Multiple Blood Pressure loci on rat Chromosome 13 attenuate the development of hypertension in the Dahl S Hypertensive rat

Carol Moreno\textsuperscript{1,2}, Mary L. Kaldunski\textsuperscript{1}, Tao Wang\textsuperscript{2,3}, Richard J. Roman\textsuperscript{1,4}, Andrew S. Greene\textsuperscript{1,5}, Jozef Lazar\textsuperscript{1,6}, Howard J. Jacob\textsuperscript{1,2,7} and Allen W. Cowley Jr.\textsuperscript{1}

\textsuperscript{1}Department of Physiology, \textsuperscript{2}Human and Molecular Genetics Center, \textsuperscript{3}Division of Biostatistics, \textsuperscript{4}Kidney Disease Center, \textsuperscript{5}Biotechnology and Bioengineering Center, \textsuperscript{6}Department of Dermatology, \textsuperscript{7}Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA.

\textbf{Short title:} Multiple hypertensive loci in the Dahl S chromosome 13

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\textbf{Author of correspondence:} Allen W. Cowley Jr, cowley@mcw.edu
Abstract

Previous studies have indicated that substitution of chromosome 13 of the salt-resistant Brown Norway BN/SsNHsdMcwi (BN) rat into the genomic background of the Dahl salt-sensitive rat SS/JrHsdMcwi (SS) attenuates the development of salt-sensitive hypertension and renal damage. In order to identify the regions within chromosome 13 that attenuate the development of hypertension during a high salt diet in the SS rat, we phenotyped a series of overlapping congenic lines covering chromosome 13, generated from an intercross between the consomic SS-13BN and the SS rat. Blood pressure was determined in chronically catheterized rats after 2 weeks of high salt diet (8% NaCl) together with microalbuminuria as an index of renal damage. Four discrete regions were identified, ranging in size from 4.5 to 16 Mbp, each of which independently provided significant protection from hypertension during high salt diet, reducing blood pressure by 22 to 32 mmHg. Protection was more robust in female than in male rats in some of the congenic strains, suggesting a sex interaction with some of the genes determining blood pressure during high salt diet. Among the 23 congenic strains, several regions overlapped. When three of the “protective” regions were combined onto one broad congenic strain, no summation effect was seen, obtaining the same decrease in blood pressure as with each one independently. We conclude from these studies that there are four regions within chromosome 13 containing genes that interact epistatically and influence arterial pressure.
Introduction

Essential hypertension is a multifactorial disease determined by the interaction of genetic and environmental factors (22, 23), making the identification of the causal genes a daunting task. In humans, genome-wide scans have identified many blood pressure quantitative trait loci (QTLs) (19, 24, 29, 37, 38), but confirmation among different populations has been difficult.

The rat is a useful model for the study of complex diseases such as hypertension because multiple hypertensive strains each carry some phenotypic similarities to the human disease (21, 42-44). The Dahl S rat is a commonly used model for the study of salt-sensitive hypertension and has remarkable similarities with phenotypic traits seen in many African Americans with hypertension, including low renin, salt-sensitivity, hyperinsulinemia, and early end-stage renal disease (3, 13).

Genetic studies have demonstrated the polygenic nature of salt-sensitive hypertension in the SS rat, and a number of blood pressure QTLs have been identified in different genomic regions, including chromosome 13 (6, 34). Utilization of chromosomal substitution approaches have recently provided even stronger evidence of this, showing that substitution of different chromosomes from the Brown Norway BN/SsNHsdMcwi (BN) strain into the Dahl SS/JrHsdMcwi (SS) strain attenuates the development of salt-induced hypertension (www.pga.mcw.edu). Of particular interest to our laboratory has been consomic strain SS-13BN, which has chromosome 13 from the BN introgressed into the isogenic genetic background of the SS strain. These rats exhibit a marked reduction of salt-induced hypertension and proteinuria (6). However, the specific regions of chromosome 13 that harbor the genes related to salt-sensitive hypertension in the SS rat are unknown (15, 47).

Different regions of chromosome 13 have been previously linked to blood pressure. Since
Rapp *et al* first found linkage between the renin locus and blood pressure (36), many studies have studied the role of the renin gene in the development of hypertension in the Dahl S rat. Studies by St. Lezin *et al* and Jiang *et al* found that the renin gene participates in hypertension in the Dahl S rat (15, 40), but Zhang *et al* and DiPaola *et al* failed to demonstrate any effect on blood pressure sensitivity when the renin allele from the Dahl R rats was transferred to the Dahl S rat (8, 47). This group found instead a blood pressure QTL in a region above renin. Other groups have found broad QTLs for blood pressure in other regions of chromosome 13 (17, 39, 47).

To better define the regions of chromosome 13 involved in blood pressure regulation during a high salt diet, overlapping congenic strains were derived from a cross between SS-13\textsuperscript{BN} consomic and SS rats, and phenotyped for arterial pressure and proteinuria. The congenic strains also enabled determination of sexual dimorphism and interaction among the different genomic regions in chromosome 13 in the control of blood pressure.
Methods

Protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. Experiments were performed on male and female rats of SS, SS-13\textsuperscript{BN} consomic, and congenic strains. The SS colony at MCW was rederived from SS/JrHsd rats originally obtained as a congenic control strain from Dr. T. Kurtz (UCSF) in 1991. This colony has been maintained at MCW by strict brother-sister mating for more than 46 generations. Chromosome 13 from the salt-resistant BN rat, raised at MCW with strict brother-sister mating for over 30 generations, was substituted for chromosome 13 of the salt-sensitive SS rat through selective breeding as described previously (6). The SS-13\textsuperscript{BN} consomic strain has since been maintained by brother-sister mating for 20 generations. The rats were raised on a purified AIN76 diet containing 0.4% NaCl (Dyets Inc, Allentown, PA). Experimental rats were switched to an 8.0% NaCl diet at 10 weeks of age, 2 weeks prior to surgical implantation of catheters.

Generation of congenic population: Consomic SS-13\textsuperscript{BN} rats were crossed to SS females, and the first generation progeny were intercrossed to create the second generation (F2) that captured different regions of chromosome 13 due to recombination events. These F2 rats were backcrossed to SS females for further development of the congenic strains for chromosome 13. Progenitors of each generation were selected by marker assisted breeding, whereby rats were genotyped with 44 simple sequence length polymorphism (SSLP) genetic markers for chromosome 13 (~1 marker/cM), genetic recombination evaluated and the selection of breeders completed to further generate the congenic strains.

A total of 26 congenic strains were developed covering the entire length of chromosome 13, of which 23 strains were phenotyped. As a positive control an SS strain
was re-derived from three overlapping congenic strains, in which all alleles for chromosome 13 were fixed to the SS strain. This strain showed no differences in blood pressure or proteinuria compared to the SS parental inbred strain in response to a high salt diet, and it was defined and used in the current study as the SS control strain for phenotyping.

**Fluorescent Genotyping:** A portion of an ear from each animal was collected and incubated overnight in a lysis buffer containing 100 µg/ml of Proteinase K. DNA was precipitated with isopropyl alcohol and pelleted by centrifugation at 12000 g for 10 min. The pellet was washed with 75% ethanol, air dried, resuspended in TE buffer, pH 7.5. The concentration of DNA in the samples was determined by measurement of 260 to 280 nm absorption ratios (Beckman DU640). The rats were genotyped using fluorescent M13 labeled primers, as previously described (28). In brief, genomic DNA (25 ng) was amplified by PCR in a 6 µL reaction containing 150 nmol/L of primer-dye conjugate, 10nm/L Trizma Base, 1.5 mmol/L MgCl2, 50mmol/L KCl (pH 8.3), 200 µmol/L dNTP, and 1 U/µl Taq DNA polymerase. A “touchdown” PCR reaction was performed, as previously described (9). Samples were run on an ABI 377-96 DNA sequencer for 2 hours, and data analyzed with ABI Genescan and Genotyper. Data was stored in our colony management database.

**Chronic phenotyping protocol.** Experiments were performed in control SS and SS-13BN and congenic rats (n = 10-15 for each sex of each strain). Animals were anesthetized with an i.m. injection of ketamine (40 mg/kg), xylazine (2.5 mg/kg) and acepromazine (0.6 mg/Kg) and a microrenathane catheter was implanted in the left femoral artery for measurement of arterial blood pressure. The catheter was tunneled to the back of the neck and brought out through a stainless steel spring and attached to a swivel device. After surgery, the rats were given 10
mg/Kg Baytril to prevent infections and 1 mg/Kg Buprenex to prevent pain. The rats were placed in stainless-steel metabolic cages and food and water were provided *ad libitum*. After a 2 day recovery period, heart rate, systolic, diastolic and mean arterial pressure (MAP) were recorded between 9 AM and 1 PM on 3 consecutive days and averaged. A urine sample was collected for 24 hours during the second day of the blood pressure recording for measurement of urine volume, sodium and potassium and total protein and albumin excretion.

Statistics: Mean values ± SE are presented and significance of differences in corresponding values between SS, SS-13BN and congenic rats were determined by analysis of variance followed by a Dunnett’s posthoc test (SigmaStat ver 2.03, SPSS Inc., Chicago Ill).
Results

Figure 1 shows a schematic representation of the 23 overlapping congenic strains from the SS-13$^{BN}$ consomic phenotyped in the present study. The size of the congenic regions ranged from 4.6 Mbp (congenic strain 9) to 50 Mbp (congenic strain 4). Some of the congenic strains covered large regions of the chromosome enabling evaluation of possible gene-gene interactions. Other strains covered small regions of chromosome 13 representing a possible step towards positional cloning of the genes involved in the development of hypertension.

As expected, the SS strain developed hypertension after 2 weeks of high salt diet (181 ± 2.9 mmHg in male and 177 ± 5 mmHg in female rats). Substitution of the entire chromosome 13 from the BN rat in the SS-13$^{BN}$ consomic greatly attenuated the development of hypertension after high salt diet, in both male and female rats (142 ± 1.3 mmHg and 137 ± 1.9 mmHg in male and female consomic rats, respectively, p<0.05 from SS), validating our previous observations (6).

The average MAP values for control and 23 congenic strains are detailed in Table 1. In male rats there were six congenic strains (lines 1, 2, 5, 10, 17 and 18) in which MAP was significantly lower than the SS strain after two weeks on high salt diet (Figure 2a). In contrast, in the female congenics a total of 11 strains exhibited lower MAP than the SS strain (Figure 2b). Although there were no sex differences in blood pressure in the control SS and SS-13$^{BN}$ strains, there were some congenic strains which exhibited significant differences between male and female rats. In some congenic strains the values of blood pressure were higher in the males than in the females, suggesting an interaction between gender and genetic determinants of blood pressure salt sensitivity in the
congenic regions. The specific congenic strains that showed sexual dimorphism in the development of hypertension were strains 1, 4, 7, 8, 9, 10, 11, 13, 16, 19, 22 and 26 (see Table 1).

Four discrete non-overlapping regions were identified in female congenic rats that had an effect reducing MAP. These are represented by congenic strains 1 (12.4 Mbp), 5 (14 Mbp), 9 (4.5 Mbp) and 26 (16 Mbp). Each of these congenic strains independently provided protection from salt-induced hypertension, and reduced blood pressure by 22 to 32 mmHg compared to the control SS female rats. Figure 3 represents a schematic of the different congenic strains showing protection from hypertension. Two of the four congenics/regions identified in the female congenics were also evident in the male congenic population (congenic lines 1 and 5). Nevertheless, the congenic strains harboring the renin gene (congenic strains 7, 8 and 9), and congenic line 26 showed no differences in blood pressure from the SS rat in the male rats, although they did exhibit protection from hypertension in the female rats.

When two or three of the “protective” regions were combined on one broad congenic strain of female rats (Figure 3), no summation effect was observed in congenic strains 4, 7 and 11, which obtained a similar decrease in blood pressure as with each smaller congenic strain independently (Table 1). This suggests that there is an epistatic interaction among these regions in the control of blood pressure.

Epistasis was more dramatically illustrated by the fact that the decrease in blood pressure achieved by the some of the smaller congenic strains was comparable to that observed in the consomic strain, which carries the entire chromosome (and therefore all four regions) from the BN rat. The sum of effects of each individual strain was much
greater than that obtained when the four regions were combined, and even greater than
the consomic rat (*Figure 4*). These observations indicate the existence of epistatic
interactions between the different genetic regions of chromosome 13 that affect blood
pressure. There are a number of possibilities that can explain this observation. Blood
pressure regulation is a complex, multi-factorial trait that is regulated by a variety of
physiological systems. Many pathways have been identified that can alter blood pressure
regulation, in particular those impacting the kidney. In the SS rat, alterations in renal,
vascular, nervous, and cardiac function have been described, each of which could
independently impact blood pressure. Connections between each of the congenic regions
and these independent intermediate effectors of blood pressure would provide a
mechanism by which apparent epistatic interactions could occur. Epistasis occurring
through a direct gene-gene interaction at two or more loci has been observed in a number
of studies of complex phenotypes and has been proposed to be a common feature in
human disease (26). Given the extent of the congenic regions in this study and the
number of genes in each of the regions, such interactions are likely. However it is
unlikely that these direct gene-gene interactions are responsible for the observed
phenomenon since there is certainly not one final common biochemical pathway for
blood pressure reduction. Instead it is most likely that given the central role of the kidney
in blood pressure regulation, the observed epistatic effect is determined by genes that
commonly alter intrinsic or extrinsic structure-function relationships in the kidney’s
ability to excrete sodium and water. Appendix 1 lists the genes located within the four
regions identified in our study. We used GeneInfo, a literature data mining tool that
identifies genes associated with specific disease, to search Pubmed for genes related to
blood pressure regulation and/or hypertension (48), and we found that from the 361 genes in the region, only a handful of genes have been related to blood pressure or hypertension (none in congenic line 1; *Cxcr4* and *Ctse* in congenic line 5; *Ren1, Adora1, Adipor1* and *Rnpep* in congenic line 9, and *Pl2g4a, Ptgs2, Fmo3* and *Sele* in congenic line 26). It is notable that within the strain #1 congenic region there are no genes that are known to influence arterial blood pressure. This is consistent with our understanding that the complex trait of arterial blood pressure is indeed importantly influenced by a variety of biochemical and cellular signaling pathways that can ultimately influence vascular, cardiac, neural, endocrine and renal functions. The absence of known connections of any of the genes within this congenic to arterial pressure emphasizes that there is considerably more to learn about the complex biochemical and cellular regulation of cardiovascular function.”

Albumin excretion was also measured in parental and congenic strains after two weeks of high-salt diet (*Table 2*). Higher albumin excretion was observed in the SS compared to the SS-13<sup>BN</sup> consomic, in both male and female rats. None of the congenic strains showed a significant reduction in albumin excretion compared to the SS rat, despite the fact that blood pressure was reduced in many of the congenic strains. A clear sex effect in this parameter was observed however, as albumin excretion was much higher in males than in females in the parental SS and in 17 of the 23 congenic strains (*Table 2*); but not in the SS-13<sup>BN</sup> consomic strain where albumin excretion in both male and female was low.
We also measured sodium excretion to assure ourselves that all rats had similar food intake, and therefore similar salt loading. There were no differences among the strains, within gender, in this parameter.
Discussion

Previous studies indicate that transfer of chromosome 13 from BN to SS rats greatly attenuates the development of hypertension and renal disease (6) but the genomic regions involved remain to be determined. In the present study, we developed and phenotyped a series of 23 overlapping congenic strains across the entire length of chromosome 13, to narrow the region containing the genes that protect against the development of hypertension during a high salt diet.

The four narrow regions on chromosome 13 identified in our study, represented by congenic strains 1, 5, 9 and 26, each show an effect on blood pressure salt sensitivity, and represent genomic regions that range in physical size from 4.6 to 16 Mbp. The renin region, represented by strain 9 (4.6 Mbp), has been identified in previous studies to be related to blood pressure (20, 33, 36). Although renin is an obvious candidate for this region due to the important role that the renin-angiotensin system plays in the regulation of blood pressure, the role of the renin gene in the development of hypertension in the Dahl S rat has been an issue of controversy. Since Rapp et al initially reported linkage of the renin gene to blood pressure in the Dahl S rat, (36), the renin region has been linked to the development of hypertension in other hypertensive strains (10, 33) and some human populations (1, 12), although there is no evidence of functional sequence variants within the renin gene itself (8, 15, 40, 47). The present study implicates a role for the region around the renin gene in the development of hypertension in female rats, but this region was not protective in males.

The genomic region identified in the p end of the chromosome (congenic strain 1) and the chromosomal region above renin (congenic strain 5) have not previously been
reported to be related to blood pressure in any rat strain. The region of chromosome 13 above renin corresponds, by comparative mapping, to a region in human chromosome 2 that has been linked to systolic blood pressure in the Quebec Family Study (38), and in a sib-pair study as part of the Family Blood Pressure Program (19). Also, a homologous region corresponding to congenic strain 1 has been found to be associated with blood pressure in two different human study populations (18, 32).

With respect to the region found toward the q-end of the chromosome, represented by congenic strain 26, there have been previous studies where QTL for blood pressure spanned this genomic area. For instance, SS/Rapp rat by Zhang et al (47) found that when this region of chromosome 13 from the Dahl R rat was introgressed onto the genomic background of the SS/Rapp rat, there was a 24 mm of Hg attenuation in blood pressure sensitivity to a high salt diet. Also, a QTL for blood pressure was found in this region in a cross between the Lyon Normotensive and the Lyon Hypertensive strains (10), although the peak of this QTL was located at the renin gene. QTL covering the region have also been found in crosses from SHR and WKY rats (39), SHR and diabetic BB rats (17). It is interesting and relevant that the region of congenic strain 26 on chromosome 13 corresponds to a genomic region in human chromosome 1 which has been found to harbor several blood pressure-related QTLs in French Canadian (14) and African American populations (16).

Linkage analysis has very little power to detect QTL adjacent to each other within a genomic region. One of the most important findings of the present study is that the congenic strains have enabled the clear detection of four separate genomic regions involved in blood pressure during a high salt diet on chromosome 13 of the SS rat. While
the phenotypic effect of the four genomic regions defined by the congenic rat strains 1, 5, 9 and 26 was similar (in the female population), the genes responsible are obviously different. Moreover, the sum of the protective effects of each of the discrete regions of Chr 13 (-116 mmHg) was greater than the protective effects of the whole consomic SS-13\textsuperscript{BN} strain (-40 mmHg), as seen in the female rats (\textit{Figure 4}). This suggests the existence of epistatic interactions between these genomic regions in the regulation of blood pressure. Gene interactions in the regulation of blood pressure was initially suggested by Deng et al\cite{7}, and later confirmed in several other studies, mainly by the same group\cite{4, 11, 25, 31, 35}.

The present congenic rat study indicates that there are multiple regions that can attenuate the development of hypertension in SS rats. These observations differ markedly from previous QTL analysis studies. Indeed there has been considerable controversy about the localization of blood pressure loci on chromosome 13 in the Dahl S rat. The initial linkage analysis that launched the search for genes of hypertension was a study by Rapp et al in 1989 indicating that the renin gene co-segregated with blood pressure in a cross of Dahl S and R rats\cite{36}. A previous linkage study performed by our group, in an F2 population derived from SS and BN rats, failed to find significant linkage for blood pressure on chromosome 13 in either male or female rats\cite{27, 41}. However, in a subsequent study, we found that transfer of chromosome 13 from BN to SS had a very large protective effect on salt-induced hypertension\cite{6}. In the current study, at least four loci located in neighboring regions of chromosome 13 were found (see Figures 1 and 4). In the initial F2 linkage study, the presence of several QTL segregating at other chromosomes could have reduced the power for QTL detection of interval mapping\cite{2}.
We hypothesize that multiple loci within a single chromosome could reduce the ability of a linkage analysis to predict their exact location, and would require very large numbers of rats to achieve adequate recombination. This was tested during the process of developing the overlapping congenic strains for this study. We performed a linkage analysis for blood pressure in an F2 population (n=187 rats phenotyped) derived from backcrossing the SS-13\textsuperscript{BN} consomic rats with SS rats (unpublished observations). In this F2 population, the whole genome was SS and segregation of alleles could only occur on chromosome 13. This linkage analysis resulted in only a single, albeit broad but highly significant QTL (LOD score of 9.41), with its peak at 45 Mbp. This broad QTL with 95% confidence limits of nearly 60 Mbp fell over the margins of congenic strain 26, but failed to detect the other three loci on chromosome 13. This data suggests that QTL mapping, although useful in the initial detection of genetic regions linked to a phenotype/disease, can lack precision when multiple loci are located nearby on a chromosome.

**Influence of gender on the development of hypertension and renal injury.** There were sex differences in the effect of some genomic regions on blood pressure. The most noticeable was the lack of effect of the renin region in male rats, while significant attenuation of blood pressure during a high salt diet was found in the female rats. Although a mechanistic study of this phenomenon is beyond the scope of this present study, it suggests that some genes affecting blood pressure are sex specific, and that sex should be taken into account in linkage and association studies. These observations are consistent with sex-specific gene effects that have been described in linkages studies with
the SS rat (27, 41), and in other rat models of hypertension like the Sabra (46), or the Genetically Hypertensive rat (5). In humans, association and genetic linkage performed for different complex diseases, including hypertension, have also been found to be sex specific (30, 45).

Protein excretion follows blood pressure in susceptible strains, but it is known to be also determined by different genetic loci (27, 41). The SS-13^BN consomic strain showed both reduced blood pressure and protein excretion; however to our surprise, none of the congenic strains evidenced a significant reduction of albumin excretion, including those that had a decreased blood pressure with respect to the SS strain. The reason for this is not apparent, although one can speculate that a combination of genes maybe at opposite locations in chromosome 13 may be needed to protect the kidney from renal damage. It is possible that the necessary gene combination was not achieved in the present study with these overlapping congenic strains.

In summary, we have confirmed that development of overlapping congenic strains is a very powerful tool for narrowing genomic regions containing blood pressure related genes. We have identified four distinct regions in chromosome 13 containing genes that participate in the regulation of blood pressure during high salt diet. These regions act epistatically in the regulation of blood pressure with two of them being sex specific. This could provide an explanation of why it has been so difficult in the past to map blood pressure QTLs to this chromosome. Finally, it should be emphasized that although these studies were completed in strains derived from SS and BN rats, these QTL may translate to humans, providing a guide for candidate regions to look for disease mutations.
Acknowledgments

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**Figure and Table Legends**

**Figure 1.** Schematic representation of the overlapping congeneric strains developed, in which segments of various lengths of chromosome 13 from the Brown Norway rat (black bars) were introgressed in the genetic background of the SS rat, by marker assisted breeding. Delimitation of the congeneric regions is defined by the first outward SS marker allele. Thin bars represent chromosome crossover regions.

**Figure 2a.** Mean arterial pressure (mmHg) values after 2 weeks high salt for male SS, SS-13\textsuperscript{BN} consomic, and overlapping congeneric strains of chromosome 13. *p*<0.05 vs SS strain.

**Figure 2b.** Mean arterial Pressure (mmHg) values after 2 weeks high salt for female SS, SS-13\textsuperscript{BN} consomic, and overlapping congeneric strains of chromosome 13. *p*<0.05 vs SS strain.

**Figure 3.** Schematic representation of the overlapping congeneric strains for chromosome 13, with bars representing the BN allele regions. Four regions for blood pressure were identified in females, represented by congeneric strains 1, 5, 9 and 26. Black bars indicate congeneric strains that showed no reduction in blood pressure. Grey bars represent congeneric strains in which protection from salt-induced hypertension was observed in both males and females (hashed bars) or was gender specific (solid bars).

**Figure 4.** Impact on salt-induced hypertension of the 4 congeneric regions on chromosome 13 of the BN rat introgressed into the background of the SS. *p*<0.05 compared to SS.
Table 1: Blood pressure in male and female congenic and parental strains after two weeks of high-salt diet. Statistics for sex difference within strain presented. * p<0.05 compared to SS within sex. # p<0.05 compared to female within a strain.

Table 2: Microalbuminuria in male and female congenic and parental strains after two weeks of high-salt diet. * p<0.05 compared to SS within sex. # p<0.05 compared to female within a strain.

Appendix 1: List of known genes located in the four candidate regions for blood pressure regulation. Highlighted are the genes that have been related to blood pressure and/or hypertension.
Figure 2b

Females

Mean Arterial Pressure (mmHg)

Strain

SSBW13 11 17 26 45 78 10 9 215 19 20 27 22 23 39 414 18
Figure 3
**Figure 4**

<table>
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<tr>
<th>Females</th>
<th>Males</th>
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<tr>
<td>SS = 177 mmHg</td>
<td>SS = 181 mmHg</td>
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<tr>
<td>SS-13$^{3N}$ = 137 mmHg</td>
<td>SS-13$^{3N}$ = 142 mmHg</td>
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<tr>
<td>$\Delta$ 40 mmHg*</td>
<td>$\Delta$ 39 mmHg*</td>
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</table>

- **Congenic strain 1** (12.4 Mbp): 148 (Δ 29)* mmHg
- **Congenic strain 5** (14 Mbp): 146 (Δ 31)* mmHg
- **Congenic strain 9** (4.5 Mbp): 146 (Δ 31)* mmHg
- **Congenic strain 26** (16 Mbp): 155 (Δ 22)* mmHg

$\Delta$ 113 mmHg

- **Males**
  - 165 (Δ 16)* mmHg
  - 159 (Δ 22)* mmHg
  - 171 (Δ 10) mmHg
  - 172 (Δ 9) mmHg

$\Delta$ 57 mmHg
### Table 1

<table>
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<th>Male MAP (mmHg)</th>
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<td>SS-13BN</td>
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<tr>
<td>SS</td>
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<td>181 ± 2.9</td>
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* = p < .05 from reconstructed SS control strain; within gender
# = p < .05 from female within strain
Table 2

<table>
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<th>Male Microalbuminuria (mg/day)</th>
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*=p<.05 from reconstructed SS control strain; within gender
#=p<.05 from female within strain
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