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Differentially Expressed Genes in the Locus Associated with Relative Kidney Weight and Resting Blood Pressure in Hypertensive Rats of the ISIAH Strain

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Abstract—The comparative full-genome sequencing of transcriptomes of the renal cortex and medulla from hypertensive ISIAH rats and normotensive WAG rats revealed the differential expression of genes in the locus of chromosome 11 associated to the traits of resting blood pressure and relative kidney weight. Six differentially expressed genes (*Kcne1*, *Rcan1*, *Mx1*, *Mx2*, *Tmprss2*, and *RGD1559516*) were identified in the renal cortex, and three genes (*Rcan1*, *Mx2*, and *Tmprss2*) were identified in the renal medulla. An analysis of the functions of these genes pointed at the *Rcan1* gene as the most relevant candidate gene associated with both the traits of resting blood pressure and relative kidney weight in ISIAH rats. The elevation of the transcription levels of the *Mx1* and *Mx2* genes in hypertensive ISIAH rats may represent an adaptation that contributes to the alleviation of inflammatory processes in the kidneys.

Keywords: hypertension, relative kidney weight, differentially expressed genes, QTL analysis, ISIAH rat strain
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The analysis of molecular genetic mechanisms that underlie arterial hypertension is among the highly relevant tasks of medical genetics. Arterial hypertension may emerge spontaneously or be induced by various factors, such as oxidative stress, excessive consumption of salt, or emotional stress [1]. Hypertensive rat models are used in research on the molecular genetic mechanisms that underlie the emergence of various forms of the disease. The ISIAH rat strain has been generated at the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (Novosibirsk, Russia) by means of long-term selection. This rat strain is used to model the stress-dependent form of arterial hypertension. A dramatic increase (to 200–220 mmHg) in the blood pressure (BP) of animals exposed to mild stress, i.e., animals confined to a small wire cage for 30 min, is characteristic of this rat strain [2], and even the basal BP level is elevated in rats aged 2 months and older. The further progression of hypertension in ISIAH rats is accompanied by changes that are characteristic of arterial hypertension in humans, such as left ventricle hypertrophy, thickening of the walls and the shrinkage of the lumen of the small arteria of the heart, changes in the ECG pattern, elevated reactivity of the sympathetic nervous system and the hypophyseal-adrenocortical axis, glomerulosclerosis, and the increase in kidney weight [3–7]. Emotional stress often leads to BP elevation in humans; therefore, the elucidation of molecular genetic mechanisms that underlie the hypertension development in ISIAH rats will expand the knowledge on the key factors in the genetic control of the stress-dependent form of arterial hypertension.

The arterial BP level is a quantitative trait controlled by multiple genes. Genes associated with quantitative traits are often identified using the search for genetic loci associated with the trait under investigation (QTL analysis) and the detection of differentially expressed genes (DEGs) in the loci identified. We have previously demonstrated that a locus that showed statistically significant association with the basal BP level in 3–4-month-old ISIAH rats was situated on chromosome 11 (36–64 Mbp, with a peak near the microsatellite marker D11Rat7) and coincided with the locus associated with the trait of relative kidney weight. The BP level in F_2 (ISIAH × WAG) male rats that carried two ISIAH rat alleles in the locus under investigation was significantly lower than in animals homozygous for the Wistar allele, and the relative kid-

Abbreviations: ISIAH, rats with inherited stress-induced arterial hypertension; WAG, Wistar Albino Glaxo rat strain; BP, blood pressure; QTL, quantitative trait locus; DEG, differentially expressed genes; PCR, polymerase chain reaction; RGD, Rat Genome Database; SNP, single nucleotide polymorphism.

ney weight was decreased in heterozygous animals [8]. The locus in question is reportedly associated with the BP level in rats with salt-sensitive hypertension. Importantly, the presence of alleles from rats with salt-sensitive hypertension resulted in lower BP levels, similar to the phenomenon observed in ISIAH rats [9].

The search for genes located in the QTL of chromosome 11 and associated with resting BP value and relative kidney weight was performed in the present work. The experiment consisted of full-genome sequencing of the transcriptome (RNA-seq) of the renal cortex and medulla from ISIAH rats and control normotensive WAG (Wistar Albino Glaxo) rats. As a result, genes differentially expressed in the kidneys of ISIAH and WAG rats and localized in the QTL of chromosome 11 associated with the traits of resting BP and relative kidney weight were identified and single-nucleotide polymorphisms (SNPs) in the mRNA of DEGs found in the chromosomal locus under investigation (on chromosome 11) were characterized.

EXPERIMENTAL

Animals. Hypertensive rats of the ISIAH (inherited stress induced arterial hypertension) strain and animals of the normotensive WAG (Wistar Albino Glaxo) strain were used in the study. The work was performed at the Resource Center of Laboratory Animal Genetics of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (RFMEFI61914X0005 and RFMEFI62114X0010). The rats were housed under standard conditions with unlimited access to food (a balanced diet) and water. The kidney transcriptome of three-month male rats was analyzed. Systolic BP was measured under light ether narcosis using the indirect tail-cuff method so that emotional stress caused by the procedure could be avoided. Basal systolic BP was 171.7 ± 1.22 mm Hg in ISIAH rats and 116.33 ± 1.86 mm Hg in WAG rats. Each experimental group consisted of three animals.

The rats were quickly decapitated, the kidneys were dissected, and cortex and medulla fragments were isolated from cross-sectioned kidneys and analyzed separately. Tissue samples were stored in RNA Later (Qiagen, the United States) at −70°C until use. The experiments were performed in accordance with the international rules of laboratory animals use and were approved by the bioethics committee of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences.

Samples of renal cortex and medulla from ISIAH and WAG rats were subjected to full-genome sequencing of the transcriptome and bioinformatic identification of DEGs; this part of the work was performed at JSC Genoanalytica (Moscow, Russia). All samples were analyzed as experimental replicates.

RNA-seq analysis of differential expression of genes. The mRNA fraction was isolated from total RNA using the Dynabeads mRNA Purification Kit (Ambion, the United States) according to the manufacturer's protocol. cDNA libraries were prepared using the NEBNext mRNA Library Prep Reagent Set for Illumina (New England Biolabs, the United States). Sequencing of cDNA libraries was performed on the Illumina platform. The number of reads for each sample exceeded 10 million. TopHat2 software [10] was used to map the fastq files to the reference genome (Rnor_5.0\rn5) available on the website of the Rat Genome Sequencing Consortium (RGSC) and to annotate the genes listed in the NCBI RefSeq database. The CollectRnaSeqMetrics module of the Picard software package (http://broadinstitute.github.io/picard/) was used to assess the quality of mapped data. The level of gene expression defined as the number of gene fragments normalized to transcript length and the depth of library sequencing (FPKM, fragments per kilobase of transcript per million mapped reads) was determined using the Cufflinks/Cuffdiff software; the same software was used to assess differential expression of genes [11]. Genes were considered DEGs at the statistical significance level (q value) < 0.05.

DEGs associated with disease were identified using the Rat Genome Database (RGD, http://rgd.mcw.edu/).

Detection of polymorphisms. SAMtools software was used to combine the bam-files for each rat after mapping of the genes to the reference genome. The sequencing depth for all five files combined for each rat exceeded 10 million reads per file. Polymorphisms were identified using the Genome Analysis Toolkit Unified Genotyper (GATK) software package. The analysis was limited to single-nucleotide polymorphisms (SNPs) that met the following GATK criteria: GQ > 20, DP > 10, QD > 2, and FS < 30. Only the polymorphisms present in both alleles of all ISIAH rats analyzed and absent from the genome of control rats of the WAG strain were used in the present work. The putative effect of the substitution of an amino acid residue on protein function was assessed using the SIFT software [12].

QTL analysis was performed as described previously [8]. Three-month male F_2 ISIAH × WAG rats (n = 103) were used in the study. Genomic DNA was isolated from liver tissue according to the standard protocol that involved digestion by proteinase K and phenol-chloroform extraction [13]. The DNA was precipitated and re-dissolved in deionized water. QTL analysis was performed using 145 polymorphic microsatellite markers. The position of the markers on the chromosome was determined using the RGSC Genome Assembly v 5.0. database and expressed in megabases (Mb) from the end of the chromosome.

Genotyping was performed using PCR in a standard buffer (67 mM Tris-HCl (pH 8.9); 16 mM $(NH_4)_2SO_4$; 1.5 mM $MgCl_2$; 0.01% Tween 20; and 10 mM β-mercaptoethanol) supplemented with 200 μM of all of the four dNTPs, 3 μM of each primer, and

1 activity unit of Taq polymerase (Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia). The amount of the DNA template was 50–100 ng. Amplification was performed according to the following scheme: initial denaturation at 95°C for 5 min and 38 amplification cycles with denaturation at 94°C for 20 s, primer annealing for 15 s, and elongation at 72°C for 20 s. The elongation time was the same for all reactions, since the length of the amplicons did not exceed 300 bp. The duration of the final elongation was 5 min. The PCR fragments were analyzed using electrophoresis in 6–8% polyacrylamide gel. Electrophoresis was performed in Tris-borate buffer at a voltage density of 10 V/cm. The gels were stained with ethidium bromide and photographed in a Biometra gel imager (Germany) to visualize the PCR fragments.

Linkage of the markers to hypothetical genes that controlled the trait under investigation was assessed by calculating the linkage probability criterion (the ratio of probabilities of the presence and absence of linkage). The LOD score is conventionally used as the criterion for QTL probability.

Linkage analysis was performed using the MAPMAKER/EXP 3.0 and MAPMAKER/QTL 1.1 software [14]. Locus boundaries were defined using the change in the LOD score per unit as described in [1]. Threshold values of statistical significance of the LOD score were calculated using the QTL Cartographer Version 1.17 and JZmapqtl software (statgen.ncsu.edu) [15, 16]. The permutation test involved 1000 permutations of experimental data [17]. The linkage was considered statistically significant if the experimental value of the LOD score exceeded the 5% threshold value used in genome analysis (experiment-wise threshold) [18]; linkage was considered suggestive if the experimental LOD score exceeded the threshold value of 5% in the permutation test for an individual chromosome (chromosome-wise threshold).

Real-time PCR. Total RNA isolation from samples of kidney cortex and medulla of ISIAH and WAG rats and DNAse treatment of the RNA was performed using the SV Total RNA Isolation system (Promega, United States) according to the manufacturer's protocol. Samples from five animals were included into each group.

Reverse transcription was performed in the volume of 50 μL. The solution contained 1 μg of total RNA, 0.25 nmol primers (random nonanucleotide primers N_9, Biosan, Novosibirsk, Russia), 36 μL reverse transcription buffer, 40 activity units of MoMLV reverse transcriptase (Vector-Best, Russia), and 0.4 mM dNTPs. The cDNA synthesis mixtures were incubated at 37°C for 1 h, then at 42°C for 30 min, and at 50°C for 10 min. The enzyme was inactivated by incubating the reaction mixture at 75°C for 5 min.

Aliquots (5 μL) were drawn from all cDNA samples and pooled. The pooled samples were used as standard cDNA for the construction of calibration curves in real-time PCR. Real-time PCR was performed in an iQ5 amplifier (Bio-Rad Laboratories, the United States) using the dye SYBR Green I. Oligonucleotide primers were selected using PrimerBLAST software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and produced by Biosan (Russia). The following primers (5' → 3') were used: for *Rcan1,* F: TGGCAAACGGTGATGTCTTC, R: TGTAAAGTCTGGGCAAAGTACA; for the reference gene *Rpl30* F: CATCTTGGCGTCTGATCTTG, R: TCAGAGTCTGTTTGTACCCC. Real-time PCR was performed under the following conditions: incubation at 95°C for 1 min; 40 amplification cycles of 15 s at 95°C, 20 s at 63°C, and 20 s at 72°C with detection of the fluorescence of PCR products during 10 s at 83°C; the melting curve was recorded in the temperature range of 63–94°C. The number of cDNA samples processed using primers for the gene *Rcan1* ranged from eight to ten per plate, with four replicates for each cDNA sample. The same samples were used in the reaction mixtures with primers for the gene *Rpl30* loaded onto the same plate (four replicates per sample), and standard cDNA diluted 1 : 1, 1 : 4, 1 : 16 and 1 : 64 with the same primers was loaded onto the same plates (at two replicates per dilution). PCR with each cDNA sample was performed at least twice. The relative amounts of the cDNAs under investigation were inferred from the calibration curves constructed using dilutions of standard cDNA. The values for the target genes were normalized to the amounts of cDNA derived from reference genes, and thus the differences in expression levels of the genes under investigation in animals of the different strains were assessed.

Statistical processing of the results was performed using the Mann-Whitney U-test implemented in Statistica 6.0 (StatSoft, the United States) software package. The data are presented as arithmetic mean values and standard error of the mean (M ± SEM), with the expression level in WAG rats assumed to be 100%.

RESULTS

The number of the genes mapped to the locus of chromosome 11 associated with relative kidney weight and BP level and differentially expressed in the renal cortex of ISIAH and WAG rats was six, whereas the number of such genes differentially expressed in the renal medulla of rats of the two strains was three (Table 1). The exact position of the genes in the locus is illustrated in Fig. 1. The differential expression of three genes (*Rcan1*, *Mx2*, and *Tmprss2*) was characteristic of both kidney structures. The transcription level of the gene *Rcan1* in both renal cortex and medulla of ISIAH rats was lower than in control rats. The transcription levels of the rest of the genes were elevated in both kidney structures of ISIAH rats (Table 1).

No associations with pathological arterial hypertension or kidney disease were previously documented

Table 1. Genes localized in the locus of chromosome 11 associated with the traits of relative kidney weight and resting blood pressure and differentially expressed in the kidneys of ISIAH and WAG rats

Gene			Mb**	ISIAH/WAG***	*q*-value
symbol	ID	name*			
Renal cortex					
Kcne1	XM_006248034.1	Potassium voltage-gated channel, Isk-related family, member 1, transcript variant X2	36.10	2.89	0.0018
***Rcan1*#**	NM_153724.2	Regulator of calcineurin 1	36.15	–0.88	0.0018
Mx1	NM_001271061.1	Myxovirus (influenza virus) resistance 1, transcript variant 5	41.40	1.32	0.0018
Mx2	XM_006248150.1	Myxovirus (influenza virus) resistance 2, transcript variant X1	41.55	3.40	0.0018
Tmprss2	XM_006248126.1	Transmembrane protease, serine 2, transcript variant X3	41.57	0.91	0.0018
RGD1559516	NC_005110.4	Similar to ribosomal protein S2, pseudogene	43.72	1.23	0.0059
Renal medulla					
***Rcan1*#**	NM_153724.2	Regulator of calcineurin 1	36.15	–0.58	0.0094
Mx2	XM_006248150.1	Myxovirus (influenza virus) resistance 2, transcript variant X1	41.55	1.33	0.0016
Tmprss2	XM_006248126.1	Transmembrane protease, serine 2, transcript variant X3	41.57	0.45	0.0383

*The complete official name of the gene in the GenBank database (http://www.ncbi.nlm.nih.gov/gene/).
**The position of the gene on chromosome 11 (million base pairs (megabases) from the 5'-end of the chromosome).
*** $\log_2$ values of the ISIAH/WAG ratio are shown.
#Genes associated with diseases of the nervous system.

for genes listed in Table 1, as evident from the RGD database; however, one of the genes (*Rcan1*) was associated with pathologies of the nervous system. A statistically significant decrease in the transcription level of the gene *Rcan1* in the renal cortex and medulla of ISIAH rats has been confirmed by real-time PCR (Fig. 2).

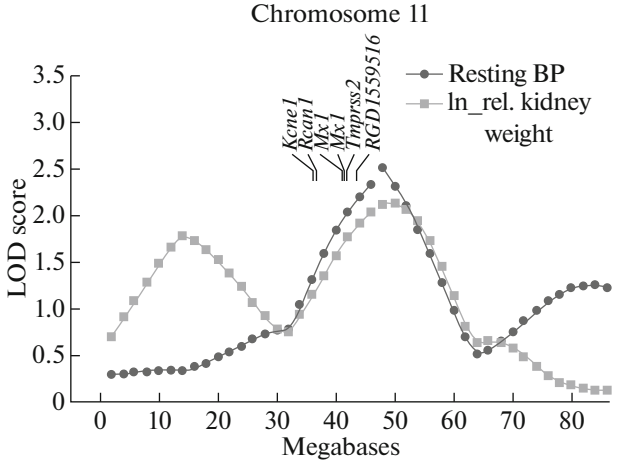

Fig. 1. Genes localized in the QTL of chromosome 11 associated with the traits of resting blood pressure and relative kidney weight and differentially expressed in the kidneys of ISIAH and WAG rats.

Nucleotide substitutions characteristic of ISIAH rats and not occurring in rats of the control WAG strain (Table 2) were identified in the nucleotide sequence of mRNAs of three genes (*Rcan1*, *Mx2*, and *Tmprss2*). Polymorphisms in mRNAs of *Rcan1* and *Mx2* have not been reported previously. The only nucleotide substitution that led to a nonsynonymous amino acid substitution was detected in the mRNA of the gene *Mx2*. The analysis that used the SIFT software did not predict any significant changes in the function of the Mx2 protein due to the aforementioned change in the amino acid sequence.

All polymorphisms in the mRNA of the *Tmprss2* gene were located in the 3'-untranslated region and were termed as downstream gene variants by the SIFT software. This identifier is assigned to polymorphisms with incompletely characterized functional significance; these polymorphisms are assumed to modify the expression of the downstream gene. The annotation of the results shows that five polymorphisms of the *Tmprss2* gene (in positions 1408, 1270, 1224, 1198, and 874) (Table 2) can potentially affect the expression of the *Mx2* gene.

Table 2. Polymorphisms in DEGs localized in the QTL on the chromosome 11

Gene	Genbank ID	Position	rs	Substitution	Substitution type
Rcan1	NM_153724	36149810	novel	p.Pro98Pro/c.294G > A	Synonymous variant
Mx2	NM_134350	41546241	novel	c.-67A > G	5'-UTR variant
		41556180	novel	p.Asn202Lys/c.606C > A	Missense variant
		41568301	novel	p.Val543Val/c.1629G > C	Synonymous variant
Tmprss2	NM_130424	41574536	rs13451606	c.*1408T > C	3'-UTR variant
		41574674	rs13451607	c.*1270A > C	Same
		41574720	rs13451540	c.*1224T > C	"
		41574745	–	c.*1198delT	"
		41575070	rs13451539	c.*874A > T	"
		41575105	rs13451605	c.*839A > G	"
		41575252	rs107011669	c.*692T > C	"

5'- and 3'-UTR—5'- and 3'-untranslated region, respectively.

DISCUSSION

Full-genome sequencing of the transcriptome of the renal cortex and medulla of hypertensive ISIAH rats and normotensive WAG rats has been performed. Genes differentially expressed in ISIAH and WAG rats and situated in the locus of chromosome 11 associated with traits of resting BP and relative kidney weight have been identified [8]. If a locus is common for two or more traits, the genes found in this locus may be supposed to have pleiotropic effects on these traits or to be closely linked and to affect individual traits.

A statistically significant decrease in BP was detected previously in F_2 hybrid (ISIAH × WAG) male rats that carried two ISIAH alleles in the QTL under investigation, whereas the rats heterozygous for the alleles of the locus had a lower relative kidney weight [8], mostly due to the higher body weight (Table 3).

The locus under investigation included five previously characterized genes with significantly different transcription levels in the kidneys of hypertensive ISIAH rats and normotensive WAG rats.

One of these genes (*Kcne1*) is involved in the regulation of potassium homeostasis in the organism and in the control of ion transport in the proximal tubules of the kidney. Mice that lack this gene (*kcne1*$^{-/-}$) presented a reduced volume of liquid in the body and elevated hematocrit [19], although there were no statistically significant differences in the BP level in *kcne1*$^{-/-}$ and control mice [20]. However, the elevated expression of this gene in the renal medulla of ISIAH rats may lead to an increase in the volume of the extracellular fluid and blood plasma and, ultimately, to an increase in blood pressure.

Rcan1 (regulator of calcineurin 1) is a negative regulator of calcineurin, a calcium-dependent phosphatase that plays an important role in the development of glomerular hypertrophy and extracellular matrix synthesis in diabetic nephropathy [21]. Calcineurin is among the primary factors that cause myocardial hypertrophy [22], a characteristic feature of ISIAH rats [3]. The development of myocardial hypertrophy in mice was reportedly accompanied by a decrease in the level of transcription of the gene *Rcan1* [23].

Calcineurin inhibitors enhance sympathetic neurotransmission in the kidneys and promote the elevation of BP [24], whereas the attenuation of the inhibitory effect of the gene *Rcan1* observed in the present study is expected to have an opposite effect. Thus, the decrease in the expression level of the gene *Rcan1* in ISIAH rats is likely to have high adaptive significance, since it prevents excessive sympathetic stimulation of the kidney upon the elevation of sympathetic tone in ISIAH rats. Lower BP in F_2 rat hybrids that are homozygous for the ISIAH allele of the locus in question is apparently due to this phenomenon.

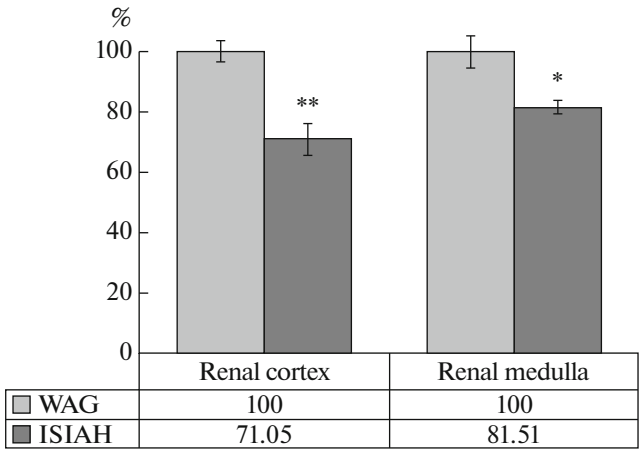

Fig. 2. Transcription level of the gene *Rcan1* in the renal cortex and medulla of ISIAH rats as determined by real-time PCR. *$p = 0.016$; **$p = 0.008$.

Table 3. Effect of ISIAH and WAG alleles on phenotypic features (body weight and kidney weight) in the group of 3–4-month-old F_2 (ISIAH × WAG) male rats

Chromosome, marker	Trait	Genotype		
		I/I M ± SEM *n*	I/W M ± SEM *n*	W/W M ± SEM *n*
11, D11Rat7	Resting BP	157.12 ± 2.72* 26	156.27 ± 2.15 55	169.50 ± 4.09†† 20
	Relative kidney weight	0.618 ± 0.011†† 26	0.582 ± 0.006 54	0.615 ± 0.016 20
	Body weight	253.03 ± 6.58 26	260.31 ± 5.10 54	241.25 ± 6.62† 20
	Kidney weight	1.56 ± 0.04* 26	1.52 ± 0.03 56	1.44 ± 0.04 20

* $p < 0.05$ relatively to the W/W genotype (Student's *t*-test).
† $p < 0.05$.
†† $p < 0.01$ relatively to the I/W genotype (Student's *t*-test).
I/I is rat homozygous for the ISIAH allele; I/W is heterozygous rat; W/W is rat homozygous for the WAG allele.

The above-described features of the *Rcan1* gene allow for the assumption of elevated calcineurin activity in the kidneys of ISIAH rats due to a lower transcription level of *Rcan1*. The ultimate result of this change consists in an increase in kidney weight, attenuation of sympathetic activation, and a decrease in the BP. Thus, the gene *Rcan1* can be regarded as a candidate gene potentially associated with the traits of resting blood pressure and relative kidney weight, and further research on the role of this gene in the pathogenesis of arterial hypertension is justified.

Two DEGs (*Mx1* and *Mx2*) from the locus of chromosome 11 are involved in control of resistance to viral infections. The protein encoded by the gene *Mx1* is a marker of interferon activity [25]. The activation of the *Mx1* gene may be triggered by exogenous interferon [26] or by the activation of the innate immune system; this occurs during viral infections of the kidney that lead to the development of glomerulonephritis [27].

The *Mx2* gene is also activated by interferon [28]; however, the protein encoded by *Mx2* is also involved in processes not related to viral infections, unlike the protein encoded by *Mx1*. For instance, MX2 (MxB) can regulate the efficiency and or/kinetics of transport between the nucleus and the cytoplasm and participate in the control of cell cycle in cultured human HeLa cells [29].

Transcription levels of the *Mx1* and *Mx2* genes were elevated in autoimmune disease [30]. The involvement of the immune system in the development of pathological arterial hypertension was discovered long ago and proved using rats with salt-sensitive and spontaneous hypertension as the experimental models [31]. The connection between autoimmune disease and the development of essential hypertension has been revealed [32]. Our earlier studies demonstrated that the locus that showed the most significant association with the BP level in 6-month old ISIAH rats was identical to the locus significantly associated with relative spleen weight [33]. Thus, we assume that the immune system is also involved in the development of stress-dependent hypertension in ISIAH rats.

Elevated transcription levels of *Mx1* and *Mx2* genes in ISIAH rats allow for the assumption of elevated levels of interferon, a putative marker of inflammatory processes, in the kidneys of ISIAH rats. The progression of pathological arterial hypertension is often associated with the development of inflammatory processes in the kidneys [34, 35]. Electron microscopic studies of the kidney revealed structural changes evident of early stages of glomerular and medullar sclerosis in 6-month-old ISIAH rats [4, 7]. A microarray analysis of gene expression levels in the kidneys of 6-month-old ISIAH and WAG rats revealed a statistically significant elevation of the expression level of the *Mx2* gene in the kidney cortex of ISIAH rats as compared to WAG rats [36]. Thus, elevated expression levels of *Mx1* and *Mx2* genes in kidney tissues of ISIAH rats may be indicative of an inflammatory process that is followed by the development of nephrosclerosis resulting in a stable elevation of blood pressure.

The *Tmprss2* gene encodes the transmembrane serine protease 2. Mice that lack this gene (*Tmprss2*$^{-/-}$) are viable and do not have the apparent phenotype related to the loss of gene function; in particular, there were no histological changes in the kidneys of the animals. The TMPRSS2 protease is supposed to participate in specific processes not related to the basic vital activity of *Tmprss2*$^{-/-}$ mice but involved in response to stress or disease [37]. The protease TMPRSS2 has recently been shown to play a key role in the activation

of the influenza virus in vivo [38]. No information on the putative role of this gene in the regulation of the BP level is currently available.

Some of the polymorphisms found in mRNA products of *Rcan1* and *Mx2* genes were not reported in previous studies of other rat strains. One of the substitutions in the nucleotide sequence of the mRNA of the gene *Mx2* is nonsynonymous. Analysis that employed the SIFT software did not predict significant changes in the function of the mutated Mx2 protein. However, recent studies showed that even synonymous single-nucleotide substitutions may affect mRNA stability, as well as protein conformation and regulation [39]. Therefore, the polymorphisms in the genes *Rcan1* and *Mx2* detected in the present study should be considered candidate sites for experimental assessment of their contribution to changes in gene expression and the possible effect on the functioning of the protein products of these genes.

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