Defining a rat blood pressure quantitative trait locus to a <81.8 kb congenic segment: comprehensive sequencing and renal transcriptome analysis

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Submitted 25 June 2010; accepted in final form 12 August 2010

Gopalakrishnan K, Saikumar J, Peters CG, Kumarasamy S, Farms P, Yerga-Woolwine S, Toland EJ, Schnackel W, Giovannucci DR, Joe B. Defining a rat blood pressure quantitative trait locus to a <81.8 kb congenic segment: comprehensive sequencing and renal transcriptome analysis. Physiol Genomics 42A: 153–161, 2010. First published August 17, 2010; doi:10.1152/physiolgenomics.00122.2010.—Evidence from multiple linkage and genome-wide association studies suggest that human chromosome 2 (HSA2) contains alleles that influence blood pressure (BP). Homologous to a large segment of HSA2 is rat chromosome 9 (RNO9), to which a BP quantitative trait locus (QTL) was previously mapped. The objective of the current study was to further resolve this BP QTL. Eleven congenic strains with introgressed segments spanning <81.8 kb to <1.33 Mb were developed by introgressing genomic segments of RNO9 from the Dahl salt-resistant (R) rat onto the genome of the Dahl salt-sensitive (S) rat and tested for BP. The congenic strain with the shortest introgressed segment spanning <81.8 kb significantly lowered BP of the hypertensive S rat by 25 mmHg and significantly increased its mean survival by 45 days. In contrast, two other congenic strains had increased BP compared with the S. We focused on the <81.8 kb congenic strain, which represents the shortest genomic segment to which a BP QTL has been mapped to date in any species. Sequencing of this entire region in both S and R rats detected 563 variants. The region did not contain any known or predicted rat protein coding genes. Furthermore, a whole genome renal transcriptome analysis between the S and the <81.8 kb S.R congenic strain revealed alterations in several critical genes implicated in renal homeostasis. Taken together, our results provide the basis for future studies to examine the relationship between the candidate variants within the QTL region and the renal differentially expressed genes as potential causal mechanisms for BP regulation.

MATERIALS AND METHODS

Animals. All animals were cared for as per approved protocols by the University of Toledo Health Science Campus Institutional Animal Care and Use Committee (IACUC), and all animal study protocols were reviewed and approved by the IACUC. S rats were from our animal colony. The congenic substrains for this study were derived from either S.R(9)x3x2C or S.R(D9Mc95-Resp18), which was previously referred to as S.R(9)x3x2Bx1(11). In brief, the parental congenic strain was crossed with S to generate a population of F1 animals. These F1 animals were then intercrossed to generate an F2 population. The F2 animals were genotyped using microsatellite markers throughout the region on RNO9 from 73140156–74554694 (Fig. 1). The recombinant F2 animals with various introgressed regions of the R alleles were backcrossed with S to “trap” the recombinant chromosome, genotyped, and intercrossed to obtain animals that are homozygous for the introgressed R region on the S genomic background.

BP measurements. Each set of congenic substrains (n = 20 males) and control S rats (n = 20 males) were bred, housed, and studied concomitantly to minimize environmental effects. Rats were weaned at 30 days of age and given a low-salt diet (0.3% NaCl, Harlan Teklad). At 40–42 days of age rats were fed 2% NaCl diet (Harlan Teklad) for 24 days. Systolic BP was measured using the tail-cuff method commencing on the 25th day (4). Briefly, conscious restrained rats were warmed to 28°C. The BP of each rat was measured for 4 consecutive days by two blinded operators. BP values for each day were the mean of three or four consistent readings. The final BP value used was the mean of the four daily BP values. The day after the last

INHERITANCE OF HYPERTENSION in both humans and mammalian models is a well-established observation yet one of the most difficult to comprehend with respect to the causal elements on the genome. A recent thrust in genome-wide association studies (GWAS) has attempted to address this challenge by two measures, i.e., by increasing sample size and by increasing the density of single nucleotide polymorphisms (SNPs) analyzed. While these designs favor identification of additional loci associated with blood pressure (BP), such associations require further experimental validation for cause-effect relationships.

Rat models of hypertension are used to delineate the genetic contributions to BP. Evidence through classical linkage fol-

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BP measurements, rats were euthanized and heart weights were recorded.

BP was also collected using a telemetry system (Data Sciences International, St. Paul, MN) as explained in detail previously (20). Briefly, 4 days after the BP measurements by the tail cuff method, rats S(n/H11005-10) and congenic rats (n/H11005-11) were surgically implanted with transmitters into the left flanks, and the probes were inserted through each animal’s femoral artery and advanced to the lower abdominal aorta. Rats were allowed to recover from surgery for 1 wk before the transmitters were turned on and BP data were collected for 4 consecutive days. All statistical analyses were as previously reported (20).

**Urinary protein excretion.** We fed 40-day-old S and S.R(D9Mco95-D9Mco98) rats (n = 18/group) with 2% NaCl diet (Harlan Teklad diet) for 12 days. On day 13 these animals were caged individually in metabolic cages (Lab Products, Seaford, DE) with free access to water to collect urine samples. Urine was collected in the presence of

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**Fig. 1. Substitution mapping of rat chromosome 9 (RNO9).** Polymorphic microsatellite markers along with their locations on the rat genome according to the Ensembl database (http://www.ensembl.org, Nov. 2009) are shown to the left. Congenic strains are represented as colored bars: green, strains that lowered blood pressure (BP) of the S rat; black, strains that increased the BP of the S rat; and grey, strains that did not show a change in BP compared with the S. White boxes flanking the colored bars are regions of recombination. The BP effect of each strain is given at the bottom except for S.R(D9Mco95-Resp18), the data for which have been previously published. Details of the effects are given in Table 1. S, Dahl salt sensitive; R, Dahl salt resistant; S.R, congenic strains.
~0.01% sodium azide over a period of 24 h, and their volumes were recorded. Total protein and blood urea nitrogen (BUN) levels were analyzed using an automated analyzer at the Department of Pathology, University of Toledo College of Medicine.

Survival studies. S (n = 7) and S.R(D9Mco95-D9Mco98) (n = 7) rats were raised and administered 2% salt as described under the BP measurements. These rats were continued on the 2% salt diet until their death. Data collected were analyzed statistically using the SPSS software.

Isolation of DNA and genotyping. DNA was extracted from rat tail tissue samples in 96-well formats using the Wizard SV 96 Genomic DNA purification system (Promega). Polymorphic microsatellite markers between S and R within the desired genomic segment were PCR amplified from tail DNA samples and resolved on acrylamide gels as per previously published procedures (37).

Sequencing: Primers were designed to amplify PCR products that were ~800–1100 bp covering the entire 81.8 kb region (http://frodo.wi.mit.edu/primer3/). Each primer was attached with an M13 forward transcription to cDNA using Superscript III (Invitrogen) followed by PCR using two sets of primers designed manually to amplify the overlapping expressed sequence tags (ESTs): CB585071, CB581681, CB750247, AA874825, and AI599340. The primer sequences were as follows: sense primer 1 5’ATGAATGGTAGTATCTGAAATG3’ and antisense primer 1 5’GGCCTACAGTTTATTAATACACAT3’; sense primer 2 5’ATGATGACCTCAGTTGAAATG3’ and antisense primer 2 5’CAACGCCTTACACTGAGCTAA3’.

GeneChip microarray experiment and data analysis. Six S and 6 S.R(D9Mco95-D9Mco98) rats were randomly selected from the group of animals used for urine collection described above. The day after urine collection, these rats were euthanized for isolation of kidneys. RNA was isolated from the kidneys using TRIzol and RNeasy kits (Qiagen). The isolated RNA from two animals were pooled together and considered as one biological sample. Three such RNA samples from S and congenic rats were used for the cRNA preparation. cRNA was prepared and fragmented as suggested by the Affymetrix technical manual and simultaneously hybridized (15 μg adjusted cRNA for each chip) to Rat Expression Array 230 2.0 (3’IVT Expression Analysis). Statistical analyses of the microarray data were performed using R statistical package (version 2.8.1), and the data were further analyzed using Ingenuity Pathway Analysis software. The microarray dataset at the Gene Expression Omnibus database is assigned the accession number GSE22515.

RESULTS

R rat alleles within 81.8 kb decrease BP of the S rat. A total of 11 new congenic substrains were developed for this study as detailed in MATERIALS AND METHODS (Fig. 1). Only one of the 11 congenic substrains had a significant lowering effect on BP. The congenic strain S.R(D9Mco95-D9Mco98), which demonstrated the BP lowering effect of −25 mmHg (P < 0.001, Table 1) compared with S, had a very short introgressed segment of <81.8 kb (Fig. 1). The BP effect of S.R(D9Mco95-D9Mco98) was also tested by the telemetry method. The systolic, diastolic, and mean arterial pressure (MAP) of S.R(D9Mco95-D9Mco98) rats was statistically different from that of S rats.

Table 1. Observed effects of rat chromosome 9 congenic strains on systolic BP

<table>
<thead>
<tr>
<th>Congenic Strain</th>
<th>n</th>
<th>Blood Pressure, mmHg</th>
<th>Effect</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.R(D9Mco72-Resp18)</td>
<td>30</td>
<td>220 [3.34]</td>
<td>-5</td>
<td>0.988</td>
</tr>
<tr>
<td>S.R(D9Mco14-Resp18-1)</td>
<td>30</td>
<td>212 [4.52]</td>
<td>-3</td>
<td>0.969</td>
</tr>
<tr>
<td>S.R(D9Mco14-Resp18-2)</td>
<td>20</td>
<td>208 [4.08]</td>
<td>-2</td>
<td>0.905</td>
</tr>
<tr>
<td>S.R(D9Mco14-Resp18-3)</td>
<td>20</td>
<td>212 [4.52]</td>
<td>-1</td>
<td>1.000</td>
</tr>
<tr>
<td>S.R(D9Mco95-D9Mco98)</td>
<td>20</td>
<td>219 [4.43]</td>
<td>-25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S.R(D9Mco98-Resp18-1)</td>
<td>19</td>
<td>190 [6.32]</td>
<td>18</td>
<td>0.044</td>
</tr>
<tr>
<td>S.R(D9Mco98-Resp18-2)</td>
<td>20</td>
<td>199 [5.19]</td>
<td>18</td>
<td>0.032</td>
</tr>
<tr>
<td>S.R(D9Mco95-D9Mco100-1)</td>
<td>20</td>
<td>204 [4.97]</td>
<td>4</td>
<td>0.533</td>
</tr>
<tr>
<td>S.R(D9Mco95-D9Mco100-2)</td>
<td>20</td>
<td>219 [4.43]</td>
<td>-7</td>
<td>0.638</td>
</tr>
<tr>
<td>S.R(D9Mco95-D9Mco102)</td>
<td>20</td>
<td>199 [5.19]</td>
<td>4</td>
<td>0.789</td>
</tr>
<tr>
<td>S.R(D9Mco101-Resp18)</td>
<td>20</td>
<td>208 [4.08]</td>
<td>-5</td>
<td>0.641</td>
</tr>
</tbody>
</table>

Blood pressure (BP) values are means ± SE. Effect, congenic value–Dahl salt-sensitive (S) value. Negative values indicate a decrease in BP compared with the S rat, whereas positive values indicate an increase in BP compared with the S rat. Only male rats were used. Independent t-test was used to compare the means when only 2 strains were in an experiment and ANOVA was used to compare the means when there were more than 2 strains in a single experiment.

The online version of this article contains supplemental material.
BP of this strain was significantly lower than that of the S as measured by telemetry ($P < 0.001$, Fig. 2). Heart weights of S.R(D9Mco95-D9Mco98) (1.25 ± 0.034) were significantly lower than that of the S (1.37 ± 0.018) ($P = 0.003$).

Alleles of the R rat adjacent to the 81.8 kb region increase BP of the S rat. A 1.33 Mb interval distal to the 81.8 kb region on RNO9 described above is represented by two of the congenic strains, S.R(D9Mco98-Resp18-1) and S.R(D9Mco98-Resp18-2). BP of both of these strains was significantly higher than that of the S by 18 mmHg ($P = 0.044$ and 0.032, respectively) (Fig. 1, Table 1). These strains do not overlap with S.R(D9Mco95-D9Mco98), which lowers BP. The BP of the other nine strains shown in Fig. 1 was not significantly different from that of the S.

Congenic rats with R alleles in the 81.8 kb region survive longer than the S. In addition to having lower BP compared with the S, the S.R(D9Mco95-D9Mco98) congenic rats survived significantly longer than the S (Fig. 3). The mean survival of the S.R(D9Mco95-D9Mco98) congenic rats was 137 days, whereas that of the S rat was 92 days. The difference in survival of 45 days was highly significant ($P = 0.0004$).

The <81.8 kb critical region. The <81.8 kb critical region in the context of the logarithm of the odds (LOD) plot obtained by linkage analysis of S and R rats and subsequent substitution mapping studies is shown in Fig. 4. This region lies between 73140156 and 73221915 bp on the q33 cytogenetic band of RNO9 and is homologous to 218561830–218650679 bp on human chromosome 2q35 and 73856916–73949046 bp on mouse chromosome 1. VISTA plots suggest that there are stretches of evolutionarily conserved sequences across mammals within this region (supplementary Fig. S1). There is one reported variant within the critical <81.8 kb region between S and R (G/C, ENSRNOSNP2795799 at 73186288 bp, http://snplotyper.mcw.edu). Sequencing of the <81.8 kb region detected 563 allelic variants between S and R including the reported SNP (Supplemental Table S2). The <81.8 kb region of S.R(D9Mco95-D9Mco98) contains no rat protein coding gene annotations but has six ESTs. One of the six ESTs, BF397395, contains two SNPs between S and R at 73160114 and 73160151 (Supplementary Table S2), but this EST does not align with any known genes in any species. The remaining five ESTs are overlapping. Interestingly, all the five overlapping ESTs were located within a 640 bp genomic segment on RNO9, which was identical to a genomic segment between 57762964 and 57763603 bp on RNO2 except at two loci, i.e., T on RNO2 at 57763252 to G on RNO9 at 73260976 and T on RNO2 at 57763416 to G on RNO9 at 73207139. Since all the five overlapping ESTs were assembled only on RNO9 (none on RNO2), we designed primers to amplify any expressed sequences from cDNA of both S and R rats, anticipating amplification of sequences that matched the nucleotides on RNO9, i.e., G on RNO9 at 73206976 and G on RNO9 at 73207139. Instead, sequencing of the PCR products revealed that all the ESTs amplified from both S and R rats...
contained T on RNO2 at 57763252 and T on RNO2 at 57763416, suggesting that these ESTs listed on RNO9 may indeed be transcribed from RNO2 but not from RNO9. 5’-RACE experiments were designed to amplify extended transcripts from each of these ESTs. While the positive control resulted in amplification of the entire transcript of GAPDH, there were no products obtained from any of the ESTs. These results are in concordance with the observation that there are no reported protein coding genes identified by comparative mapping with the mouse genome. However, the homologous region on human chromosome 2 contains a gene, DIRC3 (disrupted in renal carcinoma 3, Entrez Gene ID: 729582). The protein coding sequence of human DIRC3 has 4 exons. Only the fourth exon has two alignments with 83–90% homology to two stretches of sequences within the <81.8 kb region (73146699–73146743 and 73146751–73146792, respectively). The critical region does not contain any known miRNAs but contains several SINE and LINE elements as reported by the UCSC browser (http://genome.ucsc.edu). Some of these elements are highly conserved between rat, mouse, and human (Supplementary Fig. S1). There are four gaps in the reference sequence assembly of the <81.8 kb region. These are between 73178596 and 73178645 bp, 73187710 and 73187759 bp, 73206637 and 73206686 bp, and 73209272 and 73209321 bp (http://genome.ucsc.edu).

Renal transcriptome analysis. Young, S.R(D9Mc095-D9Mco98) congenic rats that were 54 days of age had an early trend for
lower total urinary protein and BUN levels (54.9 and 96.15 mg/ml/day, respectively) compared with the S (80.5 and 145.2 mg/ml/day, respectively). We therefore examined early changes in the renal transcriptomes between the S and S.R(D9Mco95-D9Mco98) congenic rats. Whole genome microarray analysis of S and S.R(D9Mco95-D9Mco98) revealed that 1,000 genes were differentially expressed. The top list of up- and downregulated genes is given in Table 2. The most upregulated gene (10-fold) in the kidney of the congenic strain was aldo-keto reductase family 1, member B7 (Akr1b7) (Table 2). Akr1b7 was associated with a network involved in acute phase/inflammatory response signaling including transcripts of heat shock proteins (Hspa1a, Hspa1b, Hsp1); Serpine1, complement factor B (Cfb); Stanniocalcin-1 (Stc1), a known renal protective anti-inflammatory molecule (16, 45); Gremlin-1 (Grem1), associated with TGF-β signaling and kidney development; and another member number C14 of the aldo-keto reductase family 1 (Akr1c14) (Fig. 5). The most downregulated transcript in the congenic strain compared with the S was

![Fig. 5. The list of upregulated genes in the congenic strain compared with S given in Table 2 were modeled using Ingenuity Pathway Analysis. Upregulated genes are shown in shades of red based on the fold-change in expression.](http://physiolgenomics.physiology.org/)

Table 2. Differentially expressed genes in the kidneys of S.R(D9Mco95-D9Mco98) compared with S

<table>
<thead>
<tr>
<th>Probe Set ID</th>
<th>Fold-change</th>
<th>P Value</th>
<th>Gene Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1368569_at</td>
<td>10.450</td>
<td>0.0283</td>
<td>Akr1b7, aldo-keto reductase family 1, member B7</td>
</tr>
<tr>
<td>1396101_at</td>
<td>2.051</td>
<td>0.0296</td>
<td>Stc1, stanniocalcin 1</td>
</tr>
<tr>
<td>1368247_at</td>
<td>2.050</td>
<td>0.0008</td>
<td>Hspa1a, heat shock 70 kDa protein 1A</td>
</tr>
<tr>
<td>1377404_at</td>
<td>1.854</td>
<td>0.0073</td>
<td>Stc1, stanniocalcin 1</td>
</tr>
<tr>
<td>1370912_at</td>
<td>1.733</td>
<td>0.0005</td>
<td>Hspa1b, heat shock 70 kDa protein 1B (mapped)</td>
</tr>
<tr>
<td>1385620_at</td>
<td>1.568</td>
<td>0.0105</td>
<td>Hsp1, heat shock 105 kDa/110 kDa protein 1</td>
</tr>
<tr>
<td>13770708_a_at</td>
<td>1.699</td>
<td>0.0071</td>
<td>Akr1c14, aldo-keto reductase family 1, member C14</td>
</tr>
<tr>
<td>1369633_s_at</td>
<td>1.548</td>
<td>0.0144</td>
<td>Akr1c14, aldo-keto reductase family 1, member C14</td>
</tr>
<tr>
<td>1368519_at</td>
<td>1.534</td>
<td>0.0080</td>
<td>Serpine1, serine (or cysteine) peptidase inhibitor, clade E, member 1</td>
</tr>
<tr>
<td>1389470_at</td>
<td>1.511</td>
<td>0.0213</td>
<td>Cfb, complement factor B</td>
</tr>
<tr>
<td>1369113_at</td>
<td>1.505</td>
<td>0.0015</td>
<td>Greml1, gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis) melan-A</td>
</tr>
<tr>
<td>1390596_at</td>
<td>1.500</td>
<td>0.0012</td>
<td>Mlap, DnaJ (Hsp40) homolog, subfamily B, member 1</td>
</tr>
<tr>
<td>1383302_at</td>
<td>1.493</td>
<td>0.0030</td>
<td>DnaJ, uncoupling protein 1 (mitochondrial, proton carrier)</td>
</tr>
<tr>
<td>1387033_at</td>
<td>-1.975</td>
<td>0.0414</td>
<td>Ucp1, ST3 beta-galactoside alpha-2,3-sialyltransferase 2</td>
</tr>
<tr>
<td>1369732_a_at</td>
<td>-1.664</td>
<td>0.0079</td>
<td>St3gal2, 3-phosphoinositide-dependent protein kinase-1</td>
</tr>
<tr>
<td>1370052_at</td>
<td>-1.484</td>
<td>0.0133</td>
<td>Pdpk1, signal recognition particle 72</td>
</tr>
<tr>
<td>1375552_at</td>
<td>-1.481</td>
<td>0.0213</td>
<td>Snr72, similar to transcription factor 7-like 2, T-cell specific, HMG-box</td>
</tr>
<tr>
<td>1392592_at</td>
<td>-1.456</td>
<td>0.0467</td>
<td>LOC679869, small proline-rich protein 1A-like</td>
</tr>
<tr>
<td>1371248_at</td>
<td>-1.426</td>
<td>0.0052</td>
<td>Sprr1al, solute carrier family 36 (proton/amino acid symporter), member 2</td>
</tr>
<tr>
<td>1393663_at</td>
<td>-1.418</td>
<td>0.0187</td>
<td>St3gal2, similar to hepatocellular carcinoma-associated antigen 58 homolog</td>
</tr>
<tr>
<td>1377532_at</td>
<td>-1.399</td>
<td>0.0177</td>
<td>RGD1305020, UDP glucuronosyltransferase 2 family, polypeptide B36</td>
</tr>
<tr>
<td>1368397_at</td>
<td>-1.369</td>
<td>0.0091</td>
<td>Ugt2b36, E74-like factor 5</td>
</tr>
<tr>
<td>1383974_at</td>
<td>-1.365</td>
<td>0.0075</td>
<td>Elf5, similar to transcription factor 7-like 2, T-cell specific, HMG-box</td>
</tr>
</tbody>
</table>

For the purpose of comparison, S rat array sets were arbitrarily assigned as the base line and the S.R(D9Mco95-D9Mco98) (congenic rat) array sets as the experimental arrays. Thus, a positive number for fold-change indicates that the expression in the congenic strain was higher than that in the S and a negative number indicates that the expression in the congenic strain was lower than that in the S.
uncoupling protein 1 (Ucp1). Other downregulated genes are listed in Table 2. Pathway analysis points to downregulation of at least eight transcripts (Table 2) in a network associated with several renal phenotypes (Fig. 6).

DISCUSSION

Genetic analysis of inherited hypertension in humans has largely expanded in recent years through large-scale GWAS. Results from multiple methods to assess the genomic risk for development of hypertension including whole genome (2, 28, 30, 42, 47), haplotypic (3), and pathway-based (44) GWAS analyses and gene-based analyses (42, 47) converge with strong evidence to suggest that genetic contributions to BP is largely explained by a previously underestimated number of alleles, most lacking major effects per se. Modeling genetics of hypertension in rats has indicated a similar scenario (7, 9, 13, 18, 19, 22, 33, 43).

Ascertaining whether the contribution of a locus to BP is causal or consequential is crucial to understanding the etiology of hypertension. This has not been achieved by any other means to date other than by substitution mapping (5, 6, 8, 10, 12, 21, 32, 35). The current study represents one such sustained, rigorous substitution mapping of a BP QTL on RNO9, which has resulted in “trapping” alleles of the R rat on the S rat genetic background that results in lowering of BP compared with that of the S rat. The significance of this study is that the critical mapped segment of 81.8 kb is unparalleled in terms (1) resolution of mapping BP QTLs that are the sole introgressed segments within congenic strains; 2) the introgressed segment not containing any known rat protein coding genes; 3) sequencing of a critical QTL interval for identifying candidate variants; and 4) renal transcriptome depicting alterations in renal homeostasis.

While the current mapping study provides evidence for the presence of a BP QTL between S and R rats within a very short segment of 81.8 kb, it also suggests that there are additional, yet undetected allelic variants of S and R as BP QTLs on RNO9. Two of the strains shown in Fig. 1 that possess R alleles other than within the <81.8 kb region increase BP of the S rat. This indicates the presence of at least one other R allele in the immediate vicinity of the <81.8 kb region, which has an opposite effect of increasing BP of the S rat.

In a previous report (11), a 117 kb region on RNO9 between 74.584 (D9Mco14) and 74.701 Mb (Resp18-Intron2) was prioritized as the BP QTL based on this region being shared between two congenic strains [S.R(D9Mco95-Resp18) and S.R(9)x3x2C], both of which demonstrated a BP lowering effect. This method of mapping is described as the common-segment method by a recent report (38). However, congenic strains that were constructed either to span or to represent segments within the 117 kb region did not validate this localization [Fig. 1, S.R(D9Mco72-Resp18), S.R(D9Mco14-Resp18-1), S.R(D9Mco14-Resp18-2) or S.R(D9Mco14-Resp18-3)]. Our data therefore clearly support the demerits of the common-segment method of mapping QTLs using congenic strains (38) and suggest that BP lowering effect of the two congenic strains S.R(D9Mco95-Resp18) and S.R(9)x3x2C is attributed to the R alleles that are not common between the two strains. The finding of a BP lowering effect demonstrated by a substrain of S.R(D9Mco95-Resp18), i.e., S.R(D9Mco95-D9Mco98), which does not share R alleles with S.R(9)x3x2C, supports this interpretation.

Another interesting observation is that, similar to a previous substitution mapping study from our laboratory, the location of the critical <81.8 kb region (Fig. 4) is not within the confidence interval of the LOD peak for linkage to BP detected in the original analysis between S and R rats. The reasons for this are detailed elsewhere (21) and also applicable to the current study. Briefly, this observation suggests that the LOD peak is configured as a “ghost” peak generated between at least two

Fig. 6. The list of downregulated genes in the congenic strain compared with S given in Table 2 were modeled using Ingenuity Pathway Analysis. Downregulated genes are shown in shades of green based on the fold-change in expression.
adjacent QTLs instead of directly over either one of the QTLs (14, 23, 46), once again providing evidence for the existence of more than one BP QTL on RNO9.

Congenic strains continue to be used extensively to map several complex traits. It should be mentioned, however, that congenic strains are not devoid of residual heterozygosity or passenger cryptic loci (40, 41). The minimal congenic strain reported in our study has undergone a total of 18 backcrosses. Nevertheless, it is still possible that residual heterozygosity may also be contributing to the observed phenotype. In evaluating the applicability of congenic strains, Shao et al. (38) have recently proposed a sequential method as being a better method than the common-segment method. Both of these methods compare and contrast introgressed segments of two congenic strains to locate the QTL. Given that a number of QTL mapping studies are reporting the identification of multiple closely linked QTLs, some with opposing effects and/or epistasis, our view is that both the common-segment method and the sequential method ultimately require proof with minimal congenic strains such as the <81.8 kb congenic strain described in the current study.

Most, if not all, of the genetic studies of hypertension are typically conducted with a “gene-centric” view, i.e., focused on finding candidate protein coding genes as candidate BP QTLs. However, GWAS of hypertension in humans suggest that variants associated with BP are not necessarily all within protein coding genes. Although our study is by no means exhaustive so as to completely exclude the presence of a rat protein coding gene within the critical interval, the current observation of localization of a rat BP QTL to a region with very limited evidence for the presence of a protein coding gene lends support to noncoding variants to be also examined closely as BP QTLs within the critical <81.8 kb region.

The homologous regions of the <81.8 kb region lies on chromosomes 1 and 2 of mice and humans, respectively (Fig. 4). There are no GWAS that point to the homologous region of the rat <81.8 kb critical region as regions associated with BP. However, among the human linkage studies that point to HSA2, LOD peaks reported for BP in the Samouns (1), Amish (15), and, to some extent, the Finnish (31) populations are in the homologous region of the rat <81.8 kb BP QTL. Although the rat data, as per the current predictions, do not indicate a protein-coding candidate gene, the homologous human segment contains a gene, DIRC3, and therefore it cannot be excluded as a candidate for the homologous human BP QTL. The function of DIRC3 is unknown. Subtractive hybridization or RNA capture in sequence-capture chips needs to be applied to this project to detect any genes that are not yet annotated.

Proteinuria between the S and S.R(D9Mco95-D9Mco98) was not significantly different but only had a decreased trend in the congenic strain compared with S. Because this was observed in young, 54-day-old rats, it was possible that early changes in the kidney transcriptome could reflect on pathways that are affected by the BP QTL. The results suggested that some molecules involved in the renal acute phase/inflammatory response (17, 27, 29, 36, 39, 45) and other renal phenotypes were differentially expressed. It is admittedly quite difficult to interpret these data as the causative pathway due to the limited nature of examination of all other organs and tissues involved in BP regulation. Further, extensive work will be required to understand the mechanism of the fine-mapped BP QTL.

Overall, the mapping and extensive sequencing results of the current study provide the basis for future studies on variants detected within the minimal congenic BP QTL region to be explored as novel candidate quantitative trait nucleotides (QTNs). This study provides evidence for localization of a BP QTL to a high degree of resolution. Further resolving the mapped <81.8 kb region solely by the congenic approach is clearly not an expedient approach. While bioinformatic methods may suggest new protein-coding gene annotations in future or predict potential functionality of noncoding elements, functional testing of such elements as BP QTLs would require the development and application of complementary genetic engineering approaches such as transgenesis, zinc finger nuclease-based knock-out, or knock-in models to test the variants within the critical interval as BP QTNs.

ACKNOWLEDGMENTS

The authors thank the Rat Genome Database team for support with bioinformatic analysis.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-020176 and HL-076709 (to B. Joe).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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