Published in final edited form as:
Hypertension. 2013 September ; 62(3): . doi:10.1161/HYPERTENSIONAHA.113.01708.

Female-specific hypertension loci on rat chromosome 13

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Abstract

A 3.7 Mb region of rat chromosome 13 (45.2–49.0 Mb) affects blood pressure (BP) in females only, indicating the presence of gender-specific BP loci in close proximity to the *Renin* locus. In the present study, we used a series of Dahl salt-sensitive/Mcwi (SS)-13 Brown Norway (BN) congenic rat strains to further resolve BP loci within this region. We identified 3 BP loci affecting female rats only, of which the 2 smaller loci (line9$_{BP3}$ and line9$_{BP4}$) were functionally characterized by sequence and expression analysis. Compared with SS, the presence of a 591 Kb region of BN chromosome 13 (line9$_{BP3}$) significantly lowered BP by 21 mmHg on an 8% NaCl diet (153±7 vs 174±5 mmHg, $P<0.001$). Unexpectedly, the addition of 23 Kb of BN chromosome 13 (line9$_{BP4}$) completely erased the female-specific BP protection on 8% NaCl diet, suggesting that BN hypertensive allele(s) reside in this region. The congenic interval of the protective line 9F strain contains 3 genes (*Optc*, *Prelp*, and *Fmod*) and the hypertensive line 9E contains 1 additional gene (*Btg2*). Sequence analysis of the 2 BP loci revealed a total of 282 intergenic variants, with no coding variants. Analysis of gene expression by RT-qPCR revealed strain- and gender-specific differences in *Prelp*, *Fmod*, and *Btg2* expression, implicating these as novel candidate genes for female-specific hypertension.

Keywords

Hypertension; Genetics; Gender; Blood Pressure; Kidney

Introduction

Hypertension risk is defined by interaction of both environmental and genetic factors.[1] Gender has historically been regarded as a covariate, but recent studies have begun analyzing male and female populations separately, and found that some genetic variants confer susceptibility to hypertension in a gender-specific manner.[2–7] Such variants may define genetically and etiologically distinct subgroups of men and women with hypertension and have implications for rational selection of gender-specific treatments. However, reports on female cohorts are strikingly limited and greatly restricted by population sample size, emphasizing the need for additional strategies to identify gender-specific loci.

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Disclosures: No

In a previous study,[8] we used congenic mapping to identify gender-specific blood pressure (BP) loci on rat chromosome 13. This revealed a 3.7 Mb region of BN chromosome 13 (45.2–49.0 Mb) that reduced BP by 20 mmHg in females only, indicating the presence of female-specific BP allele(s).[8] This SS-13^{BN} congenic strain (referred to as line 9) contains 83 genes in the 3.7 Mb congenic interval (chr13:45.2–49.0 Mb), including the BP mediator *Renin*.[8] In the present study, we refined the line 9 congenic interval to 3 female-specific BP loci and have functionally characterized the 2 smallest QTL (line9_{BP3} and line9_{BP4}), which contained a total of 4 genes (*Btg2*, *Prelp*, *Fmod*, and *Optc*). Although no nonsynonymous coding variants were identified, we detected strain- and gender-specific differences in *Btg2*, *Prelp*, and *Fmod* expression. Collectively, we have identified specific candidate genes for BP that differentially impact salt-sensitive hypertension in females only.

Materials and Methods

Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin (MCW). Full descriptions of the 5 rat strains used in this study (Figure 1) are provided in the Expanded Materials and Methods.

Blood Pressure Measurement

Mean arterial pressure (MAP) was measured by telemetry transmitter implantation with a catheter inserted into the abdominal aorta, as described previously.[9]

Measurement of Albumin Excretion

After 16 days of 8% NaCl diet, rats were acclimated in metabolic cages for 24 hours, followed by a 24-hour urine collection and measurement of albuminuria, as described previously.[9]

RT-qPCR

Total RNA from renal cortex and medulla of SS and SS-13^{BN} congenic rats fed 0.4% NaCl (low salt) or 8% NaCl (high salt) diets for 7 days (n=4–6 per group) was synthesized to cDNA and transcript expression measured using an ABI HT7900 Real-Time machine (Applied BioSystems, Foster City, CA), as described previously.[10] Primers are listed in Table S1.

Inflammation and Fibrosis RT-qPCR Array

Inflammatory and fibrotic gene expression was examined using a rat inflammatory cytokines and receptors RT2 Profiler PCR Array (PARN-120ZE-4, SABiosciences, Fredrick, MD), according to the manufacturer's protocol.

Sequence Analysis

Genomic DNA sequence of BN (rn4 assembly) and SS/JrHsD/Mcwi were accessed from the RGD website and analyzed using, as described previously.[9] Sequence analysis was performed using TargetScan,[11] Variant Effect Predictor,[12] Polyphen2,[13] TRANSFAC, and MatInspector softwares.

Statistical analysis

Statistical analyses were performed using Sigma Plot 12.0 software. Data are presented as mean ± SEM. BP data were analyzed by 1-way ANOVA on Ranks followed by the Dunn's post-hoc test. Albuminuria data were analyzed by 1-way ANOVA followed by the Holm-

Sidak post-hoc test. Gene expression was analyzed by 1-way ANOVA followed by Tukey's post-hoc test.

Expanded Materials and Methods are provided in the online-only Data Supplement.

Results

Blood Pressure

A series of SS-13^{BN} congenic rat strains were phenotyped for MAP and urinary albumin excretion as depicted in Figure 1. After 21 days on 8% NaCl diet, the MAP of line 9 (173±7 mmHg, n=11), line 9C (169±6 mmHg, n=21), line 9E (187±6 mmHg, n=11), and line 9F (180±7 mmHg, n=12) male rats were not significantly different from SS (Figure 1). In contrast, after 21 days on 8% NaCl diet, the MAP of female rats from line 9 (149±7 mmHg, $P<0.05$, n=11), line 9C (152±3, $P<0.05$, n=28), and line 9F (153±7 mmHg, $P<0.05$, n=9) were significantly lower than line 9E (186±3 mmHg, n=8) and SS (174±5 mmHg, n=13) (Figure 1). The decreased MAP in line 9F females, but not line 9F males, indicates that female-specific BN protective allele(s) reside in the 591 Kb line 9F interval (chr13: 46,420,127–47,010,821 bp). Elevated BP in line 9E females suggests that BN hypertensive allele(s) are located within the 23 Kb region (chr13:47,008,948–47,031,810 bp) that differentiates line 9E from line 9F (Figure 1). However, because line 9E males were also hypertensive, it is not possible to establish whether the hypertensive BN allele(s) within the 23 Kb region are gender-specific based on BP alone. A protective locus also likely exists within the 2.0 Mb region (chr13:47.0–49.0 Mb) that lowered BP in line 9C.

Renal Damage

To assess renal damage, urine was collected from SS and SS-13^{BN} congenic rats on 8% NaCl diet for 17 days and total urinary albumin excretion was quantified. Similar to BP, male albuminuria in lines 9 (255±49 mg), 9C (242±25 mg), 9E (343±41 mg) and 9F 224±31 mg) were not statistically different from male SS rats (198±36 mg) (Figure 1). Albuminuria in female lines 9 (45±12 mg, $P<0.001$), 9C (70±8 mg, $P<0.01$), and 9F (60±11 mg, $P<0.01$) was lower than SS (135±15 mg; Figure 1), suggesting that renal protection is secondary to significantly decreased BP in females of these congenic lines. In comparison, female line 9E rats had much higher albuminuria (230±32 mg, $P=0.066$) compared with SS (Figure 1), but was just below the threshold for statistical significance. Because line 9E and SS female rats are similarly hypertensive on 8% NaCl diet (186±3 vs. 174±5 mmHg), these data suggest that the BN allele(s) within the 23 Kb region (chr13:47,008,948–47,031,810 bp) may also modify renal damage independently of BP.

Sequence Analysis

Compared with the BN reference, a total of 5,143 variants were previously identified over the entire line 9 congenic interval.[10] Further congenic mapping to narrow the protective 2.0 Mb BN interval (chr13:47.0–49.0 Mb) in line 9C to a more manageable size will be require before a more in depth analysis can be pursued. Therefore, below we have focused our analysis specifically on the smaller $line9_{BP3}$ and $line9_{BP4}$ quantitative trait loci (QTL) defined by the line 9E and 9F congenic strains.

$Line9_{BP3}$—The $line9_{BP3}$ QTL is a 591 Kb protective region of BN (chr13: 46,420,127–47,010,821 bp) that contains 3 genes (*Optc*, *Prelp*, and *Fmod*) and 264 total variants, with no coding variants and none predicted to interfere with 3′UTR. Of note, 10 variants were predicted to interfere with consensus transcription factor binding sites of the putative 5′ promoters of *Optc*, *Prelp*, and *Fmod* (Table S2).

Line9$_{BP4}$—The line9$_{BP4}$ QTL is a 23 Kb hypertensive region of BN (chr13:47,008,948–47,031,810 bp) that contains only *Btg2* and 18 total variants, none of which are located in coding regions of *Btg2* (Table S3). Of note, 9 variants were predicted to change consensus transcription binding sites and 2 variants were located in the 3′UTR, but were not predicted to interfere with any miRNA binding sites (Table S3).

Candidate Gene Expression

The BP and albuminuria (Figure 1) indicate that variant(s) modifying BP in a female-specific manner likely reside in the line9$_{BP3}$ and line9$_{BP4}$ QTLs. However, neither QTL had any predicted nonsynonymous variants within the protein coding regions that could account for BP changes. Instead, we hypothesized that noncoding variants could impact gene expression. To test this possibility, we performed RT-qPCR analysis of candidate gene expression (*Optc*, *Prelp*, *Fmod*, and *Btg2*) in kidneys of SS, line 9E, and line 9F congenic strains on low salt diet (0.4% NaCl) and after 7 days on high salt diet (8% NaCl). After 7 days of high salt diet, BP was not significantly different between strains (Figure S1), thus representing an early stage of the development of hypertension, at which point the underlying mechanisms can be assessed independently of BP changes.[14]

Line9$_{BP3}$—The line9$_{BP3}$ QTL significantly decreased BP in line 9F females, but not males (Figure 1). Line9$_{BP3}$ contains 3 validated genes (*Fmod*, *Prelp*, and *Optc*) that do not have nonsynonymous changes. *Optc* is a class III small leucine-rich repeat protein (SLRP) that is largely restricted to the eye[15] and has no reported role in BP or renal function. Based on this, and the relatively low *Optc* expression in the kidney that did not change in females, we have deprioritized *Optc* on the list of candidate genes. Compared with SS, both male and female line 9F rats on low (0.4% NaCl) and high (8% NaCl) salt diets had significantly decreased medullary expression of Prelp (male: −81 to −87% vs SS; $P<0.001$ and female: −83 to −89% vs SS; $P<0.001$) and Fmod (male: −97 to −99% vs SS; $P<0.001$ and female: −96 to −97% vs SS; $P<0.001$) (Table 1). Compared with SS, cortical expression in line 9F males and females also showed similar trends to the renal medulla, except for elevated Prelp expression in the renal cortex of line 9F females on both diets (Table 1).

Line9$_{BP4}$—The line9$_{BP4}$ QTL in line 9E females increased BP by 34mmHg compared with line 9F (Figure 1), indicating the presence of hypertensive BN allele(s). Only *Btg2* resides in the line9$_{BP4}$ congenic interval. In males, *Btg2* expression was not significantly different in any of the groups tested (Table 1). In contrast, baseline *Btg2* expression in renal cortex of line 9E females was 1.9±0.2-fold ($P<0.05$) higher than line 9F, suggesting that BN allele(s) in Line9$_{BP4}$ increase baseline *Btg2* expression independently of BP. After 7 days of 8% NaCl diet, *Btg2* expression significantly increased 2.6±0.2- and 2.9±0.7-fold ($P<0.05$) in the cortex of both lines 9E and 9F, respectively (Table 1), indicating that *Btg2* responds to salt challenge in females only. Similar trends were also observed in the renal medulla, but did not reach statistical significance.

Renin Expression

The renin-angiotensin system (RAS) is widely implicated in gender-specific BP.[16–18] Moreover, BN allele(s) in a SS-13^{BN} congenic that overlaps with lines 9E and 9F was reported to restore normal renin expression/function,[10] prompting us to determine whether renin expression differed in lines 9E and 9F. After 7 days of 8% NaCl diet, renin expression was decreased 60–80% ($P<0.05$) by salt-challenge in all strains tested, but no differences between males or females of either strains were detected (Table S4). This indicates that gender-specific BP differences between line 9E and 9F rats are likely independent of *Renin*.

Tissue Remodeling Pathways

Dysfunction in the renal outer medulla has been suggested as a primary cause of salt-sensitive hypertension in the SS rat, leading to renal damage and fibrosis.[19] Since *Fmod*, *Prelp*, and *Btg2* have been implicated in tissue remodeling,[20–25] we hypothesized that the differences in candidate gene expression would coincide with downstream changes in inflammatory and fibrosis pathways that mediated tissue remodeling. To test this possibility, we analyzed expression of 88 remodeling genes by RT-qPCR array in the renal medullas of SS, line 9E, and line 9F congenic strains on low salt diet (0.4% NaCl) and after 7 days on high salt diet (8% NaCl).

Line9$_{BP3}$—Compared with SS, 38 out of 88 genes were uniquely downregulated (−1.5 to −61.5-fold) during salt-challenge in the protected line 9F females only (Table S5). The downregulated pathways included metalloproteases (6 out of 7), interleukins (6 out of 7), and members of the TGF-β pathway (12 out of 20) (Table S5), suggesting that multiple factors in tissue remodeling are actively suppressed in the protected line 9F females. Of the 38 genes downregulated in the line 9F females, 26 were also decreased (−1.5 to −13.3-fold) in the unprotected line 9F males during salt-challenge (Table S5).

Line9$_{BP4}$—We also tested whether tissue remodeling pathways were upregulated in the hypertensive line 9E females compared with the protected line 9F. Surprisingly, 24 out of 88 genes were uniquely upregulated (1.5 to 5.6-fold) in line 9E females compared with line 9F females, of which only 1 was shared by the line 9E males (Table S5). Even more strikingly, 18 out of the 24 genes that were upregulated by salt-challenge in line 9E females were actively suppressed in the line 9F females (Table S5).

Discussion

Multiple human[4, 7, 26] and rat[27–36] studies have identified gender-specific BP loci; however, the majority of these have failed to translate to mechanistic discoveries. Previously, we identified a 3.7 Mb QTL on rat chromosome 13 (45.2–49.0 Mb) that attenuated salt-sensitive BP in female rats only.[8] Here, we identified at least 3 QTL that mediated BP in females only (Figure 1). In the 2 smallest congenics (lines 9E and 9F), we reduced the total congenic intervals from 3.7 Mb to 614 Kb and identified 2 BP loci (line9$_{BP3}$ and line9$_{BP4}$), which contained a total of 3 differentially expressed candidate genes (*Fmod*, *Prelp*, and *Btg2*). Based on these findings, *Fmod*, *Prelp*, and *Btg2* are novel BP candidate genes and potential therapeutic targets for female-specific genetic hypertension.

How does gender influence genetic hypertension?

Estrogen and the X chromosome are associated with lower BP,[16–18] but it is largely unknown how these factors interact with genetic loci. Despite this, there is clear indication that some BP loci differ between males and females[4, 7, 26–36] and it is highly likely that several mechanisms exist. One possibility is that promoter response elements (RE) for hormone receptors (e.g., estrogen or androgen receptors) or X-linked transcription factors are disrupted by genetic variants, which modifies gender-specific expression of a candidate gene that influences disease pathogenesis. For example, genetic variants frequently disrupt the estrogen RE in the promoter of the tumor suppressor gene, *BRCA1*.[37] The resulting downregulation of BRCA1 protein then increases breast cancer risk.[38]

Similar to *BRCA1*,[37] our data suggest that *Btg2* expression is likely regulated by estrogen and/or X-linked factors, because changes in *Btg2* expression were specific to females only (Table 1). This fits with previous evidence of *BTG2* promoter regulation by estrogen receptor (ER) response elements.[39–41] By sequence analysis, we also identified multiple

conserved ER response elements in the rat *Btg2* promoter and in close proximity to sequence variants; however, none were predicted to be disrupted by sequence variants. Several other transcription factors (TF) that were predicted to bind the *Btg2* promoter (ERRα, Gata-1/-2/-3, and Myb) also interact with ER signaling,[42–44] suggesting that estrogen could also indirectly regulate the *Btg2* promoter by interacting with other transcriptional machinery. Because ER forms TF complexes (e.g., with ERRα,[44] Gata-1/-2/-3,[42] and Myb[43]), it is also possible that variation in binding sites of these TF in the *Btg2* promoter (Table S3) could influence ER-mediated *Btg2* expression in the kidney. We also detected a putative binding site for the gender-determining transcription factor Sry in the *Btg2* promoter (Table S3). However, although Sry is widely implicated in male hypertension,[18] it is Y-linked and therefore likely not accountable for the female-specific changes in Btg2 expression that we observed in line 9E (Table 1).

Gender might also influence response to the effects of a genetic locus (i.e., hypersensitivity in one gender versus the other). Our data suggest that only line 9F females are protected by low *Prelp* and *Fmod* expression (Figure 1), despite these genes being strongly downregulated in both genders (Table 1). This fits with evidence that multiple mouse knockouts (e.g., COX2,[45] LDLR,[46] and eNOS[47]) and the mRen2.Lewis transgenic rat model[48] show sexual dimorphism in BP and hypertension risk. Although transgenic models do not constitute a "genetic locus" per se, one could argue that these transgenic models demonstrate dimorphic phenotypes based on gender and genotype interactions.

Candidate Genes

Line9$_{BP3}$—Compared with SS, the BN-derived allele(s) in line9$_{BP3}$ significantly decreased *Fmod* and *Prelp* expression in line 9F females (Table), which predisposed only the line 9F females to lower BP and albuminuria (Figure 1). *Fmod* and *Prelp* belong to the class II family of SLRPs, which has been previously implicated in renal damage and fibrosis.[20] *Fmod* is expressed in the peritubular kidney,[23] is elevated during renal fibrosis,[49] and interacts directly with the profibrotic mediator, TGF-β.[50] *Prelp* is also highly expressed in the kidney[50] and in other tissues regulates the inflammatory mediator, NF-κB.[51] Additionally, both *Fmod* and *Prelp* are also implicated in complement pathway activation,[52] which has been associated with increased kidney damage.[53] Collectively, these data support that the downregulation of remodeling pathways in line 9E (Table S5) are likely due to decreased expression of *Fmod* and *Prelp* (Table 1).

Line9$_{BP4}$—*Btg2* is the only gene residing in line9$_{BP4}$ and is elevated in female line 9E rats prior to salt-challenge (Tab1e 1), suggesting that elevated *Btg2* expression predisposes line 9E females to hypertension (Figure 1). *Btg2* is highly expressed in the kidney proximal tubules,[54] but has no previously reported role in BP or renal damage. *Btg2* is a transcriptional co-regulator involved in cell-cycle,[41, 55] apoptosis,[55] inflammation,[24] and fibrosis.[56] In addition to regulation by ER,[24] The *Btg2* promoter is also regulated by NF-κB[24] and p53[57, 58] in response to multiple inflammatory stimuli, including TGF-β[56] and TNF-α.[24] Collectively, these data suggest that similar *Btg2*-dependent mechanism(s) might influence female-specific renal inflammation and fibrosis.

Line 9 is a compound BP QTL

The existence of both line9$_{BP3}$ and line9$_{BP4}$ demonstrate that line 9 is a compound BP QTL. Moreover, the hypertensive line9$_{BP3}$ QTL (*Btg2*) offsets the BP lowering effects of the protective line9$_{BP4}$ QTL (*Fmod* and *Prelp*) (Figure 1), indicating that these are interdependent BP loci. However, at present it is unclear whether line9$_{BP3}$ and line9$_{BP4}$ are merely subtractive, or have an epistatic relationship (i.e., phenotypic manifestation of one variant is dependent on the other).[59] Based on the literature,[24, 50, 51, 56] it is possible that

Btg2, *Fmod*, and/or *Prelp* could indirectly interact through common pathways (e.g., TGF-β, NF-κB, and complement factor). However, epistatic interaction(s) between *Btg2*, *Fmod*, and *Prelp* have yet to be tested empirically.

In addition to line9$_{BP3}$ and line9$_{BP4}$, at least one other BP QTL resides within the original line 9 congenic interval,[8] because the addition of flanking BN genome (chr13:47.0–49.0 Mb) lowered BP (−Δ22 mmHg) in the female line 9C rats compared with SS (Figure 1). This additional BP locus offset the hypertensive effects of line9$_{BP4}$, indicating the existence of potentially 3 epistatic BP loci within a 2.6 Mb interval (chr13:46.4–49.0Mb). Our data also suggest that the third BP locus in line 9C (chr13:47.0–49.0 Mb) is unique to females only. We recently published that at least 2 other BP loci reside in the line 9 congenic interval using male rats only.[60] One BN locus (chr13:47.2–49.0 Mb; referred to as line9$_{BP2}$) was hypertensive and the other BN locus, containing *Renin* (chr13:46.1–47.2; referred to as line9$_{BP1}$), lowered BP in male rats.[60] At present, it appears that the male BP QTL identified previously[60] and the female BP QTL identified here (Figure 1) are likely not shared. Although the protective line9$_{BP1}$ (male) and line9$_{BP3}$ (female) QTL overlapped, the line 9E males were not protective, suggesting that protective BP loci in this region are gender-specific. Likewise, the overlapping hypertensive line9$_{BP2}$ (male) and protective line9$_{BP4}$ (female) QTL had opposite phenotypes, indicating that BP loci in these QTL are very likely not shared. Further congenic mapping, ZFN-mutagenesis, and molecular analysis will be required to *(1)* narrow the protective BP allele(s) in line 9C (chr13:47.0–49.0 Mb) and *(2)* test whether epistasis or purely additive effects regulate overall hypertension risk for females in the line 9 congenic region.

Perspectives

The observed gender differences in BP regulation are longstanding,[18] but the mechanism(s) specific to genetic hypertension in females are largely unresolved. Despite this, the majority of BP studies have focused primarily on male hypertension. Here, we identified a 614 Kb region on rat chromosome 13 that contains 2 female-specific BP loci. Within these loci we identified 3 differentially expressed candidate genes (*Btg2*, *Fmod*, and *Prelp*) that are specific to female BP and offer potential therapeutic targets for treating female hypertension. Future studies will be needed to test the functional roles of *Btg2*, *Fmod*, and *Prelp* in the kidney and potentially other tissues involved in the development of hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Elisabeth Metzger, Elleanor Christenson, Rebecca Schilling, Allison Zappa, Anne Temple, Marc Casati, Nadia Barreto, Jaime Wendt-Andrae, and Michael Tschannen for their technical support.

Sources of Funding

M.J.F. is supported by a National Heart, Lung, and Blood Institute training grant (5T32HL007792). This study was supported by National Heart, Lung, and Blood Institute grant 5P01HL082798 to A.S.G. and C.M.Q.

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Novelty and Significance

1) What Is New?

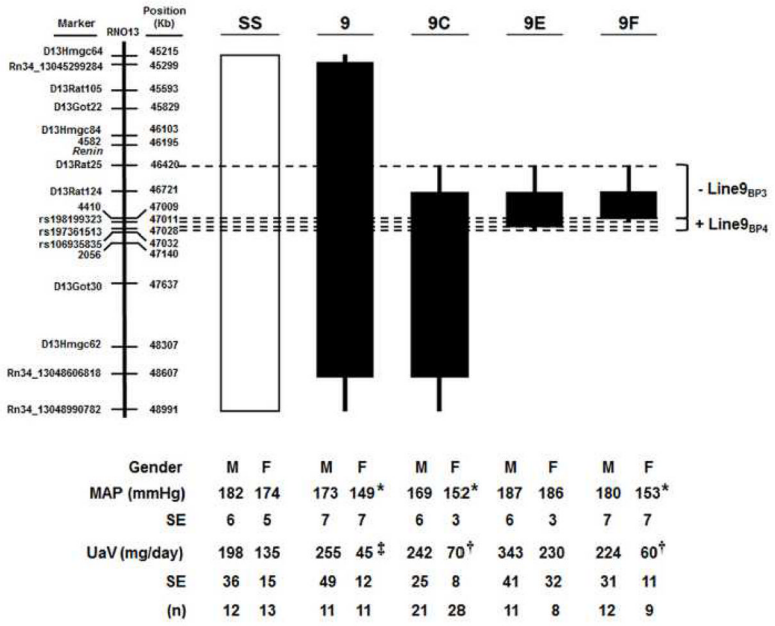
- We identified 3 novel female-specific BP loci: the protective line9$_{BP3}$ BP QTL (chr13:46,420,127–47,010,821 bp), the adjacent hypertensive line9$_{BP4}$ QTL (chr13:47,008,948–47,031,810 bp), and another protective region (chr13:47.0–49.0) that has yet to be refined.
- The BP lowering line9$_{BP3}$ QTL contains 2 candidate genes (*Fmod* and *Prelp*) that were downregulated in both males and females, but attenuated BP in females only.
- The BP elevating line9$_{BP4}$ QTL contains 1 candidate gene (*Btg2*) that was upregulated in females only and increased BP in females only.

2) What Is Relevant?

- Gender differences in BP are long recognized, but the mechanism(s) specific to genetic hypertension in females are largely unresolved.
- Here we identified 2 novel BP loci (line9$_{BP3}$ and line9$_{BP4}$) that contain 3 differentially expressed candidate genes (*Fmod*, *Prelp*, and *Btg2*).
- Although several others have identified larger BP QTL in females with many candidate genes, this is the first report of female-specific BP loci being reduced to 1 or 2 gene resolution.

3) Summary

- We refined the line 9 congenic interval (chr13:45.2–49.0 Mb) to 2 female-specific BP QTL (591 Kb and 23 Kb) that contain a total of 3 differentially expressed candidate genes. We also discovered a third female-specific BP locus (chr13:47.0–49.0 Mb) that will require further congenic mapping to narrow the causative allele(s).



Gender	M	F	M	F	M	F	M	F	M	F
MAP (mmHg)	182	174	173	149*	169	152*	187	186	180	153*
SE	6	5	7	7	6	3	6	3	7	7
UaV (mg/day)	198	135	255	45‡	242	70†	343	230	224	60†
SE	36	15	49	12	25	8	41	32	31	11
(n)	12	13	11	11	21	28	11	8	12	9

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 chromosomal regions that could be BN or SS. *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 8 to 28 animals per group. *P<0.05 vs. SS, †P<0.01 vs. SS, and ‡P<0.001 vs. line vs. SS, as determined by a 1-way ANOVA on Ranks followed by a Dunn's post-hoc test (for MAP) or by a 1-way ANOVA followed by a Holm-Sidak post hoc test (for albuminuria). Data for male line 9C has been published previously.[60]

Table

Candidate Gene Expression in the Renal Medulla and Cortex of SS, Line 9F, and Line 9E Rats on Low-or High-Salt Diets

	Medulla		Cortex	
Gene Groups	**LS**	**7 Days HS**	**LS**	**7 Days HS**
Prelp				
SS(male)	1±0.1	0.7±0.04†	1.4±0.5	0.6±0.1
Line 9F (male)	0.2±0.02*	0.1±0.02*	0.3±0.1*	0.5±0.1
SS (female)	1±0.1	0.6±0.1†	1.5±0.4	2.3±0.2
Line 9F (female)	0.2±0.02*	0.1±0.01*	1.6±0.2	1.9±0.2
Fmod				
SS(male)	1.0±0.1	0.8±0.1	1.1 ±0.2	1.5±0.3
Line 9F (male)	0.01±0.01*	0.03±0.02*	0.3±0.2*	0.5±0.1*
SS (female)	1.0±0.2	2.6±0.8*†	1.3±0.3	1.3±03
Line 9F (female)	0.03±0.01*	0.03±0.01*	0.7±0.2	0.8±0.1
Btg2				
Line 9F (male)	1.0±0.1	1.1±0.1	1.0±0.1	1.3±0.1
Line 9F (male)	1.3±0.2	1.0±0.3	1.2±0.2	1.1±0.2
Line 9F (female)	1.0±0.1	2.1±0.5	1.1±0.2	2.9±0.7†
Line 9E (female)	1.3±0.3	2.1±0.5	1.9±0.2*	2.6±0.2†

Data are presented as mean fold-expression±SEM. Statistical significance was determined by 1-way ANOVA followed by Tukey post hoc test. HS indicates high salt; LS, Low salt, and SS, salt-sensitive.

*Statistically significant between strains.

†Statistically significant within strains.