A Quantitative Trait Loci–Specific Gene-by-Sex Interaction on Systolic Blood Pressure Among American Indians

The Strong Heart Family Study


Abstract—Age-adjusted systolic blood pressure is higher in males than females. Genetic factors may account for this sex-specific variation. To localize sex-specific quantitative trait loci (QTL) influencing blood pressure, we conducted a genome scan of systolic blood pressure, in males and females, separately and combined, and tested for aggregate and QTL-specific genotype-by-sex interaction in American Indian participants of the Strong Heart Family Study. Blood pressure was measured 3 times and the average of the last 2 measures was used for analyses. Systolic blood pressure was adjusted for age and antihypertensive treatment within study center. We performed variance component linkage analysis in the full sample and stratified by sex among 1168 females and 726 males. Marker allele frequencies were derived using maximum likelihood estimates based on all individuals, and multipoint identity-by-descent sharing was estimated using Loki. We detected suggestive evidence of a QTL influencing systolic blood pressure on chromosome 17 at 129 cM between markers D17S784 and D17S928 (logarithm of odds [LOD]=2.4). This signal substantially improved when accounting for QTL-specific genotype-by-sex interaction (P=0.04), because we observed an LOD score of 3.3 for systolic blood pressure in women on chromosome 17 at 136 cM. The magnitude of the linkage signal on chromosome 17q25.3 was slightly attenuated when participants taking antihypertensive medications were excluded, although suggestive evidence for linkage was still identified (LOD=2.8 in women). Accounting for interaction with sex improved our ability to detect QTLs and demonstrated the importance of considering genotype-by-sex interaction in our search for blood pressure genes. (Hypertension. 2006;48:266-270.)

Key Words: epidemiology ■ blood pressure ■ gender

Sexual dimorphism in the regulation of blood pressure has been demonstrated in several population studies1–3 and in experimental animal models.2 Age-adjusted blood pressure is consistently higher in men than women, but these differences are attenuated when women enter menopause.1 These findings suggest the presence of distinct mechanisms of blood pressure regulation in males and females and stress the importance of the sex hormonal environment in determining blood pressure.

Genetic factors account for 30% to 40% of the blood pressure variation in a population,1 and the effect of some genes may be apparent only in the setting of appropriate sex hormonal milieu. Several genome scans of blood pressure variation have been published, but limited success has been achieved in identifying genes influencing blood pressure in the general population. One reason that few studies have identified significant linkage to blood pressure variation may be genotype-by-sex interaction, which, when present, could reduce the power to localize quantitative trait loci (QTL). Indeed, none of the previous gene mapping studies have accounted for genotype-by-sex interaction on blood pressure variation. In this article, we examine the evidence for genotype-by-sex interaction on resting systolic blood pressure (SBP) in American Indian participants of the Strong Heart Family Study (SHFS). The identification of sex-specific QTL may allow us to identify functional genes that influence the variation in blood pressure not recognized previously because of sex differences in the expression of the phenotype.

Methods

Population
The Strong Heart Study (SHS), supported by the National Heart, Lung, and Blood Institute, is a population-based observational study...
of cardiovascular disease and its risk factors among American Indians. Subjects were recruited from 3 field centers located in Arizona, North and South Dakota, and Oklahoma and have been followed since 1989. The family component of the SHS, known as the SHFS, was initiated in 1998 and has enrolled ~1200 participants from each center. This study uses family data of participants recruited from 2001 to 2003. Participating communities are tribes from Southwestern Oklahoma, 3 tribes from Arizona, and 3 tribes from South/North Dakota. The SHS and SHFS protocols were approved by the Indian Health Service Institutional Review Board, by the institutional review boards of the participating institutions, and by the 13 American Indian tribes participating in these studies. All of the subjects gave informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46.

**Phenotyping**

During a clinic visit, family members were interviewed to obtain clinical history and environmental exposures, and a physical examination was performed. After 5 minutes of rest, forearm seated blood pressure was measured 3 times by a trained technician using a mercury sphygmomanometer (WA Baum Co) and size-adjusted cuffs. The first and fifth Korotkoff sounds were recorded. The average of the last 2 measures was used for all of the analyses. Anthropometric measures of height, weight, and waist circumference were also obtained at the clinic visit. Waist circumference was measured using a standard protocol. Body mass index (BMI) was calculated as weight (kg)/height (m²). Body fat mass was measured using an RJL bioelectric impedance meter (RJL Systems) and estimated by the RJL formula based on total body water. Fasting blood samples were obtained for measurements of lipids, glucose, insulin, glycohemoglobin, and serum creatinine. Albumin and creatinine were measured in a random urine sample using nephelometric immunochemistry and alkaline picrate methods, respectively. Urinary albumin excretion was estimated by the albumin:creatinine ratio (mg/g).7

**Diabetes**

Diabetes was defined using the American Diabetes Association criteria as fasting plasma glucose levels ≥126 mg/dL or treatment for diabetes.8

**Genotyping**

The SHFS genotyping procedures have been described previously. All of the family members were genotyped for ~400 markers spaced at intervals that averaged 10 cM. Marker allele frequencies were derived using maximum likelihood methods estimated from all of the individuals, and multipoint identity-by-descent sharing was estimated using Loki.12 Pedigree relationships have been verified using the pedigree relationship statistical tests (PREST) package, which uses likelihood-based inference statistics for genome-wide identity-by-descent allele sharing. Mendelian inconsistencies and spurious double recombinants were detected using the SimWalk2 package.14 The overall blanking rate for both types of errors was <1% of the total number of genotypes for Arizona, North and South Dakota, and Oklahoma.

**Statistical Analysis**

Heritability and genetic correlations were estimated using maximum likelihood variance decomposition methods that have been implemented in SOLAR (version 2.1.2). Genome scans were performed using multipoint variance component models. The method tests for linkage between marker loci and the trait by partitioning the phenotypic variance of blood pressure distribution into its additive genetic and environmental variance components. 

To examine the evidence for genotype-by-sex interaction on blood pressure levels, we implemented a 3-stage strategy. First, we tested for additive genotype-by-sex interaction. For these analyses, the univariate variance component model is extended to include the genetic covariance between relative pairs in 2 environments. For this analysis, the 2 environments are taken to be male and female. The likelihood of a model including a genotype-by-sex interaction is compared with the likelihood of restricted models in which such interactions are excluded. Three restricted models are tested: one in which the genetic correlation (ρg) between the 2 groups is constrained to 1.0 (allowing for a test of differential additive genetic effects among males and females); one in which the genetic variance (σg) among groups is constrained to be equal (allowing for a test of differences in the magnitude of the genetic effects among males and females); and one in which the environment (residual) variances (σe) among the 2 groups are constrained to be equal (allowing for a test of residual environmental interaction with sex status). Second, we performed separate linkage analysis of males and females (sex-stratified subsets) and compared with the results of an analysis including both males and females (combined sample) to restrict the number of regions considered in the QTL-specific genotype-by-sex interaction analysis. Finally, we examined the evidence for a QTL-specific genotype-by-sex interaction at regions identified in the linkage analysis. The likelihood of the model including QTL-specific genotype-by-sex interaction was compared with the likelihood of the restricted model in which such interaction was excluded using a likelihood ratio test.18

SBP was rank transformed separately for men and women and within study centers, to normalize its distribution. Linear regression models were used to adjust for the effects of age, age², sex, age-by-sex interaction, and hypertension medication usage within each center using SAS 8.02 (SAS Institute). BMI was a significant predictor of SBP levels. However, adjustment for the effects of BMI and other covariates, such as percentage of body fat, waist circumference, history of diabetes, serum creatinine, lipid measures, fasting glucose, and urine albumin excretion, did not substantially change the results of the linkage analyses, and, therefore, were not included in the final model (data not shown). Different models were explored to account for hypertension treatment (which changes blood pressure levels), including models restricted to nontreated individuals (combined, n=1481; females, n=907; males, n=574) and models adjusting for hypertension treatment as a covariate. In comparing results from different models, we looked for consistency of the QTL signal. We calculated the empirical distribution of logarithm of odds (LOD) scores under the assumption of multivariate normality, using 10 000 replicates and simulation methods. We determined the robust LOD score by multiplying the observed LOD score by a correction coefficient, calculated by regressing the expected LOD scores on the observed simulated LOD scores. In addition, we determined the 1-LOD unit drop support interval for all of the linkage results with an LOD score ≥1.8.

**Results**

Women comprised 60% of 1894 genotyped individuals, and the mean age was 42 years (Table 1). The prevalences of hypertension and diabetes were 34% and 24%, respectively. Hypertension was more prevalent in men (40%) than women (30%), and men were less often treated with medications (52% of hypertensive men versus 74% of hypertensive women). Resting SBP was higher in men than in women, even when excluding treated individuals (Table 1). Average SBP increased with age in both sexes but was consistently higher in men than women until ages 55 to 60 years (data not shown). On average, participants were obese (BMI >30 kg/m²), and ~85% of men and women were overweight (BMI >25 kg/m²). Urine albumin:creatinine ratio was ≥30 mg/g in 19% of participants (n=363).

Genetic data were available for >18 000 relative pairs, with ~7000 female-female relative pairs, 2700 male-male relative pairs, and 8300 male-female relative pairs (Table 1, available online at http://hyper.ahajournals.org). Estimated heritability (h²) for SBP was 0.28 ± 0.06 for the combined sample, 0.28 ± 0.04 for women, and 0.35 ± 0.10 for men, after accounting for the covariate effects of age, age², center, and antihypertensive medications. SBP genetic
effects were higher in models restricted to untreated individuals (\(n=1481\), \(\text{h}^2=0.49\pm0.06\) for the combined sample, \(\text{h}^2=0.46\pm0.08\) for women, and \(\text{h}^2=0.53\pm0.12\) for men). These differences may be because of a more homogenous study group after removing participants with high blood pressure.

We tested for an additive genotype-by-sex interaction using the combined sample of subjects. The estimated genetic correlation between men and women for SBP was not significantly different from 1 (\(\text{rhoG(female/male)}=0.82, P=0.19\)). The genetic SD for women (\(\sigma_g\), females) was 0.47 and for men (\(\sigma_g\), males) was 0.55, but they were not significantly different from the fit of a model in which the sex-specific SDs were constrained to be equal (\(P=0.42\)).

To further investigate genotype-by-sex interaction, we compared linkage analysis results in sex-stratified analysis to those in the combined sample (Table 2). We identified 1 chromosome region with a robust LOD score of 3.3 in women on chromosome 17 at 136 cM (Table 2 and Figure), with a 1-LOD unit support interval spanning 17 cM from 122 to 139 cM (q-terminus). This linkage signal on 17q25.3 was consistently localized, although the magnitude of effect was attenuated in models not accounting for drug treatment and in models restricted to untreated individuals (Table 2). In addition, the signal on 17q was identified at the same genome location in the combined sample, but the LOD score was smaller. In contrast, no signal on 17q25 was detected in men. Analysis of the QTTL-specific genotype-by-sex interaction on chromosome 17 at 136 cM revealed a significant QTTL-specific interaction (\(P=0.04\)).

Five additional regions with LOD scores \(\geq1.8\) were identified (Table 2). The regions on chromosomes 1, 2, and 8 were limited to men; a second region on chromosome 2 was identified only in women. All of these 4 regions showed significant QTTL-specific gene-by-sex interaction (\(P<0.01\)). A linkage on chromosome 9 was observed in the combined sample.

### Discussion

Gender differences in blood pressure are well-documented in different populations1–3 and have been largely attributed to sex hormonal effects.21 Estradiol and testosterone affect several pathways of blood pressure regulation, including the autonomic nervous system22 and the kidneys,2,23 but also have direct effects on blood vessels.24 However, the underlying genetic mechanism of blood pressure variation in men and women has not been explored. Sex-dependent genetic effects on blood pressure may be because of genes located on sex chromosomes. For example, Harrap et al25 have described suggestive linkage of SBP to the X chromosome in the Vitoria Family Heart Study. Alternatively, the effects of autosomal genes involved in blood pressure regulation may be modulated by the hormonal environment, which may differentially affect blood pressure in men and women.

Sex-specific QTTLs have been identified for obesity traits26 and have been extended recently to other traits including blood pressure phenotypes.27 In this study, we identified a QTTL-specific gene-by-sex interaction on resting SBP on chromosome 17 at 136 cM. The linkage signal on chromosome 17q25.3 was identified in women but not in men. The magnitude of the signal was greatly attenuated in the combined sample of women and men, demonstrating the importance of accounting for gene-by-
sex interactions in the identification of QTLs influencing blood pressure variation.

Several other studies have identified genetic effects on SBP in the same or nearby regions on chromosome 17 but none have accounted for sex-specific genetic effects (Table II, available online at http://hyper.ahajournals.org). Suggestive linkage to SBP was identified at region 17q25.3 for age of onset of hypertension among blacks from the Hypertension Genetic Epidemiology study (HyperGEN; LOD = 1.7).28 This is the same region identified in our study in women. Near our peak linkage signal at 17q24.2, suggestive linkage to pulse pressure was identified in Hispanics participating in the National Heart, Lung, and Blood Institute Family Blood Pressure Program (FBPP).29 In addition, genome-wide evidence for linkage was identified at region 17q23.2 for blood pressure factor in Hispanic participants of the FBPP (LOD = 3.6). Interestingly, some evidence for linkage was also observed in white HyperGEN participants (which is 1 of the 4 networks of the FBPP) in this same region (LOD = 1.5).30 Levy et al13 described linkage of longitudinal SBP to 17q21.2 (LOD = 4.7) and 17q21.3 (LOD = 2.2) in the Framingham Heart Study. A significant gene-by-age interaction for SBP at the 17q21.2 region was later described by Diego et al32 in the same cohort. Suggestive linkage to the region 17q21.3 has also been described in a sibling-pair analysis of essential hypertension among United Kingdom and French families33 and for SBP among Icelandic hypertensive families.34

When restricting the analysis to subjects untreated for high blood pressure, the linkage signal on 17q25.3 was decreased by 0.5 LOD units. Similar findings have been reported previously.35 These reductions are partly related to the individuals who were removed from analyses when excluding treated participants (n = 413 exclusions). Nonetheless, even with decreases in LOD score, we find suggestive evidence for linkage on chromosome 17. This evidence offers strong support for the presence of a blood pressure–related QTL on chromosome 17 and speaks to the robustness of this signal. Moreover, our results show a high degree of overlap with other studies and may indeed provide probable locations for candidate gene follow-up studies.

Approximately 182 genes underlie the 1 LOD unit drop support interval (17 cM) of the 17q signal. A candidate gene, urotensin II receptor or orphan G protein–coupled receptor (GPR14), is located at 17q25.3. Urotensin II is a potent systemic vasoconstrictor but has natriuretic and vasodilatory effects in the kidneys.36 Urotensin II has been associated with hypertension and heart failure. The expression of GPR14 is confined to neuronal and cardiovascular tissues, and this distribution suggests that it contributes to blood pressure regulation.

Another plausible candidate gene, angiotensin I converting enzyme (ACE) gene, is located at 17q23.3, which is ≈19.5 million base pairs from the peak LOD score. ACE is a key component of the renin–angiotensin–aldosterone system, which influences vascular tone and salt and fluid retention and is an important player in blood pressure regulation. ACE product converts angiotensin I to angiotensin II, a potent vasoconstrictor, and promotes aldosterone secretion. In addition, ACE inactivates bradykinin, a vasodilatory peptide. The ACE deletion/deletion (D/D) polymorphism has been associated with hypertension in men but not in women.37 ACE gene may enhance the hypertensive effects of Angiotensinogen gene variants, another component of the renin–angiotensin–aldosterone system.38 ACE variants have also been associated with increased SBP among smokers.39

Although no other genome-wide significant evidence of linkage was detected, suggestive evidence of linkage to SBP was detected on chromosomes 1p, 2p, 2q, 8p, and 9p. Some of these regions have been described previously. For example, linkage to chromosome 2p22.3 has been identified by Krushkal et al40 for SBP (P < 0.01) and by Angius et al41 (LOD = 2.0) and Rao et al42 (LOD = 2.08) for hypertension traits. Although these signals do not meet the genome-wide significance threshold, they suggest regions worthy of further study and may help to distinguish between true and false positives.

**Perspectives**

Our findings suggest that 1 or more genes on chromosome 17q regulate variation in SBP, particularly among female participants of the SHFS. Indeed, QTL-specific genotype-by-sex interaction on blood pressure variation was identified, which suggests that the effects of some autosomal genes for blood pressure variation may be modulated by sex-dependent factors. This region on chromosome 17q has been identified by several studies and may, therefore, have broad significance for blood pressure regulation, given the general lack of previous genome-wide evidence for linkage to SBP. Thus, future research should pursue this region with comprehensive linkage disequilibrium mapping. Identification of the risk alleles underlying this linkage peak may suggest novel mechanisms in the development and regulation of blood pressure.

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Disclosures
None.

References
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