Analysis of Sex Hormone Genes Reveals Gender Differences in the Genetic Etiology of Blood Pressure Salt Sensitivity: The GenSalt Study

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BACKGROUND
We examined the association between 799 single-nucleotide polymorphisms in 39 sex hormone genes and blood pressure (BP) responses to a dietary-sodium intervention.

METHODS
A 7-day low-sodium feeding study (51.3 mmol sodium/day) followed by a 7-day high-sodium feeding study (307.8 mmol sodium/day) was conducted among 1,906 Han Chinese participants. Nine BP measurements were obtained at baseline and the end of each intervention period using a random-zero sphygmomanometer.

RESULTS
Among men, absolute BP responses to sodium interventions decreased with the number of minor alleles of estrogen receptor 1 (*ESR1*) markers rs9340844, rs9397453, rs9371562, rs9397459, and rs9383951. For example, mean diastolic blood pressure (DBP) responses to low-sodium intervention (95% confidence interval) were −2.67 (−3.13, −2.22) mm Hg among those with the rs9397453 C/C genotype, −1.23 (−1.98, −0.48) mm Hg among those with the C/T genotype, and 0.08 (−2.31, 2.47) mm Hg among those with the T/T genotype ($P = 1 \times 10^{-4}$; false discovery rate (FDR)-q = 0.04). Mean DBP responses to high sodium according to the rs9397453 genotypes were 1.46 (1.03, 1.89) mm Hg among those with C/C, 0.19 (−0.54, 0.91) mm Hg among those with C/T, and −1.10 (−2.82, 0.61) mm Hg among those with T/T ($P = 2 \times 10^{-4}$; FDR-q = 0.04). Similar trends were noted for the association between these *ESR1* variants and SBP responses to the dietary intervention. There were no significant associations between sex hormone gene variants and salt sensitivity in women, with genotype-gender interactions noted for the *ESR1* markers that achieved significance in men.

CONCLUSIONS
We identified strong, consistent associations between *ESR1* gene variants and salt sensitivity in men. Our results support a gender-specific role for *ESR1* in the etiology of this complex trait.

Keywords: blood pressure; genetics; polymorphism; dietary sodium; salt sensitivity; gender; hypertension.

doi:10.1093/ajh/hps018

Hypertension is a global public health challenge because of its high prevalence and the concomitant increase in risk of cardiovascular disease.[1,2] As a complex trait, blood pressure (BP) is influenced by the interaction of multiple environmental and genetic determinants.[3] Among environmental determinants, dietary sodium intake is the most important modifiable risk factor for hypertension.[4–6] The causal relationship between dietary sodium intake and elevated BP has been documented extensively in clinical trials.[7,8] Clinical trials have also shown that BP responses to sodium intervention vary substantially among individuals, a phenomenon known as BP salt sensitivity.[9] A risk factor for hypertension, cardiovascular disease, and premature death,[10–12] several determinants of salt sensitivity have been clearly established, including elevated BP, African-American race, metabolic syndrome, and older age.[13–16] Recently, gender differences in BP responses to low- and high-sodium interventions were identified, with women showing increased salt sensitivity compared with men.[15] Although reasons for this gender difference are not well understood, animal and human studies have documented associations between sex hormones and both renal sodium handling and vascular function.[17–19] These data suggest that

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Clinical Trial Registry Number: NCT00721721

Initially submitted April 06, 2012; date of first revision July 18, 2012; accepted for publication August 10, 2012.

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genes encoding sex hormones could have important influences on BP salt sensitivity and may help to explain the observed gender differences in BP response to sodium intake.

The objective of the current study was to examine the association between genes involved in sex hormone biosynthesis, metabolism, and degradation and systolic BP (SBP) and diastolic BP (DBP) responses to a dietary sodium intervention among 1,906 Han Chinese participants of the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study. In addition, we conducted subgroup analyses to examine the possibility of a gender-specific role of sex hormone genes in the etiology of the salt-sensitivity phenotypes.

METHODS

Study population

The GenSalt study was conducted in a Han Chinese population living in rural areas of northern China where habitual salt intake is high.[20] A community-based BP screening was conducted among persons aged 18–60 years in the study villages to identify potential probands and their families. Those with a mean SBP between 130 and 160 mm Hg and/or a DBP between 85 and 100 mm Hg and no use of antihypertensive medications and their spouses, siblings, and offspring were recruited as volunteers for the dietary intervention study. Detailed eligibility criteria for the probands and siblings/spouses/offspring have been presented elsewhere.[21] Individuals who had stage-2 hypertension, secondary hypertension, a history of clinical cardiovascular disease or diabetes, used antihypertensive medications, or were pregnant, heavy alcohol drinkers, or currently on a low-sodium diet were excluded from the study. Among the 1,906 eligible participants, 1,871 (98.2%) and 1,860 (97.6%) completed the low-sodium and high-sodium interventions, respectively, and were included in the current analysis.

Institutional review boards at all of the participating institutions approved the GenSalt study. Written informed consents for the baseline observation and for the intervention program were obtained from each participant.

Dietary intervention

The study participants received a 7-day low-sodium diet (3 grams of sodium chloride or 51.3 mmol of sodium per day) followed by a 7-day high-sodium diet (18 grams of sodium chloride or 307.8 mmol of sodium per day). During the period of sodium intervention, dietary potassium intake remained unchanged. Total energy intake was varied according to each participant's baseline energy intake. All study foods were cooked without salt, and prepackaged salt was added to the individual study participant's meal when it was served by the study staff. To ensure study participants' compliance with the intervention program, they were required to have their breakfast, lunch, and dinner at the study kitchen under supervision of the study staff during the entire study period. The study participants were instructed to avoid consuming any foods that were not provided by study personnel. Three timed urinary specimens (one 24-hour and two overnight) were collected at baseline and at the end of each intervention phase (days 5, 6, and 7) to monitor each participant's compliance with their dietary sodium intervention. Overnight urinary sodium excretion measures were converted to 24-hour values using formulas developed from a random subsample of 238 subjects who collected overnight and 24-hour urine samples on the same days. The mean (standard deviation) of 24-hour urinary excretions of sodium and potassium were 242.4 (66.7) mmol and 36.9 (9.6) mmol at baseline, 47.5 (16.0) mmol and 31.4 (7.7) mmol during the low-sodium intervention, and 244.3 (37.7) mmol and 35.7 (7.5) mmol during the high-sodium intervention, respectively. Baseline 24-hour urinary sodium excretions were not significantly different from those of the high-sodium intervention phase, showing that sodium intake during the high-sodium diet was similar to the habitual sodium intake of this population.

Phenotype measurement

A standard questionnaire was administered by trained staff at the baseline examination to collect information on family structure, demographic characteristics, personal and family medical history, and lifestyle risk factors including alcohol consumption, cigarette smoking, and physical activity. Three morning BP measurements were obtained according to a standard protocol during each of the 3 days of baseline observation and on days 5, 6, and 7 of each intervention period. All BP readings were measured by trained and certified observers using a random-zero sphygmomanometer.[22] BP was measured with the participant in the sitting position after 5 minutes of rest. In addition, participants were advised to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 minutes prior to their BP measurements. All BP observers were blinded to the participant's dietary intervention. Body weight and height were measured twice with participants wearing light indoor clothing without shoes during the baseline examination. Body mass index (BMI) was calculated as kilograms per meters squared (kg/m^2).

Salt-sensitivity phenotypes were defined continuously as the absolute changes in SBP and DBP when switching from baseline to low-sodium and from low-sodium to high-sodium intervention. Mean BP responses to low sodium were calculated as the mean of 9 measurements on days 5, 6, and 7 during the low-sodium intervention minus the mean of 9 measurements at baseline. Responses to high sodium were calculated as the mean of 9 measurements on days 5, 6, and 7 during the high-sodium intervention minus the mean of 9 measurements on days 5, 6 and 7 during the low-sodium intervention.

Candidate gene and single-nucleotide polymorphism selection and genotyping

We conducted a Medline literature search using Medical Subject Heading (MeSH) terms "gonadal steroid hormones" or keyword "sex hormones" and MeSH terms "genes" or "polymorphism, single nucleotide" and utilized the Kyoto Encyclopedia of Genes and Genomes steroid hormone biosynthesis pathway map to identify genes encoding sex hormones. References of articles used to identify genes can be found at http://www.nature.com/ajh. Table 1 provides the names, chromosomal locations, and functions of the 44

Table 1. Genes involved in sex steroid hormone biosynthesis, metabolism, and degradation

Gene symbol	Gene name	Chr	Physical position ± 5,000 bp	SNPs	Function
GSTM1	Glutathione S-transferase mu 1	1	(110225436, 110241367)	1	Involved in the excretion of catechol estrogen.[1]
HSD3B2	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	1	(119952554, 119970658)	6	Crucial early role in the biosynthesis of hormonal steroids.[2]
HSD3B1	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	1	(120044821, 120062681)	10	Crucial early role in the biosynthesis of sex steroid hormones.[3]
EPHX1	Epoxide hydrolase 1, microsomal	1	(225992794, 226038260)	20	Regulates cholesterol metabolism.[4]
SRD5A2	Steroid-5-alpha-reductase, alpha polypeptide 2	2	(31742550, 31811136)	13	Metabolizes testosterone.[5]
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	2	(38289116, 38342044)	27	Metabolizes estradiol to hydroxyl/methoxy estrogens.[5]
LHCGR	Luteinizing hormone/choriogonadotropin receptor	2	(48854428, 48987880)	33	Mediates function of LH and hCG.[6]
FSHR	Follicle stimulating hormone receptor	2	(49184296, 49386676)	93	Mediates function of FSH.[7]
UGT1 Family[a]	UGD glucosyltranferase 1	2	(234540100, 234686951)	55	Catalyzes the glucuronidation of estrone.[8,9]
FOXL2	Forkhead box L2	3	(138658066, 138670982)	2	Negatively regulates CYP17, a steroidogenic enzyme involved in the biosynthesis of sex steroid hormones.[10]
UGT2 Family[b]	UDP glucuronosyltransferase 2 family, polypeptide B17	4	(69397902, 70523967)	70	Catalyzes the glucuronidation of estrone.[8,9]
SULT1E1	Sulfotransferase family 1E, estrogen-preferring, member 1	4	(70671498, 70730870)	9	Catalyzes the sulfate conjugation of estrogen.[11]
SRD5A1	Steroid-5-alpha-reductase, alpha polypeptide 1	5	(6628456, 6674675)	16	Catalyzes the sulfate conjugation of estrogen.[11]
ESR1	Estrogen receptor 1	6	(151972826, 152455754)	130	Mediates effects of estrogens; interacts with testosterone and estrogens to activate SRD5A2.[5,12]
CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5	7	(99240817, 99282621)	3	Catalyzes the hydroxylation of testosterone.[13]
CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4	7	(99349604, 99386888)	0	Metabolizes estradiol to hydroxyl/methoxy estrogens.[5]
STAR	Steroidogenic acute regulatory protein	8	(37996167, 38013783)	3	Facilitates the movement of cholesterol into the inner mitochondrial membrane, an early step in steroidogenesis.[8]
HSD17B3	Hydroxysteroid (17-beta) dehydrogenase 3	9	(98992588, 99069434)	31	Converts androstenedione to testosterone.[5]
AKR1C3	Aldo-keto reductase family 1, member C3	10	(5072549, 5154878)	37	Involved in the conversion of androstenedione to estrone and testosterone to estradiol.[7]
CYP17A1	Cytochrome P450, family 17, subfamily A, polypeptide 1	10	(104585288, 104602290)	4	Converts pregnenolone to 17α-hydroxypregnenolone and DHEA. Converts progesterone to 17α-hydroxyprogesterone and androstenedione.[3]
FSHB	Follicle stimulating hormone, beta polypeptide	11	(30247563, 30261808)	6	Regulates follicular development and sex steroid production.[14]
GSTP1	Glutathione S-transferase pi 1	11	(67346066, 67359131)	1	Involved in the excretion of catechol estrogen.[1]
PGR	Progesterone receptor	11	(100895355, 101006255)	23	Mediates the function of progesterone.[2]
ESR2	Estrogen receptor 2	14	(64545950, 64810268)	52	Mediates the effect of estrogens.[12]

(*Continued*)

Table 1. (Continued)

Gene symbol	Gene name	Chr	Physical position ± 5,000 bp	SNPs	Function
CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	15	(51495254, 51635807)	60	Rate-limiting step in estrogen production.[3]
CYP11A1	Cytochrome P450, family 11, subfamily A, polypeptide 1	15	(74625100, 74665081)	7	Involved in the formation of pregnenolone from cholesterol.[3]
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	15	(75006883, 75022951)	0	Metabolizes estradiol to hydroxyl/methoxy estrogens.[5]
PDE8A	Phosphodiesterase 8A	15	(85518671, 85687376)	40	Expressed in the ovary and testis; involved in hormone signaling pathway.[15]
SULT1A1	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	16	(28611903, 28639907)	2	Catalyzes the sulfate conjugation of estrogen.[11]
HSD17B2	Hydroxysteroid (17-beta) dehydrogenase 2	16	(82063866, 82137139)	28	Responsible for the oxidation of estrogen to a less active form.[3]
SHBG	Sex hormone-binding globulin	17	(7526287, 7541701)	1	Binds to sex steroids (testosterone, estradiol) to regulate their bioavailability.[7]
HSD17B1	Hydroxysteroid (17-beta) dehydrogenase 1	17	(40698984, 40712857)	0	Responsible for the reduction of estrogen to a more active form.[3]
LHB	Luteinizing hormone beta polypeptide	19	(49514237, 49525347)	1	Promotes spermatogenesis and ovulation by stimulating the testes and ovaries to synthesize steroids.[6]
COMT	Catechol-O-methyltransferase	22	(19924130, 19962498)	15	Involved in the excretion of catechol estrogen.[1]
GSTT1	Glutathione-S-transferase theta 1	22	(24371133, 24389680)	0	Involved in the excretion of catechol estrogen.[1]
AR	Androgen receptor	X	(66758874, 66955461)	0	Mediates the effect of estrogens.[12]

Abbreviations: Chr, Chromosome; LH, Luteinizing hormone; hCG, human chorionic gonadotropin; FSH, Follicle stimulating hormone; CYP17, Cytochrome P450, family 17; UGD, UDP-glucose-6-dehydrogenase; SRD5A2, steroid-5-alpha-reductase, alpha polypeptide 2; DHEA, Dehydroepiandrosterone.

[a]UGT1 family includes a cluster of related genes: UGT1A10, UGT1A9, UGT1A7, UGT1A3, and UGT1A1. [b]UGT2 family includes a cluster of related genes: UGT2B17, UGT2B15, UGT2B7, UGT2B4, and UGT2A1.

Supplementary references 1–15 are available at www://www.nature.com/ajh.

Table 2. Baseline characteristics of 1,906 GenSalt dietary intervention participants

Characteristic	Men (N = 1,010)	Women (N = 896)	*P* for gender difference
	Mean ± SD		
Age, y	39.3 ± 9.6	38.1 ± 9.4	0.008
BMI, kg/m^2	23.1 ± 3.1	23.5 ± 3.2	0.009
Baseline BP, mm Hg			
Systolic	118.7 ± 12.8	114.9 ± 15.4	< 0.0001
Diastolic	75.6 ± 9.9	71.7 ± 10.5	< 0.0001
BP response to low salt, mm Hg			
Systolic	–5.3 ± 7.0	–5.7 ± 7.1	0.20
Diastolic	–2.4 ± 5.7	–3.2 ± 5.3	0.001
BP response to high salt, mm Hg			
Systolic	4.5 ± 5.9	5.3 ± 6.1	0.004
Diastolic	1.4 ± 5.4	2.6 ± 5.3	< 0.0001

Abbreviations: BMI, body mass index; BP, blood pressure.

candidate genes that were identified and examined in the current study. Single-nucleotide polymorphism (SNP) data, genotyped as part of the Affymetrix platform (Affymetrix 6.0, Inc., Santa Clara, CA), were available for 948 SNPs in 44 of the selected candidate genes and their 5,000 base pair flanking regions. Quality control, including checks of Mendelian consistency, genotyping call rate, minor allele frequency (MAF), and Hardy-Weinberg equilibrium, was performed using PLINK software. After exclusion of 133 SNPs with low MAF (< 0.01), 5 SNPs with low call rate (< 0.95), and 11 SNPs with low MAF and call rate, 799 SNPs from 39 genes remained for the analysis.

Statistical analysis

Baseline characteristic and BP response variables were calculated for men and women separately and compared in univariable analyses using *t* tests. Additive associations between single SNPs and BP responses to low- and high-sodium interventions were assessed using a mixed linear regression model to account for the nonindependence of family members. Age, gender, BMI, BP measurement at room temperature, and study site were adjusted in multivariable analyses. Because the expression of genes encoding sex hormones may vary substantially between men and women, a similar but gender-stratified analysis was also conducted. To adjust for multiple comparisons, the false discovery rate (FDR) q value was calculated for all SNPs. For SNPs with a FDR-q < 0.05, we estimated the mean effect size and 95% confidence interval (CI) for each genotype using a mixed linear regression model. For SNPs significant in any of the gender-stratified analyses, a test for gender–genotype interaction was conducted. These analyses were conducted using SAS statistical software (version 9.1; SAS Institute Inc). We used Haploview software (version 4.2; http://www.broadinstitute.org/haploview) to estimate the extent of linkage disequilibrium (LD), defined by the pairwise r^2 value between SNPs.[23]

RESULTS

The baseline characteristics of GenSalt study participants according to gender are presented in Table 2. Approximately 53% of study participants were male. On average, men were older and leaner than their female counterparts. Although men had higher average baseline SBP and DBP measures compared with women, their BP responses to dietary sodium intake were generally lower in magnitude (Table 2).

Analysis of 799 SNPs in 39 genes encoding sex hormones revealed significant associations between genetic variants in the estrogen receptor 1 (*ESR1*) gene and BP responses to dietary sodium intake in the overall and gender-stratified analyses (Supplementary Figures a-f at http://www.nature.com/ajh). Among all GenSalt study participants, the minor alleles of 2 common, moderately correlated intronic *ESR1* markers, rs9397453 and rs9383951 (pairwise $r^2 = 0.68$), were strongly associated with decreased DBP responses to low-sodium intervention (Figure 1a and 1b). With MAFs = 0.11, rs9397453 attained *a* $P = 1 \times 10^{-4}$ (FDR-q = 0.04; Figure a) and rs9383951 attained $P = 4 \times 10^{-5}$ (FDR-q = 0.04; Figure b). Although not significant after adjustment for multiple comparisons, similar trends were observed for SBP response to low-sodium intervention for these 2 markers.

Analysis of GenSalt men showed results similar to those of the overall analysis, with 5 *ESR1* variants attaining statistical significance for the salt-sensitivity phenotypes (Table 3). The minor alleles of *ESR1* markers rs9397453 and rs9383951 were significantly associated with decreased DBP response to the low-sodium intervention (both $P = 0.0001$; both FDR-q = 0.04). In addition, intronic *ESR1* variant rs9340844 (MAF = 0.14), which was in moderate LD with both rs9397453 (pairwise $r^2 = 0.62$) and rs9383951 (pairwise $r^2 = 0.41$), also significantly associated with DBP response to low sodium ($P = 0.0001$; FDR-1 = 0.04). Furthermore, 3 highly correlated *ESR1* SNPs (all pairwise $r^2 > 0.90$), which included rs9397453 plus rs9371562 and rs9397459, were significantly associated with DBP response to the

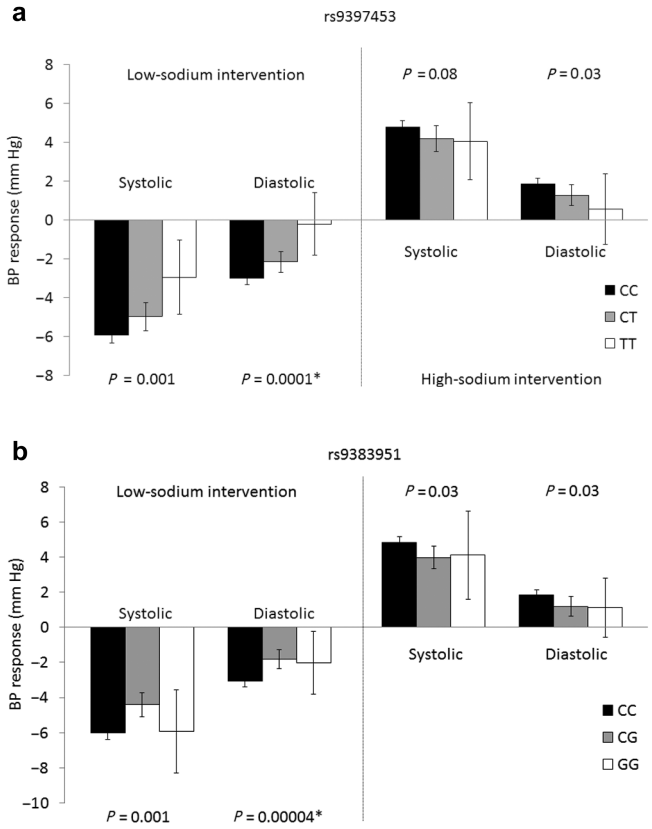

Figure 1. Systolic blood pressure and diastolic blood pressure responses to the low- and high-sodium dietary interventions according to estrogen receptor 1 rs9397453 (a) and rs9383951 (b) genotypes. Asterisk indicates significant after adjustment for multiple comparisons.

high-sodium intervention (all $P = 0.0002$; all FDR-q = 0.05). Consistent trends were observed for rs9340844 ($P = 0.002$; FDR-q = 0.10) and rs9383951 ($P = 0.0005$; FDR-q = 0.25).

In contrast, there was no evidence of association between any of the variants in genes encoding sex hormones, including *ESR1*, with salt-sensitivity phenotypes among women (Supplementary Figures e and f at http://www.nature.com/ajh). For those *ESR1* markers that were highly significant among men but not women (rs9340844, rs9397453, rs9371562, rs9397459, and rs9383951), we tested for gender–genotype interactions, noting highly significant interactions for most BP response phenotypes (Table 3). These data suggest that the results of the overall analysis were strongly driven by the associations observed in men.

DISCUSSION

The current study provides the first evidence of a relationship among variants of the *ESR1* gene and salt sensitivity of BP. Although *ESR1* markers rs9397453 and rs9383951 were associated with salt-sensitivity phenotypes in overall analyses, gender-stratified findings suggested that these results were primarily driven by the strong associations identified in male GenSalt participants. The minor alleles of *ESR1* rs9397453 and rs9383951, plus additional markers rs9340844, rs9371562, and rs9397459, were shown to significantly decrease BP responses to sodium in men but not women, with highly significant gender–genotype interactions noted. These findings may have important public health and clinical implications. Despite previous work that identified increased BP responses to sodium intake in women compared with men,[15] the current work provides the first evidence of sexual dimorphism in the genetic etiology of this complex trait. Our research highlights the importance of considering gene–environment interaction in the context of BP salt sensitivity and also suggests novel genetic mechanisms underlying BP response to sodium intake in men.

To date, GenSalt is the first dietary intervention study to examine the association between genetic variants encoding sex hormones and BP response to dietary sodium intervention. Study attributes, including the recruitment of all Han Chinese participants, should make the analysis robust to population stratification. The study participants were also similar with respect to lifestyle risk factors, including diet and physical activity, minimizing environmental variation and increasing statistical power to detect genotype–phenotype associations. The majority of participants completed the dietary intervention (96.8%), and compliance with the study interventions, as assessed by urinary excretion of sodium and potassium during each intervention period, was excellent. Measurement error was reduced and power enhanced by the large number of BP measurements that were collected for each participant. Finally, stringent quality control procedures were used during measurement of BP and the other study covariables, conduct of the dietary interventions, genotyping, and marker data cleaning.

Table 3. Blood pressure responses to the dietary sodium interventions, according to gender and *ESR1* genotypes

			Blood pressure response to low sodium					Blood pressure response to high sodium				
			Men		Women			Men		Women		
SNP	Genotype	N_{Men}/N_{Women}	Absolute change, mm Hg, (95% CI)	*P*	Absolute change, mm Hg, (95% CI)	*P*	Interaction *P*[b]	Absolute change, mm Hg, (95% CI)	*P*	Absolute change, mm Hg, (95% CI)	*P*	Interaction *P*[b]
Systolic blood pressure												
rs9340844	CC	717/649	–5.86 (–6.37, –5.34)	0.0008	–6.01 (–6.62, –5.41)	0.14	0.01	4.63 (4.18, 5.07)	0.001	4.99 (4.51, 5.46)	0.72	0.001
	CT	231/204	–4.26 (–5.13, –3.38)		–5.64 (–6.48, –4.79)			3.64 (2.88, 4.41)		5.18 (4.28, 6.09)		
	TT	29/19	–3.89 (–6.11, –1.67)		–3.63 (–6.17, –1.08)			2.53 (0.98, 4.08)		5.04 (2.06, 8.02)		
rs9397453	CC	768/695	–5.70 (–6.19, –5.20)	0.002	–6.03 (–6.62, –5.45)	0.15	0.14	4.56 (4.12, 4.99)	0.003	5.06 (4.60, 5.53)	0.77	0.03
	CT	185/160	–4.34 (–5.37, –3.31)		–5.58 (–6.55, –4.60)			3.47 (2.63, 4.30)		4.97 (3.96, 5.98)		
	TT	17/11	–2.65 (–5.15, –0.16)		–3.42 (–6.06, –0.79)			1.97 (–0.49, 4.43)		6.77 (3.09, 10.46)		
rs9371562	TT	769/707	–5.77 (–6.28, –5.27)		–6.08 (–6.66, –5.50)			4.62 (4.18, 5.05)		5.02 (4.56, 5.48)		
	TA	209/170	–4.33 (–5.30, –3.37)	0.001	–4.94 (–5.87, –4.01)	0.07	0.22	3.48 (2.65, 4.31)	0.001	4.75 (3.75, 5.75)	0.53	0.007
	AA	15/11	–2.70 (–5.48, 0.09)		–6.41 (–9.82, –2.99)			1.17 (–1.07, 3.41)		9.21 (6.30, 12.13)		
rs9397459	GG	769/707	–5.77 (–6.27, –5.26)		–6.08 (–6.66, –5.50)			4.62 (4.19, 5.06)		5.02 (4.56, 5.48)		
	GA	210/170	–4.37 (–5.33, –3.40)	0.002	–4.94 (–5.87, –4.01)	0.07	0.24	3.48 (2.66, 4.31)	0.001	4.75 (3.75, 5.75)	0.53	0.006
	AA	15/11	–2.70 (–5.49, 0.09)		–6.41 (–9.82, –2.99)			1.16 (–1.08, 3.41)		9.21 (6.30, 12.13)		
rs9383951	CC	765/684	–5.78 (–6.28, –5.28)	0.005	–6.14 (–6.73, –5.56)	0.04	0.12	4.65 (4.22, 5.08)	0.0009	5.03 (4.56, 5.50)	0.77	0.0005
	CG	196/176	–4.07 (–5.02, –3.11)		–4.69 (–5.66, –3.71)			3.21 (2.31, 4.10)		4.93 (3.95, 5.91)		
	GG	16/12	–5.00 (–8.47, –1.53)		–6.98 (–9.94, –4.02)			1.94 (–0.64, 4.53)		6.74 (2.26, 11.21)		
Diastolic blood pressure												
rs9340844	CC	717/649	–2.78 (–3.25, –2.31)	0.0001[a]	–3.11 (–3.59, –2.63)	0.74	3.7×10^{-6}	1.45 (1.02, 1.89)	0.002	2.04 (1.60, 2.48)	0.21	1.8×10^{-6}
	CT	231/204	–1.24 (–1.91, –0.56)		–3.25 (–3.97, –2.54)			0.57 (–0.11, 1.26)		2.60 (1.89, 3.30)		
	TT	29/19	–0.92 (–2.77, 0.93)		–1.73 (–3.40, –0.05)			–0.66 (–2.10, 0.79)		2.22 (0.40, 4.04)		
rs9397453	CC	768/695	–2.67 (–3.13, –2.22)	0.0001[a]	–3.21 (–3.69, –2.73)	0.18	0.03	1.46 (1.03, 1.89)	0.0002[a]	2.17 (1.74, 2.60)	0.70	0.005
	CT	185/160	–1.23 (–1.98, –0.48)		–2.98 (–3.71, –2.24)			0.19 (–0.54, 0.91)		2.26 (1.49, 3.03)		
	TT	17/11	0.08 (–2.31, 2.47)		–0.86 (–2.61, 0.90)			–1.10 (–2.82, 0.61)		2.78 (–0.18, 5.73)		
rs9371562	TT	769/707	–2.68 (–3.15, –2.22)		–3.23 (–3.70, –2.76)			1.50 (1.07, 1.94)		2.17 (1.76, 2.58)		
	TA	209/170	–1.33 (–2.07, –0.59)	0.0004	–2.63 (–3.39, –1.87)	0.14	0.09	0.14 (–0.57, 0.85)	0.0002[a]	2.03 (1.17, 2.88)	0.69	0.005
	AA	15/11	–0.32 (–2.93, 2.28)		–2.61 (–5.55, 0.33)			–0.73 (–2.53, 1.07)		4.40 (1.76, 7.03)		
rs9397451	GG	769/707	–2.69 (–3.16, –2.22)		–3.23 (–3.70, –2.76)			1.51 (1.07, 1.95)		2.17 (1.76, 2.58)		
	GA	210/170	–1.29 (–2.03, –0.55)	0.0003	–2.63 (–3.39, –1.87)	0.14	0.08	0.13 (–0.57, 0.84)	0.0002[a]	2.03 (1.17, 2.88)	0.69	0.004
	AA	15/11	–0.32 (–2.93, 2.28)		–2.61 (–5.55, 0.33)			–0.73 (–2.53, 1.07)		4.40 (1.76, 7.03)		
rs9383951	CC	765/684	–2.73 (–3.18, –2.28)	0.0001[a]	–3.28 (–3.75, –2.81)	0.08	0.003	1.49 (1.05, 1.92)	0.0005	2.15 (1.73, 2.57)	0.74	1.3×10^{-5}
	CG	196/176	–1.06 (–1.82, –0.30)		–2.42 (–3.20, –1.65)			0.09 (–0.66, 0.84)		2.21 (1.37, 3.06)		
	GG	16/12	–1.02 (–3.45, 1.41)		–3.27 (–5.83, –0.72)			–0.22 (–2.05, 1.61)		2.87 (0.07, 5.67)		

Abbreviation: CI, confidence interval.
[a]FDR-q < 0.05. [b]*P* value for gender-genotype interaction.

Certain limitations should be addressed. Because our research was conducted in a Han Chinese population, the findings may not be generalizable to populations with distinct LD structure. However, it should be noted that transethnic replications of genomic associations have been increasingly reported as unique populations are examined.[3] In addition, although the Affymetrix 6.0 platform generally provides good genomic coverage of common polymorphisms in the Han Chinese population (approximately 75%),[24] genotype data were not available for 5 genes encoding sex hormones (see Table 1). Therefore, future research to examine the association between these genes and BP salt sensitivity is needed. Furthermore, with MAFs of significant *ESR1* SNPs ranging from 11% to 14%, mean BP responses among participants homozygous for the variant alleles were not very precise, with relatively large CIs. However, we were able to confirm the veracity of the genotype calls through checks of Mendelian consistency among family members in addition to other standard quality control procedures. Further, the MAFs were more than sufficient to identify significant dose-response associations in our data.

The *ESR1* gene is a particularly attractive candidate for genetic study of BP-related traits due to its well-established role in vascular function. Studies have long documented the expression of this gene in endothelial and smooth muscle cells of men and women.[19] Estrogen binding to *ESR1* has been shown to cause rapid vasodilation of blood vessels through the activation of endothelial nitric oxide synthase and also has long-term effects on gene expression in vascular cells.[25–27] *ESR1* variants have already been linked to SBP, DBP, hypertension, hypertensive pregnancy, left ventricular hypertrophy, and cardiovascular diseases,[28–37] with marker rs2234693 (widely known as the PvuII variant) by far the most commonly associated SNP in the *ESR1* gene.[29–31,33,34,36,37] We examined rs2234693 for its association with salt-sensitivity phenotypes in the current study, observing minimum raw *P* values of 0.04 and 0.07 for its association with DBP responses to low- and high-sodium interventions, respectively. Although noteworthy, these modest associations did not remain significant after adjustment for multiple testing.

In contrast, we identified significant associations between *ESR1* SNPs rs9397453 and rs9383951 and BP salt sensitivity, findings that appeared to be driven by the strong associations observed in male GenSalt participants. Among men, we identified consistent inverse associations between novel *ESR1* variants rs9340844, rs9397453, rs9371562, rs9397459, and rs9383951 with DBP responses to the dietary sodium interventions, noting similar trends for SBP responses. These 5 intronic *ESR1* variants showed little evidence for conservation across species or regulatory action, making it unlikely that they are causally associated with the salt-sensitivity phenotype.[38] It is more plausible that the associations reflect LD of these SNPs with a functional but still undiscovered variant. Although rs9397453, rs9371562, and rs9397459 were in very strong LD, there were only moderate pairwise correlations between this group of variants and rs9340844 and rs9383951. Therefore, it is unclear whether the identified variants reflect the signal of only 1 or more causal *ESR1* variants. While we await replication and functional study to elucidate the true nature of the observed relationship in humans, the results provide promising evidence for a gender-specific role of the *ESR1* gene in salt sensitivity of BP.

Our finding of a relationship between the *ESR1* gene and BP response to sodium in men but not women is of particular interest. Although we are the first to identify such an association with salt sensitivity, past studies have noted gender-specific associations of *ESR1* with other BP-related phenotypes. For example, Ellis and colleagues identified significantly increased SBP among male carriers of the minor allele of *ESR1* SNP rs2234693 compared with men who were homozygous for the major allele.[29] Similarly, Peter and colleagues identified significant associations between *ESR1* rs2234693 and moderately correlated SNP rs2077647 with SBP and pulse pressure among male participants of the Framingham Heart Study offspring cohort.[30] Neither study identified corresponding associations among their female participants.[29,30] Male-specific associations of *ESR1* variants have also been reported for cardiovascular diseases,[31,37] with Shearman and colleagues reporting a 2.9-fold increased risk of myocardial infarction among male carriers of the rs2234693 minor allele compared with those homozygous for the major allele ($P < 0.001$).[37] Despite the accumulating evidence from genetic studies that suggest gender differences in the association between *ESR1* and BP-related phenotypes, there is a paucity of physiologic research to support such relationships. Given gender differences in concentrations of circulating estrogens[26] as well as the *ESR1* protein,[39,40] it is plausible that there may be gender differences in vascular function mediated by estrogen and its alpha receptor (encoded by *ESR1*). Future research in this area could provide important insights into observed gender differences in BP salt sensitivity and other BP-related traits.

In summary, we provide the first evidence of a role for *ESR1* variants in BP salt sensitivity. Gender-stratified analyses showed that these findings were driven by the strong associations observed in men, with no significant associations in women and highly significant gender–genotype interactions noted. Despite these promising results, additional work is needed. Replication efforts will be necessary to confirm our findings. Furthermore, sequencing studies to identify the functional alleles responsible for the reported associations are also warranted. Still, the current report provides an important contribution to the accumulating body of evidence that suggests a gender-specific role of *ESR1* in the genetic etiology of BP-related traits.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at the American Journal of Hypertension online (http://www.oxfordjournals.org/our_journals/ajh/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

ACKNOWLEDGMENTS

The Genetic Epidemiology Network of Salt Sensitivity is supported by research grants (U01HL072507, R01HL087263, and R01HL090682) from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. Dr Kelly was supported by award number K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and American Heart Association award number 11SDG5130026.

DISCLOSURE

We have no conflicts of interest to declare.

REFERENCES

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: Analysis of worldwide data. *Lancet* 2005;365:217–223.
2. Danaei G, Finucane MM, Lin JK, Singh GM, Paciorek CJ, Cowan MJ, Farzadfar F, Stevens GA, Lim SS, Riley LM, Ezzati M, Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating G. National, regional, and global trends in systolic blood pressure since 1980: Systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet* 2011;377:568–577.
3. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, Sim X, Tay W, Chen CH, Zhang Y, Yamamoto K, Katsuya T, Yokota M, Kim YJ, Ong RTH, Nabika T, Gu D, Chang L, Kokkubo Y, Huang W, Ohnaka K, Yoamori Y, Nakashima E, Lee JY, Seielstad M, Isono M, Hisxon JE, Chen YT, Miki T, Zhang X, Sugiyama T, Jeon JP, Liu JJ, Takayanagi R, Kim SS, Aung T, Sung YJ, Jin L, Wong TY, Han BG, Kobayashi S, Ogihara T, Zhu D, Iwai N, Wu JY, Teo YY, Tai ES, Cho YS, He J. Meta-analysis identifies five novel loci associated with blood pressure in East Ssians. *Nat Genet* 2011;43:531–538.
4. Whelton PK, He J, Appel LJ, Cutler JA, Havas S, Kotchen TA, Roccella EJ, Stout R, Vallbona C, Winston MC, Karimbakas J, National High Blood Pressure Education Program Coordinating C. Primary prevention of hypertension: Clinical and public health advisory from the National High Blood Pressure Education Program. *JAMA* 2002;288: 1882–1888.
5. He J, Whelton PK. Salt intake, hypertension and risk of cardiovascular disease: An important public health challenge. *Int J Epidemiol* 2002;31:327–331.
6. Chobanian AV, Hill M. National heart, lung, and blood institute workshop on sodium and blood pressure: A critical review of current scientific evidence. *Hypertension* 2000;35:858–863.
7. Cutler JA, Follmann D, Allender PS. Randomized trials of sodium reduction: An overview. *Am J Clin Nutr* 1997;65:643S–651S.
8. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, 3rd, Simons-Morton DG, Karanja N, Lin PH, Group DA-SCR. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (dash) diet. Dash-Sodium Collaborative Research Group. *NEJM* 2001;344:3–10.
9. Weinberger MH. Salt sensitivity of blood pressure in humans. *Hypertension* 1996;27:481–490.
10. Barba G, Galletti F, Cappuccio FP, Siani A, Venezia A, Versiero M, Della Valle E, Sorrentino P, Tarantino G, Farinaro E, Strazzullo P. Incidence of hypertension in individuals with different blood pressure salt-sensitivity: Results of a 15-year follow-up study. *J Hypertens* 2007;25:1465–1471.
11. Morimoto A, Uzu T, Fujii T, Nishimura M, Kuroda S, Nakamura S, Inenaga T, Kimura G. Sodium sensitivity and cardiovascular events in patients with essential hypertension. *Lancet* 1997;350:1734–1737.
12. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension* 2001;37:429–432.
13. Wright JT, Jr., Rahman M, Scarpa A, Fatholahi M, Griffin V, Jean-Baptiste R, Islam M, Eissa M, White S, Douglas JG. Determinants of salt sensitivity in black and white normotensive and hypertensive women. *Hypertension* 2003;42:1087–1092.
14. Obarzanek E, Proschan MA, Vollmer WM, Moore TJ, Sacks FM, Appel LJ, Svetkey LP, Most-Windhauser MM, Cutler JA. Individual blood pressure responses to changes in salt intake: Results from the dash-sodium trial. *Hypertension* 2003;42:459–467.
15. He J, Gu D, Chen J, Jaquish CE, Rao DC, Hixson JE, Chen J-c, Duan X, Huang J-f, Chen C-S, Kelly TN, Bazzano LA, Whelton PK, GenSalt Collaborative Research G. Gender difference in blood pressure responses to dietary sodium intervention in the gensalt study. *J Hypertens* 2009;27:48–54.
16. Chen J, Gu D, Huang J, Rao DC, Jaquish CE, Hixson JE, Chen C-S, Chen J, Lu F, Hu D, Rice T, Kelly TN, Hamm LL, Whelton PK, He J, GenSalt Collaborative Research G. Metabolic syndrome and salt sensitivity of blood pressure in non-diabetic people in china: A dietary intervention study. *Lancet* 2009;373:829–835.
17. Pechere-Bertschi A, Maillard M, Stalder H, Brunner HR, Burnier M. Renal segmental tubular response to salt during the normal menstrual cycle. *Kidney Int* 2002;61:425–431.
18. Hinojosa-Laborde C, Lange DL, Haywood JR. Role of female sex hormones in the development and reversal of dahl hypertension. *Hypertension* 2000;35:484–489.
19. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *NEJM* 1999;340:1801–1811.
20. Zhao L, Stamler J, Yan LL, Zhou B, Wu Y, Liu K, Daviglus ML, Dennis BH, Elliott P, Ueshima H, Yang J, Zhu L, Guo D, Group IR. Blood pressure differences between northern and Southern Chinese: Role of dietary factors: The international study on macronutrients and blood pressure. *Hypertension* 2004;43:1332–1337.
21. GenSalt Collaborative Research G. Gensalt: Rationale, design, methods and baseline characteristics of study participants. *J Hum Hypertens* 2007;21:639–646.
22. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ. Human blood pressure determination by sphygmomanometry. *Circulation* 1993;88:2460–2470.
23. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of ld and haplotype maps. *Bioinformatics* 2005;21:263–265.
24. Nishida N, Koike A, Tajima A, Ogasawara Y, Ishibashi Y, Uehara Y, Inoue I, Tokunaga K. Evaluating the performance of affymetrix snp array 6.0 platform with 400 Japanese individuals. *BMC Genomics* 2008;9:431.
25. Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW. Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest* 1999;103:401–406.
26. Mendelsohn ME, Rosano GMC. Hormonal regulation of normal vascular tone in males. *Circ Res* 2003;93:1142–1145.
27. Pare G, Krust A, Karas RH, Dupont S, Aronovitz M, Chambon P, Mendelsohn ME. Estrogen receptor-alpha mediates the protective effects of estrogen against vascular injury. *Circ Res* 2002;90:1087–1092.
28. Lehrer S, Rabin J, Kalir T, Schachter BS. Estrogen receptor variant and hypertension in women. *Hypertension* 1993;21:439–441
29. Ellis JA, Infantino T, Harrap SB. Sex-dependent association of blood pressure with oestrogen receptor genes eralpha and erbeta. *J Hypertens* 2004;22:1127–1131.
30. Peter I, Shearman AM, Zucker DR, Schmid CH, Demissie S, Cupples LA, Larson MG, Vasan RS, D'Agostino RB, Karas RH, Mendelsohn ME, Housman DE, Levy D. Variation in estrogen-related genes and cross-sectional and longitudinal blood pressure in the framingham heart study. *J Hypertens* 2005;23:2193–2200.
31. Xu H, Hou X, Wang N, Hui B, Jin J, Yun S, Wang X, He X, He J, Zhang S, Han Y. Gender-specific effect of estrogen receptor-1 gene polymorphisms in coronary artery disease and its angiographic severity in Chinese population. *Clin Chim Acta* 2008;395:130–133.
32. Tamura M, Nakayama T, Sato I, Sato N, Izawa N, Hishiki M, Mizutani Y, Furuya K, Yamamoto T. Haplotype-based case-control study of estrogen receptor alpha (esr1) gene and pregnancy-induced hypertension. *Hypertens Res* 2008;31:221–228.

33. Hayashi K, Maeda S, Iemitsu M, Otsuki T, Sugawara J, Tanabe T, Miyauchi T, Kuno S, Ajisaka R, Matsuda M. Sex differences in the relationship between estrogen receptor alpha gene polymorphisms and arterial stiffness in older humans. *Am J Hypertens* 2007;20:650–656.
34. Molvarec A, Ver A, Fekete A, Rosta K, Derzbach L, Derzsy Z, Karadi I, Rigo J, Jr. Association between estrogen receptor alpha (esr1) gene polymorphisms and severe preeclampsia. *Hypertens Res* 2007;30:205–211.
35. Figtree GA, Kindmark A, Lind L, Grundberg E, Speller B, Robinson BG, Channon KM, Watkins H. Novel estrogen receptor alpha promoter polymorphism increases ventricular hypertrophic response to hypertension. *J Steroid Biochem Mol Biol* 2007;103:110–118.
36. Shearman AM, Cooper JA, Kotwinski PJ, Humphries SE, Mendelsohn ME, Housman DE, Miller GJ. Estrogen receptor alpha gene variation and the risk of stroke. *Stroke* 2005;36:2281–2282.
37. Shearman AM, Cupples LA, Demissie S, Peter I, Schmid CH, Karas RH, Mendelsohn ME, Housman DE, Levy D. Association between estrogen receptor alpha gene variation and cardiovascular disease. *JAMA* 2003;290:2263–2270.
38. Xu Z, Taylor JA. Snpinfo: Integrating gwas and candidate gene information into functional snp selection for genetic association studies. *Nucleic Acids Res* 2009;37:W600–W605.
39. Ma Y, Qiao X, Falone AE, Reslan OM, Sheppard SJ, Khalil RA. Gender-specific reduction in contraction is associated with increased estrogen receptor expression in single vascular smooth muscle cells of female rat. *Cell Physiol Biochem* 2010;26:457–470.
40. Hodges YK, Tung L, Yan XD, Graham JD, Horwitz KB, Horwitz LD. Estrogen receptors alpha and beta: Prevalence of estrogen receptor beta mrna in human vascular smooth muscle and transcriptional effects. *Circulation* 2000;101:1792–1798.