Two Linked Blood Pressure Quantitative Trait Loci on Chromosome 10 Defined by Dahl Rat Congenic Strains

Michael R. Garrett, Xiaotong Zhang, Oksana I. Dukhanina, Alan Y. Deng, John P. Rapp

Abstract—A quantitative trait locus (QTL) for blood pressure was previously detected on rat chromosome 10 (RNO10) by linkage analysis and confirmed by the construction of congenic strains that encompass large regions of RNO10. In the present study, the rat RNO10 blood pressure QTL was dissected by the further construction of congenic substrains. The original congenic region was shown to contain 2 blood pressure QTLs (QTL 1 and QTL 2) 24 cM apart. These were localized to a <2.6-cM region between markers D10Rat27 and D10Rat24 for QTL 1 and to a <3.2-cM region between D10Rat12 and D10Mco70 for QTL 2. Comparative mapping suggests that the rat RNO10 QTL 2 could be localized very close to a blood pressure QTL described by sib-pair analysis on human chromosome 17, but this is not definitively established because of multiple and complex chromosomal rearrangements between rodents and humans. (Hypertension. 2001;38:779-785.)

Key Words: sodium ■ genetics ■ blood pressure

Rat chromosome 10 (RNO10) has been shown by linkage analysis to contain a quantitative trait locus (QTL) that influences blood pressure (BP). This result was obtained in experiments using spontaneously hypertensive rats (SHR) or stroke-prone SHR crossed with various normotensive strains,1–6 with Dahl salt-sensitive (S) rats crossed with various normotensive strains7–10 and with a cross of the strains,1–6 with Dahl salt-sensitive (S) rats crossed with normotensive strains.7–10 Inbred Dahl salt-sensitive (SS/Jr) rats were bred in our colony15 and followed procedures in accordance with institutional guidelines. All rats used in the present study were bred in our colony, and we established because of multiple and complex chromosomal rearrangements between rodents and humans. Congenic substrains were constructed from progenitor congenic strains as follows. The congenic strain was crossed to S, and the F1 population was intercrossed to produce a large F2 population of 250 rats. The F2 rats were genotyped using microsatellite markers throughout the congenic region. DNA for genotyping was prepared from tail biopsy tissue using the QiAamp Tissue Kit (Qiagen Inc). Congenic substrains were constructed from rats that had useful congenic strains constructed to verify the existence of a QTL span relatively large segments of chromosome to ensure that the QTL is encompassed. The purpose of the present study was to develop congenic substrains from the existing congenic strains on RNO10 to define the BP QTL location for ultimate gene identification. These substrains are constructed in such a way as to systematically reduce the introgressed donor chromosomal segment. A reduced segment that still retains a BP effect in the congenic strain refines the position of the QTL on the genetic map.

In our previous studies with linkage analysis that involved S rats crossed with various normotensive strains, we concluded that RNO10 was likely to contain two linked BP QTLs.8 This was based mainly on the very broad region with significant linkage to BP in a cross of S with the Milan normotensive strain (MNS). The present work proves that this is in fact the case, and one of these QTLs aligns within reason to the homologous region of HSA17 that reportedly contains a BP QTL.

Methods

All rats used in the present study were bred in our colony, and we followed procedures in accordance with institutional guidelines. Inbred Dahl salt-sensitive (SS/Jr) rats were bred in our colony13 and are referred to as S rats. Congenic strains used as starting strains in this work were reported previously. Congenic strain S.MNS(10b) contains a segment of RNO10 from the MNS on the S rat genetic background.12 Congenic strain S.LEW(10) contains a segment of RNO10 from Lewis (LEW) rats on the S rat genetic background.9 Congenic substrains were constructed from progenitor congenic strains as follows. The congenic strain was crossed to S, and the F1 population was intercrossed to produce a large F2 population of 250 rats. The F2 rats were genotyped using microsatellite markers throughout the congenic region. DNA for genotyping was prepared from tail biopsy tissue using the QiAamp Tissue Kit (Qiagen Inc). Congenic substrains were developed from rats that had useful recombinant chromosomes in the region of interest. Such rats were crossed with S to duplicate the recombinant chromosome and then selectively bred to fix the recombinant chromosome in the homozygous state on the S background. Techniques for microsatellite genotyping by the polymerase chain reaction and construction of linkage maps have been described previously.7,9,12

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BP was measured by the tail-cuff method on conscious restrained rats warmed to 28°C using semiautomatic equipment (IITC, Inc, Life Science Instruments) as described in detail previously. Briefly, 20 male S and 20 male congenic rats were age matched, housed in the same cages, and raised and studied concomitantly. Starting at 40 to 42 days of age, the rats were fed a 2% NaCl diet (Teklad diet TD94217; Harlan Teklad). BP measurements were taken on 4 consecutive days starting on the 24th day on the 2% NaCl diet. The final BP of a rat was taken as the average of the 4 consecutive day determinations.

Radiation Hybrid and Comparative Mapping

The radiation hybrid maps were compiled from data obtained from the Rat Genome Database at the Medical College of Wisconsin (www.rgd.mcw.edu) and The Wellcome Trust Center for Human Genetics (www.well.ox.ac.uk/rat_mapping_resources). Markers not already on the radiation hybrid map from these two sources, including markers denoted as D10Mco (Medical College of Ohio microsatellite markers) and genes Pdk2, Mapt, Srp68, Galr2, and Aanat, were mapped by testing all 106 samples in the rat radiation hybrid panel (Research Genetics). Primers for these markers are available on our Web site (www.mco.edu/depts/physiology/research). The regions in mice and humans homologous to our BP QTL regions were identified using The Mouse Genome Informatics Database (www.informatics.jax.org) and Online Mendelian Inheritance in Man (OMIM) at The National Center for Biotechnology (www.ncbi.nlm.nih.gov).

Development of New Gene Markers

For a better comparison of the QTL region across species, all of the genes that map to our QTL regions in mice and humans were checked for the availability of rat sequence data. If the rat sequence was available for any gene that mapped to the QTL region in mice or humans, a primer set was developed and placed on the rat radiation hybrid map. The genes placed on the rat radiation hybrid map in this way were Pdk2, Mapt, Srp68, Galr2, and Aanat.

Results

Two series of congenic strains that define BP QTLs on RNO10 are presented. In the first case, the donor strain was LEW on the S rat genetic background. The linkage map is at the right, and the numbers denote map distances in cM. The centromere is toward the top of the map. The filled bars to the left of the linkage map indicate the extent of the LEW donor regions for each congenic strain. The open bars on the ends of these congenic segments indicate the interval in which recombination occurred. The effect on BP of each strain compared with S rats is shown on the graph at the bottom. BP effect is defined as the BP of congenic rats (n=20) minus BP of concomitantly studied S rats (n=20); the standard error of this difference is indicated. Filled columns indicate a significant effect on BP (at least P≤0.001) between the congenic strain and S. An open column indicates that the BP effect was not significant (P>0.05). A negative BP deviation means that the congenic strain had a lower BP than concomitantly studied S rats.

Figure 1. Congenic strains for RNO10 with LEW as the donor strain on the S rat genetic background. The linkage map is at the right, and the numbers denote map distances in cM. The centromere is toward the top of the map. The filled bars to the left of the linkage map indicate the extent of the LEW donor regions for each congenic strain. The open bars on the ends of these congenic segments indicate the interval in which recombination occurred. The effect on BP of each strain compared with S rats is shown on the graph at the bottom. BP effect is defined as the BP of congenic rats (n=20) minus BP of concomitantly studied S rats (n=20); the standard error of this difference is indicated. Filled columns indicate a significant effect on BP (at least P≤0.001) between the congenic strain and S. An open column indicates that the BP effect was not significant (P>0.05). A negative BP deviation means that the congenic strain had a lower BP than concomitantly studied S rats.
**Table 1**: HW/BW Ratio for Comparison of S and Congenic Strains

<table>
<thead>
<tr>
<th>Congenic Strain</th>
<th>HW/BW Ratio, mg/g</th>
<th>t Test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Congenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.LEW(10)</td>
<td>4.161 (0.046)</td>
<td>3.758 (0.027)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.LEW×1</td>
<td>4.265 (0.032)</td>
<td>3.890 (0.034)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.LEW×5</td>
<td>4.048 (0.034)</td>
<td>4.008 (0.053)</td>
<td>0.53</td>
</tr>
<tr>
<td>S.LEW×6</td>
<td>4.386 (0.054)</td>
<td>3.885 (0.021)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.LEW×11</td>
<td>4.346 (0.066)</td>
<td>4.005 (0.038)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.LEW×12</td>
<td>4.260 (0.067)</td>
<td>4.020 (0.036)</td>
<td>0.003</td>
</tr>
<tr>
<td>S.MNS(10b)</td>
<td>4.308 (0.061)</td>
<td>3.839 (0.043)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.MNS×1</td>
<td>4.105 (0.043)</td>
<td>3.944 (0.033)</td>
<td>0.005</td>
</tr>
<tr>
<td>S.MNS×2</td>
<td>4.221 (0.064)</td>
<td>4.016 (0.047)</td>
<td>0.014</td>
</tr>
<tr>
<td>S.MNS×3</td>
<td>4.086 (0.031)</td>
<td>3.968 (0.033)</td>
<td>0.013</td>
</tr>
<tr>
<td>S.MNS×3×2</td>
<td>4.239 (0.087)</td>
<td>4.281 (0.046)</td>
<td>0.67</td>
</tr>
<tr>
<td>S.MNS×3×4</td>
<td>4.162 (0.037)</td>
<td>3.995 (0.035)</td>
<td>0.003</td>
</tr>
<tr>
<td>S.MNS×3×5</td>
<td>4.084 (0.038)</td>
<td>4.193 (0.049)</td>
<td>0.088</td>
</tr>
<tr>
<td>S.MNS×4</td>
<td>4.297 (0.094)</td>
<td>3.817 (0.024)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.MNS×5</td>
<td>4.266 (0.054)</td>
<td>3.978 (0.035)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>S.MNS×12</td>
<td>4.418 (0.061)</td>
<td>4.161 (0.058)</td>
<td>0.005</td>
</tr>
<tr>
<td>S.MNS×13</td>
<td>4.241 (0.033)</td>
<td>4.219 (0.043)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

S and congenic strains were always studied concomitantly; there were 20 male rats in each group. Values are group mean values with SEM in parentheses.

Figure 1 shows the progenitor strain S.LEW(10) and 5 congenic strains derived from the progenitor strain. This original strain had a 74-cM segment from LEW rats introgressed into the S genetic background and showed a major lowering of BP by ~43 mm Hg compared with that of S. This has been reported previously. Congenic strains S.LEW×1 and S.LEW×6 were derived from opposite ends of S.LEW(10) and overlap in the middle of S.LEW(10) (Figure 1). Both of these overlapping strains also have lower BP by >40 mm Hg compared with that of S, implying that a BP effect of a congenic strain, this was corroborated by HW/BW data (Table). Thus, when there is a significant decrease in BP of a congenic strain compared with S, the HW/BW ratio also decreases. Conversely, when a strain had no BP effect, the HW/BW ratio also shows no effect.

It is noted that the QTL 2 region defined in Figure 4 was seen only in the congenic strain series where the donor strain was MNS. When the donor strain was LEW, QTL 2 was not detected. In Figure 1, the strain S.LEW×5 derived from the LEW donor rat spans the QTL 2 region but does not have a BP effect.

**Discussion**

Genetic linkage analysis to discover BP QTLs has been widely practiced using hypertensive rat strains. This work was recently reviewed; in particular, a detailed analysis of previous work on RNO10 is contained therein. Linkage analysis emphasizes the present data and compares them with pertinent data on HSA17.

Linkage analysis of QTL is inherently imprecise at localization of the gene or genes responsible for a QTL. If construction of a congenic strain confirms the presence of a QTL, subsequent construction of congenic substrains allows more precise QTL localization and the identification of multiple QTLs in a region that are impossible to define by linkage analysis.

Previously, we studied F2 populations obtained by crossing S with MNS7 or by crossing S with LEW9. Linkage analysis indicated the presence of BP QTLs on RNO10 in both F2 populations, and initial congenic strains confirmed
the presence of QTLs with either MNS$^{12}$ or LEW$^9$ as the donor of large regions of RNO10. In the present study, the congenic series developed with MNS as the donor of segments of RNO10 revealed two BP QTLs, called QTL 1 and QTL 2. The QTL 1 region from the MNS congenic series overlaps with the QTL defined with the LEW congenic series. This is a uniquely powerful result for the existence and localization of a QTL from two independent congenic series. It means that both LEW and MNS probably carry functionally important contrasting alleles compared with S for a gene (or genes) in the QTL 1 region. In contrast to QTL 1, the QTL 2 region was identified in the congenic series derived from MNS but not from LEW. This implies that at QTL 2, the LEW and S alleles are not functionally different with regard to BP.

Although the production of multiple congenic substrains is tedious, it does provide a systematic way to localize the QTL. In the case of the MNS-derived congenic series, the localization was improved ~25-fold, from 74 cM in the original congenic strain to <2.6 cM for QTL 1 and <3.2 cM for QTL 2. This required two iterations of substrain construction. Noteworthy is the fact that these localizations do not include important candidate loci ACE (ACE) and the inducible form of NO synthase (Nos2).

It is not particularly surprising to find evidence for multiple QTLs by congenic strain analysis of a region in which only 1 QTL peak was detected on linkage analysis. This has been observed with QTLs for BP on rat chromosome 2 (RNO2) (M.R.G. and J.P.R., unpublished observations), polycystic kidney disease in mice,$^{17}$ trypansomiasis resistance in mice,$^{18}$ systemic lupus erythematosus in mice,$^{19}$ and epilepsy in mice.$^{20}$ There also is evidence for 3 BP QTLs on rat chromosome 1 (RNO1) based on congenic analysis.$^{21}$ In this case, linkage analysis had clearly suggested multiple loci,$^{9, 22, 23}$ because some of the loci were far enough apart on the chromosome.
The region of HSA17 that is homologous to RNO10 has been studied for linkage to BP. Julier et al. used a sib-pair analysis and found significant linkage of BP to markers in the region of HSA17 homologous to the BP QTL region of RNO10 as initially crudely defined by linkage analysis. In particular, human marker D17S934 was near the center of the region on HSA17 associated with BP that overlapped with the rat BP QTL. Similar data obtained by Baima et al. confirmed this result.

With the markedly improved QTL localization on RNO10 presented here, it is of interest to reexamine how closely the human BP QTLs align with the human linkage data on HSA17. It is emphasized that although the present congenic rat data are relatively precise, the human QTL localizations are necessarily less well defined. Thus, the following discussion should be interpreted with caution. Figure 5 shows comparative maps for the BP QTL region of interest for rats, mice, and humans. Because RNO10 and mouse chromosome 11 (MMU11) are well conserved and because the mouse map contains many more known loci than the rat map, the mouse map serves as a good bridge for comparisons between rats and humans. Although there certainly are many loci common to MMU11 and HSA17, the order of the loci is not completely identical between humans and mice. This has an impact on how closely the human and rat BP QTLs can be colocalized.

In Figure 5, the critical human marker D17S934 maps between rat BP QTL 1 and QTL 2 on the mouse/rat maps. This is based on the map position of D17S934 on the human gene sequence map and the association of the majority of loci in this human region with a comparable mouse region that clearly lies between the rat BP QTL 1 and QTL 2. This region includes GFAP (glial fibrillary acidic protein), which is in a human BAC (bacterial artificial chromosome) contig with D17S934; the two loci are ~64 kb apart. Note, however, that two other genes in the human region around GFAP in Figure 5 (ITGA2B and ITGB3) are located in the mouse (and by inference, in the rat) in a different location very close to rat BP QTL 2. Thus, it is at least conceivable that because of rearrangements of small chromosomal regions between species, the same QTL is in a different relative position between species. That is, in Figure 5, the human QTL that is near human loci ITGA2B and ITGB3 could be the same QTL that is near mouse/rat loci Itga2b and Itgb3. The other alternative is that the human and rat QTL are different and ~7 cM apart on the mouse map (ie, the distance on the mouse map from Gfap at 62 to 69 cM at approximately the center of the RNO10 QTL 2) (Figure 5). The present data do not permit a
conclusion as to which possibility is correct because one cannot tell which mouse/rat locus, \textit{Gfap} or \textit{Itga2b} / \textit{Itgb3}, is the appropriate one to locate the human QTL on the mouse/rat maps.

In a recent study, Levy et al.\textsuperscript{24} used longitudinal family BP data from the Framingham Heart Study to perform a genome scan. A major BP QTL was found on HSA17, which spans a large interval that includes the region just discussed.\textsuperscript{13,14} The best localization we can discern from their data are from markers with the highest 2-point LOD scores. These are D17S1299 and D17S2180 (ATC6A06), which are at 46.5 and 56.2 Mb on the human gene sequence map. This obviously spans the critical segment that contains \textit{GFAP} and D17S934, which are at 51.2 Mb.

Another locus of interest in humans is pseudohypoaldosteronism type II, which is associated with hypertension. One locus for this syndrome has been localized to HSA17 near marker D17S250 located at 42.2 Mb.\textsuperscript{25} This places it \(\approx 10\) Mb from \textit{GFAP} and the marker D17S934 in Figure 5 and does not allow one to definitively include or exclude the pseudohypoaldosteronism type II locus from the BP QTL discussed here.

Other studies by sib-pair analysis in humans have not found BP QTLs that match with either of the RNO10 BP QTLs. Krushkal et al.\textsuperscript{26} and Perola et al.\textsuperscript{27} found no linkage to HSA17. Xu et al.\textsuperscript{28} did detect BP linkage to HSA17, but this was located in the p arm of HSA17, \(\approx 40\) cm from the region noted here, which was in the q arm.

\textbf{Acknowledgments}

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\textbf{References}


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