Multiple Quantitative Trait Loci for Blood Pressure Interacting Epistatically and Additively on Dahl Rat Chromosome 2

Julie Dutil, Vasiliki Eliopoulos, Johanne Tremblay, Pavel Hamet, Sophie Charron, Alan Y. Deng

Abstract—Our previous work demonstrated 2 quantitative trait loci (QTLs), C2QTL1 and C2QTL2, for blood pressure (BP) located on chromosome (Chr) 2 of Dahl salt-sensitive (DSS) rats. However, for a lack of markers, the 2 congenic strains delineating C2QTL1 and C2QTL2 could not be separated. The position of the C2QTL1 was only inferred by comparing 2 congenic strains, one having and another lacking a BP effect. Furthermore, it was not known how adjacent QTLs would interact with one another on Chr 2. In the current investigation, first, a critical chromosome marker was developed to separate 2 C2QTLs. Second, a congenic substrain was created to cover a chromosome fragment thought to harbor C2QTL1. Finally, a series of congenic strains was produced to systematically and comprehensively cover the entire Chr 2 segment containing C2QTL2 and other regions previously untested. Consequently, a total of 3 QTLs were discovered, with C2QTL3 located between C2QTL1 and C2QTL2. C2QTL1, C2QTL2, and C2QTL3 reside in chromosome segments of 5.7 centiMorgan (cM), 3.5 cM, and 1.5 cM, respectively. C2QTL1 interacted epistatically with either C2QTL2 or C2QTL3, whereas C2QTL2 and C2QTL3 showed additive effects to each other. These results suggest that BP QTLs closely linked in a segment interact epistatically and additively to one another on Chr 2. (Hypertension. 2005;45:557-564.)

Key Words: hypertension ■ comparative congenics

Ever since the revelation of a quantitative trait locus (QTL) for blood pressure (BP) on chromosome 2 (Chr 2) of Dahl salt-sensitive (DSS) rats,1,2 Chr 2 seems to play a role in the development of hypertension in several of the hypertensive strains.3-13 In our initial work, 2 BP QTLs designated C2QTL1 and C2QTL2 were localized to regions on Chr 2 of the DSS rat.5,6 C2QTL1 was found between the markers D2Rat303 and D2Rat166,6 and C2QTL2 was found between the markers D2Rat166 and D2Rat131.5 However, at the time, the position of C2QTL1 was solely inferred from comparing 2 overlapping congenic strains, one having and the other lacking a BP effect.6 It was uncertain whether this deduction was valid in localizing a QTL for a polygenic trait. Another issue was that C2QTL1 defined by S.M16 and C2QTL2 defined by S.M5 and S.M65 shared a polygenic trait. Another issue was that C2QTL1 and C2QTL2 could not be separated. The position of the C2QTL1 was only inferred by comparing 2 congenic strains, one having and another lacking a BP effect. Furthermore, it was not known how adjacent QTLs would interact with one another on Chr 2. In the current investigation, first, a critical chromosome marker was developed to separate 2 C2QTLs. Second, a congenic substrain was created to cover a chromosome fragment thought to harbor C2QTL1. Finally, a series of congenic strains was produced to systematically and comprehensively cover the entire Chr 2 segment containing C2QTL2 and other regions previously untested. Consequently, a total of 3 QTLs were discovered, with C2QTL3 located between C2QTL1 and C2QTL2. C2QTL1, C2QTL2, and C2QTL3 reside in chromosome segments of 5.7 centiMorgan (cM), 3.5 cM, and 1.5 cM, respectively. C2QTL1 interacted epistatically with either C2QTL2 or C2QTL3, whereas C2QTL2 and C2QTL3 showed additive effects to each other. These results suggest that BP QTLs closely linked in a segment interact epistatically and additively to one another on Chr 2. (Hypertension. 2005;45:557-564.)

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Materials and Methods

Animals

Congenic strains, S.M, S.M1, S.M2, S.M5, and S.M6 and DSS strains are the same as used previously5,6 (Figure 1).

Constructions of Congenic Substrains

S.M, S.M1, S.M5, and S.M65,6 were used to derive congenic substrains. The basic design was to systematically and as completely as possible cover the entire Chr 2 region of interest. The goal is to define each QTL unambiguously.

The procedure was similar to that published previously.15 In brief, rats of the DSS and S.M (or S.M1 or S.M5 or S.M6) (Mispro, Montreal, Canada) were used to derive congenic substrains (see the online supplement for details, available at http://www.hypertensionaha.org). In the end, the authenticity of each congenic substrain has been established by genotyping the markers for the region of interest and 57 additional markers scattered throughout the rat genome (data not shown). The chromosome region homoygous MM for each congenic substrain is depicted by a solid bar in Figures 1 and 2. New congenic substrains produced in the present work are: DSS.MNS-(D2Rat183-D2Chm113)/Lt (abbreviated as C2S.M7), DSS.MNS-(D2Chm25-D2Mit14)/Lt (C2S.M8), DSS.MNS-(D2Chm25-D2Rat131)/Lt (C2S.M9), DSS.MNS-(D2Wox27-Adh)/Lt (C2S.M10), DSS.MNS-(D2Chm51-D2Rat341)/Lt (C2S.M11), and DSS.MNS-(D2Chm25-Fgg)/Lt (C2S.M12).

BP Measurements

BP studies on the congenic strains were essentially the same as reported previously.5,6,15-21 In brief, male rats were weaned at 21 days of age, maintained on a low-salt diet (0.2% NaCl; Harlan Teklad 7034), and then fed a high-salt diet (2% NaCl; Harlan Teklad 7034), and then fed a high-salt diet (2% NaCl; Harlan Teklad 7034).
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 to 320 grams. BPs for all the strains were measured at least at 2 different times to exclude seasonal and environmental influences. Thus, the BP data were pooled from separately reproducible measurements for each strain.

**Statistical Analysis**

Repeated measures analysis of variance (ANOVA) followed by Dunnett test (which permits a correction for multiple comparisons and sample sizes) was used to compare a parameter between 2 groups as presented previously.\(^5,6,15-21\) During the BP comparison, ANOVA was first used to analyze the data to see if there was any difference among the groups. If the difference is significant, then the Dunnett test was followed-up to see which group is different and how much is significantly different from the DSS strain.

The 2×2 ANOVA determines a QTL–QTL interaction (or a lack of it) by evaluating whether the observed BP effect of a congenic strain combining 2 separate congenic strains is significantly different from a predicted sum of BP effects from each individual congenic strain.

**Results**

**A Chromosome Marker Defining Separate QTLs**

The marker designated as D2Chm90 was instrumental in separating QTLs (Figure 1 and Table) (see the online supplement for the detail as to how these markers were generated). It came from the same supercontig containing Mme and is homozygous MM for S.M, but SS for S.M1, S.M2, and S.M6 (Figure 1). Thus, D2Chm90 effectively separated S.M1 and S.M2 from S.M6. Consequently, there are definitively at least 2 different BP QTLs present (Figure 1). Because S.M1 had a BP 22 mm Hg lower (\(P<0.03\)) than that of DSS, whereas S.M2 is different (\(P>0.82\)) from DSS by 2 mm Hg, C2QTL1 should be present between D2Rat303 and D2Chm90 (Figure 1). Because BP of S.M6 was 48 mm Hg lower (\(P<0.001\)) than that of DSS, C2QTL2 should exist between D2Chm90 and D2Mgh10 (Figure 1). BP of S.M (ie, 120 mm Hg) is not different from that of S.M6 (ie, 122 mm Hg) (\(P>0.5\)).
Constructions of New Congenic Substrains to Fine-Map Multiple BP QTLs

C2S.M7, C2S.M8, C2S.M9, C2S.M10, C2S.M11, and C2S.M12 span ≈ 5.7 cM, 11.5 cM, 8 cM, 28 cM, 1 to 2 cM, and 5.5 cM, respectively.

Figure 3 shows the actual tracings of systolic arterial pressure, diastolic arterial pressure, and mean arterial (MAP) of DSS and congenic strains by telemetry. For the simplicity of comparisons among the strains, averaged MAPs are shown at the bottom of Figures 1 and 2.

C2S.M7 was produced by targeting the chromosome segment between D2Rat303 and D2Rat166 of S.M1 (Figure 1). C2S.M7 exhibited a BP lower (P < 0.03) than that of DSS (Figures 2 and 3).

When C2S.M9 (Figure 2) were produced from S.M6, its MAP of 139 mm Hg was 31 mm Hg lower (P < 0.02) than that of 170 mm Hg of DSS rats (Figure 2). This lowering in BP of 31 mm Hg by C2S.M9 could only explain ≈65% (ie, 31/48) of that observed in S.M6 (ie, 170 to 122 = 48 mm Hg), suggesting that there might be another QTL, C2QTL3, that would have an additive BP effect on C2QTL2. To prove this prediction, a new congenic substrain, C2S.M11 (Figure 2), was constructed specially targeting the subsegment in S.M6 not overlapping with C2S.M9 (Figures 1 and 2). MAP of C2S.M11 was 147 mm Hg (Figures 2 and 3), which was lower (P < 0.03) than that of DSS.

From C2S.M9, C2S.M12 was produced (Figure 2), and its BPs were not different (P > 0.3) from those of DSS (Figures 2 and 3). As a result, the interval for C2QTL2 can be further narrowed to the section not overlapping between C2S.M9 and C2S.M12 by subtracting the segment shared between them (Figure 2).

The genes for the soluble subunits (Gucy1a) and (Gucy1b) of guanylate cyclase 1 are located in the chromosome fragment in C2S.M12 (Figure 2), which had a BP not different (P > 0.3) from that of DSS.

Epistatic and Additive QTL–QTL Interactions

Figure 4 summarizes the relationships among the 3 C2QTLs, assuming that 1 QTL was involved in each QTL interval. A
2×2 factorial ANOVA indicated that there is an epistatic interaction \((P<0.03)\) between C2QTL1 and a combination of C2QTL2 and C2QTL3 (Figure 4a). This interaction can be viewed in another way. That is, S.M (Figure 1), which harbors 3 QTLs, C2QTL1, C2QTL2 and C2QTL3, possessed a similar BP (ie, 120 mm Hg) as that of S.M6 (ie, 122 mm Hg), which contained only 2 QTLs, C2QTL2 and C2QTL3 (Figure 1). The BP effect of C2QTL1 was apparently masked when combined with C2QTL2 and C2QTL3.

C2QTL3 and C2QTL2 appear to act additively to each other, because a 2×2 factorial ANOVA demonstrated that there is no epistatic interactions between them \((p\text{ interaction }0.5\); Figure 4b). In other words, a combined BP effect of C2QTL2 and C2QTL3 is greater than each of the 2 QTLs acting alone.

**Discussion**

Major findings from the current studies are: (1) a comprehensive and systematic congenic coverage unraveled multiple QTLs closely linked in a segment on Dahl rat Chr 2; and (2) certain of these QTLs demonstrated epistatic interactions, whereas others exhibited additive effects.

**Comprehensive Congenic Coverage Divulging Multiple BP QTLs in a Closely Linked Region**

All the available markers in the rat database for the region between D2Rat166 and Mme were tested, but none was either homozygous SS or MM for S.M1 and S.M6 (Figure 1). Therefore, potential overlaps could not be ruled out between the lower segment for C2QTL16 and the upper segment for C2QTL25 (Figure 1). Two approaches of proving that C2QTL1 and C2QTL2 are separate genes were that a marker between D2Rat166 and Mme should be SS for both S.M1 and S.M6, and/or nonoverlapping congenic strains separately covering C2QTL1 and C2QTL2 would each show a BP effect.

The new marker, D2Chum90, turned out to be SS for S.M1 and S.M6 (Figure 1). Moreover, both C2S.M8 and C2S.M9 are clearly separate from C2S.M7 (Figure 2), and all 3 showed BP effects (Figure 2). Combining both sets of
evidence, C2QTL1 and C2QTL2 indisputably represent distinctive genetic loci.

In addition to C2S.M7, C2S.M8, and C2S.M9, congenic strain C2S.M11 (Figure 2) also showed a BP significantly lower \( (P < 0.03) \) than that of DSS (Figure 3). In contrast, C2S.M10 and C2S.M12 (Figures 2 and 3) had BPs not statistically different \( (P > 0.3) \) from that of DSS (Figure 3). Because congenic strains C2S.M7, C2S.M9, and C2S.M11 unequivocally do not overlap in the chromosome segments that they cover (Figure 2) and all showed BP effects, consequently, 3 separate QTLs, C2QTL1, C2QTL2, and C2QTL3, must be located in C2S.M7, C2S.M9, and C2S.M11, respectively. C2QTL1, C2QTL2, and C2QTL3 reside in intervals of 5.7, 3.5, and 1.5 cMs respectively.

Because C2S.M10 and C2S.M12 as “negative” controls did not manifest significant alterations in BP from DSS, the BP effects of the 3 QTLs observed could not be caused by the genetic background in congenic strains C2S.M7, C2S.M9, and C2S.M11. It is a QTL that is responsible for lowering BP in each congenic strain.

Because of a lack of congenic strain specifically made for the fragment between D2Chm57 and D2Wox27 (Figure 2), it cannot be ruled out that an additional QTL could still exist in that region.

Our results are consistent with those of other investigators,\(^3\,^7\,^8\) who showed the existence of several BP QTLs in similar regions. Specifically, in comparing our current results with those of Garrett and Rapp,\(^7\) several features are evident. First, Milan normotensive strain (MNS) and Wistar Kyoto strains may or may not share the same alleles at each QTL on Chr 2. For example, C2QTL1 seems unique to the DSS and MNS contrast. Second, C2QTL2 and C2QTL3 shared the same chromosome segment with QTL1 and QTL2 in Garrett and Rapp,\(^7\) implying that the same QTL alleles could be in common between MNS and Wistar Kyoto. Third, the C2QTL3 region in our current work is now small enough (ie, 1.5 cM) for positional cloning to identify the gene. Finally, our telemetry affords more accurate measurements to assess a QTL effect and QTL–QTL interactions. In sum, our current work fine-mapped the 3 C2QTLs and presented evidence for both epistatic and additive QTL–QTL interactions among them.

**Epistatic and Additive QTL Interactions Among C2QTL1, C2QTL2, and C2QTL3**

From Figure 4, it is apparent that C2QTL1 is epistatic to either C2QTL2 or C2QTL3. It is unknown if the interaction was between C2QTL1 and C2QTL2, or between C2QTL1 and C2QTL3.
and C2QTL3. Mechanistically, it is probable that C2QTL1 belongs to the same pathway/cascade as either C2QTL2 or C2QTL3. Increasingly, epistasis in determining BP has been recognized as an important organizational hierarchy among BP QTLs, not simply a background genetic noise. It will be worthwhile to ascertain with which of the 2 QTLs, ie, C2QTL2 or C2QTL3, C2QTL1 interacts epistatically. One way to accomplish this task is to make “double” congenic strains between both C2QTL1 and C2QTL2, and C2QTL1 and C2QTL3, much as S.M6 being for C2QTL2 and C2QTL3. Because these 3 QTLs are located in close proximity on the same chromosome (Figure 2), any “double” congenic strain as such will require a crossover between 2 closely linked markers. So far, no such crossings have been achieved (data not shown). Another way to study these epistatic interactions is to construct “double” congenic strains between a congenic strain from a different chromosome and every one of the single congenic strains harboring the 3 QTLs on Chr 2. In this case, no chromosome crossovers are required so that a production of such a “double” congenic strain is practically achievable. This line of work is ongoing.

The phenomenon of additive QTL interactions between C2QTL2 and C2QTL3 is not an isolated instance. Several BP QTLs adjacent to one another acting additively have also been detected on another chromosome using congenic strains. In a mechanistic sense, it is possible that QTLs in an additive relationship belong to different pathways/cascades.

### Perspectives

There are 3 BP QTLs closely linked in a chromosome segment of 19.6 cM of the DSS rat in contrast to the MNS rat. C2QTL1 interacted with either C2QTL2 or C2QTL3 epistatically, whereas C2QTL2 and C2QTL3 seemed to be additive in affecting BP. By extrapolation, the identifications of these QTLs and how they interact with one another could have an impact on the revelation of mechanisms governing certain forms of human essential hypertension.

### Newy Generated Polymorphic Chr 2 Markers

<table>
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<tr>
<th>Rat Marker</th>
<th>Supercontig</th>
<th>Primer Sequences (5′→3′)</th>
<th>Size (bp)</th>
<th>Temperature (°C)</th>
<th>Condition (P or A)</th>
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A indicates agarose gel electrophoresis; bp, base pairs; DSS, Dahl salt-sensitive strain; MNS, the Milan normotensive strain; P, polyacrylamide gel electrophoresis.

Supercontigs are those obtained from blasting an existing marker on the map to the rat genome database at http://www.ncbi.nlm.nih.gov/blast/. Only polymorphic markers used in our present work are listed.

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References


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