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Comparative transcriptional profiling of renal cortex in rats with inherited stress-induced arterial hypertension and normotensive Wistar Albino Glaxo rats

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Abstract

Background: The renal function plays a leading role in long-term control of arterial pressure. The comparative analysis of renal cortex transcriptome in ISIAH rats with inherited stress-induced arterial hypertension and normotensive WAG rats was performed using RNA-Seq approach. The goal of the study was to identify the differentially expressed genes (DEGs) related to hypertension and to detect the pathways contributing to the differences in renal functions in ISIAH and WAG rats.

Results: The analysis revealed 716 genes differentially expressed in renal cortex of ISIAH and WAG rats, 42 of them were associated with arterial hypertension and regulation of blood pressure (BP). Several Gene Ontology (GO) terms significantly enriched with DEGs suggested the existence of the hormone dependent interstrain differences in renal cortex function. Multiple DEGs were associated with regulation of blood pressure and blood circulation, with the response to stress (including oxidative stress, hypoxia, and fluid shear stress) and its regulation. Several other processes which may contribute to hypertension development in ISIAH rats were: ion transport, regulation of calcium ion transport, homeostatic process, tissue remodeling, immune system process and regulation of immune response. KEGG analysis marked out several pathways significantly enriched with DEGs related to immune system function, to steroid hormone biosynthesis, tryptophan, glutathione, nitrogen, and drug metabolism.

Conclusions: The results of the study provide a basis for identification of potential biomarkers of stress-sensitive hypertension and for further investigation of the mechanisms that affect renal cortex function and hypertension development.

Keywords: Stress-sensitive hypertension, Renal cortex, Transcriptional profiling, RNA-Seq, ISIAH rats

Background

The etiology of essential hypertension is multifactorial. Many researchers agree that the renal function plays a leading role in long-term control of arterial pressure. It is thought that hypertension may be a consequence of abnormal water-electrolyte balancing by kidney [1, 2]. However, the number of factors involved in the pathogenesis of hypertension is constantly increasing [3], and the molecular mechanisms of essential hypertension development still remain uncertain.

The use of experimental animal models provides valuable information to elucidate the nature of polygenic traits [4]. One of these is the ISIAH (Inherited Stress-Induced Arterial Hypertension) rat strain which was developed to study the mechanisms of the stress-sensitive hypertension and its complications. The ISIAH rats were selected from

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an outbred Wistar rat population for a systolic arterial blood pressure (SABP) increase induced by 30 min restraint stress in a small cylindrical wire mesh cage. More than 30 generations of close inbreeding by brother-sister mating resulted in a high genetic homogeneity of the ISIAH strain [5]. Starting from the age of 6 weeks, the ISIAH rats have elevated SABP at basal condition (175.0 ± 3.5 mmHg in males and 165.0 ± 3.0 mmHg in females) and exhibit a dramatic increase in SABP (up to 210 mmHg and more) when restrained [6, 7]. The ISIAH rats also show a number of other characteristic features of hypertensive state: hypertrophy of the left ventricle, increase in the wall thickness of the small arteries, and changes in the electrocardiographic pattern [7]. ISIAH rats are also characterized by increased kidney mass [8] and some alterations in kidney histology indicative of increase in filtration barrier functional load and of initial stages of glomerular [9] and renomedullar sclerosis [10]. These features, the genetically determined enhanced responsiveness to stressful stimulation, and the predominant involvement of the neuroendocrine hypothalamic-pituitary-adrenocortical (HPA) and sympathoadrenal systems during the disease development let to consider the ISIAH rat strain as an advantageous model of the human stress-sensitive hypertensive state [11].

Recently, the next-generation sequencing technologies became very useful in providing deep insights into molecular mechanisms underlying the complex diseases development [12]. In the current work, the RNA sequencing (RNA-Seq) technology was used for comparative analysis of renal cortex transcriptome in hypertensive ISIAH and normotensive control WAG (Wistar Albino Glaxo) rats. The goal of the study was to identify the differentially expressed genes (DEGs) related to hypertension and to detect the pathways contributing to the differences in renal functions in hypertensive ISIAH and normotensive WAG rats.

The study revealed multiple DEGs in renal cortex of hypertensive ISIAH and normotensive WAG rats, including 42 DEGs known as related to hypertension and regulation of BP. These DEGs were associated with the diversity of biological processes and pathways which might contribute to development of stress-sensitive hypertension. Two of them (*Ephx2 and Glp1r*) were in the list of the top 40 genes showing the highest differences in expression in ISIAH and WAG renal cortex.

Results

The analysis revealed 716 genes differentially expressed in the renal cortex of hypertensive ISIAH and normotensive WAG rats. About the half of DEGs (372 genes, i.e. 52,0 %) were downregulated in ISIAH renal cortex. The expression of nine of these genes was detected in renal cortex of WAG rats but not in ISIAH. Alternatively, only one gene was expressed in ISIAH and silent in WAG renal cortex (Table 1). No one of these genes expressed in renal cortex of only one rat strain is known as related to hypertension development. The list of the top 40 genes showing the highest differences in expression in ISIAH and WAG renal cortex contained two genes (*Ephx2 and Glp1r*) associated with hypertension (Table 2).

Altogether, the study revealed 39 DEGs annotated in RGD as related to hypertension (Table 3). Six of these genes (*Ace, Cyp2j4, Gja1, Mmp9, Ppara*, and *Ren*) were described as genes associated with renal hypertension. According to functional annotation in DAVID, three additional DEGs (*Guca2b, P2rx4, and Pcsk5)* might be associated with regulation of BP. These DEGs may be considered as potential candidate genes related to blood pressure complications in ISIAH rats. Most of these genes were downregulated in hypertensive kidney. The majority of the DEGs associated with hypertension were related to insulin resistance and diabetic nephropathy and about half of them were associated with the immune system diseases (Table 3). Altogether, the study revealed 60 DEGs referred in RGD as associated with renal diseases, including renal fibrosis, renal insufficiency, glomerulonephritis, diabetic nephropathy, and nephrosclerosis (Table 4).

Table 1 Genes expressed at detectable levels in renal cortex of only one of rat strains

Gene symbol	Acc.#	Value, FPKM		Gene name	q-value
		WAG	ISIAH		
Cyp2c24	NM_001271354.1	10.30	0	Cytochrome P450, family 2, subfamily c, polypeptide 24	0.002
Hpse2	NM_001135762.1	1.34	0	Heparanase 2	0.002
LOC100362965	XM_002728491.2	5.57	0	SNRPN upstream reading frame protein-like	0.002
LOC102546948	XR_352663.1	7.28	0	Uncharacterized LOC102546948, transcript variant X2	0.002
LOC102547398	XR_358422.1	9.97	0	Uncharacterized LOC102547398	0.002
LOC102553584	XR_362425.1	1.87	0	Uncharacterized LOC102553584	0.002
RGD1309362	NM_001024884.1	2.45	0	Similar to interferon-inducible GTPase	0.002
Sfta2	NM_001166020.1	8.22	0	Surfactant associated 2	0.003
Slpil2	NM_001008872.1	2.25	0	Antileukoproteinase-like 2	0.029
Rpl38-ps1	XR_593941.1	0	2.45	Ribosomal protein L38, pseudogene 1	0.030

Table 2 Top 40 genes with the greatest difference in expression between ISIAH and WAG renal cortices

Gene symbol	Acc. #	Gene name	log2 fold change ISIAH/WAG
RGD1565131	XM_006248902.1	60S ribosomal protein L15-like	8.57
Fam111a	NM_001109163.1	Family with sequence similarity 111, member A	5.35
Stk32c	XM_006230485.1	Serine/threonine kinase 32C, transcript variant X1	4.91
Resp18	NM_019278.1	Regulated endocrine-specific protein 18	4.82
Ubd	NM_053299.2	Ubiquitin D	4.55
Ephx2[a]	XM_006252147.1	Epoxide hydrolase 2, cytoplasmic, transcript variant X1	4.48
Akr1b8	XM_006236251.1	Aldo-keto reductase family 1, member B8, transcript variant X1	4.45
Hpgd	NM_024390.2	Hydroxyprostaglandin dehydrogenase 15 (NAD)	4.30
Ly6al	XM_006241767.1	Lymphocyte antigen 6 complex, locus A-like, transcript variant X1	4.14
Spta1	NM_001011908.3	Spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	4.07
Tcerg1l	NM_001130077.1	Transcription elongation regulator 1-like	3.91
Glp1r[a]	XR_362044.1	Glucagon-like peptide 1 receptor, transcript variant X1	3.79
LOC686967	XM_003749071.2	Similar to olfactory receptor 1442	3.72
LOC102551856	XR_353697.1	Uncharacterized LOC102551856, transcript variant X1	3.46
Mx2	XM_006248150.1	Myxovirus (influenza virus) resistance 2, transcript variant X1	3.40
Krt19	NM_199498.1	Keratin 19	3.37
Ppp2r2c	NM_057116.1	Protein phosphatase 2, regulatory subunit B, gamma	3.27
G6b	XM_006256069.1	G6b protein, transcript variant X1	3.09
LOC102555352	XR_350674.1	Uncharacterized LOC102555352, transcript variant X4	3.05
RGD1564278	XM_003749906.2	RNA-binding protein with serine-rich domain 1-like	3.02
Kcne1	XM_006248034.1	Potassium voltage-gated channel, Isk-related family, member 1, transcript variant X2	2.89
LOC102546968	XM_006256098.1	RT1 class I histocompatibility antigen, AA alpha chain-like	2.87
Grhl1	XM_234006.7	Grainyhead-like 1 (Drosophila)	2.85
Fat3	XM_006242552.1	FAT atypical cadherin 3, transcript variant X1	−2.86
Fabp4	NM_053365.1	Fatty acid binding protein 4, adipocyte	−3.23
Rbp4	NM_013162.1	Retinol binding protein 4, plasma	−3.29
LOC361914	NM_001017465.1	Similar to solute carrier family 7 (cationic amino acid transporter, y + system), member 12	−3.32
Cyp24a1	XM_006235672.1	Cytochrome P450, family 24, subfamily a, polypeptide 1, transcript variant X1	−3.47
B3gat1	XM_006242733.1	Beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P), transcript variant X1	−3.47
LOC102552001	XR_350962.1	Uncharacterized LOC102552001, transcript variant X1	−3.60
Hhip	NM_001191817.1	Hedgehog-interacting protein	−3.64
Slc22a13	XM_006244092.1	Solute carrier family 22 (organic anion/urate transporter), member 13, transcript variant X1	−3.69
LOC102546318	XR_361882.1	Uncharacterized LOC102546318	−3.97
LOC102548532	XR_360708.1	Uncharacterized LOC102548532	−4.00
LOC102553060	XR_362149.1	Uncharacterized LOC102553060, transcript variant X1	−4.10
LOC102550987	XR_360671.1	Uncharacterized LOC102550987	−4.11
LOC501110	NM_001024361.1	Similar to Glutathione S-transferase A1 (GTH1) (HA subunit 1) (GST-epsilon) (GSTA1-1) (GST class-alpha)	−4.26
Pcdh9	NM_001191688.1	Protocadherin 9	−4.67
Pdilt	NM_001013902.1	Protein disulfide isomerase-like, testis expressed	−5.06
LOC100360791	XM_003748668.2	Tumor protein, translationally-controlled 1	−7.45

[a]-genes associated with hypertension

Table 3 Genes differentially expressed in ISIAH versus WAG renal cortex and annotated in databases as associated with hypertension and blood pressure regulation

Gene symbol	Acc. #	Gene name	log2 fold change ISIAH/WAG
Rat genome database			
Ace[abc]	NM_012544.1	Angiotensin I converting enzyme	−1.18
Acsm3[c]	XM_006230106.1	Acyl-CoA synthetase medium-chain family member 3, transcript variant X2	2.51
Adra1b[b]	NM_016991.2	Adrenoceptor alpha 1B	−1.24
Adra2a	NM_012739.3	Adrenoceptor alpha 2A	−1.01
Alas1	NM_024484.2	Aminolevulinate, delta-, synthase 1	0.47
Angpt1	XM_006241609.1	Angiopoietin 1, transcript variant X1	0.69
Apob[abc]	NM_019287.2	Apolipoprotein B	−2.52
Arg2[ac]	NM_019168.1	Arginase 2	−0.58
Cdo1	XM_006254698.1	Cysteine dioxygenase type 1, transcript variant X1	−0.78
Clu[ac]	XM_006252094.1	Clusterin, transcript variant X1	−1.75
Comt	NM_012531.2	Catechol-O-methyltransferase	−0.82
Cst3[abc]	NM_012837.1	Cystatin C	−0.50
Cyp1a1[c]	NM_012540.2	Cytochrome P450, family 1, subfamily a, polypeptide 1	−1.04
Cyp2j4[a]	NM_023025.2	Cytochrome P450, family 2, subfamily j, polypeptide 4	−0.81
Cyp4a8	NM_031605.2	Cytochrome P450, family 4, subfamily a, polypeptide 8	−0.55
Ephx1[c]	NM_012844.3	Epoxide hydrolase 1, microsomal (xenobiotic), transcript variant 2	0.59
Ephx2[ab]	XM_006252147.1	Epoxide hydrolase 2, cytoplasmic, transcript variant X1	4.48
Gja1[c]	XM_006256503.1	Gap junction protein, alpha 1, transcript variant X2	0.54
Glp1r	XR_362044.1	Glucagon-like peptide 1 receptor, transcript variant X1	3.79
Hsd11b2[c]	NM_017081.2	Hydroxysteroid 11-beta dehydrogenase 2	0.60
Itgav[b]	XM_006234437.1	Integrin, alpha V	−0.62
Klk1c12[ab]	NM_001005382.1	Kallikrein 1-related peptidase C12	−1.86
Klkb1[ac]	XM_006253121.1	Kallikrein B, plasma 1, transcript variant X1	2.22
Mif[bc]	NM_031051.1	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	0.50
Mmp9[ac]	XM_006235619.1	Matrix metallopeptidase 9, transcript variant X1	−2.49
Mthfr[ac]	XM_006239414.1	Methylenetetrahydrofolate reductase (NAD(P)H), transcript variant X2	0.49
Ppara[bc]	XM_006242154.1	Peroxisome proliferator activated receptor alpha, transcript variant X4	−0.56
Ptgds[ab]	NM_013015.2	Prostaglandin D2 synthase (brain)	−0.67
Ptk2b[bc]	XM_006252145.1	Protein tyrosine kinase 2 beta, transcript variant X3	−0.51
Ren[a]	NM_012642.4	Renin	−0.49
RT1-Bb[c]	NM_001004084.2	RT1 class II, locus Bb	−0.78
Slc26a4	XM_006239978.1	Solute carrier family 26 (anion exchanger), member 4, transcript variant X3	0.46
Slc2a4[b]	NM_012751.1	Solute carrier family 2 (facilitated glucose transporter), member 4	−0.77
Slc9a3r2	XM_006245893.1	Solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3 regulator 2, transcript variant X2	−0.48
Sncg	NM_031688.1	Synuclein, gamma (breast cancer-specific protein 1)	−1.25
Sts	XM_006256960.1	Steroid sulfatase (microsomal), isozyme S, transcript variant X1	−0.70
Tf [abc]	NM_001013110.1	Transferrin	−1.50
Vwf[bc]	NM_053889.1	Von Willebrand factor	0.77
Xdh[ac]	NM_017154.1	Santhine dehydrogenase	−0.77

Table 3 Genes differentially expressed in ISIAH versus WAG renal cortex and annotated in databases as associated with hypertension and blood pressure regulation *(Continued)*

David (regulation of blood pressure)			
Guca2b	NM_022284.2	Guanylate cyclase activator 2B	2.04
P2rx4	NM_031594.1	Purinergic receptor P2X, ligand-gated ion channel 4	−1.65
Pcsk5	XM_006231145.1	Proprotein convertase subtilisin/kexin type 5, transcript variant X2	0.53

Genes associated with: [a]-diabetic nephropathy; [b]-insulin resistance; [c]-immune system diseases

Thirty one transcription factor genes were differentially expressed in ISIAH and WAG renal cortex (Table 5). One of these (*Ppara*) is known as associated with hypertension, glomerulonephritis, insulin resistance, and immune system diseases. Its expression was downregulated in ISIAH rats.

Several genes which might play a key role in hypertension development in ISIAH rats were chosen for technical validation of the difference in their transcriptional activity in ISIAH and WAG renal cortex by real-time PCR (Fig. 1). The correlation coefficient between the results of two methods was 0.99.

Gene Ontology (GO) terms for biological processes found to be significantly enriched are represented in Additional file 1. As it is marked in this file, the majority of gene groups defined by GO terms contain DEGs associated with hypertension. Several groups of DEGs which might be important for the development of the stress-sensitive hypertension are given in bold in the file. The genes in these groups are listed in Additional file 2. Among the most significantly enriched GO terms, there are several (regulation of hormone levels, hormone metabolic processes, response to hormone and to insulin stimuli) which suggest the existence of the hormone dependent interstrain differences in renal cortex function.

Multiple DEGs were related to the response to stress and regulation of response to stress. The particular types of stress were specified by several GO terms such as response to oxidative stress, response to hypoxia, and response to fluid shear stress. In these groups, the DEGs associated with hypertension might have a special role in stress-sensitive hypertension development in ISIAH rats.

The two most essential groups of DEGs related to regulation of biological processes were associated with regulation of blood pressure and blood circulation. Several other processes which may have an important role in hypertension development were: nitrogen compound biosynthetic process, ion transport, regulation of calcium ion transport, homeostatic process (including ion homeostasis and particularly calcium ion homeostasis), and tissue remodeling.

The functional annotation revealed multiple DEGs associated with the immune system process and with regulation of immune response. KEGG analysis also marked out several pathways significantly enriched with DEGs related to immune system function (Table 6). The other significantly enriched metabolic pathways were related to steroid hormone biosynthesis, tryptophan, glutathione, nitrogen, and drug metabolism. The DEGs in the identified KEGG pathways are listed in Additional file 3.

Discussion

It was well established that kidney is one of the target organs in hypertension development [13]. In the current study, RNA-Seq approach was performed to identify genes with altered transcriptional activity in the renal cortex of hypertensive ISIAH as compared to normotensive WAG rats and to reveal those, which might be responsible for hypertension development in ISIAH rats. Multiple DEGs associated with hypertension and renal diseases were found, and the functional annotation of DEGs helped to define the main biological processes and pathways which might contribute to stress-sensitive hypertension in ISIAH rats.

GO annotation results pointed out that the hormonal regulation might have strong influences on renal function. So, the group of 16 DEGs participating in the regulation of hormone level might play an important role in orchestration of the changes in physiological and metabolic processes in ISIAH kidney (Additional file 2). Several of them (*Ace, Comt, Cyp1a1, Glp1r, Hsd11b2*, and *Ren*) are widely known as associated with hypertension development.

The angiotensin I-converting enzyme and renin (*Ace* and *Ren*) are the key components of the renin-angiotensin system (RAS). Their low expression found in the current experiment is in a good agreement with the previous study when real-time PCR showed the decreased mRNA level of these genes in kidney cortex of 4-month old ISIAH males [14]. Low-renin hypertension usually implies increased retention of Na(+) [15]. As the statistically significant plasma sodium increase was found in ISIAH rats as compared to WAG [16], the low-renin hypertension in ISIAH rats may arise due to the suppression of the RAS by the sodium retention and elevated blood pressure.

Comt encodes the enzyme catechol-O-methyltransferase metabolizing catecholamines. The inhibition of COMT induces dopamine-dependent natriuresis [17]. The catechol-O-methyltransferase-gene-disrupted mice were resistant to salt-induced hypertension [18]. So, the decreased expression of *Comt* in the renal cortex of ISIAH rats may lead to increase in renal dopaminergic effects and sodium

Table 4 Genes differentially expressed in ISIAH versus WAG renal cortex and annotated in rat genome database as associated with kidney diseases

Gene symbol	Acc. #	Gene name	log2 fold change ISIAH/WAG
Abcb1a[b]	NM_133401.1	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	−0.82
Abcc2[b]	NM_012833.1	ATP-binding cassette, subfamily C (CFTR/MRP), member 2	−0.47
Ace[abcd]	NM_012544.1	Angiotensin I converting enzyme	−1.18
Acsm3[c]	XM_006230106.1	Acyl-CoA synthetase medium-chain family member 3, transcript variant X2	2.51
Aif1[c]	NM_017196.3	Allograft inflammatory factor 1	0.59
Angpt1	XM_006241609.1	Angiopoietin 1, transcript variant X1	0.69
Apob[d]	NM_019287.2	Apolipoprotein B	−2.52
Apoc2[b]	XM_006228403.1	Apolipoprotein C-II, transcript variant X1	−1.20
Apoh[d]	NM_001009626.1	Apolipoprotein H (beta-2-glycoprotein I)	−2.73
Arg2[bd]	NM_019168.1	Arginase 2	−0.58
Atp6v1b1	XM_006236748.1	ATPase, H transporting, lysosomal V1 subunit B1, transcript variant X1	0.57
Bak1	NM_053812.1	BCL2-antagonist/killer 1	0.54
Bsnd	NM_138979.2	Bartter syndrome, infantile, with sensorineural deafness (Barttin)	0.45
C1qa[c]	NM_001008515.1	Complement component 1, q subcomponent, A chain	0.59
Cfb[abc]	NM_212466.3	Complement factor B	−1.09
Clu[abcd]	XM_006252094.1	Clusterin, transcript variant X1	−1.75
Cndp1[cd]	NM_001007687.1	Carnosine dipeptidase 1 (metallopeptidase M20 family)	−1.01
Col3a1[ab]	NM_032085.1	Collagen, type III, alpha 1	−0.60
Comt	NM_012531.2	Catechol-O-methyltransferase	−0.82
Cst3[d]	NM_012837.1	Cystatin C	−0.50
Cyp1a1[b]	NM_012540.2	Cytochrome P450, family 1, subfamily a, polypeptide 1	−1.04
Cyp2j4[ad]	NM_023025.2	Cytochrome P450, family 2, subfamily j, polypeptide 4	−0.81
Cyp4a8	NM_031605.2	Cytochrome P450, family 4, subfamily a, polypeptide 8	−0.55
Ephx2[bd]	XM_006252147.1	Epoxide hydrolase 2, cytoplasmic, transcript variant X1	4.48
Fga[b]	NM_001008724.1	Fibrinogen alpha chain, transcript variant 1	1.00
Fhit	NM_021774.1	Fragile histidine triad	0.80
Fmod[d]	XM_006249885.1	Fibromodulin, transcript variant X1	−0.94
Gatm[b]	NM_031031.2	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	0.74
Gfpt2[d]	NM_001002819.2	Glutamine-fructose-6-phosphate transaminase 2	1.75
Gja1	XM_006256503.1	Gap junction protein, alpha 1, transcript variant X2	0.54
Gtpbp4[b]	XM_006254146.1	GTP binding protein 4	1.77
Hao1	XM_006235096.1	Hydroxyacid oxidase (glycolate oxidase) 1, transcript variant X1	−1.68
Igfbp1[d]	NM_013144.1	Insulin-like growth factor binding protein 1	1.09
Itgal	XM_006230269.1	Integrin, alpha L, transcript variant X1	0.71
Kit	XM_006250909.1	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, transcript variant X1	0.62
Klk1c12[d]	NM_001005382.1	Kallikrein 1-related peptidase C12	−1.86
Klkb1[cd]	XM_006253121.1	Kallikrein B, plasma 1, transcript variant X1	2.22
Lgals1	NM_019904.1	Lectin, galactoside-binding, soluble, 1	0.91
Mif[c]	NM_031051.1	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	0.50
Mme[c]	NM_012608.2	Membrane metallo-endopeptidase	0.55
Mmp9[acd]	XM_006235619.1	Matrix metallopeptidase 9, transcript variant X1	−2.49
Mthfr[bcde]	XM_006239414.1	Methylenetetrahydrofolate reductase (NAD(P)H), transcript variant X2	0.49
Muc20[bc]	XM_006248449.1	Mucin 20, cell surface associated, transcript variant X1	0.63

Table 4 Genes differentially expressed in ISIAH versus WAG renal cortex and annotated in rat genome database as associated with kidney diseases *(Continued)*

Pla2g7[abc]	XM_006244606.1	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma), transcript variant X1	0.71
Ppara[c]	XM_006242154.1	Peroxisome proliferator activated receptor alpha, transcript variant X4	−0.56
Ptgds[de]	NM_013015.2	Prostaglandin D2 synthase (brain)	−0.67
Ptk2b[c]	XM_006252145.1	Protein tyrosine kinase 2 beta, transcript variant X3	−0.51
Ren[bd]	NM_012642.4	Renin	−0.49
Rhcg[b]	NM_183053.1	Rh family, C glycoprotein	0.66
RT1-Bb[a]	NM_001004084.2	RT1 class II, locus Bb	−0.78
Serpinf1[d]	NM_177927.2	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	−1.34
Slc17a2	NM_001107353.1	Solute carrier family 17, member 2	0.52
Slc19a3[b]	NM_001108228.1	Solute carrier family 19 (thiamine transporter), member 3	0.66
Slc4a1	XM_006247255.1	Solute carrier family 4 (anion exchanger), member 1, transcript variant X1	0.86
Tf[cd]	NM_001013110.1	Transferrin	−1.50
Tgfbi[d]	NM_053802.1	Transforming growth factor, beta induced	−0.93
Ttc21b	NM_001191737.1	Tetratricopeptide repeat domain 21B	−0.48
Vwf[abc]	NM_053889.1	Von Willebrand factor	0.77
Wfs1	NM_031823.1	Wolfram syndrome 1 (wolframin)	0.50
Xdh[abd]	NM_017154.1	Xanthine dehydrogenase	−0.77

Genes associated with: [a]-renal fibrosis; [b]-renal insufficiency; [c]-glomerulonephritis, [d]-diabetic nephropathies; [e]-nephrosclerosis

excretion, and may be considered as an adaptive mechanism. Earlier, the significantly decreased transcription of *Comt* was also detected in kidney of 6-month old ISIAH rats [19].

Cyp1a1 knockout mice are hypertensive. Cyp1a1 metabolizes omega-3 polyunsaturated fatty acids to vasodilators and the loss of these vasodilators may lead to increases in BP [20]. So, the decreased level of *Cyp1a1* transcription in ISIAH renal cortex suggests its contribution to hypertension development in these rats.

The enzyme encoded by 11β-hydroxysteroid dehydrogenase (*Hsd11b2*) oxidizes glucocorticoids to the inactive metabolite cortisone. In aldosterone target tissues, 11βHSD2 is coexpressed with mineralocorticoid receptors and protects the receptor from activation by glucocorticoids. It was found that decreased HSD11B2 activity is related to hypertension [21] and *Hsd11b2* null mice are also hypertensive [22]. So, the increased *Hsd11b2* transcription in ISIAH renal cortex may lead to decreased glucocorticoid action and be protective against excessive elevation of blood pressure.

GLP-1 receptor (*Glp1r* gene) was shown to be expressed in glomerular capillary and vascular walls in the mouse kidney. Its signaling plays a crucial role in protection against increased renal oxidative stress [23]. So, *Glp1r* upregulation in renal cortex of ISIAH rats may be adaptive against the oxidative stress.

Several DEGs participating in the regulation of hormone level are related to retinol metabolism (*Cyp26b1, Rbp4,* and *Retsat*) and intracellular transport (*Rbp1*). Retinoids (vitamin A and its analogs) are highly potent regulators of cell differentiation, cell proliferation, and apoptosis. Retinoids and/or retinoid-related proteins play important role in the development of metabolic diseases, primarily obesity, diabetes, and dyslipidemia [24]. Earlier, several signs of metabolic syndrome, such as dislipidemia, increased glucose content, and increased body weight were described in ISIAH rats [25]. The elevated RBP4 was reported in chronic kidney disease [26] and may contribute to insulin resistance in spontaneously hypertensive rats [27]. Based on that, we suggest that the upregulation of *Rbp4* may be related to development of metabolic syndrome in ISIAH rats, too.

RetSat saturates all-trans-retinol to all-trans-13,14-dihydroretinol which is transiently oxidized to all-trans-13,14-dihydroretinoic acid before being oxidized further by Cyp26 enzymes [28]. Cyp26b1 catalyzes the inactivation of retinoic acid (RA) to hydroxylated forms and helps to maintain tissue RA concentrations within appropriate bounds [29]. The particular role of elevated transcription of *Retsat* and *Cyp26b1* in ISIAH kidney function remains to be determined.

According to the functional annotation, four genes responsible for regulation of hormone level *(Ace, Glp1r, Hsd11b2* and *Ren*) are also associated with the regulation

Table 5 List of genes encoding transcription factors differentially expressed in ISIAH versus WAG renal cortex

Gene symbol	Acc. #	Gene name	log2 fold change ISIAH/WAG
Bcl6	NM_001107084.1	B-cell CLL/lymphoma 6	−2.47
Btbd11	XM_006241173.1	BTB (POZ) domain containing 11	−0.98
Etv1	XM_006240048.1	Ets variant 1, transcript variant X2	−0.85
Etv5	XM_006248542.1	Ets variant 5, transcript variant X1	−0.84
Foxi1	NM_001105776.1	Forkhead box I1	0.48
Grhl1	XM_234006.7	Grainyhead-like 1 (Drosophila)	2.85
Hdac9	XM_006240037.1	Histone deacetylase 9, transcript variant X14	−0.89
Hes6	XM_006245407.1	Hes family bHLH transcription factor 6, transcript variant X1	−0.72
Hr	XM_006252284.1	Hair growth associated, transcript variant X2	1.24
Irf4	XM_006253900.1	Interferon regulatory factor 4, transcript variant X2	−1.51
Irf7	NM_001033691.1	Interferon regulatory factor 7	1.52
Ivns1abp	XM_006249989.1	Influenza virus NS1A binding protein, transcript variant X2	−0.59
Klf12	NM_001007684.1	Kruppel-like factor 2	−0.69
Mybl1	XM_006237749.1	Myeloblastosis oncogene-like 1, transcript variant X1	−1.37
Nfkbil1	XM_006256046.1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1, transcript variant X1	0.94
Nkd2	NM_001107454.1	Naked cuticle homolog 2 (Drosophila)	−0.84
Osr2	XM_006241542.1	Odd-skipped related transciption factor 2, transcript variant X1	1.03
P8	XM_006230201.1	Nuclear proten 1	−1.86
Pou2af1	NM_001109599.1	POU class 2 associating factor 1	2.60
Ppara	XM_006242154.1	Peroxisome proliferator activated receptor alpha, transcript variant X4	−0.56
Ppargc1b	NM_176075.2	Peroxisome proliferator-activated receptor gamma, coactivator 1 beta	−0.62
Prox1	XM_006250454.1	Prospero homeobox 1, transcript variant X1	−0.74
Satb2	XM_006244931.1	SATB homeobox 2, transcript variant X1	−1.13
Sox9	XM_003750950.2	SRY (sex determining region Y)-box 9	−0.80
Spry4	XM_006254656.1	Sprouty homolog 4 (Drosophila), transcript variant X2	−0.70
Tcerg1l	NM_001130077.1	Transcription elongation regulator 1-like	3.91
Tcf4	NM_053369.1	Transcription factor 4	−0.90
Zbtb16	XM_006243015.1	Zinc finger and BTB domain containing 16, transcript variant X1	−1.20
Zdhhc2	NM_145096.2	Zinc finger, DHHC-type containing 2	0.67
Zfp36	NM_133290.3	Zinc finger protein 36	−0.61
Zfp385b	NM_001107736.1	Zinc finger protein 385B	0.67

of BP. Two other genes in the group 'regulation of BP' (*Ephx2, and Guca2b) were earlier described as* common genes related to regulation of BP in several rat models of programmed hypertension [30]. In ISIAH renal cortex these genes were upregulated. *Ephx2* encodes the soluble epoxide hydrolase (sEH) which metabolizes the epoxyeicosatrienoic acids (EETs) having antihypertensive properties. EETs also possess anti-inflammatory actions that could protect the kidney vasculature from injury during renal and cardiovascular diseases [31, 32]. sEH is considered as a main effector of angiotensin II-induced [31] and salt-sensitive hypertension [33]. Besides, it was considered as one of the gatekeeper genes contributing to programmed hypertension [30]. The uroguanylin (*Guca2b* encoded protein) deficiency results in impaired ability to excrete an enteral load of NaCl, primarily due to an inappropriate increase in renal Na + reabsorption, and in increased mean arterial pressure in uroguanylin knockout animals [34]. Based on this information, we may suggest that upregulation *of Ephx2* exerts the pressor effect and *Guca2b* exerts an opposite effect on systemic BP and renal function in ISIAH rats.

Three other DEGs related to regulation of BP (*Adra1b*, *P2rx4*, and *Ppara*) were downregulated in ISIAH renal cortex. The alpha1B-adrenoceptors (*Adra1b*) are involved in blood vessel remodeling [35] and mediate the vasoconstrictor actions of the renal sympathetic nerves in rats with renal failure [36, 37]. PPARα is a nuclear transcription

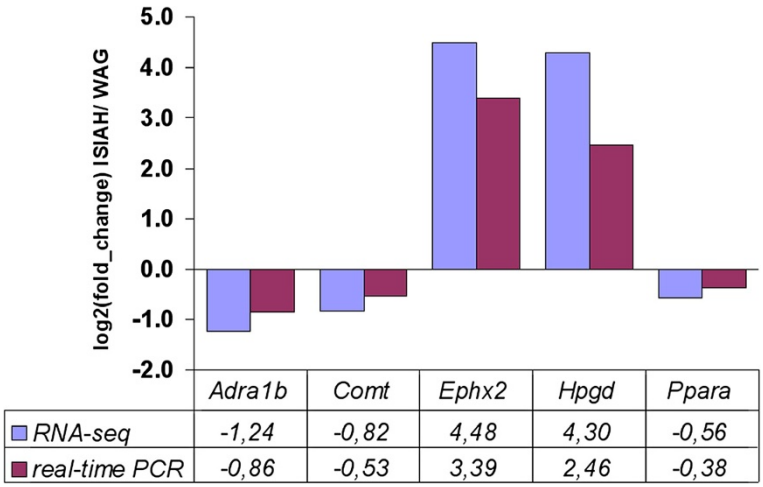

Fig. 1 Comparison of gene expression level measurements obtained by RNA-seq and qPCR

factor. It contributes to regulation of BP and vascular reactivity in SHR [38]. PPARα deficiency appears to aggravate the severity of diabetic nephropathy through an increase in extracellular matrix formation, inflammation, and circulating free fatty acid and triglyceride concentrations [39]. Alternatively, the PPAR-alpha and -gamma agonists attenuate diabetic kidney disease in the apolipoprotein E knockout mice [40]. P2rx4(-/-) mice have higher BP and excrete smaller amounts of NO products in their urine than do wild-type mice. They have impaired flow-dependent control of vascular tone and remodeling [41]. Besides, the lack of P2X4R expression leads to increased renal fibrosis [42]. So, we may suggest that downregulation of *Adra1b* may be adaptive and protect against the excessive sympathoexcitation, and *P2rx4*, and *Ppara* deficiency may contribute to development of kidney pathology in ISIAH rats.

In the current study, we found multiple DEGs related to response to different stimuli and to stress. This is well consistent with the previous observation that HPA and sympathoadrenal systems are activated in ISIAH rats [11] and that the changes in kidney function of 6-month old ISIAH rats are based on altered expression of many genes working in stress-related mode [19]. The stress response (or stress cascade) is considered as disruptions in homeostasis which result in a series of neural and endocrine adaptations. The stress cascade is responsible for allowing the body to make the necessary physiological and metabolic changes required to cope with the demands of a homeostatic challenge [43]. In the current study, the functional annotation helped to identify multiple DEGs involved in homeostatic process. Several DEGs in this group were associated with both hypertension and kidney diseases. That is in a good agreement with the opinion that essential hypertension is one of the "syndromes of impaired genetic homeostasis" [44] and that homeostatic process might be impaired in patients with chronic kidney disease [45].

Table 6 Metabolic pathways significantly enriched with genes differentially expressed in ISIAH and WAG renal cortices

Term	Count	*P*-value
Complement and coagulation cascades	9	0.003
Type I diabetes mellitus	8	0.005
Cell adhesion molecules (CAMs)	13	0.006
Drug metabolism	8	0.014
Steroid hormone biosynthesis	6	0.018
Tryptophan metabolism	6	0.018
Metabolism of xenobiotics by cytochrome P450	7	0.020
Glutathione metabolism	6	0.032
Antigen processing and presentation	8	0.037
Nitrogen metabolism	4	0.047

Earlier, the comparative electron microscopic study of glomerular apparatus in 6-month old ISIAH and Wistar rats showed hypertrophy of renal corpuscles in hypertensive kidney, accompanied by multiple structural changes such as capillary narrowing or dilation, endothelial flattening, podocyte hypertrophy and flattening of their cytopodia, thickening of basal lamina, mesangial volume expansion and increase in the number of intercapillary processes of mesangial cells [9]. Complex of these signs suggested a disturbance of glomerular capillary blood circulation and a functional podocyte stress, compensating the microcirculatory disturbances. Changes in basal membranes and mesangium were considered as indicative of increase in filtration barrier functional load, and of initial stages of glomerular sclerosis [9]. In the current study, we used younger rats, nevertheless, we found groups of DEGs associated with blood circulation, renal hypertension, and with the development of

nephrosclerosis. Probably, the changes in their expression may be potentially important for the appearance of the microcirculatory and structural disturbances in aging kidney.

The particular types of stress specified by GO terms were associated with oxidative stress, hypoxia, and fluid shear stress (Additional file 1). The oxidative stress is considered to be the pathogenic outcome of oxidant overproduction, which occurs as a result of imbalance between prooxidants and antioxidants [46]. Several genes in this group showed reduced transcription and several were upregulated.

The protein encoded by *Abcc2* mediates transport of various molecules across extra- and intra-cellular membranes, including the transport of prostaglandin E2 [47], which affects multiple segments of the preglomerular vascular tree in a different ways [48]. ABCC2 deficiency may be associated with increased oxidative stress, leading to renal tubular cell damage [49].

Two other genes associated with response to oxidative stress (*Clu* and *Mmp9*) are known as genes related to hypertension and kidney diseases. Clusterin (*Clu*) upregulation attenuates renal fibrosis in obstructive nephropathy [50]. Alternatively, the loss of clusterin expression worsens renal ischemia-reperfusion injury [51]. Overexpression of MMP9 could alter glomerular basement membrane components thereby causing podocyte structural changes [52]. MMP9 is also known to cleave podocalyxin in podocytes, which is a charge barrier to prevent microalbuminuria [53]. Loss of MMP9 reduces atherosclerotic burden [54] and, alternatively, the elevated urine values of MMP-9 was recognized as a marker of atherosclerotic disease [55]. So, the decrease in *Clu* and *Mmp9* expression may be protective in ISIAH renal cortex.

Another gene repressed in ISIAH renal cortex and related to oxidative stress was *Hao1.* It encodes a peroxisomal enzyme that oxidizes glycolate to glyoxylate with concomitant production of H_2O_2. Downregulation of Hao1 expression during oxidative stress may provide a mechanism to prevent excessive H_2O_2 formation [56]. Alpha B-crystallin (*Cryab* gene) is a ubiquitous stress inducible molecular chaperone. CRYAB is promoting angiogenesis and preventing apoptosis [57]. Expression of cystatin C has protective effects against various oxidative stresses that induce cell death [58]. Its decreased transcription in ISIAH renal cortex may contribute to oxidative damage and, probably, to hypertension development.

It is known that chronic hypertension can occur if there is an abnormality of kidney function that shifts pressure natriuresis so that sodium balance is maintained at elevated blood pressures [59]. Tubular sodium reabsorption depends on the activity of ion transport systems, which are modulated by neural, endocrine, paracrine, and physical factors [60]. In our study, the functional annotation revealed 32 DEGs related to ion transport. The changes in their transcriptional activity suggest that different mechanisms of osmoregulation may contribute to function of hypertensive kidney. These results are in a good agreement with the statement that ion transport is one of the major processes that are vital for functions of kidney and organism as a whole.

KEGG analysis showed an overrepresentation of DEGs involved in several metabolic pathways (Table 6 and Additional file 3). The most significantly enriched one was pathway associated with complement and coagulation cascades. Inflammation and coagulation play pivotal roles in the pathogenesis of vascular diseases. Increasing evidence points to an extensive cross-talk between these two systems, whereby inflammation leads not only to activation of coagulation, but coagulation also considerably affects inflammatory activity [61].

One of the DEGs related to this pathway (*Fga*) is involved in platelet aggregation and has been recognized as biomarker for acute kidney injury [62]. Its upregulation may contribute to enhanced coagulation and exert negative effect on ISIAH kidney function. The other upregulated gene related to complement and coagulation cascades (*Serpinc1*) contributes to negative regulation of inflammatory response and to fibrinolysis. *Serpinc1* deficiency is significantly associated with a tendency toward thrombosis formation in the kidney [63]. So, its upregulation may exert a protective effect on ISIAH kidney function.

Two another upregulated DEGs (*Klkb1* and *Vwf*) in the complement and coagulation cascades are known as associated with both hypertension and kidney diseases. Plasma prekallikrein (*Klkb1* gene) was considered as a risk marker for hypertension and nephropathy in type 1 diabetes. Its level was elevated in association with increased blood pressure, and positively correlated with urinary albumin excretion rate [64]. As for *Vwf* gene, it was demonstrated that immobilization stress exposure was followed by a rise in von Willebrand factor concentrations, adrenocorticotropic hormone and corticosterone release in saline pretreated rats [65]. The enhanced *Vwf* gene transcription in ISIAH renal cortex suggests that it may be one of the genes working in stress-related mode in renal cortex of ISIAH rats.

Several pathways found in the current study were closely related to the immune system function. It is long known that the immune system changes play a role in hypertension and an extensive bidirectional interactions between the sympathetic nervous system and the immune system exist [66, 67]. Recent studies have shown that both innate and adaptive immunity contribute to hypertension [68]. Major histocompatibility complex (MHC) class I molecules are ligands for the killer-cell immunoglobulin-like receptors, which are expressed by natural killer (NK) cells and T cells. The interactions

between these molecules contribute to both innate and adaptive immunity [69]. MHC class-II molecules are key participants in immune activation events in autoimmunity [70]. It was shown that mice lacking adaptive immune cells, including recombinase-activating gene-deficient mice and rats and mice with severe combined immunodeficiency have blunted hypertension to stimuli such as ANG II, high salt, and norepinephrine [71]. The current work helped to reveal several genes related to MHC class I and MHC class II which might be useful for further studies of immune system changes during hypertension development in ISIAH rats.

Several other pathways were significantly enriched with DEGs related to steroid hormone biosynthesis, tryptophan, glutathione, nitrogen, and drug metabolism. The most DEGs associated with hypertension in these pathways were related to steroid hormone biosynthesis and were discussed above. The other pathways (glutathione, nitrogen, and drug metabolism) didn't contain the DEGs directly associated with hypertension, however, these also might play important role in pathology development in ISIAH rats. This may be true, at least for the glutathione metabolism. It is known that glutathione is an important intracellular antioxidant that protects against a variety of different oxidant species. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats [72]. In our study, we found several DEGs involved in glutathione metabolism. GPx2 is a key enzyme in the antioxidant system of the cells [73]. The glutathione S-transferases provide cellular protection against the toxic effects of a number of environmental toxicants and products of oxidative stress by conjugation with glutathione [74]. So, we may suggest that downregulation of glutathione S-transferases may weaken oxidative defense and mediate the pathological processes in ISIAH kidney and the upregulation of *Gpx2* seems to play a protective role.

Conclusion

The results of the study revealed multiple genes differentially expressed in renal cortex of hypertensive ISIAH and normotensive WAG rats, including 42 genes associated with hypertension and regulation of BP. Their functional annotation showed that many different processes might be brought into play. Two DEGs associated with hypertension (*Ephx2 and Glp1r*) were in the list of the top 40 genes showing the highest differences in expression in ISIAH and WAG renal cortex. These DEGs may be considered as potential candidates for further studies to better understand the mechanisms of the hypertension development in the ISIAH rats. The results of the discussion suggested that the interstrain differences in ISIAH and WAG renal function may probably arise from the imbalance in processes leading to the development of pathology from one side and the processes trying to restore the homeostasis from the other side. As the number of hypertensive and the other potentially relevant genes was considerable, we were not able to discuss all of them in details.

Our findings provide a basis for identification of potential biomarkers of stress-sensitive hypertension and further investigation of the signaling mechanisms that affect kidney function and contribute to hypertension development.

Methods

Animals

The work was carried out on hypertensive ISIAH and normotensive WAG rats bred in the Center for Genetic Resources of Laboratory Animals at the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, (Novosibirsk, Russia, RFMEFI61914X0005 and RFMEFI61914X0010). All rats were maintained in the standard conditions with free access to food and water. The systolic arterial blood pressure (BP) was measured indirectly by the tail-cuff method. The BP level was determined under short-term ether anesthesia to exclude the effect of psychological stress induced by the measuring procedure. In RNA-seq experiments the 3-month old ISIAH ($n = 3$), and WAG ($n = 3$) males were used. Their systolic arterial BP was 171.7. ± .1.22 mmHg in ISIAH and 116.33 ± 1.86 mmHg in WAG males. The kidney of the decapitated rats was immediately removed and sectioned to get the samples of renal cortex which were stored in RNA Later (Qiagen, Chatsworth, CA) at −70 °C until use. All animal experiments were approved by the Institute's Animal Care and Use Committee.

RNA-seq analysis

The collected samples of renal cortex were sent to JSC Genoanalytica (Moscow, Russia), where mRNA was extracted using Dynabeads mRNA Purification Kit (Ambion, USA). cDNA libraries were constructed using NEBNext mRNA Library Prep Reagent Set for Illumina (NEB, USA) following the manufacturer's protocol and were subjected to Illumina single-end sequencing. Three renal cortex samples from ISIAH and three renal cortex samples from WAG rats were run as experimental replicates. The resulting fastq-formatted sequencing data were mapped to the RGSC Rnor_5.0\rn5 reference genome using Tophat2 aligner [75] and NCBI RefSeq gene annotation. Quality assessment of the mapped data was performed using the module 'CollectRnaSeqMetrics' from Picard tools suite (http://broadinstitute.github.io/picard/). The summary statistics for each sequenced library is given in Additional file 4. The Cufflinks/Cuffdiff programs were then used to estimate gene expression levels in FPKM (fragments per kilobase of transcript per million

mapped reads), and to perform differential expression analysis [76]. Genes were considered to be differentially expressed at q value < 0,05.

Functional annotation

The functional analysis of DEGs was performed using DAVID (The Database for Annotation, Visualization and Integrated Discovery) tool (http://david.abcc.ncifcrf.gov/) [77, 78]. The Gene Ontology option was utilized to determine the significantly ($p < 0.05$) enriched biological processes and groups of genes possibly contributing to hypertensive phenotype in ISIAH rats. The Kyoto Encyclopedia of Genes and Genomes Pathway Database (KEGG, http://www.genome.jp/kegg/) was used to identify pathways that were most significant to the data set. The genes related to hypertension and renal diseases were detected according to the DEGs annotation in Rat Genome Database (RGD, http://rgd.mcw.edu/). The detection of transcription factors among DEGs was performed using gene annotations from GenBank (http://www.ncbi.nlm.nih.gov/gene/), an atlas of combinatorial transcriptional regulation in mouse and man [79] and Panther classification system (http://www.pantherdb.org/).

Quantitative real-time PCR (qPCR)

The relative amount of target mRNA was measured by qPCR. Samples of renal cotrtex were analyzed in 3-month old ISIAH and WAG rats. Each group contained five rats. Total RNA was extracted using the TRI reagent (Molecular research center, USA). Remaining traces of genomic DNA were removed from the RNA samples using DNase I (Promega, USA) treatment, according to the manufacturer's instructions.

Reverse transcription was performed in 50 μl of RT buffer containing 3 μg of total RNA, 0.25 nmol of random nonanucleotide primers (Biosan, Russia), 0.4 mM dNTP, and 40 units of MoMLV (Vektor-Best, Russia). The cDNA was synthesized at 37 °C (1 h), 42 °C (30 min), and 50 °C (10 min). The enzyme was inactivated by heating the mixture at 75 °C for 5 min.

qPCR was performed in a final volume of 20 μl. The reaction volume contained master mix with SYBR Green, forward and reverse primers (0,15 mM each), 1 unit of HotStart Taq polymerase (Vektor-Best, Russia), and the cDNA template. The housekeeping gene *Rpl30* encoding ribosomal protein L30 was used as a reference gene. Primer's sequences, their annealing temperatures, and the temperatures of fluorescence signal acquisition are given in Additional file 5.

qPCR was carried out in an iCycler iQ4 Real-Time PCR Detection System (Bio-Rad Laboratories, USA) with an initial denaturation of 1 min at 94 °C followed by 40 cycles of 15 s at 94 °C, 20 s at primer's annealing temperatures (see Additional file 5), 20 s at 72 °C, fluorescence signal acquisition for 10 s, and then generation of melting curve from 65 to 94 °C. The standard-curve quantitation method was applied [80]: the relative amount of the tested cDNA was determined using calibration curves derived from the dilutions of the standard cDNA. Standard cDNA solution for plotting calibration curves was obtained by mixing aliquots from each of the synthesized cDNA samples. In each experiment, cDNA samples with primers for the target gene (four replicates per cDNA sample), the same samples with primers for the reference gene (four replicates), and the standard cDNA dilutions (1 : 1, 1 : 4, 1 : 16, and 1 : 64) with the primers for the target gene (two replicates), and with the primers for the reference gene (two replicates) were placed on the same plate. The value for the target gene was further normalized against the qPCR level of the reference gene.

Statistical calculations for qPCR data were performed with the software package Statistica v.6.0 (Statsoft, USA) using nonparametric statistics, Mann-Whitney U-test. Differences were considered statistically significant when P was less than 0.05. The data were presented as means and their standard errors (M ± S.E.M.).

Availability of supporting data

The data sets supporting the results of this article are included within the article and its additional files.

Additional files

Additional file 1: GO Terms and DEGs. (XLS 188 kb)

Additional file 2: DEGs related to GO terms. (XLS 92 kb)

Additional file 3: DEGs in the KEGG pathways. (XLS 27 kb)

Additional file 4: The number of mapped reads in hypothalamuses of ISIAH and WAG rats*. (XLS 22 kb)

Additional file 5: Primers used in real-time PCR. (DOC 36 kb)

Abbreviations

AA: Arachidonic acid; BP: Blood pressure; DAVID: Database for Annotation, Visualization and Integrated Discovery; DEG: Differentially expressed genes; EETs: Epoxyeicosatrienoic acids; FPKM: Fragments per kilobase of transcript per million mapped reads; GO: Gene Ontology; HPA: Hypothalamic-pituitary-adrenal; ISIAH: Inherited Stress-Induced Arterial Hypertension; KEGG: Kyoto Encyclopedia of Genes and Genomes Pathway Database; MHC: Major histocompatibility complex; NK: Natural killer; qPCR: Quantitative real time polymerase chain reaction; RA: retinoic acid; RAS: renin-angiotensin system; RGD: Rat Genome Database; RNA-seq: RNA sequencing; SABP: Systolic arterial blood pressure; sEH: Soluble epoxide hydrolase; WAG: Wistar Albino Glaxo.

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

Authors' contributions

LF participated in interpretation of data, and helped to draft the manuscript; MR performed quantitative real time PCR; NE has made substantial contribution to bioinformatics analysis; AM has made substantial contributions to conception and design of the study and participated in interpretation of data; OR participated in interpretation of data and drafted the manuscript. All authors read approved the final manuscript.

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Declarations
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