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Congenic Interval Mapping of RNO10 Reveals a Complex Cluster of Closely-Linked Genetic Determinants of Blood Pressure

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Abstract—Genetic dissection of the rat genome for identifying alleles that cause abnormalities in blood pressure (BP) resulted in the mapping of a significant number of BP quantitative trait loci (QTLs). In this study we mapped at least one such BP QTL on rat chromosome 10 (RNO10) as being within the introgressed segment of a S.LEW congenic strain S.LEWx12x2x3x8 spanning 1.34 Mb from 70 725 437 bp to 72 063 232 bp. BP of 3 congenic strains that span shorter segments of this region was additionally examined. Results obtained indicate that LEW alleles that comprise a 375-kb introgressed segment of the congenic strain S.LEWx12x2x3x5 (70 725 437 bp to 71 100 513 bp) increase BP. The magnitude of change in BP exhibited by the 2 strains, S.LEWx12x2x3x8 and S.LEWx12x2x3x5, is the net phenotypic effect of the underlying genetic determinants of BP. In this respect, the current data are superior to previous QTL localization of BP QTL1, which was hypothesized based on differential congenic segments. Genetic dissection using these 2 congenic strains as tools is expected to facilitate further dissection of the regions. Meanwhile, differential congenic segments were used to predict and thereby prioritize regions for candidate gene analysis. Using this approach, 2 distinct regions of 401 kb and 409 kb within S.LEWx12x2x3x8 and a 104 kb region within S.LEWx12x2x3x5 were prioritized for further consideration. Because all of these genetic elements are located within a 1.06-Mb region of RNO10, our study has revealed a remarkable insight into a genomic module comprising very closely-linked, opposing genetic determinants of BP. (***Hypertension*. 2007;50:891-898.**)

Key Words: hypertension ■ gene ■ linkage

Despite the description of a number of blood pressure (BP) quantitative trait loci (QTLs) in humans, rats, and mice, identities of the underlying genetic determinants conferring susceptibility to hypertension in any species remain largely unknown,[1–4] with the exception of rat 11-β-hydroxylase[5,6] and CD36.[7] Linkage analysis and substitution mapping using various rat strain comparisons provide conclusive evidence for the existence of multiple genetic determinants of BP on rat chromosome 10 (RNO10).[8–32] Located on this chromosome are genes coding for angiotensin-I converting enzyme, nerve growth factor receptor, skeletal myosin heavy polypeptide 3, nitric oxide synthase-2, ATPase-Na^+/K^+ transporting-β 2 polypeptide, nicotinic-cholinergic receptor-beta polypeptide 1, and protein kinase-lysine deficient 4, all of which are appealing candidates for causally controlling BP. However, fine-mapping and/or DNA sequencing has not provided evidence for most of these genes as candidates for BP QTLs, implicating that the identities of the genetic determinants of BP on RNO10 remain elusive.[8–31]

By replacing progressively shorter segments of RNO 10 of the hypertensive Dahl Salt-sensitive (S) rat with corresponding segments from the Lewis (LEW) rat genome, we have previously fine-mapped a BP quantitative trait locus (S.LEW BP QTL1) to a 1.17-Mb region containing 18 genes.[32] None of these genes have any known function related to the regulation of BP. Although all of them are positional candidate genes, nonsynonymous variants are present only within 3 [Chemokine (C-C motif) ligand 5 (*Ccl5*), ATP-dependent RNA helicase (*Ddx52*), and a novel gene (RGD1559577)] of the 18 positional candidates.[32]

Because the previous localization was based on differential congenic segments, there was a need to locate the BP genetic determinant or determinants within congenic strains with shorter introgressed segments. The present study has resulted in generating and testing a new iteration of congenic substrains. A BP lowering effect is captured in the 1.34-Mb introgressed segment contained within the S.LEWx12x2x3x8 congenic strain. These data provide evidence for further localization of at least 1 genetic determinant of BP within this congenic strain. Data collected from 3 other congenic strains that span this 1.34-Mb interval indicate that there are additional genetic determinants of BP located within S.LEWx12x2x3x8. Inter-

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From the Department of Physiology and Pharmacology, University of Toledo College of Medicine, 3035 Arlington Ave, Ohio.

Correspondence to Bina Joe, PhD, Physiological Genomics Laboratory, Department of Physiology and Pharmacology, University of Toledo College of Medicine, 3035 Arlington Avenue, Toledo, Ohio 43614-5804. E-mail bina.joe@utoledo.edu

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estingly, 1 of these additional genetic determinants of BP has a BP increasing effect demonstrated by LEW alleles.

Materials and Methods

Strains

All animal studies were conducted as per approved IACUC protocols. The inbred Dahl salt-sensitive (SS/Jr) rat strain, designated as S, was from our colony. The LEW/NCrlBR rat strain was originally obtained from Charles River Laboratories (Wilmington, Mass) and is referred to as LEW. All congenic strains/substrains had the LEW chromosomal region of interest introgressed onto the genetic background of the S rat strain. For details on the construction of these congenic substrains please see the online data supplement available at http://hyper.ahajournals.org.

Markers

The markers with "D10Mco" as the prefix were developed at the University of Toledo Health Science Campus (former Medical College/University of Ohio) using rat genomic DNA sequence obtained from the Ensembl website (www.ensembl.org). These can be accessed on our web site (http://hsc.utoledo.edu/depts/physiology/research/rat/marker.html). For further details on other markers, please see the data supplement.

Genotyping

Polymerase chain reaction (PCR)-based genotyping was carried out using microsatellite marker primers on DNA extracted from a tail biopsy as elaborated in the online supplemental section.

Phenotyping

BP of the animals was measured by both the tail-cuff and telemetry methods as elaborated in the data supplement.

Real-Time PCR Quantification of Gene Expression

Three male S and 3 male S.LEWx12x2x3x8 rats were euthanized at 50 days of age after they were placed on the 2% NaCl diet for 7 days before sacrifice and tissue collection. The rats used in the study were a subset of the same rats used in the telemetry study. Sample preparation for real-time PCR is provided in the data supplement.

Data Collection and Statistical Analyses

In addition to BP, heart weight (HW) and body weights of all strains were measured and compared with S. Relative heart weight was calculated as the ratio of HW to body weight. All statistical analyses were done using the SPSS software.

Results

"Trapping" the BP Effect of a RNO10 BP QTL Within a Congenic Strain Spanning 1.34 Mb

The congenic strain S.LEWx12x2x3, shown in Figure 1, is the previously published strain with the shortest introgressed LEW region that encompasses the differential QTL segment flanked by the microsatellite markers D10Mco88 and D10Mco89 (Figure 1; 71 513 116 bp to 72 684 774 bp). Of the 3 strains adjoining S.LEWx12x2x3 in Figure 1, S.LEWx12x2x1 and S.LEWx12x2x3x7 did not have a BP lowering effect, but S.LEWx12x2x3x8 did (Figure 1 and Table 1). The HW effect observed in these congenic strains is congruent with the observed BP effect (Figure 1 and Table 1). Thus, the limits of the introgressed segment of S.LEWx12x2x3x8 currently define the shortest interval within which at least 1 genetic determinant that lowers BP in the S rat can be located. This localization now spans 1 337 795 bp between 70 725 437 bp and 72 063 232 bp.

Corroboration of the BP Effect by Telemetry

Figure 2 shows the 24-hour average for the S rat versus the S.LEWx12x2x3x8 congenic rat systolic BP measured by telemetry. Throughout the 4-day period of measurement, the systolic BP effect was significantly different between S and S.LEWx12x2x3x8. The overall mean systolic BP of the S rat was 213 mm Hg (±7.47 mm Hg), and that of the S.LEWx12x2x3x8 was 188 mm Hg (±4.27 mm Hg). The observed BP lowering effect of 25 mm Hg (±9.04 mm Hg) was significant at $P=0.014$, thus corroborating the BP effect observed by the tail-cuff method. Telemetry data collected also demonstrated a diastolic BP lowering effect of 19 mm Hg (±7.64 mm Hg, $P=0.025$), a mean arterial pressure lowering effect of 23 mm Hg (±8.24 mm Hg, $P=0.017$), and a pulse pressure lowering effect of 6 mm Hg (±1.78 mm Hg, $P=0.003$) in S.LEWx12x2x3x8 compared with the S rat. Heart rates were not significantly different between S and S.LEWx12x2x3x8.

Multiple QTLs Within a Small 1.34-Mb Genomic Module

In addition to S.LEWx12x2x3x8, 3 new strains, each spanning a shorter segment within the introgressed region of S.LEWx12x2x3x8, were phenotyped for BP. These 3 strains are represented on the right hand side of Figure 1 as S.LEWx12x2x3x4, S.LEWx12x2x3x5, and S.LEWx12x2x3x1. Of these 3 strains, only one (S.LEWx12x2x3x5) demonstrated a BP effect. Importantly, the BP of S.LEWx12x2x3x5 (D10Mco83 to D10Mco147) was significantly higher by 15 mm Hg than that of the S (Figure 1 and Table 1), suggesting that LEW alleles introgressed within S.LEWx12x2x3x5 cause an increase in hypertension of the S rat. Similar to BP, HW of S.LEWx12x2x3x5 was also significantly higher by 130 mg compared with the S rat.

Unlike S.LEWx12x2x3x5, the congenic strain S.LEWx12x2x3x1, did not demonstrate a BP effect or HW effect (Figure 1, Table 1). Therefore, the net BP increasing effect of the genetic elements within the congenic region of S.LEWx12x2x3x5 is not traceable as the net effect of LEW alleles retained within the congenic region of S.LEWx12x2x3x1. These data allow for construction of the hypothesis that the observed BP increasing effect of LEW alleles could be sought within the region identified in Figure 1 as Region 2.

Further complicating this inference is that the BP and HW effects that were observed in S.LEWx12x2x3x5 were lost in the S.LEWx12x2x3x4 congenic substrain, which had a longer introgressed segment compared with S.LEWx12x2x3x5 (Figure 1 and Table 1). These results suggest that the differential segment between S.LEWx12x2x3x4 and S.LEWx12x2x3x5 harbor alleles that negate the BP increasing effect of S.LEWx12x2x3x5. The negating effect observed may be the result of BP counteracting alleles. If so, then we reasoned that these counteracting alleles could be searched for within the chromosomal segment flanked by D10Rat58 to D10Mco43 (409 404 bp). This genomic segment of interest is hitherto named as Region 3 (Figure 1).

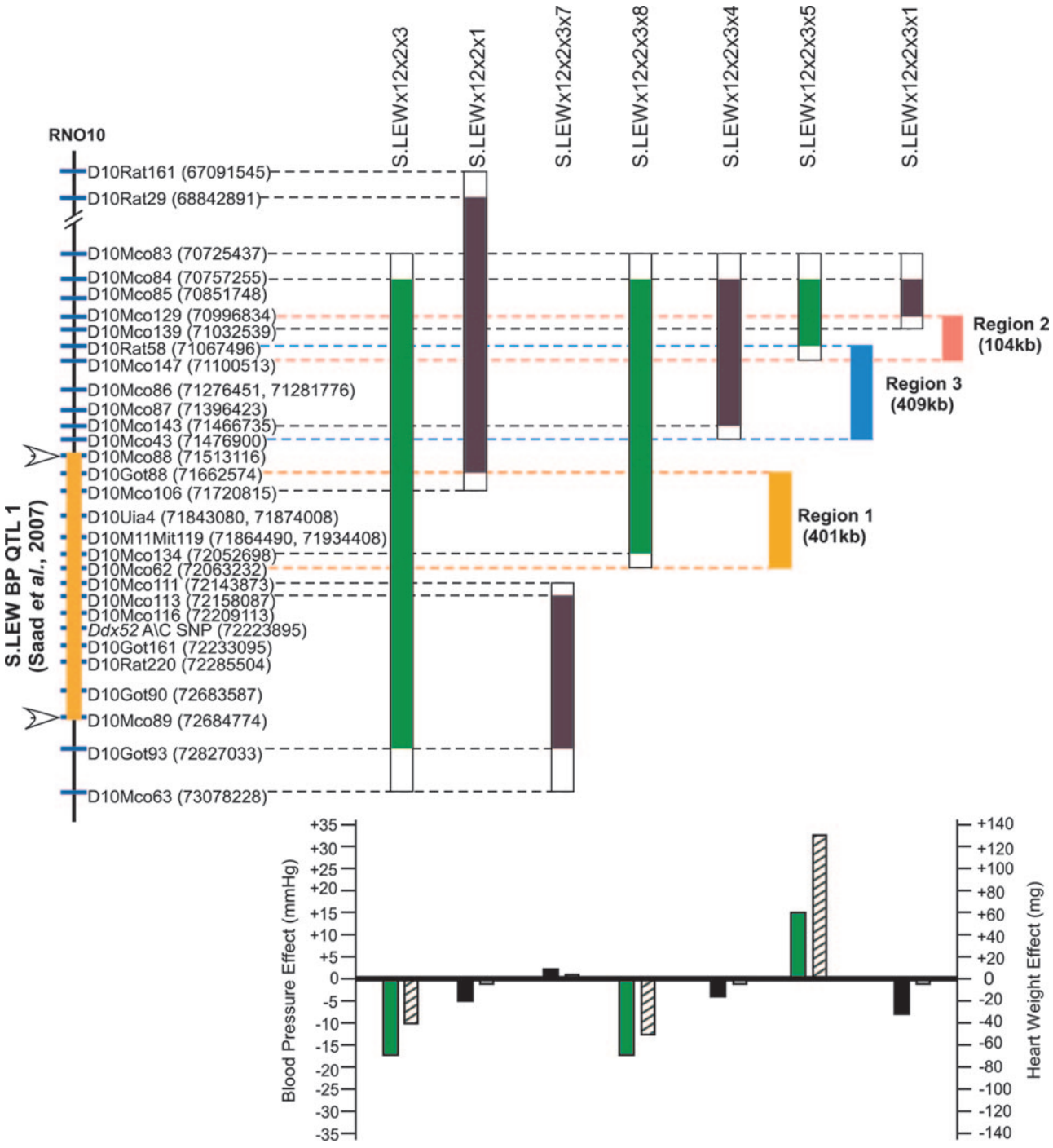

Figure 1. Congenic substrains and their phenotypic effects. The relevant section of the physical map of RNO10 is shown to the left of the figure. Values in parenthesis next to the marker names indicate their physical locations in base pairs. The previously mapped BP QTL1 region is shown as the orange bar flanked by 2 open arrows. Congenic strains are shown as solid colored bars flanked by open bars. Solid color bars illustrate the LEW segment introgressed onto the background of S. The open bars at the end of each introgressed segment represent the region of recombination. Green colored bars represent the LEW introgressed segment in congenic substrains with a BP effect, whereas the dark gray colored bars represent the LEW introgressed segment in congenic substrains without a BP effect. The congenic strains S.LEWx12x2x1 and S.LEWx12x2x3 were previously published[32] and are presented here for illustrative purposes. The locations of the newly determined BP Regions 1, 2, and 3 are respectively presented at the right of the illustration as orange, peach, and blue bars. The bottom portion of the figure illustrates the BP and HW effect observed for each congenic substrain compared with that of the S rat BP (effect=Congenic substrain BP or HW − S rat BP or HW): Green bars represent a significant BP effect, black bars represent a BP effect with a probability value >0.05, hatched bars represent a significant HW effect, and open bars represent a HW effect with a probability value >0.05.

Table 1. Observed Effects of Rat Chromosome 10 Congenic Strains on Blood Pressure, Body Weight, Heart Weight, and Relative Heart Weight

	Blood Pressure (mm Hg)				Body Weight (g)			
Strain Comparison	S	Congenic	Effect*	*t* Test	S	Congenic	Effect*	*t* Test
S.LEWx12x2x3x7 Vs S	197 [3.74]	199 [2.04]	+2 (4.26)	0.668	337 [2.36]	337 [2.09]	0 (3.15)	0.912
S.LEWx12x2x3x8 Vs S	204 [3.72]	187 [2.67]	−17 (4.48)	0.001	302 [5.58]	306 [2.34]	+4 (6.05)	0.558
S.LEWx12x2x3x4 Vs S	198 [3.62]	194 [4.18]	−4 (5.53)	0.514	331 [2.08]	335 [3.00]	+4 (3.65)	0.345
S.LEWx12x2x3x5 Vs S	221 [3.95]	236 [4.70]	+15 (6.12)	0.015	310 [3.38]	311 [4.21]	+1 (5.38)	0.911
S.LEWx12x2x3x1 Vs S	221 [3.95]	213 [5.37]	−8 (6.52)	0.253	310 [3.38]	308 [5.30]	−2 (6.03)	0.732
	Heart Weight (g)				Relative Heart Weight			
Strain Comparison	S	Congenic	Effect*	*t* Test	S	Congenic	Effect*	*t* Test
S.LEWx12x2x3x7 Vs S	1.31 [0.01]	1.31 [0.01]	0 (0.02)	0.916	3.90 [0.04]	3.91 [0.04]	+0.01 (0.06)	0.908
S.LEWx12x2x3x8 Vs S	1.26 [0.01]	1.21 [0.01]	−0.05 (0.01)	0.006	4.20 [0.07]	3.95 [0.02]	−0.25 (0.07)	0.003
S.LEWx12x2x3x4 Vs S	1.36 [0.01]	1.36 [0.02]	0 (0.02)	0.764	4.12 [0.04]	4.06 [0.06]	−0.06 (0.08)	0.453
S.LEWx12x2x3x5 Vs S	1.32 [0.01]	1.45 [0.01]	+0.13 (0.02)	<0.0001	4.27 [0.05]	4.67 [0.09]	+0.40 (0.10)	0.001
S.LEWx12x2x3x1 Vs S	1.32 [0.01]	1.32 [0.02]	0 (0.02)	0.974	4.27 [0.05]	4.32 [0.14]	+0.05 (0.14)	0.697

*Effect=Congenic value–S value. Negative values indicate a decrease in the congenic effect compared to the S rat, whereas positive values indicate an increase in the congenic effect compared to the S rat. Standard error of the mean is in brackets. Standard error of the mean difference is in parenthesis. Number of rats in each group ranged from 17 to 32. Only male rats were used. Independent *t* test was used to compare the means (significance at $P<0.05$). Relative heart weight is the ratio of heart weight to body weight multiplied by 1000. Data for two strains illustrated in Figure 1 (S.LEWx12x2x1, and S.LEWx12x2x3) are not presented in the table because the data were previously published (Saad et al, 2007).

Candidate Genes Within the 3 Regions of Interest for Further Investigation

Figure 3 illustrates all the iterations of substitution mapping conducted using 21 congenic strains developed by our laboratory that resulted in fine-mapping of the region contained in the original congenic strain, S.LEW(10), to the multiple regions discussed in this report (Regions 1 through 3). Note that the current localization of all these Regions is within the 2-LOD support interval of the original linkage analysis.[14] Figure 3 also shows the genes contained within each of the 3 Regions prioritized for further investigation. Region 1 contains a total of 7 genes (*Ccl6*, *Ccl3*, *Ccl4*, LOC689133, *Expi*, LOC360228, and RGD1566204). Region 2 contains a total of 4 genes (LOC688779, *Lig3, Rffl*, and *Rad51l3*), and Region 3 contains 18 genes (*Rad51l3*, RGD1310708, *Nle1*, *Unc45b*, *Slfn5*, LOC688477, *Slfn8*, LOC688857, LOC688871, *Slfn2*, LOC688900, LOC688910, *Slfn3*, LOC688925, RGD1564411, LOC287569, *Pex12*, and *Ap2b1*).

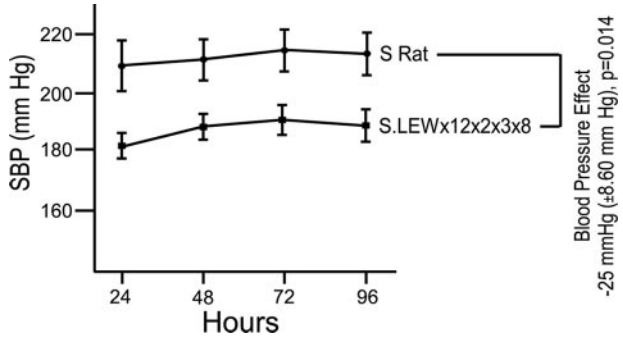

Figure 2. Corroboration of tail-cuff measurements by telemetry. Twenty-four-hour averages of systolic blood pressure (SBP) values for S rats vs S.LEWx12x2x3x8 congenic rats. Error bars represent the standard error of the mean. Standard error of the mean difference is given in parenthesis.

Expression Analysis of the Genes Contained Within Region 1

Heart and kidney gene expression for the 7 genes within Region 1 was characterized using the S rat versus S.LEWx12x2x3x8 comparison (Table 2). Based on fold-change of expression, heart and kidney LOC360228 and *Expi* expression was greater in the S rat than that in the S.LEWx12x2x3x8 congenic strain. Gene expression of *Ccl9* and *Ccl5* were determined to evaluate the chemokine gene cluster. *Ccl9*, which is not within Region 1, showed the greatest level of fold-change difference in both the heart and kidney of S.LEWx12x2x3x8 compared with that of the S rat.

Discussion

Overall, this study resulted in the following 3 major findings: (1) The previously inferred location of BP QTL1 which was based on differential congenic segments, was traceable within the confines of the congenic strain S.LEWx12x2x3x8 spanning 1.34 Mb (D10Mco83-D10Mco62); (2) A second novel BP increasing effect of LEW alleles on the S genetic background was located within the limits of the congenic strain S.LEWx12x2x3x5 spanning 375 kb (D10Mco83-D10Mco147), which is in the vicinity of Region 1; and (3) A third novel closely-linked genomic segment, wherein LEW alleles lower BP of the S rat, was located within the congenic strain S.LEWx12x2x3x4 spanning 751 kb.

For further localization, newer iterations of congenic substrains derived from S.LEWx12x2x3x8 and S.LEWx12x2x3x5 are required. Because these 2 congenic strains are already with very short introgressed segments of 1.34 Mb and 375 kb, respectively, development and testing of further congenic substrains will take a considerable amount of time and resources. In the meantime, assessment of differential congenic segments allows for prioritization of the

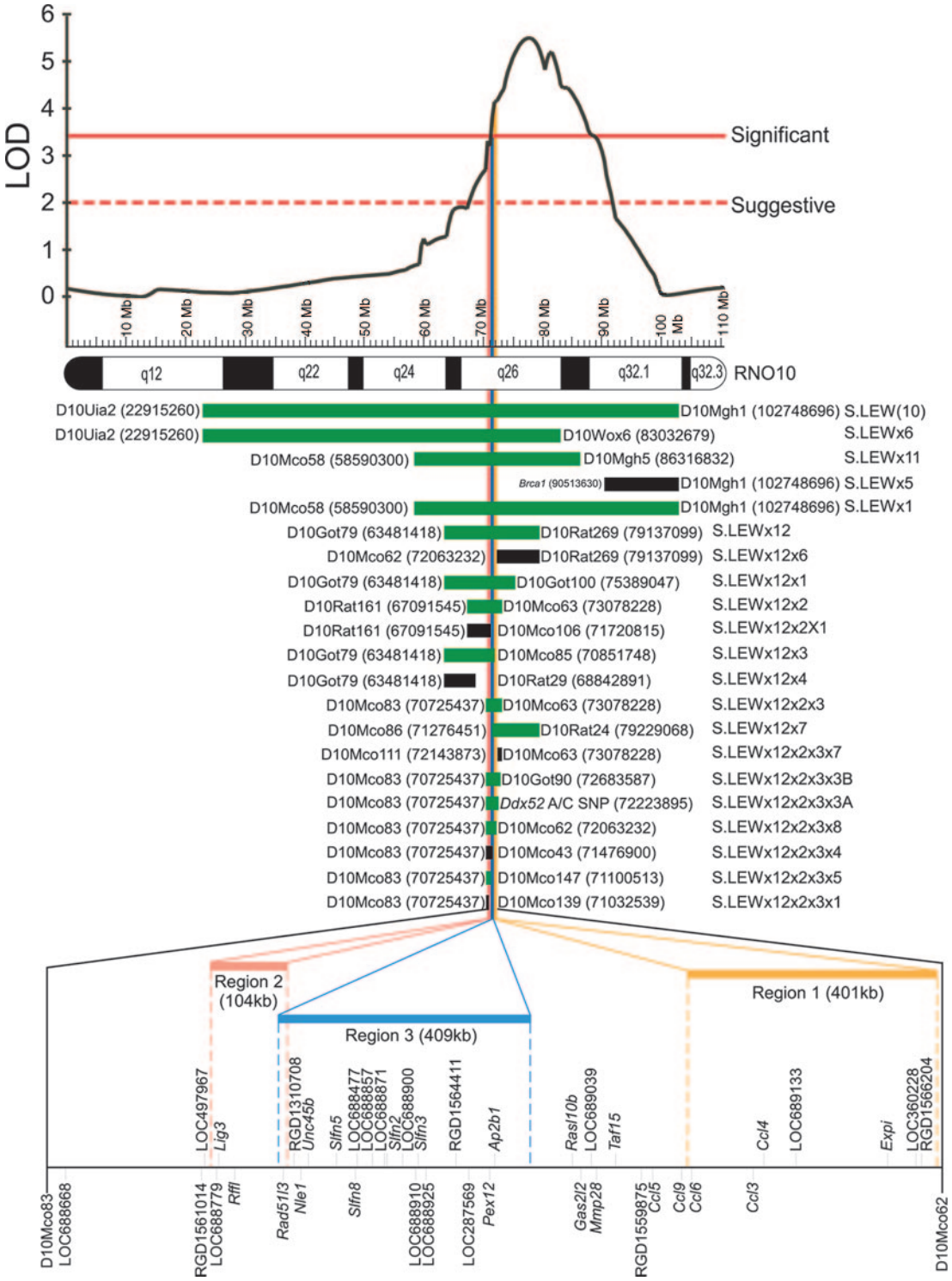

Figure 3. Comprehensive illustration of linkage and substitution mapping on RNO10 using S and LEW rats. The LOD plot for BP derived by the study of an F_2(S×LEW) population[14] is shown alongside the physical and cytogenetic maps of RNO10. End markers of 21 congenic strains and their current physical locations are shown flanking bars that represent congenic strains. The physical location for each marker was obtained from uniSTS (www.ncbi.nlm.nih.gov) using the *Rattus norvegicus* strain BN/SsNHsdMCW chromosome 10 accession number CM000081 (version CM000081.1; GI: 56554742), except for D10Wox6, which was not mapped on CM000081 and was therefore positioned by searching for the deposited sense primer sequence in the 4-Mb region from RNO10 79 Mb to RNO10 83 Mb and calculating its location based on the findings. Green bars indicate that the congenic strains had a BP effect and black bars indicate that the congenic strains had no BP effect. All congenic strains except the ones reported in the current article are previously published.[32] The black line at the very bottom of this figure depicts the entire length of S.LEWx12x2x3x8 with all the genes within the introgressed segment. Colored vertical lines (peach, blue, and orange) collectively depict the genomic module containing all the BP QTL Regions described in this study. The limit for each Region is indicated at the bottom of the diagram. Annotated genes found within each region are also illustrated toward the bottom of the figure.

Table 2. Real-Time PCR Results for S.LEW QTL1 Genes

	ΔC_t			$\Delta\Delta C_t$		Fold-Change		
Gene	S	C	*t* Test		CI (95%)	S>C	C>S	CI (95%)
Heart								
RGD1566204	8.17 [0.630]	8.60 [0.448]	0.389	0.43 [0.773]	(1.30, −0.44)	1.34		(2.47, 0.73)
LOC360228	6.38 [0.651]	8.23 [0.135]	0.009*	1.85 [0.665]	(2.60, 1.09)	3.6		(6.07, 2.13)
Expi	14.8 [0.376]	16.2 [1.255]	0.340	1.44 [1.310]	(3.30, −0.42)	2.71		(9.88, 0.74)
LOC689133	14.5 [0.795]	14.9 [1.824]	0.780	0.34 [1.990]	(2.59, −1.91)	1.27		(6.06, 0.26)
Ccl4	9.95 [0.376]	8.99 [0.473]	0.052	−0.95 [0.605]	(−0.27, −1.64)		1.94	(3.12, 1.20)
Ccl3	11.1 [0.329]	11.1 [0.766]	0.949	−0.03 [0.834]	(0.91, −0.97)		1.02	(1.97, 0.53)
Ccl6	6.09 [0.141]	5.84 [0.350]	0.309	−0.25 [0.378]	(0.17, −0.68)		1.19	(1.60, 0.88)
Ccl9	13.5 [0.552]	10.8 [0.686]	0.006*	−2.6 [0.880]	(−1.65, −3.64)		6.28	(12.54, 3.14)
Ccl5	7.25 [0.189]	7.03 [0.051]	0.229	−0.21 [0.196]	(0.03, −0.47)		1.16	(1.38, 0.97)
Kidney								
RGD1566204	6.44 [0.196]	6.77 [0.336]	0.194	0.32 [0.389]	(0.76, −0.11)	1.25		(1.70, 0.92)
LOC360228	8.30 [0.481]	9.78 [1.424]	0.161	1.49 [1.504]	(3.19, −0.21)	2.81		(9.16, 0.86)
Expi	15.1 [0.603]	16.9 [0.560]	0.049*	1.73 [0.824]	(2.80, 0.66)	3.23		(6.98, 1.58)
LOC689133	14.3 [1.651]	14.5 [2.396]	0.907	0.20 [2.910]	(3.50, −3.09)	1.15		(11.37, 0.117)
Ccl4	7.77 [0.162]	7.54 [0.242]	0.246	−0.23 [0.292]	(0.10, −0.56)		1.17	(1.47, 0.93)
Ccl3	9.59 [0.462]	9.80 [0.507]	0.624	0.21 [0.686]	(0.98, −0.56)	1.15		(1.98, 0.67)
Ccl6	5.20 [0.754]	4.39 [0.299]	0.159	−0.81 [0.811]	(0.10, −1.73)		1.75	(3.31, 0.92)
Ccl9	10.6 [0.563]	8.66 [0.428]	0.009*	−1.92 [0.707]	(−1.12, −2.73)		3.8	(6.63, 2.18)
Ccl5	5.66 [0.277]	5.17 [0.406]	0.161	−0.48 [0.492]	(0.06, −1.04)		1.4	(2.06, 0.95)

S is the S Rat and C is the congenic strain used for real-time PCR, which was S.LEWx12x2x3x8. Heart and kidney expression were analyzed using n=3 for both strains compared. The ΔC_t was calculated as: [group mean of the gene C_t − group mean of the GAPDH C_t]. The $\Delta\Delta C_t$ was calculated as: [congenic ΔC_t−S rat ΔC_t]. Fold-change in expression was determined using the $2^{-\Delta\Delta C_t}$ method. All *t* tests and confidence intervals (CI) were obtained by SPSS (significance at $P<0.05$, indicated by an *). Standard deviation (SD) is given in brackets. Standard deviation for the values were determined by propagation of the standard errors; $[(\text{S Rat}-\Delta C_t-\text{SD})^2+(\text{C Rat}-\Delta C_t-\text{SD})^2]^{1/2}$. S>C indicates that the fold-change in expression is greater in the S rat compared to the congenic S.LEWx12x2x3x8. C>S indicates that the fold-change in expression is greater in the S.LEWx12x2x3x8 congenic compared to the S rat.

3 candidate regions labeled as Regions 1, 2, and 3 in Figure 1. There are 7 positional candidate genes in Region 1. None of these genes contain nonsynonymous variants between the S and LEW alleles. Organ-specific differences in gene expression provide evidence for genetic elements modulating transcription of genes both within or outside the Region 1 as candidate genetic determinants of the BP QTL effect.

At this stage of substitution mapping, it is of interest to relate the location of genetic determinants of BP to the original LOD plot. As illustrated in Figure 3, the entire genomic module of 1.34 Mb described in our study is close to but not directly under the apex. This observation suggests that the practice of analyzing candidate genes physically close to the point of highest statistical evidence for linkage[33,34] may miss the identification of the underlying genetic determinants.

There is a clear-cut difference between mapping congenic intervals as QTL intervals and mapping differential congenic segments as QTL intervals. Because the net effect of alleles within the introgressed segment of any congenic strain is what is measured during phenotyping a congenic strain, the underlying contributing factors to the observed net effect remain unknown. Therefore, mapping using congenic intervals as QTL intervals is more appropriate for definitive conclusions about localization of QTLs. Mapping by differential segments as QTL intervals is appropriate as a hypothesis, which requires further validation. Our study has used the limits of the introgressed segments of 3 congenic strains for defining the limits of 3 different genetic determinants. Further, to prioritize searching for the underlying QTL effectors, we have prioritized 3 regions based on differential congenic segments. These regions undoubtedly require further corroboration with minimal strains encompassing each of the proposed regions only. A typical example is Region 1, which is localized with data obtained from S.LEWx12x2x3x8, which we now know, also contains additional closely-linked BP QTLs trapped in S.LEWx12x2x3x4 and S.LEWx12x2x3x5. It is possible that the LEW alleles within S.LEWx12x2x3x4 and S.LEWx12x2x3x5, cancel the effect of each other and thereby the net BP effect of S.LEWx12x2x3x8 is that of Region 1 only. Additional evidence for this interpretation is the lack of a BP effect in S.LEWx12x2x3x4, which contains LEW alleles at Regions 2 and 3. On the other hand, Region 1 may not be a stand-alone QTL effecter, but a modifier of another, yet unidentified closely-linked allele within S.LEWx12x2x3x8. At the very least, construction and characterization of a minimal congenic strain with introgressed LEW alleles in Region 1 will be necessary to sort these possibilities.

Among all the regions of interest reported here, Region 1 was best characterized because it lies within the previously

inferred QTL1 segment. Among the previously listed 18 candidate genes[32] for QTL1, the current Region 1 retains only 7 candidate genes, 3 of which are chemokines. None of these candidate genes have any nonsynonymous variants, indicating that changes in the protein sequence of the genes located within this region is not the cause of the BP effect. Causal polymorphisms could however be synonymous polymorphisms that alter mRNA stability, rate of translation, and/or protein function.[35,36] The genes located within Region 1 did not have any synonymous changes either. Genetic elements causing changes in gene expression was the next obvious suspect, which have been previously shown to be causal factors in a number of recent positional cloning projects on a variety of polygenic traits across species from plants to mammals.[37,38] Unfortunately, S.LEWx12x2x3x8 was the only reasonable strain to test for differential gene expression of genes within Region 1 as candidates. Differential expression of LOC360228 in the heart and *Expi* in the kidney is of interest for follow-up studies. Interestingly, the expression of none of the positional candidate chemokines, *Ccl3*, *Ccl4*, and *Ccl6*, were differentially expressed in either kidneys or heart, but the expression of a chemokine outside the limits of the QTL1 interval, *Ccl9*, was significantly different between S and the congenic strain. It remains to be determined whether transcriptional modulators of *Ccl9* or other genes outside Region 1 are present within Region 1.

While mapping QTLs with fine resolution, because every nucleotide is a candidate quantitative trait nucleotide (QTN), it is critical to evaluate the structure and properties of the genomic sequence in question. There are 3 noticeable gaps in the assembly of the rat genome within Region 1 (http://genome.ucsc.edu/cgi-bin/hgTracks). The sizes of the 3 largest gaps are 39 741 bp (71 892 260 bp to 71 932 000 bp), 13 077 bp (71 694 974 bp to 71 708 050 bp), and 7510 bp (71 879 835 bp to 71 887 344 bp). There are 13 other minor gaps of $<$50bp. These gaps are a concern that we cannot resolve without improvements in the quality of the currently available physical map of the rat. Segmental duplications are reported on RNO 10.[39] Chen et al have demonstrated segmental duplications flanking the multiple sclerosis locus on human chromosome 17q and suggested that segmental duplications could affect the biological activity of the genes on a chromosome.[40] However, such segmental duplications may not be genetic determinants underlying Region 1 because it does not appear to contain segmental duplications (http://ratparalogy.gs.washington.edu/cgi-bin/hgTracks). Similarly, QTNs within microRNAs are also not predicted as present within the physical map spanning Region 1 (www.ensembl.org). These observations coupled with the data obtained from the gene expression analysis suggest that single nucleotide polymorphisms, either individually or collectively as a haplotype, that regulate the expression of LOC360228, *Expi*, and/or *Ccl9* can be prioritized as potential effecters of Region 1.

Compared with Region 1, the analysis of the other 2 regions detected in this study is lagging because candidate gene sequencing and transcript quantitation are not yet undertaken. Similar to Region 1, however, neither Region 2 nor Region 3 contain any microRNAs or segmental duplications (www.ensembl.org; http://ratparalogy.gs.washington.edu/cgi-bin/hgTracks). Nevertheless, unlike the genomic segment spanning Region 1, the physical maps of Regions 2 and 3, shown in Figure 2, are without any large gaps in the sequence assembly, which is favorable for further mapping of these regions.

Perspectives

The quest for genetic determinants of BP within a 1.34 Mb region between 70 725 437 bp and 72 063 232 bp (D10Mco83-D10Mco62) on RNO10 is presented. To our knowledge, this study is unique as it represents the shortest genomic module determined to harbor 3 closely-linked BP QTLs with opposing action. Similar complex clustering of closely-linked QTLs are reported for genetic susceptibility to systemic lupus erythematosus in mice[41] and in humans.[42] Seeking the identities of each of the underlying QTL effecters is not only of relevance to delineate the molecular underpinnings of hypertension, but also of interest to understand the basic design with respect to the organization of disease-causative genetic factors on the rat genome.

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