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Striatin Gene Variants are Associated with Salt Sensitivity of Blood Pressure by Mechanisms that Differ in Women and Men

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Abstract

Background: Salt sensitivity of blood pressure (**SSBP**) is a substantial risk factor for cardiovascular morbidity and mortality. Striatin is critical for estrogen and aldosterone's non-genomic signaling. However, the role of biological sex on the SSBP phenotype associated with striatin gene variants remains unexplored.

Method: Data from 1306 subjects participating in the Hypertensive Pathotype (**HyperPATH**) consortium were used to identify striatin gene single nucleotide variants (**SNVs**) associated with SSBP. Haploblock analysis revealed a novel diplotype in striatin's upstream regulatory region (rs888083 and rs6744560), with 31% of subjects being homozygous for the risk diplotype.

Results: Individuals homozygous for the risk diplotype had significantly greater SSBP than non-risk diplotypes ($p<0.009$). While a significant genotype/SSBP association was present in both sexes, their potential mechanisms differed. Women, but not men homozygous risk diplotypes, had significantly greater aldosterone levels than non-risk diplotypes (5.8±0.4 vs 3.2±0.7 ng/dl, $p=0.01$, liberal Na^+ diet [**LIB**], adjusted). Men, but not women, homozygous risk diplotypes, had significantly reduced renal plasma flow (**RPF**) response to Angiotensin II than non-risk diplotypes (delta 95.2±5.2 vs 122.9±10.2 ml/min/1.73m^2, $p=0.01$, LIB, adjusted). The SNVs composing the risk diplotype were associated with lower striatin mRNA expression in human tissues (*in silico*).

Conclusion: In women, the primary driver of SSBP is increased aldosterone, while in men, it is reduced RPF responses. Thus, despite a common hypertensive phenotype (SSBP) in both sexes, the *specific* treatment approaches might differ to increase therapeutic gain and mitigate adverse

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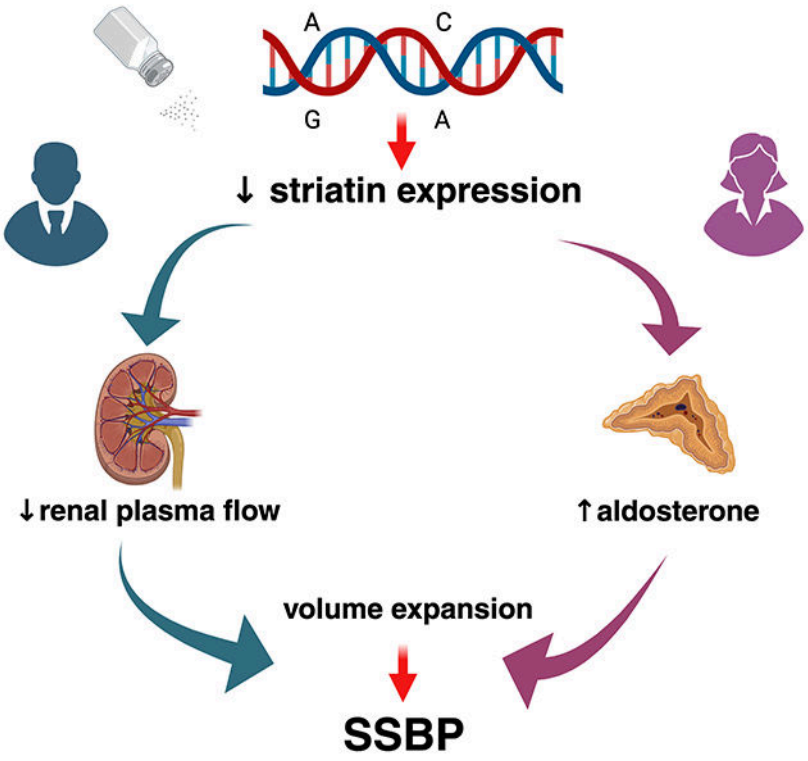
Disclosures

None

effects. These genetic and sex-based observational results require confirmation in a prospective clinical study.

Graphical Abstract



Keywords

Aldosterone; Estrogen; Hypertension; Salt Sensitive Blood Pressure; Renal Plasma Flow

Introduction

Striatin (STRN) is a caveolin-1 binding scaffolding protein that interacts with estrogen receptors (ER) and the mineralocorticoid receptor (MR)[1,2]. Data from our group and others have shown that STRN plays a central role in the interaction between estrogen (E2)-ER and aldosterone (ALDO)-MR. MR and ER require STRN for their rapid, non-genomic effects on ERK1/2 and eNOS phosphorylation in human endothelial cells[1–4]. In addition, we reported an important role in STRN-dependent development of salt sensitive blood pressure (SSBP), increased thrombotic responses, and MR blockade-mediated renoprotection in mice[5–7]. Karas' group documented a role for STRN-dependent, E2-mediated vasoprotection in mice[3]. These preclinical results raise the possibility that STRN may have cardiovascular effects that differ by sex in humans.

We and others have documented that women have increased magnitude and frequency of SSBP than men through mechanisms that are not entirely clear[8,9]. Using a candidate gene approach, we reported that a polymorphism in the STRN gene (rs2540923) in

humans was associated with SSBP[5]. This association was confirmed in a larger cohort of Caucasian, diabetic, hypertensive, and normotensive people[10]. However, studies comparing and contrasting the association of rs2540923 with SSBP in men and women have yet to be reported. Because the minor allele frequency (MAF) of rs2540923 is 4%, the assessment of biological sex on SSBP and the potential mechanisms involved are limited.

The goal of the present study was three-fold: first, to identify if other single nucleotide variants (SNV) within the STRN gene exist that had higher MAF and were informative; second, to characterize sex-specific differences in SSBP in those patients with STRN gene variants; and third, to evaluate potential sex-specific mechanisms underlying the SSBP.

Method

International Hypertension Pathotype (HyperPATH) study design and protocol

Subjects participating in the HyperPATH consortium were consented well before the development of guidelines to promote openness. Therefore, requests for select de-identified study data and analytical methods will be considered case-by-case from qualified researchers with Institutional Review Board approvals and executed Institutional data transfer agreements. The data set will be available from Prof. Gordon H. Williams, Principal Investigator for the HyperPATH consortium, by e-mail request (gwilliams@bwh.harvard.edu). We assessed the role of STRN gene variants in women and men from the HyperPATH cohort. Details have been previously reported [5,8,10–13] and are provided in Supplement.

SSBP Calculation

Systolic blood pressure (SBP) was measured for 30 mins, every 5 mins supine at the end of seven days of a liberal sodium (Na^+) diet (LIB) or a restricted Na^+ (RES) diet as we previously reported[5,8,10]. The average SBP value was used to calculate SSBP as SBP on LIB minus SBP on a RES diet. Protocol details are provided in the Supplement.

Identification of risk STRN haplotype using our previously published data set

In a subset of 366 individuals in the HyperPATH cohort, we reported the genetic structure related to the STRN gene using a candidate gene approach assessing SNVs located within the coding region and 5 kB upstream and 5 kB downstream from it[5]. These analyses identified 40 informative SNVs that passed quality control assessment as previously reported[5]. These were then submitted for haplotype analysis (Haploview program 4.1) with SSBP as the phenotype. The SNVs were divided into three blocks; only one (block 1) had a significant global p-value (0.04). Associations between SSBP and haplotypes within the STRN gene were assessed using PLINK. Only one of the seven haplotypes in block one was significant (p=0.002). The haplotype was comprised of the following 7 SNVs: rs2540923, rs888083, rs11678303, rs6744560, rs7562109, rs10490658 and rs7573966[5]. The rs2540923 was used in the previous two publications[5,10]. However, this SNV has low MAF, which hindered its utility from assessing any associated mechanisms beyond SSBP. Two other SNVs also had low MAF, and two others were not present in our genomic dataset (Table S1). We then assessed the relationship of SSBP to each of the two remaining SNVs

(rs888083 and rs6744560 with 41% and 34% MAF frequency, respectively). The trend analysis with SSBP was p=0.056 and p=0.041, respectively. The linkage disequilibrium analysis between the two SNVs was 60%. The relationship between SSBP and a diplotype of the two SNVs was significant (p=0.011).

Identification of the risk STRN haplotype in our new genomic data set

For the current study, the genomic data were determined in 1,306 individuals in the HyperPATH cohort (see Supplement). We used the MEGA (multiethnic genotyping array) Illumina BeadChip (Illumina, Inc, San Diego, California, USA), arrayed to 1.7 million genetic markers across the human genome. We extracted 32 SNVs in the region of the STRN gene and 5 kB downstream and upstream from the coding region. After quality control of the data using 3 filters (MAF<0.01; genotype-rate>95%; and Hardy-Weinberg test), we then performed haplotype analysis, as described above, for the new genomic data set using PLINK.

In silico analysis of rs888083 and rs6744560

Three approaches to potentially identify the functionality of the SNVs (e.g., eQTLS, mRNA levels, transcription factors) were used – GTEx portal[14], Haploreg[15], and Unibind[16]. Details are provided in the Supplement.

Statistical Analyses

All statistical analyses were performed using STATA version 17.1. Baseline analyses of demographic information were done using one-way ANOVA. In addition, we performed a multivariate linear regression model on the association between the diplotype STRN gene and outcome variables. The model was adjusted for age, sex, body mass index (BMI), disease status (hypertensive, normotensive, and diabetic), race (Caucasians and African descent), and study sites. To create the diplotype structure, we combined the three genotypes of each candidate SNV into three groups: **G0**, when individuals had no risk allele from both SNVs; **G1**, when individuals carried one risk allele from both SNVs; **G2**, when individuals were homozygous for the risk allele from both SNVs. Data are presented as least square mean and standard error unless stated otherwise. The main objective was to assess the effect of risk diplotype carriers compared to non-risk diplotype carriers. Hence risk diplotype carriers (G1 and G2) are only compared with non-risk diplotype carriers (G0), and no comparison was made between homozygous and heterozygous risk diplotype carriers. Because of multiple comparisons, p<0.025 is considered statistically significant.

Results

Haplotype analysis using SNVs at STRN region

Haplotype analysis, performed using SNVs at the STRN gene region, revealed four blocks within the STRN gene (Figure S1). Block one, which contained 16 SNVs, was the only block with a significant association with SSBP (global p-value = 0.026). This block includes ten haplotypes, two significantly associated with SSBP (CTAGTGGGTC**A**CTCT**C**, p=0.0095; CTAGTGGGTC**G**CTCT**A**, p=0.012). The two candidate SNVs (rs888083 [**A**, **G**], rs6744560 [**C**, **A**]) were in both haplotypes but present in opposite pairs. Notably, the

beta for each haplotype was the opposite – one negative and one positive. These were the only haplotypes in this block with this structure for our two candidate SNVs (Table S2). Moreover, in our haplotype regression model, these two SNVs (with frequencies of 0.28 and 0.46, respectively) account for 40% of the variance associated with SSBP.

Risk SNVs and diplotype associated with SSBP

Using multivariate linear regression analysis (adjusted for sex, age, race, disease status, BMI, and site), we assessed the association between each SNV and our primary outcome, SSBP. With SNV rs888083, the genotype was not significant (p=0.09; Figure 1A). With SNV rs6744560, it was (p=0.049; Figure 1B). However, when we performed the analysis with the three diplotypes in the model [homozygous non-risk diplotype (G0), heterozygous risk diplotype (G1), and homozygous risk diplotype (G2)], there was a significant relationship between diplotype and SSBP (p=0.017; Figure 1C). Furthermore, the SSBP in G2 diplotypes was highly significantly different than in G0 (p=0.009; Table 2)

Demographic characteristics of the diplotypes

Of 1306 individuals in the genomic dataset, 924 carried the diplotype, and 606 subjects had available data for our primary outcome, SSBP. The demographic characteristics of individuals in the three diplotype groups are presented in Table 1. The target population comprised 46% women, 20% individuals of African descent, and 64% of individuals diagnosed with hypertension. No significant differences among the three diplotypes were observed for sex, race, disease status, age, and BMI.

Our haplotype linear regression model (adjusted for sex, age, race, disease status, BMI, and site) accounted for 18% of the variance of SSBP in our population. Further, G1 and G2 had significantly greater SSBP than G0, with β coefficients of 3.63 and 4.36, respectively (Table 2). Thus, these diplotype results confirm the previous findings that STRN gene tagging SNV variance was associated with SSBP [5,10]. We then assessed the relationship between haplotype and SBP, ALDO, plasma renin activity (PRA), and ALDO/renin ratios in the entire population. No significant differences were observed. In contrast, the relationships between SSBP and sex, disease status, and age, but not race, were significant (Table S3). As anticipated, SSBP was significantly higher in hypertensives than normotensives and in older vs. younger individuals. However, it was only in individuals less than 50 years of age where the haplotype effect was significant (G0 vs G2, p=0.011). In older individuals (≥50 years of age), there was no haplotype effect (G0 vs G2, p=0.55).

In the main regression model, the effect of sex was highly significant (p<0.0001) with a β regression coefficient of 4.22. We then separately assessed the relationship between SSBP and diplotype status in men and women. In men, compared to the G0 no-risk haplotype group, men in the G2 homozygous risk diplotype groups had greater SSBP (β coefficient 4.65, p=0.018 [Figure 2A]). Women exhibited a similar trend as men with a significant genotype effect between G0 and G2 (β coefficient 4.79, p=0.025 [Figure 2B]). Further, women homozygous risk diplotypes had greater SSBP than men homozygous risk diplotypes (p=0.02, t-test).

Potential mechanisms associated with the SSBP and STRN risk diplotypes

Maladaptive responses to a sodium load by one or more volume homeostatic pathways have been proposed to cause SSBP[12]. We investigated two of these: altered renal plasma flow (RPF) and dysfunction in the Renin-Angiotensin-Aldosterone System (RAAS), particularly ALDO.

Evaluation of ALDO levels indicated that G2 women had higher baseline ALDO on LIB Na^+ than G0 with β coefficient of 2.07 (p=0.014) (Table 3). The differences in LIB Na^+ ALDO levels were not mediated by differences in PRA or serum cortisol (a surrogate for ACTH) or serum or 24-hour urine potassium levels. However, there were no significant differences in ALDO levels in men between either G1 or G2 haplotype groups and the G0 group (Table 3).

RPF based on p-aminohippurate (PAH) clearance was assessed basally and in response to in vivo angiotensin II (ANGII) stimulation on the LIB and RES Na^+ diets. Men diplotype G0 subjects had a significant (p=0.02) increase in RPF when dietary Na^+ intake was changed from RES to LIB. Men in G1 and G2 groups and women in all three groups did not (Table 4). Thus, in men, the absolute change in RPF in G1 was nearly 75% less compared to G0 and more than 50% of that in G2. Both men and women in all three diplotype groups have significant (p<0.01) reductions in RPF in response to ANGII. Additionally, the response in men in the diplotype G0 group was significantly greater than in the G1 group (p=0.017) and G2 group (p=0.035; Table 4).

Sex-genotype interaction

Next, we assess the interaction between biological sex and STRN diplotype. For SSBP, the interaction was not significant (between G0 and G1 [p=0.25] and between G0 and G2 [p=0.12]). However, on the LIB Na^+ diet, robust sex-diplotype interactions were observed for baseline plasma ALDO (between G0 and G1 [p=0.023] and between G0 and G2 [p=0.015]) and RPF (between G0 and G1 [p=0.035] and between G0 and G2 [p=0.018]).

SNV in silico analyses

In this study, we employed three different in silico approaches to assess the candidate SNVs association with SSBP: GTEx, Halporeg, and Unibind. First, using GTEx, we evaluated the expression levels of our two candidate SNVs (rs888083 and rs6744560) in various human tissues. The risk allele of either SNV exhibited significantly lower STRN expression levels than the non-risk allele (Table S4). Second, using Haploreg v4.2 to explore annotation of these SNVs, rs6744560 changed the YY1 motif and had three enhancer histone marks, none of which were associated with pathways that might affect RAAS or blood pressure. However, rs888083 was associated with 10 potential binding proteins, including upstream transcription factor 1 (USF-1). Third, using the Unibind website, we identified 57 potential transcription factor binding sites associated with the STRN gene region. One of these, the Hypoxia-inducible factor 1 subunit alpha (HIF1A), was identified within haplotype block 1 (Chr2: 36,846,965 – 36,903,515) and 10 base pairs (36,889,930 – 36,889,940) from rs888083 according to GRCh38 assembly.

Discussion

The goal of the present study was three-fold: first, to determine if other SNVs within the STRN gene exist that had higher MAF and were informative relative to SSBP. We identified a novel STRN diplotype significantly associated with SSBP and decreased STRN expression levels. Thirty-one percent of our cohort were homozygotes for the risk diplotype. Second, we documented that both women and men diplotype risk homozygotes (G2), compared to non-risk homozygotes (G0), had significantly increased SSBP. While there was no sex-genotype interaction with SSBP, women diplotype risk homozygotes had higher SSBP than men. Third, we determined that ALDO levels were increased in women, suggesting that the mechanism associated with SSBP in women was ALDO mediated.

In contrast, there was a blunted rise in RPF with a shift to a LIB Na^+ diet in men, suggesting that the mechanism associated with SSBP in men was RPF-mediated. Thus, there were significant sex-genotype interactions for both ALDO and RPF levels. In summary, while both men and women risk diplotype carriers had SSBP, the likely mechanisms for this SSBP differed by sex, suggesting a need for different genotype and sex-specific, precision therapeutic and prevention strategies.

We documented that as many as 60% of hypertensives and 47% of normotensives have SSBP, depending on the population studied[12]. Women have more SSBP than men, those of African descent more than Caucasians, and older versus younger individuals[8,9,17]. While not all studies reported similar findings, usually, the effect of biological sex was consistent[8,9]. Finally, relevant to the present study, a positive family history of hypertension was more likely observed in those with SSBP than those without SSBP[8,12,18,19]. However, reports of an association between genetics and SSBP are infrequent, even though a recent review suggests that genetic factors may be associated with as many as 50% of hypertensive subjects[12]. In the current study, women also had a significantly greater SSBP than men, particularly those who were homozygous for the STRN risk diplotype (G2). Given the frequency of the G2 risk diplotype in our population (~1/3), the fact that women have greater SSBP than men may be driven by those who carry the risk STRN diplotype.

In our cohort, the SSBP associated with the diplotype status was driven primarily by those with hypertension. In normotensives, the SSBP/diplotype association was not significant. There were no differences in SSBP by race (Caucasian vs African), although our sample size was too small in the African cohort to confirm this statistically. While older subjects had significantly higher SSBP than younger subjects, only individuals under 50 had a significant relationship between SSBP and diplotype status.

In general, mechanisms underlying SSBP have been divided into two major categories – those related to dysfunction in ALDO/MR levels or function and those related to renal vascular dysfunction, most commonly RPF alterations[9,17,20–23]. We and others documented that, as compared to men, women had greater ALDO responses to ANGII, contributing to their SSBP[8,24]; results that are consistent with a lack of suppression of RAAS by dietary salt and increased ALDO production in women contributing to their SSBP[9,11,25]. Increased activity of the ALDO biosynthetic pathway may account for greater ALDO

production in women. There is evidence that ALDO synthase (CYP11B2) expression is increased in female Balb/C mice compared to male mice on a high salt diet[21]. In addition, in Wistar rats on a RES diet, we reported increased enzymatic activity of the early pathway of ALDO's biosynthetic pathway (greater CYP11A1 and StAR) in rat zona glomerulosa cells[26]. Moreover, there is evidence that E2 regulates ALDO production in zona glomerulosa cells that express ERα, ERβ, and G protein-coupled estrogen receptors[27,28].

Additional molecular mechanisms are suggested by our in silico analyses. These revealed two potential new transcription factor binding sites in the STRN risk diplotype – rs888083 results in motif binding sites for HIF1A and USF-1 transcription factors. USF-1 and HIF1A are part of a multiprotein complex of transcription factors that can regulate ER transcription[29]. Furthermore, E2 regulates HIF1A transcription[30]. Consequently, the relationship between USF-1, HIF1A, and STRN may be influenced by E2 and could contribute to the sexual dichotomy in ALDO and RPF associated with STRN risk diplotypes. If so, the pathophysiological phenotype (pathotype) associated with the STRN risk diplotype in women may depend on estrogen, i.e., more prominent in premenopausal women than men. This possibility could be assessed by studying the effect of ovariectomy on the pathotype in STRN deficiency mice or by detailed studies in an appropriate group of post-menopausal women. However, whether these specific molecular mechanisms contribute to the STRN deficiency pathotype remains to be determined.

Clinically, our results suggest that women G2 may derive more significant therapeutic benefit from MR antagonists than men. Several studies reported a more significant fall in BP with MR blockade in women than men or in female rats[31–33], which may be due to greater expression of MR compared to males[9]. The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) reported that women had a more significant decrease in diastolic BP than men[31]. Further, in the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival (EPHESUS) trial, MR blockade significantly reduced all-cause mortality in women but not men[34]. These studies were not designed to compare sex differences following MR antagonist treatment directly. Nonetheless, they are consistent with the mechanisms-based outcome of the current study.

In contrast to an ALDO-associated mechanism in women, STRN G2 have blunted RPF in men in response to salt intake or following stimulation with ANGII on the LIB Na^+ diet. Under normal physiologic conditions, RPF is reduced with a RES Na^+ diet to minimize Na^+ excretion and increased on a LIB diet to increase the excretion of Na^+. Changes in tissue ANGII levels mediate these changes. When ANGII is increased (RES diet), this results in vasoconstriction, and RPF is reduced. On the LIB Na^+ diet, when ANGII levels are normally low, infusing ANGII will lead to an enhanced reduction in RPF (classical receptor theory response). Thus, genetically engineered mice or humans that carry a gene associated with non-suppressed ANGII levels on a LIB Na^+ diet have reduced increments in RPF going from a RES to LIB diet and decreased RPF response to infused ANGII on a LIB diet[23,35].

In the current study, men risk diplotype carriers showed prominent renal vascular dysfunction, potentially secondary to increased tissue ANGII, resulting in SSBP. In support of this hypothesis are studies in other hypertensives with SSBP with a similar RPF

phenotype to the G2 subjects. When given angiotensin-converting enzyme inhibitors (ACEi), their abnormal RPF phenotype was corrected[35]. In contrast, women STRN risk diplotypes, whose SSBP was secondary to an increased ALDO and therefore had suppressed ANGII, had an appropriately enhanced RPF response to a LIB salt diet and ANGII infusion.

These results suggest that angiotensin receptor blockers (ARB) or ACEi may benefit male diploid carriers more than women. Consistent with this hypothesis, a systematic review of 13 ACEi and 9 ARB clinical trials reporting sex-specific effects: ACEis and ARBs may be more effective in men[36]. In addition, the VALUE clinical trial comparing valsartan and amlodipine on cardiac morbidity and mortality among 15,245 hypertensive patients showed that amlodipine was superior in women and valsartan was better for men [37]. Also, the results showed excess cardiac events with valsartan treatment in women but not in men, suggesting that valsartan may be harmful in women. However, these findings were only sometimes consistent depending on the population studied and the study designed used[34,38]. No published reports have associated STRN risk haplotypes with long-term cardiovascular risk factors.

Clinically, the data presented herein and in our previous publications strongly support the hypothesis that STRN risk diplotypes, resulting in reduced STRN expression, are associated with SSBP in both men and women. However, the mechanisms related to SSBP differ by sex. Thus, with confirmation of the findings reported herein, therapeutic and preventive strategies should vary by sex. In men, they are renal vascular and ANGII mediated, while in women, they are ALDO mediated. Clinically, not only do clinicians potentially have a specific way (a genetic biomarker) to identify a substantial fraction of individuals with SSBP, but they also will know precisely what the first-line therapy should be. Further, giving an inappropriate agent to the wrong sex may be ineffective and harmful.

Our study has all of the limitations of an observational study. However, in contrast to most observational studies in this area, the structure of the HyperPATH cohort does ameliorate some of the limitations of an observational study. Thus, additional prospective studies are required to validate the proposed mechanisms. Second, STRN is the mediator of steroid signaling pathways, and the E2 and ALDO levels and MR modulate their function and levels. Hence, additional studies are required to measure other steroid levels, such as E2, progesterone, or testosterone. Third, ex vivo cell studies should be done to assess the STRN, ER, and MR levels in tissue of the risk and non-risk diplotypes. Further, we did not measure tissue ANGII levels but instead inferred them. Direct measurements would be necessary.

In conclusion, STRN risk diplotypes are associated with SSBP in both men and women, but women have greater SSBP. The mechanisms leading to SSBP are sex-dependent: in women, with salt loading inappropriately, higher ALDO levels lead to SSBP; in men, blunted RPF responses to salt loading lead to volume expansion and SSBP. Thus, while both men and women risk diplotype carriers had SSBP, their potential mechanisms differed, suggesting a need for different genotype and sex-specific, precision therapeutic, and prevention strategies. These genetic and sex-based observational results require confirmation in a prospective study/clinical trial.

Perspectives

We and others have documented that women have greater SSBP than men through unclear mechanisms. We now have identified a novel STRN risk diplotype that is significantly associated with SSBP in both men and women, primarily driven by individuals <50 years of age. The SNVs forming this risk diplotype were also associated with decreased STRN expression levels. Thirty-one percent of our cohort were homozygotes for the risk diplotype. While there was no sex-genotype interaction with SSBP, women diplotype risk homozygotes had higher SSBP than men. We also determined that the mechanism associated with SSBP in women was inappropriately increased ALDO levels. In contrast, the mechanism associated with SSBP in men was inappropriately reduced renal plasma flow levels. Hence, both men and women risk diplotype carriers had SSBP, but their associated mechanisms likely differ, resulting in significant sex-genotype interactions for both ALDO and RPF. These contrasting mechanisms suggest a need for different genotype and sex-specific, precision therapeutic, and prevention strategies. Thus, women carrying the STRN risk diplotype will respond better to MR antagonists, whereas men STRN risk diplotype carriers will respond better to ARB or ACEi. Furthermore, using an inappropriate sex-based therapy may not only be less effective but also could be contraindicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

STRN	Striatin
ALDO	Aldosterone
SSBP	Salt sensitivity of blood pressure
E2	Estrogen
ER	Estrogen receptor
MR	Mineralocorticoid receptor
LIB	Liberal Na^+
RES	Restricted Na^+

ANGII	Angiotensin II
SNV	Single nucleotide variant
G0	Homozygous non-risk diplotype carriers
G1	Heterozygous risk diplotype carriers
G2	Homozygous risk diplotype carriers
MAF	Minor allele frequency
PAH	p-aminohippurate
RPF	Renal plasma flow
SBP	Systolic blood pressure
PRA	Plasma renin activity
RAAS	Renin-angiotensin-aldosterone system
ARB	Angiotensin Receptor Blocker
ACEi	Angiotensin-converting enzyme inhibitor

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Novelty and Relevance

What is new?

- A novel STRN risk diplotype was significantly associated with SSBP in both sexes (primarily driven by individuals <50 years), with SSBP in women being higher than in men.
- The mechanism underlying the SSBP differed by sex; in women, inappropriately increased ALDO, and in men, inappropriately reduced renal plasma flow.

What is relevant?

These contrasting mechanisms suggest a need for different genotype and sex-specific, precision therapeutic, and prevention strategies.

Clinical/Pathophysiological Implications

Women STRN risk diplotypes will respond better to MR antagonists, whereas men will respond better to ARB or ACEi. Furthermore, using sex-based therapy inappropriately may not only be less effective but also could be contraindicated.

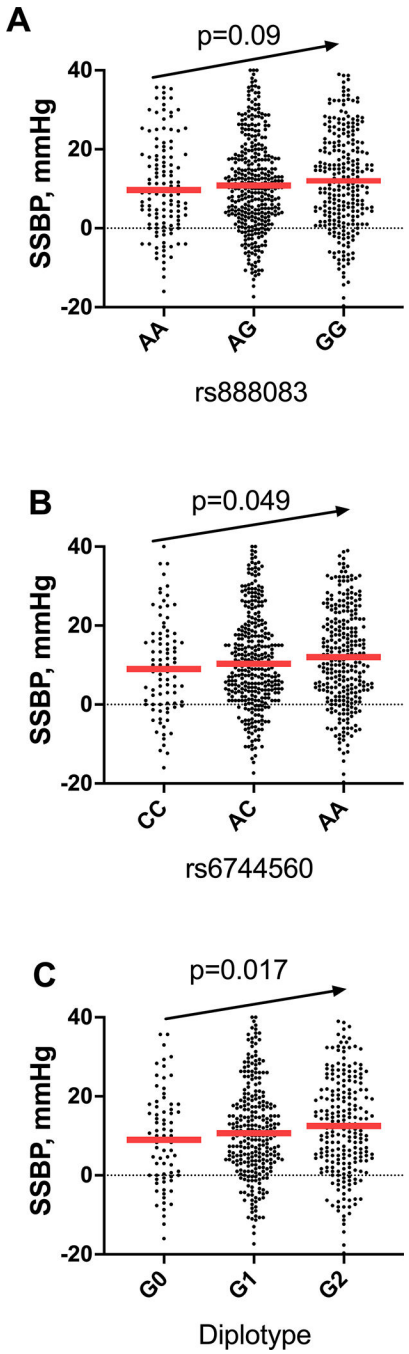

Figure 1. Relationship between SNVs and their diplotype with SSBP in HyperPATH cohort.

Multivariate regression analyses, adjusting data for study site, disease status, genotype, BMI, race, age, and sex, were performed on rs888083 (A), rs6744560 (B), and the diplotype that carry both risk alleles (C). Analyses were done on AACC - homozygous non-risk diplotype carriers (G0); AGAC - heterozygous risk diplotype carriers (G1) who have one risk allele from each SNV; and GGAA - homozygous risk diplotype carriers (G2) who carry both risk allele. The red line represents the median.

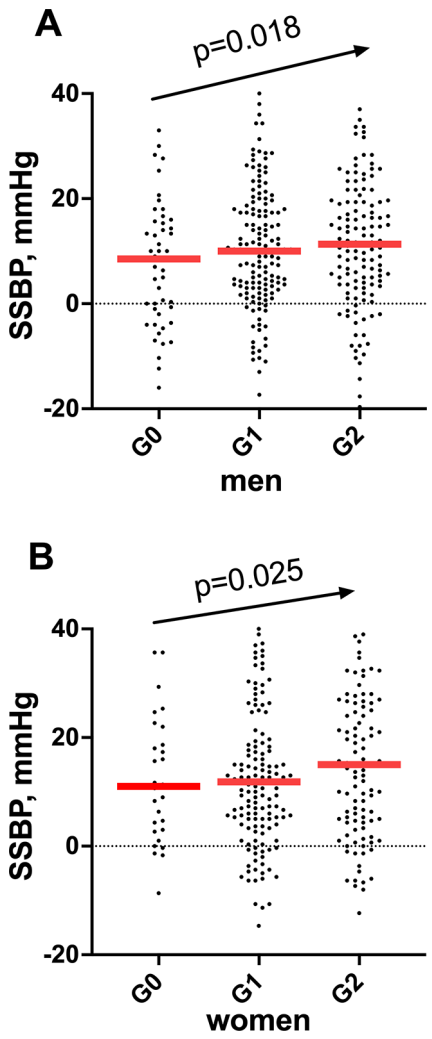

Figure 2: SSBP in men (A) and women

(B). Data are analyzed by multivariate regression, adjusted for site, disease status, BMI, age, genotype, and race within each sex. Analyses were done on AACC - homozygous non-risk diplotype carriers (G0); AGAC - heterozygous risk diplotype carriers (G1) who have one risk allele from each SNV; and GGAA - homozygous risk diplotype carriers (G2) who carry both risk allele. The red line represents the median.

Table 1.

Baseline characteristics.

Data is shown as mean ± SD. The difference between groups is calculated by Kruskal-Wallis for categorical variables and one-way ANOVA for continuous variables. Homozygous non-risk diplotype carriers (G0), heterozygous risk diplotype carriers (G1), homozygous risk diplotype carriers (G2), liberal Na^+ diet (LIB), restricted salt diet (RES).

Outcomes	G0	G1	G2	p-value
Women (n)	28	146	105	0.70
Men (n)	48	150	129	
African-American (n)	15	55	49	0.60
Caucasians (n)	61	241	185	
Hypertensive (n)	56	184	149	0.09
Normotensive (n)	19	83	58	
Diabetes (n)	1	29	27	
Age	45.4±10.9	45.5±10.6	46.5±10.5	0.47
BMI	28.1±4.8	28.0±4.6	28.0±4.5	0.98

Table 2.

Blood pressure and RAAS characteristics.

Data were analyzed by multivariate regression, and outcomes were adjusted for study site, sex, age, race, genotype, disease status, and BMI. Data is shown as adjusted mean ± SEM.

outcome	G0	G1	G2
SSBP, mmHg	9.1±1.4	12.8±0.7 *	13.5±0.8 †
SBP-LIB, mmhg	133.4±1.9	136.0±0.9	137.4±1.1
SBP-RES, mmHg	124.3±1.6	123.2±0.8	123.9±0.9
DBP-LIB, mmHg	80.4±1.1	81.6±0.6	80.9±0.6
DBP-RES, mmHg	75.9±1.0	74.1±0.5	74.9±0.6
aldosterone-LIB ng/dL	4.8±0.4	4.6±0.2	5.3±0.2
aldosterone-RES/ng/dL	15.6±1.2	17.3±0.6	17.2±0.7
PRA-LIB, ng/mL/h	0.5±0.1	0.4±0.0	0.5±0.0
PRA-RES, ng/mL/h	2.2±0.3	2.6±0.1	2.3±0.2
ALDO/renin ratio-LIB	30.8±4.5	24.0±2.3	22.2±2.5

* p=0.025, and

† p=0.009 compared to SSBP in G0 group. G0: homozygous non-risk diplotype carriers, G1: heterozygous risk diplotype carriers, G2: homozygous risk diplotype carriers. Salt sensitive Blood Pressure (SSBP), systolic blood pressure (SBP), diastolic blood pressure (DBP), plasma renin activity (PRA), aldosterone (ALDO), and ALDO/renin ratio.

Table 3:
Aldosterone and plasma renin activity (PRA) levels in men and women.

Data are analyzed by multivariate regression, adjusted for site, disease status, BMI, age, and race within each sex, and presented as adjusted mean ± SEM.

Outcome	men			women		
	G0	G1	G2	G0	G1	G2
Aldosterone-LIB, ng/dL	5.6±0.5	4.8±0.3	5.4±0.3	3.2±0.7	4.5±0.3	5.3±0.4 *
PRA-LIB, ng/mL/h	0.6±0.1	0.5±0.0	0.5±0.0	0.5±0.1	0.5±0.0	0.5±0.1
Cortisol-LIB, mg/dL	11.6±0.6	11.7±0.4	11.4±0.4	10.2±0.8	10.2±0.4	10.5±0.4
Serum potassium, mmol/L	4.3±0.1	4.2±0.0	4.1±0.0	4.1±0.1	4.1±0.0	4.2±0.0
24-hr urine potassium, mmol	77.0±3.6	74.7±2.0	72.9±2.2	80.30±4.7	67.1±2.0	67.9±2.4

* p=0.014 vs G0 women. G0: homozygous non-risk diplotype carriers, G1: heterozygous risk diplotype carriers, G2: homozygous risk diplotype carriers.

Table 4.

Renal plasma flow (RPF) in men and women according to diploid status.

Data represents least square mean ± SEM following multivariate regression analysis and data adjusted for study site, disease status (hypertensive, normotensive, and diabetes), race, age, BMI, and genotype in each sex.

outcome	men			women		
	G0	G1	G2	G0	G1	G2
Δin RPF (LIB-RES)	40.7±16.0 *	10.5±8.5	18.3±8.7	−2.0±16.0	16.5±7.1	23.2±8.9
Δ in RPF (ANGII on LIB)	122.9±10.2	95.2±5.2 †	98.5±5.3 ‡	110.0±11.6	116.0±5.3	103.7±6.4

In men, the G0 group difference in RPF from LIB to RES is significant (*p=0.02, t-test). Changes in RPF in response to angiotensin II stimulation on LIB diet are reduced in G1 and G2 compared to men G0 (The p-values are † p=0.017, ‡ p=0.035). There is no difference between groups of women. G0: homozygous non-risk diplotype carriers, G1: heterozygous risk diplotype carriers, G2: homozygous risk diplotype carriers. LIB: liberal Na^+ diet; RES: restricted Na^+ diet.