Original Article

Use of a Panel of Congenic Strains to Evaluate Differentially Expressed Genes as Candidate Genes for Blood Pressure Quantitative Trait Loci

Soon J. LEE, Jun LIU, Nianning QI, Ross A. GUARNERA,* Si Y. LEE, and George T. CICILA

Candidate gene(s) for multiple blood pressure (BP) quantitative trait loci (QTL) were sought by analysis of differential gene expression patterns in the kidneys of a panel of eight congenic strains, each of which carries a different low-BP QTL allele with a genetic composition that is otherwise similar to that of the hypertensive Dahl salt-sensitive (S) rat strain. First, genes differentially expressed in the kidneys of one-month-old Dahl S and salt-resistant (R) rats were identified. Then, Northern filter hybridization was used to examine the expression patterns of these genes in a panel of congenic strains. Finally, their chromosomal location was determined by radiation hybrid (RH) mapping. Seven out of 37 differentially expressed genes were mapped to congenic regions carrying BP QTLs, but only one of these genes, L-2 hydroxy acid oxidase (*Hao2*), showed the congenic strain-specific pattern of differential kidney gene expression predicted by its chromosomal location. This data suggests that *Hao2* should be examined as a candidate gene for the rat chromosome 2 (RNO2) BP QTL. (*Hypertens Res* 2003; 26: 75−87)

***Key Words*: hypertension, quantitative trait loci, differential display of RNA, cDNA array, Dahl rats**

Introduction

Human essential hypertension is a complex, multifactorial disorder resulting from the interplay of multiple environmental and genetic factors. Several rat models of genetic hypertension have been developed to study the genetic determinants of blood pressure (BP) regulation (*1*−*3*). The inbred Dahl salt-sensitive (SS/Jr or S) rat is the most widely studied genetic model of salt-sensitive hypertension (*4*). At least 14 different chromosomal locations associated with BP, *i.e.*, BP quantitative trait loci (QTL), have been identified in this model by genetic linkage analysis (*4*). Eight of these BP QTLs were subsequently confirmed by constructing congenic strains (*5*−*11*) (Table 1). These eight congenic strains, each containing a different low BP QTL allele but having an otherwise similar genetic composition derived from S rats, constitute a powerful and unique tool for identifying strong candidate genes for BP QTL (*1*).

The toughest impediment to understanding the molecular basis of hypertension is proceeding from chromosomal locations associated with BP QTL to determining what genes in those chromosomal regions are causative for the observed differences in BP. A methodology must be developed to rapidly identify and characterize strong candidate genes for QTLs. The development of congenic strains and substrains has become a key method for limiting the chromosomal region that contains gene(s) responsible for the effect of a given QTL. Many congenic strains have already been developed to study the genetic basis of quantitative traits of both clinical and commercial importance, including BP. As described above, a panel of such congenic strains could be used to help

From the Department of Physiology and Molecular Medicine, Medical College of Ohio, Toledo, USA, and *Department of Biology, Sungkyunkwan University, Seoul, Korea.

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Address for Reprints: Soon Jin Lee, Ph.D., Department of Physiology and Molecular Medicine, Medical College of Ohio, Block Health Science Bldg., 3035 Arlington Ave., Toledo, OH 43614, USA. E-mail: sjlee@mco.edu

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Table 1. Selected Characteristics of Congenic Strains Containing BP QTL from Dahl Rats

Chr.	Congenic strain	Donor strain[a]	BP[b] effect (mmHg)	*p* (*t* test)	Ref.
1	S.LEW(chr 1)	LEW	26	<0.0001	(*6*)
2	S.WKY-D2N35/Nep	WKY	44	<0.0001	(*11*)
3	S.R-Edn3	R	21	0.0001	(*10*)
5	S.LEW(chr 5)	LEW	15	<0.0001	(*6*)
7	S.R-Cyp11b	R	63[c]	<0.0001[c]	(*5*)
9	S.R (chr 9)	R	19	<0.0001	(*7*)
10	S.M(10b)	MNS	35	<0.0001	(*8*)
13	S.R-Ren	R	24	0.002	(*9*)
2 and 10	S.WKY(2)M(10b)	WKY/MNS	47	<0.0001	(*29*)

[a] Inbred rat strain names are abbreviated as follows: LEW, Lewis; WKY, Wistar-Kyoto; R, Dahl salt-resistant; MNS, Milan normotensive strain; S, Dahl salt-sensitive. [b] The blood pressure (BP) effect was assessed after 37-day-old male rats were fed a 2% NaCl diet for 24 days. It is expressed as the decrease in systolic BP of the congenic strain compared to S rats, tested at the same time. [c] These rats were fed a 4% NaCl diet.

identify strong candidate genes for BP QTL.

BP differences observed between Dahl S rats and the congenic strains derived from them can arise from 1) the BP-lowering effect of alleles introduced from a normotensive strain into the S-rat strain, *i.e.*, the allele from the normotensive strain is the one responsible, in part, for the observed BP effect or 2) the BP-lowering effect of the substitution of S-rat alleles, that were causative of BP elevation, with alleles from the normotensive strain, *i.e.*, the S-rat allele is the one responsible, in part, for the observed BP effect.

The RNO7 congenic strain appears to be of the former type—*i.e.*, the allele carried by the normotensive (Dahl salt-resistant) strain differs from the S-rat allele (*12*, *13*). In this case, the R-rat *Cyp11b1* allele, present in the introgressed region of R-rat RNO7 in both the original congenic strain and subsequent congenic substrains, was associated with both lower BP and a decreased capacity for synthesis of the mineralocorticoid 18-OH-DOC. The S-rat *Cyp11b1* allele is apparently a "wild-type" allele, functionally similar to alleles carried by many normotensive rat strains other than R. If the incoming allele, derived from a normotensive strain, is responsible for the lower BP observed in the congenic strain, we can expect that only alleles from certain inbred rat strains (the R-rats in this case) could replace S-rat alleles, as is the case with the *Cyp11b1* locus. The *cd36* locus on rat chromosome 4 (RNO4) and insulin-resistance (*14*) would be an example of the second situation. Here, the insulin-resistant SHR rat carries a defective allele encoding an inactive protein. In this case, it may not matter from what strain the replacement allele is derived, so long as it is functional. Indeed, transgenic SHR rats carrying a functional *cd36* gene have been shown to be less insulin-resistant than SHR rats that carried a defective *cd36* gene (*15*).

In the present work, our strategy was to screen for candidate genes for the many BP QTLs identified in the Dahl rat model by identifying genes differentially expressed between inbred Dahl salt-sensitive (S) and salt-resistant (R) rats in the kidney using the differential display of mRNA (*16*, *17*) and cDNA array filter hybridization techniques (*18*). The pattern of kidney mRNA expression of such differentially expressed genes was then examined in a panel of one-month old male rats from eight different congenic strains (Table 1).

We hypothesize that genes responsible for the effect of a given BP QTL may show a congenic strain-specific differential expression pattern in a target organs. Target organs for BP regulation would include those that are critical for BP homeostasis maintenance and/or show alterations in their structural/functional properties during the progression of hypertension. The kidney, brain, heart, aorta and other blood vessels, adrenal glands and ganglia of the sympathetic nervous system clearly would be suitable as target organs for such analysis. Genes that are differentially expressed in target organs should also map to the chromosomal interval carried by that particular congenic strain in order to be considered a strong candidate gene for that interval. All of the other congenic strains in the panel would carry a different allele for that particular BP QTL, *i.e.* from the common recipient strain (S in our case) used to construct the congenic strains. Thus we would expect that mRNA expression for that gene should be similar in both the parental, S stain and all of these other congenic strains. We have chosen to study differential expression in the kidney because of its key role in maintaining the balance between the salt excretion and resorption required for maintaining volume homeostasis.

An examination of the differences in kidney gene expression between S and R rats should allow us to screen for candidate genes whose effects on BP (and differential gene expression) stem from a mutation present in the S-rat allele. In this case, congenic strains carrying an interval of the normotensive rat chromosome corresponding to the location of this particular QTL and gene would not carry the mutant, S-rat, allele and would have a lower BP compared to the parental S strain.

This comparison would also directly screen for candidate

genes whose effects on BP (and differential gene expression) stem from a mutation present in the R-rat allele. In this case, congenic strains carrying the introgressed R-rat chromosome corresponding to the location of this particular QTL and gene would substitute the mutant, R-rat, allele for that normally carried by the S strain and cause this congenic strain to have a lower BP compared to the parental S strain. This scenario would apply to identifying candidate genes for QTLs present in the rat chromosome 3, 7, 9, and 13 (Table 1) congenic strains in which the R-rat derived chromosomal region was introgressed into the S strain.

Kidney mRNA levels were examined in weanling rats that were maintained on a low NaCl diet to minimize differences in gene expression resulting from differences in BP. This should allow us to primarily study alterations in gene expression stemming from genetic differences, rather than those arising from a secondary response to the BP differences inherent in these inbred strains. Radiation hybrid (RH) mapping was used to determine the chromosomal location of differentially expressed genes and to determine whether these genes were associated with intervals containing BP QTLs. The kidney was chosen as a target organ because of its importance in maintaining volume homeostasis and salt excretion/resorption (*19*, *20*). Genes showing a congenic strain-specific pattern of differential gene expression in the kidney, and which mapped to a QTL-containing region in their respective congenic strain, were to be considered candidate genes, and worthy of additional attention.

Methods

Animal Studies/Sample Preparation

Male inbred Dahl S, R and congenic rat strains (described in Table 1) were from the colony at the Medical College of Ohio. Rats were weaned 30 days after birth and maintained on a low-salt (0.3% NaCl, diet 8640; HarlanTeklad, Indianapolis, USA) diet. For each strain, 3−4 rats of 31−34 days of age were killed by pentobarbital, and kidney samples were collected and processed immediately for total RNA. All procedures were carried out with approval from our Institutional Animal Care and Use Committee and were conducted in accordance with the National Research Council's guidelines.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from tissues as described previously (*21*). Tissues were homogenized in a guanidine thiocyanate/phenol solution (Ultraspec; Biotecx Laboratories, Houston, USA), extracted with chloroform and isolated using RNA Tack Resin (Biotecx Laboratories). cDNA was synthesized from total RNA as previously described (*22*). Total RNA (5 μg) pooled from 3−4 rats per strain was heat-denatured for 10 min at 68 °C and incubated at 37 °C for 1 h with 0.5 μg oligo(dT)$_{20}$, 0.5 mmol/l of each dNTP, 50 mmol/l Tris-HCl (pH 8.3), 10 mmol/l $MgCl_2$, 20 units of placental ribonuclease inhibitor (RNAsin; Promega, Madison, USA) and 200 units of M-MLV reverse transcriptase (SuperScript II; Life Technologies, Grand Island, USA) in a 20 μl volume.

Differential Display of mRNA

We used a kit (Delta Differential Display kit; ClonTech, Palo Alto, USA) for differential display of mRNA, with some modifications of the protocol. Differential display of mRNA was performed with kidney RNA prepared from S and R rats. For each group, cDNA was synthesized from RNA, poled in equal amounts from three to four rats, using M-MLV reverse-transcriptase and oligo(dT)$_{20}$ as described above. The amounts of cDNA used for differential display were normalized by reverse transcriptase-polymerase chain reaction (RT-PCR) for glyceraldehyde-3-phosphate dehydrogenase (*Gapd*) expression levels (*22*).

Two volumes of cDNA, one of which was 3 times larger than the other, were chosen for each sample based on the normalized cDNA amount, and were PCR-amplified as follows. Various combinations of a) ten arbitrarily chosen 5′25-mer oligonucleotide primers and b) nine 3′oligo(dT) anchor 29-mer oligonucleotide primers were used for differential display. PCR mixtures contained 20 μmol/l of 3′-oligo(dT) anchor primers, 20 μmol/l of the appropriate upstream primer, 50 μmol/l of dNTPs, 5 μCi (50 nmol/l) [α-^{33}P]-dATP (1,000−3,000 Ci/mmol; New England Nuclear, Boston, USA), 40 mmol/l Tricine-KOH (pH 9.2 at 25 °C), 15 mmol/l KOAc, 3.5 mmol/l $Mg(OAc)_2$, 75 μg/ml bovine serum albumin, and Advantage KlenTaq polymerase mix (ClonTech) in a total volume of 20 μl. A two-phase PCR-amplification protocol was used for differential display. The first phase (first 3 cycles) utilized the following low-stringency conditions to allow the primers to bind to template at multiple sites such that a moderate number of distinct PCR-products will be amplified: one cycle of 94 °C for 5 min, 40 °C for 5 min, and 68 °C for 5 min; followed by two cycles of 94 °C for 30 s, 40 °C for 5 min, and 68 °C for 5 min. The PCR-products resulting from the preceding phase were then specifically-amplified using the following higher-stringency conditions: 25 cycles of 94 °C for 15 s, 60 °C for 1 min, and 68 °C for 2 min; and ending with a final cycle of 68 °C for 7 min. PCR-amplified ^{33}P-labeled cDNAs were loaded in adjacent lanes and electrophoretically size-fractionated through a 0.4 mm-thick 6% polyacrylamide non-denaturing gel.

Recovery and Re-Amplification of cDNA

PCR products of interest were located on the gel, and the bands were excised with a razor. The gel slice and filter paper were incubated in 50 μl dH_2O for 10 min and boiled for 15 min. The eluate was re-amplified using the same primer set and PCR conditions as used for differential display, but

Table 2. Kidney mRNA Expression in a Panel of Congenic Rat Strains and Radiation Hybrid Mapping of cDNAs

Group	cDNA	Rat strain											Chr.	cDNA identity	GenBank acc.#
		S	R	1	2	3	5	7	9	10	13	2/10			
I	2S	—	−18	170	29	34	**−13**	35	14	17	91	13	M	cytochrome c oxidase I, *CoxI*	BF228365
I	6G-a	—	−41	85	75	69	95	**−61**	285	129	49	56	2*	similar to rat brain EST (AW527159)	BF228364
I	10B	—	−73	286	**−77**	107	—	−15	84	127	49	130	2	similar to mouse solute carrier family 7 member 12, *Slc7a12*	BF228356
I	5S-a	—	−47	124	**−17**	—	—	80	54	48	24	27	5*	aldehyde reductase 1	BF228366
I	5S-b	—	−72	—	−43	**24**	−78	−50	−59	−56	—	−34	3*	sodium dependent dicarboxylate transporter (*Slc13az*)	BF228367
I	6G-b	—	11	12	14	28	**−11**	109	31	28	45	24	7	poly-A binding protein	BF228361
II	1G	—	−55	−37	—	—	−19	—	—	20	—	12	18	H^+-ATPase subunit	BF228358
II	2R	—	−25	−43	—	—	−48	−21	—	—	—	—	8	cytochrome c oxidase Va, *Cox5a*	BF228363
II	2G	—	−19	−37	−15	38	−29	−50	—	12	—	19	3	cryptochrome 2, *Cry2*	BF228359
II	2B	—	−35	−49	−28	25	—	−81	17	15	−23	−18	6	proton-phosphate symporter	BF228371
II	6B	—	−34	−34	—	−12	−13	−57	—	—	−12	18	14	follistatin-like *mac*25	BF228372
II	9G	—	−55	208	−26	119	—	−28	41	27	—	13	20	prolyl 4-hydroxylase α subunit	BF228362
II	12B	—	−51	36	—	33	−14	−26	—	—	−20	—	N.D.	GTP-specific succinyl CoA synthetase β	BF228357
II / IV	7B	—	−41	44	27	−53	−15	−35	—	—	27	−37	20	nucleoside diphosphate kinase, *Nme2*	BF228373
II / IV	8S	—	−22	−40	−34	409	−28	39	330	16	58	203	N.D.	unknown EST	BF228368
II / IV	5G	—	−39	—	209	23	−53	49	−11	−20	—	−48	8	rat PC-12 cell EST (H32003)	BF228360
III	5R	—	55	44	72	63	24	—	32	72	65	54	7	unknown EST	BF228369

cDNAs identified in differential display analysis were used as probes for Northern filter hybridization to confirm their differential expression between S and R rats and to examine their expression patterns in a panel of congenic rat strains. Abbreviations of rat strains are as in Fig. 1 except for that of strain 1, which is S.LEW(chr1). Kidney mRNA expression values for the genes shown were adjusted for expression of *Gapd* mRNA to normalize for differences in the RNA amounts loaded on the gel. Differences are expressed as the % difference [(congenic strain level−S rat level)/S rat level]×100, with differences <10% indicated by — Bold numbers indicate the values for genes showing a congenic strain-specific pattern of differential mRNA expression in the kidneys of a single congenic strain in the panel compared to the S strain. The chromosomal locations (Chr.) determined by radiation hybrid mapping for 15 cDNAs are shown. * cDNAs that map within the region of normotensive rat strain chromosomes introgressed into the congenic strain. M, mitochondria; N.D., location not determined. The threshold value for linkage in the radiation hybrid mapping was an LOD score of 12. GenBank accession numbers for sequences showing similarity to the differentially expressed kidney cDNAs are shown in parentheses. The last column provides the GenBank accession number for the differentially expressed kidney cDNAs. Differentially expressed genes were divided into four groups based on their expression pattern in the congenic strain panel. Group I genes showed differential kidney mRNA expression only in a single congenic strain in the panel, group II genes in a subset of the congenic rat strain panel, and group III genes in all but one of the congenic rat strains, compared to S rats. Group IV genes showed altered mRNA expression levels in the “double” congenic strain, S.WKY(2)M(10b), compared to mRNA expression levels observed in either congenic strain from which it was derived.

Table 3. Primer Sequences of Differentially Expressed cDNAs for RH Mapping

cDNA	5 ′primer	3 ′primer
6G-a	TGCAGATGGGTAGGAGTGGCTATG	CCTTCGCAACACCTGGTTCATC
10B	CTGGGCTTGTGCTGTTAGTGATTG	CCTCTGTATGCAAAACATCCTTGGAG
5S-a	CATGATTACGGTGGATGGGAAG	GGCAATGCTTAGCAGGAGAACTG
5S-b	AAGAGAATCCCAGGCATCTTGG	AGCTAGAGGTCGGCTTCCATTG
1G	ATGTGGCAGGCACCATGAAG	CTTGCTTTAGCAGCTCGGTCGAG
2R	CCAGGCAATCTGAGATGAATCC	AAGTAGCATGTCCCTGACTGAAGC
2G	AGAGTGTCTTGGAGCCCTGGAATG	GCTCTTGCCTGCAACCATCTCTTC
2B	GAGGCTTGGCTTTAGAGGTGTATGGAAG	AGAGACTCTGGCATCTCAGGTGGAGGAG
6B	GTGCTCAGCTATAACCTGCGAATACATTAGC	GGAATATGTCAGGCAGGAGTGGGGTTAC
9G	TAACGCACCAGGCATGATCG	GTCCACAGATGAAACATGGGATG
7B	TCCGTCTGTCTCAGGTGTGTCCAT	GCATGTGTGTGCCCACTCCATA
5G	CCTCACTAAATGCTGGTGGACC	TATAGGTGCGAGGTCAGGTTGG
6G-b	AACTGCTTCCATGTTGGCATCTGC	TTTCGCTTGGTGGGCTTGTAGTACAG
array1	GACTTTCATCTGAATGCCAC	TTCCCTCCGTGTCTGTACTC
array3	TGCGAGAGATGCCAGTGGAC	GGCCATGTGCTCCAAGTCAG
array4	ATCATCAAATGCCAGTAATG	GGCCAAACTGATAACAGAGA
array5	ACTAGCTGCTCACTCCATGC	CGAGGGATTGGACTCACTTA
array7	CTAATTGGGCTTGCTCAGGG	CGGGTTCACCAGGCTTTAAT
array8	GACTTTCATCTGAATGCCAC	TTCCCTCCGTGTCTGTACTC
array9	TAGCAAGAGGCTCACACCCA	TCCAGGCTCCAGCATTTAGT
array11	TTGCCTTAACCCTACTGAAG	GATGCCAGTTGGAAGTCAGA
array12	GCGGCTAGCATTACCACTT	GAGATTTGCCACTTGGGTGA
array14	CGCCATGGCTGACAAGGAAG	CACTGGGCAGTTAGGCTGGG
array15	GTGACAACATGTGCAAACGC	TTACAGGAAGCTGCTCTTGG
array16	GTCGGAGGGTTAATTAGTTT	CTCGGGACTTGTTGTGGCAT
array17	TCGTGTTCTGAGGGCCAACC	ATCCTGCCGATGCCACATCT
array18	GGATGCATGATATTCCCTAA	GTGTAAATGGTGAGATGGCT
array19	AGGTAATTGGCATGTCACAC	GAGCCCTCTACCTTGAGAAA
array20	AACACCCGATATATCACAGA	CTCGTCCCTAGCTGGTCAGA

with a decreased number of cycles, increased dNTP concentrations (200 μmol/l), and no radioisotope. Re-amplified PCR-products were electrophoresed through low melting agarose gels and the appropriate bands were excised. Differential-display products isolated in this manner were cycle-sequenced directly. Differential-display products where sequencing resulted in a single "ladder" of bases were radiolabeled for use as probes for Northern filter hybridization. Differential-display bands containing multiple PCR-products were subcloned into plasmids (pCRII; InVitrogen, Carlsbad, USA) and then sequenced. Plasmid inserts were then purified, radiolabeled, and used as a probes for northern hybridization.

Characterizing Differentially Expressed cDNA Bands

Re-amplified cDNAs were cycle-sequenced directly using a ThermoSequenase radiolabeled terminator cycle sequencing kit (USB, Cleveland, USA) according to the manufacturer's instructions. Sequencing reactions was heat-denatured for 3 min at 94 °C prior to fractionation on 0.4 mm-thick 6% polyacrylamide gels containing 7 mol/l urea and a glycerol-tolerant gel buffer. Following electrophoresis, gels were dried before exposure to X-ray film (BioMax MR; Kodak, Rochester, USA). The obtained sequences were compared to the sequences in the GenBank or dbEST databases using the computer programs BLAST (National Center for Biotechnology Information, Bethesda, USA) and MacVector (Kodak). The sequences of differentially expressed cDNAs have been deposited in the GenBank Database and their accession numbers can be found in Table 2.

Measurement of RNA Expression Levels

RNAs for Northern filters were pooled from samples isolated from 3−4 rats of each group (S, R, and 9 congenic strains) as described above for cDNA synthesis. Northern filter hybridization analysis was performed according to the standard procedures. Briefly, 10 μg of total RNA for each sample was fractionated by 1% formaldehyde agarose gel electrophoresis and capillary-transferred onto a nylon membrane (Nytran Plus, 0.2 μm pore size; Schleicher and Schuell, Keene,

USA). Hybridization and washing were performed as described previously (*23*) using re-amplified cDNAs as probes. Filters were then exposed to X-ray film and the signals on film were normalized for *Gapd* RNA expression levels. cDNAs that did not produce signals on Northern hybridization were studied using the standard RT-PCR protocol as described in the differential display section using specific primer sets designed from the sequence of the differential display products (Table 3).

Hybridization Using cDNA Array Filters

Total kidney RNA samples, pooled as described above for differential display of mRNA, were reverse-transcribed with ^{33}P-dCTP using a modified mouse MLV reverse transcriptase (SuperScript II; Life Technologies, Rockville, USA) from 2 μg of RNA. Rat GeneFilters (GF 300; Research Genetics, Huntsville, USA) that contained 5,184 genes from the Unigene database (http://www.ncbi.nlm.nih.gov/UniGene/Rn.Home.html) arrayed on a nylon membrane, were prehybridized for at least 2 h at 42 °C in hybridization solution (MicroHyb, Research Genetics) containing Cot-1 DNA and poly(dA) as blocking agents. Next, ^{33}P-labeled cDNA probes from the S and R rats were heat-denatured, added separately to the hybridization solution, and allowed to hybridize with filters for 18 h at 42 °C. Filters were washed twice with 2 × saline sodium citrate (SSC), 1% sodium dodecyl sulphate (SDS) at 50 °C for 20 min, and once at room temperature with 0.5 × SSC, 1%SDS for 15 min, and then exposed to a phosphor-imager screen (Molecular Dynamics, Sunnyvale, USA). Screens were scanned using a phosphor-imager (Molecular Dynamics), and the resulting signals on the two filters were compared using the Pathways 2.0 computer program (Research Genetics).

Radiation Hybrid Mapping of cDNAs

PCR conditions were developed for primers designed for cDNAs such that the PCR products 1) were only amplified from rat genomic DNA or 2) could be clearly distinguished from hamster PCR products based on size differences (Table 3). A rat/hamster radiation hybrid panel, consisting of DNA from 106 somatic cell hybrid lines derived from fused hamster and irradiated-rat cells (*24*) (DNA purchased from Research Genetics), was then examined with primers to determine which cell lines retained the rat sequences. To minimize typing errors, each primer set was used to amplify DNA from the radiation hybrid panel in duplicate independent experiments. Conflicting results were resolved by repeating the PCR. Rat and hamster PCR products that could not be distinguished using agarose gel electrophoresis were resolved using the single strand conformation polymorphism (SSCP) technique (*25*), using the ThermoFlow ETC System (Novex, San Diego, USA). Bands 7R and 6G-b were mapped using this technique.

The RHMAPPER 1.22 program (*26*) run under Linux 2.0.36 was used to analyze marker retention patterns and identify the chromosomal location of the differentially expressed cDNAs. A rat radiation hybrid marker database was constructed with data available from the radiation hybrid database maintained by the European Bioinformatics Institute (Hinxton, UK) (www.ebi.ac.uk/RHdb/index.html).

Mapping the *Hao2* Gene

A rat P1 artificial chromosome (PAC) library (RPCI-31 from Pieter de Jong's Laboratory, Children's Hospital Oakland Research Institute, Oakland, USA) was screened with a ^{32}P-labeled full-length rat *Hao2* cDNA probe (1.6 kb) to identify clones containing the *Hao2* gene. Positive PAC clones were obtained from Research Genetics and clone DNA was isolated using a kit (Qiagen, Valencia, USA). The presence of *Hao2* gene in the clone was confirmed by PCR-amplification using *Hao2*-specific primers and Southern filter hybridization analysis. PAC clone (441D18) DNA was digested with *Rsa*I (New England BioLabs, Beverly, USA) and subcloned into the PCR-Blunt vector (Zero Blunt PCR Cloning Kit; Invitrogen, Carlsbad, USA) to create a library that was screened with radiolabeled $(CA)_{15}$ and $(CT)_{15}$ oligonucleotides to identify clones containing dinucleotide repetitive elements. A primer set, (D2Mco47; 5′-AGGTAGG TGGAGAGCAGGTATGG and 5′-TCATATACACATACA CACATGCG) was designed to amplify a microsatellite sequence found in one genomic DNA insert.

Clones containing the 5′-untranslated portion of *Hao2* were identified using the following "overgo" probe (*27*): two 22-mer oligonucleotides (5′-TGTAGCGGGCTTTGCATTG CTG and 5′-GGTAGAGGCAGCAATGCAAAGC) having a 14 bp overlap were designed from a 30-bp region of the 5′-untranslated portion of *Hao2* that was free of repetitive DNA. "Overgo" oligonucleotides were heated for 5 min at 80 °C, chilled at 37 °C for 10 min, and labeled with ^{32}P-dCTP at 37 °C for 30 min using a kit (RadPrime; Life Technologies).

Statistical Analysis

One-way analysis of variance (ANOVA) was calculated using the program StatView 5.1 (Abacus Concepts, Berkeley, USA).

Results

Differential Display of Kidney RNA from One-Month-Old Dahl S and R rats

Differential display of mRNA was used to screen for genes that were differentially expressed in the kidneys of Dahl S and R rats. cDNA was synthesized from pooled kidney RNA isolated from one-month-old S and R rats, and glyceralde-

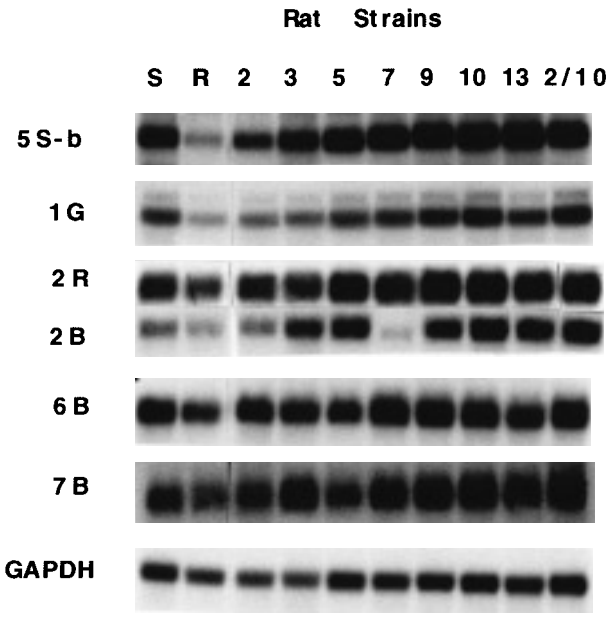

Fig. 1. *Representative Northern filter hybridization analysis of differentially expressed cDNAs. cDNAs identified by differential display of mRNA analysis were used as probes for Northern filter hybridization to confirm their differential expression between S and R rats and to examine their expression patterns in a panel of congenic rat strains. Rat strains in this figure are abbreviated as follows: S, Dahl salt-sensitive; R, Dahl salt-resistant; 2, S.WKY-D2N35/Nep; 3, S.R-Edn3; 5, S.LEW(chr5); 7, S.R-Cyp11b; 9, S.R(chr 9); 10, S.M(10b); 13, S.R-Ren; 2/10, S.WKY(2)M(10b). Northern analysis was performed using filters prepared with 10 μg samples of total kidney RNA pooled from 4 rats/strain with differentially expressed cDNAs and* Gapd *as probes. Results are shown using six different cDNAs.* Gapd *mRNA expression levels were used to normalize for differences in RNA loading. Note: cDNA probes were analyzed on different filters and the amounts of RNA sample loaded were determined and normalized separately for each filter. The* Gapd *result shown in this figure is representative for one filter and does not necessarily reflect the RNA amount present on the filters used with other probes shown here.*

hyde-3 phosphate dehydrogenase (*Gapd*) mRNA expression levels were used to normalize the cDNA amount (see Methods). Similar amounts of S and R rat kidney cDNAs were then used for differential display PCR using an anchor primer and an arbitrary primer.

The first 20 pairs of differential display primers amplified about 15% of the approximately 12,000 genes expected to be expressed in the kidney (*28*). This process identified 35 cDNA bands that were differentially expressed in the kidneys of S and R rats, which were then isolated and characterized. Eighteen of these cDNA bands were LINE (long interspersed nucleotide element) repeats that were not studied further because their multiple copies in the genome prevent them from being mapped. Fifteen cDNA bands showed sequence similarity to previously described genes and ESTs. Two differentially expressed cDNAs showed no significant similarity to any previously described genes or ESTs present in the GenBank or dbEST databases (Table 2).

Kidney mRNA Expression of Differentially Expressed cDNAs in a Panel of Congenic Rat Strains

Northern filter hybridization confirmed the differential mRNA expression of 17 non-LINE genes in kidneys of S and R rats and was used to examine their expression in the kidneys of eight different congenic rat strains. These congenic strains have portions of rat chromosomes 1 (*6*), 2 (*11*), 3 (*10*), 5 (*6*), 7 (*5*), 9 (*7*), 10 (*8*) and 13 (*9*), respectively, from normotensive rat strains introgressed into the S strain, with all congenic strains having lower BP compared to the S strain (Table 1). Kidney mRNA expression was also examined in S.WKY(2)M(10b), a "double" congenic strain, where portions of RNO2 and chromosome 10 from WKY and MNS rats, respectively, that contain epistatic loci, were introgressed into an S-rat genetic background (*29*). Northern filter hybridization results with the differentially expressed cDNAs are summarized in Table 2, with representative Northern filter hybridization results shown in Fig. 1.

Four groups of differentially expressed genes were identified based on their pattern of differential kidney mRNA expression in the congenic strain panel. Six of these genes showed differential kidney mRNA expression only in a single congenic strain in the panel (expression pattern I), compared to S rats. This group included: 1) an unknown gene with 99% sequence similarity to a rat brain-derived EST (band 6G-a), differentially expressed only in S.R-Cyp11b; 2) aldehyde reductase 1 (band 5S-a) differentially expressed only in S.WKY-D2N35/NEP; 3) a gene similar to mouse Slc7a17 (solute carrier family 7; member 12, band 10B) differentially expressed only in S.WKY-D2N35/NEP; 4) cytochrome c oxidase subunit 1 (*CoxI*; band 2S), a mitochondrial enzyme, differentially expressed only in S.LEW (chr5); 5) sodium-dependent dicarboxylate transporter (band 5S-b), differentially expressed only in S.R-Edn3 (chr3); and 6) poly-A binding gene (band 6G-b), differentially expressed only in S.LEW (chr5).

Ten genes showed another expression pattern, wherein differential kidney mRNA expression was observed in R rats and a subset of the congenic rat strain panel, compared to S rats (expression pattern II). This group included two ESTs (band 8S, a previously undescribed EST; and band 5G, a rat PC-12 cell EST) and eight known genes, cytochrome c oxidase subunit Vα (*Cox5a*; band 2R), proton-phosphate symporter (band 2B), H^+-ATPase subunit α (band 1G), follistatin-like mac25 protein (band 6B), succinyl CoA synthetase β (band 12B), nucleoside diphosphate kinase (band 7B), cryptochrome 2 (band 2G) and prolyl 4-hydroxylase α subunit (band 9G).

We also identified one gene, a previously undescribed

Table 4. Radiation Hybrid Mapping Results for the 20 cDNAs in Rat cDNA Array Filter with Largest Expression Differences

cDNA array clone	Chr.	cDNA identity	GenBank accession #
1	13	TREK-1, K^+ channel, subfamily K, member 2 (*Kcnk2*)	AA817837
2	2	similar to mouse endophilin (*Sh3glb1*)	AA817771
3	10	similar to mouse plasma glutathione peroxidase	AA818827
4	2*	rat lung EST	AA859109
5	18†	rat spleen EST	AA818361
6	7	similar to methyl CpG binding domain	AA819660
7	7	similar to mouse calponin 2 (*Cnn2*)	AI385133
8	M	mitochondrial cytochrome c oxidase 2 (*Cox2*)	AA859075
9	4	rat kidney EST, similar to mouse Unigene cluster Mn.21501	AA866308
10	N.D.	similar to heat shock binding protein 1	AA818369
11	4	kidney-specific androgen-regulated protein KAP	AI070884
12	20	inositol polyphosphate multikinase (*Ipmk*)	AA901117
13	16	lipoprotein lipase (*Lpl*)	L03294
14	2*	similar to mouse retinoblastoma binding protein 4 (*Rbbp4*)	AI112905
15	15	similar to mouse annexin7 (*Anxa7*)	AA900396
16	20	Nudix, (nucleotide diphosphate linked moiety X)-type motif 3 (*Nudt3*)	AA817821
17	18	rat brain EST	AI111946
18	2*	lecithin retinol acyltransferase (*Lrat*)	AA899076
19	2*	L-2 hydroxy acid oxidase (*Hao2*)	AI029336
20	6	similar to mouse EST AA675043	AI111879

The chromosomal locations, as determined by radiation hybrid mapping, of the 20 cDNAs showing the largest difference in renal mRNA expression between Dahl S and R rats are shown. Array clones 2, 6, and 13 were mapped by researchers at the University of Iowa (Sheffield/Soares group). * cDNA clones mapped within chromosomal regions introgressed into congenic strains. † Clone 5 mapped near a reported BP QTL (*42*). M, mitochondria. An LOD score of 12 was set as the threshold value for linkage in RH mapping. N.D., location not determined.

cDNA (band 5R) for which altered RNA expression was observed in all but one of the congenic rat strains and R rats, compared to S rats (expression pattern III).

Three cDNAs showed altered mRNA expression levels in the "double" congenic strain, S.WKY(2)M(10b), compared to mRNA expression levels observed in either congenic strain from which it was derived (expression pattern IV). The three genes showing this pattern of kidney mRNA expression included a previously undescribed cDNA (band 8S), a rat PC-12 cell EST (band 5G) and nucleoside diphosphate kinase (band 7B).

Radiation Hybrid Mapping of Differential Display cDNAs

Radiation hybrid (RH) mapping (*30*) was used to determine whether differentially expressed kidney cDNAs localized to BP QTL-containing chromosomal regions introgressed into congenic strains. RH mapping results for 14 of the differentially expressed cDNAs are shown in Table 2.

Three cDNAs, bands 5S-a, 5S-b and 6G-a, were linked to markers located in regions of normotensive-strain chromosomes that were introgressed into congenic rat strains (mapping to rat chromosomes 5, 3, and 2, respectively). One cDNA, band 2R, mapped near a known BP QTL on chromosome 8. Four cDNAs, bands 10B, 5R, 2G and 6G-b, mapped to chromosomes carrying BP QTL, but outside of the region of the normotensive-strain chromosome introgressed into those congenic rat strains. Six differentially expressed genes mapped to chromosomes where no congenic strains were available.

Candidate Gene Screening Using Rat cDNA Array Filters and Radiation Hybrid Mapping

Differentially expressed kidney genes in one-month-old S and R rats were also screened using the cDNA array technique (*18*). Duplicate filters containing an array of 5,184 rat cDNAs (Rat GeneFilter 300; Research Genetics) were hybridized with ^{33}P-labeled cDNA probes synthesized from kidney total RNA isolated from one-month-old S and R rats separately, and the expression profiles of the two filters were compared using the Pathways 2.0 computer program (Research Genetics). The 20 genes showing the greatest differences in mRNA expression between S and R rats were selected for further study (Table 4). A rat EST database (http://ratest/eng.uiowa.edu) was searched to identify differentially expressed cDNA clones having known chromosomal locations. Radiation hybrid (RH) mapping analysis was carried out for cDNAs lacking such mapping data using primers specific for each cDNA (Table 3). Table 4 summarizes the

Table 5. Renal *Hao2* Expression in One-Month-Old S, R and S.WKY-D2N35/Nep Rats on a Low Salt Diet

Strain	*N*	*Hao2* RNA expression
S	4	1.00＋0.04
R	3	0.75＋0.03
S.WKY-D2N35/Nep	4	0.50＋0.03
		p value
S *vs.* R		0.01
S *vs.* S.WKY-D2N35/Nep		＜0.0001

One-month-old S, S.WKY-D2N35/Nep, and R rats, maintained on a low salt (0.3% NaCl) diet from weaning, were fed either on low (0.3% NaCl) or high (4% NaCl) salt diets for 24 h. Northern filters of kidney RNA from these rats were hybridized with radiolabeled probes for *Hao2* and *Gapd*. *Hao2* mRNA expression levels were normalized for *Gapd* mRNA expression levels. The *Hao2* expression levels shown are relative values, where the value for S rats on a low salt diet was set at 1.00. *P* values were calculated using one-way ANOVA.

mapping results for the 18 genes.

Four array clones (4, 14, 18, and 19; Table 4) mapped to the region of the introgressed WKY-rat chromosome present in congenic strain S.WKY-D2N35/Nep, and were selected for examination of their mRNA expression pattern in the congenic strain panel. Three array clones (4, 14, and 18) showed a differential pattern of kidney mRNA expression in R rats and a subset of the congenic strain panel, compared to S rats (data not shown). However, none of the above three genes showed the congenic strain-specific pattern of differential mRNA expression predicted by their chromosomal location. In other words, we did not observe differential kidney mRNA expression in the S.WKY-D2N35/Nep congenic strain that carries the low BP allele(s) for the RNO2 BP QTL, compared to the S strain.

One gene (array clone 19), L-2 hydroxy amino oxidase (*Hao2*), mapped to the region of introgressed WKY-rat RNO2 present in the S.WKY-D2N35/Nep congenic strain, and showed differential kidney mRNA expression in R rats and only in a single congenic strain in the panel [S.WKY-D2N35/Nep], compared to S rats (Fig. 2, Table 5). Northern filter analysis of rat kidney samples using a rat *Hao2* cDNA probe showed the presence of two strong bands of 1.8 kb and 2.0 kb, similar in size to that reported by Belmouden *et al*. (*31*), but the origin of the size difference between the two transcripts is unknown (*31*). Their expression levels were similar within a given sample and the values for the 2.0 kb band are presented. Another gene (array clone 5), inositol polyphosphate multikinase (*Ipmk*), mapped to a region of rat chromosome 18 that was shown to contain a BP QTL in an F_2 (S×BN) population (*32*).

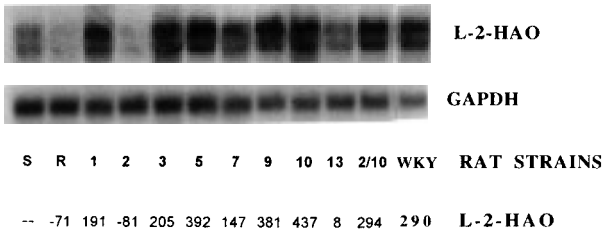

Fig. 2. *Expression of the RNO2 BP QTL candidate gene, L-2 hydroxy acid oxidase (*Hao2*), in a panel of congenic rat strains. Northern filters of kidney RNA from S, R, and congenic rats raised on low NaCl diets were hybridized with radiolabeled probes for* Hao2 *and* Gapd. Hao2 *mRNA expression levels were normalized for* Gapd *mRNA expression levels. Differences are expressed as a % difference [(congenic strain level−S rat level) / S rat level]×100. Abbreviations for rat strains and the protocols for Northern filter hybridization are the same as in Fig. 1.*

Genetic Mapping of *Hao2*

We used genetic linkage analysis to further refine the location of *Hao2* with respect to the endpoints of the regions of WKY-derived RNO2 in congenic strains S.WKY-D2N35/Nep and substrains (*33*). A full-length rat *Hao2* cDNA probe was used to identify an *Hao2*-containing PAC clone (441D18) from a rat PAC library. A library containing sequences from this PAC was screened to identify clones containing 1) dinucleotide repeats and 2) the 5′portion of *Hao2* that were then sequenced. We identified a clone containing the 5′portion of *Hao2* (nucleotides 1−90 numbered as in reference) (*31*), thereby confirming that the PAC clone did contain the *Hao2*.

A primer set, *D2Mco47*, was designed to amplify a sequence containing dinucleotide repeats and distinguished alleles carried by the S and WKY strains. *D2Mco47* was mapped in an F_2 (S×WKY) population to a similar location compared to that by RH mapping with primers specific for the 3′region of *Hao2* (Table 3), between the *D2Mgh10* and *Atp1a1* markers. *D2Mco47*, and by extension, the closely linked *Hao2* gene, mapped within the introgressed regions of two congenic substrains, S.WKY×3 and S.WKY×12, which contain, respectively, 20.1−21.7 cM and 6.6−7.6 cM of WKY-rat RNO2. The S.WKY×3 and S.WKY×12 congenic strains had BPs which were significantly lower than that of the S strain (*33*).

Discussion

Aitman and coworkers used differential gene expression in a congenic strain, in conjunction with RH mapping, to identify *cd36* as a candidate gene for an insulin-resistance QTL in the SHR model (*14*). In the present study we extended this approach, using a congenic strain panel, in which each strain

carried a different low BP QTL allele within an allelic composition that was otherwise similar to that of Dahl S rats, to identify candidate genes for BP QTL. We hypothesize that a gene responsible, in part, for the effect of a given BP QTL might be differentially expressed in a target organ, such as the kidney. If so, this gene should map to the chromosomal region introgressed into the congenic strain carrying that particular BP QTL.

No comparison of two inbred strains having contrasting phenotypes, *i.e.*, normotension and hypertension, will be ideal. This is because of the epistatic gene interactions that occur in the different genetic backgrounds carried by each strain, as well as the fixation of different sets of genes that are unrelated to the strain-differences in BP in the inbred strains compared. We used the Dahl R rat as a representative normotensive strain to screen for kidney genes that were differentially expressed, compared to Dahl S rats, because the R rat was the donor strain for half of the congenic strains in our panel (Table 1). As discussed above, strain differences between the hypertensive S strain and the congenic strains derived from it arise from 1) substitution of a "wild-type" allele for a mutant S-rat allele that is directly-causative of a BP QTL effect in the congenic strain, resulting in a reduction in BP and/or 2) substitution of a "mutant" allele from a normotensive strain for a "wild type" S-rat allele, where the gene product encode by the normotensive strain allele is directly-responsible for the BP reduction. Comparison of the gene expression between Dahl S and R rats, in theory, will allow us to analyze the first class of mutant alleles. However, we can use gene expression comparisons between Dahl S and R rats to analyze the second class of mutant alleles only for congenic strains derived by introgressing R-rat *chromosomes* or in which the R-rat allele and the normotensive strain allele present in the congenic region are functionally similar (as appears to be the case for *Hao2*).

Evaluating Candidate Genes

Two complementary techniques, differential display of mRNA and cDNA array hybridization, were used to identify differentially expressed kidney genes. Differential display of mRNA, which examines all the genes present in the tissue in an unbiased manner, was initially used to identify differentially expressed genes, which were then radiation hybrid-mapped to determine their chromosomal location. Later, we used cDNA array hybridization as a more rapid way to screen large numbers of genes. However, this technique is limited to examining the expression of those genes and ESTs that are presently known and available in the array. For our cDNA array study, only differentially expressed genes that mapped to chromosomal regions containing BP QTLs were examined to determine their kidney mRNA expression pattern in the congenic strain panel. This approach was taken because genes that do not map to the introgressed region of a congenic strain cannot be considered potential low BP QTL alleles carried by that congenic strain. This approach also allowed us to search the University of Iowa Rat EST database (http://ratest.uiowa.edu/) for the chromosomal location of some of the differentially expressed genes.

We evaluated differentially expressed cDNAs as candidate genes for BP QTL based on their chromosomal location rather than the magnitude of their observed differences in mRNA expression for the following reasons. Differential mRNA expression can be evaluated in four ways to judge genes as potential candidates: 1) by the magnitude of the differences in mRNA expression observed between the samples compared; 2) by the chromosomal location of the differentially expressed gene; 3) by the importance of the tissue/organ in regulating the expression of the quantitative trait; and 4) by the temporal/developmental point when gene expression is studied. Clearly, identifying genes differentially expressed in a particular tissue is only useful if the tissue is critical in determining the expression of the quantitative trait. And it is also clear that a gene must map to a chromosomal region containing a QTL to be considered a candidate gene responsible for, at least in part, the effect of that particular QTL. The magnitude of expression differences is the most problematic of the three criteria to apply, because there is no clear way to set thresholds for differences in the magnitude of expression for use as a criterion for selecting genes for further study. The chromosomal location and the organ in which mRNA expression was studied are, therefore, much more important for selecting candidate genes. Finally, the time selected for the analysis of differential gene expression will be critical, particularly for identifying the genes responsible for the differences in the quantitative trait, rather than studying genes whose expression is altered as a consequence of the differences in the quantitative trait. Thus, gene expression was studied in weanling inbred and congenic strains maintained on a low NaCl diet prior to the accumulation of organ damage resulting from BP elevation in the S strain.

Hao2 as a Candidate Gene

We identified seven genes showing congenic-strain specific patterns of expression (Table 2 and Fig. 2). Five of these genes (2S, 6G-a, 10B, 5S-a, and array clone 19) showed an altered expression similar to that of R rats in terms of the direction of the change, while two genes did not. Only three (10B, 5S-b and array clone 19) of these seven genes mapped to the chromosomes predicted by our hypothesis (*34*). While clone 10B showed a congenic strain-specific pattern of differential expression in the kidney and mapped to RNO2 as was expected (*34*), it did not map to the introgressed region of WKY-derived RNO2 present in congenic strain S.WKY-D2N35/Nep. On the other hand, clone 5S-b mapped to the region of the R-rat derived chromosome carried by the S-R-Edn3 congenic strain and showed a rat chromosome 3 (RNO3) congenic-specific expression pattern. However, clone 5S-b was subsequently eliminated as a potential candi-

date gene because it did not map in the congenic regions of several subsequently derived congenic substrains that had a decreased BP compared to the parental S strain (unpublished observations). Clone 5S-b also mapped to a region of R-rat derived RNO3 introgressed into a congenic substrain whose BP was not decreased compared to the S strain (unpublished observations).

Only one gene (array clone 19), *Hao2*, showed both a congenic strain-specific pattern of differential expression in the kidney (only in S.WKY-D2N35/Nep), compared to the S strain, and mapped to the region of WKY-rat RNO2 introgressed into that congenic strain. Thus, *Hao2* can be considered a candidate gene that may be responsible, in part, for this RNO2 BP QTL. The candidacy of this gene is further supported by the presence of *Hao2* in the introgressed regions of smaller congenic substrains (S.WKY×3 and S.WKY×12) derived from S.WKY-D2N35/NEP, both of which substrains show lower BP than the parental S strain.

Hao2 is an FMN-dependent enzyme that oxidizes a variety of L-2 hydroxy acids into keto acids at the expense of O_2, forming H_2O_2 in the process. In rats, this peroxisomal enzyme is only expressed in the kidney (*31*), although humans possess two homologous *Hao2* genes that are expressed in both the liver and kidney (*35*). The rat Hao2 enzyme showed broad substrate specificities *in vitro*, although its natural substrates remain unknown (*36*). Methyl guanidine synthase, recently reported as identical in sequence to *Hao2* (*37*), has been previously implicated in hypertension. Its product, methyl guanidine, inhibits nitric oxide synthase, which produces the potent vasodilator, nitric oxide (*38*). In the present study, the high expression of *Hao2* RNA observed in S-rat kidneys may have been associated with an increased production of methyl guanidine, which in turn could have inhibited nitric oxide production by nitric oxide synthase to a greater extent in S rats than in S.WKY-D2N35/NEP rats, the congenic strain carrying the low BP allele for the RNO2 QTL. Lower production of nitric oxide could contribute to the higher BP observed in S rats compared to S.WKY-D2N35/NEP rats (*11*). The recent report of decreased inducible nitric oxide synthase protein in the kidneys of S compared to R rats supports this notion (*39*). In the present work, however, kidney *Hao2* mRNA expression was higher in WKY than in S rats (Fig. 2), possibly due to the influence of different alleles present in the genetic background of the WKY strain. This suggests that the lower BP observed in the S.WKY-D2N35/NEP, compared to the parental S strain, may have been due to substitution of the S-rat *Hao2* allele and its associated *cis*-regulatory elements (*i.e.*, promoter and enhancer).

Six cDNAs identified by differential display of mRNA (bands 2S, 10B, 6G-a, 5S-a, 5S-b, and 6G-b; Table 2) showed a congenic strain-specific pattern of differential kidney mRNA expression but mapped neither to the congenic region of particular chromosomes predicted by our hypothesis nor to the congenic region of substrains. This congenic strain-specific pattern of expression may reflect involvement of these genes in a pathway of BP regulation specific for each congenic strain and defined by the QTL or QTLs introgressed into the specific congenic strain. These may be "downstream genes" whose expression levels were affected by different BP QTL-causative genes, perhaps representing intermediate phenotypes related to different pathways of BP regulation in the congenic strain. Interestingly, two of these genes (bands 6G-a and 5S-a) mapped to regions of normotensive-strain chromosomes that were introgressed into congenic strains and may be potential candidate genes for the BP QTLs located on RNO2 and rat chromosome 5 (RNO5). Because band 2S, *CoxI*, is mitochondrially encoded and therefore does not segregate (*40*), its altered kidney mRNA expression must be in response to another factor. This could perhaps be a gene(s) present in the region of RNO5 introgressed in S.LEW(*5*), the only congenic strain showing decreased *CoxI* mRNA expression.

Other Patterns of Differential Gene Expression

Ten genes identified by differential display of mRNA (group II; Table 2) and three genes identified by cDNA array hybridization (array clones 4, 14, 18; Table 4) showed a pattern of expression wherein altered kidney mRNA expression levels were seen in a subset of the congenic strain panel, compared to S rats. Such genes may participate in a common pathway of BP regulation shared by that subset of the congenic strain panel. The expression patterns of such genes could be influenced by several different low BP alleles—each for a different BP QTL—present in this subset of congenic strains. In this case, each BP QTL contains gene(s) that influence events in the same pathway, leading to altered expression of "downstream" genes.

Nucleoside diphosphate kinase (band 7B; Table 2), and succinyl CoA synthase (band 12B; Table 2) form complexes in which both enzyme activities are functional (*41*), and these complexes have been proposed to channel phosphate bond energy from NTPs into the tricarboxylic acid cycle, as well as to promote the reverse reaction. The kidney mRNA expression patterns of these two genes in the congenic panel were consistent with coordinated expression (Table 2), showing similarly altered RNA expression levels, in terms of both magnitude and direction, in all but three of the congenic strains. It is possible that the disruption of the coexpression for these two enzymes observed in the three congenic strains may be related to a common intermediate phenotype.

In conclusion, we have described a new approach that identified *Hao2* as a candidate gene that may be responsible, in part, for an RNO2 BP QTL. This was accomplished by identifying differentially expressed genes in a target organ, the kidney, examining their expression using a panel of congenic strains carrying low BP alleles for different QTLs, and radiation hybrid mapping. In addition, our identification of genes showing differential kidney mRNA expression pat-

terns among the congenic strains in the panel suggests that different mechanisms of BP regulation may operate in these congenic strains. This approach may also be applied to examining gene expression in other organs that play key roles in the regulation of BP.

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