Blood pressure and proteinuria effects of multiple quantitative trait loci on rat chromosome 9 that differentiate the spontaneously hypertensive rat from the Dahl salt-sensitive rat

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A blood pressure (BP) quantitative trait locus (QTL) was previously located within 117 kb on rat chromosome 9 (RNO9) using hypertensive Dahl salt-sensitive and normotensive Dahl salt-resistant rats. An independent study between two hypertensive rat strains, the Dahl salt-sensitive rat and the spontaneously hypertensive rat (SHR), also detected a QTL encompassing this 117 kb region. Dahl salt-sensitive alleles in both of these studies were associated with increased BP. To map SHR alleles that decrease BP in the Dahl salt-sensitive rat, a panel of eight congenic strains introgressing SHR alleles onto the Dahl salt-sensitive genetic background were constructed and characterized. S.SHR(9)x3B, S.SHR(9)x3A and S.SHR(9)x2B, the congenic regions of which span a portion or all of the 1 logarithm of odds (LOD) interval identified by linkage analysis, did not significantly alter BP. However, S.SHR(9), S.SHR(9)x4A, S.SHR(9)x7A, S.SHR(9)x8A and S.SHR(9)x10A, the introgressed segments of which extend distal to the 1 LOD interval, significantly reduced BP. The shortest genomic segment, BP QTL1, to which this BP-lowering effect can be traced is the differential segment of S.SHR(9)x4A and S.SHR(9)x2B, to which an urinary protein excretion QTL also maps. However, the introgressed segment of S.SHR(9)x10A, located outside of this QTL1 region, represented a second BP QTL (BP QTL2) having no detectable effects on urinary protein excretion. In summary, the data suggest that there are multiple RNO9 alleles of the SHR that lower BP of the Dahl salt-sensitive rat with or without detectable effects on urinary protein excretion and that only one of these BP QTLs, QTL1, overlaps with the 117 kb BP QTL region identified using Dahl salt-sensitive and Dahl salt-resistant rats. *J Hypertens* 26:2134–2141 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Abbreviations: LOD, Logarithm of odds; LRS, Likelihood ratio statistic; QTL, Quantitative trait locus; RNO9, Rat chromosome 9; SHR, Spontaneously hypertensive rat; SHRsp, Stroke-prone spontaneously hypertensive rat; UPE, Urinary protein excretion; WKY, Wistar-Kyoto rat

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Introduction

Essential hypertension is a complex trait influenced by both environmental and genetic factors [1]. For example, genetics can render the blood pressure (BP) of an individual sensitive or resistant to changes in dietary salt intake [1,2]. Because of the difficulties that are inherent in genetic analyses in humans, animal models are important tools for dissecting genetic factors in disease, and, in the case of hypertension, rat models have been used most frequently [3–6]. In the past, comparisons between inbred strains for detecting BP quantitative trait loci (QTLs) have generally utilized a hypertensive strain and a normotensive strain [4,5,7–10], among which a 117 kb region was previously detected as a BP QTL between the Dahl salt-sensitive (S/jr) rat and the Dahl salt-resistant (R/jr) rat [11]. It is likely, however, that the sets of alleles predisposing two inbred strains to hypertension will differ, just as there could be different genetic determinants of hypertension between two unrelated patients with essential hypertension.

The S/jr rat and the spontaneously hypertensive rat (SHR) are hypertensive inbred strains. However, the S/jr rat was selectively bred for high BP in response to a high-salt diet [12], whereas the SHR was selected for spontaneous hypertension with no environmental stimuli [13]. Although the S/jr rat does develop spontaneous hypertension, the SHR is less sensitive to the BP-altering effects of salt loading [14]. Thus, the rationale for the use of two hypertensive strains to map BP QTLs is that it provides the means to delineate the genetic determinants responsible for differential etiology of hypertension observed between these two strains. Previously, linkage analysis of an F_2(SxSHR) population fed a high-salt diet led to the identification of BP QTLs on rat chromosomes 3, 8 and 9 (RNO3, RNO8 and RNO9, respectively) [14]. The SHR allele on RNO9, which is the focus of the

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current study, was linked to lower BP [14]. A second SxSHR linkage analysis performed by a different group also identified SHR alleles on RNO9 linked to lower BP values [15,16]. Furthermore, the 117 kb QTL region mapped between S/jr and R/jr rats is within the 2 LOD support interval of both of these S/jr vs. SHR BP QTL studies. Therefore, alleles on RNO9 may represent genetic elements exerting a causal effect on BP in multiple strain comparisons, thus warranting further corroboration and fine mapping. The purposes of the experiments reported here were to (a) confirm the existence of a salt-sensitive BP QTL on RNO9 by congenic strain analysis, (b) map the BP QTL to a shorter segment on RNO9 by linkage analysis and subsequent congenic substrain analysis, (c) study the urinary protein excretion (UPE) effects of the BP QTLs, and (d) compare and contrast the mapping data obtained with previous mapping studies reported within this region.

Methods

Animals

The inbred Dahl Salt-sensitive rat strain (SS/Jr), referred to as the S/jr rat, was from our colony. The SHR strain (SHR/Hsd) was originally obtained from Harlan Sprague–Dawley (Indianapolis, Indiana, USA) and maintained in our colony. The progenitor S.SHR(9) congenic strain was developed as previously described [17]. The congenic substrains were derived from S.SHR(9) as previously described for S.R congenic substrains [18]. All animal procedures and protocols were approved by the University of Toledo Health Science Campus Institutional Animal Care and Use Committee.

Genotyping

Genotyping was done by radioactive PCR using microsatellite markers as previously described [19].

Microsatellite marker development

New microsatellite markers *D9Mco72* through *D9Mco94* were designed using sequence information from the rat genome sequence (RGSC 3.4, December 2004, Ensembl Release 49 in March 2008) available at Ensembl (www.ensembl.org). Information on these markers is available at the website http://hsc.utoledo.edu/depts/physiology/research/rat/marker.html. Primers were designed around dinucleotide or trinucleotide repeats using Primer3 software (http://frodo.wi.mit.edu/primer3/). Primer pairs were tested for polymorphisms between S/jr and SHR DNA. Those that were polymorphic were used to genotype the progenitor congenic strain and substrains as above.

Tail-cuff blood pressure measurement

Depending on the number of congenic strains concomitantly tested with S/jr rat, two experimental designs were used for BP measurement. In the first design, male S/jr rat ($n = 20$) and one congenic strain ($n = 20$) were concomitantly tested. Rats were weaned onto a low-salt (0.3% NaCl) Harlan Teklad 7034 diet, placed on a *2%* NaCl (Harlan Teklad) diet at 40–42 days of age and maintained on this diet for 28 days. The rats were housed in cages such that there was one S/jr rat and one congenic rat per cage. Systolic BP was measured on days 24 to 28 of the high salt regimen, by the tail-cuff method on conscious, restrained rats warmed to 28°C. Each rat had its BP measured once per day for 4 days by operators blinded to the study. Daily BP was the average of three consistent readings. The four daily BP readings of a rat were averaged for the final BP. The reported BP for each strain is the average of the final BP readings for all rats of that strain.

The second experimental design utilized three or four strains per experiment. Twenty male rats from each strain were weaned at 30 days onto the Harlan Teklad 7034 diet. Rats were placed on the *2%* NaCl (Harlan Teklad) diet at 40–42 days of age and maintained on it for 28 days. The rats were housed in cages containing two rats from two different strains, and the strains were randomized. Systolic BP was measured as above, except that each rat had its BP measured twice, once by each operator, over the final 4 days on the *2%* NaCl diet. Following the week of BP measurement, five or six S/jr, S.SHR(9), S.SHR(9)x7A and S.SHR(9)x8A rats were implanted with radiotelemetry probes as described below. At the end of the experiment, rats were killed using CO_2 hypoxia and their body and heart weights were recorded.

Blood pressure measurement by radiotelemetry

Surgeries were performed as previously described [20,21]. Measurements spanned 48 h. A moving average over a period of 4 h was calculated for each rat. The values for rats of the same strain were then averaged before analysis. The overall average BP and heart rate was the mean of each 4 h moving average.

Urinary protein excretion

UPE was determined as described previously [17,22]. Briefly, S/jr, S.SHR(9)x8A and S.SHR(9)x10A rats previously used in BP experiments were caged individually in metabolic cages. Four days after the completion of the BP measurements by the tail-cuff method, urine was collected in the presence of sodium azide, over a 24-h period and their volumes recorded. This time point corresponds to day 32 after commencement of feeding the high-salt (*2%*NaCl) diet. The pyrogallol red-based QuanTtest Red Total Protein Assay (Quantimetrix, Redondo Beach, California, USA) was used to estimate the protein concentration in the urine samples in a 96-well format. The absorbance of each sample at 600 nm was determined using a VERSAmax tunable microplate reader from Molecular Devices (Sunnyvale, California, USA) and compared with those of the standard curve. The standard curve was produced by measuring absor-

bance of the QuanTtest human protein standards (25–200 mg/dl). The linear regression of this standard curve was used to calculate the concentration (mg/dl) of protein in the urine samples. Twenty-four hour UPE was the product of the urinary protein concentration and the volume collected.

Quantitative trait locus(QTL) analysis of F_2 [(S.SHR(9)x8A) × S/jr]

Male rats from an F_2([S.SHR(9)x8A] × S/jr) population ($n = 53$) were phenotyped for UPE and tail-cuff BP at 80 and 130 days, respectively, as described above. One-way analysis of variance (ANOVA) followed by Kolmogorov–Smirnov post-hoc test indicated that the BP and UPE values were distributed normally in this segregating population. The F_2 rats were genotyped for nine polymorphic microsatellite markers located within the S.SHR(9)x8A congenic region and the Map Manager QT program was used to detect and localize QTLs using both unconstrained (free) and constrained (dominant, additive or recessive) genetic models for regression analysis to indicate the dominance properties of each QTL. Likelihood ratio statistics (LRS) generated within Map Manager QT as measures of the significance of a QTL [23] were converted into LOD scores (LOD = LRS/4.6) for reporting purposes. Threshold values for 'suggestive' and 'significant' linkage follow the definitions described by Lander and Kruglyak [24].

Statistical analyses

All statistical analyses were performed using the SPSS software package (SPSS, Chicago, Illinois, USA) with results presented as the mean ± SE. All analyses were done by *t*-test when a single congenic strain was compared with S/jr strain or by ANOVA when multiple congenic strains were concomitantly phenotyped along with Dahl salt-sensitive rats. A P value less than 0.05 was used to assess statistical significance.

Results

Effect of RNO9 spontaneously hypertensive rat alleles on blood pressure of the Dahl salt-sensitive rat

Figure 1 illustrates all the congenic strains constructed and characterized in the present study. The limits of the introgressed segments within these congenic strains as defined by the physical map of the rat genome are provided in Table 1. The BP of S/jr and S.SHR(9) rats were compared after 28 days on a 2% NaCl diet (Table 2). The BP of the S/jr rats averaged to 207 (±4.10) mmHg, whereas the BP of the S.SHR(9) congenic rats was 180 (±2.07) mmHg. There was a 27 (±4.64) mmHg reduction in BP ($P < 0.001$) in the S.SHR(9) congenic strain as compared with that in the S/jr rat. These data indicate that the congenic strain S.SHR(9) contains SHR allele(s) responsible for decreasing BP identified previously by linkage analysis [14] (Fig. 1 and Table 2).

Like S.SHR(9), the BP of six S.SHR(9) congenic substrains [S.SHR(9)x2B, S.SHR(9)x3A, S.SHR(9)x3B, S.SHR(9)x4A, S.SHR(9)x7A and S.SHR(9)x8A] were measured and compared with the parental S/jr strain on a 2% NaCl diet. Compared with the BP in concomitantly raised S/jr rats, the BP in S.SHR(9)x2B, S.SHR(9)x3A and S.SHR(9)x3B rats lowered by 4 (±6.14), 11 (±6.78) and 9 (±6.03) mmHg, respectively. None of these BP-lowering effects was statistically significant (Table 2). However, BP was reduced by 25 (±6.13) mmHg in S.SHR(9)x4A rats, by 28 (±5.22) mmHg in S.SHR(9x)7A and by 37 (±4.84) mmHg in S.SHR(9)x8A rats compared with that in S/jr rats. These three strain differences were statistically significant ($P \leq 0.001$) (Table 2). The proximal part of the congenic region of S.SHR(9)x4A overlaps that of S.SHR(9)x2B, which lacks a BP effect. Therefore, it is reasonable to conclude that the shortest discernable limits of the QTL1-containing region is within the differential congenic segment of S.SHR(9)x4A and S.SHR(9)x2B, between D9Mco76 (53,933,081 bp) and D9Mco85 (76,455,867 bp) (Fig. 1).

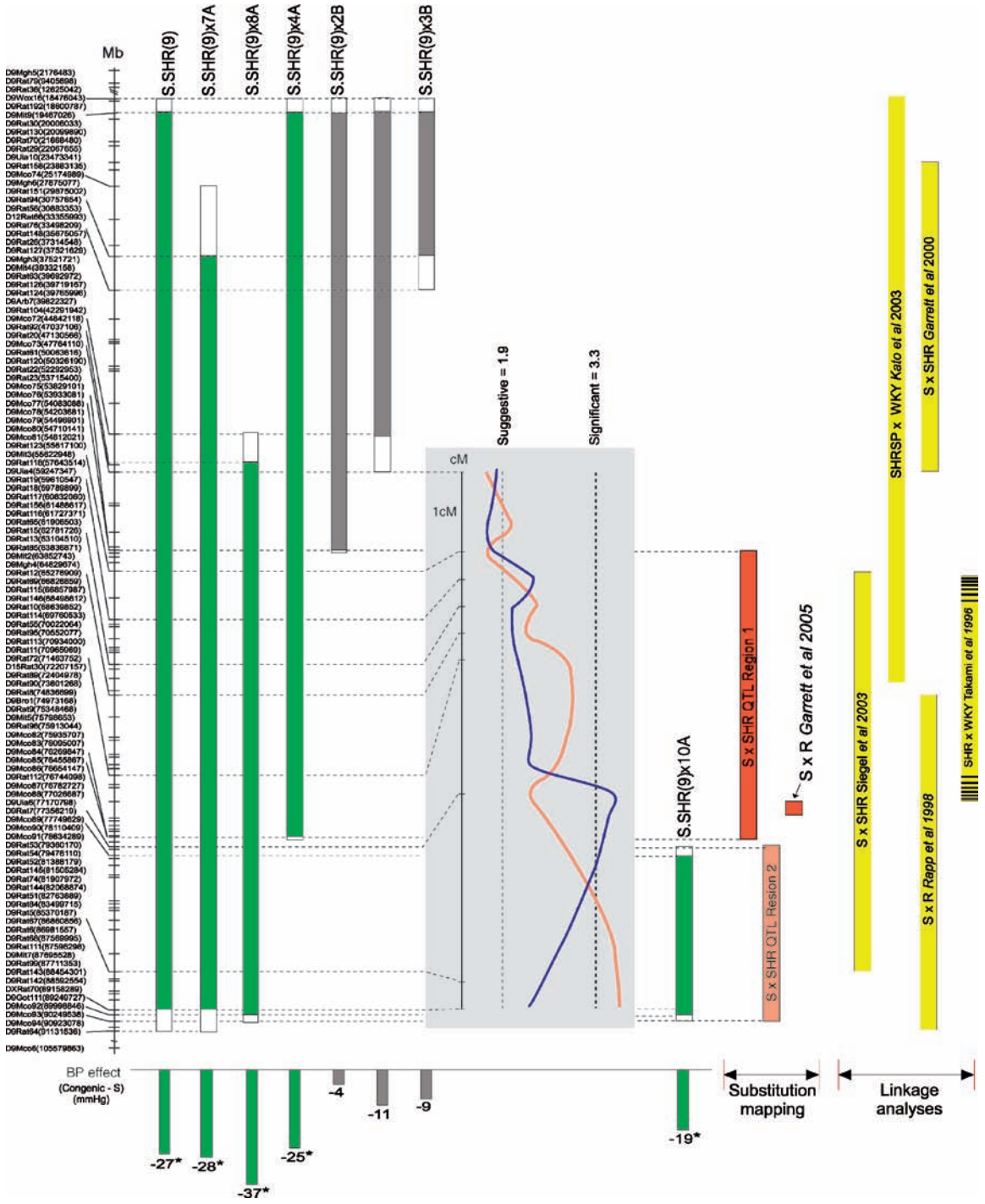
Blood pressure effects of congenic substrains ascertained by telemetry

To confirm the BP data obtained by tail-cuff, a telemetry experiment was performed using S/jr, S.SHR(9), S.SHR(9)x7A and S.SHR(9)x8A rats (Fig. 2). The systolic BP of S/jr rats over the 48 h of the experiment averaged 185 (±1.97) mmHg. The S.SHR(9), S.SHR(9)x7A and S.SHR(9)x8A congenic strain rats all had similar systolic BPs that were significantly lower than that in S/jr rats [147 (±1.56), 156 (±1.73) and 146 (±1.44), respectively]. A similar pattern was observed for diastolic BP and mean arterial BP (Fig. 2). The heart rates of the four strains did not significantly differ from one another (Fig. 2).

Further linkage using F_2 recombinants derived from [(S.SHR(9)x8A) × S/jr]

To improve the resolution of mapping the BP QTL present within the S.SHR(9)x8A congenic interval, this congenic strain was backcrossed to S/jr rats to obtain an F_1 population, which was intercrossed to generate an F_2 population of male rats ($n = 53$). Linkage of the F_2 population to BP indicated that there are two distinct peaks that map within the introgressed segment of S.SHR(9)x8A (Fig. 1). One peak, which surpasses the threshold for suggestive linkage on the additive mode of inheritance, co-localizes with the BP QTL1 region prioritized by the differential segments of S.SHR(9)x4A and S.SHR(9)x2B. The second peak passes the threshold for significant linkage on additive, dominant and free modes of inheritance and is at the distal end of the congenic region with a peak LOD score of 3.70 at marker *D9Got111* (89,249,727) (Fig. 1). This raises the possibility that there are multiple BP QTLs within the limits of the congenic strain S.SHR(9)x8A. In contrast, however, the linkage for

Fig. 1



Summary of RNO9 BP quantitative trait locus analysis. The physical map of RNO9 is shown on the left. Locations of microsatellite markers in base pairs (bp) are shown in parenthesis after the name of each marker. These locations were as reported in the rat genome assembly available at the Ensembl database (RGSC 3.4, December 2004; www.ensembl.org). Green and gray bars represent spontaneously hypertensive rat (SHR) alleles introgressed onto the S/jr genetic background. White boxes represent regions of recombination. The S.SHR(9), S.SHR(9)x4A, S.SHR(9)x7A, S.SHR(9)x8A and S.SHR(9)x10A strains in green had a blood pressure-lowering effect when compared to S/jr rats. The strains depicted in gray, S.SHR(9)x2B, S.SHR(9)x3A and S.SHR(9)x3B did not alter blood pressure. The graph depicts a logarithm of odds (LOD) plot from a linkage analysis performed on and F_2[(S.SHR(9)x8A)xS] population using the additive mode of inheritance. The red line indicates blood pressure LOD scores, whereas the blue line indicates urinary protein excretion LOD scores. The vertical thin black dashed line on the LOD plot is the threshold for suggestive linkage at that locus, whereas the thick black dashed line is the threshold for significant linkage. Orange bars indicate blood pressure quantitative trait loci confirmed and mapped by congenic strain analysis. Yellow bars indicate 1 LOD intervals identified by linkage analysis. The dashed box labeled Takami *et al.* [40] centers on the location of the marker with the peak LOD score.

Table 1 S.SHR(9) congenic intervals

Congenic strain	Microsatellite markers		Congenic interval (bp)
	From	To	
S.SHR(9)	*D9Wox16* (18,476,043)	*D9Rat64* (91,131,536)	72,655,493
S.SHR(9)x2B	*D9Wox16* (18,476,043)	*D9Mco77* (54,083,088)	35,607,045
S.SHR(9)x3A	*D9Wox16* (18,476,043)	*D9Mco73* (47,764,110)	29,288,067
S.SHR(9)x3B	*D9Wox16* (18,476,043)	*D9Rat76* (33,498,209)	15,022,166
S.SHR(9)x4A	*D9Wox16* (18,476,043)	*D9Mco85* (76,455,867)	57,979,824
S.SHR(9)x7A	*D9Mco74* (25,174,989)	*D9Rat64* (91,131,536)	65,956,547
S.SHR(9)x8A	*D9Mco72* (44,842,118)	*D9Mco93* (90,249,538)	45,407,420
S.SHR(9)x10A	*D9Uia6* (77,170,798)	*D9Mco93* (90,249,538)	13,078,740

Genotypes at all microsatellite markers are homozygous Dahl salt-sensitive. Markers 'from' and 'to' in each row span the current furthest limits of each congenic strain. Physical location of each end marker in base pairs (bp) is given in parentheses (RGSC 3.4, December 2004; www.ensembl.org, release 49, March 2008).

UPE suggested that there is only one QTL for UPE within the S.SHR(9)x8A congenic region, thus confirming the results previously published in a genome scan [25]. The peak score of the UPE LOD plot is 3.57 at *D9Uia6* (77,170,798). This score is above the significance threshold on the additive mode of inheritance (Fig. 1) and above the suggestive threshold on free and dominant modes of inheritance (data not shown).

Confirmation for the existence of multiple blood pressure quantitative trait loci

A new congenic substrain, S.SHR(9)x10A, was constructed to trap the region exclusively spanning the significant BP QTL peak detected by the linkage analysis of F_2[(S.SHR(9)x8A) × S]. The congenic interval of S.SHR(9)x10A is between *D9Uia6* and *D9Mco93* (Fig. 1 and Table 1). The BP of S.SHR(9)x10A rat was significantly lower than that of the S/jr rat by 19 (±6.21) mmHg ($P = 0.01$). Note that the limits of the S.SHR(9)x10A congenic interval are outside those of S.SHR(9)x4A. Therefore, it is clear that there are at least two BP QTLs with SHR alleles lowering BP of the S/jr rat in both QTLs. These two QTLs are QTL1 trapped within the distal portion of S.SHR(9)x4A and QTL2 trapped within S.SHR(9)x10A (Fig. 1).

Detection of disparate urinary protein excretion effects of BP QTL1 and BP QTL2

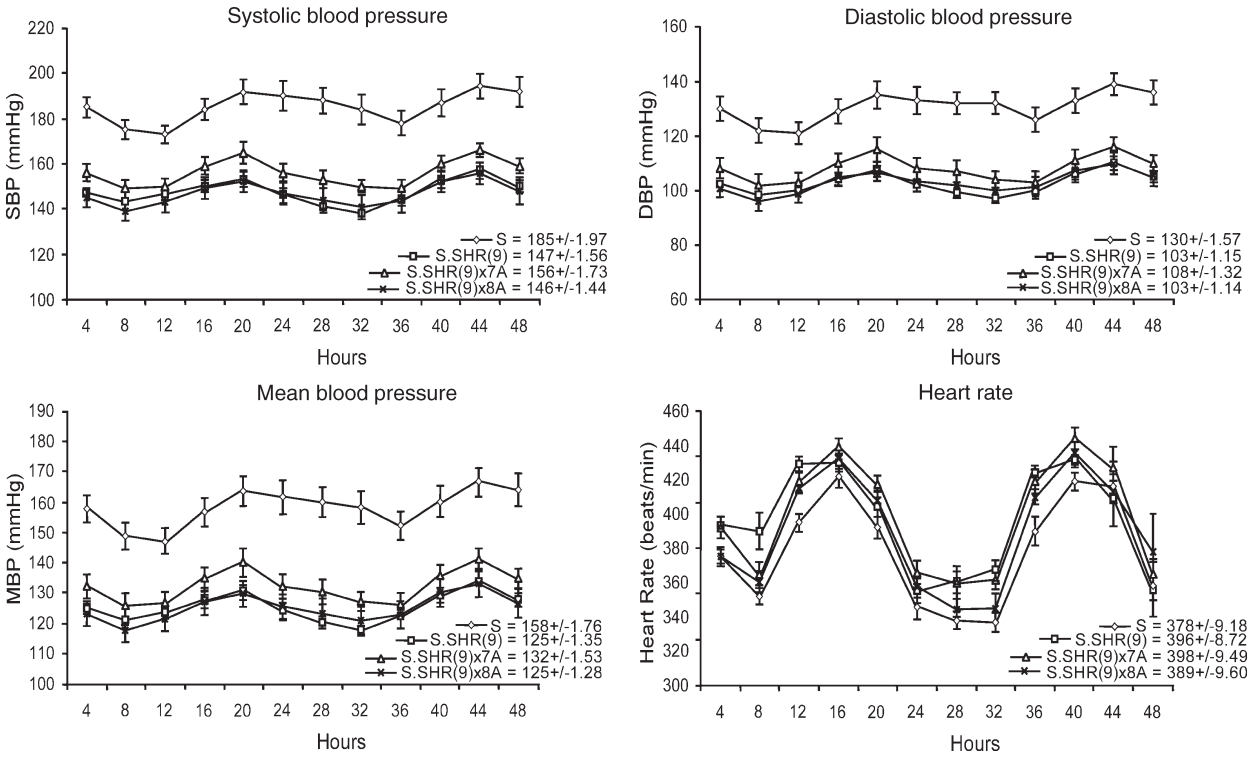
Because the introgressed segment of the congenic strain S.SHR(9)x8A aligns with both of these QTL regions, it should contain SHR alleles from both BP QTLs 1 and 2, in contrast to S.SHR(9)x10A, which only contains SHR alleles from QTL2. Although both congenic substrains S.SHR(9)x8A and S.SHR(9)x10A had lower BP compared with S/jr rats, only one of these two strains, S.SHR(9)x8A, also had a significant reduction of UPE.

Table 2 Phenotypic effects of RNO9 congenic strains

Congenic strain	Blood pressure (mmHg)				Body weight (g)				Heart weight (g)				Relative heart weight			
	Dahl salt-sensitive	Congenic	Effect	*P*	Dahl salt-sensitive	Congenic	Effect	*P*	Dahl salt-sensitive	Congenic	Effect	*P*	Dahl salt-sensitive	Congenic	Effect	*P*
S.SHR(9)	207 [4.10]	180 [2.07]	−27 [4.64]	<0.001	316 [3.93]	311 [3.45]	−5 [5.23]	0.331	1.31 [0.017]	1.17 [0.018]	−0.14 [0.025]	<0.001	4.17 [0.063]	3.77 [0.054]	−.40 [0.083]	<0.001
S.SHR(9)x2B	213 [4.85]	209 [3.78]	−4 [6.14]	0.486	311 [1.63]	301 [5.65]	−10 [5.88]	0.113	1.30 [0.012]	1.23 [0.016]	−0.07 [0.020]	0.001	4.19 [0.044]	4.11 [0.071]	−0.08 [0.084]	0.348
S.SHR(9)x3A	213 [4.63]	202 [4.95]	−11 [6.78]	0.209	339 [3.00]	328 [7.67]	−11 [8.23]	0.386	1.48 [0.067]*	1.35 [0.021]	−0.13 [0.060]	0.140	4.32 [0.240]*	4.15 [0.081]	−0.17 [0.228]	0.132
S.SHR(9)x3B	213 [4.63]	204 [3.88]	−9 [6.03]	0.393	339 [3.00]	366 [3.96]	+27 [4.97]	0.001	1.48 [0.067]*	1.50 [0.021]	+0.02 [0.058]	0.989	4.32 [0.240]*	4.10 [0.076]	−0.22 [0.216]	0.222
S.SHR(9)x4A	205 [4.91]**	180 [3.71]†	−25 [6.13]	0.001	318 [2.09]**	295 [3.95]†	−23 [3.95]	0.041	1.33 [0.023]**	1.13 [0.016]†	−0.20 [0.028]	<0.001	4.18 [0.067]**	3.82 [0.055]†	−.36 [0.088]	0.001
S.SHR(9)x7A	203 [4.11]	175 [3.22]	−28 [5.22]	<0.001	343 [5.20]	321 [6.99]	−22 [8.21]	0.069	1.39 [0.033]‡	1.11 [0.033]◇	−0.28 [0.044]	<0.001	4.04 [0.080]‡	3.47 [0.057]◇	−.57 [0.094]	<0.001
S.SHR(9)x8A	203 [4.11]	166 [2.57]	−37 [4.84]	<0.001	343 [5.20]	329 [5.81]	−14 [7.83]	0.320	1.39 [0.033]‡	1.14 [0.030]‡	−0.25 [0.042]	<0.001	4.04 [0.080]‡	3.48 [0.042]‡	−.56 [0.089]	<0.001
S.SHR(9)x10A	214 [5.61]	195 [4.32]	−19 [6.21]	0.010	316 [2.83]	314 [2.76]	−2 [4.26]	0.852	1.40 [0.026]	1.29 [0.011]	−0.11 [0.033]	0.003	4.44 [0.092]	4.11 [0.044]	−.33 [0.118]	0.019

The number of rats in each group was 20 except **$n = 16$, †$n = 14$, ◇$n = 5$, and *$n = 3$. S.SHR(9)x3A and S.SHR(9)x3B rats were concomitantly tested, as were S.SHR(9)x7A and S.SHR(9)x8A rats. The effect is defined as difference between the congenic value and the Dahl salt-sensitive value. Standard error of each value is in brackets. Relative heart weight is defined as heart weight divided by body weight times 1000. Effects were considered significant if *P* value is less than or equal to 0.05.

Fig. 2



Blood pressure measured by telemetry. Graphs are presented for (a) systolic blood pressure, (b) diastolic blood pressure, (c) mean blood pressure and (d) heart rate. The data points represent a moving average. The 4 h time point is the average of hours 1–4, the 8 h time point is the average of hours 5–8 and so on. Data presented on the graphs are the average of each time point over the 48-h period of measurement. The average blood pressures and heart rates for each 24-h period did not differ within strains.

S/jr and S.SHR(9)x10A rats excreted 91.29 (±6.05) and 90.99 (±6.70) mg/24 h, respectively. S.SHR(9)x8A excreted 61.64 (±4.56) mg/24 h ($P = 0.002$ vs. S/jr rat). This observation further supports the existence of two different BP QTLs, that is, QTL1 with an associated effect on UPE and QTL2 with a BP effect independent of UPE.

Discussion

The work reported here has accomplished the following five objectives: (1) The existence of a salt-sensitive BP QTL on RNO9 was corroborated by congenic strain analysis. (2) The location of the BP QTL was further mapped within a shorter introgressed segment of a congenic substrain S.SHR(9)x8A. (3) This BP effect was associated with at least two distinct BP QTLs, each with SHR alleles lowering BP. (4) The two BP QTLs had disparate effects on renal function. (5) BP QTL2 was isolated within the shorter limits of a new congenic strain, S.SHR(9)x10A, which now serves as a tool for further fine mapping.

Inbred rat strains are utilized to study the genetic determinants of hypertension with the end goal of identifying genes with a role in human disease [4,7,10]. The genomic composition of each hypertensive strain can be viewed as a distinct pool of alleles, the net effect facilitating susceptibility to the development of high BP. This does not mean that all alleles of a hypertensive strain confer susceptibility to hypertension. Hypertensive strains also contain alleles that confer resistance to the development of high BP. For example, using congenic strains, the S/jr rat was found to contain genetic elements that account for a lowering BP effect [19,26]. The effect of these 'protective' alleles is overcome by the effect of the susceptibility alleles, thereby resulting in the phenotype of hypertension. The existence of such protective alleles in both S/jr and SHR rats is well documented by independent laboratories [19,26,27]. These studies also provide proof for the existence of differential causative mechanisms for the observed high BP in each of these hypertensive strains. Our previous linkage studies identified such BP QTLs that differentiate the hypertensive S/jr rats from two other hypertensive rat strains, SHR [14] and Albino Surgery [28]. Similarly, Llamas *et al.* [29] also detected BP QTLs by linkage analysis using parental strains without contrasting BP. All of these studies are linkage analyses that require additional corroboration by substitution mapping. Wendt *et al.* [30] and Schulz *et al.* [31] have recently reported two such validatory studies of

QTLs using consomic strains. Congenic strains were also produced between SHR and stroke-prone SHRsp, but no significant difference in BP was found [32]. The current work is the first to use congenic strains to validate a BP QTL detected using two hypertensive rat strains. The S.SHR(9) congenic strain not only allowed for corroboration of the BP QTL but also served as a genetic tool for further substitution mapping. Contrary to the expectation of finding evidence for a single BP QTL, there were two distinct closely linked BP QTLs detected. Considering that the regions are still very large, encompassing several megabases, this is not surprising. Several studies of QTL mapping for BP suggest clustering of QTLs underlying LOD peaks detected by linkage analysis [19,33–36].

In mapping BP QTLs, it is of additional interest to determine whether there are also linked phenotypes potentially related to the change in BP. If these linked phenotypic differences occur before BP changes are detected, such phenotypes could be prioritized for further analysis as causative of the BP effect. Heart weights of the congenic strains with BP-lowering effects are, as expected, consistently lower than that of the S/jr rat. These heart weight changes corroborated the changes observed in BP. For several of the mapping projects in our laboratory, heart weight has similarly demonstrated concordance with the BP effect [11,19,20,21]. In contrast to the heart weight effects, the effects of RNO9 BP QTL1 and QTL2 on UPE constitute an interesting and important first step towards the assessment of linked phenotypes. Based on the data presented here, the cause–consequence association of UPE with BP is relatively more applicable for QTL1 than for QTL2. It should be noted, however, that based on the magnitude of change in BP effect being larger between S/jr vs. S.SHR(9)x8A (−37 mmHg) compared with that between S/jr vs. S.SHR(9)x10A (−19 mmHg), the expected proteinuria effect would be similarly greater for the S/jr vs. S.SHR(9)x8A than for that between S/jr vs. S.SHR(9)x10A. Therefore, an alternate interpretation for the lack of proteinuria effect between S/jr and S.SHR(9)x10A could be that it is not detectable under the given set of experimental conditions wherein a BP effect is indeed detectable.

Besides our S/jr vs. SHR comparison, which is an extension of the linkage analysis from our laboratory [14], four other linkage studies identified BP QTLs on RNO9 (Fig. 1), underlying the importance of genetic elements on RNO9 in BP control. Of these, our study corroborates the QTL interval reported by Siegel *et al.* [16] better than the interval reported by Garrett *et al.* [14], both of which were conducted on F_2(SxSHR) rats administered a high-salt diet. Although the reason for this discrepancy is not known, it is clear that linkage studies almost always require additional corroboration. However, the regions of interest described in the present work overlap those of two other strain comparisons, the S/jr vs. R/jr rats [37] and the SHR vs. WKY [38]. However, corroboration for the linkage studies is only available from the S/jr vs. R/jr rats comparison [11]. The fine-mapped location of the S/jr vs. R/jr rats comparison is within the QTL1 region of the current report (Fig. 1). It remains to be determined whether the R/jr and SHR alleles that contrast the BP-increasing effect of S/jr alleles within these overlapping QTL segments are the same or not. Besides these studies on low-salt-fed or high-salt-fed animals, significant linkage of pharmacologically induced BP changes on RNO9 has also been reported using a female F_2(SxBN) population [39]. In addition, linkage analysis in a large Kyrgyz family has identified a BP QTL on human chromosome 2, part of which is homologous to RNO9 [40]. All of these data together, along with a significant amount of work from our laboratory in the form of S.SHR(9) and S.R(9) congenic strain analysis [11,18,37], makes BP QTLs on RNO9 interesting targets for further fine mapping.

Dietary salt as a factor influencing the BP and UPE QTLs between S/jr and SHR was assessed by previous linkage studies, which indicated that the BP QTL effect of SHR alleles on RNO9 was predominantly observed when the animals were administered a high-salt diet [14,16] but not they were administered a low-salt diet [25]. Therefore, all of the substitution mapping studies reported here were conducted on animals administered a high (2% NaCl) salt diet. However, the linkage analysis shown in Fig. 1 was conducted on rats maintained on a low-NaCl diet. Interestingly, similar to the previous report [25], this QTL analysis on low salt is in conformity with the effect of UPE observed largely proximal to the marker *D9Uia6* and the BP effect independent of the UPE effect located distal to *D9Uia6*.

This project is at a point where candidate gene analysis would be premature. BP QTL1, which spans 22.52 Mb from *D9Mco76* to *D9Mco85*, contains 211 genes (www.ensembl.org). BP QTL2, which measures 13.08 Mb between *D9Uia6* and *D9Mco93*, contains 120 genes (www.ensembl.org). Experience from our laboratory points to the likely scenario of multiple genetic elements, some with opposing action, underlying the observed BP effect in other rat congenic segments that are 1.5 Mb or less [19]. At this point, there is no clear reason for prioritization of any of the genes. Novel genes implicated in the control of BP can be missed if one were to approach the current efforts of mapping with bias for similar detection of previously unknown genes implicated in BP control. To our knowledge, there are only three examples of mapping by congenic strains leading closest to the strict definition of positional cloning [41,42,43]. Similar to these studies, further refinements through the substitution mapping approach are clearly warranted for the current study to progress toward the final goal of

positional cloning the underlying genetic determinants of BP.

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