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Congenic mapping of a blood pressure QTL region on rat chromosome 10 using the Dahl salt-sensitive rat with introgressed alleles from the Milan normotensive strain

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Abstract Multiple blood pressure (BP) quantitative trait loci (QTLs) are reported on rat chromosome 10 (RNO10). Of these, QTLs detected by contrasting the genome of the hypertensive Dahl salt-sensitive (S) rat with two different relatively normotensive strains, Lewis (LEW) and the Milan normotensive strain (MNS), are reported. Because the deduced QTL regions of both S vs. LEW and S vs. MNS comparisons are within large genomic segments encompassing more than 2 cM, there was a need to further localize these QTLs and determine whether the QTLs are unique to specific strain comparisons. Previously, the S.MNS QTL1 was mapped to less than 2.6 cM as a differential segment between two congenic strains. In this study, multiple congenic strains spanning the projected interval were studied. The BP effect of each strain was interpreted as the net effect of alleles introgressed within that congenic strain. The results suggest that the MNS alleles within the previously proposed differential segment (*D10Rat27-D10Rat24*) do not independently lower BP of the S rat. However, another congenic strain, S.MNS(10) × 9, containing introgressed MNS alleles that are outside of the previously proposed differential segment is of interest because (1) it demonstrated a BP-lowering effect, (2) it is contained within a single congenic strain and is not based on the observed effect of a differential segment, and, more importantly, (3) it overlaps with the previously identified S.LEW BP QTL region. Identification of the same QTL affecting BP in multiple rat strains will provide further support for the QTL's involvement and importance in human essential hypertension.

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Introduction

Despite the knowledge that genetic factors are causally involved in the etiology of essential hypertension, establishing the identities of such genetic factors in humans has been a difficult task, thus prompting genetic analysis in model organisms (Cowley 2006). Because of the advantage of the ability to generate and test consomic and congenic strains, genetic analysis using several rat models of hypertension has enabled precise localizations of blood pressure (BP) quantitative trait loci (QTLs) on the rat genome (Deng 2007, Joe and Garrett 2006, Rapp 2000). Rat chromosome 10 (RNO10) harbors multiple BP QTLs that are corroborated by the construction and characterization of congenic strains (Saad et al. 2007a, 2007b). Our laboratory has contributed to this research by comparing the genome of the hypertensive Dahl salt-sensitive (S) rat with that of two relatively normotensive strains, Lewis (LEW) and the Milan normotensive strain (MNS) (Dukhanina et al. 1997, Garrett et al. 2001, Saad et al. 2007a). The fine mapping of BP QTLs identified by the S and LEW comparison is presented elsewhere (Saad et al. 2007a, 2007b). There are two QTLs, QTL1 and QTL2, identified using the S vs. MNS comparison (Garrett et al. 2001). Of the two QTLs, QTL1 had previously been mapped to a shorter interval of less than 2.6 cM on the genetic linkage map of RNO10 between the microsatellite markers *D10Rat27* and *D10Rat24*. This localization was inferred based on the differential segment between two congenic strains, only one of which demonstrated a BP-lowering

effect compared to the S rat (Garrett et al. 2001). The goal of the present study was to (1) confirm the presence of a BP QTL within the limits of *D10Rat27* and *D10Rat24*, as previously claimed, and (2) examine the region between *D10Mco1* and *D10Rat27* for the presence of another BP QTL, a region that has not been previously investigated for independent effects on BP.

Materials and methods

Strains

All rats used in the present study were bred in-house and used as per approved IACUC protocols. The inbred Dahl salt-sensitive (SS/Jr) rat and the Milan normotensive strain are designated in this report as S and MNS, respectively. The congenic substrains used in this study, namely, S.MNS(10) × 8 and S.MNS(10) × 9, were derived from the congenic strain S.MNS(10b), previously developed by our group (Garrett et al. 2001). The congenic substrains S.MNS(10) × 12 × 1A, S.MNS(10) × 12 × 2E, S.MNS(10) × 12 × 2B, S.MNS(10) × 12 × 2C, and S.MNS(10) × 12 × 2F were derived from the congenic strain S.MNS × 12 (Garrett et al. 2001), referred to here as S.MNS(10) × 12. S.MNS(10) × 5 and S.MNS(10) × 13 are the same strains as the previously reported S.MNS × 5 and S.MNS × 13, respectively (Garrett et al. 2001). All congenic strains/substrains in the current study had the MNS chromosomal region of interest introgressed onto the genetic background of the S rat strain. To obtain these congenic substrains, the S.MNS(10b) and S.MNS(10) × 12 were crossed to the S strain to yield F_1 rats that were heterozygous for the original introgressed chromosomal segment. F_1 rats were intercrossed to obtain F_2 rats that were genotyped for markers in the congenic segment to look for recombinants. Rats with appropriate recombinant chromosomes were crossed to S again to duplicate the recombinant chromosome. The offspring were genotyped to select rats retaining the desired recombinant region. Two rats with the same recombinant chromosomal segment were crossed and the litters were genotyped to obtain rats that were homozygous throughout the recombinant region of interest. The homozygous rats were crossed to fix the recombinant chromosomal segment in a new congenic substrain.

Markers

The markers with *D10Mco* as the prefix were developed at the University of Toledo Health Science Campus (formerly Medical College/University of Ohio) using rat genomic DNA sequence obtained from the Ensembl website (http://www.ensembl.org). These can be accessed from our website (http://www.hsc.utoledo.edu/depts/physiology/research/rat/marker.html). The *D10Rat* marker sequences were obtained from the Whitehead Institute/MIT Center for Genome Research (http://www.broad.mit.edu/rat/public), Rat Genomic Mapping Project. The *D10Got* marker sequences were obtained from the Wellcome Trust Center for Human Genetics (http://www.well.ox.ac.uk/rat_mapping_resources). The *D10Uia* marker sequences were obtained from the University of Iowa and the primer sequences are available at the Rat Genome Database (http://www.rgd.mcw.edu/).

Genotyping

DNA was extracted from a tail biopsy using the QIAamp Tissue Kit (Qiagen, Valencia, CA). Polymerase chain reaction (PCR) genotyping with microsatellite markers was done as per established procedures (Saad et al. 2007a).

Tail-cuff BP measurement

The BP of a congenic strain was compared to its own separate group of control S rats that were bred, housed, and studied concomitantly. Rats were weaned at 30 days of age to a low-salt (0.3% NaCl) Harlan Teklad diet 7034 (Madison, WI). Twenty male congenic substrain rats were matched by age and weight with 20 male S control rats. Rats were housed in 20 cages with each cage containing one congenic substrain rat and its matched S control rat. At 40–42 days old, the rats were fed a 2% NaCl Harlan Teklad diet, TD94217, for 24 days. Systolic blood pressure (SBP) was measured by two blinded operators using the tail-cuff method on conscious restrained rats warmed to 28°C. SBP was measured on each rat once a day for four consecutive days. The SBP value of each day was the average of three to four consistent readings. The final SBP value used was the averaged SBP value of the four days. Rats were killed with CO_2 and body and heart weights were measured.

BP measurements by telemetry

For the S.MNS(10) × 8 congenic substrain, BP was also collected using a telemetry system (Data Sciences International, MN, USA). Briefly, after the tail-cuff measurements were completed, six S and six congenic rats were randomly selected from the whole group. At 70–73 days of age a transmitter was surgically implanted into the left flank of each rat and the probe was inserted through the femoral artery and advanced to the lower abdominal

aorta. The rats continued on the 2% NaCl diet for the duration of the experiment. The animals were allowed to recover for 1 week before BP data were collected for a period of 4 days. SBP, diastolic blood pressures (DBP), mean arterial pressures (MAP), pulse pressures (PP), and heart rates (HR) were collected when the rats were between 80 and 84 days of age. All readings were made at 5-min intervals throughout the 4-day period of data collection.

Data collection and statistical analyses

For all the strains tested, we collected BP (mmHg), HW (g), and body weight (g). Relative HW was calculated as the ratio of HW to body weight multiplied by a factor of 1000. Statistical analysis was done using SPSS software (SPSS Inc., Chicago, IL, USA). All means were compared using the independent-samples *t* test.

Results

Tracking the RNO10 BP S.MNS QTL1 region

As previously described and illustrated here (Fig. 1), the BP-lowering effect demonstrated by the congenic strain S.MNS(10) × 5 was denoted as S.MNS QTL1, which was subsequently further localized using additional congenic strains to be within the genomic segment flanked by the microsatellite markers *D10Rat27* (77,091,892 bp) and *D10Rat24* (79,229,068 bp) (Garrett et al. 2001). At that time, these markers represented the differential segment between S.MNS(10) × 12, which had the BP-lowering effect, and S.MNS(10) × 13, which did not have a BP-lowering effect (Garrett et al. 2001). Based on the same reasoning, but with additional genotyping of the crossover segments of the top ends of S.MNS(10) × 12 and S.MNS(10) × 13, BP QTL1 was further localized to the 459-kb region between *D10Rat193* (77,219,627 bp) and *D10Rat112* (77,679,198 bp) (Fig. 1). A new iteration of congenic substrains, shown with the prefix S.MNS(10) × 12 in Fig. 1, was developed with the goal of "trapping" the QTL1 region within a congenic substrain whose introgressed segment included the newly refined QTL1 region. All five congenic substrains spanned the newly refined QTL1 region because they were all derived from a single strain that was previously shown to exhibit a lowering effect on BP. Surprisingly, none of the five congenic substrains had a significant BP-lowering effect compared with the S rat (Fig. 1, Table 1). Note that the BP of the S rats is highly variable, and therefore every experiment had to be performed with its own group of S rats. Therefore, data presented are analyzed based on comparisons between the congenic strains that were concomitantly tested along with a control group of S rats. Furthermore, the relative heart weights of the five congenic substrains corroborated the observed changes in BP (Table 1). Also, the fact that three separate yet apparently similar minimal strains [S.MNS(10) × 12 × 2B, S.MNS(10) × 12 × 2C, and S.MNS(10) × 12 × 2F] containing the newly refined QTL1 region lacked the BP-lowering effect supports the hypothesis that this region either lacks a BP QTL or at least harbors a gene that is dependent on the presence of another region, potentially the region spanning from *D10Got112* to *D10Wox23*. There is also the possibility that the point of crossover found in the region between *D10Rat193* and *D10Mco30* of S.MNS(10) × 12 is different from the crossover points of all five congenic substrains that were derived from S.MNS(10) × 12 and the BP QTL happens to be located within this differential segment. This scenario is unlikely because of the low probability that all five separate strains derived from the same parental strain have a crossover end that is separate from that of the parental strain, especially when one takes into consideration the relatively short (327 kb) crossover segment.

Evidence for the presence of another BP QTL contained within a single congenic strain located upstream of BP QTL1

S.MNS(10) × 5 had a large introgressed segment upstream of the proposed QTL1 region. S.MNS(10) × 8 is a congenic strain that has introgressed MNS alleles within the proposed QTL1 region (*D10Rat193-D10Rat112*, 459,571 bp) and further upstream of it (*D10Mco84-D10Rat193*, 6,462,372 bp) (Fig. 1). The BP of S.MNS(10) × 8 was significantly lower than that of S as measured by both tail-cuff (Fig. 1, Table 1) and telemetry (Fig. 2). S.MNS(10) × 9 is a congenic strain with an introgressed region from *D10Rat84* to *D10Got98* (6,196,208 bp), which is exclusive of the previously proposed differential congenic segment. The BP of S.MNS(10) × 9 was significantly lower than that of the S rat (Fig. 1, Table 1). Based on these findings, the region spanning from *D10Mco84* to *D10Got98* has been prioritized for the presence of an important BP QTL and future refinement to identify the BP QTL in the S vs. MNS comparison.

Discussion

Contrary to our expectations of further refining the previously determined location of the S.MNS QTL1, the results obtained in this study indicate that the BP effect of QTL1

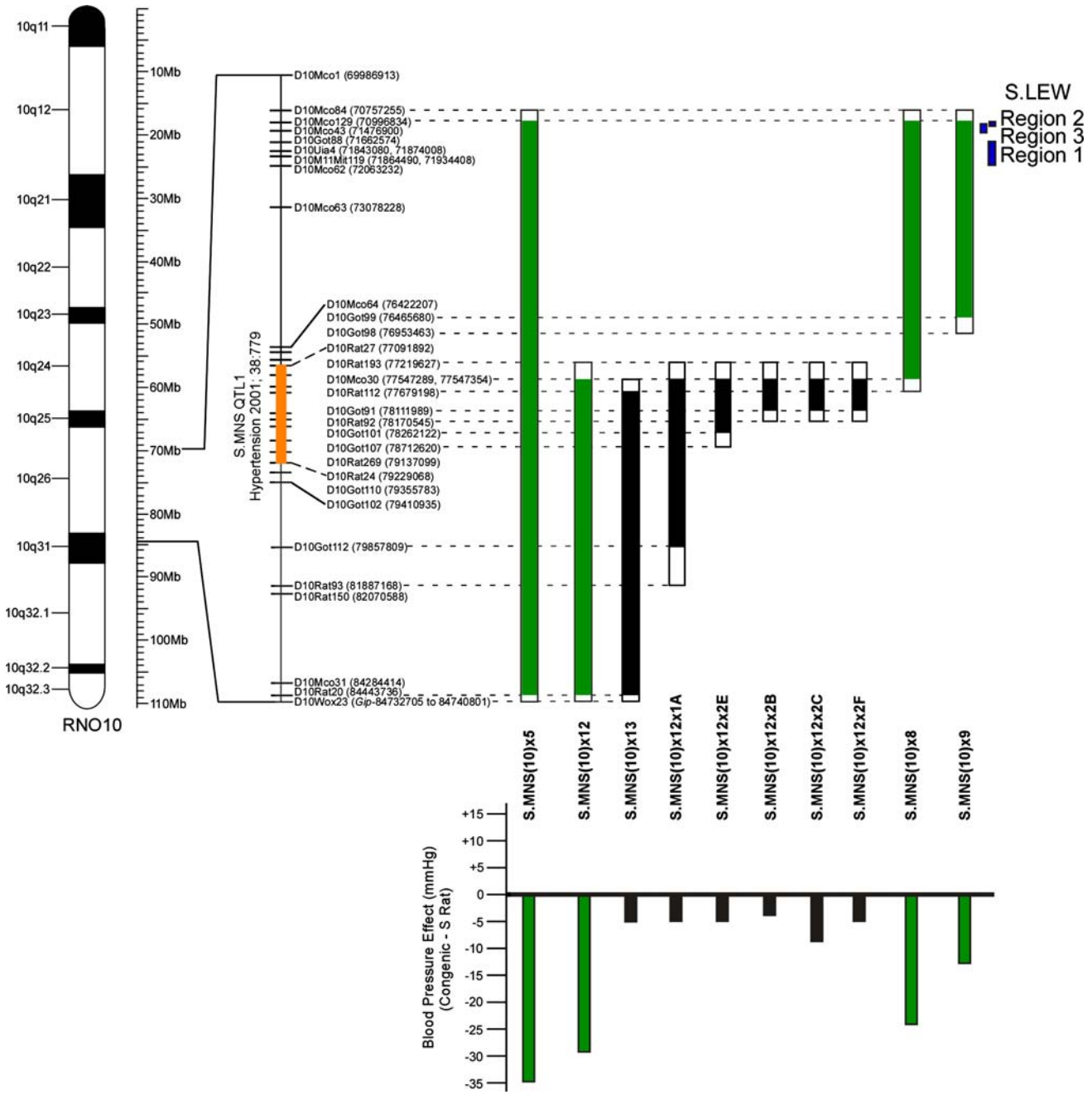

Fig. 1 Congenic substrains and their BP effect compared with the S rat. The relevant section of the physical map of RNO10 is shown to the left of the figure. Values in parenthesis next to the marker names indicate their physical locations in base pairs. The previously mapped S.MNS BP QTL1 region is shown as the orange bar. Congenic strains are shown as solid colored bars flanked by open bars. Solid color bars illustrate the MNS segment introgressed onto the background of S. The open bars at the end of each introgressed segment represent the region of recombination. Green bars represent the MNS introgressed segment in congenic substrains with a BP effect, whereas the black bars represent the MNS introgressed segment in congenic substrains without a BP effect. The congenic strains S.MNS(10) × 5, S.MNS(10) × 12, and S.MNS(10) × 13 were previously published as S.MNS × 5, S.MNS × 12, and S.MNS × 13 (Garrett et al. 2001), respectively, and are presented here for illustrative purposes. The lower portion of the figure illustrates the BP effect observed for each congenic substrain compared with that of the S rat BP (effect = congenic substrain BP - S rat BP): Green bars represent a significant ($p < 0.05$) BP effect; black bars represent a BP effect with a $p > 0.05$. At the top right of the figure are the regions shown in blue that we are currently tracking in the S.LEW comparison (Saad et al. 2007b)

could not be "trapped" within any congenic strain that spans the previously projected QTL1 interval. From the point of view of fine mapping this QTL, this is not a desirable result. However, this important observation provided the opportunity to critically reexamine the data and construct an alternate hypothesis for the initially observed

Table 1 Observed effects of rat chromosome 10 congenic strains on blood pressure, body weight, heart weight, and relative heart weight

	Blood pressure (mmHg)				Body weight (g)			
Congenic strain	S	Congenic	Effect*	*t* test	S	Congenic	Effect*	*t* test
S.MNS(10) × 8	204 [2.84]	180 [2.86]	−24 (4.03)	<0.0001	340 [5.26]	345 [7.09]	+5 (8.83)	0.614
S.MNS(10) × 9	209 [3.51]	197 [3.04]	−12 (4.65)	0.015	308 [1.28]	304 [2.10]	−4 (2.46)	0.133
S.MNS(10) × 12 × 1A	195 [3.35]	190 [2.55]	−5 (4.21)	0.200	312 [3.22]	299 [2.78]	−13 (4.26)	0.005
S.MNS(10) × 12 × 2E	195 [3.35]	190 [2.40]	−5 (4.12)	0.199	312 [3.22]	297 [1.47]	−15 (3.55)	<0.0001
S.MNS(10) × 12 × 2B	210 [4.60]	206 [5.06]	−4 (6.84)	0.529	306 [3.09]	306 [2.31]	0 (3.86)	0.959
S.MNS(10) × 12 × 2C	210 [4.60]	201 [2.71]	−9 (5.34)	0.104	306 [3.09]	296 [3.60]	−10 (4.75)	0.033
S.MNS(10) × 12 × 2F	198 [3.96]	193 [3.50]	−5 (5.29)	0.281	307 [2.02]	300 [3.15]	−7 (3.75)	0.074
	Heart weight (g)				Relative heart weight			
Congenic strain	S	Congenic	Effect*	*t* test	S	Congenic	Effect*	*t* test
S.MNS(10) × 8	1.45 [0.04]	1.37 [0.03]	−0.08 (0.05)	0.106	4.29 [0.10]	3.97 [0.04]	−0.32 (0.11)	0.014
S.MNS(10) × 9	1.31 [0.01]	1.24 [0.01]	−0.07 (0.01)	<0.0001	4.28 [0.03]	4.09 [0.03]	−0.19 (0.05)	0.001
S.MNS(10) × 12 × 1A	1.18 [0.01]	1.17 [0.01]	−0.01 (0.01)	0.781	3.83 [0.04]	3.94 [0.02]	+0.11 (0.05)	0.043
S.MNS(10) × 12 × 2E	1.18 [0.01]	1.16 [0.01]	−0.02 (0.01)	0.292	3.83 [0.04]	3.86 [0.05]	+0.03 (0.06)	0.689
S.MNS(10) × 12 × 2B	1.25 [0.01]	1.21 [0.02]	−0.04 (0.02)	0.263	4.08 [0.05]	3.98 [0.05]	−0.10 (0.08)	0.211
S.MNS(10) × 12 × 2C	1.25 [0.01]	1.18 [0.01]	−0.07 (0.02)	0.016	4.08 [0.05]	4.02 [0.04]	−0.06 (0.07)	0.371
S.MNS(10) × 12 × 2F	1.17 [0.01]	1.13 [0.01]	−0.04 (0.01)	0.023	3.83 [0.04]	3.77 [0.02]	−0.06 (0.05)	0.285

*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 was 20. 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

QTL effect. The data obtained collectively seem to suggest that the MNS BP-lowering QTL alleles within the *D10Rat193-D10Rat112* region may require the presence of other MNS alleles within the region spanning from *D10Got112* to *D10Wox23*. The lack of either the *D10Rat193-D10Rat112* region, as in the S.MNS(10) × 13 strain, or the *D10Got112-D10Wox23* region, as in all the other strains derived from S.MNS(10) × 12, abolishes the BP-lowering effect. The genetic dissection at this stage is insufficient to examine this possibility. Therefore, the presence of a BP QTL that lowers the BP of the S rat within the previously proposed QTL segment cannot be entirely ruled out, but its ability to lower BP independently appears to be unlikely because of the lack of a BP effect in the minimal strains containing the region spanning *D10Rat193-D10Rat92*. Mapping of this potentially complex allelic interdependence is of interest to us. A similar scenario was previously described in the genetic dissection of BP QTLs on RNO10, using the congenic strain S.LEW × 12 × 2 × 3 × 4 (Saad et al. 2007b), and on RNO5 (Garrett and Rapp 2002).

We are also tracking another BP QTL in a region that was previously trapped within the S.MNS(10) × 5 strain but was never previously examined in a separate congenic substrain. Data obtained from S.MNS(10) × 9, which is entirely outside of the previously proposed BP QTL1, suggests the presence of novel and previously undetected MNS alleles that lower BP. The location of these alleles can be defined by the limits of the congenic substrain S.MNS(10) × 9 (*D10Mco84-D10Got98*), which contains 106 genes (http://www.ncbi.nlm.nih.gov) and 182 S.MNS single nucleotide polymorphisms (http://www.ensembl.org). Tracking this BP QTL is preferred because it is contained within a congenic strain and is not defined by the difference between two or more congenic strains or intervals.

The introgressed region of S.MNS(10) × 9 is an interesting region to further fine map because of our findings that multiple QTLs with opposing BP effects are detectable within shorter segments of less than 1 Mb within this region by comparing the S and LEW genomes (Fig. 1) (Saad et al. 2007a, 2007b). Multiple-strain comparisons with the S genome as a common denominator allow for detection of QTLs that may or may not be identical at the resolution of single quantitative trait nucleotides. Further fine mapping of the S.MNS QTL region that overlaps with our S.LEW BP QTL region is expected to facilitate this level of resolution. At the current stage of mapping, which is still within a large genomic segment of 6,196,208 bp, it is not yet practically feasible for candidate gene sequence analysis. This conclusion is based partly on our recent experience with the fine mapping of the overlapping S.LEW BP QTL. At the stage when the S.LEW BP QTL

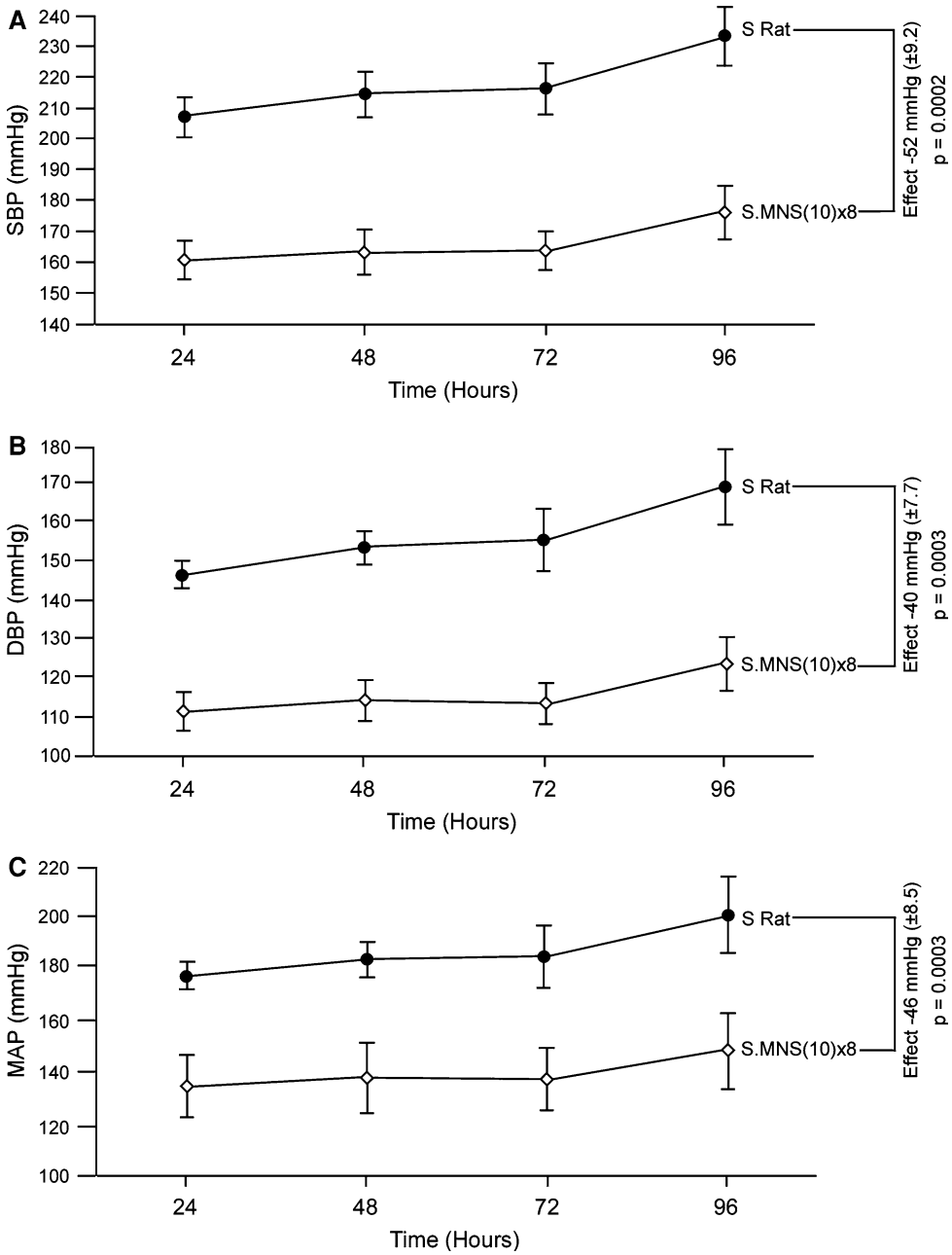

Fig. 2 Corroboration of tail-cuff measurements by telemetry. Twenty-four-hour averages of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) values for S rats vs. S.MNS(10) × 8 congenic rats. Error bars represent the standard error of the mean. Standard error of the mean difference is given in parenthesis. The effect is determined as the overall S rat SBP, DBP, or MAP value subtracted from the overall congenic substrain SBP, DBP, or MAP value (effect = congenic – S rat)

was within 1.17 Mb, we comprehensively sequenced all potential transcripts and identified three nonsynonymous variants (Saad et al. 2007a). However, our recent observations suggest that none of these three variants are within the further fine-mapped QTL interval, suggesting that variants outside of the coding regions of genes are potential candidates for the S.LEW BP QTL (Saad et al. 2007b).

The BP QTLs of our study are also within the regions identified as BP QTLs by using S.LEW congenic strains (Charron et al. 2005) and SHRSP.WKY congenic strains (Kreutz et al. 1995b; Monti et al. 2003; Saad et al. 2007a). Apart from these substitution mapping studies, there are ten other linkage analyses that have detected BP QTLs on RNO10. Of these, varying degrees of overlaps are noticeable with the QTLs described in our study (Saad et al. 2007a). A linkage analysis between MNS, which is the relatively normotensive strain used in our study, and the Milan hypertensive strain (MHS) has also located a BP QTL that broadly overlaps with all the QTLs reported in our study (Zagato et al. 2000). It will be of interest to discern whether the hypertension causative alleles of the S rat and the MHS, within this region of RNO10, are similar.

Finally, based on the results obtained in this study, a technical note on the methodology of mapping QTLs using congenic strains is worth mentioning. As part of substitution mapping, it has become standard practice to designate

differential congenic segments of two congenic strains (one with the QTL effect and the other without) as the proposed QTL interval. The results of our study wherein one such proposed differential congenic segment designated as the QTL region (QTL1) could not be confirmed with a congenic strain that spans the QTL region serves as a typical example that reiterates the need to corroborate QTL localizations determined as differential segments of two congenic strains, by constructing and phenotyping congenic strains that actually represent the differential segment.

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