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Insilico Test of Functional Role of rs8068318 Polymorphism of Arterial Hypertension-Associated *TBX2* Candidate Gene

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ABSTRACT
Arterial hypertension is the most common disease, and cardiovascular pathology has for a long time topped the list of causes of global mortality. Despite the large number of scientific works devoted to this problem, the etiology of essential arterial hypertension is unknown. However, in recent years, genetic factors have become highly important, namely, the contribution of individual single nucleotide polymorphisms of various genes encoding single biological pathways that are involved in the development of hypertension. In the course of this study, the functional role of rs8068318 polymorphism of the *TBX2* candidate gene associated with the development of arterial hypertension was studied. The selection of the polymorphic locus was based on the data of the catalog of genome-wide association study (GWAS) of the National Human Genome Research Institute. The functional role was evaluated using online software: HaploReg (v4.1), GTExportal, and PolyPhen-2. The already conducted 68 genome-wide studies detected 382 single nucleotide hypertension-associated polymorphisms. The polymorphic locus - rs8068318 - of the *TBX2* candidate gene is associated with the development of hypertension in 4 full-genomic studies, two of which examined its involvement in the development of hypertension in the European population. It was established that this SNP is in the area of hypersensitivity to DNase, the region of regulatory DNA motifs to 4 transcription factors; in the field of histones marking promoters and enhancers in 8 tissues; significantly associated with the expression of the *TBX2-AS1* gene and the *TBX2* gene in various organs and tissues. The polymorphic locus rs8068318 was found to be in disequilibrium in linkage ($r^2 \geq 0.8$) with 15 SNPs, which have significant epigenetic effects. Thus, the polymorphic locus rs8068318 of the *TBX2* candidate gene, which is GWAS-significant for AH, and the 15SNPs linked to it have a significant functional role in the body.

Keywords: arterial hypertension, rs8068318, *TBX2*, epigenetic effects.

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INTRODUCTION
The term "arterial hypertension" (AH) is understood to mean an increased systolic blood pressure syndrome (SBP) ≥140 mm Hg. and/or diastolic blood pressure (DBP) ≥90 mmHg. [Chazova et al., 2019]. The share of patients with so-called essential or primary hypertension is high and ranges from 80 to 95%, depending on the studied population and the completeness of the examination. The remaining cases of increased blood pressure can be attributed to secondary, or symptomatic, hypertension, which are symptoms of the underlying disease.

Today, the etiology and pathogenesis of hypertension remains quite relevant, since AH is the most common (about one third of the world's population) non-infectious disease [Ionov et al., 2018]; by 2025, more than 1.5 billion people with hypertension are expected [Johnson, Richard et al., 2015]. It should be noted that a persistent increase in systolic-diastolic blood pressure significantly increases the overall risk of cardiovascular disease, and is also a key link in the development of diseases associated with arterial hypertension [Park, Jeong Bae et al., 2015]. In addition, an increase in SBP by every 10 mmHg. increases the risk of developing coronary heart disease by 23%, ischemic stroke by 43%, and hemorrhagic stroke by 74% [Kengne et al., 2007].

The molecular mechanisms of the development of hypertension are complex and insufficiently studied yet. However, over the entire period of studying this pathology, there was a consensus about such links in the hypertension pathogenesis as: activation of the renin-angiotensin-aldosterone system (RAAS), which leads to increased vascular resistance, impaired water-salt metabolism, heart and blood vessel remodeling; endothelial dysfunction, which disturbs the synthesis of various biologically active substances that directly or indirectly affect the level of blood pressure [Polonikov et al., 2015]; insulin resistance, accompanied with transmembrane ion-exchange mechanism blockage by developing hyperinsulinism, which leads to an increase in the sensitivity of the vascular wall to pressor impacts [Simonenko et al., 2014]; as well as dysregulation in the central nervous system, hyperhomocysteinuria, obesity, etc. [Polonikov et al., 2017a, Reshetnikov et al., 2019].

Arterial hypertension is classified as a multifactorial disease, as well as most other diseases [Reshetnikov et al., 2017, Yarosh et al., 2015], therefore, special attention is paid to genetic predisposition, as one of the main risk factors for the development of hypertension, which is devoted to a number of scientific works both abroad [Koch, 2016; Stoll et al., 2018], and in the Russian Federation [Polonikov et al., 2017b; Sirotina et al., 2018; Moskalenko et al., 2019a; Moskalenko et al., 2019b]. A large number of single nucleotide polymorphic loci that are involved in the development of hypertension have been identified, however, their functional significance has been poorly studied [Moskalenko et al., 2018, Moskalenko et al., 2019c; Moskalenko et al., 2020].

OBJECTIVE
To evaluate the functional significance of the polymorphic locus rs8068318 of the TBX2 arterial hypertension-associated gene, according to genome-wide studies.

MATERIAL AND METHODS
The polymorphic locus was selected based on data from the catalog of genome-wide association study (GWAS) of the

National Human Genome Research Institute (http://www.genome.gov/gwastudies/). We considered significant associations of polymorphisms with hypertension in the European population [Ponomarenko I et al., 2019]. The results were considered significant at $p< 5\times10^{-8}$. In addition, the researchers examined the presence of associations with phenotypes having arterial hypertension, common biological pathways; regulatory potential (regSNP) and effects on gene expression (eSNP); association with non-synonymous substitutions (nsSNPs), as well as the functional effects of these nsSNPs; the frequency of occurrence of polymorphic loci in the study population minimum 5%; functional effects (regSNP, eSNP, nsSNP) of linkage disequilibrium polymorphisms ($r2\geq0.8$) with SNPs selected for analysis [Neskubina et al., 2019]. Regulatory potential was estimated using the HaploReg online software (v4.1) (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php), GTExportal (http://www.gtexportal.org/) and Ensembl (www.ensembl.org).

RESULTS AND DISCUSSION

The catalog of genome-wide association study (GWAS) of the National Human Genome Research Institute presents the results of 68 studies on the study of hypertension. In the course of these studies, 382 single nucleotide polymorphisms (SNPs) associated with hypertension were detected. We selected the polymorphic locus rs8068318 of the TBX2 AH-associated gene, according to the GWAS catalog, satisfying the above criteria. This polymorphic locus showed its significance in 4 genome-wide studies, of which two studies examined its involvement in the development of hypertension in the European population.

The regulatory value of SNP was evaluated using HaploReg (v4.1) and GTExportal. This polymorphism has 15 SNPs that have strong adherence ($r2\geq0.8$) and significant regulatory potential (Table).

Table 1: Regulatory potential of the polymorphic locus rs8068318 of the TBX2 gene and its adherent SNPs (at $r2\geq0.8$) according to Haploreg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php).

chr	pos (hg38)	LD (r^2)	SNP	Ref	Alt	EUR freq	Promoter histonemarks	Enhancer histonemarks	DNAse	Proteinsbound	Motifs changed	GENCODE genes	dbSNP funcannot
17	61394696	0.91	rs2286526	C	T	0.72	LIV, LNG	13 tissues	4 tissues	POL2,GATA2		1.9kb 3' of BCAS3	intronic
17	61398281	0.97	rs1000423	C	T	0.73			15 tissues	SUZ12, POL2		1.6kb 5' of TBX2	intronic
17	61400993	0.97	rs34446110	G	C	0.73	22 tissues	6 tissues	19 tissues	SUZ12	LXR,Myc,Rad21	TBX2	intronic
17	61401283	0.97	rs12952625	G	A	0.73	22 tissues	8 tissues	16 tissues		AP-3	TBX2	intronic
17	61401415	0.9	rs2270114	G	C	0.72	22 tissues	8 tissues	11 tissues		GATA	TBX2	intronic
17	61404807	0.93	rs201348033	GC	G	0.73	13 tissues	10 tissues	11 tissues		27 altered motifs	TBX2	intronic
17	61406405	1	rs8068318	C	T	0.73	8 tissues	8 tissues	6 tissues		4 altered motifs	TBX2	intronic
17	61406955	1	rs7215775	A	G	0.73	6 tissues	8 tissues	4 tissues		5 altered motifs	TBX2	intronic
17	61407472	1	rs8073698	C	T	0.73	4 tissues	11 tissues	9 tissues		5 altered motifs	TBX2	intronic
17	61407656	1	rs8074151	G	A	0.73	4 tissues	12 tissues	8 tissues	POL2	T3R	TBX2	intronic
17	61407759	1	rs8078036	G	A	0.73	4 tissues	12 tissues	11 tissues	POL2	Hsf, Pax-4	TBX2	intronic
17	61408032	1	rs2240736	C	T	0.73	4 tissues	12 tissues	6 tissues		Smad4	TBX2	intronic
17	61408194	1	rs1057987	C	G, T	0.73			12 tissues			TBX2	missense
17	61409371	0.93	rs1058004	C	T	0.72			14 tissues		9 altered motifs	TBX2	3'-UTR
17	61409438	1	rs729781	A	G	0.73	19 tissues	10 tissues	40 tissues	POL2	5 altered motifs	TBX2	3'-UTR
17	61412874	0.99	rs11871637	C	T	0.73	21 tissues	4 tissues	27 tissues	CHD2		C17orf82	

Notes: SNP- polymorphic locus, LD - linkage disequilibrium, Chr-chromosome, Pos- Position, Ref –reference alleles, Alt - alternate non-reference alleles, Freq EUR – frequency European, DNAse–deoxyribonuclease, dbSNPfuncannot - annotated as synonymous, missense or

nonsense, changing the consensus sequence at splice sites, or residing in introns or UTRs.

Regulatory potential of the polymorphic locus rs8068318 of the TBX2 gene and its adherent SNPs (at $r2 \geq 0.8$) according to Haploreg v4.1. Polymorphism rs8068318 of the *TBX2* gene is in the region of hypersensitivity to DNase, the region of regulatory DNA motifs to transcription factors: MZF1::1-4, Pax-5, RXRA, SPIB. SNP is located in the region of histones marking promoters in 8 tissues, and enhancers also in 8 tissues. In addition, 7 out of 15 adherent polymorphisms are located in DNA regions interacting with 4 regulatory proteins: POL 2, GATA2, SUZ12, CHD 2. This polymorphism is significantly associated with the expression of the *TBX2-AS1* gene in brain tissues (Brain – Frontal Cortex (BA9), $p=0.0000092$; Brain - Putamen (basalganglia), $p=0.000033$; Brain – Nucleus accumbens (basalganglia), $p=0.0000010$), arteries and aorta ($p=0.0000016$), left ventricle of the heart ($p=0.0000091$), in adipose tissue (Subcutaneous p=4.6e-7 and Visceral (Omentum) p=9.1e-11) and others, as well as with the expression of the *TBX2* gene in brain tissues (Brain - Cortex, p=0.0000036, Brain - Cerebellum, p=0.0000013), in adipose tissue (Subcutaneous p=2.4e-7 and Visceral (Omentum) p=2.8e-8), in the left ventricle of the heart (p=0.000046), etc. The directly studied locus does not belong to non-synonymous substitutions, but one of its adherent SNPs (rs1057987) is nsSNP ($r^2=1$, D´=1), which leads to a replacement in the amino acid sequence of serine arginine in the polypeptide.

Our results indicate that rs8068318 of the *TBX2* gene, associated with the development of hypertension, according to genome-wide studies, plays an important functional role in the body: it is located in the region of hypersensitivity to DNase; the region of DNA regulatory motifs to transcription factors: MZF1::1-4, Pax-5, RXRA, SPIB; in the field of histones marking promoters and enhancers in 8 tissues; significantly associated with the expression of the TBX2-AS1 and TBX2 genes in brain tissues, arteries and aorta, in adipose tissue. The transcription factors associated with this polymorphic locus are involved in various biological processes: according to information in the UniProt database (www.uniprot.org), the transcription factor RXRA (a retinoic acid receptor that acts as a transcription factor) influences a fairly large number of different processes (on their own or as a partner of a number of nuclear receptors - RARA, RARB and PPARA): it participates in the regulation of lipid metabolism and adipocyte differentiation and the regulation of anti-inflammatory processes, including as a heterodimer with RARA (positively regulates the expression of miRNA-10a, thereby inhibiting the GATA6/VCAM1 signaling response to hemodynamic changes in vascular endothelial cells), and also promotes phagocytosis and myelin by macrophages and is involved in the regulation of calcium signaling, by suppressing the expression of the *ITPR2* gene, thereby controlling cell aging; the RXRA/PPARA heterodimer is required for the transcriptional activity of PPARA on fatty acid oxidation genes such as *ACOX1* and on the genes of the P450 cytochrome system; The transcription factor PAX-5 (paired box 5) affects the differentiation and activation of B-lymphocytes and the processes of switching to the synthesis of immunoglobulin E, as well as the participation of factor PAX-5 in tumor processes [Mineev et al., 2011]; the transcription factor SPIB (belongs to the family of transcription factors Ets) plays an important role in the differentiation of mature B-lymphocytes into plasma cells and the differentiation of plasmacytoid dendritic cells [Takagi et al., 2016].

CONCLUSION

Thus, it was found that the polymorphic locus rs8068318 of the *TBX2* gene, selected from 382 single nucleotide polymorphisms (SNPs), associated with the development of arterial hypertension, according to genome-wide studies, has a significant functional role in the human body (it is located in the area of hypersensitivity to DNase, the region of regulatory DNA motifs to 4 transcription factors; in the field of histones marking promoters and enhancers in 8 tissues; significantly associated with the expression of the *TBX2-AS1* gene and the *TBX2* gene in various organs and tissues (brain, aorta, arteries, adipose tissue). Also, this polymorphic locus is in adherence disequilibrium ($r^2 \geq 0.8$) with 15 SNPs, and one of its adherent SNP (rs1057987) is a non-synonymous substitution ($r^2=1$, D´=1), which leads to a change in the sequence of amino acids in the polypeptide).

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