Physiol Genomics 45: 729–736, 2013.
First published June 11, 2013; doi:10.1152/physiolgenomics.00077.2013.

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Isolation and high-throughput sequencing of two closely linked epistatic hypertension susceptibility loci with a panel of bicongenic strains

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Submitted 7 May 2013; accepted in final form 10 June 2013

Pillai R, Waghulde H, Nie Y, Gopalakrishnan K, Kumarasamy S, Farms P, Garrett MR, Atanur SS, Maratou K, Aitman TJ, Joe B. Isolation and high-throughput sequencing of two closely linked epistatic hypertension susceptibility loci with a panel of bicongenic strains. *Physiol Genomics* 45: 729–736, 2013. First published June 11, 2013; doi:10.1152/physiolgenomics.00077.2013.—Interactions or epistasis between genetic factors may contribute to "missing heritability." While linkage analyses detect epistasis, defining the limits of the interacting segments poses a significant challenge especially when the interactions are between loci in close proximity. The goal of the present study was to isolate two such epistatic blood pressure (BP) loci on rat chromosome 5. A panel of S.LEW bicongenic strains along with the corresponding monocongenic strains was constructed. BP of each set comprising of one bicongenic and two corresponding monocongenic strains were determined along with the parental Salt-sensitive (S) strain. Epistasis was observed in one out of four sets of congenic strains, wherein systolic blood pressures (SBP) of the two monocongenic strains S.LEW(5)x6Bx9x5a and S.LEW(5)x6Bx9x5b were comparable to that of S, but the SBP of the bicongenic strain S.LEW(5)x6Bx9x5 (157 ± 4.3 mmHg) was significantly lower than that of S (196 ± 6.8 mmHg, $P < 0.001$). A two-way ANOVA indicated significant interactions between the LEW alleles at the two loci. The interacting loci were 2.02 Mb apart and located within genomic segments spanning 7.77 and 4.18 Mb containing 7,360 and 2,753 candidate variants, respectively. The current study demonstrates definitive evidence for epistasis and provides genetic tools for further dissection of the isolated epistatic BP loci.

epistasis; blood pressure; congenic; QTL; rat; genetics; mapping; linkage; GWAS; association

MANY BLOOD PRESSURE (BP) quantitative trait loci (QTLs) have been successfully located and fine mapped to short genomic segments spanning a few megabases or kilobases of the rat genome (8, 11–13, 17, 18, 20–22, 35, 36, 40), (http://rgd.mcw.edu/). While a majority of the fine-mapped QTLs act independently of each other, very few BP QTLs have been identified as interacting or epistatic loci (1–4, 6, 7, 10, 14, 24, 29–33, 38, 39). The work described here involves the study of two such QTLs on rat chromosome 5 (RNO5) that are closely linked and interact with each other to influence BP. The interacting QTLs, QTL1 and QTL2 linked to BP, were previously inferred based on differential segments between congenic strains constructed from the parental congenic strain S.LEW(5). These introgressed Lewis rat (LEW) segments were 6.3 and 4.6 cM on the genetic background of the Dahl salt-sensitive (S) rat. Both these QTLs were captured in a single congenic strain S.LEW(5)x6x9 that was used as a parental strain for the current study (14, 25).

Isolating QTL1 and QTL2 as individual introgressed segments in two different monocongenic strains and reconstituting them in a single bicongenic strain would provide direct evidence that LEW alleles within these two BP QTLs interact with each other to cause a BP lowering effect in the S rat. To obtain this important evidence, in the current report, we generated a series of four different groups of congenic strains. Each group consisted of the following: *1*) one bicongenic strain containing introgressed LEW alleles on RNO5 at QTL1 and QTL2 separated by an S genomic segment, *2*) the two corresponding monocongenic strains, and *3*) the parental S strain. Each of these bicongenic strains were unique because they were developed by combining novel lines of monocongenic substrains containing introgressed LEW segments potentially different in their regions of recombination with the S genome. Data obtained from these new groups of congenic strains will be presented. This includes *1*) definitive evidence for the presence of epistasis between the two closely linked QTLs, *2*) capture of the two proposed epistatic QTL segments in independent congenic strains within better resolved intervals, *3*) sequencing of the QTL regions, and *4*) enlistment of potential candidate genetic elements.

METHODS

Animals. The inbred S rat was from our colony maintained at the University of Toledo Health Science Campus. LEW/NCrlBR (LEW rat) was originally obtained from the Charles River Laboratories (Wilmington, MA) and maintained in our colony. These animals were maintained and studied as per the Institutional review committee's approved protocol.

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Construction of the panel of monocongenic and bicongenic strains. The bicongenic strains used for this study were derived from the parental strain S.LEW(5)x6x9 developed in our laboratory (25). The parental strain S.LEW(5)x6x9 was bred with the S rat to generate an F1 population. The F1 rats were intercrossed to generate an F2 population. F2 rats that had recombinations on RNO5 between the two microsatellite markers D5Mco39 and D5Mco47 were selected and backcrossed to the parental S strain to fix the recombination to homozygosity. Additional markers were used to determine the extent of the introgressed LEW alleles containing segments retained within each of the monocongenic strains. To develop bicongenic strains, male and female pairs of the monocongenic strains, each representing one of the two QTLs, were randomly selected and intercrossed to develop F1 strains that were heterozygous at the two QTL regions. The animals containing the heterozygous alleles within each of the two QTLs were intercrossed to generate the bicongenic strains that were homozygous LEW at QTL1 and 2.

Genotyping. Genomic DNA was extracted from rat tail biopsies using the Promega Wizard SV-96 genomic DNA purification system. Primers to amplify microsatellite markers were designed from genomic DNA sequences of the region of interest obtained from the rat genome data available at the Ensembl web site (http://www.ensembl.org). Polymorphic microsatellite markers were identified by PCR of genomic DNA and used for genotyping the congenic strains (37).

Systolic blood pressure measurement by the tail-cuff method. Each group of congenic rats (consisting of one bicongenic strain and two corresponding monocongenic strains) along with the control S rats were bred, housed, and studied concomitantly. We used 15–20 male rats of each strain for this study. At 42 days of age, rats were fed high salt (2% NaCl) for 24 days. Systolic blood pressure (SBP) of all strains within a group was measured on the same four days between 7 AM and 12 PM by the tail-cuff method on conscious rats in a chamber warmed to 28°C as detailed previously (35). In brief, SBP was measured by two blinded operators once a day for 4 days, and the value for each day was the average of three or four consistent readings. The final SBP value reported was the average of the SBP data collected for 4 days.

Corroboration of tail-cuff BP measurements by telemetry. Rats from the tail-cuff study were continued on a 2% NaCl diet and surgically implanted with C40 radiotelemetry transmitters as described previously [n = 6/group for all strains except S.LEW(5)x6Bx9x5a, n = 5] (35). Briefly, rats were anesthetized under 2% isoflurane in oxygen. The ventral side of the rat was dissected to accommodate the C40 transmitter in the flank of the animal and the probe of the transmitter was introduced into the femoral artery through a small incision made in the artery. The probe was secured in place with ties of nonabsorbable suture thread, and the skin was sutured. The rats were then allowed to recover for 1 wk, and BP was recorded over a period of 3 days. During the recordings, rats were 81–83 days old. Four-hour moving average BP values were used for statistical analyses and plotted as means ± SE.

Statistical analyses. All BP data obtained by the tail-cuff and telemetry methods were statistically analyzed using the SPSS software (SPSS, Chicago, IL) by a one-way ANOVA. A P value of ≤ 0.05 was used as a threshold for statistical significance. To determine if the BP effect was due to interaction between the two QTLs, two-way ANOVA followed by Fisher's least significant difference, and the Student-Newman-Keuls post hoc test was performed. A P value of ≤0.05 was considered statistically significant for interaction.

Genomic sequencing and analyses. Genomes of the S and LEW/NCrlBR rat strains were sequenced on the next generation sequencing platform Illumina HiSeq2000 by paired end sequencing technology with 100 bp read length and 350 bp insert size. Sequence reads were mapped to the reference BN genome RGSC-3.4 (15) using short read aligner BWA-0.5.8c (26). Genomic variants in S and LEW/NCrlBR, relative to the BN reference genome, were identified with the Genome Analysis Toolkit (GATK version 1.0.6001) (9) after rigorous preprocessing of mapped reads including removal of clonal reads, indel realignment, and base call recalibration. Potential false positives were filtered by the variant quality score recalibration function of GATK, which employs the Bayes Gaussian mixture model. Genomic variants [single nucleotide polymorphisms (SNPs) and indels] polymorphic between S and LEW/NCrlBR in the two congenic intervals were then extracted with Perl script. Functional consequences of the SNPs and indels were estimated with Variant Effect Predictor version 2.4 (28) on the Ensembl v66 rat gene set. All nonsynonymous and splice-site variations in QTL 1 and 2 were independently validated with genomic DNA of S and S.LEW(5)x6Bx9x5 using primers designed to detect the variants by sequencing as described elsewhere (16). The list of primers used is provided in Table 1.

RESULTS

A panel of 12 congenic substrains including monocongenic and bicongenic strains was developed and assigned into four groups as shown in Fig. 1. Each group had a unique combination of LEW alleles within the two QTLs, QTL1 and QTL2 (Fig. 1). Each group of animals was concomitantly raised along with the control S rats and their BP was measured by the tail cuff method (n = 15–20 per group) (Fig. 1, Table 2). None of the strains in *groups 1, 2,* or *3* differed in their BP compared with the S rat (Fig. 1, Table 2). However, within *group 4*, the SBP of the two monocongenic strains S.LEW(5)x6Bx9x5a and S.LEW(5)x6Bx9x5b were comparable to that of the S, but the bicongenic strain, S.LEW(5)x6Bx9x5 had a systolic BP of 157 ± 4.3 mmHg, which was 39 mmHg lower than the SBP of the S rat (SBP =196 ± 6.8 mmHg) ($P < 0.001$, Table 2). Statistical analysis of the data obtained with the three strains in *group 4* along with the S by two-way ANOVA showed significant interaction (P = 0.041, Table 3). These data point to an interactive effect (labeled as C in Fig. 2). The interactive effect was observed between the two introgressed segments within the monocongenic strains labeled as S.LEW(5)x6Bx9x5a and S.LEW(5)x6Bx9x5b in Fig. 1. Furthermore, because this interaction was not observed with any of the other groups of congenic rats represented in Fig. 1, the introgressed segment contained within the strains S.LEW(5)x6Bx9x2a, S.LEW(5)x6Bx9x3a, and S.LEW(5)x6Bx9x4a could be eliminated as BP QTL containing segments. Therefore the most likely location of one of the interacting BP QTLs was interpreted to be within the 7.77 Mb orange-colored segment labeled as QTL1 in Fig. 1. The

Table 1. *List of primers for independent validation of nonsynonymous variations in protein-coding genes*

Gene Name	Left Primer	Right Primer	Product Size
Zcchc11	AGAATTCTCCCCAACCGTCT	AGTCACCCAACCTTTCCACA	900
Osbpl9	GCCACCATGCCTCATCTACT	AAGAATGTGATGTGAGCCTTGA	601
Raver2	CCCCTATGGGGGAAAGTAGA	AAAGCCGATTGCATAAGGAC	840
Zyg11a	TCCCCCAGGACTGAGTTACA	GAAAAGCTTCATGTGTGCTACAA	780
Zyg11b	GGAACTAAAAAGAAAAACCAACCA	CCTTTTGCCACCACAAGCTA	780
Faf1	TGCCCATGCCATGTAGATTA	CCTCCTACACGTAGGCATCC	960

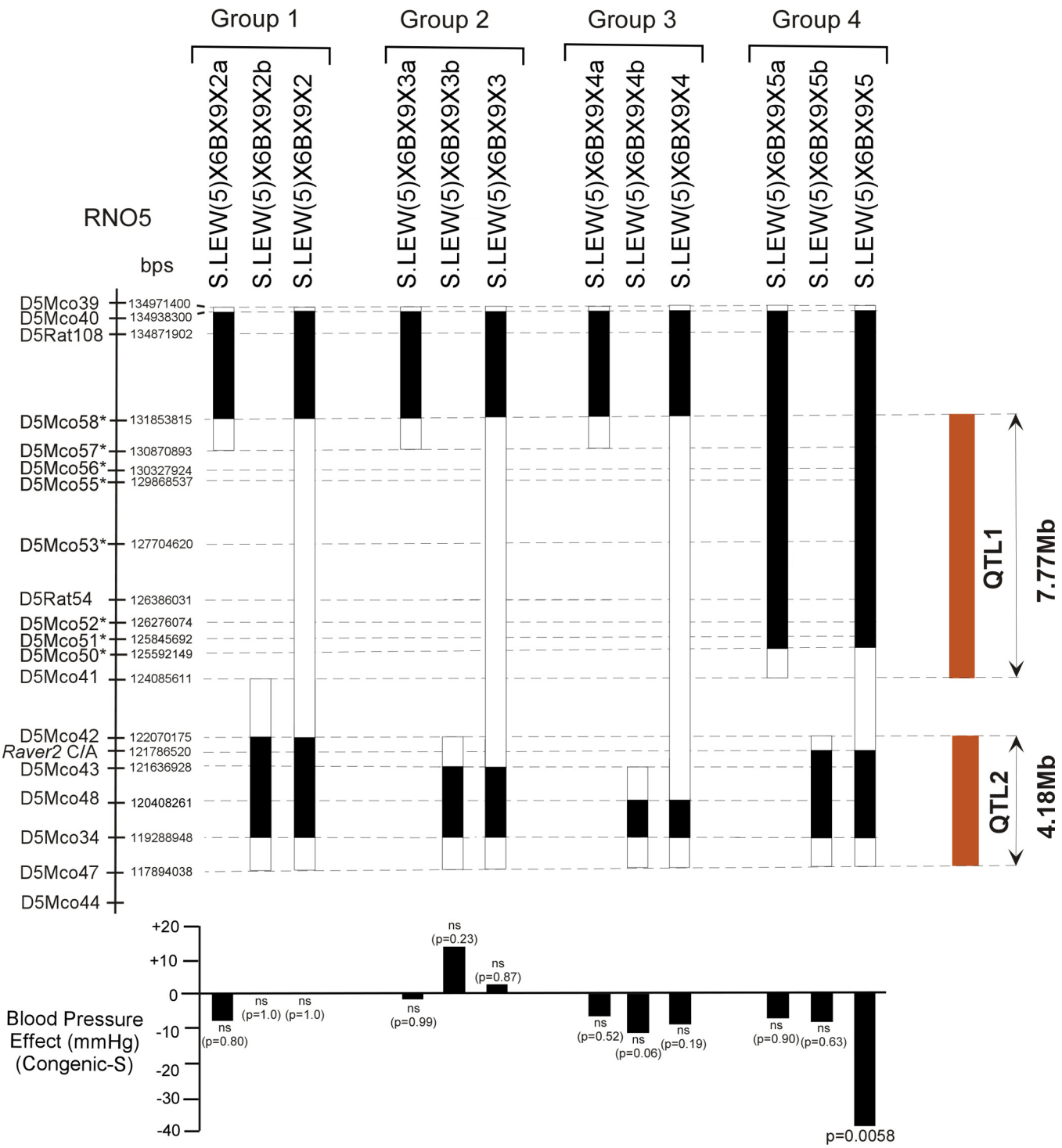

Fig. 1. Schematic representation of the monocongenic and bicongenic strains. The physical map of rat chromosome 5 (RNO5) along with the microsatellite markers and their locations according to the Ensembl database (http://www.ensembl.org, RGSC 3.4) are shown on the *left*. The vertical bars alongside of the physical map represent schematics of congenic strains. Black boxes are introgressed LEW segments. The regions of recombination are represented by white boxes flanking each of the monocongenic strains. Long white boxes connecting the 2 regions of recombination flanking each of the black boxes within a bicongenic strain are genotyped and confirmed as Dahl salt sensitive (S). The blood pressure (BP) effect of each strain compared with S (n = 15–20 per group) is shown as a black bar below the schematic of the congenic strain [not significant (ns)]. BP data were analyzed by a 1-way ANOVA for each group of congenic strains. The inferred locations of the BP QTL are indicated as orange bars on the right side.

Table 2. *BP effect of the four groups of monocongenic and their respective bicongenic strains*

Congenic Substrain	SBP (mmHg) ± SE	BP Effect (Congenic-S)	*P* Value
S.LEW(5)x6Bx9x2	205 ± 5.1	0	1.000
S.LEW(5)x6Bx9x2A	199 ± 4.2	−6	0.798
S.LEW(5)x6Bx9x2B	204 ± 5.4	−1	1.000
S	205 ± 3.4		
S.LEW(5)x6Bx9x3	226 ± 5.5	6	0.87
S.LEW(5)x6Bx9x3A	221 ± 6.3	1	0.998
S.LEW(5)x6Bx9x3B	231 ± 4.3	12	0.233
S	220 ± 5.6		
S.LEW(5)x6Bx9x4	181 ± 2.5	−10	0.194
S.LEW(5)x6Bx9x4A	184 ± 4.3	−7	0.516
S.LEW(5)x6Bx9x4B	179 ± 3.2	−12	0.059
S	191 ± 2.1		
S.LEW(5)x6Bx9x5	157 ± 4.3	−39	<0.001
S.LEW(5)x6Bx9x5A	190 ± 5.6	−6	0.899
S.LEW(5)x6Bx9x5B	187 ± 5.2	−9	0.63
S	196 ± 6.8		

Systolic blood pressure (SBP) data were collected by the tail-cuff method and statistically analyzed by 1-way ANOVA using SPSS. BP, blood pressure; S, Dahl salt-sensitive rat; LEW, Lewis rat.

second orange-colored segment labeled as QTL2 in Fig. 1 represents the BP QTL that interacts with QTL1. BP QTL2 spans 4.18 Mb, which is the introgressed segment of S.LEW(5)x6Bx9x5b.

To corroborate the result obtained by the tail-cuff method, the BP of S.LEW(5)x6Bx9x5a, S.LEW(5)x6Bx9x5b, S.LEW(5)x6Bx9x5, and S were additionally tested by the telemetry method [$n = 6$/group for all strains except S.LEW(5)x6Bx9x5a, $n = 5$]. Similar to the data obtained by the tail-cuff method, the SBP of the two monocongenic strains S.LEW(5)x6Bx9x5a and S.LEW(5)x6Bx9x5b were not different from that of the S. However, the SBP of the bicongenic strain S.LEW(5)x6Bx9x5 was significantly lower than that of the S ($P = 0.041$, Fig. 3). Similarly, the diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP) of the bicongenic strain S.LEW(5)x6Bx9x5, but not either one of the monocongenic strains, were significantly lower than that of the S (DBP $P = 0.036$, MAP $P = 0.009$, PP $P = 0.049$; Fig. 3). A two-way ANOVA of the SBP, DBP, MAP, and PP data obtained by the telemetry method further confirmed that the observed interaction was statistically significant (Table 4).

Sequence variants within the two identified QTL regions. The location of the two QTL regions were queried by BLAST searches of the sequences delimiting the two QTLs labeled as QTL1 and 2 in Fig. 1. The genomic sizes of these QTLs differed between the rat genome assembly versions RGSC 3.4 and RGSC 5.0 (Table 5). The interpretations of genomic content of the two QTLs in the current report were based on RGSC 3.4 because this assembly was used to determine the sequences of the two QTL regions. As per RGSC 3.4, QTL1 was located within 7.77 Mb containing 85 annotations (Supplemental Table S1) and QTL2 within 4.18 Mb containing 34 annotations (Supplemental Table S2).[1] Genomic DNA sequencing of the QTL regions 1 and 2 identified 7,360 and 2,753 variants, respectively, between S and LEW. These variant counts include SNPs and insertion and deletion polymorphisms. The majority of the variants in both the QTLs were outside of protein-coding genes (Supplemental Table S3–S6). However, within QTL1, variants were confirmed in three protein-coding candidate genes (Table 6). These genes are Fas (TNFRSF6) associated factor 1 (*Faf1*), whose function is to potentiate FAS-induced apoptosis (5); Zyg-11 homolog A (*Zyg11a*), which probably acts as target recruitment subunit in an E3 ubiquitin ligase complex ZYGA-CUL2-elongin BC (by similarity); and Zyg-11 homolog B (*Zyg11b*), predicted as a target recruitment subunit in the E3 ubiquitin ligase complex ZYG11B-CUL2-Elongin BC (27) (Table 6). Similarly, within QTL2, one nonsynonymous variation and one splice-site variation was confirmed in the gene ribonucleoprotein PTB-binding 2 (*Raver2*), which may bind single-stranded nucleic acids (potential) (23). Variants within *Faf1* and *Zyg11a* are predicted splice-site variants, whereas variants within *Zyg11b* and *Raver2* are nonsynonymous polymorphisms. SIFT/PROVEAN

[1] The online version of this article contains supplemental material.

Table 3. *Report of statistical analyses for interactive effects for SBP measured by the tail-cuff method by two-way ANOVA*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	7,624.999	3	2,541.666	9.718	0.000
Intercept	1,037,823.409	1	1,037,823.409	3,967.973	0.000
QTL1	3,584.586	1	3,584.586	13.705	0.001
QTL2	2,555.919	1	2,555.919	9.772	0.004
QTL1 * QTL2	1,199.786	1	1,199.786	4.587	0.041
Error	7,323.401	28	261.550		
Total	1,082,464.590	32			
Corrected Total	14,948.400	31			

QTL, quantitative trait locus.

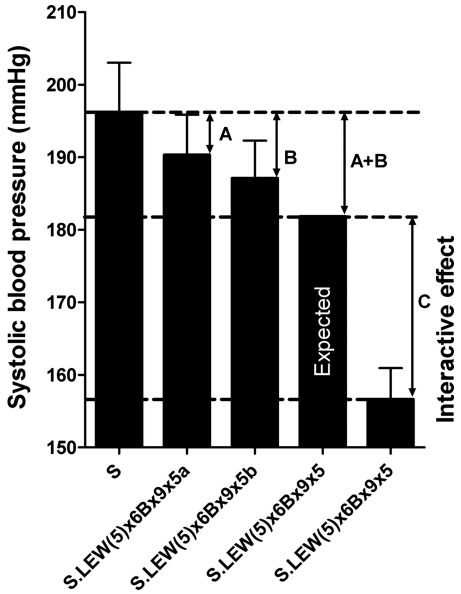

Fig. 2. Interactive systolic BP effect. Results from *group 4* are presented. The BP differences of the monocongenic strains compared with S rat are represented by *intervals A* and *B*. The additive BP effect expected in the bicongenic strain S.LEW(5)x6Bx9x5 is represented by the interval labeled A+B. The difference between the expected and the observed BP of the congenic strain S.LEW(5)x6Bx9x5 is labeled as "interactive effect" represented by the *interval C*. A 2-way ANOVA showed that this interaction was significant, $P = 0.041$.

(protein variation effect analyzer) software tool was used to analyze the consequences of nonsynonymous polymorphisms (http://sift.jcvi.org/). The results indicate a neutral effect due to the change in amino acid on the biological function of the protein coded by genes *Zyg11b* and *Raver2*. Given that the focus of the current study was to obtain definitive evidence for genetic interactions and localization of the two QTLs, constructing hypotheses around these candidate variants as the only potentially causative variants for the observed interactive effect is premature. The congenic strains reported will serve as genetic tools for further fine-mapping and positional cloning of the two interacting loci.

DISCUSSION

The locations of the interacting BP QTLs reported in this study were previously interpreted based on collective data obtained from several S.LEW congenic strains containing differential introgressed segments on RNO5 (14, 25). Such interpretations, however logical, could be misleading without definitive evidence provided through the isolation and reconstitution of each of the two interacting BP QTLs (34). Isolating interacting loci as introgressed segments within monocongenic strains and reconstituting these introgressed segments within bicongenic strains are relatively easier when the two interacting loci are on two different chromosomes. For example, two BP QTLs on chromosomes 2 and 10 are demonstrated to interact by the comparisons between two monocongenic strains with introgressed segments of chromosomes 2 and 10 and a bicongenic strain with both of the introgressed segments on chromosomes 2 and 10 (33). Compared with this example, the present study was challenging because *1*) the two epistatic loci were within a few megabases on the same chromosome and *2*) the parental strain that was used for isolating the two interacting QTLs contains a contiguous LEW introgressed segment spanning the entire genomic segment from QTL1 through QTL2. The study therefore required the

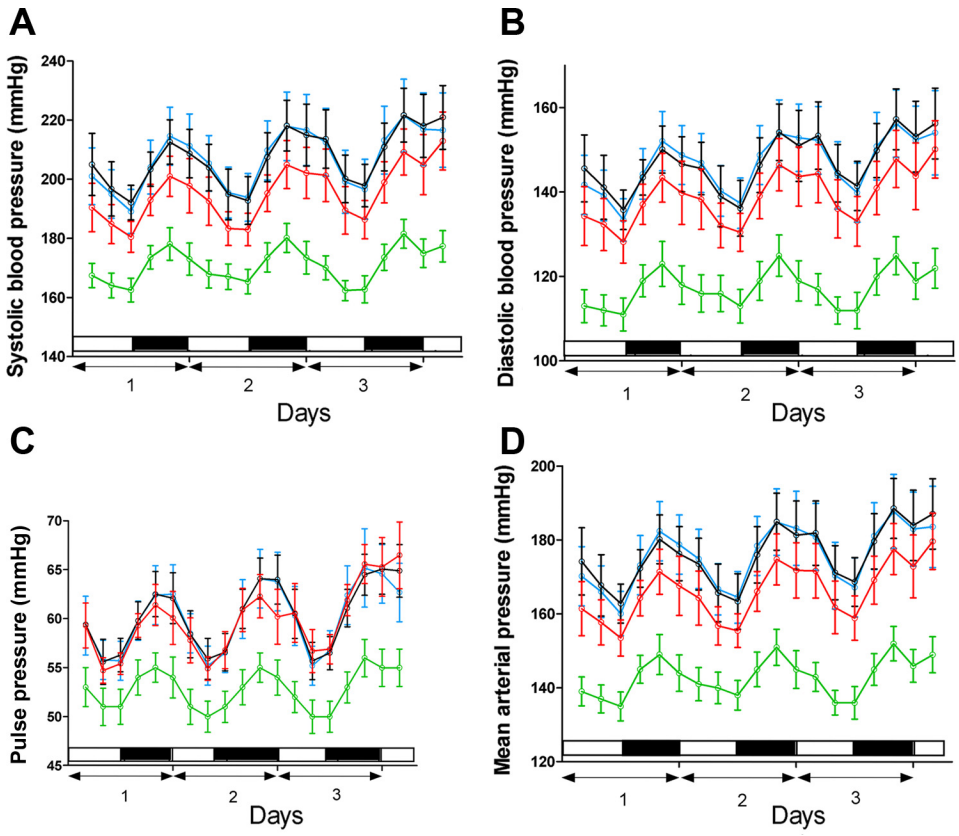

Fig. 3. Telemetry measurement of systolic, diastolic, mean arterial and pulse pressures of S and congenic strains. [$n = 6$/group for all strains except S.LEW(5)x6Bx9x5a, $n = 5$]. Green, S.LEW(5)x6Bx9x5; blue, S.LEW(5)x6Bx9x5a; black, S.LEW(5)x6Bx9x5b; red, S.

Table 4. *Report of statistical analyses by two-way ANOVA for SBP, DBP, MAP, and PP measured by the telemetry method*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
		Dependent Variable: SBP			
Corrected model	5,036.072	3	1,678.691	4.912	0.011
Intercept	869,142.857	1	869,142.857	2,543.049	0.000
QTL1	892.857	1	892.857	2.612	0.123
QTL2	845.873	1	845.873	2.475	0.132
QTL1 * QTL2	3,200.635	1	3,200.635	9.365	0.006
Error	6,493.667	19	341.772		
Total	881,431.000	23			
Corrected total	11,529.739	22			
		Dependent Variable: DBP			
Corrected model	10,313.070	3	3,437.690	14.477	0.000
Intercept	479,136.311	1	479,136.311	2,017.729	0.000
QTL1	88.406	1	88.406	0.372	0.549
QTL2	3,552.406	1	3,552.406	14.960	0.001
QTL1 * QTL2	6,185.200	1	6,185.200	26.047	0.000
Error	4,511.800	19	237.463		
Total	496,372.000	23			
Corrected total	14,824.870	22			
		Dependent Variable: MAP			
Corrected model	4,070.713	3	1,356.904	5.159	0.009
Intercept	624,046.959	1	624,046.959	2,372.707	0.000
QTL1	751.340	1	751.340	2.857	0.107
QTL2	760.102	1	760.102	2.890	0.105
QTL1 * QTL2	2,472.229	1	2,472.229	9.400	0.006
Error	4,997.200	19	263.011		
Total	633,594.000	23			
Corrected total	9,067.913	22			
		Dependent Variable: PP			
Corrected model	309.556	3	103.185	10.425	0.000
Intercept	76,799.393	1	76,799.393	7,759.340	0.000
QTL1	60.460	1	60.460	6.109	0.023
QTL1 * QTL2	113.581	1	113.581	11.476	0.003
Error	188.056	19	9.898		
Total	77,599.182	23			
Corrected total	497.612	22			

DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure.

replacement of the LEW segment between the locations of the two interacting loci with S alleles. This was achieved first by the construction of several monocongenic strains, followed by grouping these strains such that there were two monocongenic strains, one containing QTL1 and the other containing QTL2. The two monocongenic strains within a group were used as progenitor strains for reconstitution into a bicongenic strain within the same group. The data collected with four such groups of monocongenic and bicongenic strains compared with the S clearly demonstrate that the two interacting BP QTLs were resolved as single introgressed segments within congenic strains that were 2.02 Mb apart.

The sizes of the two QTL regions are amenable for further dissection using the congenic approach. QTL1 is homologous to two regions on human chromosome 1 (HSA 1: 50513686–59012474 and 67390578–67600639 bp). QTL2 is homologous to chromosome 1 in humans (HSA1: 61330931–65697828 bp). Within these two regions there are no reported direct associations to blood pressure (http://www.genome.gov/gwastudies/). Genome-wide association studies (GWAS) do not typically account for gene-gene interactions, whereby data on epistasis could be easily missed (41). If similar epistasis, as described in our studies using rat models, does exist between the human homologous segments of QTLs 1 and 2, one would not expect current GWAS to demonstrate associations with either one of the homologous segments per se. Indeed, there are no known associations to BP within homologous segments of rat QTL1 or QTL2 on human chromosome 1. The current data thus points to epistasis, as described in our report, as perhaps contributing toward BP regulation by genetic elements within the homologous regions of QTLs 1 and 2 in humans. This, of course, remains to be determined.

Table 5. *Physical sizes of the QTL intervals*

	RGSC 3.4	Size of the QTL, base pairs	RGSC 5.0	Size of the QTL, base pairs
QTL1	124,085,611–131,853,815	7,768,204	126,545,004–134,002,747	7,457,743
QTL2	117,894,038–122,070,175	4,176,137	124,137,683–124,137,683	4,043,618

Table 6. *List of SNP within protein-coding genes*

Gene Name	Position (RGSC 3.4)	SNP	S	LEW	BN	Location
Faf1	131357775	SNP-splice site, intronic	A	T	T	QTL1
Zyg11a	129293730	SNP-splice site, intronic	T	C	T	QTL1
Zyg11b	129367632	SNP-nonsynonymous	T	C	C	QTL1
Raver2	121784282	SNP-splice site, intronic	G	A	G	QTL2
Raver2	121786520	SNP-nonsynonymous	C	A	C	QTL2

Variants listed were independently confirmed by sequencing as described under METHODS. SNP, single nucleotide polymorphism; BN, Brown Norway rat.

GWAS of hypertension accounts for only ~1% of the inheritance of BP. This observation is leading to recent literature pointing to epistasis as one of the factors contributing to "missing heritability" of quantitative traits such as BP (19, 26). The present study attests to epistasis as a possible factor contributing to missing heritability in human hypertension GWAS because inheritance of a protective effect of LEW alleles at either QTL1 or QTL2 by itself did not result in an observable change in BP. Therefore, the genomic segments could have been easily dismissed as ones that do not contain any genetic elements that causally alter BP. However, reconstitution of LEW alleles within QTL1 and QTL2 demonstrate that the missing heritability is indeed accounted for by the presence of LEW alleles at both loci. Our data in rat models lend support for perhaps similar epistatic loci on the homologous segments in humans.

ACKNOWLEDGMENTS

Authors appreciate helpful suggestions, especially for statistical analysis, from Prof. John P. Rapp.

GRANTS

This work was funded by National Heart, Lung, and Blood Institute (NHLBI) Grants HL-076709, HL-020176, HL-112641 to B. Joe, by funding from the Imperial College BHF Centre of Research Excellence to S. S. Atanur and T. J. Aitman, intramural funding from the MRC Clinical Sciences Centre and from the EU EURTRANS project to T. J. Aitman. M. R. Garrett is supported by NHLBI Grant HL-094446 and the Robert M. Hearin Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.P., H.W., Y.N., K.G., P.F., K.M., and B.J. performed experiments; R.P., H.W., and K.M. analyzed data; R.P. and H.W. prepared figures; R.P., H.W., M.R.G., S.S.A., K.M., T.J.A., and B.J. edited and revised manuscript; R.P., H.W., Y.N., K.G., S.K., P.F., M.R.G., S.S.A., K.M., T.J.A., and B.J. approved final version of manuscript; K.M., T.J.A., and B.J. interpreted results of experiments; B.J. conception and design of research; B.J. drafted manuscript.

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