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Received January 27, 2022; revised April 4, 2022; accepted May 4, 2022

Abstract—In this work, the associations of polymorphism of candidate genes of arterial hypertension with the development of severe preeclampsia (PE) in the population of the Central Chernozem region of Russia were studied. Genotyping of five polymorphic variants (rs1799945 of the *HFE* gene, rs8068318 of the *TBX2* gene, rs1173771 of the *AC025459.1* gene, rs932764 of the *PLCE1* gene, rs167479 of the *RGL3* gene) was performed in 217 women with severe PE and 235 pregnant women with moderate PE. It was revealed that the *G* allele and the *GG* genotype of the rs167479 polymorphic locus of the *RGL3* gene were associated with the risk of severe PE according to allelic (OR = 1.35, $p_{perm} = 0.02$), additive (OR = 1.36, $p_{perm} = 0.02$), and recessive (OR = 1.61, $p_{perm} = 0.04$) genetic models. It has been established that this polymorphic locus is localized in a functionally active region of the genome that performs the functions of enhancers and promoters in various organs and tissues, is an area of hypersensitivity to DNase-1 and a binding site with nine transcription regulatory factors, and is associated with the expression level of the *CTC-510F12.3* gene in the pituitary gland. In addition, rs167479 identifies a missense mutation that leads to the substitution of the amino acid Pro162His in the RalGDS-like3 protein and has a predictor potential of "PROBABLY DAMAGING."

Keywords: preeclampsia, polymorphic locus, *RGL3*, GWAS

DOI: 10.1134/S102279542212002X

INTRODUCTION

Preeclampsia (PE) is one of the most severe complications of pregnancy, which classically manifests itself as increased blood pressure (DBP more than 90 and/or SBP more than 140 mmHg), proteinuria (≥0.3 g/day), and the development of obvious or hidden edema and is accompanied by an impaired functioning of various organs and systems [1]. According to the worldwide statistics, PE complicates the course of pregnancy in 2–8% of all cases and is one of the main causes of maternal morbidity and mortality [2, 3]. Women with a history of PE represent a cohort of individuals with a higher risk of developing thrombosis and strokes, type II diabetes mellitus, arterial hypertension (AH), renal failure, cardiomyopathy, etc., within 15 years after childbirth [4, 5]. PE also significantly contributes to the structure of perinatal morbidity and mortality. It has been established that maternal PE significantly enhances the risk of early neonatal death, the birth of underweight children, the development of respiratory distress syndrome, and peri- and intraventricular hemorrhages in newborns [6, 7]. In addition, PE is an independent risk factor for developing cardiovascular and neuropsychiatric diseases in the future in children born after this pregnancy [8, 9].

PE refers to multifactorial diseases, as are the majority of human pathologies [10–14]. Genetic determinants correspond to at least 50% in the development of this complication of pregnancy [15]. Recently, specific attention was paid to the study of the effects of polymorphisms in various groups of candidate genes which encode proteins involved in PE pathogenesis, together with candidate genes of AH, as one of the fundamental symptoms of this complication of pregnancy [16–18]. It should be noted that despite the large number of conducted genetic studies of PE, the findings obtained are contradictory, and the results vary depending on the ethnoterritorial characteristics of the examined groups, including those observed in Russia, which determines the relevance of further studies of the molecular genetic basis of PE.

The present study aimed to evaluate the associations of polymorphisms of AH candidate genes with the development of severe PE.

MATERIALS AND METHODS

The present study involved 452 women with a pregnancy complicated by PE (mean age of patients was 27.39 ± 4.05 years), who were under the supervision of obstetricians and gynecologists at the Perinatal Center at Belgorod St. Joasaph Regional Hospital and signed

informed consents to participate in this study. The group of women with a moderate PE consisted of 235 pregnant women, while the group of women with severe PE included 217 subjects. The samples consisted of ethnically Russian women who were born and lived in the Central Chernozem region of Russia and were unrelated to each other. The diagnosis of PE was verified on the basis of the presence of arterial hypertension and proteinuria. Severe PE was diagnosed with the presence of severe hypertension (DBP higher than 110 mmHg and/or SBP higher than 160 mmHg) and daily proteinuria higher than 5 g/L combined with one or more criteria of severe PE specified in the clinical recommendations "Hypertensive Disorders during Pregnancy, Childbirth, and Postpartum Period. Preeclampsia. Eclampsia" (2016). A moderate PE was diagnosed in the case of excluded parameters of severe PE and AH presence (SBP = 140–159 and/or DBP = 90–109 mmHg) and proteinuria ≥0.3 g/L [19]. Clinical, clinical anamnestic, and clinical laboratory screening of pregnant women was carried out at the time of delivery controlled by the ethics committee at the Medical Institute at Belgorod National Research University.

All women with PE were examined via molecular genetic analysis of five candidate gene polymorphisms that were previously associated with developing arterial hypertension according to data in the catalog of genome-wide association studies (GWAS): rs1799945 *HFE*, rs8068318 *TBX2*, rs1173771 *AC025459.1*, rs932764 *PLCE1*, rs167479 *RGL3* [20–45]. DNA isolation and subsequent genotyping of polymorphic loci was conducted according to the previously described approach [46]. Single nucleotide polymorphisms (SNPs) were included on the basis of the following criteria [47, 48]: they were associated with AH development according to two or more GWAS and possessed a significant regulatory potential in conformance with the HaploReg (v.4.1) online resource (http://compbio.mit.edu/HaploReg) [49]. The data on the empirical distribution of genotypes and its correspondence to the theoretically expected one according to the Hardy–Weinberg equilibrium were obtained (the deviations were statistically significant at $p_{bonf} \le 0.01$). The association analysis of examined SNPs with developing severe and moderate PE was carried out via logistic regression analysis (four genetic models were examined: allelic, additive, recessive, and dominant) controlling for covariates (age, family history of PE, and body mass index prior the pregnancy). Genetic analysis was performed with correction for multiple testing using the adaptive permutation test (p_{perm}). The statistically significant level was estimated at $p_{perm} < 0.05$ [50]. To assess the association between SNPs and PE, the values of the odds ratio (OR) and 95% confidence interval for OR (95%CI) were determined. All calculations were conducted in PLINK v. 2.050 (http://zzz.bwh.harvard.edu/plink) [51].

Polymorphic variants which were significantly associated with the development of severe PE were estimated for their effect on the transcriptional activity (eQTL) and alternative gene splicing (sQTL) using the online tool GTExportal (http://www.gtexportal.org/) [52], while epigenetic effects were assigned using online tool HaploReg (v4.1), http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) [53], the estimate of predictive values of nonsynonymous substitutions was carried out using the PolyPhen-2 database (http://genetics.bwh.harvard.edu/pph2/) [54].

RESULTS AND DISCUSSION

The population genetic analysis of the observed distribution of the genotypes of the studied polymorphisms of AH candidate genes (rs1799945 *HFE*, rs8068318 *TBX2*, rs1173771 *AC025459.1*, rs932764 *PLCE1*, and rs167479 *RGL3*) demonstrated its correspondence with the expected distribution according to the Hardy–Weinberg equilibrium (Bonferroni correction for the number of examined loci was introduced $p_{bonf} \le 0.01$ (0.05/5)).

The association of rs167479 of the *RGL3* gene with the development of severe PE was revealed. According to the data obtained in the present study, the minor *G* allele and the *GG* genotype of rs167479 of the *RGL3* gene are significantly associated with an increased risk of developing severe PE according to the allelic (OR = 1.35, 95%CI 1.03–1.76, $p = 0.02$, $p_{perm} = 0.02$), additive (OR = 1.37, 95%CI 1.04–1.79, $p = 0.02$, $p_{perm} = 0.02$), and recessive (OR = 1.61, 95%CI 1.02–2.51, $p = 0.04$, $p_{perm} = 0.04$) genetic models (Table 1).

On the basis of the GWAS catalog, rs167479 of the *RGL3* gene was significantly associated ($p \le 5 \times 10^{-8}$) with the parameters of blood pressure (BP) and AH according to the results of eight genome-wide association studies. The *G* allele of the examined SNP was associated with enhanced SBP, DBP, PP, while the *T* allele has a "protective" effect of developing AH and is associated with a lower BP level (SBP, DBP, PP, MBP) [38–45]. This completely coincides with the obtained data on the "risky" effect of the *G* allele for PE development. It should be noted that only one of five studied GWAS-significant SNPs for hypertension demonstrated the association with PE development in the population of the Central Chernozem region of Russia, while four SNPs failed to be associated with the risk of developing PE, despite their significant effect in developing hypertension in previous GWAS. A specificity of genetic determination of AH was also reported in the population of the Central Chernozem region of Russia by other studies [55–57]. Therefore, the results of the present study once again confirm the necessity for replication studies of GWAS-significant SNPs in certain populations (including the Central Chernozem region).

Table 1. Association of GWAS-significant SNPs of arterial hypertension candidate genes with developing severe PE

SNPs (gene)	Allele, genotype	Severe PE ($n = 217$), % (n)	Moderate PE ($n = 235$), % (n)	OR (95%CI)	p
rs1799945 (*HFE*)	*G*	18.31 (78)	22.01 (103)	0.79 (0.56–1.11)	0.19
	C	81.69 (348)	77.99 (365)	1.26 (0.89–1.77)	
	GG	5.63 (12)	4.70 (11)	1.21 (0.48–3.01)	0.81
	GC	25.35 (54)	34.62 (81)	0.64 (0.41–0.98)	0.09
	CC	69.07 (147)	60.68 (142)	1.44 (0.95–2.17)	0.08
	H_O/H_E (P_{HWE})	0.253/0.299 (0.036)	0.346/0.343 (1.000)		
	Minor *G* allele (allelic model)			0.79 (0.57–1.10)	0.17
	G/G vs. *G/C* vs. *C/C* (additive model)			0.81 (0.48–1.11)	0.18
	G/G vs. *G/C* + *C/C* (dominant model)			0.69 (0.47–1.03)	0.07
	G/G + *G/C* vs. *C/C* (recessive model)			1.21 (0.52–2.80)	0.65
rs8068318 (*TBX2*)	*C*	28.47 (119)	28.38 (130)	1.01 (0.74–1.36)	1.00
	T	71.53 (299)	71.62 (328)	1.00 (0.73–1.35)	
	CC	9.09 (19)	9.61 (22)	0.94 (0.47–1.88)	0.98
	CT	38.76 (81)	37.55 (86)	1.05 (0.70–1.58)	0.87
	TT	47.85 (109)	52.84 (121)	0.97 (0.66–1.44)	0.96
	H_O/H_E (P_{HWE})	0.387/0.407 (0.498)	0.375/0.406 (0.256)		
	Minor *C* allele (allelic model)			1.00 (0.75–1.35)	0.98
	C/C vs. *C/T* vs. *T/T* (additive model)			1.00 (0.75–1.34)	0.98
	C/C vs. *T/T* + *T/T* (dominant model)			1.03 (0.70–1.49)	0.89
	C/C + *C/T* vs. *T/T* (recessive model)			0.94 (0.49–1.79)	0.85
rs1173771 (*AC025459.1*)	*A*	43.66 (186)	40.79 (186)	1.13 (0.85–1.48)	0.42
	G	56.34 (240)	59.21 (270)	0.89 (0.67–1.17)	
	AA	19.25 (41)	14.91 (34)	1.36 (0.80–2.31)	0.28
	AG	48.83 (104)	51.75 (118)	0.89 (0.60–1.32)	0.60
	GG	31.92 (68)	33.46 (76)	0.94 (0.62–1.43)	0.83
	H_O/H_E (P_{HWE})	0.488/0.492 (0.890)	0.503/0.487 (0.558)		
	Minor *A* allele (allelic model)			1.12 (0.86–1.47)	0.39
	A/A vs. *A/G* vs. *G/G* (additive model)			1.13 (0.86–1.48)	0.38
	A/A vs. *A/G* + *G/G* (dominant model)			1.07 (0.71–1.59)	0.75
	A/A + *A/G* vs. *A/G* (recessive model)			1.36 (0.83–3.24)	0.23

Table 1. (Contd.)

SNPs (gene)	Allele, genotype	Severe PE ($n = 217$), % (n)	Moderate PE ($n = 235$), % (n)	OR (95%CI)	p
rs932764 (*PLCE1*)	*A*	46.98 (202)	47.22 (221)	1.10 (0.83–1.44)	0.52
	G	53.02 (228)	52.78 (247)	0.91 (0.69–1.19)	
	AA	20.93 (45)	22.22 (52)	0.93 (0.58–1.49)	0.83
	AG	52.09 (112)	50.00 (117)	1.09 (0.74–1.60)	0.73
	GG	26.98 (58)	27.78 (65)	0.96 (0.62–1.49)	0.93
	H_O/H_E (P_{HWE})	0.520/0.498 (0.584)	0.510/0.498 (0.637)		
	Minor *A* allele (allelic model)			0.99 (0.76–1.28)	0.94
	A/A vs. *A/G* vs. *G/G* (additive model)			0.99 (0.75–1.29)	0.94
	A/A vs. *A/G* + *G/G* (dominant model)			1.04 (0.68–1.57)	0.84
	A/A + *A/G* vs. *A/G* (recessive model)			0.92 (0.59–1.45)	0.73
rs167479 (*RGL3*)	*G*	52.11 (222)	44.61 (207)	1.35 (1.03–1.78)	0.03
	C	47.89 (204)	55.39 (257)	0.74 (0.56–0.97)	
	GG	26.76 (57)	18.53 (43)	1.61 (1.00–2.58)	0.05
	GC	50.70 (108)	52.16 (121)	0.94 (0.64–1.39)	0.83
	CC	22.54 (48)	29.31 (68)	0.70 (0.45–1.10)	0.13
	H_O/H_E (P_{HWE})	0.507/0.499 (0.891)	0.521/0.494 (0.428)		
	Minor *G* allele (allelic model)			1.35 (1.03–1.76)	0.02
	G/G vs. *G/T* vs. *T/T* (additive model)			1.37 (1.04–1.79)	0.02
	G/G vs. *G/T* + *T/T* (dominant model)			1.42 (0.93–2.19)	0.10
	G/G + *G/T* vs. *T/T* (recessive model)			1.61 (1.02–2.51)	0.04

OR—odds ratio, 95%CI—95% confidence interval for odds ratio; p—significance level; H_O—observed heterozygosity; H_E—expected heterozygosity, P_{HWE}—significance level of deviation from the Hardy–Weinberg equilibrium.

The estimate of the functional effects revealed that the examined marker rs167479 of the *RGL3* gene was located in a functionally active genomic region, which exhibits various tissue/organ-specific regulatory effects. For instance, in the amnion, placenta, and mesenchymal and stem cell cultures, the DNA region including rs167479 functions as both enhancer and promoter. In the trophoblast, primary peripheral blood cells, the genomic region residing rs167479 represents an enhancer. At the same time, the same DNA region (the place of localization of rs167479) is considered an evolutionarily conservative site and the region hypersensitive to DNase-1 and a binding site of nine transcription factors: AP-1, CNT2, Rd 21, SETDB1, SP1, R4, WT 1, ZNF219, and Zinc. It should be noted that the *G* allele, which is associated with developing severe PE based on our findings, significantly increases the "sensitivity" to transcription factors CCNT2 (ΔLOD scores of *G* (ref) and *T* (alt) alleles is 11.9), Rad21 (ΔLOD scores of *G* (ref) and *T* (alt) alleles is 11.3), and ZNF219 (ΔLOD scores of the *G* (ref) and *T* (alt) alleles is 12.0). According to the GTExportal online resource, the examined polymorphic marker is associated with the expression level of the *CTC-510F12.3* gene in the pituitary gland ($p = 0.0000017$). Using the PolyPhen-2 database, it was observed that rs167479 determines a nonsynonymous proline to histidine amino acid substitution at position 162 (Pro162His) of the RalGDS-like3 polypeptide (Ral guanine nucleotide dissociation stimulator-

like 3). The predictor potential of this missense mutation corresponds to the "PROBABLY DAMAGING" class (sensitivity = 0.70; specificity = 0.97, score = 0.992).

According to the GeneCards database [58], the *RGL3* gene encodes the protein related to guanine nucleotide exchange factors (GEFs)) and activates the functioning of small guanosine triphosphate hydrolases (GTPases, small G proteins) by facilitating their dissociation with GDP [59, 60]. GEFs signaling pathways, which predominantly belong to the Rho family, represent a common part of the pathogenesis of diabetes mellitus and cardiovascular diseases, including AH and coronary and cerebrovascular pathology [61]. It was established that GEFs were expressed in endothelial and smooth muscle arterial cells and participated in the regulation of these structures, which determines the potential role of GEFs in the development of hypertensive disorders, including PE [62]. Small GTPases are characterized by a wide spectrum of effectors, thus regulating multiple biological processes (regulation of transmembrane transport, cytoskeleton reorganization, transcription activation, regulation of gene expression, etc.) [63]. Signaling pathways involving small G proteins modulate the activity of various membrane ion channels (epithelial sodium channel (ENaC), K^+ and Ca^{2+} channels). One of the key factors regulating the volume of fluid circulating in the body and, hence, the blood pressure is the reabsorption of sodium ions in the distal parts of the nephrons via ENaC [64]. In addition, the RPM/RGL3 expression inhibits the induction of the Elk-1 transcription factor involved in cell growth, differentiation, and migration [65, 66].

The present study demonstrated the association of GWAS-significant AH polymorphic locus rs167479 of the *RGL3* gene with the development of severe preeclampsia in the population of the Central Chernozem region of Russia (minor *G* allele and *GG* genotype enhanced the risk of severe PE). The polymorphic variant rs167479 of the *RGL3* gene has significant epigenetic effects (is localized in a functionally active genomic region, which can function as enhancer and promoter in various organs and tissues, and also represents a region hypersensitive to DNase-1 and a binding site with nine transcription regulatory factors) and is associated with the expression level of the *CTC-510F12.3* gene in the pituitary gland. Moreover, rs167479 determines a missense mutation resulting in the Pro162His substitution in the RalGDS-like3 protein and demonstrates a predictor potential of "PROBABLY DAMAGING."

FUNDING

The present study was supported by the Grant of the President of the Russian Federation "The Study of Genetic Factors of Women's Reproductive Health" (MD-3284.2022.1.4).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

REFERENCES

1. Michalczyk, M., Celewicz, A., Celewicz, M., et al., The role of inflammation in the pathogenesis of preeclampsia, *Mediators Inflammation*, 2020, vol. 10, p. 3864941. https://doi.org/10.1155/2020/3864941
2. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin Summary, Number 222, *Obstet. Gynecol.*, 2020, vol. 135, no. 6, pp. 1492—1495. https://doi.org/10.1097/AOG.0000000000003892
3. Serebrova, V.N., Trifonova, E.A., and Stepanov, V.A., Pregnancy as a factor of adaptive human evolution: the role of natural selection in the origin of preeclampsia, *Russ. J. Genet.*, 2021, vol. 57, no. 1, pp. 23—35. https://doi.org/10.1134/S1022795421010142
4. Turbeville, H.R. and Sasser, J.M., Preeclampsia beyond pregnancy: long-term consequences for mother and child, *Am. J. Physiol.: Renal Physiol.*, 2020, vol. 318, no. 6, pp. F1315—F1326. https://doi.org/10.1152/ajprenal.00071.2020
5. Chourdakis, E., Oikonomou, N., Fouzas, S., et al., Preeclampsia emerging as a risk factor of cardiovascular disease in women, *High Blood Pressure Cardiovasc. Prev.*, 2021, vol. 28, no. 2, pp. 103—114. https://doi.org/10.1007/s40292-020-00425-7
6. Mendola, P., Mumford, S.L., Männistö, T.I., et al., Controlled direct effects of preeclampsia on neonatal health after accounting for mediation by preterm birth, *Epidemiology*, 2015, vol. 26, no. 1, pp. 17—26. https://doi.org/10.1097/EDE.0000000000000213
7. Khader, Y.S., Batieha, A., Al-Njadat, R.A., and Hijazi, S.S., Preeclampsia in Jordan: incidence, risk factors, and its associated maternal and neonatal outcomes, *J. Matern. Fetal Neonatal Med.*, 2018, vol. 31, no. 6, pp. 770—776. https://doi.org/10.1080/14767058.2017.1297411
8. Nahum Sacks, K., Friger, M., Shoham-Vardi, I., et al., Prenatal exposure to preeclampsia as an independent risk factor for long-term cardiovascular morbidity of the offspring, *Pregnancy Hypertens.*, 2018, vol. 13, pp. 181—186. https://doi.org/10.1016/j.preghy.2018.06.013
9. Nahum Sacks, K., Friger, M., Shoham-Vardi, I., et al., Long-term neuropsychiatric morbidity in children exposed prenatally to preeclampsia, *Early Hum. Dev.*, 2019, vol. 130, pp. 96—100. https://doi.org/10.1016/j.earlhumdev.2019.01.016

10. 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
11. Severinova, O.V., Lokteva, T.I., Gureev, V.V., et al., The effect of arginase II selective inhibitors on the functional parameters of experimental animals in ADMA-like preeclampsia, *J. Int. Pharm. Res.*, 2019, vol. 46, no. 4, pp. 272—275.
12. Vennou, K.E., Kontou, P.I., Braliou, G.G., and Bagos, P.G., Meta-analysis of gene expression profiles in preeclampsia, *Pregnancy Hypertens.*, 2020, vol. 19, pp.52—60. https://doi.org/10.1016/j.preghy.2019.12.007
13. Reshetnikov, E.A., The rs34845949 polymorphism of the SASH1 gene is associated with the risk of preeclampsia, *Nauchn. Rezul't. Biomed. Issled.*, 2021, vol. 7, no. 1, pp. 44—55. https://doi.org/10.18413/2658-6533-2020-7-1-0-4
14. 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
15. Galaviz-Hernandez, C., Sosa-Macias, M., Teran, E., et al., Paternal determinants in preeclampsia, *Front. Physiol.*, 2019, vol. 9, p. 1870. https://doi.org/10.3389/fphys.2018.01870
16. Serebrova, V.N., Trifonova, E.A., and Stepanov, V.A., Evolutionary genetic analysis of the role of the *CORO2A* gene regulatory regions in the development of hereditary predisposition to preeclampsia in Russians and Yakuts, *Nauchn. Rezul't. Biomed. Issled.*, 2018, vol. 4, no. 3, pp. 38—48. https://doi.org/10.18413/2313-8955-2018-4-3-0-4
17. Reshetnikov, E., Ponomarenko, I., Golovchenko, O., et al., The VNTR polymorphism of the endothelial nitric oxide synthase gene and blood pressure in women at the end of pregnancy, *Taiwan. J. Obstet. Gynecol.*, 2019, vol. 58, no. 3, pp. 390—395. https://doi.org/10.1016/j.tjog.2018.11.035
18. Chen, G., Li, L., Wu, J., et al., Correlations of P-selectin and E-selectin gene polymorphisms with preeclampsia, *Panminerva Med.*, 2021, vol. 63, no. 1, pp. 93—94. https://doi.org/10.23736/S0031-0808.19.03672-3
19. *Hypertensive Disorders during Pregnancy, Childbirth and the Postpartum Period: Preeclampsia. Eclampsia. Clinical Guidelines (Clinical Protocol)*, Moscow, 2016, pp. 4–6.
20. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret, G.B., Munroe, P.B., Rice, K.M., et al., Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk, *Nature*, 2011, vol. 478, no. 7367, pp. 103—109. https://doi.org/10.1038/nature10405
21. Pichler, I., Minelli, C., Sanna, S., et al., Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels, *Hum. Mol. Genet.*, 2011, vol. 20, no. 6, pp. 1232—1240. https://doi.org/10.1093/hmg/ddq552
22. Astle, W.J., Elding, H., Jiang, T., et al., The allelic landscape of human blood cell trait variation and links to common complex disease, *Cell*, 2016, vol. 167, no. 5, pp. 1415—1429. e19. https://doi.org/10.1016/j.cell.2016.10.042
23. Chambers, J.C., Zhang, W., Lord, G.M., et al., Genetic loci influencing kidney function and chronic kidney disease, *Nat. Genet.*, 2010, vol. 42, no. 5, pp. 373—375. https://doi.org/10.1038/ng.566
24. Wain, L.V., Verwoert, G.C., O'Reilly, P.F., et al., Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure, *Nat. Genet.*, 2011, vol. 43, no. 10, pp. 1005—1011. https://doi.org/10.1038/ng.922
25. Ehret, G.B., Ferreira, T., Chasman, D.I., et al., The genetics of blood pressure regulation and its target organs from association studies in 342 415 individuals, *Nat. Genet.*, 2016, vol. 48, no. 10, pp. 1171—1184. https://doi.org/10.1038/ng.3667
26. Pilling, L.C., Atkins, J.L., Duff, M.O., et al., Red blood cell distribution width: genetic evidence for aging pathways in 116 666 volunteers, *PLoS One*, 2017, vol. 12, no. 9. e0185083. https://doi.org/10.1371/journal.pone.0185083
27. Raffield, L.M., Louie, T., Sofer, T, et al., Genome-wide association study of iron traits and relation to diabetes in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL): potential genomic intersection of iron and glucose regulation?, *Hum. Mol. Genet.*, 2017, vol. 26, no. 10, pp. 1966—1978. https://doi.org/10.1093/hmg/ddx082
28. Kanai, M., Akiyama, M., Takahashi, A., et al., Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases, *Nat. Genet.*, 2018, vol. 50, no. 3, pp. 390—400. https://doi.org/10.1038/s41588-018-0047-6
29. Sung, Y.J., Winkler, T.W., de Las Fuentes, L., et al., A large-scale multi-ancestry genome-wide study accounting for smoking behavior identifies multiple significant loci for blood pressure, *Am. J. Hum. Genet.*, 2018, vol. 102, no. 3, pp. 375—400. https://doi.org/10.1016/j.ajhg.2018.01.015
30. Oskarsson, G.R., Oddsson, A., Magnusson, M.K., et al., Predicted loss and gain of function mutations in ACO1 are associated with erythropoiesis, *Commun. Biol.*, 2020, vol. 3, no. 1, p. 189. https://doi.org/10.1038/s42003-020-0921-5
31. Vuckovic, D., Bao, E.L., Akbari, P., et al., The polygenic and monogenic basis of blood traits and diseases, *Cell*, 2020, vol. 182, no. 5, pp. 1214—1231. e11. https://doi.org/10.1016/j.cell.2020.08.008
32. Chen, J., Spracklen, C.N., Marenne, G., et al., The trans-ancestral genomic architecture of glycemic traits, *Nat. Genet.*, 2021, vol. 53, no. 6, pp. 840—860. https://doi.org/10.1038/s41588-021-00852-9
33. Wain, L.V., Vaez, A., Jansen, R., et al., Novel blood pressure locus and gene discovery using genome-wide association study and expression data sets from blood and the kidney, *Hypertension*, 2017. https://doi.org/10.1161/HYPERTENSIONAHA.117.09438
34. Kato, N., Loh, M., Takeuchi, F., et al., Trans-ancestry genome-wide association study identifies 12 genetic

loci influencing blood pressure and implicates a role for DNA methylation, *Nat. Genet.*, 2015, vol. 47, no. 11, pp. 1282—1293.
https://doi.org/10.1038/ng.3405

35. Shungin, D., Winkler, T.W., Croteau-Chonka, D.C., et al., New genetic loci link adipose and insulin biology to body fat distribution, *Nature*, 2015, vol. 518, no. 7538, pp. 187—196.
https://doi.org/10.1038/nature14132
36. Takeuchi, F., Akiyama, M., Matoba, N., et al., Interethnic analyses of blood pressure loci in populations of East Asian and European descent, *Nat. Commun.*, 2018, vol. 9, no. 1, p. 5052.
https://doi.org/10.1038/s41467-018-07345-0
37. Tachmazidou, I., Süveges, D., Min, J.L., et al., Whole-genome sequencing coupled to imputation discovers genetic signals for anthropometric traits, *Am. J. Hum. Genet.*, 2017, vol. 100, no. 6, pp. 865—884.
https://doi.org/10.1016/j.ajhg.2017.04.014
38. Liu, C., Kraja, A.T., Smith, J.A., et al., Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci, *Nat. Genet.*, 2016, vol. 48, no. 10, pp. 1162—1170.
https://doi.org/10.1038/ng.3660
39. Surendran, P., Drenos, F., Young, R., et al., Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension, *Nat. Genet.*, 2016, vol. 48, no. 10, pp. 1151—1161.
https://doi.org/10.1038/ng.3654
40. Hoffmann, T.J., Ehret, G.B., Nandakumar, P., et al., Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation, *Nat. Genet.*, 2017, vol. 49, no. 1, pp. 54—64.
https://doi.org/10.1038/ng.3715
41. Giri, A., Hellwege, J.N., Keaton, J.M., et al., Trans-ethnic association study of blood pressure determinants in over 750000 individuals, *Nat. Genet.*, 2019, vol. 51, no. 1, pp. 51—62.
https://doi.org/10.1038/s41588-018-0303-9
42. German, C.A., Sinsheimer, J.S., Klimentidis, Y.C., et al., Ordered multinomial regression for genetic association analysis of ordinal phenotypes at Biobank scale, *Genet. Epidemiol.*, 2020, vol. 44, no. 3, pp. 248—260.
https://doi.org/10.1002/gepi.22276
43. Wu, Y., Byrne, E.M., Zheng, Z., et al., Genome-wide association study of medication-use and associated disease in the UK Biobank, *Nat. Commun.*, 2019, vol. 10, no. 1, p. 1891.
https://doi.org/10.1038/s41467-019-09572-5
44. Sakaue, S., Kanai, M., Tanigawa, Y., et al., A cross-population atlas of genetic associations for 220 human phenotypes, *Nat. Genet.*, 2021, vol. 53, no. 10, pp. 1415—1424.
https://doi.org/10.1038/s41588-021-00931-x
45. Jeong, H., Jin, H.S., Kim, S.S., and Shin, D., Identifying interactions between dietary sodium, potassium, sodium-potassium ratios, and FGF5 rs16998073 variants and their associated risk for hypertension in Korean adults, *Nutrients*, 2020, vol. 12, no. 7, p. 2121.
https://doi.org/10.3390/nu12072121
46. Tikunova, E., Ovtcharova, V., Reshetnikov, E., et al., Genes of tumor necrosis factors and their receptors and the primary open angle glaucoma in the population of Central Russia, *Int. J. Ophthalmol.*, 2017, vol. 10, pp. 1490—1494.
https://doi.org/10.18240/ijo.2017.10.02
47. 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
48. 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
49. Ward, L.D. and Kellis, M., HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease, *Nucleic Acids Res.*, 2016, vol. 44, no. D1, pp. D877—D881.
https://doi.org/10.1093/nar/gkv1340
50. Che, R., Jack, J.R., Motsinger-Reif, A.A., and Brown, C.C., An adaptive permutation approach for genome-wide association study: evaluation and recommendations for use, *BioData Min.*, 2014, vol. 7, no. 1, p. 9.
https://doi.org/10.1186/1756-0381-7-9
51. Purcell, S., Neale, B., Todd-Brown, K., et al., PLINK: a tool set for whole-genome association and population-based linkage analyses, *Am. J. Hum. Genet.*, 2007, vol. 81, no. 3, pp. 559—575.
https://doi.org/10.1086/519795
52. GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues, *Science*, 2020, vol. 369, no. 6509, pp. 1318—1330.
https://doi.org/10.1126/science.aaz1776
53. Minyaylo, O., Ponomarenko, I., Reshetnikov, E., et al., Functionally significant polymorphisms of the MMP-9 gene are associated with peptic ulcer disease in the Caucasian population of Central Russia, *Sci. Rep.*, 2021, vol. 11, no. 1, p. 13515.
https://doi.org/10.1038/s41598-021-92527-y
54. Adzhubei, I., Jordan, D.M., and Sunyaev, S.R., Predicting functional effect of human missense mutations using PolyPhen-2, *Curr. Protoc. Hum. Genet.*, 2013, chapter 7, unit 7.20.
https://doi.org/10.1002/0471142905.hg0720s76
55. Moskalenko, M.I., Milanova, S.N., Ponomarenko, I.V., et al., Study of associations of polymorphism of matrix metalloproteinases genes with the development of arterial hypertension in men, *Kardiologiia*, 2019, vol. 59, no. 7S, pp. 31—39.
https://doi.org/10.18087/cardio.2598
56. Polonikov, A., Rymarova, L., Klyosova, E., et al., Matrix metalloproteinases as target genes for gene regulatory networks driving molecular and cellular pathways related to a multistep pathogenesis of cerebrovascular disease, *J. Cell. Biochem.*, 2019, vol. 120, no. 10, pp. 16467—16482.
https://doi.org/10.1002/jcb.28815

57. 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

58. Stelzer, G., Rosen, N., Plaschkes, I., et al., The GeneCards suite: from gene data mining to disease genome sequence analyses, *Curr. Protoc. Bioinf.*, 2016, vol. 54, pp. 1.30.1—1.30.33. https://doi.org/10.1002/cpbi.5

59. McNiven, M.A. and Razidlo, G.L., Regulation of cell migration, *Encyclopedia of Cell Biology*, 2016, vol. 3, pp. 208—215. https://doi.org/10.1016/B978-0-12-394447-4.30030-X

60. Budko, A.A., Khesina, P.A., Diakov, L.M., and Lazarevich, N.L., Small GTPase Rab3B: biological properties and possible role in carcinogenesis, *Adv. Mol. Oncol.*, 2018, vol. 5, no. 4, pp. 78—85. https://doi.org/10.17650/2313-805X-2018-5-4-78-85

61. Loirand, G., Scalbert, E., Bril, A., Pacaud, P., Rho exchange factors in the cardiovascular system, *Curr. Opin. Pharmacol.*, 2008, vol. 8, no. 2, pp. 174—180. https://doi.org/10.1016/j.coph.2007.12.006

62. Li, M., Jiao, Q., Xin, W., et al., The emerging role of Rho guanine nucleotide exchange factors in cardiovascular disorders: insights into atherosclerosis: a mini review, *Front. Cardiovasc. Med.*, 2022, vol. 8, p. 782098. https://doi.org/10.3389/fcvm.2021.782098

63. Hurd, C.A., Brear, P., Revell, J., et al., Affinity maturation of the RLIP76 Ral binding domain to inform the design of stapled peptides targeting the Ral GTPases, *J. Biol. Chem.*, 2021, vol. 296, p. 100101. https://doi.org/10.1074/jbc.RA120.015735

64. Karpushev, A.V., Mikhailova, V.B., and Abramochkin, D.V., The role of small G-proteins in the regulation of ion channels, *Usp. Fiziol. Nauk*, 2020, vol. 51, no. 1, pp. 3—17. https://doi.org/10.31857/S0301179820010063

65. Ehrhardt, G., Korherr, C., Wieler, J., et al., A novel potential effector of M-Ras and p21 Ras negatively regulates p21 Ras-mediated gene induction and cell growth, *Oncogene*, 2001, vol. 20, no. 2, pp. 188—197. https://doi.org/10.1038/sj.onc.1204053

66. Kasza, A., Signal-dependent Elk-1 target genes involved in transcript processing and cell migration, *Biochim. Biophys. Acta*, 2013, vol. 1829, no. 10, pp. 1026—1033. https://doi.org/10.1016/j.bbagrm.2013.05.004

Translated by A. Kazantseva