# Hypertension Suppression, Not a Cumulative Thrust of Quantitative Trait Loci, Predisposes Blood Pressure Homeostasis to Normotension

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***Background***—Genetics of high blood pressure (BP) has revealed causes of hypertension. The cause of normotension, however, is poorly understood. Inbred Lewis rats sustain normotension despite a genetic push in altering BP. It was unknown whether this rigid resistance to BP changes is because of an insufficient hypertensive impact from limited alleles of quantitative trait loci (QTLs) or because of an existence of a master control superseding the combined strength of hypertensive QTL alleles.

***Methods and Results***—Currently, BP-elevating QTL alleles from hypertensive Dahl salt-sensitive rats (DSS) replaced those of Lewis on chromosomes 7, 8, 10, and 17 on the Lewis background. These hypertensive QTL alleles were then merged to systematically achieve multiple combinations. Results showed that there was no quantitative correlation between BP variations and the number of hypertensive QTL alleles, and that BP was only slightly elevated from a combined force of normotensive alleles from 7 QTLs. Thus, a genetic factor aside from the known QTLs seemed to be at play in preserving normotension and act as a hypertension suppressor. A follow-up study using consecutive backcrosses from Dahl salt-sensitive rats and Lewis identified a chromosome segment where a hypertension suppressor might reside.

***Conclusions***—Our results provide the first evidence that normotension is not enacted via a numeric advantage of BP-lowering QTL alleles, and instead can be achieved by a particular genetic component actively suppressing hypertensive QTL alleles. The identification of this hypertension suppressor could result in formulating unique diagnostic and therapeutic targets, and above all, preventive measures against essential hypertension. **(*Circ Cardiovasc Genet*. 2015;8:610-617. DOI: 10.1161/CIRCGENETICS.114.000965.)**

**Key Words:** blood pressure ■ chromosomes ■ genetics ■ models, animal ■ rats



Essential hypertension is one of the most prominent disorders leading to fatal cardiovascular and renal diseases and is known to be polygenic.[1] The genetic architecture of polygenic hypertension has long been thought to be composed of individual constituents known as quantitative trait loci (QTLs) for blood pressure (BP).[2,3] These QTLs, and mostly because of them, are expected to be genetically responsible for linearly increasing or decreasing BP by a cumulative-of-miniscule-effect formula.[2,3] Although this assumption follows statistical calculations well, the actual biological impact of BP QTLs, in reality and functionally, acts according to modularity rather than accumulatively aggregating independent individual units when experimentally combined in an animal model, the hypertensive Dahl salt-sensitive rats (DSS).[4]

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A qualifying caveat in this line of work is that experimentally combining QTLs was successfully performed exclusively in the genetic background of DSS, which has lost the capacity of buffering BP changes.[5] The reverse is not expected to be informative if the work was to be done in the normotensive Lewis genetic background. How can this be? What is so special about the Lewis genetic background?

Hypertensive animal models are widely appreciated as potent tools for comprehending the pathogenesis of essential hypertension. In comparison, normotensive animals, beyond their usefulness as controls for hypertensive models, have received little recognition capable of unmasking critical mechanisms that can prevent hypertension. For example, genome regulations in Lewis are so powerful that the ability of QTLs in raising and diminishing BP can be annulled.[5] Thus, a thorough understanding of the pathogenesis of hypertension requires the identification of individual QTLs. Conversely, the elucidation of mechanisms sustaining normotension demands our comprehension of the genetic element(s) that can nullify the impact of hypertensive QTL alleles.

Although contributions from BP-increasing alleles of QTLs to hypertension are well recognized,[2,3,6] little is known about the nature of genome regulations on QTLs that can over-ride their impact on BP.[6,7] An obvious question to address is what is the genetic basis of these genome regulations?

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Because they can negate the effects of BP QTLs,[5] it seems most likely that a suppressor would function in the Lewis genome, but it would be either dysfunctional or missing in the DSS genome.[6,7] Alternatively, an inadequate accumulation of hypertensive QTL alleles could not be ruled out.

The current investigation had 3 objectives. First, an analysis was to be performed to ascertain the BP influence of various hypertensive QTL alleles on different chromosomes on the Lewis genetic background. Second, because BP is a quantitative trait, if no BP effect could be achieved from a single QTL from 1 chromosome, double and multiple QTL combinations from several chromosomes were to be made. Finally, genomic segment(s) that potentially contain a hypertension suppressor(s) (HSs), whether it is a QTL or not, was to be defined.

## Methods

### Animals

Protocols for handling as well as maintaining animals were approved by our institutional animal committee (CIPA). The DSS and Lewis strains are the same as used previously.[5]

### Construction of New Congenic Strains

Congenic strains bear different segments of Lewis chromosomes that are replaced by the homologues of DSS. The breeding and screening procedures in this process were similar to those reported previously.[4,5] For the present work, 4 new congenic strains were produced (Figure 1), designated: Lewis.DSS-(D7Rat27-D7Mgh1)/Lt (abbreviated as C7L.S), Lewis.DSS-(D8Chm12-D8Rat15)/Lt (abbreviated as C8L.S), Lewis.DSS-(D10Mgh6-D10Mgh1)/Lt (abbreviated as C10L.S), and Lewis.DSS-(D17Rat15-D17Rat51)/Lt (abbreviated as C17L.S). Rats for chromosomes 1 and 16 failed to reproduce in the last round of breeding to build LL homozygotes, and consequently no congenic strains were established for these chromosomes.

### Manufacture of Congenic Combinations

A congenic strain for 1 chromosome was coalesced with another from a separate chromosome to synthesize a double or multiple QTL aggregate.

### Generation of Backcross Animals

The process was similar to that of creating a congenic strain,[5] except that no chromosome segments were selected. Briefly, DSS was bred with Lewis to produce $F_1$ progeny. $F_1$s were backcrossed to DSS to

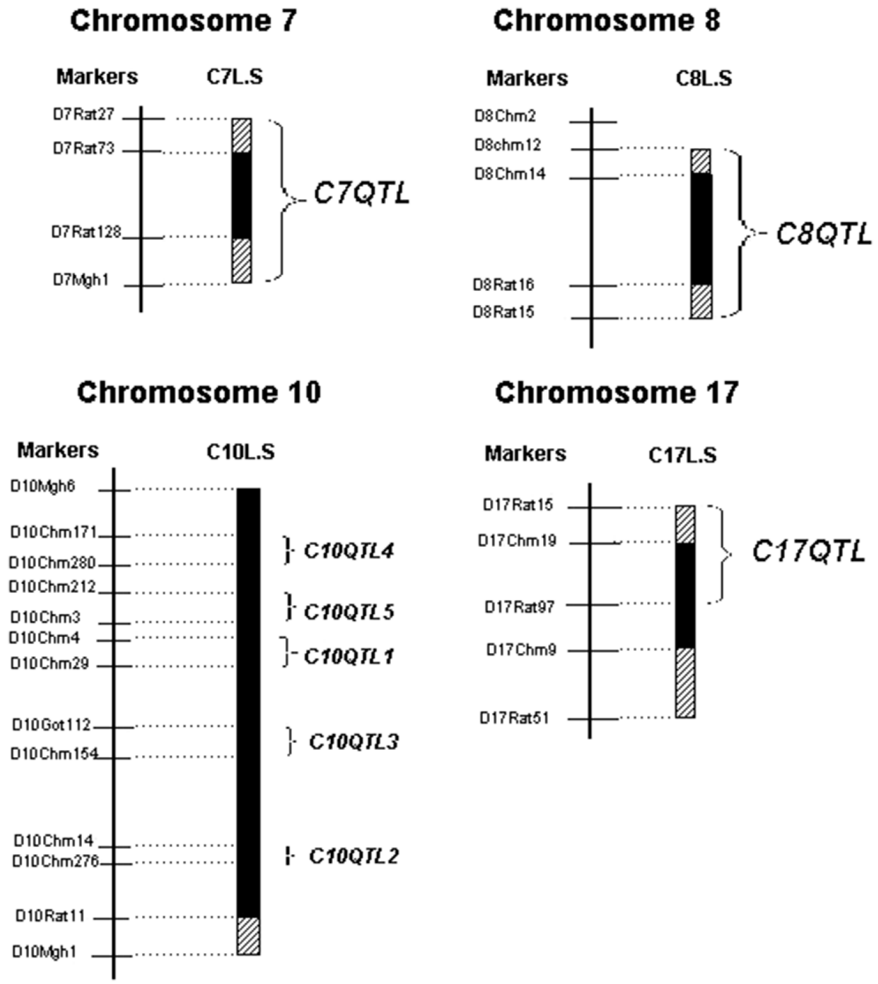


**Figure 1.** Chromosome coverage of 4 congenic strains and quantitative trait loci (QTL) placements within them. Solid bars under congenic strains symbolize the chromosome fragments that have been replaced by those of Dahl salt-sensitive rats. Hatched bars on ends of solid bars indicate the ambiguities of crossover breakpoints between markers. The actual mean arterial pressures for Lewis and congenic strains are given in Figure I in the Data Supplement. The full names are given in the Construction of new congenic strains section. The placements of the blood pressure QTLs are marked in the right by brackets. The data defining these QTLs are given in Figure II in the Data Supplement.

beget backcross 1 (BC1) offspring, then BC2, and so on. BPs of rats from $F_1$ and each BC generation were measured.

### Animal Protocols, BP Measurement, and Statistical Analyses

Breeding protocols, dietary treatments, implantation, and BP measurement schedules were virtually the same as documented previously.[5] Briefly, male rats were weaned at 21 days of age, maintained on a low-salt diet (0.2% NaCl, Harlan Teklad 7034) and followed by 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 at 56 days of age (namely 3 weeks from the time of the high-salt diet).

Repeated measures ANOVA followed by Dunnett test, which corrects for multiple comparisons and unequal sample sizes, was used to compare a parameter in mean arterial pressure (MAP) between 2 groups, a congenic and Lewis strains, as reported previously.[4,5] The Systat 9 program from SPSS Science was used. The power of such an analysis with 5 to 10 rats in each group is high enough to detect BP differences by telemetry.[4,5] The study provides 85% power for detecting BP differences between 2 strains.

## Results

### Nomenclature Clarifications

First, a single gene is presumed to be responsible for 1 QTL.[8] Second, BP, MAP, or hypertension is interchangeably used to designate a QTL, because all the QTLs in our studies simultaneously affect MAP, systolic arterial and diastolic arterial pressures.

### Rationale and Study Design

Two types of genetic factors could be a HS in short: (1) one (or several) of the Lewis BP QTL alleles themselves and (2) one (or several) of Lewis alleles other than BP QTLs. If a Lewis BP QTL allele was such a HS, a congenic strain with each of other QTLs in double and multiple combinations would provide some insights. If a HS was a gene other than a BP QTL, it would be necessary to pinpoint the chromosome segment in the genome harboring it in a manner unprejudiced toward, and nonprescribed by, QTLs.

### BP Effects of Single Congenic Strains

Figure I in the Data Supplement displays MAPs for each of 4 congenic strains, C7L.S, C8L.S, C17L.S, and C10L.S, as well as for each congenic combination. In designating a congenic strain, for example, C7 refers to chromosome 7, L preceding S indicates that the former provided the recipient background and L and S refer to Lewis and DSS chromosome segments, respectively. Because systolic arterial pressures and diastolic arterial pressures were consistent (data not shown) with MAPs of all the strains, only their MAPs are presented.

The congenic strain made in the Lewis genetic background, that is, C8L.S, did not show a BP different from that of Lewis (Figure IA in the Data Supplement; Figure 2). Similarly, C7L.S and C17L.S cannot singularly change BP from the level of Lewis (Figure I in the Data Supplement; Figure 2), despite that each of them (Figure 1) carries the hypertensive DSS alleles from at least 1 BP QTL[4] and the magnitude of the BP response from each QTL is considerable when placed in the DSS background (Figure II in the Data Supplement). This fact signifies that the HS is not the alleles from any one of the QTLs nor do they belong to any of other genes residing in the chromosome region specified by each of the 3 congenic strains, C7L.S, C8L.S, and C17L.S.

C10L.S slightly, but significantly, increased BP (ANOVA $P<0.002$) from the Lewis comparing strain (Figure IE in the Data Supplement; Figure 2), indicating that DSS alleles from 5 BP QTLs[4,9] conveyed by the congenic strain partially overcame the control of a HS. Whether a HS was Lewis alleles from 1 of 5 BP QTLs could not be established.

### Combining QTLs to Ascertain the Possibility That the Lewis Alleles From a BP QTL Could be a HS

The congenic strains built in the Lewis background could be combined progressively and with one increment at a time. First, if with a specific combination, a significant BP impact was seen, the Lewis QTL alleles on the chromosome could be implicated as a HS. Second, if a BP effect was observed

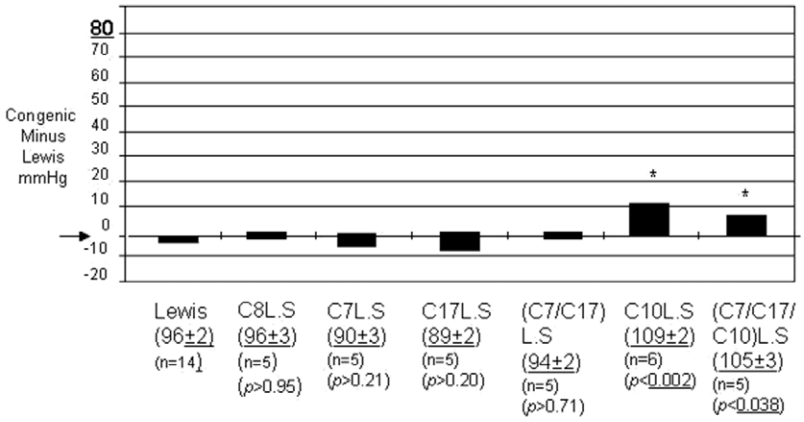


**Figure 2.** Mean arterial pressure (MAP) changes in congenic strains in the Lewis background. The arrow points at the Lewis background as the reference group. MAP of Dahl salt sensitive rats (DSS) minus Lewis is ≈80 mm Hg as underlined and in bold on the *y* axis. The actual MAPs for Lewis and congenic strains are given in Figure I in the Data Supplement. The chromosome coverage of each congenic strain is indicated in Figure 1. The quantitative trait loci (QTL) on each chromosome were defined by congenic strains made in the DSS background. Their MAPs are provided in Figure IIA in the Data Supplement. The magnitude of effects on blood pressure from these QTLs is displayed in Figure IIB in the Data Supplement. On the *x* axis, numbers underlined and below the strain indicates the averaged MAP for that rat strain as derived from the actual MAP data shown in Figure I in the Data Supplement. ± indicates SEM; n, number of rats in each group; and *P*, ANOVA+Dunnett comparing that group with Lewis in MAP.

and increased proportionately to the number of DSS alleles of QTLs added onto it, it could mean that it were the quantitative accumulations of the QTL alleles that regulated BP.

C7L.S was first combined with C17L.S to form a combination of (C7C17)L.S. Its BP was not different from that of C7L.S or C17L.S alone (ANOVA $P>0.28$; Figure 2; Figure I in the Data Supplement). This lack of effect indicates that neither C7QTL nor C17QTL Lewis alleles can be a HS for each other and HS, if anything, cannot be attributed to a quantitative accumulation of the Lewis alleles from the 2 QTLs.

Because C10L.S that lodges DSS alleles from 5 QTLs (Figure 1) exhibited an augmentation in BP compared with Lewis (Figure IE in the Data Supplement; Figure 2), 2 issues emerged. First, the alleles from each QTL might only be able to exert a minor, but insignificant amount of, influence and it was only in summation from the DSS alleles from multiple QTLs could they reach a threshold to change BP. In this scenario, one expects BP would multiply proportionately with the addition of more DSS alleles from additional QTLs in (C7/C17/C10)L.S, as the threshold seemed to have been overcome in C10L.S. Alternatively, the Lewis alleles from 1 of 5 QTLs could act as a HS. In this case, (C7/C17/C10)L.S should show an increase in BP from C10L.S.

As shown in Figure 2 and Figure I in the Data Supplement, BP of (C7/C17/C10)L.S was not different from that of C10L.S alone (ANOVA $P=0.28$; Figure IG in the Data Supplement). This outcome demonstrated that the impact of all the hypertensive alleles from 7 QTLs were blunted by the suppressive effect of a HS, and it is the action of a HS, not a quantitative aggregation of QTL alleles that controlled BP changes in the Lewis background. The HS cannot be the Lewis alleles from any of the 5 QTLs replaced in C10L.S.

Because of these results, no additional combinations of DSS QTL alleles were pursued. The HS seemed to be composed of a Lewis gene other than the Lewis alleles of the known BP QTLs. It would be more informative, then, that an approach not selecting for QTLs should be adopted to locate the HS.

### Identifying a Chromosome Region Possibly Harboring a HS

An intercross between DSS and Lewis yielded $F_1$ progeny, that is, $F_1$(DSS×Lewis), and their BP was not different from that of Lewis (Figure III in the Data Supplement). This outcome showed complete dominance of the Lewis genome and is consistent with our previous evidence that most heterozygous congenic strains possessed the same BP effect as homozygotes.[10] Thus, the most direct and efficient strategy to identify chromosome regions harboring a HS appeared to be the following.

Backcrosses were to be made to progressively increase the DSS genome content until a BP effect could be seen. One could then correlate the BP change with the genome segments that were SL heterozygotes in 1 backcross generation, but SS in the immediately after backcross generation. Consequently, one could deduce the Lewis genome composition which, when replaced by that of DSS, may have removed the suppressive control on the DSS BP QTL alleles. Consequently, one could locate a HS.

$F_1$, BC1, BC2, and BC3 generations carry, on average, 50%, 25%, 12.5%, and 6.25% of the Lewis genome, respectively. BPs of BC1 and BC2 are not significantly different from those of Lewis and $F_1$ (Figure III in the Data Supplement), indicating that the remaining 6.25% Lewis genome should contain a HS. Indeed, BP of BC3 rats turned sharply higher than that of BC2 rats (Figure III in the Data Supplement). Because each rat in BC2 or BC3 was different from one another, a thorough genotype comparison among all the rats individually was performed along with their BPs. A total genome scan (Table) revealed SL and SS regions in BC2 and BC3 rats. BC2 rats were further divided into 2 groups on the basis of their BP readings, that is, BC2 high BP (HBP) rats and BC2 low BP (LBP) rats.

The criterion of selecting the appropriate chromosome fragments potentially carrying a HS was established as follows: all the rats with low BP comparable with that of BC1 should be SL in BC2, which still possessed a suppressive capability, and all the rats with a BP increase in BC2 and BC3 should be SS, which seemed to have lost the suppressive capability.

According to this strict criterion, only 1 chromosome region qualified for a lodging a HS. This segment was designated as C18HS, and its size was ≈20 cM[5] between 2 newly generated markers, C18Chm238 and C18Chm126, without accounting for the ambiguous regions. C18Chm126 and D18Chm238 are situated between D18Mit8 and D18Mgh3, and between D18Rat1 and the end of Chr 18, respectively.[5] The Table shows that C18HS was SS in all BC3 rats as well as in the 3 BC2 HBP rats, that is, BC2 HBPa, BC2 HBPb, and BC2 HBPc. C18HS was SL in all BC2 LBP rats, namely BC2 LBPa, BC2 LBPb, and BC2 LBPc. Thus, being SL seemed to be required for C18HS to retain its suppressive capacity, which was lost when C18HS became SS. Coincidentally, a congenic strain, C18L.S, was LL in C18HS,[5] thus explaining its lack of BP impact. The extent of C18HS is delimited to the section between C18Chm238 and not lower than C18Mit8 (Table), because BC2 HBPb, which has lost the suppressive capability, was SS for C18Chm126, but SL for D18Mit8.

The section between C18Mit8 and C18Wox7 can be excluded to harbor a HS, because C18L.S did not exhibit a BP influence,[5] in spite of the presence of DSS alleles from 2 separate BP QTLs. If any of these 2 QTLs were a HS, one would expect to see a BP increase in C18L.S, because these 2 QTL alleles were SS in genotypes.

Based on the criterion of selecting a HS-containing segment defined previously, most regions did not qualify because they were either SS in BC2 LBP rats or SL in BC3, or both (Table). An analysis of contradictory correlation disqualified other chromosome segments. For instance, D1Uia12 was SL for BC2 LBPa and BC2 LBPb, but SS for BC2 LBPc. D1Uia12 was SL for BC2 HBPa and BC2 HBPb, despite being SS for BC3 rats. A similar process of disqualification was applied to the chromosome fragments marked by chromosome markers such as D3Rat66, D6Mit1, D7Mgh1, and D11Mit1.

## Discussion

Principal revelations from the current work are (1) a singular or aggregated impact toward hypertension from DSS QTL

**Table. Identification of a Chromosome 18 Region Potentially Containing a Hypertension Suppressor in the Lewis Genome by a Total Genome Scan of Backcross Rats With Dahl Salt-Sensitive Rats**

| | | BC2 | | | | | | BC3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chr | Markers | LBPa | LBPb | LBPc | HBPa | HBPb | HBPc | a | b | c | d | e | f | g |
| MAP | | 87 | 105 | 105 | 131 | 125 | 132 | 133 | 130 | 130 | 157 | 137 | 140 | 136 |
| Chr 1 | D1Wox25 | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS | SS | SS |
| | D1Rat304 | SS | SS | SS | SS | SS | SL | SS | SS | SS | SL | SS | SS | SS |
| | D1Mco27 | SS | SL | SS | SS | SL | SS | SS | SS | SS | SS | SS | SL | SL |
| | *Sa* | SS | SL | SS | SS | SL | SS | SS | SS | SL | SS | SS | SS | SS |
| | D1Uia12 | SL | SL | SS | SL | SL | SS | SS | SS | SS | SS | SS | SS | SS |
| | D1Arb33 | SS | SS | SS | SS | SS | SS | SS | SL | SS | SL | SL | SL | SS |
| | D1Rat19 | SS | SS | SL | SS | SS | SS | SS | SS | SS | SL | SL | SL | SS |
| Chr 2 | *Camk* | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | *Gca* | SL | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS | SS |
| | D2Rat302 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS |
| | *Cpb* | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D2Rat199 | SS | SL | SS | SL | SS | SL | SS | SS | SL | SS | SS | SS | SS |
| | D2Uia5 | SS | SL | SL | SL | SS | SL | SL | SL | SL | SS | SL | SS | SS |
| Chr 3 | D3Rat107 | SS | SS | SL | SS | SS | SL | SS | SS | SS | SL | SS | SS | SS |
| | D3Rat17 | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS | SL |
| | D3Rat24 | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D3Rat66 | SS | SL | SL | SL | SS | SS | SS | SS | SS | SS | SS | SL | SS |
| | D3Wox3 | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D3Rat52 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | L |
| Chr 4 | D4Mgh1 | SL | SS | SS | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS |
| | D4Mgh16 | SS | SS | SL | SS | SL | SS | SS | SL | SS | SL | SS | SL | SS |
| | D4Uia1 | SS | SL | SS | SS | SL | SS | SS | SL | SS | SL | SL | SL | SS |
| | D4Mit17 | SS | SL | SS | SS | SL | SS | SS | SL | SS | SL | SL | SS | SS |
| | D4Uia4 | SS | SS | SL | SS | SL | SS | SS | SL | SS | SS | SL | SS | SS |
| Chr 5 | D5Rat130 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D5Uia8 | SS | SS | SS | SS | SS | SS | SS | SS | SL | SL | SS | SS | SS |
| | D5Mco2 | SS | SS | SS | SS | SS | SL | SS | SS | SL | SS | SS | SL | SS |
| | *Ela2* | SS | SS | SS | SS | SS | SL | SS | SS | SL | SS | SS | SS | SS |
| Chr 6 | D6Rat105 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D6Mit1 | SL | SL | SS | SL | SS | SL | SS | SS | SS | SS | SS | SS | SS |
| | D6Mgh3 | SS | SL | SL | SS | SS | SS | SS | SL | SS | SS | SL | SS | SS |
| Chr 7 | D7Mgh1 | SL | SL | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D7Rat152 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D7Rat44 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D7Rat110 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D7Rat18 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D7Rat128 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D7Rat115 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| Chr 8 | D8Mgh11 | SS | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D8Rat134 | SS | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D8Rat43 | SS | SL | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D8Uia2 | SS | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D8Rat55 | SS | SS | SL | SL | SS | SS | SS | SL | SS | SS | SS | SS | SS |
| Chr 9 | D9Rat64 | SS | SS | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SS |
| | D9Uia9 | SS | SS | SL | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS |
| | D9Wox23 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |

(*Continued*)

**Table. Continued**

| | | BC2 | | | | | | BC3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chr | Markers | LBPa | LBPb | LBPc | HBPa | HBPb | HBPc | a | b | c | d | e | f | g |
| Chr 10 | D10Mco10 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SL |
| | D10Mco17 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS |
| | D10Mgh6 | SS | SS | SS | SS | SS | SS | SL | SL | SS | SS | SL | SS | SS |
| | D10Wox6 | SL | SS | SL | SL | SS | SS | SL | SL | SS | SS | SL | SS | SS |
| | D10M11Mit58 | SL | SS | SS | SL | SL | SS | SS | SL | SS | SS | SL | SS | SS |
| Chr 11 | D11Rat50 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D11Mit1 | SL | SL | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D11Uiai | SS | SS | SL | SS | SL | SS | SL | SS | SS | SS | SS | SS | SS |
| Chr 12 | D12Mit6 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D12Rat32 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D12Mit4 | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS | SS | SS |
| Chr 13 | D13Mgh4 | SL | SL | SL | SS | SS | SL | SS | SL | SS | SL | SL | SS | SS |
| | D13Uia3 | SS | SS | SS | SS | SS | SS | SS | SS | SL | SL | SS | SS | SS |
| | D13Uia8 | SS | SS | SS | SS | SS | SS | SS | SS | SL | SL | SS | SL | SL |
| Chr 14 | D14Wox10 | SL | SS | SS | SL | SS | SL | SS | SS | SS | SS | SS | SS | SS |
| | D14Uia2 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D14Uia1 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| Chr 15 | D15Uia8 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS |
| | D15Mgh2 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D15Rat126 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS | SS |
| Chr 16 | D16Rat14 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D16Rat67 | SL | SL | SL | SS | SS | SS | SS | SL | SS | SS | SL | SS | SS |
| | D16Uia2 | SL | SL | SL | SS | SS | SS | SS | SL | SS | SS | SL | SS | SL |
| | D16Mit2 | SS | SS | SL | SS | SS | SS | SS | SL | SS | SS | SL | SS | SS |
| Chr 17 | D17Rat65 | SS | SS | SS | SL | SS | SS | SL | SS | SS | SL | SL | SS | SS |
| | D17Mit5 | SS | SS | SS | SS | SS | SS | SL | SS | SS | SS | SL | SS | SS |
| | D17Mgh5 | SS | SS | SS | SS | SS | SL | SS | SS | SL | SS | SS | SS | SS |
| | *Edn1* | SS | SL | SS | SS | SS | SL | SS | SS | SL | SL | SS | SS | SS |
| Chr 18 | D18Chm237 | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS | SS |
| | D18Chm238 | SL* | SL* | SL* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* |
| | D18Rat1 | SL* | SL* | SL* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* |
| | D18Uia6 | SL* | SL* | SL* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* |
| | D18Rat45 | SL* | SL* | SL* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* |
| | D18Mgh3 | SL* | SL* | SL* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* |
| | D18Chm126 | SL* | SL* | SL* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* | SS* |
| | D18Mit8 | SL | SL | SL | SS | SL | SS | SS | SS | SL | SS | SS | SL | SS |
| | D18Mco6 | SL | SL | SL | SS | SL | SS | SS | SS | SS | SS | SS | SS | SL |
| | D18Wox7 | SL | SL | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS | SS |
| Chr 19 | D19Rat82 | SS | SS | SS | SS | SS | SL | SL | SL | SL | SS | SS | SS | SS |
| | D19Rat25 | SS | SS | SS | SS | SS | SL | SL | SL | SL | SS | SS | SS | SS |
| | D19Rat57 | SS | SS | SS | SS | SS | SL | SL | SL | SL | SL | SL | SS | SS |
| Chr 20 | D20Wox3 | SS | SS | SS | SS | SS | SS | SS | SS | SL | SS | SS | SL | SS |
| | D20Wox1 | SS | SS | SS | SS | SL | SS | SL | SL | SS | SS | SS | SS | SS |
| | D20Mgh1 | SS | SS | SS | SS | SS | SS | SL | SL | SS | SS | SS | SS | SL |
| Chr X | DXUia2 | LL | LL | SS | SS | SS | SS | SS | SS | SS | LL | SS | SS | SS |
| | DXMco1 | SS | LL | SS | SS | SS | SS | SS | SS | LL | SS | SS | SS | SS |
| | DXRat93 | SS | LL | SS | SS | SS | SS | SS | SS | SS | SS | SS | LL | SS |

The sequence of marker appearances reflects their order on the map of each chromosome. BC indicates backcross; HBP, high blood pressure; LBP, low blood pressure; and MAP, mean arterial pressure.

*The genotypes define C18HS and are SL in genotype for all the BC2 low BP rats, but SS in genotype for all the BC2 high BP rats and all the BC3 rats. MAP for each rat is given in millimeter of mercury.

alleles is suppressed by the Lewis background, indicating that BP as a quantitative trait is not strictly determined by the cumulative-of miniscule-effect assumption. (2) A HS exists in the Lewis genome and does not belong to the alleles of any of the known BP QTLs. (3) A previously undefined segment on chromosome 18 might harbor such a HS and no BP QTL is known to be responsible for it.

## HS Regulates the Function of BP QTLs

A systematic assembly of BP-raising alleles from multiple QTLs originating from DSS revealed that a hierarchical genetic regulation plays a dominant part in how BP QTLs behave. The genetic study in backcross animals with a gradual removal of the suppressive control (Table) accentuated and provisionally isolated a chromosome section possibly harboring a HS. The unified results from the 2 lines of investigation established and then reinforced the existence of a HS and thus represents a significant advancement in revealing potential mechanisms beyond a simple description of a QTL dependence on a genetic background.

As to the nature of the HS, it could serve as a regulator at any level controlling BP QTLs ranging from transcription, post-transcription, translation, post-translation to protein interactions, although a possibility cannot be ruled out that the HS could play a dual role in regulating other QTLs as well as being a QTL itself. This HS might be absent or defective in the DSS so that the stabilizing mastery of QTLs on BP variations is lost.

The emerging conceptual HS may conflict with the prevailing conventional wisdom. Namely, the determinant to achieve either hypertension or normotension might be the balancing act of the number of alleles in BP-increasing and BP-lowering QTLs in the genome. In the DSS genome, BP-raising QTL alleles outnumber BP-reducing QTL alleles,[4] possibly rendering DSS hypertensive. Conversely, in the Lewis genome, BP-decreasing QTL alleles exceed BP-rising QTL alleles,[4] tilting the overall BP toward normotension. Although the numeric account of BP-raising and diminishing QTL alleles may be true, the genetic machinery that forces the functional change in actual BP performs differently.

Evidently, the replacement of BP-increasing by BP-decreasing alleles at 1 QTL alone (Figure II in the Data Supplement), for example, on Chr 17,[11] is sufficient to decrease the overall BP, in spite of an overwhelming computational advantage of BP-augmenting QTLs presumably counteracting it in the DSS background.[6] Thus, the balancing theory remains mostly conjectural. In contrast, the presence of a HS is experimentally prescribed and should expedite our understanding of the master control that stands higher in the regulatory hierarchy than hypertension QTL alleles in accumulation.

## Cumulative Aggregation of QTLs Does Not Correlate Quantitatively With a BP Consequence

As BP is a quantitative trait, every time when no BP effect can be generated from a congenic strain replacing QTL alleles known to decrease BP, an intuitive suspicion falls instantly on the possibility that not enough BP-increasing QTL alleles may be involved. As proved in the current work, amassing more BP-augmenting alleles from more QTLs such as C7L.S plus C17L.S could not budge BP. Therefore, in the Lewis background, the BP quantitative trait is not summarily determined by additivity of QTL alleles. The marginal BP rise in C10L.S can be interpreted as an additive influence of QTL alleles from 2 epistatic modules, not by a mere increment in QTL numbers.[4]

## Creative Genetic Approach Leads Us to Tentatively Locating a HS

Although sophisticated permutation tests are available for comprehensively analyzing interactions in permutation between all pairs of markers in a $F_2$ population (www.jax.org/research/churchill), this approach was not adopted for the following reason. Because the Lewis genome is completely dominant over that of DSS shown in $F_1$(DSS×Lewis; Figure III in the Data Supplement), we were seeking, instead, to demonstrate a direct cause–effect relationship between a chromosome fragment and the presence of a HS by removing a L allele from it. In locating the HS (Table), the correlation was qualitative and 100%, not merely statistically supported, between the chromosome segment and the presence or absence of a BP suppressive effect. The interim assignment of a HS residing in C18SH validated the proximate applicability of this approach.

Although several BP QTLs have been defined by DSS-based congenic strains[5,12] in the vicinity of the region containing the putative HS, they cannot be responsible for the action of the HS. This is because a congenic strain made in the Lewis background, C18L.S, cumulatively carries hypertensive alleles of 3 QTLs, *C18QTL1*, *C18QTL2*, and *C18QTL3*.[5,12] In spite of it, no change in BP ensued. This result has 2 implications. First, although it cannot be excluded that the HS may still be a BP QTL, it cannot be any of *C18QTL1*, *C18QTL2*, and *C18QTL3*. Second, an accumulation of BP-lowering alleles from multiple QTLs such as *C18QTL1*, *C18QTL2*, and *C18QTL3* cannot be a cause of normotension.

The location of the HS (Table) falls in the section not covered by C18L.S,[5] and thus represents a new region.

## Prospect

The existence of a HS or a QTL regulator supplies a prerequisite first step both conceptually and experimentally toward its ultimate identification, much like the QTL identification itself.[8]

## Summary

An ordered analysis of QTLs and their combinations have unveiled that, beyond QTL modularity, performances of QTLs can actually be over-ridden by genetic regulatory elements above them in the hierarchy. In the context of BP homeostasis, the undertaking of these elements prohibits hypertension. Further genetic analyses have corroborated this notion and provisionally pinpointed the genome location where a HS might reside. A molecular identification of a HS will reveal a novel mechanism that could provide a master control over the QTLs directly modulating the BP internal stability. It is, therefore, likely that genetic, molecular, and physiological studies of the HS may yield innovative diagnostic tools, relief measures, and, more importantly, preventive means against essential hypertension.

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## Disclosures

None.

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## CLINICAL PERSPECTIVE

Most of the scientific and clinical research in hypertension focuses on uncovering causes of hypertension. As an antithesis to hypertension, normotension seems more frequent than hypertension in ≈70% of the general population and is sustained. Despite of this fact, little attention has been paid to understanding mechanisms of achieving and maintaining normotension as well as opposing hypertension. The current article documents the results of an original approach in the genetics of normotension. First, by systematically increasing the number of hypertensive quantitative trait loci alleles in the resistant background, a slight blood pressure augmentation was seen, but not in proportion to the quantity of hypertensive quantitative trait loci alleles. Second, by gradually decreasing the resistant genome in backcrosses between the susceptible and resistant genetic backgrounds, we were able to detect a chromosome region associated with blood pressure changes. Thus, a potential hypertension suppressor is thought to exist in the resistant genetic background. Thus, a cumulative thrust from multiple hypertensive quantitative trait loci alleles does not drive blood pressure changes and cannot overcome the power of the genome that resists the rise in blood pressure. A novel concept emerges that a hypertension suppressor exists in the normotensive genome. Consequently, an antihypertensive genetic locus may exist. Its molecular identification will likely result in formulating a novel diagnostic and therapeutic strategy in preventing or resisting hypertension. This strategy may over-ride the hypertensive influences from genetic elements. Consequently, a novel and hierarchical avenue of antihypertensive drugs can be developed beyond targeting renin–angiotensin systems, β-blockers, and diuretics.

# Hypertension Suppression, Not a Cumulative Thrust of Quantitative Trait Loci, Predisposes Blood Pressure Homeostasis to Normotension

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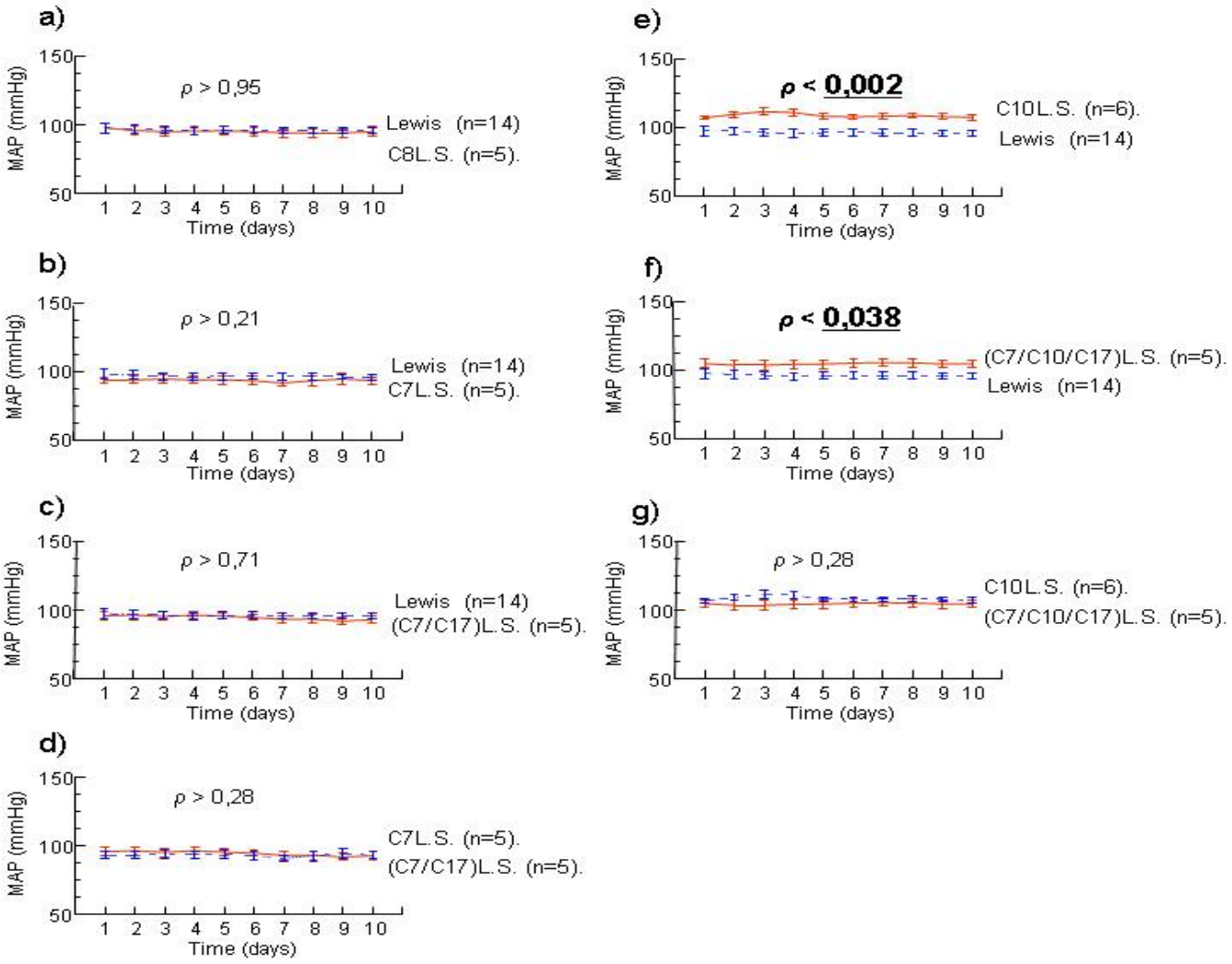
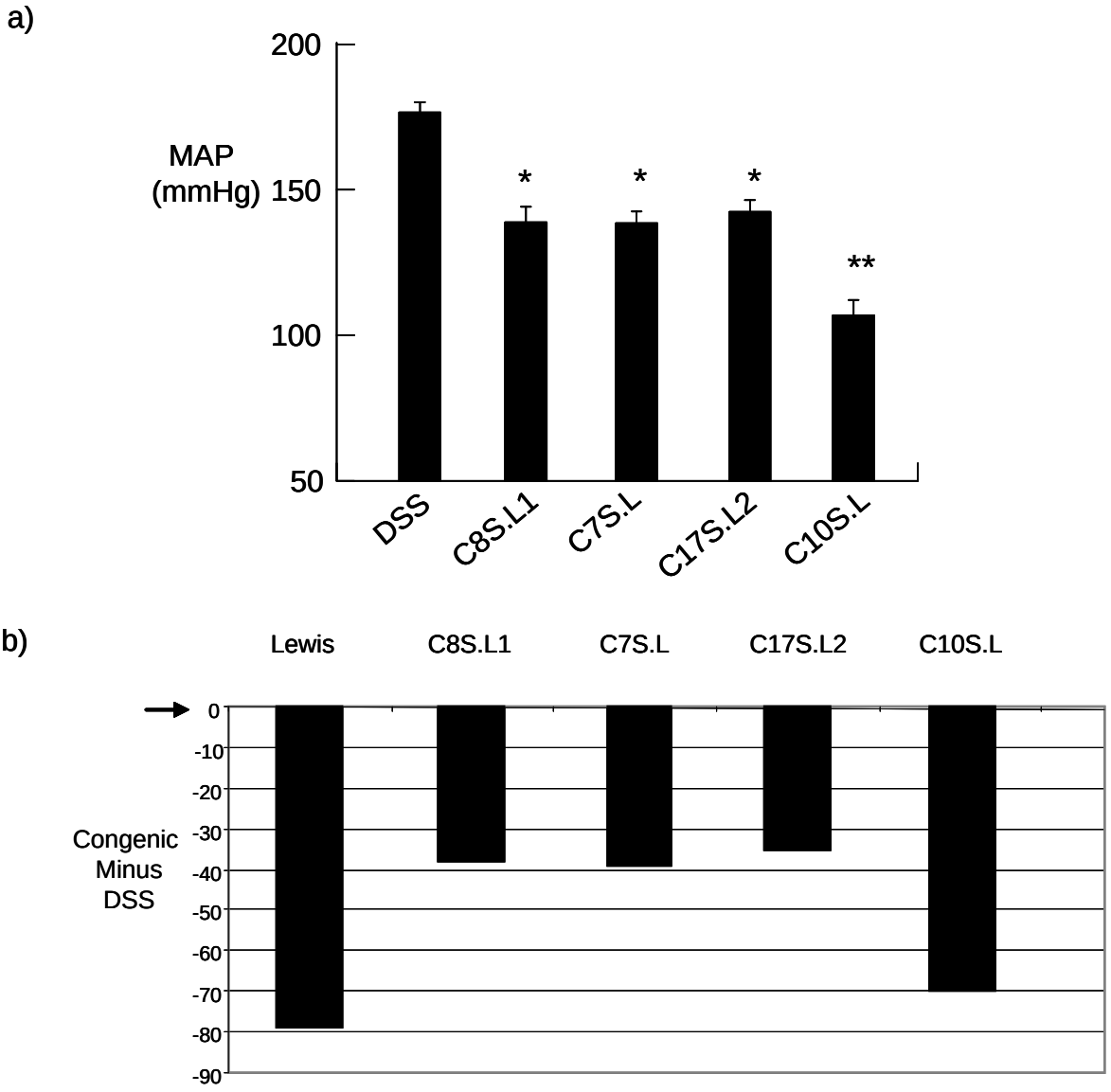
**Supplement 1. Mean arterial pressures (MAPs) of single, double and multiple congenic combinations.** The MAPs were averaged readings for the rat strains during the course of the measurements. The congenic strain designations were given in detail in the method section under Construction of new congenic strains. The chromosome span for each congenic strain is denoted in Figure 1. Statistic analyses were given in methods. p, repeated measures ANOVA followed by Dunnett; n, number of rats, error bars represent SEM for each rat strain.



**Supplement 2. Mean arterial pressures (MAPs) and their changes in congenic strains built in the Dahl-salt sensitive (DSS) background.** MAPs were averaged readings for the strains during the course of the measurements. The number of rats for the strains ranged from 6-15. The BP results were published for Chromosome (Chr) 8 (*J. Hypertens* 2004;22:1495), 17 (*Physiol. Genomics* 2005;21:112) and 10 (*Hypertension* 2005;46:1300). The chromosome span lodging each QTL is denoted in Figure 1. a). The actual MAPs measured. * denotes a difference in MAPs between DSS and a congenic strain. ** signifies differences in MAP between that congenic strain and other congenic strains as well as DSS. b) A change in the MAP (mmHg) of congenic minus DSS rats. The arrow indicates the DSS background as 0. The Lewis column indicates the MAP difference in Lewis minus DSS as a point of reference.



**Supplement 3. Changes in mean arterial pressures (MAPs) in successive backcrosses (BC) to DSS.** MAPs were measured in Dahl salt-sensitve (DSS) rats, Lewis rats, $F_1$(DSS x Lewis), and each BC generation. Rats in $F_1$(DSS x Lewis), BC1, BC2 and BC3 were randomly chosen. Error bars represent SEM. n refers to the number of rats. Each point on the BP tracings represents a 24-hour readings. On average throughout the measurements, MAPs for Lewis, $F_1$(DSS x Lewis), BC1, BC2 and BC3 were 98 ± 2, 97 ± 4, 106 ± 2, 106 ± 7, and 133 ± 2 respectively (± SEM). There seemed to be a gradual increase in MAP from BC1 to BC2, although MAPs among Lewis, $F_1$(DSS x Lewis), BC1 and BC2, are not significant ($p>0.089$). In contrast, MAPs between BC2 and BC3 are significantly different ($p<0.01$). Individuals BC2 and BC3 rats were genotyped by a total genome scan and their genotypes and MAPs are presented in the Table in the text of the manuscript.