Original Article

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A new approach to identifying hypertension-associated genes in the mesenteric artery of spontaneously hypertensive rats and stroke-prone spontaneously hypertensive rats

Takashi Ikawa[a], Yuko Watanabe[a], Daisuke Okuzaki[b], Naohisa Goto[b,c], Nobutaka Okamura[d], Kyosuke Yamanishi[d,e], Toshihide Higashino[f], Hiromichi Yamanishi[a], Haruki Okamura[d], and Hideaki Higashino[a]

Objective: Hypertension is one of the most prevalent diseases in humans who live a modern lifestyle. Alongside more effective care, clarification of the genetic background of hypertension is urgently required. Gene expression in mesenteric resistance arteries of spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP) and two types of renal hypertensive Wistar Kyoto rats (WKY), two kidneys and one clip renal hypertensive rat (2K1C) and one kidney and one clip renal hypertensive rat (1K1C), was compared using DNA microarrays.

Methods: We used a simultaneous equation and comparative selection method to identify genes associated with hypertension using the Reactome analysis tool and GenBank database.

Results: The expression of 298 genes was altered between SHR and WKY (44 upregulated and 254 downregulated), while the expression of 290 genes was altered between SHRSP and WKY (83 upregulated and 207 downregulated). For SHRSP versus SHR, the expression of 60 genes was altered (36 upregulated and 24 downregulated). Several genes expressed in SHR and SHRSP were also expressed in the renovascular hypertensive 2K1C and 1K1C rats, indicative of the existence of hyper-renin and/or hypervolemic pathophysiological changes in SHR and SHRSP.

Conclusion: The overexpression of *Kcnq1*, *Crlf1*, *Alb* and *Xirp1* and the inhibition of *Galr2*, *Kcnh1*, *Ache*, *Chrm2* and *Slc5a7* expression may indicate that a relationship exists between these genes and the cause and/or worsening of hypertension in SHR and SHRSP.

Keywords: DNA microarray, gene expression profile, hypertension, mesenteric artery, spontaneously hypertensive rat, stroke-prone spontaneously hypertensive rat, two kidneys and one clip renal hypertensive rat, one kidney and one clip renal hypertensive rat

Abbreviations: 1K1C, 1-kidney, one-clip renovascular hypertensive rat; 2K1C, 2-kidney, one-clip renovascular hypertensive rats; SHR, spontaneously hypertensive rat; SHRSP, stroke-prone SHR; WKY, normotensive Wistar Kyoto rat; SNP, single nucleotide polymorphism

INTRODUCTION

Hypertension is one of the most prevalent diseases in humans who live a modern lifestyle. It is a silent disease and does not markedly affect quality of life when moderate. When left untreated, however, it leads to life-threatening diseases associated with atherosclerosis, including myocardial infarction, renal failure and stroke [1–3]. In Japan, where general medical care should be sufficient to treat hypertension, more than 300 000 patients die each year from diseases related to hypertension [4,5]. Alongside more effective care, clarification of the genetic background of hypertension is urgently required.

A number of studies have investigated the genetic background of hypertension to date. The polygenic nature of essential hypertension in humans presents a challenge to the identification of genes involved in the genesis of the disease. DNA microarrays are a potentially powerful tool for investigating the genetics of hypertension, as they facilitate the measurement of the expression of thousands of genes

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[a]Hirakata General Hospital for Developmental Disorders, Hirakata, [b]Genome Information Research Center, [c]Department of Infection Metagenomics, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, [d]Institute for Advanced Medical Sciences, [e]Department of Neuropsychiatry, Hyogo College of Medicine, Nishinomiya, Hyogo and [f]Graduate School of Information Science and Technology, Hokkaido University, Sapporo, Hokkaido, Japan

Correspondence to Hiromichi Yamanishi, MD, PhD, Chancellor of Hirakata General Hospital for Developmental Disorders, 2–1–1 Tsuda-higashi, Hirakata, Osaka 573–0122, Japan; Tel: +81-72-858-0373; fax: +81-72-858-9321; e-mail: hirochan@hirakataryoiku-med.or.jp

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simultaneously [6–8]. Inbred homozygous rodent models of human essential hypertension are ideal for microarray research, and animal models of essential hypertension such as spontaneously hypertensive rats (SHR) [9] and stroke-prone SHR (SHRSP) [10] have been studied using microarrays [11]. Until now, most studies have been performed using tissues such as kidney [12–14], liver [15], adrenal gland [16,17] and brain [18], which are relatively easy to isolate for mRNA extraction. However, constituents of mRNAs expressed in each tissue depend on the biochemical and physiological roles of the target organ in the body and the stage of the disease. Therefore, the importance of analysing mRNA from the tissues responsible for hypertension is clear, despite the tissues causing or associated with hypertension having not yet been fully elucidated. Resistance arteries [19], including the mesenteric and femoral arteries, appear to be the most relevant tissues for analysing the cause of hypertension. For these reasons, we selected the mesenteric artery for analysing mRNA in hereditary hypertensive models of rats.

In this study, we used DNA microarray methodology in two hypertensive substrains of rats, SHR and SHRSP, together with normotensive Wistar Kyoto (WKY) rats [20] and two models renovascular hypertensive rats, two kidneys and one clip (2K1C) [21] and one kidney and one clip (1K1C) [22], surgically established from WKY, to identify genes differentially expressed between hereditary hypertension and artificially induced renal hypertension.

We also implemented a novel approach to identifying candidate genes in this study using simultaneous equations and comparative selection to identify commonalities in overlapping genes. The comparison of differences in gene expression between two groups, for example SHR and WKY, is relatively straightforward, but comparisons across three or more groups can present a challenge, as shown in Fig. 1a,b. In this study, we sought to select differentially expressed using our novel approach, that is more group selective choosing with simultaneous equations and a comparative selection method.

MATERIALS AND METHODS

Animals

Three-week-old male SHR/Izm [9], SHRSP/Izm [10] and WKY/Izm [20] rats were purchased from SLC Co. (Shizuoka, Japan) under license of the Disease Model Cooperative Research Association (Kyoto, Japan) and maintained for 3 weeks in our animal facility. Two surgical models of renovascular hypertension were established in WKY rats, two kidneys and one clip (2K1C), and one kidney and one clip (1K1C) renovascular hypertensive rats [21,22], and the models were maintained for a further 3 weeks until the animals reached 6 weeks of age. All rats were provided with a standard rodent chow diet and drinking water ad libitum. Each rat group (WKY, 2K1C, 1K1C, SHR and SHRSP) contained eight animals. All animals used in this study were handled with due care according to the guidelines established by the Japanese Association for Laboratory Animal Science, which complies with international rules and policies. This study was performed under approval (HAME-13–071 issued on 24 March 2014) of the Animal Care and Use Committee of Hyogo College of Medicine.

Establishment of rat models of renovascular hypertension

WKY rats aged 3 weeks were anaesthetized by isoflurane inhalation (1–2% in air) using a vaporizer. The right kidney exposed through a small flank incision in the abdominal skin wall was carefully secured using an ophthalmic chalazion forceps. The renal artery was exposed over a short segment by blunt dissection, and a silver plate clip of $0.18\,\text{mm} \times 2\,\text{mm} \times 6\,\text{mm}$ was placed on the right renal artery. To establish the 1K1C renovascular hypertensive model, two ligatures were passed around the left renal vascular pedicle and ureter and tied. The left kidney was removed, with the adrenal gland preserved. To establish the 2K1C renal hypertension model, this process was omitted.

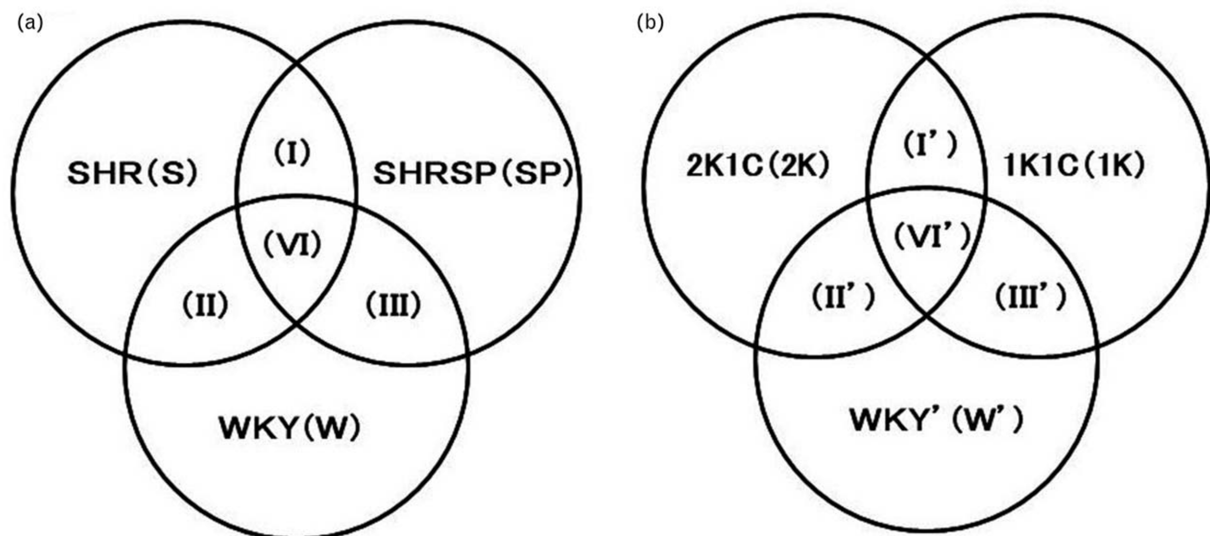

FIGURE 1 (a, b) Overview of the method for comparing differentially expressed genes among three groups. Left scheme (a): Comparison between SHR, SHRSP and WKY. (i) SHR:WKY→(S)+(W)+(I)+(III), (ii) SHRSP:WKY→(SP)+(W)+(I)+(II), (iii) SHR:SHRSP→(S)+(SP)+(II)+(III). Right scheme (b): Comparison between 2K1C, 1K1C, and WKY. (iv) 2K1C:WKY→(2K)+(W')+(I')+(III'), (v) 1K1C:WKY→(1K)+(W')+(I')+(II'). (vi) 2K1C:1K1C→(2K)+(1K)+(II')+(III'). 1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

The muscle layer was sutured shut, and the skin incision was closed using surgical staples. A sham procedure, which included the entire surgery but with arterial clipping omitted, was performed in control rats. All rats were maintained in the animal facility for 3 weeks after surgery, during which body weight and blood pressure were measured every week.

SBP measurement

SBP was measured by the tail-cuff method using an UR-5000 instrument (Ueda, Tokyo, Japan). Briefly, three consecutive SBP readings were taken between 0900 and 1100 h after warming the body at 35°C for 5 min in a heater box. SBP values were expressed as the mean ± SEM.

Tissue processing and RNA isolation

The tissues of mesenteric arteries were harvested from each rat body under sodium pentobarbital anaesthesia [50 mg/kg intraperitoneal (i.p.)] at 6 weeks of age. Fat and connective tissues were quickly removed from the samples using forceps and scissors, and the isolated arteries were stored in RNAlater stabilization solution (Qiagen GmbH, Hilden, Germany) at −20°C until use. Two days after harvesting, two to three pieces of arterial tissue were cut to 2–3 mm in size, homogenized using a BioMasher (Takara Bio Inc., Shiga Prefecture, Japan) with 5 mm diameter glass beads and a Qiagen TissueLyser (Qiagen GmbH). Total RNA was extracted with a miRNeasy Mini kit (Qiagen) according to the manufacturer's protocol. RNA quality was evaluated using a RNA Nano Chip and Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbornn, Germany), and RNA of sufficient quality was used for microarray experiments. Tissue samples taken from four of the eight rats per group were used in the microarray analyses.

DNA microarray analysis of gene expression profiles

To examine the gene expression profiles of rat mesenteric arteries, cRNA labelled with cyanine 3-CTP (PerkinElmer, Boston, Massachusetts, USA) was synthesized from 100 ng of total RNA using a Low RNA Input Quick Amp Labeling kit (Agilent Technologies) and hybridized by incubating with a SurePrint G3 Rat GE 8x60K Microarray (Agilent Technologies) in a rotor oven (Agilent Technologies) for 17 h at 65°C, followed by washing with Gene Wash Buffer 1 and 2 (Agilent Technologies). The hybridized slides were scanned with an Agilent G2505C Scanner 4000 (Agilent Technologies). The expression data were extracted, and the overall raw signal intensities on each array were normalized to the median value of all rat probes using Feature Extraction software ver. 10.7.3.1. (Agilent Technologies). For comparisons among rat strains and renovascular hypertensive models, changes in gene expression were defined as those with signal ratios increased or decreased by 4.0-fold or more. Raw data from these experiments have been submitted to the Gene Expression Omnibus (GEO, accession no. GSE74288).

To investigate gene expression between SHR, SHRSP and renovascular hypertensive rat models, genes with two-fold or more increased expression or 0.5-fold or less decreased expression in 1K1C/WKY or 2K1C/WKY were evaluated, with or without statistical significance in the measurement errors of each sample group for taking a precise information regarding the relationship between SHRs and renovascular hypertensive rats.

Annotation of differentially expressed genes

A BLASTN search of the NCBI RefSeq database was performed, employing corresponding 60-nucleotide probes (NCBI, GEO accession: GPL15084) to identify homologous genes with functional annotations [23]. Annotated gene and protein names are shown in italics and regular font, respectively.

Novel strategies to survey candidate genes related to hypertension

A novel analysis method was adopted to identify candidate genes related to or causing hypertension, as not all genes identified in the microarray experiments were associated with hypertension. As shown in Fig. 1a, in a comparison of genes differentially expressed between SHR, SHRSP and WKY, expressed genes were grouped as SHR only (S), SHRSP only (SP), WKY only (W), both SHR and SHRSP (I), both SHR and WKY (II), both SHRSP and WKY (III), and both SHR, SHRSP and WKY (VI). Therefore, (S)+(W)+(I)+(III) groups were included in the comparison of SHR and WKY, (SP)+(W)+(I)+(II) were included in the comparison of SHRSP and WKY, and (S)+(SP)+(II)+(III) were included in the comparison of SHR and SHRSP. The genes in each group were identified using simultaneous equations and a comparative selection method for genes that were common or not between the groups. Similar comparisons were performed between WKY, 2K1C and 1K1C as shown in Fig. 1b.

Functional analysis of gene expression

Among the candidate genes identified in the previous section, there remained genes related to processes other than hypertension. Therefore, the candidate genes were further analysed using a function of the Reactome pathway database to identify genes expressed in biochemical pathways using the Reactome tool [24], a free and open-source database (http://www.reactome.org/) offered on the website of the Cold Spring Harbor Laboratory, The European Bioinformatics Institute and The Gene Ontology Consortium. In the Reactome analysis tool, relationships between each gene and function in the biochemical map agreed at present were investigated. Identified genes were further analysed for functions closely related to and overlapped with signal transduction, muscle contraction, neuronal system, transmembrane small molecules, haemostasis, gene expression, metabolism and developmental biology using a Reactome analysis, while those not recognized by the Reactome database were omitted. Gene biochemical information were mainly obtained from the GenBank database (the NIH genetic sequence database, https://ncbi.nlm.nih.gov).

Validation of mRNA identification by RT-qPCR

To validate the results obtained from the microarray analysis, the six genes most likely to be closely related to

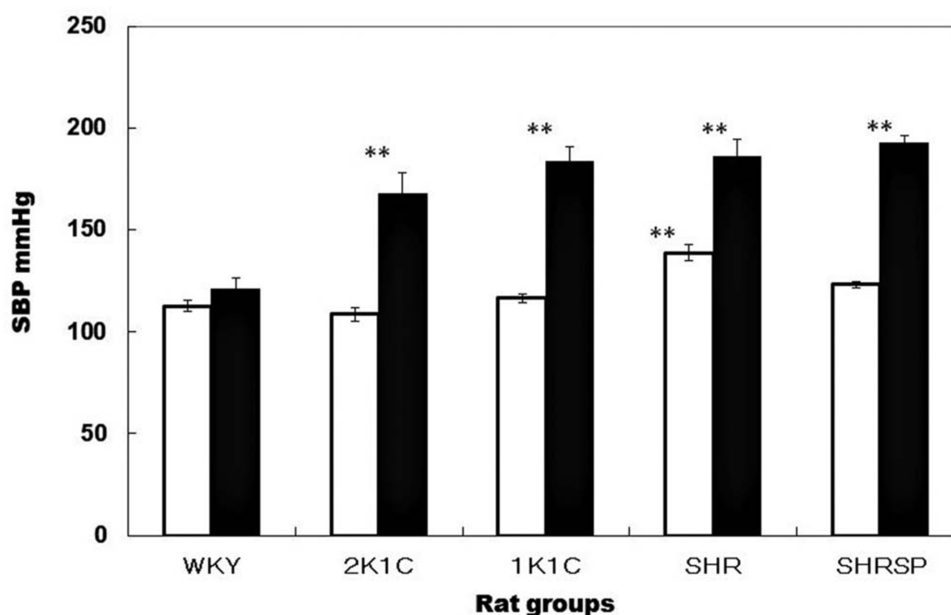

FIGURE 2 SBP in five experimental rat groups at 3 (□) and 6 (■) weeks of ages. Significant differences were observed between 3 and 6 weeks of ages in each group at $^{**}P<0.01$, respectively. **attached on the left shoulder of 3-week-old SHR column showed significantly higher blood pressure than the other groups at 3 weeks of age. 1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

hypertension were selected from the 43 identified genes, which consisted of 13 enhanced and 30 inhibited genes, and RT-qPCR was performed under three different experimental conditions using PrimePCR Probe Assay Kits (Bio-Rad Laboratories, Inc., Hercules, California, USA) for *Alb*, *Chrm2*, *Crlf1*, *Kcnh1* and *Kcnq1*, using *Gapdh* as a reference gene according to the manufacturer's instructions. For *Ache*, RT-qPCR was performed using a TaqMan Assay Kit (Thermo Fisher Scientific Instruments LLC., Waltham, Massachusetts, USA), as no cDNA was detected when a PrimePCR Probe Assay Kit for *Ache* was used.

Statistical analyses

All statistical analyses were performed using SPSS v.22.0J software (IBM Japan Inc., Tokyo, Japan). Comparisons between the means of the data in each group were performed using one-way analysis of variance (ANOVA) [25] and Games–Howell's multiple comparisons test. Comparisons of mean SBPs in each rat group between animals at 3 weeks of age with those at 6 weeks of age were performed using a *t*-test [26]. Differences were considered significant at P value less than 0.05 and P value less than 0.01 for BP measurements and DNA array measurements, respectively. Statistical comparisons between microarray and RT-qPCR data were performed using Spearman's rank correlation test [27].

RESULTS

Blood pressure values at 3 and 6 weeks of ages in the five rat groups

SBPs were measured in the WKY, SHR and SHRSP strains and the 2K1C and 1K1C models every week from 3 to 6 weeks of age (Fig. 2). At 3 weeks of age, SBP levels in SHR rats were already significantly higher than in WKY rats and other groups. At 6 weeks of age, SBP levels in 2K1C, 1K1C, SHR and SHRSP rats were significantly increased compared with the same groups at 3 weeks of age. When the differences in SBP among hypertensive animals at 6 weeks of age were compared, SBP values of the 1K1C, SHR and SHRSP groups tended to be higher than those of the 2K1C group.

Genes differentially expressed in spontaneously hypertensive, stroke-prone spontaneously hypertensive, two kidneys and one clip renovascular hypertensive and one kidney and one clip renal hypertensive rats compared with Wistar Kyoto rats

The numbers of genes with significantly different expression in the mesenteric arteries of SHR and SHRSP rats compared with WKY rats were as follows: 298 genes (44 upregulated, 254 downregulated) were identified between SHR and WKY; 290 genes (83 upregulated, 207 downregulated) were identified between SHRSP and WKY; and 60 genes (36 upregulated, 24 downregulated) were identified between SHRSP and SHR. Given the high numbers of differentially expressed genes, a new method was used to identify genes related to hypertension using simultaneous equations and comparative selection between common and noncommon genes in the conditional groups. When this approach was implemented in addition to the Reactome analysis tool, as shown in Fig. 1a, the (S) area was 3; the (SP) area was 14; the (W) area was 19; the (I) area, comprising genes common to SHR and SHRSP, was 43; of the (II) area, comprising genes common to SHR and WKY, was 23; the (III) area, comprising genes common to SHRSP and WKY, was 30; and the (IV) area, comprising genes common to SHR, SHRSP and WKY, was 69 genes.

TABLE 1. Upregulated genes associated with the occurrence and/or maintenance of hypertension in the mesenteric arteries of spontaneously hypertensive rats and stroke-prone spontaneously hypertensive rats compared with Wistar Kyoto rats, an area of (I), at 6 weeks of age

Gene symbol	Protein name	Fold changes		Remarks
		SHR/WKY	SHRSP/WKY	
1. *Inhba*	inhibin, beta A	4.447	4.092	(#2)
2. *Alb*	albumin	4.591	5.319	(#1)
3. *Adtrp*	androgen-dependent TFPI-regulating protein	4.717	5.618	
4. *Scube3*	signal peptide, CUB domain, EGF-like 3	4.787	5.111	(#2)
5. *Kcnq1*	potassium voltage-gated channel, KQT-like subfamily, member 1	4.913	4.540	#2
6. *Crtf1*	cytokine receptor-like factor 1	6.258	5.664	(#2)
7. *Serpine1*	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	7.116	6.723	
8. *Atf3*	activating transcription factor 3	7.845	8.272	(#2)
9. *Timd4*	T-cell immunoglobulin and mucin domain containing 4	7.888	6.723	(#2)
10. *Xirp1*	xin actin-binding repeat-containing protein 1-like	8.460	7.828	(#2)
11. *Hspa1b*	heat shock 70-kDa protein 1B	8.474	9.162	(#2)
12. *Abra*	actin-binding Rho activating protein	12.088	7.057	(#2)
13. *Cldn19*	claudin-19	22.979	21.647	(#2) (#1)

#2, and (#2) or (#1) in the Remarks column mean the gene enhanced by more than four times in 2K1C/WKY, and the genes enhanced by more than two times in 2K1C/WKY or 1K1C/WKY with or without statistical significance in the measurement errors of each sample group, respectively (refer to the data in Table 2).
1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

For the comparison of 2K1C with WKY and 1K1C with WKY, the numbers of genes with significantly different expression were as follows: 83 genes (36 upregulated, 47 downregulated) were identified between 2K1C and WKY; 224 genes (five upregulated and 219 downregulated) were identified between 1K1C and WKY; and 61 genes (33 upregulated and 28 downregulated) were identified between 2K1C and 1K1C. Following our novel method of analysis (Fig. 1b) in conjunction with the Reactome analysis tool, the (2K) area was 6; the (1K) area was 20, the (W') area was 19, and the (I') area, comprising genes common to 2K1C and 1K1C, was 9.

Significantly enhanced genes in the mesenteric arteries of spontaneously hypertensive rats or stroke-prone spontaneously hypertensive rats compared with Wistar Kyoto rats

Significantly enhanced genes in the mesenteric arteries of either SHR or SHRSP compared with WKY, an area of (I) in Fig. 1a, were identified as summarized in Table 1 and Fig. 3. The expression of 13 genes, *Inhba*, *Alb*, *Adtrp*, *Scube3*, *Kcnq1*, *Crlf1*, *Serpine1*, *Atf3*, *Timd4*, *Xirp1*, *Hspa1b*, *Abra* and *Cldn19*, was increased more than four-fold in both SHR and SHRSP compared with WKY.

Of note, the same genes as those identified in 2K1C versus WKY or 1K1C versus WKY were increased more than two-fold with or without statistical significance in the measurement errors of group. Those are noted in the remarks column of Tables 1 and 2. Ten of 13 genes were the same as those enhanced in (I) and 2K1C/WKY, and two were the same as those enhanced in (I) and 1K1C/WKY, with or without statistical significance as partly shown also in the heatmap of Fig. 3. Therefore, most of the genes with enhanced expression in area (I) were also present in 2K1C and/or 1K1C renovascular renal hypertensive rats.

Significantly inhibited genes in the mesenteric arteries of spontaneously hypertensive rats or stroke-prone spontaneously hypertensive rats compared with Wistar Kyoto rats

Significantly inhibited genes in the mesenteric arteries of either SHR or SHRSP compared with WKY, an area of (I) in Fig. 1a, were identified as summarized in Table 3 and Fig. 4. The expression of 30 genes, *Panx2*, *Pcsk2*, *RGD1559482*, *Ptgr1*, *RGD1562667*, *Wasf1*, *Galr2*, *Kcnh1*, *Sctr*, *Tacr1*, *Syt1*, *Ache*, *Syp*, *Acot5*, *Chrm2*, *Cacna1b*, *Slc5a7*, *MAST1*, *C4bpa*, *Dync1i1*, *P2rx2*, *Rgs9*, *Sult4a1*, *Htr3a*, *Rit2*, *Nefl*, *Vgf*, *Chrna3*, *Sst* and *Snap25*, in order of decreasing fold change, was downregulated in both SHR and SHRSP less than 0.25-fold compared with WKY. The same genes as those identified in 2K1C versus WKY or 1K1C versus WKY were downregulated less than 0.5-fold with or without statistical significance in the measurement errors of a group summarized in Table 4, as noted in the remarks column in Table 3. Twenty-seven of the 30 genes were the same as those inhibited less than 0.5-fold in the comparison of 2K1C/WKY, and 25 were the same as those inhibited less than 0.5-fold in 1K1C/WKY with or without statistical significance, as partly shown also in the heatmap of Fig. 4.

Significantly enhanced and inhibited genes in the mesenteric arteries of spontaneously hypertensive rats compared with Wistar Kyoto rats

Genes differentially expressed in the mesenteric arteries of SHR compared with WKY, an area of (S), were identified (Table 5). Two significant genes, *Lilrb3l* and *Fam216b*, were enhanced more than four-fold, and the *Vegfb* gene was inhibited less than 0.25-fold in SHR compared with WKY.

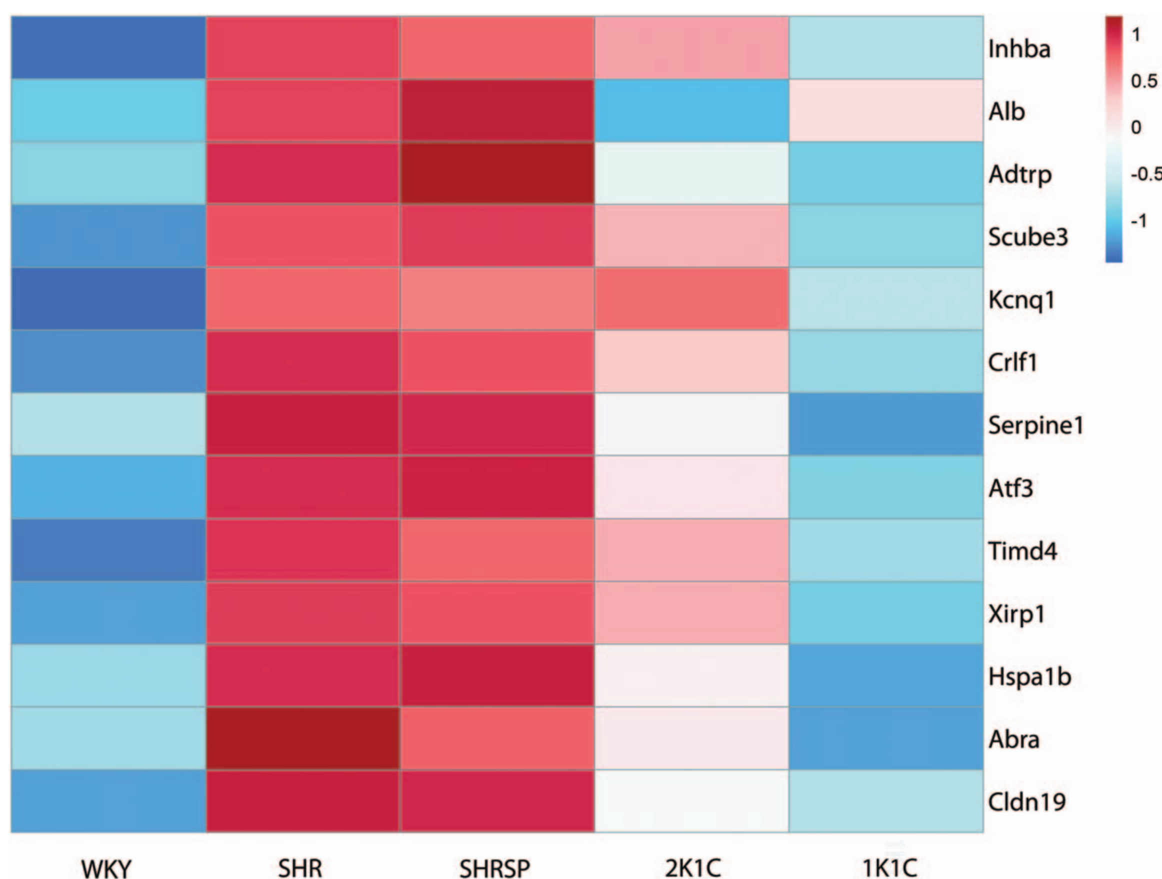

FIGURE 3 Heatmap regarding a relationship between significantly upregulated genes in SHR/WKY and SHRSP/WKY and 2K1C/WKY and 1K1C/WKY based on the data of Tables 1 and 2. Scale bar represents the degree of fold changes (FC) in gene expressions, and colour darkness represents the degree of similarity or nonsimilarity. SHR, spontaneously hypertensive rats; 1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

Significantly enhanced and inhibited genes in the mesenteric arteries of stroke-prone spontaneously hypertensive rats compared with Wistar Kyoto rats

Genes differentially expressed in the mesenteric arteries of SHRSP compared with WKY, an area of (SP), are summarized in Table 6. Seven significant genes, *Zfp451*, *Pcmtd1*, *Rps16*, *Rgs11*, *Sult1b1*, *Zfp597* and *RGD1563302*, were enhanced more than four-fold, and seven genes, *Csap1*, *Micb*, *Dlg5*, *Haghl*, *Samd14*, *Agtrap* and *Ephx2*, were inhibited less than 0.25-fold in SHRSP compared with WKY.

TABLE 2. Genes upregulated more than two-fold in 2K1C/ Wistar Kyoto rats or 1K1C/ Wistar Kyoto rats compared with significantly expressed genes in spontaneously hypertensive rats and stroke-prone spontaneously hypertensive rats

	2K1C/WKY			1K1C/WKY		
Gene symbol	Fold change	*P*	Remarks	Fold change	*P*	Remarks
Inhba	3.427	0.048	(#2)	1.618	0.458	
Alb	−1.108	0.812		2.414	0.012	(#1)
Adtrp	1.651	0.211		−1.068	0.527	
Scube3	3.500	0.019	(#2)	1.337	0.591	
Kcnq1	4.817	0.037	#2	1.798	0.451	
Crlf1	3.702	0.021	(#2)	1.486	0.483	
Serpine1	1.968	0.143		−1.919	0.488	
Atf3	3.163	0.136	(#2)	1.256	0.767	
Timd4	5.105	0.061	(#2)	1.731	0.599	
Xirp1	5.277	0.052	(#2)	1.300	0.788	
Hspa1b	2.469	0.172	(#2)	−1.634	0.504	
Abra	2.633	0.062	(#2)	−1.827	0.559	
Cldn19	4.344	0.163	(#2)	2.062	0.527	(#1)

#2 or #1, and (#2) or (#1) in the Remarks column indicates genes enhanced more than four-fold with statistical significance, and genes enhanced more than two-fold with or without statistical significance in each sample group in 1K1C/WKY or 2K1C/WKY (refer to the data of Table 1).
1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; FC, fold change in gene expression; *P*-value: *P*-values of measurement errors in a sample group; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

TABLE 3. Downregulated genes associated with the occurrence and/or maintenance of hypertension in the mesenteric arteries of spontaneously hypertensive rats and stroke-prone spontaneously hypertensive rats compared with Wistar Kyoto rats, an area of (I), at 6 weeks of age

Gene symbol	Protein name	Fold changes		Remarks
		SHR/WKY	SHRSP/WKY	
1. *Panx2*	pannexin 2	−4.510	−6.088	(#2) (#1)
2. *Pcsk2*	proprotein convertase subtilisin/kexin type 2	−4.793	−4.595	(#2) #1
3. *RGD1559482*	Similar to immunoglobulin superfamily, member 7	−5.183	−5.712	
4. *Ptgr1*	prostaglandin reductase 1	−5.230	−4.757	
5. *RGD1562667*	Similar to leukocyte mono-Ig-like receptor2	−5.458	−4.117	(#2)
6. *Wasf1*	WAS protein family, member 1	−6.515	−5.952	(#2) (#1)
7. *Galr2*	galanin receptor 2	−7.029	−6.422	(#2) (#1)
8. *Kcnh1*	potassium voltage gated channel, subfamily H (eag-related), member 1	−7.597	−8.214	(#2) #1
9. *Sctr*	secretin receptor	−9.174	−8.777	#2
10. *Tacr1*	tachykinin receptor 1	−9.287	−8.263	(#2) #1
11. *Syt1*	synaptotagmin I	−10.054	−8.950	(#2) #1
12. *Ache*	acetylcholinesterase	−10.676	−7.064	(#2) #1
13. *Syp*	synaptophysin	−13.768	−15.821	(#2) #1
14. *Acot5*	acyl-CoA thioesterase 5	−14.018	−8.514	(#2) (#1)
15. *Chrm2*	cholinergic receptor, muscarinic 2	−14.222	−13.445	(#2) #1
16. *Cacna1b*	Calcium channel, voltage-dependent, N type, alpha 1B subunit	−14.533	−13.016	(#2) #1
17. *Slc5a7*	Solute carrier family 5 (sodium/choline cotransporter), member 7	−16.209	−13.608	(#2) (#1)
18. *MAST1*	Microtubule-associated serine/threonine kinase 1	−16.845	−14.868	(#2) #1
19. *C4bpa*	Complement component 4 binding protein, alpha	−17.428	−10.686	
20. *Dync1i1*	dynein cytoplasmic 1 intermediate chain 1	−17.973	−12.475	(#2) #1
21. *P2rx2*	purinergic receptor P2X, ligand-gated ion channel, 2	−18.179	−14.688	#2 #1
22. *Rgs9*	Regulator of G-protein signalling 9	−20.957	−15.243	(#2) #1
23. *Sult4a1*	sulfotransferase family 4A, member 1	−25.120	−32.918	(#2) #1
24. *Htr3a*	5-hydroxytryptamine (serotonin) receptor 3A, ionotropic	−32.536	−24.132	(#2) #1
25. *Rit2*	Ras-like without CAAX 2	−34.014	−32.978	(#2) #1
26. *Nefl*	neurofilament, light polypeptide	−39.227	−27.010	(#2) #1
27. *Vgf*	VGF nerve growth factor inducible	−40.556	−34.402	(#2) #1
28. *Chrna3*	cholinergic receptor, nicotinic, alpha 3 (neuronal)	−47.824	−46.430	(#2) #1
29. *Sst*	somatostatin	−53.368	−72.883	(#2) #1
30. *Snap25*	synaptosomal-associated protein 25	−83.046	−66.857	(#2) #1

#2 or #1, and (#2) or (#1) in the Remarks column mean the genes inhibited by less than 0.25-fold in 2K1C/WKY or 1K1C/WKY, and the genes inhibited by less than 0.5-fold in 2K1C/WKY or 1K1C/WKY with or without statistical significance in the measurement errors of each sample group, respectively (refer to Table 4).
1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

Significantly enhanced and inhibited genes in the mesenteric arteries of two kidneys and one clip renovascular hypertensive and/or one kidney and one clip renal hypertensive renovascular hypertensive rats compared with Wistar Kyoto rats, two kidneys and one clip renovascular hypertensive renovascular hypertensive rats compared with Wistar Kyoto rats and one kidney and one clip renal hypertensive renovascular hypertensive rats compared with Wistar Kyoto rats

These above data and findings are summarized in the Supplemental data 3–5 with Tables, http://links.lww.com/HJH/B79 (I'), (2K) & (1K).

Validation of microarray data compared with RT-qPCR data

To validate the findings obtained by microarray analysis, RT-qPCR was performed in the six key identified genes selected from the significantly upregulated and downregulated genes in SHR and SHRSP compared with WKY, *Alb*, *Crlf1*, *Kcnq1*, *Chrm2*, *Kcnh1* and *Ache*, with *Gapdh* as a reference gene, among 43 identified genes consisting of 13 enhanced and 30 inhibited genes. As summarized in Table 7, a significant positive correlation between the microarray data and the RT-qPCR data was obtained using Spearman's rank correlation test [28] (SHR versus WKY, $r = 0.943$, $P < 0.01$; SHRSP versus WKY, $r = 0.943$, $P < 0.01$).

DISCUSSION

The SHR model [9] was established at the Kyoto University School of Medicine, Japan, in about 1963 through continuous brother–sister mating for six generations of normotensive WKY rats [20] with a slightly higher blood pressure. Using this strain of SHR, numerous studies on hypertension, including the pathophysiology related to hypertension and the environmental effects of factors such as food and maintenance conditions, have been carried out [28,29]. SHRSP [10] was subsequently created through continuous brother–sister mating in a closed colony of SHR.

Given the recent identification of all genes in humans [30], rats [31] and mice [32] by advances in technology, focus has increased on the causative genes of diseases, including hypertension. Two analytical methods of gene identification are available, DNA gene analysis in relation to the hypertension, and determination of mRNA expressed in tissue cells using DNA microarrays [11]. DNA microarrays are a powerful tool for studying genetics, as they facilitate

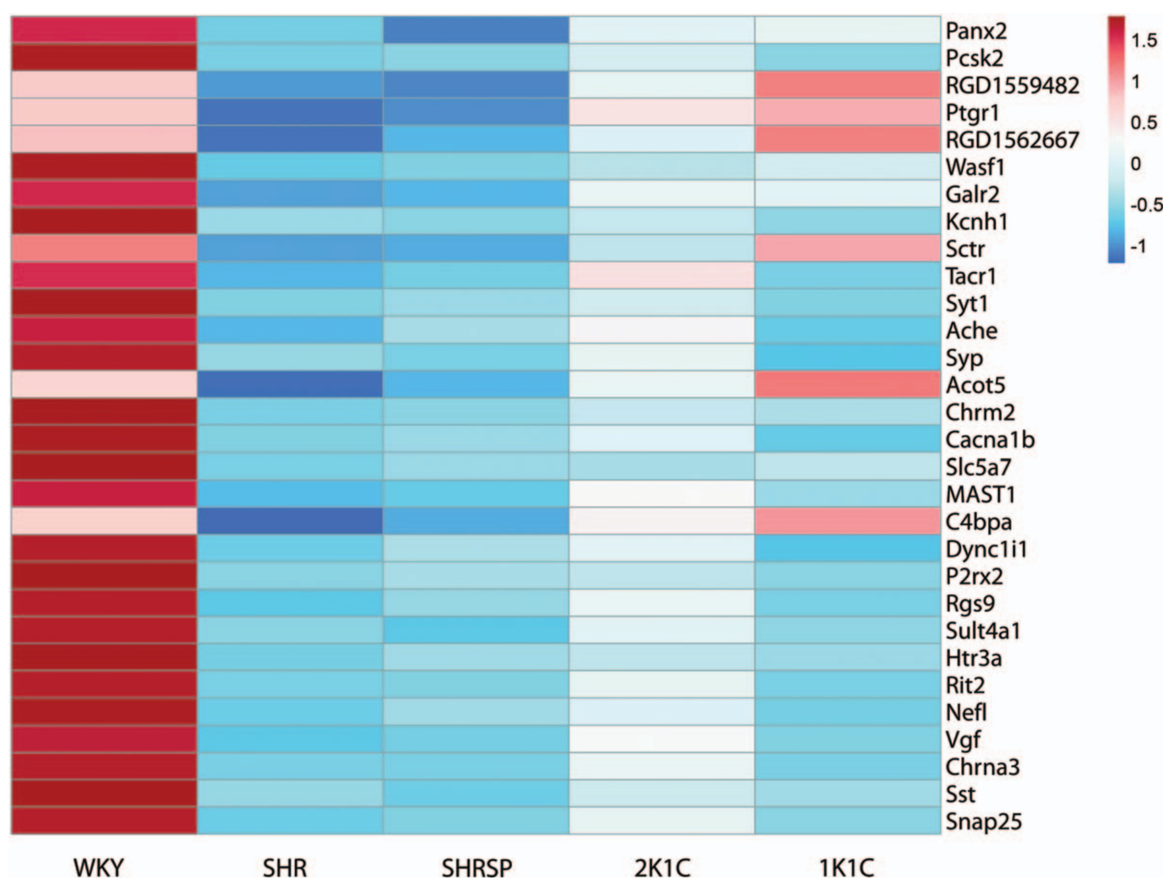

FIGURE 4 Heatmap regarding a relationship between significantly downregulated genes in SHR/WKY and SHRSP/WKY and 2K1C/WKY and 1K1C/WKY based on the data of Tables 3 and 4. Scale bar represents the degree of fold changes (FC) in gene expressions, and colour darkness represents the degree of similarity or nonsimilarity. 1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

the measurement of the expression of thousands of genes simultaneously [11,33,34]. As inbred homozygous rodent models of human essential hypertension, SHR and SHRSP are ideal tools for microarray research.

For the DNA microarray method, it is important to analyse the mRNA expressed in the tissues responsible for the disease of interest. Although the responsible tissues causing or associated with hypertension have not yet been fully elucidated, analysis of the peripheral arteries, especially those that are resistant, is appropriate. Therefore, we selected the mesenteric artery for the analysis of mRNA expressed in the resistance artery. For harder tissue containing more fibre, we extracted mRNA from the mesenteric artery and applied DNA array methodology to tissue samples from SHR and SHRSP, in comparison with normotensive WKY rats, and two more fundamentally different types of renovascular hypertensive rat, 2K1C and 1K1C [21,22]. Our aim was to identify hereditary hypertensive genes by comparing differences in expression between spontaneous and artificially induced renovascular hypertension. The genes obtained from the analysis of gene expression profiling contained several genes that were not related to hypertension. Therefore, we used simultaneous equations and comparative selections to identify commonalities in overlapping genes.

Two models of renovascular hypertension were successfully established in normotensive WKY (Fig. 2). Both 2K1C and 1K1C rats had increased blood pressures to levels comparable with SHR and SHRSP 3 weeks after surgery. Renovascular hypertensive 2K1C and 1K1C mice were reported by Wiesel *et al.* [21] as hyper-renin and hypervolemic nonrenin-dependent hypertensive models, respectively, given that plasma renin activity (PRA) was shown to be elevated more in 2K1C and atrial natriuretic peptide (ANP) mRNA expression elevated more in 1K1C at 4 weeks after surgery compared with the intact mice. In our study, at 6 weeks of age (3 weeks after surgery), SBP levels in 2K1C, 1K1C, SHR, and SHRSP were significantly increased compared with levels at 3 weeks of age and at 6 weeks of age in WKY, as shown in Fig. 2.

Validation of DNA microarray data

Given the importance of reliability in DNA microarray data, RT-qPCR was performed in the six key genes *Alb, Crlf1, Kcnq1, Chrm2, Kcnh*1 and *Ache*, with *Gapdh* as a reference gene. As summarized in Table 7, a significant positive correlation between the microarray data and the RT-qPCR data was identified. The DNA microarray data were therefore considered reliable.

TABLE 4. Genes downregulated less than 0.5-fold in 2K1C/ Wistar Kyoto rats or 1K1C/ Wistar Kyoto rats compared with significantly expressed genes in spontaneously hypertensive rats and stroke-prone spontaneously hypertensive rats

	2K1C/WKY			1K1C/WKY		
Gene symbol	Fold change	*P*	Remarks	Fold change	*P*	Remarks
Panx2	−2.800	0.016	(#2)	−2.687	0.027	(#1)
Pcsk2	−3.342	0.112	(#2)	−4.656	0.041	#1
RGD1559482	−1.884	0.088		1.460	0.258	
Ptgr1	−1.262	0.089		1.144	0.303	
RGD1562667	−2.072	0.177	(#2)	1.330	0.424	
Wasf1	−4.785	0.075	(#2)	−4.210	0.094	(#1)
Galr2	−2.987	0.054	(#2)	−3.114	0.038	(#1)
Kcnh1	−6.208	0.115	(#2)	−7.961	0.039	#1
Sctr	−4.651	0.004	#2	−1.223	0.639	
Tacr1	−2.577	0.354	(#2)	−7.882	0.043	#1
Syt1	−6.578	0.098	(#2)	−10.215	0.033	#1
Ache	−3.454	0.204	(#2)	−9.386	0.019	#1
Syp	−6.624	0.137	(#2)	−18.959	0.017	#1
Acot5	−2.191	0.027	(#2)	2.038	0.122	(#1)
Chrm2	−9.418	0.069	(#2)	−10.897	0.047	#1
Cacna1b	−7.741	0.150	(#2)	−17.091	0.034	#1
Slc5a7	−12.685	0.054	(#2)	−10.939	0.073	(#1)
MAST1	−4.701	0.253	(#2)	−11.301	0.048	#1
C4bpa	−1.695	0.132		1.623	0.131	
Dync1i1	−7.140	0.149	(#2)	−20.003	0.036	#1
P2rx2	−12.725	0.008	#2	17.877	0.003	#1
Rgs9	−7.131	0.123	(#2)	−18.187	0.025	#1
Sult4a1	−10.729	0.170	(#2)	−23.872	0.043	#1
Htr3a	−19.887	0.068	(#2)	−25.475	0.046	#1
Rit2	−11.465	0.199	(#2)	−33.738	0.040	#1
Nefl	−14.469	0.155	(#2)	−37.145	0.035	#1
Vgf	−8.401	0.244	(#2)	−31.272	0.039	#1
Chrna3	−13.123	0.122	(#2)	−47.736	0.014	#1
Sst	−32.764	0.105	(#2)	−48.145	0.050	#1
Snap25	−19.520	0.175	(#2)	−65.562	0.029	#1

#2 or #1, and (#2) or (#1) in the Remarks column indicate genes inhibited less than 0.25-fold with statistical significance, and genes inhibited less than 0.5-fold with or without statistical significance in each sample group in 1K1C/WKY or 2K1C/WKY (refer to the data in Table 3).
1K1C, one kidney and one clip renovascular hypertensive rats; 2K1C, two kidneys and one clip renovascular hypertensive rats; FC, fold change in gene expression; *P*-value: *P*-values of measurement errors in a sample group SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

The most important genes related to causality and/or relationship to hypertension were expected to be found among those common to SHR and SHRSP compared with those of WKY [an area of (I) as shown in Figs. 1a].

Upregulated genes in the area of (I)

Among the 13 upregulated genes in an area of (I), *Kcnq1* and *Crlf1* were strongly suggested to have a causal relationship and/or potentiate hypertension from the biochemical and physiological information described in Supplemental data 1, http://links.lww.com/HJH/B79. *Inhba*, *Alb*, *Scube3* and *Atf3* might be related to the development of the hypermetabolic condition, while *Adtrp*, *Timd4*, *Hspa1b* and *Cldn19* might be related to the maintenance of or compensation for hypertensive conditions in the body, while *Serpine1* might facilitate clot formation, causative for thrombosis in the brain of SHR and SHRSP.

As summarized in Table 2, *Kcnq1* was one of four upregulated genes in the comparison of 2K1C/WKY. Furthermore *Inhba, Scube3, Crlf1, Atf3, Timd4, Hspa1b, Abra, Xirp1* and *Cldn19* were also enhanced more than two-fold with or without statistical significance in each sample group expressed in comparison with 2K1C/WKY, as summarized in Table 2. These findings showed that SHR, SHRSP and 2K1C rats had many overexpressed genes in common in the mesenteric artery functionally worked as the resistance artery. Given that hypertensive 2K1C rats established from normotensive WKY are considered to arise from hyper-renin conditions [21], these conditions and related pathophysiological changes must also exist in SHR and SHRSP, at

TABLE 5. Genes associated with the occurrence and/or maintenance of hypertension in the mesenteric arteries of spontaneously hypertensive rats compared with Wistar Kyoto rats, an area of (S), at 6 weeks of age

Gene symbol	Protein name	Fold changes
1. *Lilrb3l*	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3-like	14.994
2. *Fam216b*	Family with sequence similarity 216, member B	6.498
3. *Vegfb*	Vascular endothelial growth factor B	−755.189

SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats.

TABLE 6. Genes associated with the occurrence and/or maintenance of hypertension in the mesenteric arteries of stroke-prone spontaneously hypertensive rats compared with Wistar Kyoto rats, an area of (SP), at 6 weeks of age

Gene symbol	Protein name	Fold changes
1. *Zfp45l*	zinc finger protein 45-like	31.060
2. *Pcmtd1*	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 1	20.797
3. *Rps16*	ribosomal protein S16	5.855
4. *Rgs11*	regulator of G-protein signalling 11	5.525
5. *Sult1b1*	sulfotransferase family, cytosolic, 1B, member 1	5.022
6. *Zfp597*	zinc finger protein 597	4.612
7. *RGD1563302*	RGD1563302	4.040
8. *Csap1*	common salivary protein 1	−6.126
9. *Micb*	MHC class I polypeptide-related sequence B	−8.215
10. *Dlg5*	similar to discs large homolog 5 (Placenta and prostate DLG) (Discs large protein P-dlg)	−11.020
11. *Haghl*	hydroxyacylglutathione hydrolase-like	−14.991
12. *Samd14*	sterile alpha motif domain containing 14	−17.020
13. *Agtrap*	angiotensin II receptor-associated protein	−27.343
14. *Ephx2*	epoxide hydrolase 2, cytoplasmic	−29.555

SHRSP, stroke-prone spontaneously hypertensive rats; WKY, Wistar Kyoto rats.

least in part. Among these genes, *Kcnq1* might be the most relevant upregulated gene to cause hypertension in SHR and SHRSP, as it was enhanced significantly in SHR, SHRSP and 2K1C rats in functional processes associated with hypertension through advanced repolarization in muscle and neuronal cells. Two other genes, *Alb* and *Cldn19*, were also overexpressed more than two-fold with or without statistical significance for each sample group in 1K1C compared with WKY as summarized in Tables 1 and 2. As hypertensive 1K1C rats established from normotensive WKY are considered to arise from hypervolemic conditions [21], these conditions or hypertension itself might induce overexpression of these genes in SHR and SHRSP as well. That is, *Alb* and *Cldn19* might be overexpressed in relation to hypervolemic conditions or to hypertension itself.

Inhibited genes in the area of (I)

Significantly inhibited genes in the mesenteric arteries of either groups of SHR or SHRSP compared with those of WKY, an area of (I) in Fig. 1a, were described in Supplemental data 2, http://links.lww.com/HJH/B79 in detail. As shown in the remarks column of Tables 3 and 4, 27 out of 30 genes were also inhibited by less than 0.5-fold with or without statistical significance in the comparison of 2K1C/WKY, and 25 out of the 30 genes were the same as those inhibited by less than 0.5-fold with or without statistical significance in the comparison of either 1K1C/WKY or 2K1C/WKY. That is, most genes identified as inhibited genes in the mesenteric arteries of SHR and SHRSP were common to the two artificial renal hypertensive models, indicating that SHR and SHRSP also had characteristics of hyper-renin and/or hypervolemic pathophysiological changes. Among these 30 genes, five were strongly suggested to cause and/or potentiate hypertension based on their biochemical action: *Galr2* that may potentiate arterial contraction of the artery; *Kcnh1* that may reduce arterial relaxation; *Ache* that potentiates the sympathetic nervous system; *Chrm2* that increases arterial contraction; and *Slc5a7* that decreases acetylcholine synthesis, resulting in increased blood pressure. Each other rest gene must have some fundamental functions such as an increase of blood pressure or maintain the body condition in good against hypertension-induced disorder. However, any reasonable idea was not obtained in this step.

Therefore, many genes expressed in either SHR or SHRSP were commonly expressed in 2K1C and 1K1C renovascular hypertensive rats, as summarized in Tables 1–4. These findings indicate that hyper-renin and/or hypervolemic pathophysiological changes may also occur in hereditary hypertensive SHR and SHRSP.

TABLE 7. Comparison of the expression of selected genes between microarray data and RT-qPCR data

	SHR/WKY		SHRSP/WKY	
Gene symbol	FC (RT-qPCR)	FC (microarray)	FC (RT-qPCR)	FC (microarray)
Three main enhanced genes				
Alb	4.308	4.591	7.467	5.319
Crlf1	14.690	6.258	17.700	5.664
Kcnq1	9.271	4.913	13.380	4.540
Three main inhibited genes				
Chrm2	−188.679	−14.222	−225.225	−13.445
Kcnh1	−7.962	−7.597	−9.132	−8.214
Ache	−5.459	−10.676	−3.038	−7.064

Significantly positive correlation between the microarray data and the RT-qPCR data was obtained using Spearman's rank correlation test in comparison of SHR/WKY with $r = 0.943$ at $P < 0.01$, and SHRSP/WKY with $r = 0.943$ at $P < 0.01$, respectively.
FC (RT-qPCR), fold changes based on the results obtained with RT-qPCR; FC (microarray), fold changes based on the results obtained with microarray analyses; *r*, correlation coefficient; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; WKY, Wistar Kyoto rats.

Significantly enhanced or inhibited genes in the area of (S) expressed only in spontaneously hypertensive rats

As summarized in the Table 5, three genes were identified as associated with the occurrence and/or maintenance of hypertension in SHR compared with WKY, including two that were upregulated, *Lilrb3l* and *Fam216b*. Although these genes are related to the adaptive immune system, their relationship with hypertension is unknown. Therefore, they were not considered as hypertension-associated gene in this study. The product of the third gene, *Vegfb* has a function in VEGF ligand–receptor interactions, platelet degranulation and haemostasis. Therefore, a significant decrease of this gene in the SHR for WKY remains unclear at this moment. Further studies are therefore necessary to explore the function of this gene. Taken together, no new genes related to hypertension were identified in this area of (S).

Significantly enhanced or inhibited genes in the area of (SP) expressed only in stroke-prone spontaneously hypertensive rats

We identified genes that may potentiate blood pressure in SHRSP to a greater extent than in SHR, or blood vessel fragility in the stroke-prone SHRSP rats, as summarized in the Table 6. Seven genes had increased expression: *Zfp45l*, *Pcmtd1*, *Rps16*, *Rgs11*, *Sult1b1*, *Zfp597* and *RGD1563302*, three of which (*Zfp45l, Pcmtd1* and *Zfp597*) are regulator genes that affect certain transcription processes. Three genes in these ones appear to be related to metabolic control: *Rps16* has a function in the translation process, *Rgs11* regulates G protein signalling and the product of *Sult1b1* catalyzes the sulfate conjugation of hormones and neurotransmitters. The function of *RGD1563302* was unknown. No genes with a direct relationship to hypertension were identified from the seven upregulated genes of the area of (SP). Among the seven downregulated genes, *Csap1*, *Micb*, *Dlg5*, *Hagh-1*, *Samd14*, *Agtrap* and *Ephx2*, 2 (*Agtrap* and *Ephx2*) might be relate to the greater potentiation of blood pressure in SHRSP than in WKY and SHR via the reduction in negative regulation of angiotensin II signalling and increased blood flow. *Dlg5* may be related to arterial fragility in SHRSP through the reduction in transmission of extracellular signals to the cytoskeleton. The functions of the remaining four genes were unknown.

Genes associated with models of renovascular hypertensions (2K1C and 1K1C) in the mesenteric arteries at 6 weeks of age: an area of (I’), genes associated with 2K1C renovascular hypertension: an area of (2K) and genes associated with 1K1C renovascular hypertension: an area of (1K)

These above data and findings are presented in the Supplemental data 3–5, http://links.lww.com/HJH/B79 with Tables (I’), (2K) & (1K).

To date, the following genes are reported to be associated with hypertension in the kidneys of SHR or SHRSP by use of the DNA microarray: *Ephx2* (upregulated) and *Ela1* (downregulated) [12]; *Edg1* and *Vcam1* (upregulated) [34]; *Sah, Hsp70, Mct1, Rbt, Idl1* and *Prion* (upregulated) and *thrombin, Dyn, Sod3, Ela1* and *Gst Y(b)* (downregulated) [13]. In research using brain tissue, *Oprm1, Calcyon, Calmodulin, Lhx1* and *Hes6* were altered [18], while in adrenal glands, *Crem, Fosl1, Nts, Apln, Epln, Ephx2* and *Agt* were enhanced [17]. In mesenteric arteries, *Cx43* [35], *CaV β3a* and *α2δ1* [36], and *At1aR* [37] were enhanced, and *Nrf2* [38] was inhibited. Therefore, no conclusive findings have been obtained thus far, although each candidate gene may have some relationship with hypertension. We expected to identify candidate genes reliably related to hypertension from our new approach of comparing the differences between the expressed genes of SHR, SHRSP and renal hypertension models.

In humans, the following findings were reported from the DNA meta-analyses.

(1) GWAS + Global BPgen consortiums (2010) reported that *CYP 17A 1* and *CYP 1A 2* seemed to be associated with hypertension, *EGF* (EGF-like domain), *SH2B3 SH2B* (adaptor protein 3), *PLCD3* and *MTHFR* seemed to be associated with cell growth and regulation, and *c10orf 107* and *ZNF652* seemed to be associated with carcinogenesis as hypertension-associated genes [39].

(2) CHARGE + Global BPgen consortiums (2010) reported that *ATP 2 B 1*, *CACNB 2* and *CYP 17 A 1* seemed to be associated with hypertension, *ULK 4*, *TBX 3* and *SH 2 B 3* seemed to be associated with cell function, and *PLEKHA 7* and *CSK* seem to be involved in cancer or immune reactions as hypertension-associated genes [40].

(3) The AGEN-BP consortium study in East Asia (2011) reported that *NPR 3* and *TBX 3* seems to be associated with hypertension, *FIGN* seemed to be associated with tissue development and *ST7L* and *ENPEP* seemed to be associated with carcinogenesis as hypertension-associated genes [41].

(4) In addition, European ICBP-GWAS study (2012) reported that *ADRB 1*, *MAP 4*, *NPR 3*, *ADM* and *GOSR 2* seemed to be associated with hypertension, and *FIGN*, *NOV*, *PDGFRA*, *CHIC 2*, *PIK3CG*, *ADAMTS 8*, *GUCY 1 A 1*, *FURIN* and *GNAS* seemed to be associated with cell regulation or carcinogenesis as hypertension-associated genes [42,43].

Thus, few were matched between the expression data of mRNA analysed by use of DNA microarray in SHR and SHRSP rats and the analytical data obtained from SNP analysis using DNA in human when hypertension-associated genes were investigated. Although detail of these discrepancies between the two species is unknown, the following reasons might be considered. That is, first, hypertensive patients in humans will consist of several types of hypertensions, which includes essential hypertension and secondary hypertensions in addition to the ageing effects, in contrast with analysis of mRNA expressions in SHR and SHRSP rats seemed to be essential hypertension models. Therefore, direct hypertension-related genes such as catecholamines, steroids and Ca^{2+} channel proteins might be easily picked up from a DNA analysing method in humans. Second, significantly expressed mRNAs are analysed in responsive organs such as kidney, brain and mesenteric artery considered to the cause of hypertension in any stages of hypertensive rat models, whereas significantly existing SNP genes from whole DNA constitution are picked up in relation to the high blood pressure in humans. Therefore, the difference of methodologies between the two species might pick up the different genes; moreover, which

organs, tissues or cells are responsive for hypertension is unknown. Third, as rats and humans are different animal species, causing factors for hypertension might be different in the two species.

Therefore, we must continue the analysis using both species such as SHRs and humans to solve the true hypertension-related genes. To confirm which genes are definitively responsible for hypertension, further studies using new genetic modification technologies such as knock-out [44], knock-in [45] and sophisticated genome editing methods [46] are required, based on the findings of this study. The application of new technologies in this research area will provide a clearer understanding of hypertensive pathophysiology and further the advancement of novel treatments of hypertension.

In conclusion, four upregulated genes, *Kcnq1, Crlf1, Alb* and *Xirp1*, along with five downregulated genes, *Galr2, Kcnh1, Ache, Chrm2* and *Slc5a7*, were identified as genes highly associated with the cause and/or worsening of hypertension in either SHR or SHRSP rats in this study.

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Conflicts of interest

There are no conflicts of interest.

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