Gene expression in the adrenal glands of three spontaneously hypertensive rat substrains

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Abstract. We examined gene expression profiles in rat adrenal glands using genome-wide microarray technology. Gene expression levels were determined in four rat strains, including one normotensive strain [Wistar-Kyoto (WKY)] and three substrains derived from WKY rats: spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP) and malignant SHRSP (M-SHRSP). This study represents the first attempt at using microarrays to compare gene expression profiles in SHR, SHRSP and M-SHRSP adrenal glands, employing WKY as controls. Expression measurements were made in these four rat strains at 6 and 9 weeks of age; 6 weeks of age covers the pre-hypertensive period in SHR and SHRSP, and 9 weeks of age is the period of rapidly rising blood pressure (BP). Since the aim of this study was to identify candidate genes involved in the genesis of hypertension in the SHR substrains, we identified genes that were consistently different in their expression, isolating 87 up-regulated genes showing a more than 4-fold increase and 128 down-regulated genes showing a less than 1/4-fold decrease in at least two different experiments. We classified all these up- or down-regulated genes by their expression profiles, and searched for candidate genes. At 6 weeks of age, several BP-regulating genes including sparc/osteonectin (*Spock2*), kynureninase (*Kynu*), regulator of G-protein signaling 2 (*Rgs2*) and gap junction protein α1 (*Gja1*) were identified as up-regulated, and urotensin 2 (*Uts2*), cytoplasmic epoxide hydrolase 2 (*Ephx2*), apelin (*Apln*), insulin-like growth factor 1 receptor (*Igf1r*) and angiotensin II receptor-associated protein (*Agtrap*) were identified as down-regulated. The *Kynu* and *Ephx2* genes have previously been reported by other groups to be responsible for hypertension in SHR; however, our present approach identified at least seven new candidate genes.

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Abbreviations: BP, blood pressure; NA, non-annotated clones; NFA, non-functionally annotated clones; RefSeq, reference mRNA sequence; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; M-SHRSP, malignant SHRSP; WKY, normotensive Wistar-Kyoto rat, a wild-type control strain

Key words: adrenal gland, gene expression, hypertension, spontaneously hypertensive rats, stroke-prone spontaneously hypertensive rats, malignant spontaneously hypertensive rats

Introduction

The polygenic nature of human essential hypertension has made it difficult to isolate the genes involved in the genesis of the disease. Microarrays are a potentially powerful tool for studying the genetics of hypertension, as they facilitate the simultaneous measurement of the expression of thousands of genes. Since inbred homozygous rodent models of human essential hypertension are ideal for microarray research, animal models of essential hypertension have recently been studied using microarrays (1).

In this study, we present a comparison of adrenal gland gene expression in three strains of hypertensive rats: spontaneously hypertensive rats (SHR) as well as two substrains derived from SHR, stroke-prone SHR (SHRSP) and malignant SHRSP (M-SHRSP) (2-4). SHR, the current paradigm for essential hypertension research, were developed in a breeding program with selection based solely on elevated blood pressure (BP) in Wistar rats (2). Normotensive descendants of Wistar rats [Wistar-Kyoto rats (WKY)], from which the SHR strain was derived, were used as controls (2,3). SHRSP were established from SHR by selective inbreeding for stroke-proneness (3), and M-SHRSP were established through the brother-sister mating of selected SHRSP showing higher BP at an early stage of development for 20 generations (4). An inbred strain of M-SHRSP exhibited BP as high as 250 mmHg or more before 14 weeks of age, and, compared to SHRSP, experienced more rapid and severe increases in BP (4).

Table I. Blood pressure and gene expression in SHR, SHRSP and M-SHRSP.

Rats	Blood pressure (mmHg)	>4-fold up-regulated probes	<1/4-fold down-regulated probes
Six weeks of age			
WKY	130±6		
SHR	136±5	4	4,407
SHRSP	132±3	59	149
M-SHRSP	160±4[a]	112	74
Nine weeks of age			
WKY	137±5		
SHR	158±7	84	42
SHRSP	180±10[a,b]	71	256
M-SHRSP	217±10[a,b]	146	19
Total up- and down-regulated probes		476	4,947

Significant differences at P<0.001 vs. [a]WKY of the same age, and vs. [b]the same subgroup of rats aged 6 weeks.

Adrenal gland secretory products, both medullary and cortical, are logical candidates for the study of hypertension, since they can directly influence cardiovascular, endocrine and sympathetic functions (5). This study represents the first attempt at using microarrays to compare gene expression profiles in SHR, SHRSP and M-SHRSP adrenal glands, employing WKY as controls. The aim of the study was to identify candidate genes involved in the genesis of hypertension in the SHR substrains.

Materials and methods

Animals and measurements. Experiments were performed using rats aged 6 and 9 weeks. WKY/Izm were employed as a wild-type control strain, and SHR/Kpo, SHRSP/Kpo and M-SHRSP/Kpo as hypertension model rats (2-4). The WKY/Izm strain was purchased from SLC Co. (Shizuoka, Japan), and the other three substrains from the Kinki University Animal Center. All animals used in the experiment were handled with due care according to the guidelines established by the Japanese Association for Laboratory Animal Science, which comply with international rules and policies. This study was performed with the approval of the Animal Care and Use Committee of Kinki University (KAME-19-078, April 1, 2007). All possible measures were taken to minimize the pain and discomfort of the experimental animals.

Systolic blood pressure (SBP) measurements were performed using the tail-cuff method with a UR-5000 instrument (Ueda, Tokyo, Japan). Briefly, three consecutive BP readings were taken, and BP values were expressed as the mean ± SEM. Comparisons between the means of groups were made using one-way analysis of variance (ANOVA) and Scheffe's multiple comparisons test, with differences considered significant at P<0.05.

Tissue processing and RNA isolation. After the adrenal glands were harvested, the organs were homogenized at a pitch speed of 22 strokes/sec for 2 min (2 times) in a 2-ml plastic tube with a 5-mm-diameter glass bead using the Qiagen Tissue Lyser (Retsch Gmbh & Co., Haan, Germany). Total RNA was extracted using an RNeasy Mini Kit (Qiagen Sciences, Germantown, MD, USA) according to the manufacturer's protocol. Prior to its use in the microarray experiments, RNA quality was verified with RNA Nano Chips (Agilent Technologies, Waldbornn, Germany) using an Agilent 2100 Bioanalyzer. Three rats each from all hypertensive and normotensive strains were used in each experiment.

Analysis of gene expression profiling using oligonucleotide arrays. To examine gene expression profiles in the adrenal glands of rats, cDNA was synthesized from 1 μg of the DNase I-treated total RNA using a Low RNA Input Amplification kit (Agilent Technologies), and was subjected to hybridization through incubation using a Whole Rat Genome Microarray kit (4x44K) (Agilent Technologies) with a rotor oven (Sure-print Technology, USA) for 17 h at 65˚C, followed by a washing microarray. The hybridized slides were scanned with the Agilent GenPix Scanner 4000 (Agilent Technologies). Data were extracted, and the overall raw signal intensities on each array were normalized to the median value of all rat probes using BRB-Array Tool software ver. 3.7.0. (Biometric Research Branch) (6). A significance level (P<0.05) for each probe set was calculated using the Student's t-univariate test.

Annotation of differentially expressed genes. A BLASTN search was preformed using the NCBI RefSeq database employing the corresponding 60-nucleotide-long probes (NCBI, GEO accession, GPL7294) in order to identify homologous genes with functional annotations (7). After running the BLASTN search, clones showing a score >50 or an E-value <5e-05 were defined as annotated clones, while the remaining clones were defined as non-annotated. Some of the annotated clones, either encoding hypothetical proteins or corresponding to uncharacterized cDNA clones, were defined as non-functionally annotated. Annotated gene and protein symbols were written in italics and plain text, respectively.

Results

Blood pressure measurements. SBP in WKY and the three SHR substrains was measured at 6 and 9 weeks of age (Table I). Although SBP was significantly higher in M-SHRSP at 6 weeks of age than in WKY, SHR and SHRSP, no significant difference was noted in SBP among WKY, SHR and SHRSP at this age (Table I). However, at 9 weeks of age, SBP in the SHR substrains gradually increased in the order of WKY < SHR < SHRSP < M-SHRSP (Table I).

Microarray statistical results. Since the aim of this study was to identify candidate genes involved in the genesis of hypertension in SHR substrains, gene expression profiles were compared using chips containing 41,012 probes and mRNAs extracted from the adrenal glands of SHR substrains at 6 and 9 weeks of age. The number of >4-fold up-regulated probes was found to be increased from 4 in SHR to 59 in SHRSP and to 112 in M-SHRSP at 6 weeks of age (Table I). By contrast, the number of <1/4-fold down-regulated probes was as high as 4,407 in SHR, and was markedly decreased to 149 in SHRSP and to 74 in M-SHRSP at 6 weeks of age (Table I).

Expression levels of each probe were estimated at 6 and 9 weeks of age, meaning each probe generated at least six different bits of data (Table II). The number of up- or down-regulated genes was less than the number of probes, due to redundancy in the probe sets (i.e., in some cases, two or three probes represent one gene). Genes exhibiting >4-fold or <1/4-fold expression in SHR substrains in comparison to WKY in at least two out of the six independent experiments were defined as up- and down-regulated genes, respectively. The 476 up-regulated probes identified 87 up-regulated genes, and the 4,947 down-regulated probes identified 128 down-regulated genes (Tables I and II). As it has been reported that only a few major loci play a role in the pathogenesis of hypertension in SHR (at least three) (8,9), we presumed that these 87+128 genes embody most of the candidate genes involved in the genesis of hypertension in SHR substrains, and expected the expression of candidate genes to be up- or down-regulated well before the elevation of BP, i.e., by 6 weeks of age in SHR and/or SHRSP (Table I). Accordingly, we examined the expression profiles of the 87 up-regulated and 128 down-regulated genes, and classified these genes by their expression profiles (Table II).

Classification of the 87 up-regulated genes. The 87 up-regulated genes showed 24 expression profiles, and were classified into 5 groups (Table IIA, U1 to U5). The U1 group included 4 genes expressed in SHR at 6 weeks of age, and the U2 group, 24 genes expressed in SHRSP at 6 weeks of age. The U3 group included 33 genes expressed in M-SHRSP at 6 weeks of age, the U4 group, 10 genes expressed in SHR at 9 weeks of age, and the U5 group, 16 genes expressed in SHRSP at 9 weeks of age (Table IIA).

Taking expression profiles and levels of up-regulation into consideration, each one of the 87 up-regulated genes was classified together with the results of their annotations, summarized in Table III. We focused on the 28 genes of the U1 and U2 groups, since these were up-regulated in SHR and/

Table II. Gene expression profiles in SHR, SHRSP and M-SHRSP.

A, 87 up-regulated genes showing 24 expression profiles.

Group	6H	6S	6M	9H	9S	9M
U1 (4)	1	1	1	1	1	1
	1	1	1	0	0	0
	1	0	1	1	1	1
	1	0	1	0	0	0
U2 (24)	0	3	3	3	3	3
	0	2	2	2	2	0
	0	3	3	3	0	3
	0	1	1	0	1	0
	0	1	1	0	0	1
	0	11	11	0	0	0
	0	1	0	1	1	1
	0	1	0	1	0	1
	0	1	0	1	0	0
U3 (33)	0	0	6	6	6	6
	0	0	4	4	4	0
	0	0	3	3	0	3
	0	0	4	4	0	0
	0	0	4	0	4	4
	0	0	2	0	2	0
	0	0	10	0	0	10
U4 (10)	0	0	0	4	4	4
	0	0	0	2	2	0
	0	0	0	4	0	4
U5 (16)	0	0	0	0	16	16

B, 128 down-regulated genes showing 18 expression profiles.

Group	6H	6S	6M	9H	9S	9M
D1 (98)	2	2	2	2	2	2
	1	1	1	0	0	1
	24	24	24	0	0	0
	52	52	0	0	0	0
	1	0	1	1	1	1
	1	0	1	0	1	0
	15	0	15	0	0	0
	1	0	0	1	0	1
	1	0	0	0	1	0
D2 (9)	0	1	1	1	1	1
	0	1	1	0	0	1
	0	6	6	0	0	0
	0	1	0	1	1	0
D3 (1)	0	0	1	1	1	0
D4 (18)	0	0	0	7	7	7
	0	0	0	10	10	0
	0	0	0	1	0	1
D5 (2)	0	0	0	0	2	2

Up- or down-regulated genes were classified into 5 groups according to expression profile (U1-U5 and D1-D5). Numbers in parentheses indicate the number of genes belonging to each group. 6, 6 weeks of age; 9, 9 weeks of age; H, SHR; S, SHRSP; M, M-SHRSP. Values from 0 to 52 indicate the number of up- or down-regulated genes.

Table III. The 87 genes up-regulated in SHR, SHRSP and M-SHRSP.

Gr	Probe ID	6H	6S	6M	9H	9S	9M	Fold-change	Gene or protein name (symbol)
U1	A_44_P152177	1	1	1	1	1	1	23.2-4.9	*1, Sparc/osteonectin (Spock2)
U1	A_44_P863709	1	1	1	0	0	0	27.3-7.0	NA (40), hypothetical protein
U1	A_44_P245616	1	0	1	1	1	1	47.6-9.8	Nidogen 1 (Nid1)
U1	A_44_P203564	1	0	1	0	0	0	9.2 and 6.1	Otospiralin (Otos)
U2	A_44_P560710	0	1	1	1	1	1	37.1-8.7	NA (38), stem cell tumor (CpipJ_CPIJ003969)
U2	A_42_P826202	0	1	1	1	1	1	12.0-4.2	Zinc finger protein 597 (Znf597)
U2	A_42_P703548	0	1	1	1	1	1	11.9-6.5	*2, Kynureninase (Kynu)
U2	A_44_P578428	0	1	1	1	1	0	11.7-4.5	NA (38), CNE04290
U2	A_44_P640472	0	1	1	1	1	0	4.9-4.1	NA (38), ribosomal protein
U2	A_44_P495480	0	1	1	1	0	1	33.2-7.8	FBJ osteosarcoma oncogene homolog (c-Fos)
U2	A_43_P11932	0	1	1	1	0	1	25.7-4.1	Nuclear receptor subfamily 4A3 (Nr4a3)
U2	A_44_P1033521	0	1	1	1	0	1	17.9-6.6	Trichorhinophalangeal syndrome I (Trps1)
U2	A_44_P603503	0	1	1	0	1	0	21.7-10.2	Oxidoreductase NAD-binding domain containing 1 (Oxnad1)
U2	A_44_P396202	0	1	1	0	0	1	12.2-6.7	Interferon-inducible GTPase (RGD1309362)
U2	A_44_P455271	0	1	1	0	0	0	42.4 and 9.9	NA (38), CCMP1335
U2	A_44_P406636	0	1	1	0	0	0	17.9 and 7.7	ATP-binding cassette, sub-family A (ABC1), 1 (Abca1)
U2	A_44_P464942	0	1	1	0	0	0	12.5 and 8.3	NA (38), TVAG_318060
U2	A_43_P14131	0	1	1	0	0	0	9.0 and 5.0	*3, Regulator of G-protein signaling 2 (Rgs2)
U2	A_44_P472989	0	1	1	0	0	0	7.6 and 5.4	Prostaglandin-endoperoxide synthase 2 (Ptgs2)
U2	A_42_P615837	0	1	1	0	0	0	7.3 and 4.2	Creatine kinase, mitochondrial 2, sarcomeric (Ckmt2)
U2	A_44_P970369	0	1	1	0	0	0	6.4 and 5.6	Heparan sulfate 2-O- sulfotransferase 1 (Hs2st1)
U2	A_44_P473217	0	1	1	0	0	0	6.1 and 5.4	NFA, RIKEN cDNA 4930455F23 (RGD1309708)
U2	A_44_P777181	0	1	1	0	0	0	5.9 and 5.2	RAB22A, member RAS oncogene family (Rab22a)
U2	A_44_P176831	0	1	1	0	0	0	5.6 and 4.3	Met proto-oncogene (Met)
U2	A_44_P161052	0	1	1	0	0	0	4.5 and 4.3	3-Ketoacyl-CoA thiolase B peroxisomal precursor (RGD1562373)
U2	A_43_P16225	0	1	0	1	1	1	5.9-4.6	NA (40), myosin, heavy chain 11, smooth muscle (MYH11)
U2	A_44_P354699	0	1	0	1	0	1	8.5-7.3	CTD small phosphatase like 2, transcript variant 1 (CTDSPL2)
U2	A_44_P836591	0	1	0	1	0	0	7.8 and 9.6	*4, Gap junction protein, α1 (Gja1)
U3	A_44_P402980	0	0	1	1	1	1	49.6-9.0	NK6 homeobox 2 (Nkx6-2)
U3	A_42_P686756	0	0	1	1	1	1	36.4-14.7	NFA, RIKEN cDNA 1110032A04 gene (1110032A04Rik)
U3	A_44_P553498	0	0	1	1	1	1	33.8-8.7	NA (48), carbonyl reductase 1 (Cbr1)
U3	A_44_P520159	0	0	1	1	1	1	12.9-4.8	Purinergic receptor P2Y, G-protein coupled 2 (P2ry2)
U3	A_44_P670594	0	0	1	1	1	1	10.8-4.5	NA (42) hypothetical protein CBG12924 partial
U3	A_42_P537051	0	0	1	1	1	1	5.8-4.6	Family with sequence similarity 70B (Fam70b)
U3	A_44_P653949	0	0	2	1	1	0	23.3-8.4	NA (40), hypothetical protein
U3	A_44_P777328	0	0	2	1	1	0	15.2-4.9	Phosphatidylinositol 3 kinase, reg sub, polypeptide 3 (Pik3r3)
U3	A_44_P840366	0	0	1	1	1	0	10.5-7.3	NA (38), LOC100072399
U3	A_44_P702222	0	0	1	1	1	0	8.6-5.2	NFA, hypothetical protein, LOC100286928
U3	A_42_P750683	0	0	2	1	0	1	22.7-10.7	Cysteine-rich, angiogenic inducer, 61 (Cyr61)
U3	A_43_P11531	0	0	1	1	0	1	15.7-4.6	Somatostatin receptor 1 (Sstr1)
U3	A_44_P189363	0	0	2	1	0	1	8.64.5	Endothelial cell-specific molecule 1 (Esm1)
U3	A_44_P1030258	0	0	1	1	0	0	6.3 and 6.3	Cannabinoid receptor 1 (Cnr1)
U3	A_44_P142401	0	0	1	1	0	0	5.8 and 4.7	Family with sequence similarity 184B (Fam184b)
U3	A_44_P659416	0	0	1	1	0	0	5.0 and 4.5	NA (36), CD36_04040
U3	A_44_P732150	0	0	1	1	0	0	4.6 and 4.4	NFA, expressed sequence C77370
U3	A_43_P12125	0	0	1	0	1	1	34.8-10.9	Jun B proto-oncogene (Junb)
U3	A_44_P868694	0	0	1	0	1	1	15.4-5.7	NA (36), hypothetical protein (GSPATT00018902001)
U3	A_42_P784172	0	0	1	0	1	1	9.6-4.3	Vomeromodulin (LOC690507)
U3	A_44_P475661	0	0	1	0	1	1	7.1-4.3	Integrator complex subunit 7 (Ints7)
U3	A_44_P731793	0	0	1	0	1	0	6.5 and 6.2	NA (40), TVAG_113750
U3	A_44_P668233	0	0	1	0	1	0	5.6 and 4.8	NA (36), DHX9
U3	A_44_P746102	0	0	1	0	0	1	13.3 and 4.8	NA (40), pyrroline-5-carboxylate reductase partial
U3	A_42_P701060	0	0	1	0	0	1	8.4 and 8.3	One cut homeobox 1 (Onecut1)
U3	A_42_P640277	0	0	1	0	0	1	5.7 and 4.7	Ectodermal-neural cortex 1 (Enc1)
U3	A_44_P716208	0	0	1	0	0	1	5.3 and 4.2	NA (38), GH17134 (Dgri\GH17134)

Table III. Continued.

Gr	Probe ID	6H	6S	6M	9H	9S	9M	Fold-change	Gene or protein name (symbol)
U3	A_44_P652899	0	0	1	0	0	1	5.2 and 4.6	Glycoprotein m6a (Gpm6a)
U3	A_44_P900835	0	0	1	0	0	1	5.1 and 4.0	NA (38), misc_RNA (CSMD3), partial
U3	A_44_P730958	0	0	1	0	0	1	5.0 and 4.9	Eyes absent 1 homolog (*Drosophila*) (Eya1)
U3	A_44_P634618	0	0	1	0	0	1	5.0 and 4.0	Syntaxin binding protein 6 (amisyn) (Stxbp6)
U3	A_44_P715524	0	0	1	0	0	1	4.5 and 4.2	B-cell CLL/lymphoma 7A (Bcl7a)
U3	A_44_P793124	0	0	1	0	0	1	4.4 and 4.2	Zinc finger and BTB domain containing 41 (Zbtb41)
U4	A_43_P22478	0	0	0	1	1	1	14.8-8.3	Dual-specificity tyr(Y)-phosphorylation regulated kinase 3 (Dyrk3)
U4	A_44_P534791	0	0	0	1	1	1	10.6-5.7	Methyltransferase like 2 (Mettl2)
U4	A_44_P240696	0	0	0	1	1	1	7.3-4.8	C-type natriuretic peptide precursor (Cnp)
U4	A_42_P605711	0	0	0	1	1	1	4.9-4.2	Family with sequence similarity 46A (Fam46a)
U4	A_43_P13199	0	0	0	1	1	0	12.9 and 11.4	Folate receptor 1 (Folr1)
U4	A_42_P645923	0	0	0	1	1	0	5.3 and 4.2	Cystathionine β synthase (Cbs)
U4	A_44_P808922	0	0	0	1	0	1	6.4 and 4.4	POU domain, class 4, transcription factor 1 (Pou4f1)
U4	A_42_P719221	0	0	0	1	0	1	6.3 and 5.5	Vasoactive intestinal peptide (Vip)
U4	A_44_P199028	0	0	0	1	0	1	6.3 and 4.5	Dickkopf homolog 1 (Dkk1)
U4	A_42_P694679	0	0	0	1	0	1	6.0 and 4.7	Histone deacetylase 4 (Hdac4)
U5	A_44_P556895	0	0	0	0	1	1	35.8 and 15.8	DNA-damage-inducible transcript 4 (Ddit4)
U5	A_44_P351211	0	0	0	0	1	1	18.3 and 16.9	Pleckstrin homology-like domain, family A, member 1 (Phlda1)
U5	A_44_P306581	0	0	0	0	1	1	14.2 and 13.0	Growth differentiation factor 5 (Gdf5)
U5	A_42_P548410	0	0	0	0	1	1	11.3 and 10.9	Acyl-CoA thioesterase 1 (Acot1)
U5	A_44_P268915	0	0	0	0	1	1	9.7 and 6.8	Phospholipase B domain containing 1 (Plbd1)
U5	A_43_P18366	0	0	0	0	1	1	7.0 and 4.6	Torsin family 1, member B (Tor1b)
U5	A_44_P527238	0	0	0	0	1	1	6.7 and 4.9	Solute carrier family 25 (Slc25a25)
U5	A_42_P538483	0	0	0	0	1	1	6.5 and 4.9	Ring finger protein 40 (Rnf40)
U5	A_44_P506662	0	0	0	0	1	1	6.4 and 4.3	Transcriptional regulating factor 1 (Trerf1)
U5	A_43_P15888	0	0	0	0	1	1	6.2 and 4.5	Angiomotin like 2 (Amotl2)
U5	A_44_P308369	0	0	0	0	1	1	6.0 and 5.0	UDP-glucose glycoprotein glucosyltransferase 2 (Uggt2)
U5	A_42_P662710	0	0	0	0	1	1	5.6 and 4.2	Death effector domain-containing (Dedd)
U5	A_44_P445575	0	0	0	0	1	1	5.5 and 5.5	Iroquois related homeobox 2 (Irx2)
U5	A_44_P257518	0	0	0	0	1	1	5.4 and 4.6	Insulin receptor substrate 2 (Irs2)
U5	A_44_P547236	0	0	0	0	1	1	5.2 and 4.1	Tetraspanin 33 (Tspan33)
U5	A_44_P251124	0	0	0	0	1	1	5.0 and 4.3	Protein tyrosine phosphatase, non-receptor type 18 (Ptpn18)

Gr, up-regulated U1, U2, U3, U4 and U5 groups; Probe ID, Agilent probe ID (NCBI, GEO accession, GPL7294). Numbers 0, 1 and 2 indicate the number of probes showing positive results; Fold-change, >4-fold up-regulation. NA, non-annotated. Numbers in parentheses indicate homology scores <50; NFA indicates annotated with homology scores >50, but no functional information. Other symbols or abbreviations are as described in Table II.

or SHRSP at 6 weeks of age and were expected to include the candidate genes. They comprised 20 functionally annotated and 8 non-functionally annotated genes (Table III, U1 and U2). Functionally annotated genes included genes reported to participate in BP control, such as sparc/osteonectin (*Spock2*), kynureninase (*Kynu*) and regulator of G-protein signaling 2 (*Rgs2*), as well as gap junction protein α1 (*Gja1*) (Table III, *1 to *4) (10-13).

Classification of the 128 down-regulated genes. The 128 down-regulated genes showed 18 expression profiles and were classified into 5 groups (Table IIB, D1-D5). The D1 group included 98 genes expressed in SHR at 6 weeks of age, and the D2 group, 9 genes expressed in SHRSP at 6 weeks of age. The D3 group contained 1 gene expressed in M-SHRSP at 6 weeks of age, the D4 group, 18 genes expressed in SHR at 9 weeks of age, and the D5 group, 2 genes expressed in SHRSP at 9 weeks of age, respectively (Table IIB).

Taking expression profiles and levels of down-regulation into consideration, each one of the 128 genes was classified together with the results of their annotations, summarized in Table IV. We focused on the 107 genes of the D1 and D2 groups, since these were down-regulated in SHR and/or SHRSP at 6 weeks of age and were expected to include the candidate genes. They comprised 77 functionally annotated and 30 non-annotated genes (Table IV, D1 and D2). Functionally annotated genes included genes reported to participate in BP control, such as urotensin 2 (*Uts2*), cytoplasmic epoxide hydrolase 2 (*Ephx2*), apelin (*Apln*), insulin-like growth factor 1 receptor (*Igf1r*) and angiotensin II type I receptor-associated protein (*Agtrap*) (14-18) (Table IV, *1 to *5).

Table IV. The 128 genes down-regulated in SHR, SHRSP and M-SHRSP.

Gr	Probe ID	6H	6S	6M	9H	9S	9M	Fold-change	Gene or protein name (symbol)
D1	A_44_P637734	2	3	3	1	1	1	0.07-0.16	DEAD/H box polypeptide 11 (Ddx11)
D1	A_44_P345207	1	1	1	1	1	1	0.08-0.21	ER-Golgi intermediate compartment protein 1 (Ergic1)
D1	A_44_P362486	1	1	1	1	0	1	0.14-0.25	Actin-binding LIM protein 1 (Ablim1)
D1	A_42_P508921	1	1	1	0	0	0	0.01-0.02	Aquaporin 3 (Aqp3)
D1	A_44_P531908	1	1	1	0	0	0	0.02-0.07	*1, Urotensin 2 (Uts2)
D1	A_43_P11634	2	2	2	0	0	0	0.02-0.13	GDNF family receptor α1 (Gfra1)
D1	A_44_P422233	1	1	1	0	0	0	0.03-0.06	NA (38), hypothetical protein, CaO19_10805
D1	A_44_P562496	1	1	1	0	0	0	0.04-0.11	DEAD box polypeptide 42 protein (Ddx42)
D1	A_42_P803590	1	1	1	0	0	0	0.04-0.19	NFA, B0432.8 (LOC289378)
D1	A_42_P640641	1	1	1	0	0	0	0.05-0.15	Homer homolog 2 (Homer2)
D1	A_44_P213133	1	1	1	0	0	0	0.05-0.17	Endonuclease G (Endog)
D1	A_44_P398060	2	1	2	0	0	0	0.05-0.18	Neuronal cell adhesion molecule (Nrcam)
D1	A_44_P577208	1	1	1	0	0	0	0.06-0.08	NA (38), zf(c2h2)-31
D1	A_44_P531870	1	1	1	0	0	0	0.07-0.09	*2, Epoxide hydrolase 2, cytoplasmic (Ephx2)
D1	A_44_P808578	1	1	1	0	0	0	0.07-0.10	NA (38), LOC100214468
D1	A_42_P809869	1	1	1	0	0	0	0.07-0.17	Thiosulfate sulfurtransferase, mitochondrial (Tst)
D1	A_44_P147254	1	1	1	0	0	0	0.07-0.22	Cartilage intermediate layer protein 2 (Cilp2)
D1	A_44_P402948	1	1	1	0	0	0	0.08-0.15	NA (36), LOC717608
D1	A_44_P108063	1	1	1	0	0	0	0.09-0.15	Rhomboid 5 homolog 1 (Rhbdf1)
D1	A_44_P823114	1	1	1	0	0	0	0.10-0.16	NA (40), Hypothetical protein
D1	A_44_P1022458	1	1	1	0	0	0	0.12-0.24	Tubulin, β 6 (Tubb6)
D1	A_42_P460340	2	1	1	0	0	0	0.14-0.24	Mal, T-cell differentiation protein 2 (Mal2)
D1	A_44_P610294	1	1	1	0	0	0	0.15-0.20	NA (36), BRAFLDRAFT_119740
D1	A_44_P111865	1	1	1	0	0	0	0.15-0.22	Cytochrome P450, 2A, 3a (Cyp2a3a)
D1	A_44_P850331	1	1	1	0	0	0	0.17-0.23	NA (38), GI20648
D1	A_44_P298730	1	1	1	0	0	0	0.17-0.24	NA (38), Cys-rich prot.2-bind protein
D1	A_43_P10482	1	1	1	0	0	0	0.19-0.22	NA (38), CBS138
D1	A_44_P214900	1	1	0	0	0	0	0.04 and 0.15	Pregnancy-zone protein (Pzp)
D1	A_42_P637189	1	1	0	0	0	0	0.04 and 0.16	*3, Apelin (Apln)
D1	A_44_P945741	1	1	0	0	0	0	0.05 and 0.14	NA (38), TVAG_290990
D1	A_44_P119527	1	1	0	0	0	0	0.06 and 0.22	Mediator complex subunit 17 (Med17)
D1	A_44_P740817	1	1	0	0	0	0	0.07 and 0.20	Ubiquitin-conjugating enzyme E2Z (Ube2z)
D1	A_44_P387005	1	1	0	0	0	0	0.07 and 0.22	1-Aminocyclopropane-1-carboxylate synthase homolog (Accs)
D1	A_44_P234315	1	1	0	0	0	0	0.07 and 0.24	NA (38), Misc_RNA (EMR2)
D1	A_44_P1039616	1	1	0	0	0	0	0.08 and 0.20	Cd300D antigen (LOC498022)
D1	A_44_P486624	2	2	0	0	0	0	0.08 and 0.22	Pleckstrin homology domain containing, A, 6 (Plekha6)
D1	A_44_P1041460	1	1	0	0	0	0	0.08 and 0.23	Ras-related GTP binding D (Rragd)
D1	A_44_P181560	1	1	0	0	0	0	0.10 and 0.13	Coiled-coil domain containing 95 (Ccdc95)
D1	A_42_P549451	1	1	0	0	0	0	0.10 and 0.19	Fatty acid binding protein 6, ileal (Fabp6)
D1	A_44_P507343	1	1	0	0	0	0	0.10 and 0.21	NA (38), Lrp12
D1	A_44_P701521	1	1	0	0	0	0	0.10 and 0.21	NA (34), SORBIDRAFT_02g013130
D1	A_44_P792797	1	1	0	0	0	0	0.11 and 0.18	NA (38), A1CF
D1	A_44_P481160	1	1	0	0	0	0	0.11 and 0.21	Wingless-type MMTV integration site 5A (Wnt5a)
D1	A_43_P18817	1	1	0	0	0	0	0.11 and 0.21	Intraflagellar transport 52 homolog (LOC684302)
D1	A_44_P899288	1	1	0	0	0	0	0.11 and 0.22	Leucine rich repeat and sterile α motif containing 1 (Lrsam1)
D1	A_42_P548791	1	1	0	0	0	0	0.11 and 0.23	Translin (Tsn)
D1	A_44_P562830	1	1	0	0	0	0	0.12 and 0.17	Dual specificity phosphatase-like 15 (Dusp15)
D1	A_42_P520497	1	1	0	0	0	0	0.13 and 0.15	Solute carrier family 2 (facilitated glucose transporter), 5 (Slc2a5)
D1	A_44_P653343	1	1	0	0	0	0	0.13 and 0.22	NA (40), NEMVEDRAFT_v1g176880
D1	A_43_P14506	1	1	0	0	0	0	0.14 and 0.14	Ankyrin repeat domain 34B (Ankrd34b)
D1	A_42_P548889	1	1	0	0	0	0	0.14 and 0.20	Replication protein-binding trans-activator RBT1 (MGC108974)
D1	A_42_P627572	1	1	0	0	0	0	0.14 and 0.22	Mediator of DNA damage checkpoint 1 (Mdc1)
D1	A_44_P1037892	1	1	0	0	0	0	0.15 and 0.15	Citrin (RGD1565889)
D1	A_44_P274976	1	1	0	0	0	0	0.15 and 0.16	NA (38), Hypothetical protein
D1	A_44_P871196	1	1	0	0	0	0	0.15 and 0.16	NA (40), BRAFLDRAFT_277232
D1	A_44_P996952	1	1	0	0	0	0	0.15 and 0.18	Family with sequence similarity 113, member B (Fam113b)
D1	A_42_P807866	1	1	0	0	0	0	0.16 and 0.16	Reversion induced LIM gene (Ril)

Table IV. Continued.

Gr	Probe ID	6H	6S	6M	9H	9S	9M	Fold-change	Gene or protein name (symbol)
D1	A_42_P804387	1	1	0	0	0	0	0.16 and 0.22	Ly6/Plaur domain containing 3 (Lypd3)
D1	A_44_P730235	1	1	0	0	0	0	0.17 and 0.18	Tubulin tyrosine ligase-like family 9 (Ttll9)
D1	A_44_P932360	1	1	0	0	0	0	0.17 and 0.22	N-acetyltransferase 13 (Nat13)
D1	A_43_P12888	1	1	0	0	0	0	0.17 and 0.25	Platelet-activating factor acetylhydrolase 1b 3 (Pafah1b3)
D1	A_44_P925674	2	2	0	0	0	0	0.18 and 0.22	NA (34), LOC100148890
D1	A_44_P358492	1	1	0	0	0	0	0.18 and 0.23	Farnesyl diphosphate farnesyl transferase 1 (Fdft1)
D1	A_42_P719350	1	1	0	0	0	0	0.18 and 0.23	Glutamine rich 2 (Qrich2) (RGD1562974)
D1	A_44_P188275	1	1	0	0	0	0	0.19 and 0.20	Sperm associated antigen 4 (Spag4)
D1	A_44_P368153	1	1	0	0	0	0	0.19 and 0.24	Transglutaminase 3, E polypeptide (Tgm3)
D1	A_44_P1033758	1	1	0	0	0	0	0.20 and 0.22	WD repeat domain 36 (Wdr36)
D1	A_44_P125681	1	1	0	0	0	0	0.20 and 0.23	DNA (cytosine-5-)-methyltransferase 1 (Dnmt1)
D1	A_42_P621628	1	1	0	0	0	0	0.20 and 0.23	Family with sequence similarity 63, member A (Fam63a)
D1	A_44_P135657	1	1	0	0	0	0	0.20 and 0.23	Coiled-coil domain containing 113 (Ccdc113)
D1	A_44_P456599	1	1	0	0	0	0	0.20 and 0.24	NA (40), TVAG_417060
D1	A_44_P929214	1	1	0	0	0	0	0.22 and 0.23	Notch1-induced protein (LOC493574)
D1	A_43_P12828	1	1	0	0	0	0	0.22 and 0.23	Tektin 1 (Tekt1)
D1	A_42_P587156	1	1	0	0	0	0	0.22 and 0.23	NIMA (never in mitosis gene a)-related expressed kinase 1 (Nek1)
D1	A_44_P260348	1	1	0	0	0	0	0.22 and 0.24	NFA, hypothetical protein (LOC679115)
D1	A_43_P11723	1	1	0	0	0	0	0.22 and 0.25	Guanylate cyclase activator 2a (guanylin) (Guca2a)
D1	A_44_P536832	1	1	0	0	0	0	0.23 and 0.23	DENN/MADD domain containing 2A (Dennd2a)
D1	A_44_P829047	1	1	0	0	0	0	0.23 and 0.25	Glucocorticoid modulatory element binding protein 1 (Gmeb1)
D1	A_44_P534844	1	1	0	0	0	0	0.24 and 0.25	NA (38), LOC100040084
D1	A_44_P524922	1	0	1	1	1	1	0.10-0.23	Malic enzyme 1, NADP(+)-dependent, cytosolic (Me1)
D1	A_44_P395421	1	0	1	0	1	0	0.13-0.21	NA (40), GL27102
D1	A_44_P668980	1	0	1	0	0	0	0.01 and 0.15	Gene model 1614 (Gm1614)
D1	A_44_P388673	2	0	2	0	0	0	0.02 and 0.20	Haptoglobin (Hp)
D1	A_44_P464534	1	0	1	0	0	0	0.08 and 0.10	*4, Insulin-like growth factor 1 receptor (Igf1r)
D1	A_42_P602822	1	0	1	0	0	0	0.08 and 0.22	Ret proto-oncogene (Ret)
D1	A_44_P529581	1	0	1	0	0	0	0.09 and 0.23	Oxidative stress induced growth inhibitor 1 (Osgin1)
D1	A_44_P510826	1	0	1	0	0	0	0.12 and 0.25	Syntaxin 11 (Stx11)
D1	A_44_P920649	1	0	1	0	0	0	0.13 and 0.20	NA (38), RALGPS2
D1	A_44_P1037456	1	0	1	0	0	0	0.14 and 0.21	Procollagen, type XXII, alpha 1 (Col22a1)
D1	A_43_P15051	1	0	1	0	0	0	0.14 and 0.24	NA (40), Tag-115
D1	A_44_P794334	1	0	1	0	0	0	0.15 and 0.23	NA (36), An07g02430
D1	A_44_P105034	1	0	1	0	0	0	0.15 and 0.24	Arachidonate 5-lipoxygenase (Alox5)
D1	A_44_P328796	1	0	1	0	0	0	0.16 and 0.17	Sarcalumenin (Srl)
D1	A_44_P212575	1	0	1	0	0	0	0.16 and 0.21	Butyrophilin-like 7 (Btnl7)
D1	A_42_P693821	1	0	1	0	0	0	0.19 and 0.23	Fos-like antigen 1 (Fosl1)
D1	A_44_P592941	1	0	1	0	0	0	0.21 and 0.22	NA (38), GG12284
D1	A_44_P273291	1	0	0	2	0	1	0.07-0.23	Zinc finger protein 148 (Zfp148)
D1	A_44_P299167	1	0	0	0	1	0	0.13 and 0.18	UDP-glucuronate decarboxylase 1 (Uxs1)
D2	A_44_P849944	0	2	2	1	1	1	0.15-0.21	Protease, serine, 35 (Prss35)
D2	A_44_P793901	0	1	1	0	0	1	0.14-0.17	NA (36), GSPATT00022597001
D2	A_43_P21610	0	1	1	0	0	0	0.03 and 0.03	Serine (or cysteine) peptidase inhibitor, clade C 1 (Serpinc1)
D2	A_44_P541548	0	1	1	0	0	0	0.11 and 0.16	*5, Angiotensin II receptor- associated protein (Agtrap)
D2	A_44_P185703	0	1	1	0	0	0	0.13 and 0.13	Iron-sulfur cluster assembly 1 homolog (Isca1)
D2	A_43_P15517	0	1	1	0	0	0	0.15 and 0.19	Jun D proto-oncogene (Jund)
D2	A_44_P716655	0	1	1	0	0	0	0.17 and 0.19	NFA, RIKEN cDNA 1700012B09 (RGD1561795)
D2	A_44_P337441	0	1	1	0	0	0	0.23 and 0.23	Lipase, endothelial (Lipg)
D2	A_44_P813198	0	1	0	1	1	0	0.11-0.23	NA (38), GD19242
D3	A_44_P306008	0	0	1	1	1	0	0.07-0.25	EP300 interacting inhibitor of differentiation 1 (Eid1)
D4	A_44_P279419	0	0	0	1	1	1	0.10-0.25	Calcium channel, voltage-dependent, a2/d1 (Cacna2d1)
D4	A_44_P503215	0	0	0	1	1	1	0.11-0.12	CD36 (RGD1562323), fatty acid translocase
D4	A_44_P348527	0	0	0	1	1	1	0.12-0.23	Pumilio 1 (Pum1)
D4	A_44_P827889	0	0	0	1	1	1	0.14-0.23	misc_RNA (CNOT6L)
D4	A_44_P153063	0	0	0	1	1	1	0.17-0.21	Peptidylglycine α-amidating monooxygenase (Pam)
D4	A_44_P202450	0	0	0	1	1	1	0.18-0.23	NFA, C50H11.1 (LOC498962)
D4	A_44_P147751	0	0	0	1	1	1	0.22-0.24	Ring finger protein 4 (Rnf4)

Table IV. Continued.

Gr	Probe ID	6H	6S	6M	9H	9S	9M	Fold-change	Gene or protein name (symbol)
D4	A_44_P440082	0	0	0	1	1	0	0.14 and 0.15	Stearoyl-CoA desaturase (delta-9-desaturase) (Scd)
D4	A_44_P360664	0	0	0	1	1	0	0.17 and 0.17	Prolactin receptor (Prlr)
D4	A_44_P165151	0	0	0	1	1	0	0.20 and 0.23	Platelet-activating factor acetylhydrolase, 1b, 1 (Pafah1b1)
D4	A_44_P397657	0	0	0	1	1	0	0.20 and 0.23	Apolipoprotein C-IV (Apoc4)
D4	A_44_P242773	0	0	0	1	1	0	0.21 and 0.21	RAB10, member RAS oncogene family (Rab10)
D4	A_44_P242664	0	0	0	1	1	0	0.21 and 0.22	Proteasome subunit, β type 3 (Psmb3)
D4	A_44_P233399	0	0	0	1	1	0	0.21 and 0.22	Niemann-Pick disease type C2 (Npc2)
D4	A_44_P298709	0	0	0	1	1	0	0.22 and 0.22	Coagulation factor II receptor (F2r)
D4	A_44_P364929	0	0	0	1	1	0	0.22 and 0.22	ADP-ribosylation factor-like 6 interacting protein 1 (Arl6ip1)
D4	A_44_P329712	0	0	0	1	1	0	0.22 and 0.23	NA (40), BBOV_IV009770
D4	A_44_P340805	0	0	0	1	0	1	0.12 and 0.16	NA (40), 2010011I20Rik
D5	A_44_P326584	0	0	0	0	1	1	0.15 and 0.22	Complement component 7 (C7)
D5	A_44_P353041	0	0	0	0	1	1	0.21 and 0.24	NA (36), OSTLU_52010

Gr, down-regulated D1, D2, D3, D4 and D5 groups; Numbers 0, 1, 2 and 3 indicate the number of probes showing positive results. Fold-change, <1/4-fold down-regulation in at least two different experiments. Other symbols or abbreviations are as described in Tables II and III.

Discussion

General considerations. Analysis of the relationship between the gene expression profiles of the three SHR substrains at 6 and 9 weeks of age yielded strong evidence of a heritable influence on gene expression in these substrains (Table I). The number of probes corresponding to up-regulated genes at 6 weeks of age clearly increased from SHR via SHRSP to M-SHRSP; this order reflects the genesis of the three SHR substrains (Table I) (3,4,19). By contrast, the number of probes corresponding to the down-regulated genes in SHR at 6 weeks of age was as high as 4,407, and was markedly decreased to 149 in SHRSP and to 74 in M-SHRSP (Table I). These results suggest that the mutations introduced into SHRSP and M-SHRSP effectively inhibited the down-regulation of genes in these SHR, and promoted hypertension in SHRSP and M-SHRSP. Some of the genes down-regulated in SHR, but not in SHRSP and M-SHRSP, at 6 weeks of age may play roles in alleviating the stroke-prone and/or malignant symptoms observed in SHRSP and M-SHRSP. An analysis of the genes related to these symptoms will be undertaken in our future studies.

Candidate genes involved in the genesis of hypertension among the up-regulated genes. The U1 and U2 groups are expected to include the most probable candidate genes involved in hypertension pathogenesis. They consist of 20 annotated and 8 non-annotated genes. Among the annotated genes, at least 4 candidate genes were identified (Table III, *1 to *4): (i) *Spock2* was not only up-regulated in all SHR substrains at 6 and 9 weeks of age, but is also reported to be an effector of angiotensin II signaling, which plays a pivotal role in BP control. It is thus one of the most probable candidate genes involved in the pathogenesis of hypertension (10). (ii) *Kynu* is reported to be among the candidate genes that contribute to hypertension in SHR, since the injection of kynurenic acid into the rostral ventrolateral medulla of SHR has been shown to decrease arterial BP (11). Increased expression of *Kynu* is thought to decrease kynurenine levels and increase arterial BP. (iii) *Rgs2* is up-regulated in the adrenal glands of SHRSP and M-SHRSP at 6 weeks of age (Table III), and has been reported to mediate vascular smooth muscle relaxation and BP (12). Notably, *Rgs2*$^{-/-}$ mice develop marked hypertension, and their blood vessels show enhanced contraction (12). (iv) *Gja1*, called connexin43 (*Cx43*) in humans, is involved in the control of cell-to-cell communication and is thought to modulate the contractility of the vascular wall and the electrical coupling of cardiomyocytes (13).

Other than these 4 genes, several annotated genes, such as nuclear receptor subfamily 4 (*Nr4a3*), FBJ osteosarcoma oncogene homolog (*c-Fos*) and jun B proto-oncogene (*Junb*) (Table III, U2 and U3) are known to be up-regulated by angiotensin II and affect BP through signal transduction and cellular growth (20). We presume that the candidate genes are not included in the U3, U4 and U5 groups. However, these groups do apparently include genes related to BP control, such as cysteine-rich angiogenic inducer 61 (*Cyr61*), purinergic receptor P2Y G-protein coupled 2 (*P2ry2*), *Junb*, C-type natriuretic peptide precursor (*Cnp*) and transcriptional regulating factor 1 (*Trerf1*) (21-25). Our interpretation is that though these genes are not the primary candidate genes, their expression is affected by the candidate genes, thus contributing to deteriorating hypertension.

Candidate genes involved in the genesis of hypertension among the down-regulated genes. The D1 and D2 groups are expected to include the most probable candidate genes involved in hypertension pathogenesis. They consist of 77 annotated and 30 non-annotated genes (Table IV). Among the annotated genes, at least 5 candidate genes were identified (Table IV, *1 to *5): (i) *Uts2* encodes a peptide ligand that acts on the G protein-coupled urotensin receptor and elicits long-term vasoconstriction (14). Human urotensin II promotes hypertension and atherosclerotic cardiovascular diseases (26). Plasma urotensin II levels are elevated in patients with vascular endothelial dysfunction-related diseases such as

essential hypertension, diabetes mellitus, atherosclerosis, ischemic heart disease and heart failure (26). (ii) *Ephx2* leads males homozygous for a targeted null mutation to display a significant reduction in BP, both in the presence and absence of dietary salt loading (15). Monti *et al* (27) identified *Ephx2* as a heart failure susceptibility gene in SHHF rats derived from SHR Koletsky rats. (iii) *Apln* and its receptor share similarities in structure and anatomical distribution with angiotensin II and the angiotensin AT1 receptor, providing clues regarding the physiological functions of this novel signal-transduction system (16). It has now been established that the Apln system plays a role in lowering BP, as a potent cardiac inotrope, in modulating pituitary hormone release and food and water intake, in stress activation, and as a novel adipokine that is excreted from fat cells and regulates insulin. Given its broad array of physiological roles, *Apln* has attracted much interest as a target for novel therapeutic research and drug design (15) (Table IV, D1). (iv) *Igf1r* is known to cause direct vasodilation via a nitric oxide-dependent pathway (17,28). (v) *Agtrap* encodes a transmembrane protein and is a modulator of angiotensin II signaling (18). Overexpression of the novel *Agtrap* induces cellular hypertrophy in cultured rat vascular smooth muscle and renal proximal tubular cells.

Other than these 5 genes, several genes listed in the D1 and D2 groups (Table IV) are known to affect BP. For example, haptoglobin (*Hp*) is associated with increased BP (29), arachidonate 5-lipoxygenase (*Alox5*) plays a critical role in the progression of pulmonary hypertension in rats (30), and endothelial lipase (*Lipg*) probably has a role in the pathophysiology of vascular diseases (31). The Niemann-Pick disease type C2 (*Npc2*), coagulation factor II receptor (*F2r*) and complement component 7 (*C7*) genes reportedly participate in BP control (32-34). However, since they are classified into the D4 and D5 groups, we presume that they are not the primary candidate genes.

Also of note, in the D1 group, the DEAD/H box polypeptide 11 (*Ddx11*) and ER-Golgi intermediate compartment protein 1 (*Ergic1*) genes (35,36) showed down-regulation among all SHR substrains at 6 and 9 weeks of age (Table IV, D1). These genes are not currently directly associated with hypertension. However, their expression profiles suggest their participation in the genesis of hypertension in the SHR substrains.

Other possible candidate genes for hypertension in SHRs. Although more detailed analyses are necessary, it is anticipated that further investigation of the non-annotated genes will provide insights into their specific roles in the etiology of hypertension. The U1 and U2 groups included 8 functionally non-annotated genes, and the D1 and D2 groups 30 functionally non-annotated genes. Their expression profiles suggest that they encode unknown proteins with functions related to BP control.

Although current gene expression arrays permit the simultaneous analysis of thousands of rat genes, this method is not yet capable of addressing all functional genes within the genome. However, as rat genome annotation progresses and arrays continue to improve in their extent of genomic coverage, a more complete analysis should become possible. Our current approach identified at least 9 genes, including *Kynu* (10) and *Ephx2* (26), as the most probable candidate genes causing hypertension in SHR substrains. *Kynu* and *Ephx2* have previously been demonstrated to be responsible for hypertension in SHR; however, we believe that the remaining genes also participate in the genesis of hypertension in SHR substrains.

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