Published in final edited form as:
Hypertension. 2013 April ; 61(4): 850–856. doi:10.1161/HYPERTENSIONAHA.111.01008.

Congenic mapping and sequence analysis of the *Renin* locus

Michael J. Flister[1,2,*], **Matthew J. Hoffman**[1,2,*], **Prajwal Reddy**[2], **Howard J. Jacob**[1,2,3], and **Carol Moreno**[1,2]

[1]Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, Wisconsin

[2]Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin

[3]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin

Abstract

Renin was the first blood pressure (BP) quantitative trait locus (QTL) mapped by linkage analysis in the rat. Subsequent BP linkage and congenic studies capturing different portions of the renin region have returned conflicting results, suggesting that multiple interdependent BP loci may be residing in the chromosome 13 BP QTL that includes *Renin*. We used SS-13^{BN} congenic strains to map 2 BP loci in the *Renin* region (chr13:45.2–49.0 Mb). We identified a 1.1 Mb protective Brown Norway (BN) region around *Renin* (chr13:46.1–47.2 Mb) that significantly decreased BP by 32 mmHg. The *Renin* protective BP locus was offset by an adjacent hypertensive locus (chr13:47.2–49.0 Mb) that significantly increased BP by 29 mmHg. Sequence analysis of the protective and hypertensive BP loci revealed 1,433 and 2,063 variants between Dahl salt-sensitive/Mcwi (SS) and BN rats, respectively. To further reduce the list of candidate variants, we re-genotyped an overlapping SS-13^{SR} congenic strain (S/ren$_{rr}$) with a previously reported BP phenotype. Sequence comparison between SS, Dahl R (SR), and BN reduced the number of candidate variants in the 2 BP loci by 42% for further study. Combined with previous studies, these data suggest that at least 4 BP loci reside within the 30 cM chromosome 13 BP QTL that includes *Renin*.

Keywords

hypertension; genetic; congenic; Dahl salt-sensitive rat; Brown Norway

Introduction

One strategy for mapping blood pressure (BP) variants is by linkage analysis using inbred rat strains.[1] Although useful, linkage analysis is limited to localizing a large (10–30 cM) quantitative trait locus (QTL) that is associated with BP, but cannot further reduce the list of potential causative variant(s) within the QTL.[2] Causative variant(s) can be further isolated by fixing portions of the QTL from one parental strain (donor) on the background of the other (recipient) to form a congenic strain.[2] Combined, these strategies have shown that many BP QTL actually contain multiple interdependent loci that can have additive, subtractive, or epistatic effects on BP.[3] Thus, capturing different portions of a QTL that

Corresponding Author: Carol Moreno Quinn, MD PhD, Department of Physiology, 8701 Watertown Plank Rd., Milwaukee, WI 53226, Ph: (414) 955-4475, Fax: (414) 955-6546, cmorenoquinn@gmail.com.
*M.J. Flister and M.J. Hoffman contributed equally to the manuscript.

Conflict(s) of Interest/Disclosure(s)
None

contains multiple BP loci can often lead to unexpected results that are difficult to interpret. Such has been the case for the QTL containing the BP mediator *Renin*.

In 1989, a region of rat chromosome 13 including the *Renin* gene was the first BP QTL to be mapped by linkage analysis.[4] Since then, these results were replicated[5] and confirmed in multiple other hypertensive strains.[6–10] Although the hypertensive Dahl salt-sensitive (SS) *Renin* allele co-segregated with elevated BP by linkage,[6–10] the same has not been true for congenic strains that captured portions of the *Renin* QTL. For example, BP was unexpectedly elevated by a congenic interval containing the normotensive Dahl R (SR) *Renin* allele on the SS background,[11] whereas a reverse congenic (SS *Renin* on an SR background) surprisingly decreased BP.[12] Another SS-13^{SR} congenic strain, showed that the SR *Renin* allele only reduced BP when downstream regions of the SR genome were present,[5] suggesting that other variant(s) downstream of *Renin* likely contribute to BP. Notably, each of these congenic strains also carried substantial amounts of undefined congenic interval due to the limited availability of genotype markers at the time.[5, 11, 12] As such, the blood pressure regulatory elements within the relatively large region of chromosome 13 that includes the *Renin* locus have remained largely unresolved.

In this study, we performed detailed mapping of a region of chromosome 13 including the *Renin* locus (chr13:45.2–49.0 Mb) using SS-13BN congenic strains.[13–15] This revealed a protective region (chr13:46.1–47.2 Mb) promoting lower blood pressure (referred to as line9$_{BP1}$) that is offset by an adjacent region promoting higher blood pressure (chr13:47.2–49.0 Mb; referred to as line9$_{BP2}$), both derived from the same strain. We also reanalyzed archived DNA from the hypertensive S/ren$_{rr}$ congenic strain (SR *Renin* on a SS background) used in a 1996 study published in *Hypertension* by Jiang et al.[11] This reduced by 85% the undefined congenic interval of S/ren$_{rr}$, enabling us to compare overlapping sequence between SS, SR, and Brown Norway (BN) in our congenic region. By sequence comparison (SS, SR, and BN) we identified significantly enriched regions of common BN and SR alleles that overlapped with the 2 BP loci and are likely to be functionally relevant. We also identified regions of BN and SR-specific alleles that could account for subtle differences in BP phenotypes between SS-13^{BN} and SS-13^{SR} congenic strains. Combined with 2 loci from previous studies,[16, 17] these data demonstrate that at least 4 total BP loci reside in the SS *Renin* QTL.

Materials and Methods

Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. Genomic boundaries for congenic lines 9, 9A, 9B, and 9C were described previously,[13] but have been further refined here (Figure 1).

Blood Pressure Measurement

Mean arterial pressure (MAP) was measured by telemetry transmitter implantation with a catheter inserted into the abdominal aorta, as described previously.[16]

Measurement of Albumin Excretion

On day 16 of 8% NaCl diet, rats were acclimated in metabolic cages for 24 hours, followed by a 24-hour urine collection. Albuminuria was measured as described previously.[18]

Genomic Sequencing

Genomic sequence was accessed from the RGD database (http://rgd.mcw.edu/). Full details of library preparation, sequencing, and analysis are described elsewhere.[18]

Statistical analysis

Statistical analyses were performed using Sigma Plot 11.0 software. Data are presented as mean ± SEM. All data were analyzed by one-way ANOVA followed by the Holm-Sidak post-hoc test.

Results

Blood Pressure

To narrow the regions around *Renin* that modify BP, a series of SS-13^{BN} congenic strains were generated and phenotyped for BP salt-sensitivity as depicted in Figure 1. After 21 days of 8% NaCl diet, only the MAP of 1ine 9A (147±11 mmHg, $P<0.05$, n=13) was significantly less than parental SS (179±6 mmHg; n=10) and lines 9 (176±6 mmHg; n=10), 9B (172±4 mmHg; n=24), 9C (169±6 mmHg; n=21), and 9D (190±4 mmHg; n=10; Figure 1). Because the protective line 9A is genetically identical to line 9 except from 47.2–49.0 Mb, these data suggest that hypertensive BN allele(s) reside within this 1.8 Mb region. This was also supported by the further elevation of MAP in line 9D compared with SS (190±4 vs. 179±6 mmHg; Figure 1); however, this did not reach statistical significance. Since the 1.8 Mb hypertensive locus (47.2–49.0 Mb) offset BP in lines 9 and 9C to similar levels (Figure 1), it is most likely that BN protective allele(s) reside in the 1.1 Mb region that contains *Renin* (46.1–47.2 Mb) and decrease MAP in line 9A.

Renal Damage

To assess renal damage, albumin excretion was quantified from urine that was collected from SS and SS-13^{BN} congenic rats after 17 days of 8% NaCl diet. In agreement with MAP data, only line 9A rats (117±9 mg; $P<0.05$) had reduced albumin excretion compared with parental SS (231±30 mg) and lines 9 (243±30 mg), 9B (242±23 mg), 9C (242±25 mg), 9D (320±29 mg; Figure 1). Also, similar to MAP, albumin excretion in line 9D trended higher than SS (320±29 vs 231±30 mg; Figure 1). Collectively, these data suggest that renal damage is secondary to BP in lines 9, 9A, 9B, 9C, and 9D and that the line 9 congenic interval (chr13:45.2–49.0 Mb) does not harbor any additional loci for susceptibility to end-organ damage.

Sequence Analysis

Our data support that although a region of BN chromosome 13 including the *Renin* locus (46.1–47.2 Mb) is protective, a BN hypertensive locus (47.2–49.0 Mb) resides in close proximity (Figure 1). Similarly, Jiang et al found evidence that a SR-derived hypertensive locus resides in an 8.7 Mb congenic interval surrounding *Renin*.[11] They reported that the SR *Renin* region increased BP (Δ+30 mmHg; $P<0.05$) in the SS-13^{SR} congenic (referred to as S/ren$_{rr}$) compared with SS (Figure 2A).[11] Also similar to line 9[13, 14], the S/ren$_{rr}$ congenic restores plasma renin activity (PRA),[19] angiogenesis,[19] and vasoreactivity,[20] indicating that line 9 and S/ren$_{rr}$ share some functional allele(s), but not all. Most notably, the protective region around *Renin* (46.1–47.2 Mb) did not offset the hypertensive BP allele(s) in S/ren$_{rr}$,[11] as was detected in line 9 (Figure 2). This suggests that the BN protective BP allele(s) in line 9 are not shared by SR in S/ren$_{rr}$, whereas the hypertensive allele(s) are likely shared by BN and SR.

In a previous study, only a restriction fragment length polymorphism (RLFP) in *Renin*^{A} and two flanking SSLP markers were available for genotyping S/ren$_{rr}$,[11] leaving the entire 8.7 Mb region around *Renin* undefined as SS or SR (Figure 2A). We obtained archived DNA samples from the original congenic S/ren$_{rr}$ strain published in 1996 by Jiang et al[11] and narrowed the confidence intervals by >85% through re-genotyping (Figure 2B). This increased the defined SR congenic interval to 1.9 Mb (chr13:46,109,499–48,026,949 bp),

which overlapped with the line 9 congenic interval, including the entire protective region (chr13:46.1–47.2 Mb) and 88% of the adjacent hypertensive region (chr13:47.2–49.0 Mb).

We compared the SS/Jr (used by Jiang et al[11]), the SS/JrMcwi (used for our SS-13^{BN} congenics[13]), the SR, and the BN genomic sequences across the entire 3.7 Mb line 9 congenic interval to identify common and unique variants among the strains (Supplementary Table 2). Across the line 9 interval, only 2 variants (<0.0001%) were polymorphic between SS/Jr and SS/JrMcwi, and therefore, we hereafter use SS to denote both strains. In the SS, we identified 3,691 variants that differed from BN and 2,368 variants that differed from SR (Supplementary Table S2). Combined, SS had 1,003 variants that differed from both SR and BN (Supplementary Table S2).

To identify co-segregating and unique regions of the SR and BN genomes that could influence BP in the SS, we plotted the total unique and common variants per 100 kb in the SR and BN versus SS (Figure 3). Compared with the average of the entire line 9 congenic interval, two regions (46.1–46.3 and 47.6–48.4 Mb) were significantly enriched for co-segregating SR and BN alleles by 57±11 and 61±11-fold, respectively (P<0.05; Figure 3), indicating that the mechanisms for some common phenotypes are likely shared in these regions. In contrast, a large portion (46.4–47.2 Mb) of line9$_{BP1}$ was not shared by BN and SR alleles (Figure 3). Given that the protective region (46.1–47.2 Mb) does not offset the hypertensive region (47.2–49.0 Mb) in S/ren$_{rr}$ (Figure 2), these data suggest that the protective allele(s) are likely BN-specific.

Blood Pressure QTL Analysis

The following is a description of the protective region (line9$_{BP1}$) and the adjacent hypertensive region (line9$_{BP2}$):

Line9$_{BP1}$—The protective line9$_{BP1}$ region (chr13:46.1–47.2 Mb) contains 17 validated genes and 6 predicted genes (Supplementary Table S1). Compared with BN, the line9$_{BP1}$ SS sequence contains 1,433 variants, of which 16 lie in coding regions, 6 are nonsynonymous, and 4 are predicted to be damaging (Table 1). Compared with SR, the line9$_{BP1}$ SS sequence has 251 variants, of which 7 are reside in coding regions, 2 are nonsynonymous and are predicted to be damaging (Table 1). Of the BN variants, 1,180 were specific to only BN, of which 16 were coding, 6 are nonsynonymous, and 4 are predicted to be damaging (Table 1). These BN-specific variants likely include the BP lowering allele(s) that were unique to the line 9 congenic interval and not observed in S/ren$_{rr}$ by Jiang et al[11] (Figure 2). Combined, the SS had 267 variants that differed from both SR and BN, of which 1 nonsynonymous variant was predicted to be damaging. These common variants likely include shared SR and BN allele(s) that regulate renin expression,[13, 19] angiogenesis,[13, 19] and vasoreactivity[14, 20] that were restored in both line 9 and S/ren$_{rr}$.

Line9$_{BP2}$—The hypertensive line9$_{BP2}$ region (chr13:47,198,168–48,990,782 bp) contains 30 validated genes and 9 predicted genes (Supplementary Table S1). Compared with BN, the line9$_{BP2}$ SS sequence contains 2,063 variants, of which 46 lie in coding regions, 14 are nonsynonymous, and 2 are predicted to be damaging (Table 1). Compared with SR, the line9$_{BP2}$ SS sequence contains 2,362 variants, of which 64 lie in coding regions, 12 are nonsynonymous, and 3 are predicted to be damaging. Combined, SS had 987 variants that differed from both SR and BN, with 18 variants in coding regions, 8 nonsynonymous variants, of which 3 are predicted to be damaging (Table 1). Of note, 7 out of 8 nonsynonymous variants that were shared by BN and SR also resided in *Ptprv* and were located in the 47.6–48.4 region that was significantly enriched for common SR and BN

alleles. Of these shared *Ptprv* variants, 1 variant was predicted to be damaging to Ptprv function (Figure 3 and Table 1).

Discussion

A RNO13 QTL containing *Renin* has long been associated with BP in the rat[4–12] but the genetic and molecular mechanism(s) underlying this relationship are largely unknown. Here we showed for the first time that a BN hypertensive region (chr13:47.2–49.0 Mb) resides in close proximity to the protective BN region around *Renin* (chr13:46.1–47.2 Mb; Figure 1). We refined a previously identified hypertensive SS-13^{SR} congenic strain, S/ren$_{rr}$,[11] with an SR congenic interval similar to line 9 (Figure 2). The elevated BP in line 9 and S/ren$_{rr}$ suggested that causative allele(s) are likely to be shared by SR and BN within the region (47.6–48.4 Mb) that was significantly enriched for common SR and BN polymorphisms (Figure 3). By combining congenic mapping with strain sequence comparison, we were able to preliminarily reduce by 42% the number of candidate variants in this region. Finally, these data offer a more complete picture of multiple interdependent BP loci (Figure 4) that reside within the *Renin* QTL, which was first described nearly 25 years ago.[4]

Is *Renin* a BP Candidate Gene?

Renin has historically been considered a BP candidate gene, because of the physiological role of the renin-angiotensin system (RAS) in BP regulation and its strong association with BP in multiple models of genetic hypertension.[4–12] By congenic exclusion, our data suggest that the BN region around *Renin* (46.1–47.2 Mb) is responsible for the decreased BP observed in the line 9A congenic (Figure 1). At present, it still remains unclear whether *Renin* is a causative BP gene in RNO13 QTL in the SS rat. We confirmed that coding regions of the SS, SR, and BN *Renin* alleles did not differ (Supplementary Table 1). However, differences in renin expression in SS, SR, and BN have been previously reported[13, 21] and as such, allele-specific changes in *Renin* expression/activity remain a potential mechanism. The SS rat is a model of low renin hypertension[21] that responds poorly to RAS inhibition;[22–26] leading some to argue that *Renin* is unlikely a candidate BP gene.[2] However, a recent *Renin* knockout rat generated by our group on the SS background significantly lowered BP and renal function,[27] suggesting that even at relatively low expression levels in the SS rat, *Renin* is necessary for maintaining BP and renal homeostasis. Therefore, it remains plausible that a differentially expressed *Renin* allele would cosegregate with BP in the SS rat.

Genetic elements linked to the BN and SR *Renin* alleles restore normal RAS function in the line 9[13, 19] and S/ren$_{rr}$ congenic strains, indicating that BN and SR variant(s) regulate Renin expression differently than SS. In line 9, the BN *Renin* allele restored normal renin expression,[13] renin-dependent angiogenesis,[13] and renin-dependent vasoreactivity.[14] Likewise, normal PRA,[19] angiogenesis,[19] and vasoreactivity[20] were restored in the S/ren$_{rr}$ congenic rat. Introgression of the SS *Renin* allele into the SR background in a SR-13^{SS} *Renin* congenic strain significantly decreased PRA and kidney renin expression.[12] Thus, based solely on renin expression and renin-dependent physiological mechanisms (e.g., vascular density and peripheral resistance), *Renin* should remain a candidate gene for genetic hypertension. Of note however, the BN and SR *Renin* alleles in line 9 and S/ren$_{rr}$ did not lower BP (Figure 2), as would be expected to coincide with restored PRA and decreased peripheral resistance.[14, 20] Thus, in addition to *Renin*, it is likely that another gene could contribute to BP differences in the line 9 region, possibly through complex interactions with *Renin*. This is further supported by evidence that the BP protective region around *Renin* region (chr13:46.1–47.2) did not offset BP driven by the hypertensive allele(s) in line9$_{BP2}$ in S/ren$_{rr}$,[11] as was observed in line 9 (Figure 2). This indicates that despite the evidence that shared BN and SR allele(s) restore renin expression,[13, 19] angiogenesis,[13, 19] and

vasoreactivity[14, 20] in line 9 and S/ren$_{rr}$, there likely exist other renin-independent BP allele(s) that are not shared by the SR in the line9$_{BP1}$ region.

Renin is Part of a Compound BP QTL

Renin has long been suspected to reside in a compound BP QTL, with multiple normotensive and hypertensive alleles that can be derived from a single strain.[2] Our data indicate that at least 2 loci around *Renin* modulate BP (Figure 1). In addition to the BN protective *Renin* region (46.1–47.2 Mb), we also identified a BN hypertensive region (47.2–49.0 Mb), which in lines 9 and 9C masked the protective allele(s) that were observed in line 9A (Figure 1). Similar to line 9, the S/ren$_{rr}$ congenic unexpectedly failed to lower BP compared with SS.[11] The SR had a region (47.9–48.1 Mb) that was significantly enriched for common BN and SR alleles (Figure 3), suggesting that this overlapping BN and SR region are potentially functionally relevant.

The BP-associated *Renin* allele that was first identified by Rapp and colleagues in 1989,[4] turned out to be part of a much larger BP QTL (chr13:35–111 Mb)[5] that encompasses multiple BP loci on chromosome 13 in the SS[5] and other hypertensive strains (e.g., SHR[28] and LH[29]). Using overlapping SS-13^{BN} congenics, we have now identified at least 4 BP loci (published here and elsewhere[15–17]) that overlap with the *Renin* BP QTL identified by linkage.[5, 28, 29] Specifically, our data suggest the existence of BP loci at 38.2–40.4 Mb (line 5),[16] 46.1–47.2 Mb (line 9$_{BP1}$), 47.2–49.0 Mb (line 9$_{BP2}$), and 60.6–73.0 Mb (line 26).[17] Similar compound BP QTLs have also been reported for rat chromosomes 1, 2, 3, 5, 9, and 10,[3] indicating that the complex genetic architecture within a QTL has a much greater impact on BP than originally anticipated.

For purposes of illustration, we have highlighted the published chromosome 13 BP QTL and congenic strains in Figure 4. Rapp's initial SSxSR crosses were followed-up by congenic SS-13^{SR} strains: S.R-Ren(a), S.R-Ren(b), and S.R-Ren.[5] Of these strains, only S.R-Ren (1–78 Mb) decreased BP (Δ-24 mmHg) compared with parental SS, whereas S.R-Ren(a) and S.R-Ren(b) did not.[5] Based on our congenic data, the S.R-Ren region includes 2 protective BP loci (line 9$_{BP1}$ and line 26) and 1 hypertensive locus (line 9$_{BP2}$) that collectively lower BP in the SS rat. A reverse congenic (congenic R) published by St. Lezin et al[12] contains the SS *Renin* allele, yet significantly lowered BP, possibly due to the presence of the SR region overlapping with the line 26 QTL. The S/ren$_{rr}$ congenic that increased BP (Δ+34 mmHg) compared with SS[4] includes only the hypertensive locus (line 9$_{BP2}$), resulting in significantly elevated BP compared with SS parental (Figure 2). Our smallest protective congenic, line 9A (45.2–47.3 Mb), includes only the protective *Renin* region (chr13:46.1–47.2 Mb). Combined, these data support the following: *(1)* BP QTL intervals identified by linkage are not always single entities, but rather the sums of multiple interdependent loci; *(2)* unexpectedly "losing" or "gaining" a BP phenotype by congenic mapping can be due in part to different combinations of interdependent loci, not necessarily the inclusion/exclusion of a causative variant; and *(3)* within a QTL, the +BP and – BP loci are not necessarily derived from the corresponding "hypertensive" or "normotensive" parental strain. In the case of the *Renin* QTL,[5] future studies using compound congenics or multiple gene knockout strains[30] will be needed to further validate these hypotheses.

Other Candidate Genes in Line 9$_{BP1}$ and Line 9$_{BP2}$ QTLs

In addition to *Renin*, several other potential candidate genes reside in the line 9$_{BP1}$ QTL. Within the line 9$_{BP1}$ QTL, we found 5 nonsynonymous amino acid changes between SS and BN or SR that were predicted to be damaging, of which 4 were specific to BN (Table 1). *Plekha6* and *Mybph* have no previously reported roles in BP or renal function. *Plekha6* is a poorly characterized member of the pleckstrin homology domain-containing family A.

Mybph is a myosin binding protein that also interacts with Rock1[31] and the sarcomeric myosin heavy chain.[32] *Chit1* is an interesting candidate because of its reported connection to RAS;[13] however, no direct role for *Chit1* in BP has been reported. Chit1 is a pro-inflammatory protein that is secreted by activated macrophages[33] and linked to multiple inflammatory diseases,[34] indicating that Chit1 might also contribute to chronic inflammation associated with hypertension and other CVD.

In the line 9_{BP2} QTL, we identified 26 nonsynonymous amino acid changes between SS and BN or SR, of which *Lgr6, Gpr37l1, Ptprv, Rnpep*, and *Ipo9* were predicted by PolyPhen2 to be damaging (Table 1). Of these, only *Rnpep* (also known as aminopeptidase B) has a known role in BP and renal function by modulating RAS through conversion of AngIII to AngIV.[35] In comparison, *Lgr6, Gpr37l1, Ipo9*, and *Ptprv* have no reported roles in BP or renal function. *Lgr6* and *Gpr37l1* (also known as Endothelin B receptor-like protein 2) are poorly characterized G-protein coupled receptors. *Ipo9* is a nuclear importin that functions as general transporter of nuclear cargo.[36] *Ptprv* is a p53-regulted transmembrane tyrosine phosphatase involved in cell-cycle regulation.[37] Despite lack of functional evidence, *Ptprv* makes an interesting candidate because it is highly enriched for common BN and SR nonsynonymous variants (7 out of 8), of which 1 was predicted to damage Ptprv function (Table 1).

Perspectives

The chromosome 13 BP QTL that harbors *Renin* was first discovered nearly 25 years ago by Rapp and colleagues[4] and has been independently replicated by multiple other groups.[6–10] Still, the genetic mechanism(s) underlying this QTL have remained controversial because of conflicting data from several congenic mapping studies.[4, 5, 12] Here we presented congenic mapping and sequence analysis that reconciles these conflicting reports and confirms that multiple interdependent loci influence the chromosome 13 BP QTL.[6–10] Moreover, by utilizing archived DNA samples from the previously published S/ren_{rr} strain,[11] we were able to reduce the list of BP candidates that could be shared by $SS\text{-}13^{SR}$ and $SS\text{-}13^{BN}$ congenic strains with similar phenotypes. Thus, our study not only provides novel insight to the regions around *Renin* that contribute to the chromosome 13 BP QTL, it also highlights the utility of archived experimental samples that can be reanalyzed upon need or on the advent of technological advances (e.g., NextGen sequencing). With regards to the $line9_{BP1}$ and $line9_{BP2}$ QTLs, further congenic mapping or testing of candidate gene function by targeted mutagenesis[30] will be needed to resolve the loci and elucidate the specific variant(s) that impact BP in this region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Lisa Groth, Roberta Rogge, Allison Zappa, Anne Temple, Nadia Barreto, Jaime Wendt-Andrae, and Michael Tschannen for their technical support.

Sources of Funding

M.J.F. is supported by a NHLBI training grant (5T32HL007792). This study was supported by NHLBI grant 5P01HL082798-07 to C.M.Q.

References

1. Cowley AW Jr. The genetic dissection of essential hypertension. Nat Rev Genet. 2006; 7:829–840. [PubMed: 17033627]
2. Rapp JP. Genetic analysis of inherited hypertension in the rat. Physiol Rev. 2000; 80:135–172. [PubMed: 10617767]
3. Rapp JP, Joe B. Use of contiguous congenic strains in analyzing compound qtls. Physiol Genomics. 2012; 44:117–120. [PubMed: 22108210]
4. Rapp JP, Wang SM, Dene H. A genetic polymorphism in the renin gene of dahl rats cosegregates with blood pressure. Science. 1989; 243:542–544. [PubMed: 2563177]
5. Zhang QY, Dene H, Deng AY, Garrett MR, Jacob HJ, Rapp JP. Interval mapping and congenic strains for a blood pressure qtl on rat chromosome 13. Mamm Genome. 1997; 8:636–641. [PubMed: 9271663]
6. Yu H, Harrap SB, Di Nicolantonio R. Cosegregation of spontaneously hypertensive rat renin gene with elevated blood pressure in an f2 generation. J Hypertens. 1998; 16:1141–1147. [PubMed: 9794718]
7. Sun L, McArdle S, Chun M, Wolff DW, Pettinger WA. Cosegregation of the renin gene with an increase in mean arterial blood pressure in the f2 rats of shr-wky cross. Clin Exp Hypertens. 1993; 15:797–805. [PubMed: 8104625]
8. Kurtz TW, Simonet L, Kabra PM, Wolfe S, Chan L, Hjelle BL. Cosegregation of the renin allele of the spontaneously hypertensive rat with an increase in blood pressure. J Clin Invest. 1990; 85:1328–1332. [PubMed: 1969424]
9. Pravenec M, Simonet L, Kren V, Kunes J, Levan G, Szpirer J, Szpirer C, Kurtz T. The rat renin gene: Assignment to chromosome 13 and linkage to the regulation of blood pressure. Genomics. 1991; 9:466–472. [PubMed: 1674497]
10. Dubay C, Vincent M, Samani NJ, Hilbert P, Kaiser MA, Beressi JP, Kotelevtsev Y, Beckmann JS, Soubrier F, Sassard J, et al. Genetic determinants of diastolic and pulse pressure map to different loci in lyon hypertensive rats. Nat Genet. 1993; 3:354–357. [PubMed: 7981757]
11. Jiang J, Stec DE, Drummond H, Simon JS, Koike G, Jacob HJ, Roman RJ. Transfer of a salt-resistant renin allele raises blood pressure in dahl salt-sensitive rats. Hypertension. 1997; 29:619–627. [PubMed: 9040448]
12. St Lezin EM, Pravenec M, Wong AL, Liu W, Wang N, Lu S, Jacob HJ, Roman RJ, Stec DE, Wang JM, Reid IA, Kurtz TW. Effects of renin gene transfer on blood pressure and renin gene expression in a congenic strain of dahl salt-resistant rats. J Clin Invest. 1996; 97:522–527. [PubMed: 8567976]
13. Stodola TJ, de Resende MM, Sarkis AB, Didier DN, Jacob HJ, Huebner N, Hummel O, Saar K, Moreno C, Greene AS. Characterization of the genomic structure and function of regions influencing renin and angiogenesis in the ss rat. Physiol Genomics. 2011; 43:808–817. [PubMed: 21521778]
14. Durand MJ, Moreno C, Greene AS, Lombard JH. Impaired relaxation of cerebral arteries in the absence of elevated salt intake in normotensive congenic rats carrying the dahl salt-sensitive renin gene. Am J Physiol Heart Circ Physiol. 2010; 299:H1865–H1874. [PubMed: 20852041]
15. Moreno C, Kaldunski ML, Wang T, Roman RJ, Greene AS, Lazar J, Jacob HJ, Cowley AW Jr. Multiple blood pressure loci on rat chromosome 13 attenuate development of hypertension in the dahl s hypertensive rat. Physiol Genomics. 2007; 31:228–235. [PubMed: 17566075]
16. Moreno C, Williams JM, Lu L, Liang M, Lazar J, Jacob HJ, Cowley AW Jr. Roman RJ. Narrowing a region on rat chromosome 13 that protects against hypertension in dahl ss-13bn congenic strains. Am J Physiol Heart Circ Physiol. 2011; 300:H1530–H1535. [PubMed: 21257920]
17. Lu L, Li P, Yang C, Kurth T, Misale M, Skelton M, Moreno C, Roman RJ, Greene AS, Jacob HJ, Lazar J, Liang M, Cowley AW Jr. Dynamic convergence and divergence of renal genomic and biological pathways in protection from dahl salt-sensitive hypertension. Physiol Genomics. 2010; 41:63–70. [PubMed: 20009007]

18. Flister MJ, Prisco SZ, Sarkis AB, O'Meara CC, Hoffman M, Wendt-Andrae J, Moreno C, Lazar J, Jacob HJ. Identification of hypertension susceptibility loci on rat chromosome 12. Hypertension. 2012; 60:942–948. [PubMed: 22868394]
19. Amaral SL, Roman RJ, Greene AS. Renin gene transfer restores angiogenesis and vascular endothelial growth factor expression in dahl s rats. Hypertension. 2001; 37:386–390. [PubMed: 11230305]
20. Drenjancevic-Peric I, Weinberg BD, Greene AS, Lombard JH. Restoration of cerebral vascular relaxation in renin congenic rats by introgression of the dahl r renin gene. Am J Hypertens. 2010; 23:243–248. [PubMed: 19959997]
21. Rapp JP, Tan SY, Margolius HS. Plasma mineralocorticoids, plasma renin, and urinary kallikrein in salt-sensitive and salt-resistant rats. Endocr Res Commun. 1978; 5:35–41. [PubMed: 710365]
22. de Simone G, Devereux RB, Camargo MJ, Wallerson DC, Sealey JE, Laragh JH. Reduction of development of left ventricular hypertrophy in salt-loaded dahl salt-sensitive rats by angiotensin ii receptor inhibition. Am J Hypertens. 1996; 9:216–222. [PubMed: 8695019]
23. Sugimoto K, Fujimura A, Takasaki I, Tokita Y, Iwamoto T, Takizawa T, Gotoh E, Shionoiri H, Ishii M. Effects of renin-angiotensin system blockade and dietary salt intake on left ventricular hypertrophy in dahl salt-sensitive rats. Hypertens Res. 1998; 21:163–168. [PubMed: 9786599]
24. Satoh S, Ueda Y, Suematsu N, Oyama J, Kadokami T, Sugano M, Yoshikawa Y, Makino N. Beneficial effects of angiotensin-converting enzyme inhibition on sarcoplasmic reticulum function in the failing heart of the dahl rat. Circ J. 2003; 67:705–711. [PubMed: 12890915]
25. Yamamoto K, Mano T, Yoshida J, Sakata Y, Nishikawa N, Nishio M, Ohtani T, Hori M, Miwa T, Masuyama T. Ace inhibitor and angiotensin ii type 1 receptor blocker differently regulate ventricular fibrosis in hypertensive diastolic heart failure. J Hypertens. 2005; 23:393–400. [PubMed: 15662228]
26. Kobayashi N, Honda T, Yoshida K, Nakano S, Ohno T, Tsubokou Y, Matsuoka H. Critical role of bradykinin-enos and oxidative stress-lox-1 pathway in cardiovascular remodeling under chronic angiotensin-converting enzyme inhibition. Atherosclerosis. 2006; 187:92–100. [PubMed: 16214149]
27. Moreno C, Hoffman M, Stodola TJ, Didier DN, Lazar J, Geurts AM, North PE, Jacob HJ, Greene AS. Creation and characterization of a renin knockout rat. Hypertension. 2011; 57:614–619. [PubMed: 21242461]
28. Samani NJ, Gauguier D, Vincent M, Kaiser MA, Bihoreau MT, Lodwick D, Wallis R, Parent V, Kimber P, Rattray F, Thompson JR, Sassard J, Lathrop M. Analysis of quantitative trait loci for blood pressure on rat chromosomes 2 and 13. Age-related differences in effect. Hypertension. 1996; 28:1118–1122. [PubMed: 8952608]
29. Bilusic M, Bataillard A, Tschannen MR, Gao L, Barreto NE, Vincent M, Wang T, Jacob HJ, Sassard J, Kwitek AE. Mapping the genetic determinants of hypertension, metabolic diseases, and related phenotypes in the lyon hypertensive rat. Hypertension. 2004; 44:695–701. [PubMed: 15452030]
30. Geurts AM, Cost GJ, Freyvert Y, Zeitler B, Miller JC, Choi VM, Jenkins SS, Wood A, Cui X, Meng X, Vincent A, Lam S, Michalkiewicz M, Schilling R, Foeckler J, Kalloway S, Weiler H, Menoret S, Anegon I, Davis GD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jacob HJ, Buelow R. Knockout rats via embryo microinjection of zinc-finger nucleases. Science. 2009; 325:433. [PubMed: 19628861]
31. Hosono Y, Yamaguchi T, Mizutani E, Yanagisawa K, Arima C, Tomida S, Shimada Y, Hiraoka M, Kato S, Yokoi K, Suzuki M, Takahashi T. Mybph, a transcriptional target of ttf-1, inhibits rock1, and reduces cell motility and metastasis. Embo J. 2012; 31:481–493. [PubMed: 22085929]
32. Welikson RE, Fischman DA. The c-terminal igi domains of myosin-binding proteins c and h (mybp-c and mybp-h) are both necessary and sufficient for the intracellular crosslinking of sarcomeric myosin in transfected non-muscle cells. J Cell Sci. 2002; 115:3517–3526. [PubMed: 12154082]
33. Di Rosa M, Malaguarnera G, De Gregorio C, Drago F, Malaguarnera L. Evaluation of chi3l-1 and chit-1 expression in differentiated and polarized macrophages. Inflammation. 2012 In Press.

34. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, He CH, Takyar S, Elias JA. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol. 2011; 73:479–501. [PubMed: 21054166]

35. Ardaillou R, Chansel D. Synthesis and effects of active fragments of angiotensin ii. Kidney Int. 1997; 52:1458–1468. [PubMed: 9407491]

36. Dopie J, Skarp KP, Rajakyla EK, Tanhuanpaa K, Vartiainen MK. Active maintenance of nuclear actin by importin 9 supports transcription. Proc Natl Acad Sci U S A. 2012; 109:E544–E552. [PubMed: 22323606]

37. Doumont G, Martoriati A, Marine JC. Ptprv is a key mediator of p53-induced cell cycle exit. Cell Cycle. 2005; 4:1703–1705. [PubMed: 16258284]

Novelty and Significance

1. What Is New?
   - We identified 2 novel BP loci: a protective region (chr13:46.1–47.2 Mb), which is offset by an adjacent hypertensive region (chr13:47.2–49.0 Mb). These data provide direct evidence that multiple interdependent loci mediate overall susceptibility to hypertension at the much larger *Renin* QTL that was originally described by Rapp and colleagues.
   - Our re-analysis of the S/ren$_{rr}$ congenic (SR *Renin* region on a SS background), provides a novel comparison of the common and unique SS, SR, and BN variants. Based on these data we were able to preliminarily reduce by 42% the number of potential candidate variants within the 2 BP loci and prioritize these variants for further study.
2. 2) What Is Relevant?
   - *Renin* has long been associated with genetic hypertension, but the mechanism(s) underlying this relationship are largely unknown.
   - Here we presented data, in the context of +20 years of literature, which provide a more complete picture of at least 4 BP loci that reside within the *Renin* QTL, offering novel insights to the genetic elements that mediate susceptibility to hypertension.
3. 3) Summary
   - The region surrounding *Renin* (chr13:45.2–49.0 Mb) contains at least 2 counteracting loci that mediate overall susceptibility to hypertension. Combined with previous studies, these data suggest that a total of 4 BP loci reside within the 30 cM *Renin* QTL.

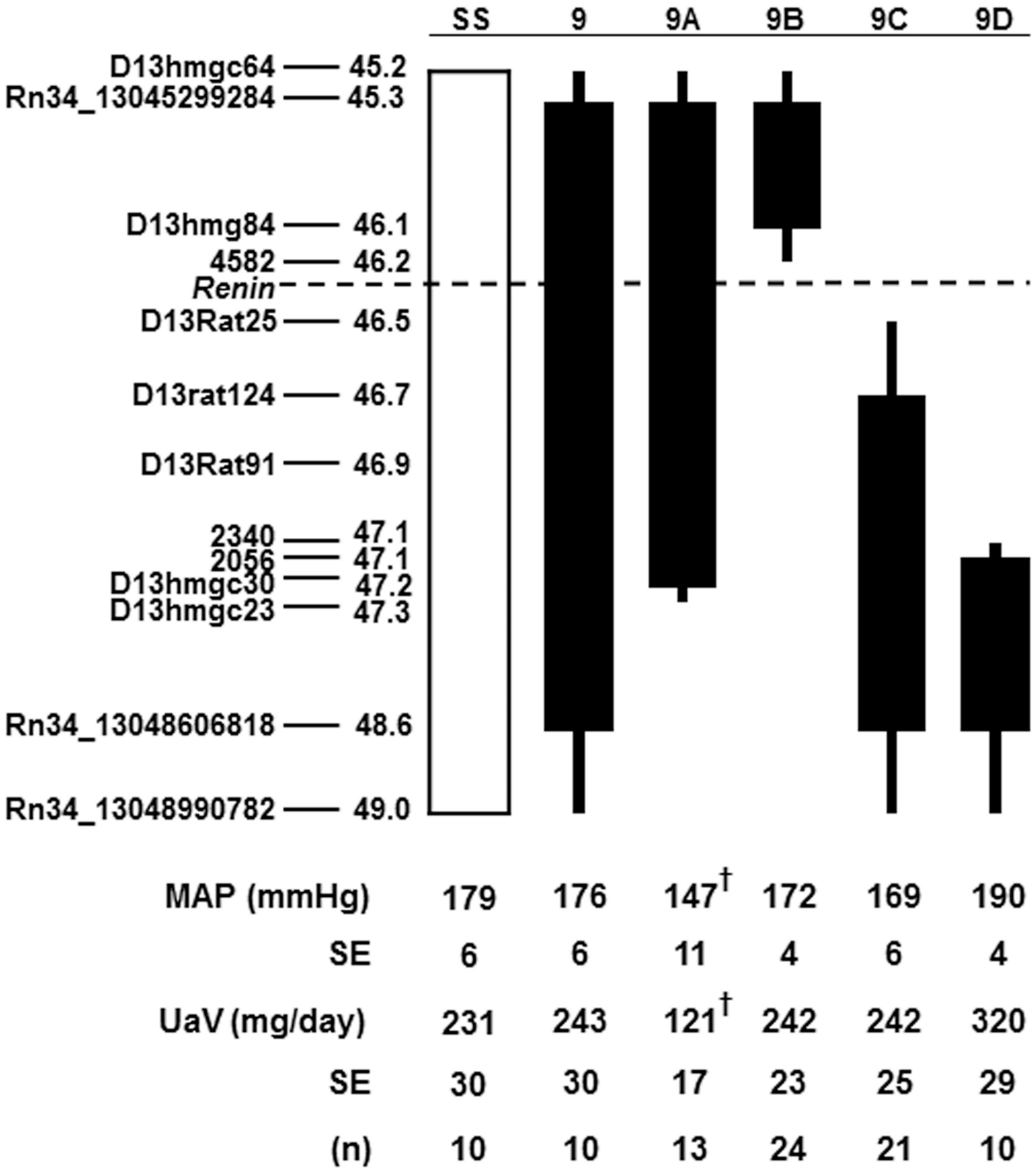

Figure 1.

Schematic representation of the SS-13^{BN} congenic strains that were generated by introgressing segments of BN chromosome 13 (black) into the genetic background of the parental SS strain (white) by marker assisted breeding. Thin black bars represent confidence intervals, which are chromosomal regions that could be BN or SS. The dashed line shows the location of *Renin* (chr13:46,262,936–46,275,213 bp). *Bottom*: Mean arterial pressure (MAP) and urinary albumin excretion (UaV) of parental SS and SS-13^{BN} congenics on 8% NaCl diet for 21 days. Values are means ± SEM from 10 to 24 animals per group. †P<0.001 vs SS, as determined by a 1-way ANOVA followed by a Holm-Sidak post hoc test.

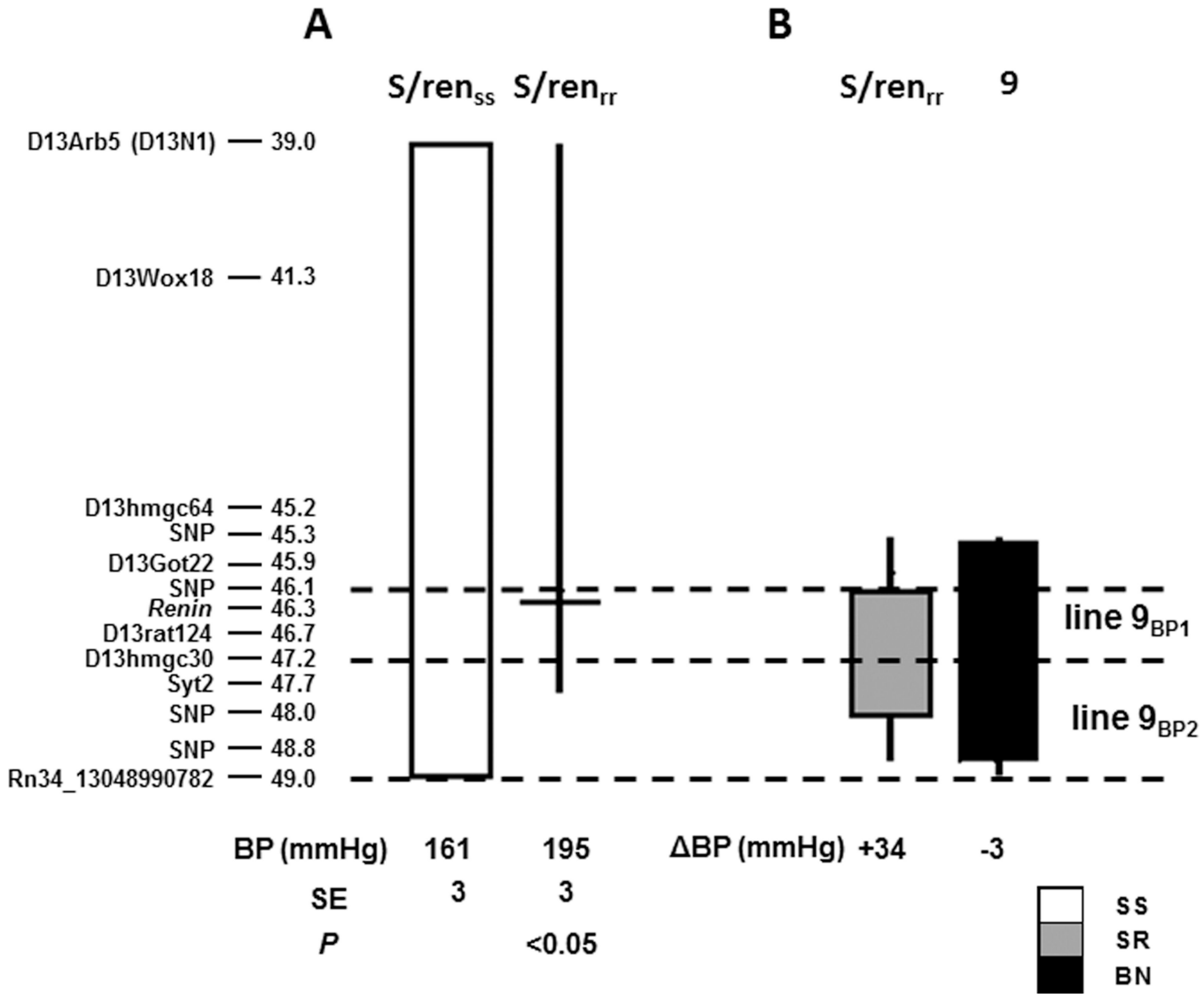

Figure 2.

Sequence analysis and comparison of the S/ren$_{rr}$ and line 9 congenic intervals. (A) As reported by Jiang et al[11], a congenic interval, marked by an RFLP in the SR *Renin* and flanked by D13Arb5 (D131N1) and Syt2, was associated with a 34 mmHg increase in BP compared with SS. (B) Archived DNA from the S/ren$_{rr}$ congenic strain was re-genotyped to better annotate the congenic interval. Genomic DNA is depicted by white (SS), grey (SR), and black (BN) bars. Thin black bars represent chromosomal regions that are unknown. Boundaries of line 9 BP QTLs are identified by the dashed lines.

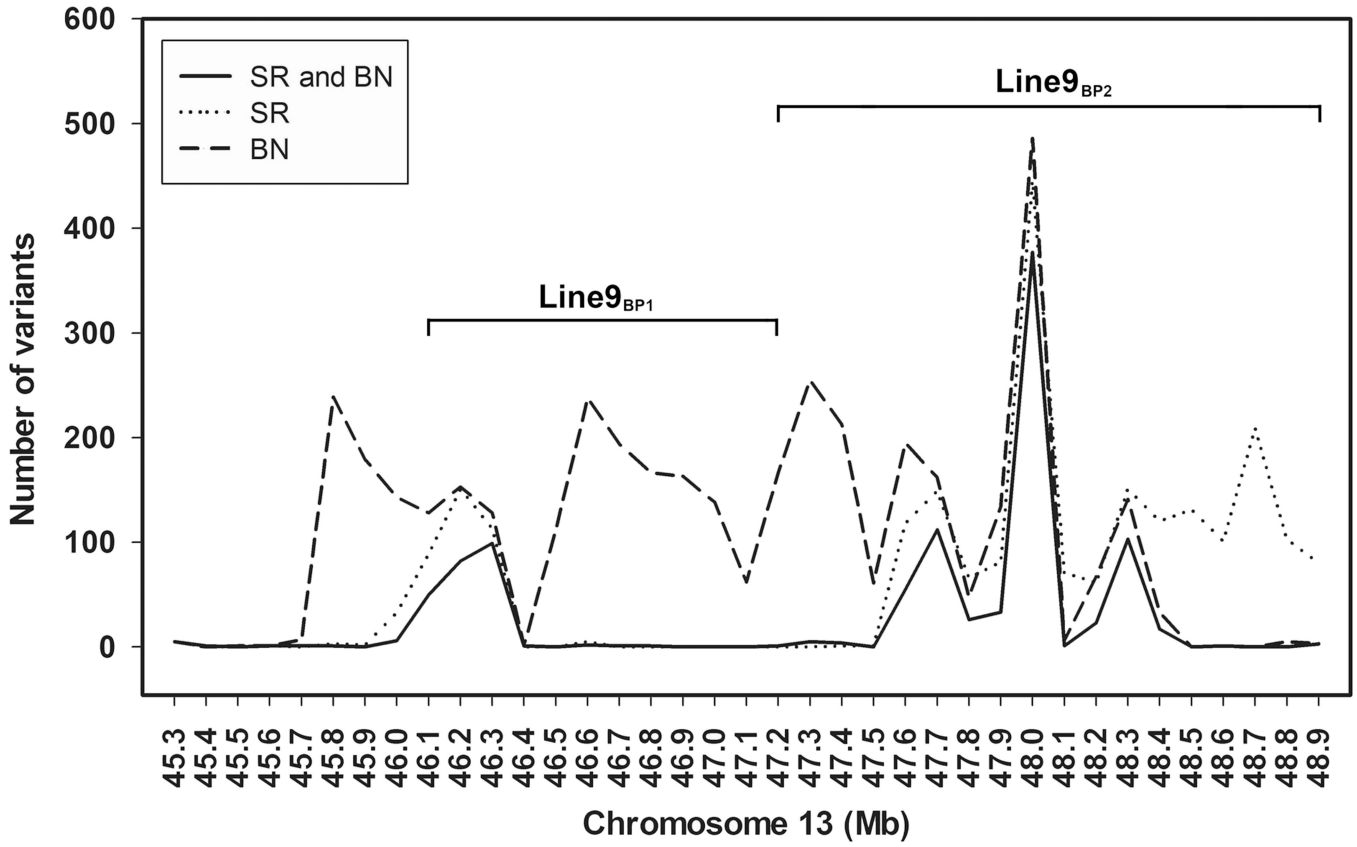

Figure 3.

Comparison of the SS, SR, and BN sequences over the line 9 congenic interval. Sequences were compared as SS variants differing from SR (dotted line), SS variants differing from BN (dashed line), and SS variants that differ from both SR and BN (solid line). Data are presented as the number of variants per 0.1 Mb bin. Boundaries of line 9 BP QTLs are identified by brackets.

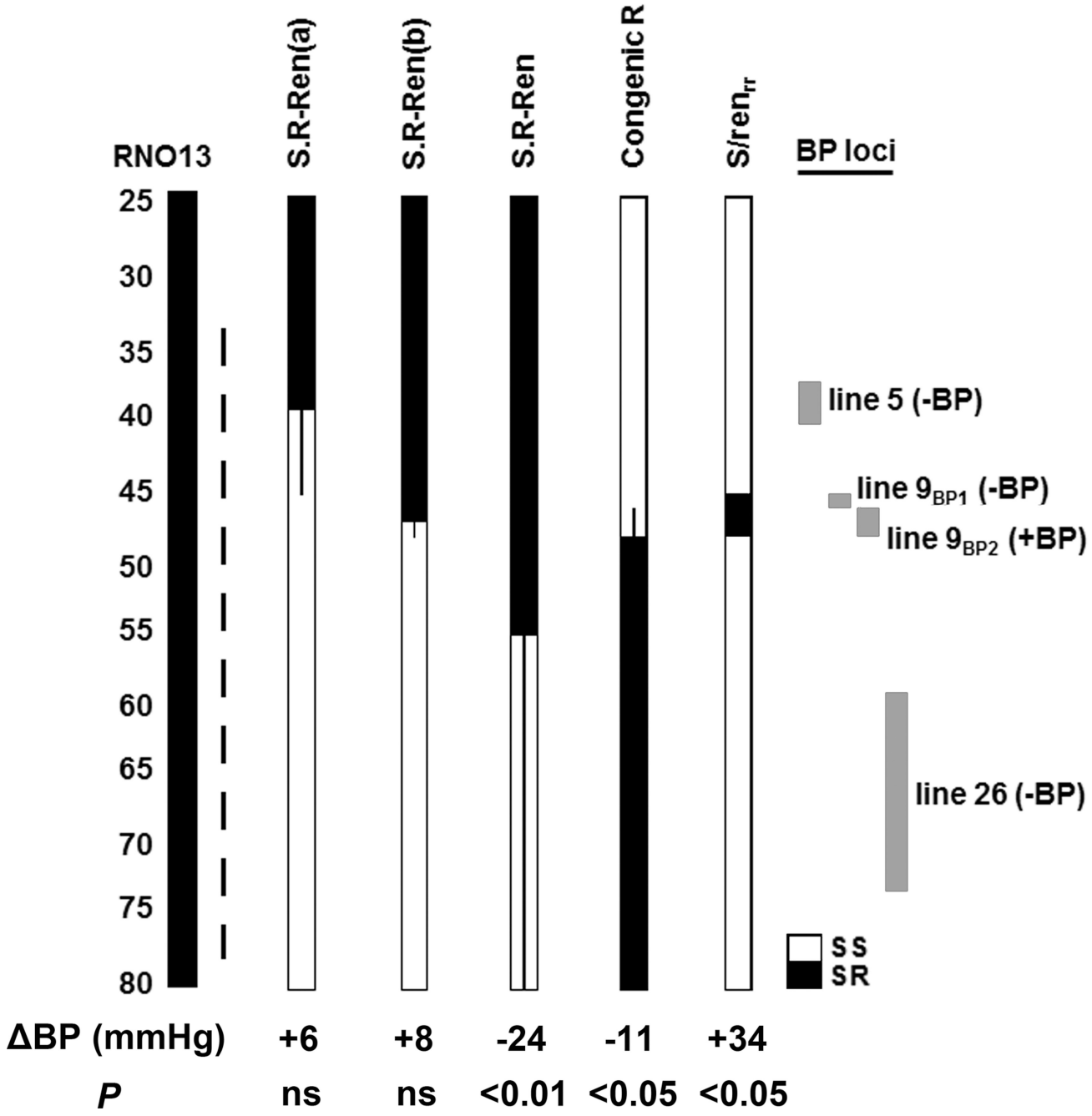

Figure 4.

A summary of the SSxSR *Renin* QTL and congenic mapping studies of the *Renin* BP loci. The BP QTL from a SSxSR cross[5] is indicated by the dashed line. Genomic DNA is depicted by white (SS) and black (SR) for the previously published congenics: S.R-Ren(a), S.R-Ren(b), and S.R-Ren;[5] Congenic R;[12] and S/ren$_{rr}$[11] Thin black bars represent chromosomal regions are unknown. The BP QTL published here and elsewhere[15–17] are represented by the grey bars (*far right*). Chromosomal positions on rat chromosome 13 are given in Mb (*far left*).

Table 1

Nonsynonymous Variants in Conserved Genes within the SS-13^{BN} Line 9 Congenic Interval

Gene Symbol	Position	BN/Mcwi nt	SS/JrMcwi nt†	SR/Jr nt	BN/Mcwi aa	SS/JrMcwi aa	SR/Jr aa	PolyPhen2 Prediction
Pik3c2b	46,010,662	C	A	A	R	H	H	damaging
Pik3c2b	46,010,672	A	G	G	T	A	A	damaging
Plekha6	*46,201,524*	*C*	*C*	*T*	*P*	*P*	*L*	*damaging*
Etnk2	*46,296,424*	*G*	*A*	*G*	*R*	*H*	*R*	*damaging*
Sox13	*46,309,763*	*T*	*C*	*C*	*Q*	*R*	*R*	*benign*
Chit1	*47,101,655*	*C*	*T*	*T*	*L*	*F*	*F*	*damaging*
Chit1	*47,101,733*	*G*	*A*	*A*	*V*	*I*	*I*	*benign*
Chi3l1	*47,146,161*	*A*	*T*	*T*	*T*	*S*	*S*	*benign*
Mybph	*47,151,306*	*T*	*C*	*C*	*I*	*T*	*T*	*damaging*
Mybph	*47,151,520*	*G*	*A*	*A*	*R*	*K*	*K*	*damaging*
Klh112	*47,428,120*	*A*	*C*	*C*	*D*	*A*	*A*	*benign*
Syt2	**47,690,620**	**T**	**C**	**C**	**V**	**A**	**A**	**benign**
Lgr6	**48,066,154**	**G**	**G**	**A**	**R**	**R**	**C**	**damaging**
Ptprv	**48,072,468**	**C**	**T**	**C**	**D**	**N**	**D**	**benign**
Ptprv	**48,080,549**	**C**	**T**	**C**	**R**	**Q**	**R**	**benign**
Ptprv	**48,081,340**	**A**	**G**	**A**	**M**	**T**	**M**	**benign**
Ptprv	**48,081,971**	**G**	**A**	**G**	**L**	**F**	**L**	**damaging**
Ptprv	**48,083,882**	**C**	**T**	**C**	**A**	**T**	**A**	**benign**
Ptprv	**48,085,207**	**G**	**C**	**G**	**E**	**A**	**E**	**benign**
Ptprv	**48,085,289**	**C**	**A**	**C**	**G**	**V**	**G**	**benign**
Ptprv	**48,086,952**	**C**	**T**	**T**	**R**	**H**	**H**	**damaging**
Gpr37l1	**48,122,109**	**C**	**C**	**T**	**V**	**V**	**I**	**damaging**
Rnpep	**48,268,174**	**T**	**G**	**G**	**E**	**A**	**A**	**benign**
Rnpep	**48,280,891**	**T**	**C**	**C**	**N**	**S**	**S**	**benign**
Ipo9	**48,405,406**	**C**	**C**	**T**	**R**	**R**	**Q**	**benign**
Lad1	48,860,329	T	T	C	I	I	T	benign

† SS/JrMcwi and SS/Jr sequence were identical for these variants.