Epistasis, Not Numbers, Regulates Functions of Clustered Dahl Rat Quantitative Trait Loci Applicable to Human Hypertension

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Abstract—Quantitative trait loci (QTLs) for blood pressure (BP) were found on chromosome 10 of Dahl salt-sensitive rats and are potentially important to human essential hypertension. But their identities and how they influence BP together were not known. Presently, we first fine mapped existing QTLs, C10QTL1, C10QTL2, and C10QTL3, by constructing congenic strains. In the process, a new QTL, C10QTL4, was identified. Because the intervals harboring C10QTL1 and C10QTL4 contain a maximum of 16 and 10 possible genes, respectively, a limited number of specific gene targets has been identified to be QTLs residing in human homologous regions on chromosome 17. Moreover, because none of these candidates encodes a gene known to influence BP, the 2 QTLs will represent novel genes for BP regulations. Second, we used congenic strains with QTL combinations to analyze the interactions between the QTLs. Consequently, a double combination of C10QTL4 and C10QTL1 possessed the same BP as each of the 2 QTLs alone. BP of a triple combination of C10QTL4, C10QTL1, and C10QTL3 was not different from BP of the C10QTL4 and C10QTL1 double combination. These results demonstrate that C10QTL4, C10QTL1, and C10QTL3 are epistatic to one another in their BP effects. In contrast, when adding C10QTL2 into the triple formation of the 3 QTLs above to create a quadruple QTL combination, BP increased proportionately, indicating that C10QTL2 acts independently of C10QTL4, C10QTL1, and C10QTL3. The epistatic and additive interactions uncovered in the animal model will help elucidate similar interactions playing a role in human essential hypertension. (Hypertension. 2005;46:1300-1308.)

Key Words: gene–gene interaction ■ fine QTL mapping ■ comparative homology ■ congenic combinations

Unraveling the pathogenesis of essential hypertension depends on revelations of functional roles of quantitative trait loci (QTLs) for blood pressure (BP) and on a comprehension of their interactions. Animal models can be used for gene discovery, as well as for providing insights into the organizational hierarchy of QTLs1 because of the availability and ethical permissibility in experimental manipulations. The list of BP QTLs localized in a Dahl salt-sensitive (DSS) strain has been accumulating.1,2 Those on chromosome (Chr) 10 especially drew our attention,3 because its homologous segments on Chr 17 also harbor QTLs for certain human populations.4–6 Identifications of individual QTLs and an unveiling of their interactions from rat Chr 10 are important in understanding the pathogenesis of essential hypertension contributed by CHR 17.7

Our previous work showed the presence of 3 QTLs on Chr 10.3 Their combined effect, as demonstrated by the congenic strain S.LC10, seemed to account for 75% of the total BP difference between DSS and the normotensive Lewis (LEW).3 Although this initial observation pointed toward an additive relationship among these QTLs, this conclusion was indirect and inferential only. More stringent experimental evidence is required to elucidate their true relationships.

Although linkage studies can provide certain clues,8 an unambiguous demonstration of an epistatic or additive interaction between 2 QTLs can only come from the study of their combinations using congenic strains.9–12 Also, fine mapping needs to progress to the point that the possibility of possessing >1 QTL in each congenic interval has been minimized.

Current studies were designed to, first, fine map individual BP QTLs on Chr 10 and to reveal limited candidates for gene discovery of a QTL that probably predisposes certain humans to hypertension. Second, systematic QTL combinations by congenic strains are to be investigated to reveal their relationships.

Methods

Animals

Protocols for handling, as well as maintaining, animals were approved by our institutional animal committee. The breeding procedure, markers used, and the screening protocol for generating congenic strains were essentially the same as reported previ-
Constructions of New Congenic Substrains
S.L.10, S.L.6, and S.L.11 were used to derive congenic substrains. The procedure was similar to that published previously. For the current work, 7 congenic substrains were produced (Figure 1) and are designated as: DSS.LEW-(D10Chm128-D10Chm182)/Lt (abbreviated as C2S.L16), DSS.LEW-(D10Chm224-D10Chm222)/Lt (abbreviated as C2S.L17), DSS.LEW-(D10Chm224-D10Chm6)/Lt (abbreviated as C2S.L20), DSS.LEW-(D10Mcc30-D10Goe92)/Lt (abbreviated as C2S.L22), DSS.LEW-(D10Rat204-D10Rat9)/Lt (abbreviated as C2S.L23), and DSS.LEW-(D10Chm128-D10Chm169)/Lt (abbreviated as C2S.L24).

BP Measurements
BP studies on the congenic strains were essentially the same as reported previously. 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 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.

BPs for all of the strains were measured at least at 2 different times to exclude seasonal, as well as environmental, influences. Thus, the BP data were pooled from separately reproducible measurements for each strain. In the presentation of telemetry data, averaged 24-hour readings are shown during the course of measurements. Because systolic and diastolic pressures were consistent with mean arterial pressures (MAP) of the strains, only MAP is presented.

Statistical Analysis
Repeated measures ANOVA followed by Dunnett (which permits a correction for multiple comparisons and sample sizes) was used to compare a parameter between 2 groups as presented previously. During the BP comparison, ANOVA was used to analyze the data to see whether there was any difference among the groups. If the difference was significant, then the Dunnett test ensued to establish which group was different and how much more significant it was. If the difference was not significant, then the ANOVA/NK test ensued to establish the presence of another QTL or simply because of the influence of the genetic background. To resolve this issue, C10S.L15 and C10S.L24 were derived (Figure 1). C10S.L17 and C10S.L20 were designed for fine mapping C10QTL1 (Figure 1). C10S.L16 and C10S.L22 were designed for fine mapping C10QTL2 and C10QTL3 (Figure 1).
Figure 1. Fine congenic mapping and QTL combinations: the linkage map is essentially the same as published previously. The contigs and marker positions within them are taken from http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=10116. Solid bars under congenic strains symbolize the DSS chromosome fragments that have been replaced by those of the Lewis rat. Open bars on ends of solid bars indicate the ambiguities of crossover break points between markers. Ace, angiotensin-converting enzyme; Nos2, the inducible form of the nitric oxide synthase; Wnk4, serine/threonine kinase WNK4. The rest of the markers are anonymous. D10Chm markers are newly designed markers based on the rat genome sequence. MAPs for DSS and congenic strains are shown at the bottom to facilitate strain comparisons. SEMs for MAPs of the all strains are between 1 and 7, and, because of space limitations, are not listed for each strain. n, number of rats measured. Intervals harboring the 4 QTLs are marked to the right by brackets with the size of each interval indicated in Mbs.
ANOVA, assuming that 1 QTL gene was involved in each QTL interval. BPs for the group of strains in Figure 3a were measured simultaneously; the same applied to those for Figure 3b and c. There is clearly an epistatic interaction between C10QTL4 and C10QTL1 (P<0.002; Figure 3a). Adding C10QTL3 into C10QTL4 and C10QTL1 double combination to form the triple combination did not increase BP (Figure 3b), indicating that C10QTL3 interacts epistatically (P<0.009), as well with both C10QTL4 and C10QTL1. The BP of the triple QTL combination was not different from that of C10QTL4 or C10QTL1 alone (data not shown). Therefore, a numerical accumulation of C10QTL4, C10QTL1, and C10QTL3 did not increase the BP effect beyond that of the single QTL of the 3 QTLs, as if being confined by a “ceiling” effect. In contrast, when C10QTL2 was added into the combination of the other 3 QTLs (Figure 3c), BP increased proportionately, indicating that there is no epistatic interaction (P<0.939) between C10QTL2 and the other 3 QTLs.

**Discussion**

A major finding from the current work is that several BP QTLs are found within a segment of Dahl rat Chr 10, which is homologous to a section of human CHR 17. The intervals for 2 of these QTLs, C10QTL4 and C10QTL1, harbor only 10 and 16 genes and undefined Locs, respectively. This limited number of candidates for each of C10QTL4 and C10QTL1 will, no doubt, facilitate the mutation screening for gene discovery of homologous QTLs in humans. Because none of these genes and Locs is known to influence BP, the human homologous genes for C10QTL4 and C10QTL1 will likely provide novel therapeutic targets and diagnostic tools for human hypertension. The second major finding from the current work is that functions of 3 BP QTLs clustered are controlled by epistasis, a feature revealed only by experimental combinations of congenic strains. Their numerical aggregation has no influence on BP. This QTL-QTL interaction will help elucidate the relationships among homologous...
Fine Congenic Mapping Identified Limited Number of Gene Candidates for Each of 2 BP QTLs on Dahl Chr 10 Potentially Relevant to Human Hypertension

Building congenic substrains for fine mapping BP QTLs has yielded 2 intervals harboring only 10 and 16 genes and Locs for each of C10QTL4 and C10QTL1 (Figures 1, 4, and 5). These candidates can serve as molecular targets for positional cloning of C10QTL4 and C10QTL1. Because they have homologous human counterparts, this limited number of candidates provides gene targets for immediate mutational screening in human populations for association studies, although the molecular identities of the 2 QTLs have not been identified.

It is worth noting that, because the obvious genes known to affect BP, such as Ace, Nos2, and Wnk4, have been excluded as candidate genes for a QTL (Figure 1), each of C10QTL4 and C10QTL1 will be a brand new gene for regulating BP homeostasis. It remains to be determined whether there could be more QTLs present in the broad intervals harboring C10QTL2 and C10QTL3 (Figure 1).

Strategy for QTL Discovery

Regarding the molecular identification of a QTL once it has been restricted to an interval harboring a limited number of genes, a brief discussion on the strategy of QTL discovery is in order. An extensive and detailed discourse has been presented recently by Deng.1

Much like the intervals harboring C10QTL1 and C10QTL4 (Figures 4 and 5), most of the genes residing in them, known or undefined, are not reputed to influence BP. Consequently, the identification of the QTL in question impinges on the strategy of positional cloning, that is, discovering the identity of the QTL based purely on its location on the chromosome without any other physiological, biochemical, or signaling clues. To accomplish positional cloning of a QTL, several approaches are available.

Fine Congenic Mapping

Ideally, if one can create a congenic strain that changes BP, and the QTL interval contains only one gene that possesses alleles contrasting between 2 comparing strains, it unambiguously proves that this gene is the QTL in question. Limitations in this line of work lie in the ability of obtaining crossovers flanking solely 1 gene. However, it is practically achievable to restrict a QTL interval to 100 to 200 kb, and such an interval can contain 1 to 2 genes.

Gene Profiling Followed by Functional Testing

One way to quickly and directly find a candidate gene for a QTL can be by gene profiling based on a genome-wide

and C10QTL3. (c) The observed BP effect of 1 congenic S.LC10 combining C10QTL4, C10QTL1, C10QTL3, and C10QTL2 is not different (P interaction >0.94) from a predicted sum of BP effects of C10QTL4, C10QTL1, and C10QTL3 plus C10QTL2. Thus, there is no QTL-QTL interaction between C10QTL2 with other 3 C10QTLs.
However, this approach depends on narrowly defined functional assumptions for the selection of a targeted tissue, time course, and age. Also, this approach assumes that the QTL in question is differentially expressed between 2 contrasting strains. As a consequence, it is prone to produce false positives. Therefore, stringent functional verifications have to follow after a candidate gene has been found by gene profiling. These functional authentications include, but are not limited to, a transgenic rescue of an appropriate phenotype and/or fine interval mapping by congenic strains.

More relevant to the intervals harboring C10QTL1 and C10QTL4, if any gene profiling is to be conducted, a region-specific microarray rather than a genome-wide microarray is appropriate. In this case, expressions of all of the genes in the intervals containing C10QTL1 and C10QTL4 will need to be examined to identify the pertinent tissues and all of the tissues in which a gene is expressed. Subsequently, the region-specific microarray for C10QTL1 and C10QTL4, respectively, will be hybridized with RNAs extracted from all of the relevant tissues. A candidate gene(s) thus found has to reside inside the C10QTL1 or C10QTL4 interval to be a valid candidate.

Region-Saturated Gene Sequencings

Allelic differences for either C10QTL1 or C10QTL4 can be present in coding rather than in the regulatory regions. In this case, mutation detections in functional domains of a gene are essential. In order to find a candidate gene with significant nucleotide differences defining differing alleles for either C10QTL1 or C10QTL2, exons and exon-intron boundaries for all of the known and undefined Locs in the interval (Figures 4 and 5) need to be sequenced and compared. If there is a significant mutation(s) found in only 1 gene in either the C10QTL1 or C10QTL4 interval, this gene will become a candidate. Functional testing on this gene to be the QTL in question will be conducted. If there are significant mutations found in multiple genes in either the C10QTL1 or C10QTL4 interval, fine definition congenic mapping will need to be accomplished to narrow the gene candidate, or/and functional testings on each of these genes need to be performed.

Functional Testing on an Individual Gene as a BP QTL

There are several techniques that allow the functional testing of a specific gene. They include fine congenic mapping presented above, transgenic rescues, gene targeting by homologous recombination, and interference RNA. In the rat, except for gene targeting, all of the other approaches can be accomplished.

Epistatic and Additive QTL Interactions among C10QTLs

It is evident that the 3 QTLs, C10QTL4, C10QTL1, and C10QTL3, do not exert an incremental BP effect when acting in concert with one another. Thus, the absolute quantity of QTLs does not control the BP of an animal. Mechanistically, it is probable that C10QTL4 belongs to the same pathway/cascade as either C10QTL1 or C10QTL3. Because most of the genes residing in the intervals harboring C10QTL1 and C10QTL4 do not have clear functions, implications in shared cellular and/or biochemical pathways between the 2 QTLs still await their molecular identities to be defined. In contrast, it is likely that C10QTL2 belongs to different pathways/cascades from C10QTL4, C10QTL1, and C10QTL3.

The revelation of epistatic interactions or a lack of it among BP QTLs has clear implications in the studies of...
human essential hypertension. First, considering the genetic bases of hypertension in a heterogeneous human population, 1 hypertensive individual can, understandably, have a defect in a QTL different from another hypertensive individual possessing a defect in another QTL on CHR 17. It is not surprising to observe that a genetic marker was found to be associated with hypertension in certain populations4–6,32,33 while not in others.34,35 Environmental influences aside, it is most likely that the varying results from analyzing various populations were attributable to the inherent genetic property of that population versus another, which is not necessarily an “inconsistency” of the genetic research. This type of genetic “population specificity” seems more of a true representation of hypertension caused by heterogeneous flaws than having a “master” gene responsible for all populations. Following a logical reasoning, in designing an antihypertensive drug and in applying clinical treatment, it seems to be more effective and efficient to administer a drug specifically targeting an appropriately designated population than aiming at most or all populations.

Second, a defect in 1 QTL will likely have a similar effect in changing BP as defects in >1 QTL sharing the same epistatic relationship. Moreover, a defect in the gene higher on the epistatic hierarchy can hide gene effects lower on the same epistatic grouping or pathway/cascade.1,11 Consequently, when analyzing the function of a gene in association with BP in human studies, a lack of an effect could also be attributable to the effect of another gene downstream in the epistatic hierarchy. In this case, separating as well as combining individual QTL effects are necessary to elucidate the relationships between the 2 QTLs.

Substitution Mapping Separating Closely Linked QTLs and Revealing Complex QTL-QTL Interactions

It is worth noting that the use of congenic strains constitutes a physical mapping of QTLs that cannot only define where exactly a QTL resides within a clearly designated interval, but also it can be combined to analyze QTL-QTL interactions. In comparison, linkage analyses are limited to indicating only an approximate location of a QTL on Chr 10.36,37 It cannot and does not define the number of QTLs present and their exact physical boundaries and unveil whether or not the QTLs act.
independently or dependently in relation to each other. Furthermore, linkage analysis cannot distinguish closely linked QTLs that have opposing BP effects. Therefore, substitution mapping is essential in achieving QTL identifications, as well as revealing QTL-QTL interactions.

Perspectives
The current work additionally refined intervals harboring 2 QTLs that have opposing BP effects. Therefore, linkage analysis cannot distinguish closely linked QTLs that have opposing BP effects. Furthermore, linkage analysis cannot distinguish closely linked QTLs that have opposing BP effects. Therefore, substitution mapping is essential in achieving QTL identifications, as well as revealing QTL-QTL interactions divulged from our analyses will provide insights into how similar QTLs will interact in humans.

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References

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