Comprehensive Congenic Coverage Revealing Multiple Blood Pressure Quantitative Trait Loci on Dahl Rat Chromosome 10

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Abstract—Chromosome mapping based on congenic strains can restrict quantitative trait loci (QTLs) for blood pressure (BP) into small intervals that are otherwise indistinguishable in linkage analysis. Also, congenic strains can be created to test a candidate gene to be a BP QTL. Taking full advantage of these features, we produced 10 congenic strains by replacing various segments of chromosome (Chr) 10 of the Dahl salt-sensitive (DSS) rat with those of the Lewis (LEW) rat. These strains were made to systematically cover an entire section of Chr 10. Three of the strains were designed to narrow the intervals that harbor previously mapped QTL1 and QTL2. Two of the strains were designed for the express purpose of testing the QTL candidacy of loci for inducible nitric oxide synthase (Nos2) and angiotensin-converting enzyme (Ace) genes. BPs of these strains were measured by telemetry and compared with those of the DSS rat. Consequently, QTL1 and QTL2 were narrowed to segments of 53.5 and 100.4 centiRays, respectively. A new QTL, QTL3, was found between QTL1 and QTL2. Both Nos2 and Ace have been disqualified as QTLs in the DSS and LEW comparison. Therefore, there are no obvious candidate genes in the segments that harbor these 3 QTLs, which represent genes previously not thought to be involved in BP regulation. These QTLs will likely have an influence on studies of human hypertension because of their homology with the human CHR 17 region in which QTLs for BP have been found. (Hypertension. 2003;42:515-522.)

Key Words: genetics ■ functional genomics ■ nitric oxide synthase ■ angiotensin-converting enzyme ■ human disease

Chromosome (Chr) 10 of the rat has been under intense investigation in the past decade. In linkage and congenic mapping studies,1–6 a region on rat Chr 10 was shown to harbor a blood pressure (BP) quantitative trait locus (QTL). Further evidence suggested that there might be >1 BP QTL present in this region.7,8 Recently, we have proved that indeed there are 2 separate QTLs in the region by using congenic strains in which chromosome segments of interest from the Dahl salt-sensitive rat (DSS) were replaced with those from the Lewis rat (LEW) and the Milan normotensive rat (MNS).9 This Chr 10 region seems particularly relevant to human genetic hypertension, because in multiple studies involving homologous regions on human CHR 17, BP QTLs have been detected.12–14

The impetus of the current work was 4-fold. First, we attempted to narrow the regions that contain the 2 QTLs detected in our previous work for the ultimate purpose of positionally cloning them. Second, large gaps on Chr 10 were left uninvestigated, and questions remained as to whether there were QTLs present in those uncovered fragments. Third, it has been debated whether or not the inducible nitric oxide synthase gene (Nos2) could be involved in genetic hypertension, especially since a mutation was found in its coding region in the DSS rat.15,16 Among the normotensive strains found to be contrasting to DSS in linkage studies,3,4,6,7 the Nos2 locus was ruled out as a BP QTL for the DSS and MNS comparison.4,5 Nevertheless, the Nos2 locus remained important as a candidate gene for the DSS and LEW comparison.4,9,11 The only way to rule it out as a QTL in the DSS and LEW comparison was to construct a congenic strain that surrounded it, and this congenic strain should not show a BP effect. Finally, the angiotensin-converting enzyme gene (Ace) had not been excluded as a QTL in either the DSS and MNS4,5,10 or the DSS and LEW comparisons.6,9–11 Similar to the test of candidacy for Nos2 mentioned earlier, we needed to create and then show that a congenic strain that harbors Ace actually does not have a BP effect to rule it out as a QTL candidate. To address these issues, we systematically developed multiple congenic strains that covered consecutive segments along an entire region of Chr 10 of interest and studied their BPs by accurate and continuous measurements via telemetry.
Methods

Animals

Two congenic strains, designated S.L4 and S.L5, that were previously shown to have trapped 2 BP QTLs, QTL1 and QTL2,9,11 were used to derive congenic substrains. First, rats of the DSS strain and each of the S.L4 and S.L5 strains were crossbred to produce F1 rats, which then were intercrossed to produce F2 progeny. The F2 rats were genotyped for markers that resided in a region of interest. Because crossovers were sought within small regions containing QTL1 and QTL2, 358 rats had to be screened. For this purpose, we bred 10 pairs of F2 rats for each cross and rebred them thereafter when no desired crossovers were found. Several rats with a crossover inside the region of interest, either for QTL1 or QTL2, were found. Each rat was then backcrossed to a DSS rat to duplicate the fragment. Male and female heterozygous backcrossed rats were intercrossed to produce progeny that were LL homozygous for the region of interest and SS homozygous for the rest of the chromosome and the rest of the genome. This was verified by genotyping >90 markers scattered throughout the rat genome.9,11 As a result, several congenic substrains were established. They were designated as follows: S.LEW-D10Rat119/D10Mgh1 (abbreviated S.L10), S.LEW-D10Rat55/D10Rat120 (S.L7), S.LEW-D10Rat55/D10Rat13 (S.L8), S.LEW-D10Rat55/D10Rat13 (S.L9), S.LEW-D10Wox51/D10Rat27 (S.L10), and S.LEW-D10Rat119/D10Rat133 (S.L13) (Figure 1). The chromosome region of homozygous LL in each strain is indicated by a solid bar in Figure 1. All of the markers in the region were genotyped for each congenic strain in question and were homozygous LL.

At the same time, a new congenic strain, S.LEW-D10Rat119/D10Mgh1 (S.LC10), was made to cover the entire Chr 10 region (Figure 1). The purpose was to derive additional congenic substrains to cover the previously untested middle region of the Chr. Consequently, 3 new congenic substrains were created from S.LC10. They were designated as follows: S.LEW-D10Rat27/Igfbp4 (S.L11), S.LEW-D10Rat24/Igfbp4 (S.L12), and S.LEW-D10Got125/D10Rat120 (S.L14) (Figure 1). These strains were made the same way as S.L4 and S.L5 by a speed congenic strategy.9,11 All of the congenic strains were newly created except for S.L1, which is the same strain as in Deng et al9 and Sivo et al.11

Figure 1. QTL mapping on DSS Chr 10. The linkage map is essentially the same as published previously,9–11 which is based on an F2(DSS×LEW) population. Numbers to the right of the map are units in centiMorgans (cM), and those to the left of the map are in centiRays (cR) between markers. Solid bars under congenic strains symbolize DSS chromosome fragments that were replaced by those of the LEW rat. The entire region indicated by solid bars and junctions between the solid and open bars are homozygous LL on the map for all markers listed in the corresponding positions. Open bars on ends of solid bars indicate the ambiguities of crossover break points between markers. Junctions between solid and open bars as well as ends of chromosome regions of interest in each strain are connected by dotted lines to marker positions on the map. The physical map refers to the alignment of rat supercontigs in an ascending order from top to bottom on Chr 10, obtained by blasting a marker to the rat genome database (http://www.ncbi.nlm.nih.gov/genome/seq/RnaBlast.html). The name of a contig starts with NW_042, followed by 3 numbers. Digits below or to the side of a contig in parentheses indicate its approximate size in bp. Aldoc indicates aldolase c gene; Igfbp4, insulin-like growth factor–binding protein 4 gene; and Ngfr, nerve growth factor receptor gene. Remaining markers are anonymous (8,14,27, http://www.genome.wi.mit.edu/rat/public/). Congenic strains are S.LEW-D10Rat119/D10Mgh1 (S.L10), S.LEW-D10Rat119/D10Rat20 (S.L7), S.LEW-D10Rat55/D10Rat13 (S.L8), S.LEW-D10Rat55/D10Rat13 (S.L9), S.LEW-D10Rat55/D10Rat13 (S.L10), and S.LEW-D10Rat119/D10Rat133 (S.L13) (Figure 1). The chromosome region of homozygous LL in each strain is indicated by a solid bar in Figure 1. All of the markers in the region were genotyped for each congenic strain in question and were homozygous LL.

Placement of the 3 QTLs is indicated by brackets to the right of the map.
BP Measurements
Male rats were weaned at 21 days of age, maintained on a low-salt diet (0.2% NaCl, Harlan Teklad 7034) for 2 weeks, and then fed a high-salt diet (2% NaCl, Harlan Teklad 94217) starting from 35 days of age until the end of the experiment. Telemetry probes were implanted when rats were 56 days old (ie, after 3 weeks of the high-salt diet), with their body weights between 250 and 320 g. After surgery, the rats were allowed 10 days to recuperate before their BPs were read. The implantation of telemetry probes and postoperative care of the animal were the same as detailed previously. 9,11,17,18

Statistical Analysis
Repeated-measures ANOVA followed by the Dunnett test in commercially available software (SYSTAT 9 program, SPSS Inc) was used. In pairwise comparisons between 2 congenic strains, ANOVA followed by the Tukey test was performed.

An expanded Methods section can be found in an online supplement available at http://www.hypertensionaha.org.

Results

Construction of Congenic Strains
Genotyping of markers was carried out to establish the Chr region that was LL homozygous in each of the congenic strains (Figure 1). A genome scan showed that all of the 90 markers outside the regions of interest were SS homozygous (data not shown). Thus, from a genetic viewpoint, these congenic strains were LL homozygous for the designated Chr segments but essentially SS homozygous for the rest of the genome. All congenic strains shown in Figure 1 are new except for S.L1, which is the same strain as in Siva et al. 11 Also, strains S.L6, S.L9, and S.L11 do not overlap (Figure 1).

S.L13 was specially designed to test Nos2 as a candidate for a BP QTL. Although the markers around Nos2 are all LL in S.L13, they fall into different contigs, with Nos2 located in the middle (Figure 1). Consequently, the Nos2 gene locus 15 is included inside S.L13. The congenic strain S.L8 was specially created to test Ace as a candidate gene for a BP QTL.

BP Studies
All BP components were measured, including mean arterial (MAP), diastolic (DAP), and systolic (SAP) pressures. For simplicity of presentation, each point on the graphs in Figures 2 and 3 represents averaged 24-hour readings taken every 4 hours. Figure 2 shows a comparison in BPs between the DSS and LEW parental strains and between S.LC10 congenic strains. MAP, DAP, and SAP of LEW were significantly lower than those of DSS (P<0.0001). DSS showed a gradual increase in BP with time, whereas the BPs of LEW stayed rather constant throughout the course of measurement. Similarly, the BPs of S.LC10 were significantly lower than those of DSS (P<0.0001) but still above those of LEW (Figure 2).

Figure 3 shows comparisons of MAP, DAP, and SAP of DSS with those of congenic strains. MAPs, DAPs, and SAPs of S.L6, S.L9, S.L10, and S.L11 strains were lower (P<0.03) than those of the DSS strain starting from day 5 until the end of the measurement. In contrast, MAPs, DAPs, and SAPs of the remaining congenic strains were not different (P>0.10) from those of the DSS strain in overall measurement. The number of rats chosen was consistent with our previous work. 9,11,17,18 The BP response patterns among the congenic strains were not different (data not shown).

Figure 4 shows comparisons of BP effects between S.LC10 and a congenic strain that demonstrated BP-lowering effects (Figures 2 and 3). Each of the S.L6
(QTL2), S.L9 (QTL1), and S.L11 (QTL3) strains had a BP different from that of DSS (Figure 3) and from that of S.LC10 ($P$<0.006; Figure 4) but not different from each other in pairwise comparisons ($P$>0.71, detailed data not shown). S.L1, S.L7, S.L8, S.L12, S.L13, and S.L14 strains did not have different BPs in pairwise comparisons with each other ($P$>0.50, detailed data not shown).

**Comparative Mapping**

Figure 5 shows systematic comparative mapping among rat, mouse, and human genomes that corresponds to the intervals containing the 3 QTLs in the rat. The goal was to discover whether there were more genes that could be considered potential candidates for BP regulation. For this purpose, the regions of rat Chr 10 that contained the 3 QTLs as mapped in Figure 1 were used to find all of the known genes in the homologous regions of mice and humans. Figure 6 synthesizes the QTL mapping results from the current and previous studies and then shows the comparative mapping of QTLs among rats, mice, and humans.

**Discussion**

The major findings in the current work are as follows: (1) The positions for QTL1 and QTL2 have been placed inside regions of 53.5 centiRay (cRs) and 100.4 cR, respectively (Figure 1); (2) A new QTL, QTL3, falls into the interval of D10Rat27 and D10Rat 93, which is $\approx 155.7$ cR (Figure 1);
There are no genes to be considered as obvious candidates for these QTLs located in the segments that harbor QTL1, QTL2, and QTL3. Nos2 is located outside QTL1, and Ace is found outside the QTL2 region (Figure 1). More significantly, both Nos2 (Figures 4m, 4n, and 4o) and Ace (Figures 4g, 4h, and 4i) have been ruled out as QTLs for the DSS and LEW comparison. Identification of these QTLs will likely lead to the discovery of new genes previously not thought to genetically influence BP (Figure 5). The positions of QTL1, QTL2, and QTL3 correspond to the conserved synteny region on a section of human CHR 17, where QTLs have been mapped in studies of several populations (Figure 6). The pathogenic mechanisms that underlie these QTLs in the rat will potentially have a direct influence on studies of certain forms of essential human hypertension.

QTL Fine Mapping
The BP difference between DSS and LEW (Figure 2) can be partially accounted for by the effects of the 3 QTLs in S.LC10 (Figure 2). QTL1, QTL2, and QTL3 are separated by S.L6, S.L9, and S.L11 congenic strains (Figure 3). A lack of an effect from S.L1, S.L7, S.L8, S.L12, S.L13, and S.L14 indicates that (1) the segments defined by them do not contain QTLs and (2) the remaining LEW genome in the DSS background did not influence BP in the S.L6, S.L9, and S.L11 congenic strains. Consequently, QTL1 exists between D10M11Mit119 and D10Rat27 markers (≈1.4 cM, or 53.5 cR, or inside the supercontig NW_042667 of ≈10 megabases [Mb]; Figure 1).

Although the BP effects of both S.L9 (P<0.01) and S.L10 (P<0.001) were different from those of DSS, S.L10 showed a slightly greater effect than did S.L9 (Figure 3). However, the BPs of S.L9 and S.L10 were not different from each other (P>0.10). It remains to be determined whether this difference is due to the existence of multiple QTLs, the effects of some QTL modifiers that are present in the region between D10Got84 and D10Rat27 markers, or simply residual genetic background variations between the 2 strains.

Because S.L1 had the same BP as DSS, QTL2 should be localized to the segment between D10Rat17 and D10Rat9.
perspective for deriving a QTL interval by comparing positive and negative congenic strains is valid, on the basis of our previous QTL mapping work on Chr 2.18 In that case, a congenic strain was later made for the nonoverlapping segment, and it turned out to have a BP effect (data not shown). Although the positions of QTL2 and QTL3 are only inferred from comparisons of S.L1, S.L6, S.L7, and S.L8 from S.L11 and S.L12, respectively, further work will be pursued to construct 2 additional congenic strains, 1 by substituting the fragment between D10Rat27 and D10Rat93, which is approximately 155.7 cR (9.4 cM) but <15 Mb. The correspondence among the distances in centiRays, centiMorgans, and megabases is not necessarily proportional for each QTL region. For the purpose of identifying a QTL, the physical distance seems to be the most appropriate assessment.

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Relation Between the QTLs
It is noteworthy that S.L10 contains at least 3 BP QTLs (Figure 1). Questions arise as to whether there are potential epistatic interactions among them or possible additive effects between them. The sum of the BP-reducing effect from each congenic strain, S.L6 (QTL2), S.L9 (QTL1), S.L11(QTL3), was 24+32+23=79 mm Hg in MAP, whereas the BP-reducing effect of S.LC10 alone was 68 mm Hg in MAP (Figure 4). Therefore, the BP difference between S.L10 alone and the sum of S.L6, S.L9, and S.L11 is 68 to 79, or -11 mm Hg. It must be stressed that MAP, as measured in millimeters of mercury used herein, is the number averaged throughout the days of measurement for the strain in question. It did not take into account that changes in MAP were not uniform among the strains. There was a tendency for MAPs to increase as the measurements progressed in S.L6, S.L9, and S.L11 (Figure 3), whereas the MAP for S.LC10 remained constant during the period of measurement (Figure 2). The averaged values, therefore, only serve as a rough estimate for MAP. With this caveat in mind, it is still not clear whether the difference in MAPs was significant between the combined, ie, S.LC10, and singularly added, ie, S.L6+S.L9+S.L11, strains because the SEM for each strain was not considered in this equation. Whether or not epistatic interactions exist among these 3 QTLs remain to be determined. A more conclusive comparison would be to construct a “triple” congenic strain from S.L6, S.L9, and S.L11 and compare the BP of this triple strain with those of S.LC10 congenic strains. It is also possible that additional QTLs could be uncovered during the process of fine-mapping the 3 QTLs.

Comparative QTL Mapping
First, comparative mapping among the rat, mouse, and human genomes showed that there are no candidate genes that are apparently known to influence BP present in the intervals that harbor the 3 QTLs (Figure 5). Thus, chances are high that the 3 QTLs will represent novel genes for BP regulation.

Based on marker position in the map (Figure 6), QTL1 in the current work is located in a region between D10M11Mit119 and D10Rat27 but goes no further down than D10Rat27. The position of QTL3 in our present study falls into the same interval as QTL1, localized between D10Rat27 and D10Rat24 in the S.MNS congenic strains.10 The region of QTL2 seems to be common in both S.LEW (present work) and S.MNS congenic strains,10 implying that the BP-lowering alleles of QTL2 and QTL3 might be the same between LEW and MNS. The phenomenon that multiple BP QTLs exist in a cluster, ie, adjacent to each other in 1 Chr fragment, has been observed for Chr 217,18 and Chr 1.19,20 Morel et al21 also found a similar occurrence for lupus erythematosus in the mouse. These QTLs could interact with each other epistatically (for more detailed explanations, see Deng22).

Perspectives
Based on accurate, direct, and continuous measurement of BP by telemetry, there are multiple QTLs on Chr 10 of the DSS rat. Both Nos2 and Ace have been excluded to be the QTLs in the DSS and LEW comparison. QTL1 is located in an interval small enough to begin positional cloning. These QTLs have the potential to be relevant to certain forms of human essential hypertension. Now that the rat genome-wide sequence is available, mutation screening will be greatly facilitated on genes found in intervals that harbor the 3 QTLs. Moreover, homology mapping among the rat, mouse, and human genomes will be useful in this endeavor.21
cleotide binding protein, α-13; HELZ, helicase with zinc finger domain; HLF, hepatic leukemia factor; Icb2, intercellular adhesion molecule 2; Igβ, immunoglobulin-associated β; KCNH6, potassium voltage-gated channel, subfamily H (eag-related) member 6; Kpn2a, karyopherin (importin) α2; Lhx1, LIM homeobox protein 1; Lpo, LIM homeobox protein 1; Mdm2, p53 suppressor of tumorigenesis 2; Mmp14, matrix metalloproteinase 14; Mrps23, karyopherin (importin)/H9251; Nog, noggin; OR4D1, olfactory receptor, family 4, subfamily D, member 1; Pclp, phosphatidylinositol transfer protein; Pcem, platelet/endothelial cell adhesion molecule; Polg2, polymerase (DNA directed), γ2, accessory subunit; Ppm1d, protein phosphatase 1D, magnesium-dependent, δ isoform; Ppm1e, protein phosphatase 1E (PP2C domain containing); Prkar1a, protein kinase, cAMP-dependent regulatory, type I, α; Prkca, protein kinase C, α; Psmc5, protease (prosome, macropain) 26S subunit, ATPase 5; Psmd12, proteasome (prosome, macropain) 26S subunit, non-ATPase, 12; Rad51c, Rad51 homolog c (S. cerevisiae); Rgs9, regulator of G-protein signaling 9; Risc, retinoid-inducible serine carboxypeptidase; Rnahp, RNA helicase-related protein; Rok1, ATP-dependent, RNA helicase; Rpskbb1, ribosomal protein S6 kinase, polypeptide 1; Scn4a, sodium channel, voltage-gated, type IV, α polypeptide; Sep4, septin 4; Sfrs1, splicing factor, arginine/serine-rich 1 (ASF/SF2); Smc2, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily d, member 2; Smurf2, E3 ubiquitin ligase SMURF2; Srascm, Src-activating and signaling molecule; Stxbp4, syntaxin-binding protein 4; Sup4, suppressor of Ty 4 homolog (S. cerevisiae); Tcf2, transcription factor 2; Tex14, testis-expressed gene 14; Tex2, testis-expressed gene 2; Tkl2, toulasii-like kinase 2; TRAP240, thyroid hormone receptor-associated protein, 240-kDa subunit; Tubd1, tubulin, δ1; Vezf1; vascular endothelial zinc finger 1; Vmp1, likely orthologue of rat vacuole membrane protein 1; Znf147, zinc finger protein 147 (estrogen-responsive finger protein); and Znf161, zinc finger protein 161.

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Figure 6. Localizations of QTLs and comparative mapping with mouse Chr 11 and human CHR 17. The rat map is essentially the same as in Figure 1. Scale is based primarily on the linkage and radiation hybrid maps. The positions of QTLs are those defined by the congenic strains shown in Figure 1. Genes in the homologous regions on mouse Chr 11 and human CHR 17 and their map positions were based on http://www.ncbi.nlm.nih.gov:80/cgi-bin/Entrez/hum_srch?chr and http://ratmap.ims.u-tokyo.ac.jp/cgi-bin/comparative_home.pl. Mbp refers to position in megabases from the top of the human CHR 17 map to a specific marker. QTL1 and QTL2 to the immediate right of the map under “current work” refer to those found in our present work. Different bar graphs are used to distinguish our present work from those found in previous works.

References
