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Identification of Hypertension Susceptibility Loci on Rat Chromosome 12

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Abstract

Previous studies have identified multiple blood pressure and renal disease quantitative trait loci located on rat chromosome 12. In the present study, we narrowed blood pressure loci using a series of overlapping SS-12^{BN} congenic lines. We found that transferring 6.1Mb of SS chromosome 12 (13.4-19.5Mb) onto the consomic SS-12^{BN} background significantly elevated blood pressure on 1% NaCl (146±6 vs. 127±1 mmHg, $P<0.01$) and 8% NaCl diets (178±7 vs. 144±2 mmHg, $P<0.05$). Compared with the SS-12^{BN} consomic, these animals also had significantly elevated albumin (218±31 vs. 104±8 mg/day, $P<0.01$) and protein excretion (347±41 vs. 195±12 mg/day, $P<0.01$) on 1% NaCl diet. Elevated blood pressure, albuminuria, and proteinuria coincided with greater renal and cardiac damage, demonstrating that SS allele(s) within the 6.1Mb congenic interval are associated with strong cardiovascular disease phenotypes. Sequence analysis of the 6.1Mb congenic region revealed 12,675 single nucleotide polymorphisms between SS and BN. Of these polymorphisms, 295 lie within coding regions and 20 resulted in nonsynonymous changes in conserved genes, of which 5 were predicted to be potentially damaging to protein function. Syntenic regions in human chromosome 7 have also been identified in multiple linkage and association studies of cardiovascular disease, suggesting that genetic variants underlying cardiovascular phenotypes in this congenic strain can likely be translated to a better understanding of human hypertension.

Keywords

Hypertension; Genetic; Dahl Salt-Sensitive Rat; Brown Norway; Congenic

Introduction

Hypertension is the most prevalent cardiovascular disease (CVD) worldwide and is a major risk factor for stroke, atherosclerosis, renal failure, and heart disease.¹ In most cases, a single cause of hypertension cannot be found (i.e., essential hypertension), but rather multiple environmental and genetic factors contribute to overall disease susceptibility.¹ This, combined with complex gene interaction,² poses significant challenges in sifting through the many variants that could potentially contribute to hypertension.

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One strategy to characterize regions of the genome that affect CVD traits is through chromosome substitution (i.e., consomics). Mattson and colleagues³ used this approach to characterize blood pressure (BP) and renal damage by transferring chromosomes from the normotensive Brown Norway (BN) strain onto the hypertensive Dahl Salt-Sensitive/Mcwi (SS) background using marker-assisted selection.⁴ The SS model exhibits CVD traits⁵ and end organ damage that are highly similar to human hypertension.⁶⁻¹⁰ This comprehensive study identified multiple chromosomes from the normotensive BN strain that attenuate salt-induced hypertension and renal damage or had no effects; whereas, a few BN chromosomes (e.g., 12 and 17) surprisingly increased susceptibility to CVD.³ Several additional mapping studies have confirmed that RNO12 harbors quantitative trait loci (QTL) for BP and renal damage,^{5, 11-13} suggesting collectively that BP QTL are located on RNO12 and may be derived from normotensive as well as hypertensive strains.

In this study we narrowed regions by introgressing SS chromosome 12 back onto the SS-12^{BN} consomic background to generate overlapping congenic strains that were phenotyped for BP, renal function, and cardiac damage. These congenics revealed that transferring a 6.1Mb region of SS chromosome 12 (13.4-19.5Mb) onto the consomic SS-12^{BN} genetic background significantly elevated BP by 20-40 mmHg. This BP phenotype coincided with ~2-fold increase in albuminuria and proteinuria and a substantial increase in kidney and heart damage. Importantly, this region overlaps with multiple human and mouse quantitative trait loci (QTL) for BP and renal damage.¹⁴⁻¹⁸ This synteny suggests that renal and cardiovascular genetic elements in this region might be shared across species, thus, the SS-12^{BN} congenic model may provide insight into genetic susceptibility to cardiovascular and renal disease in humans.

Materials and Methods

Generation of SS-12^{BN} Congenic Rats

All rats were provided food and water *ad libitum* and were housed at the Medical College of Wisconsin (MCW) Animal Resource Center. All procedures were approved by the MCW IACUC committee. The SS-12^{BN}/Mcwi consomic was made by selectively breeding the SS/JrHsd/Mcwi (SS) and BN/SsN/Mcwi (BN) strains¹⁹, and SS-12^{BN} congenic strains were subsequently generated by backcrossing SS-12^{BN}/Mcwi to SS/JrHsd/Mcwi.

Blood Pressure Measurement

Mean arterial pressure was measured by telemetry transmitter implantation with a catheter inserted into the abdominal aorta (TA11PA-C40, Data Sciences, St. Paul, MN) as previously described.²⁰

Measurement of Protein and Albumin Excretion

On post-surgical day 3, rats were acclimated for 24 hours in metabolic cages, followed by 24 hour urine collection. Urinary protein and albumin were measured as described previously^{3, 12}

Echocardiography

Echocardiography was performed using a GE Vivid 7 ultrasound machine (GE Healthcare, Waukesha, WI) equipped with a 10MHz transducer as described previously.²¹

Histology

Histological analysis of kidneys and hearts was performed as described previously.²¹

Genomic Sequencing and Analysis

SS/JrHsD/Mcwi genomic DNA was sequenced using an Illumina HiSeq 2000 and analyzed by CASAVA v1.8.1 (Illumina, San Diego, CA). Variants were analyzed by Variant Effect Predictor²² and Polyphen²³. Analysis of the SS/JrHsD/Mcwi genome can be accessed on the RGD website (<http://rgd.mcw.edu>).

Statistical analysis

Statistical analyses were performed using Sigma Plot 11.0 software. Data are presented as mean \pm SEM. Interventricular septum thickness, interstitial fibrosis, and capillary density were analyzed by unpaired Student's *t* test. All other data were analyzed by one-way ANOVA followed by the Holm-Sidak multiple comparison test. Because albumin measurements failed the equal variance test, the data were log-transformed prior to one-way ANOVA followed by the Holm-Sidak multiple comparison test.

Expanded Materials and Methods are provided in the supplement.

Results

Blood Pressure

To narrow the regions on RNO12 that modify BP, we generated a series of SS-12^{BN} congenic lines as depicted in Figure 1. At 9 weeks-of-age, SS-12^{BN} consomic and congenic male rats fed a 1% NaCl diet were implanted with radiotelemeters and MAP was recorded over 3 consecutive days. On 1% NaCl diet, the MAP of line C (144 ± 2 mmHg, $n=11$) was significantly higher than SS-12^{BN} consomic (127 ± 1 mmHg, $P<0.001$, $n=10$), line A (114 ± 2 mmHg, $P<0.001$, $n=8$), and line B (119 ± 1 mmHg, $P<0.001$, $n=8$). Likewise, after an 8% NaCl challenge for 7 days, the MAP of line C rose to 178 ± 7 mmHg, which was significantly higher than the SS-12^{BN} consomic (144 ± 2 mmHg, $P<0.001$), line A (136 ± 5 mmHg, $P<0.001$), and line B (139 ± 2 mmHg, $P<0.001$) (Figure 1). Because line C is genetically identical to the SS-12^{BN} consomic except for 6.1Mb (13.4-19.5Mb), these data indicate that allele(s) within this region strongly regulate BP.

Renal Damage and Histology

To assess renal damage, urine was collected from 9-week old SS-12^{BN} consomic and congenic rats on 1% NaCl Purina diet and total albumin and protein excretion was quantified. In agreement with BP results, line C had significantly higher albumin (218 ± 29 mg, $P<0.001$) and protein (347 ± 38 mg, $P<0.001$) excretion compared with the SS-12^{BN} consomic, line A, and line B, with the values collectively ranging from 85-125 mg and 195-236 mg for total albumin and protein excretion, respectively (Figure 2A and 2B).

The extent of renal damage was assessed histologically using trichrome-stained kidney sections of 12-week old SS-12^{BN} consomic and line C rats placed on 8% NaCl. Compared with SS-12^{BN} consomics, the morphology of line C kidneys appeared to have significantly more tubular and glomerular damage (Figure 2C). Quantitative analysis of the percent area of protein casting showed significantly higher tubular casting in the outer medulla of line C ($9.3 \pm 0.7\%$) as compared with SS-12^{BN} consomic kidneys ($6.5 \pm 0.5\%$, $P<0.05$; Figure 2D). Kidneys from line C rats also showed considerably more glomerular sclerosis and basement membrane thickening as well as increased interstitial fibrosis compared to the SS-12^{BN} consomic. Glomerular sclerosis was significantly higher in line C compared to SS-12^{BN} (1.2 ± 0.1 versus 0.8 ± 0.1 , $P<0.05$), as quantified by glomerular sclerosis scoring (Figure 2E).

Heart Function and Histology

To determine whether elevated BP in line C also coincided with increased cardiac hypertrophy and remodeling, the hearts of 4-month old line C and SS-12^{BN} consomic rats on 1% NaCl Purina diet were measured by echocardiography and then collected for histological analysis. Compared with SS-12^{BN} consomics, morphological analysis of line C hearts showed notable increase in LV wall thickness (Figure 3A) and a $24 \pm 0.1\%$ ($P < 0.01$) increase in interstitial fibrosis (Figure 3B and 3C). Cardiac hypertrophy in line C was confirmed by a $31 \pm 6\%$ ($P < 0.05$) increase in septal wall thickness compared with SS-12^{BN} consomics, as determined by echocardiography (Figure 3D).

Comparative Genomics

Our data demonstrate that genetic element(s) within the line C congenic region strongly affect BP, renal function, and cardiac remodeling (Figures 1-3). This 6.1Mb region contains 133 genes and is syntenic to two regions on human chromosome 7 and one region on mouse chromosome 5 (Figure 4). A 3.9Mb region toward the *p* terminal of line C (13.4-17.3Mb) is homologous to the *p* end of human chromosome 7 (0.2-4.1Mb) in the reverse orientation (*p* to *q*) compared with the rat. The human homolog to line C from 17.2 to 17.9Mb is also located on human chromosome 7 spanning from 99.2 to 100.3Mb in the same orientation (*p* to *q*) as the rat. The remaining 1.1Mb of the line C congenic region (16.8-17.3Mb) aligned to the human X and Y chromosomes from 1.5 to 2.1Mb and 155.0 to 155.1Mb (X chromosome) and from 1.4 to 2.1Mb and 59.1 to 59.3Mb (Y chromosome). In comparison, line C is syntenic to a contiguous region on mouse chromosome 5 (137.8-142.7Mb) in the reverse orientation (*q* to *p*) compared with the rat.

A total of 6 human QTLs^{14-18, 24}, 2 human SNP association studies^{25, 26} and 2 mouse QTLs^{27, 28} for BP or renal function have been previously mapped to regions that are syntenic to the line C congenic region (Figure 4). Of these studies, two significantly associated the 3.9Mb toward the *p* end of human chromosome 7 with elevated BP²⁵ and diabetic nephropathy²⁴, while five studies linked the marker D7S1799 (chr12:103982240) in multiple populations with elevated MAP, diastolic BP, and systolic BP.¹⁴⁻¹⁸ Two additional linkage studies in the mouse identified concordant BP QTLs on mouse chromosome 5 spanning 89-148Mb²⁷ and 126-162Mb.²⁸ On average the QTLs identified in mouse and human linkage studies spanned 30-60Mb and contained approximately 600-700 genes. In comparison, the number of candidates in the line C congenic region is considerably less at 133 genes, of which 56 are validated, 62 are predicted, and 15 belong to the nonconserved family of vomeronasal receptors (V2R)²⁹ (Supplementary Table S1). Thus, if the same genetic elements influence BP and renal damage across species, then we can use species conservation to preliminarily narrow our list to 118 candidate genes that overlap in human, mouse and rat QTLs.

Identification of Sequence Variants within the Candidate Region

The SS genome was aligned across the 6.1Mb line C congenic region using Illumina sequencing reads. We aligned 5,283,594bp (86.9% of non-gap reference) with an average depth of 20X. Compared with the BN reference, we identified a total of 12,675 variants by two or more reads that constitute at least 30% of total reads detected at that loci (Supplementary Table S2). Of the total variants, 295 lie within coding regions of known and predicted genes and 141 are expected to cause nonsynonymous amino acid changes. Of the 141 nonsynonymous variants, 20 reside in conserved validated genes and 5 of these were predicted to be damaging by PolyPhen (<http://genetics.bwh.harvard.edu/pph/>) (Table 1). Four miRNA were also predicted within this region using miRBase (<http://www.mirbase.org/>), but we found no sequence variations within these predicted miRNAs (data not shown). Likewise, using the TargetScan tool (<http://www.targetscan.org/>) to

predict miRNA-targeted genes within the line C congenic interval revealed 18 putative miRNA target sites; however, no sequence variants were found in any of these sites.

Discussion

Previous chromosome substitution strains³ and linkage analyses^{5, 12, 13, 30} have demonstrated that RNO12 impacts BP and renal damage, but the causative variants underlying these large QTLs have not yet been identified. Our goal was to narrow cardiovascular QTLs on RNO12 by screening overlapping SS-12^{BN} congenic strains for BP, renal, and cardiac phenotypes. Analysis of the line C interval showed that transferring 6.1Mb of RNO12 (13.4-19.5Mb) from the SS strain onto the SS-12^{BN} background significantly elevated BP by 20-40 mmHg (Figure 1), which coincided with extensive renal and cardiac damage compared with the SS-12^{BN} consomic (Figures 2-3). Our observation that line C develops significant hypertension, proteinuria, albuminuria, and cardiac remodeling (Figures 1-3) on 1% NaCl Purina diet demonstrates that variants within the line C congenic region have strong phenotypic effects. These data also demonstrate that we have reduced the overlapping rat BP QTLs on RNO12 at 1.8-46.6Mb,¹¹ 11.4-29.0Mb⁵ and renal function QTLs at 9.6-33.2Mb¹² and 10.7-31.4Mb¹³ by ~85%, which provides a more manageable region to screen for potentially damaging variants.

Genetic Modifiers of BP and Renal Function on RNO12

Our data provide evidence for genetic elements surrounding the 6.1Mb region (13.4-19.5Mb) that likely modulate blood pressure. The flanking SS genome in line A (1-13.4Mb) and line B (19.5-36.2Mb) significantly attenuated BP and renal phenotypes (Figures 2 and 3) compared to the line C region alone. This observation suggests that either SS alleles in these regions decrease blood pressure or BN alleles increase blood pressure. Several studies have previously demonstrated that interacting “+BP QTLs” and “-BP QTLs” can be derived from either hypertensive or normotensive strains^{2, 31} and the effect of these genes is only manifested under certain genetic combinations. Furthermore, it is possible that genes within the different regions (lines A, B, and C) interact with one another (i.e., epistasis), as has been demonstrated for BP in other regions of the rat genome (e.g., RNO3,³¹ RNO8,³² RNO10,³³ and RNO13²⁰). However, further experimentation would be required to test this hypothesis. Nevertheless, our data highlight the power of using congenic strains to map complex genetic traits that rely on allelic combinations, which may not be apparent from the parental strains.

Comparative Analysis of Line C Sequence Variants

The line C congenic region is syntenic with two regions in human chromosome 7 that have been linked with BP^{15-18, 25} and renal disease^{14, 34} by linkage and association studies (Figure 4), suggesting that common genetic elements might play a similar role in rat and human. In the line C region we identified 20 nonsynonymous amino acid changes in conserved validated genes between SS and BN, of which *Mblac1*, *Il3ra*, *Spr3*, *Elfn1*, and *Azgp1* were predicted by PolyPhen to be damaging variants (Table 1). *Mblac1* is a poorly characterized metal-binding enzyme³⁵ with no reported role in BP or renal function. *Il3ra* and *Spr3* are involved in hematopoiesis³⁶ and neurogenesis,³⁷ respectively, but also have no reported role in BP or renal function. *Elfn1*, also known in humans as PPP1R28, is an inhibiting regulatory subunit of the ubiquitous protein phosphatase, PP1.³⁸ Although the function of PPP1R28 is largely unknown, the PP1 signaling pathway has been linked in humans with hypertension³⁹, diabetes⁴⁰, and heart failure.⁴¹ This human data makes *Elfn1* an interesting candidate for our congenic model, as its associated pathway may regulate cardiovascular phenotypes across species. *Azgp1* is a lipid mobilizing adipokine that has been associated with serum lipid levels,^{42, 43} multiple inflammatory phenotypes,⁴⁴ and is

also implicated in preeclampsia⁴⁵ and renal function^{45, 46} suggesting that it might mediate disease-associated remodeling pathways.

The larger of the two syntenic regions in humans (0.2-4.1Mb of human chr7) has been linked with BP and renal disease by multiple studies.²⁴⁻²⁶ Two studies associated single nucleotide polymorphisms (SNPs) in the SDK1 (sidekick-1) locus with elevated BP in Nigerian²⁵ and Japanese²⁶ populations. The predicted rat homologue of SDK1 (RGD1560686) partially overlaps the line C congenic region, suggesting that SDK1 or variant(s) in close proximity to this gene might contribute to elevated BP across species. None of the SDK1 SNPs that were associated with elevated BP in humans^{25, 26} caused amino acid changes our line C congenic region (Supplementary Table S2), which leads us to believe that mutations of SDK1 itself are probably not causing BP changes in our model. However, we cannot rule out potential variants in non-coding regulatory regions that might affect expression levels of SDK1. Multiple studies have shown that elevation of SDK1 in podocytes has been shown to increase glomerular sclerosis in both animal models⁴⁷ and HIV-associated nephropathy,^{48, 49} making this gene an interesting candidate for complex renal disease.

Perspectives

We narrowed rat BP QTLs on RNO12 by ~85% to a 6.1Mb congenic region that contains 118 conserved genes and is syntenic with BP QTLs from multiple human and mouse studies. Further congenic mapping will be needed to narrow the loci and elucidate the specific variant(s) and mechanisms that drive increased BP and renal damage in the SS-12^{BN} strain. Additionally, with the advent of rat knockout models developed by our group⁵⁰ and others,⁵¹ it is now possible to specifically target and test functional alleles in the rat. Meanwhile, these genes serve as candidates to be tested in subsets GWAS and other species or strains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

1. What Is New?

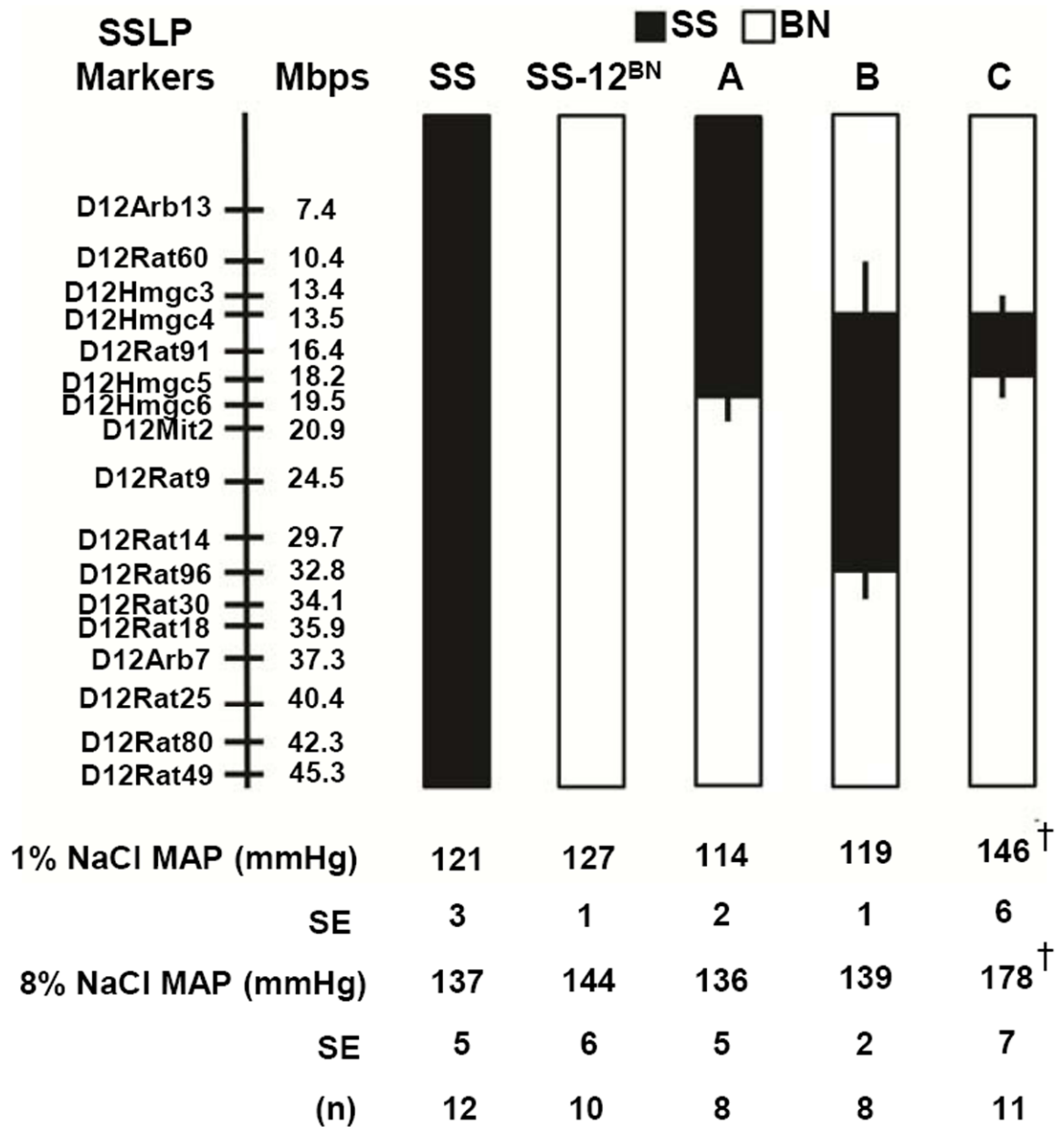
- For the first time we show that the SS-12^{BN} congenic region (13.4-19.5Mb) drives a 20-40 mmHg change in MAP and is associated with extensive renal and cardiac damage.
- We identified 12,675 SNPs, of which 295 lie within known and predicted genes and 5 are anticipated to be potentially damaging.

2. What Is Relevant?

- The SS-12^{BN} congenic region overlaps with 8 human and 2 mouse CVD QTLs, offering new insight to common genetic elements mediate susceptibility to CVD.

3. Summary

Variant(s) within the SS-12^{BN} congenic region (13.4-19.5Mb) strongly regulate BP that increases susceptibility to end-stage renal and cardiac disease.

**Figure 1.**

Schematic representation of the overlapping SS-12^{BN} congenic strains that were generated by introgressing segments of the SS chromosome 12 (black) into the genetic background of the SS-12^{BN} consomic rat (white) by marker assisted breeding. Thin black bars represent chromosomal regions that could be BN or SS. MAP of SS-12^{BN} consomic and congenic rats on 1% and 8% NaCl diets. Data represent MAP \pm SEM from 8-12 animals per group.

*** $P < 0.001$ as determined by a one-way ANOVA followed by a Holm-Sidak post hoc test.

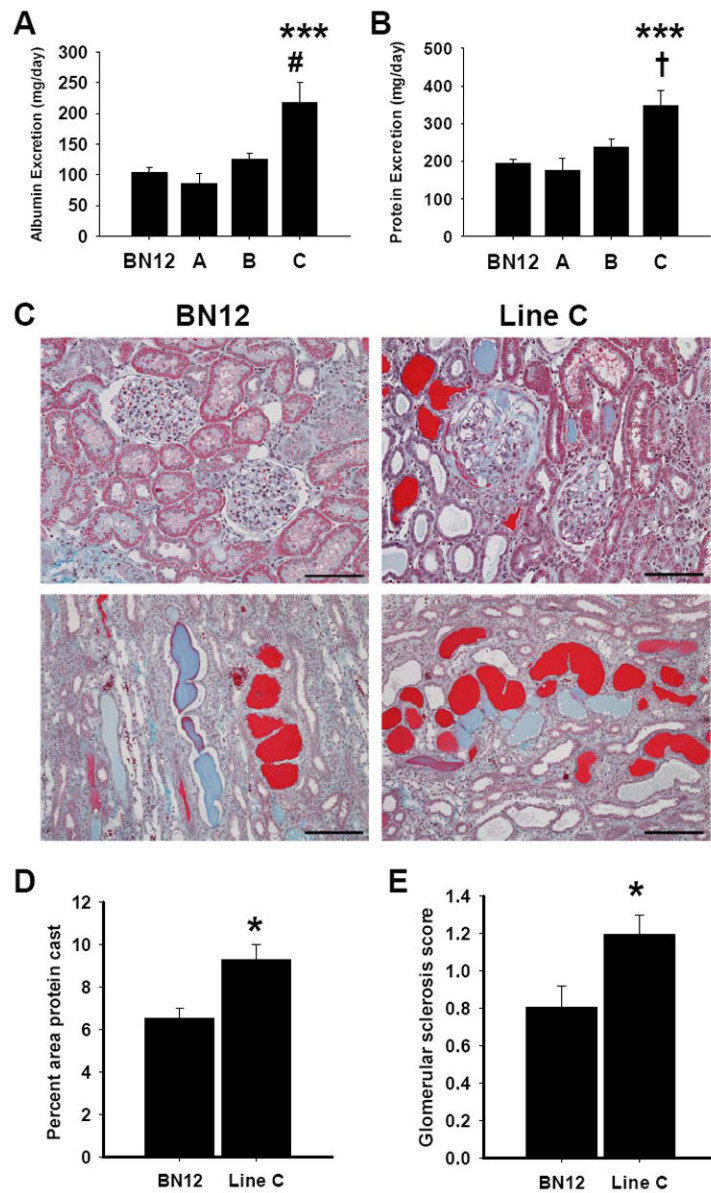


Figure 2. Urinary albumin and protein excretion of SS-12^{BN} consomic and congenic rats on a 1% NaCl diet. The rats were placed in metabolic cages overnight to acclimate, followed by a 24 hour urine collection. Data are presented as mean albumin (A) or protein excretion (B) \pm SEM from 8-12 animals per group. Data were not normally distributed, so each data point was log transformed before statistical analysis. ^{***} $P < 0.001$ vs. all groups, [#] $P < 0.01$ vs. line B, and [†] $P < 0.01$ vs. line B, as determined by a one-way ANOVA followed by a Holm-Sidak post hoc test. (C) Histology of trichrome-stained SS-12^{BN} consomic and line C kidneys. (D) Quantification of percent area of protein casting in the outer stripe of the medulla (n=4 animals per group). Data are presented as mean percent area of protein casting \pm SEM. (E) Average sclerosis score for SS-12^{BN} consomic and line C kidneys (n=4 animals per group). Data are presented as mean sclerosis score \pm SEM. For (D) and (E) ^{*} $P < 0.05$ as determined by Student's unpaired *t*-test.

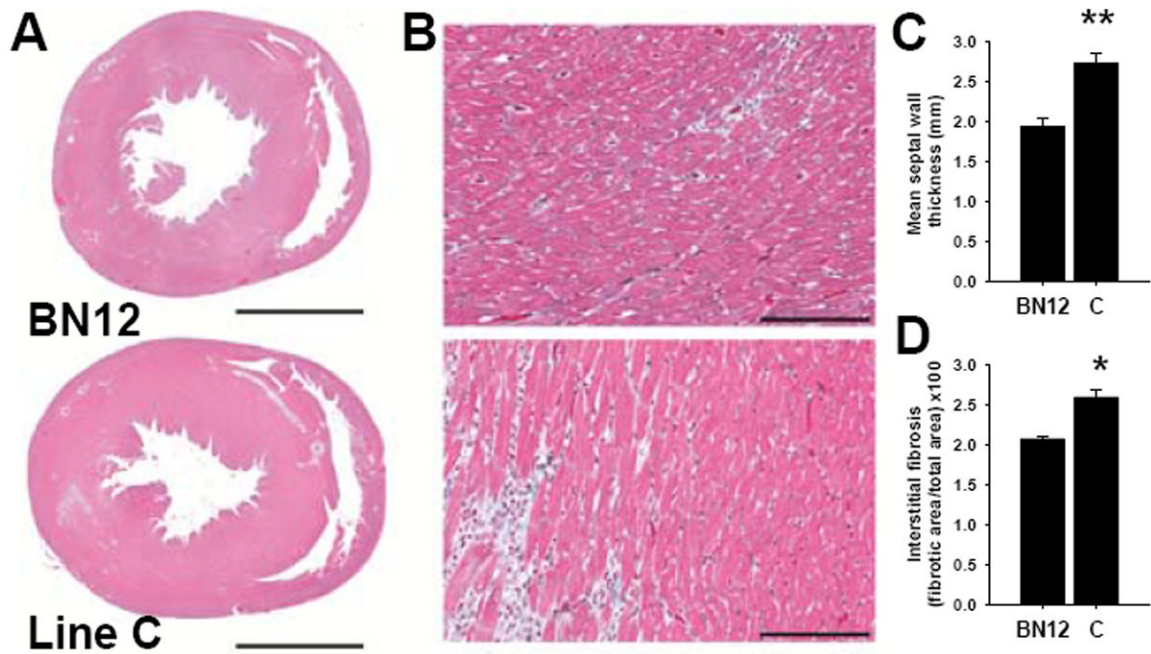


Figure 3. Left ventricular hypertrophy (LVH) and cardiac remodeling in line C compared with the SS-12^{BN} consomic. (A) Trichrome-stained mid-ventricular sections from SS-12^{BN} consomics (top) and line C (bottom) show greater LVH in line C compared with age-matched SS-12^{BN} consomics. (B) Higher magnification images (400X) of trichrome-stained mid-ventricular sections from SS-12^{BN} consomic (top) and line C (bottom) show increased interstitial fibrosis in line C as compared with the SS-12^{BN} consomic. (C) The percent fibrotic area was quantified from images of trichrome-stained mid-ventricular sections acquired at 200X magnification. Data are presented as the average % fibrotic tissue area \pm SEM (n=50 images per rat; 4-5 rats per group). (D) Septal wall thickness of SS-12^{BN} and line C hearts was assessed by echocardiography. Data represent the mean septal wall thickness \pm SEM from 3-5 rats per group. For (C) and (D), * P <0.05 and ** P <0.01 as determined by Student's unpaired t -test. Scale bars represent 3mm (A) and 150 μ m (B).

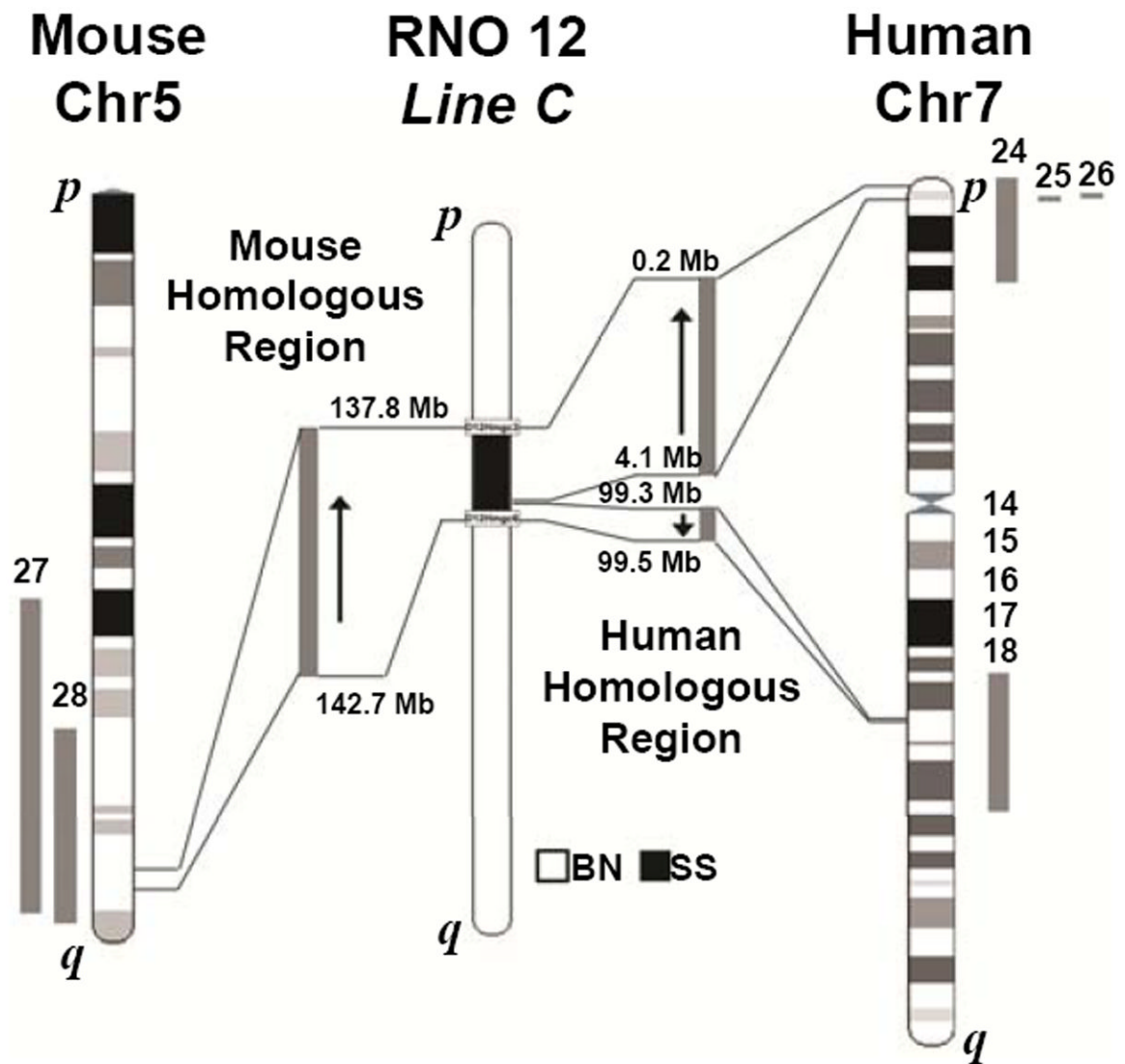


Figure 4. Schematic representation of the flanking markers on RNO12 for line C and the orthologous regions in human and mouse. The flanking markers on RNO12 for the line C congenic region are D12-Hmgc3 (RGD ID: 5683878) and D12-Hmgc6 (RGD ID: 5683884). References for previous studies showing overlapping QTLs or associations are indication in the figure.

Table 1

Nonsynonymous variants in conserved genes within the SS-12BN line C congenic region

Gene Symbol	Gene ID*	Position	Ref [†] nt	Var [†] nt	AA Change	Polyphen Prediction
Tyh3	ENSRNOT00000038773	14,480,874	A	C	N33I	Benign
E1fn1	ENSRNOT00000033551	15,139,510	C	T	M287I	Damaging
Micall2	ENSRNOT00000036744	15,395,661	T	C	V181A	Benign
Micall2	ENSRNOT00000036744	15,396,513	A	G	T280A	Benign
Adap1	ENSRNOT00000038777	15,844,453	C	T	P23L	Benign
Zfp68	ENSRNOT00000001784	16,545,529	C	T	L168F	Benign
MGC112692	ENSRNOT00000060155	16,635,046	A	C	V332G	Benign
MGC112692	ENSRNOT00000060155	16,635,542	C	T	V167I	Benign
Dhrsx	ENSRNOT00000029485	16,809,701	G	T	V78F	Benign
Akap17a	ENSRNOT00000037829	16,818,972	A	G	C476R	Unknown
Ii3ra	ENSRNOT00000001792	16,828,844	C	T	V103I	Damaging
Cyp3a9	ENSRNOT00000001863	17,176,498	A	G	I207V	Benign
Cyp3a9	ENSRNOT00000001863	17,203,077	G	A	V454I	Benign
Spry3	ENSRNOT00000011128	17,343,558	C	A	G123V	Damaging
Azgp1	ENSRNOT00000001801	17,494,146	T	C	L40P	Damaging
Zscan21	ENSRNOT000000001810	17,567,622	A	G	R51G	Benign
Mcm7	ENSRNOT00000001825	17,612,322	G	A	S74L	Benign
Mblac1	ENSRNOT00000001831	17,636,671	A	G	Q36R	Benign
Mblac1	ENSRNOT00000001831	17,636,674	G	A	G37E	Benign
Mblac1	ENSRNOT00000001831	17,636,772	G	A	G70S	Damaging

Ref nt, reference nucleotide; Var nt, variant nucleotide; AA, amino acid

* Designated by Ensembl gene ID

[†] Reference (BN/SsN/Mcwi) and variant (SS/IRHsD/Mcwi) were confirmed by Sanger sequencing