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Association of *ATP1B1*, *RGS5* and *SELE* polymorphisms with hypertension and blood pressure in African-Americans

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Abstract

Objective—Although an increasing number of hypertension-associated genetic variants is being reported, replication of these findings in independent studies has been challenging. Several genes in a human chromosome 1q linkage region have been reported to be associated with hypertension. We examined polymorphisms in three of these genes (*ATP1B1*, *RGS5* and *SELE*) in relation to hypertension and blood pressure in a cohort of African-Americans.

Methods—We genotyped 87 single nucleotide polymorphisms (SNPs) from the *ATP1B1*, *RGS5* and *SELE* genes in a well characterized cohort of 968 African-Americans and performed a case-control study to identify susceptibility alleles for hypertension and blood pressure regulation. Single SNP and haplotype association testing was done under an additive genetic model with adjustment for age, sex, BMI and ancestry-by-genotype (principal components).

Results—A total of 12 SNPs showed nominal association with hypertension and/or blood pressure. The strongest signal for hypertension was for rs2815272 in the *RGS5* gene ($P = 9.3 \times 10^{-3}$). For SBP, rs3917420 in the *SELE* gene ($P = 9.0 \times 10^{-4}$) and rs4657251 in the *RGS5* gene ($P = 9.7 \times 10^{-3}$) were the top hits. Effect size for each of these variants was approximately 2–3 mmHg. A five-SNP haplotype in the *SELE* gene also showed significant association with SBP after correction for multiple testing ($P < 0.01$).

Conclusion—These findings provide additional support for the genetic role of *ATP1B1*, *RGS5* and *SELE* in hypertension and blood pressure regulation.

Keywords

African-Americans; candidate gene; haplotype; hypertension; single nucleotide polymorphism

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Conflicts of interest

The authors have declared that no conflicts of interest exist.

Introduction

Essential hypertension, characterized by sustained elevated blood pressure (BP) with no identifiable cause, is a public health burden worldwide [1]. The condition affects over 65 million adults [2], and its prevalence in the US population increased from 24.4 to 28.9% between 1988–1994 and 1999–2004 [3]. Compared with their European–American counterparts, African–Americans are disproportionately affected [2,4] and suffer from a greater burden of disease-associated complications [5,6]. Hypertension is a multifactorial disorder likely resulting from the effect of multiple genetic variants [7] interplaying with environmental factors [8]. Linkage scans and candidate gene-based association studies have identified more than 160 candidate genomic regions for SBP, DBP and clinically diagnosed hypertension [9]. Over the last 2 years, large-scale genome-wide association studies (GWASs) have identified several hypertension candidate loci [10–12].

A chromosome 1q locus for BP-related phenotypes was reported in two independent linkage studies [13,14]. These findings were supported by observation of mouse and rat BP-related quantitative trait loci in regions syntenic to the human 1q chromosomal locus [15,16]. Genome-wide linkage scan in the GenNet Network of the Family Blood Pressure Program (FBPP) replicated a locus on chromosome 1q25 linked to DBP in European–Americans [17]. Fine mapping of this locus by the authors showed association of three genes (*ATP1B1*, *RGS5* and *SELE*) in the linked locus with hypertension [17]. In that study, *ATP1B1* variants were associated with SBP in European–Americans and with DBP in both African–Americans and European–Americans, whereas variants in *RGS5* and *SELE* were associated with SBP in African–Americans [17]. Other studies have produced supporting evidence for these observations. For example, association of a common haplotype in the *ATP1B1* gene with an increased risk of hypertension was recently reported in a Chinese population [18]. Polymorphisms in the *SELE* gene have been reported to be associated with both SBP and DBP [19]. Haplotype-based analysis showed evidence of association of *RGS5* gene polymorphisms with essential hypertension in Chinese [20]. In the present study, we conducted association studies of single nucleotide polymorphisms (SNPs) in the *ATP1B1*, *RGS5* and *SELE* genes with hypertension and BP in African–Americans enrolled from the Washington, District of Columbia metropolitan area.

Methods

Study sample

The individuals studied were all participants in the Howard University Family Study (HUFS), a population-based family study of African–Americans in the Washington metropolitan area [11]. Participants were sought through door-to-door canvassing, advertisements in local print media and at health fairs and other community gatherings. The study was conducted in accordance with the Declaration of Helsinki. Ethical approval for the study was obtained from the Howard University Institutional Review Board. All individuals provided written informed consent for the collection of samples and subsequent analysis. The participants for this study comprised 968 unrelated participants in the HUFS. During a clinical examination, demographic information was collected by interview. Weight, height, waist and hip circumference were measured using standard methods as follows: weight was measured in light clothes on an electronic scale to the nearest 0.1 kg, and height was measured with a stadiometer to the nearest 0.1 cm. BMI was computed as weight in kilograms divided by the square of the height in meters. Waist circumference was measured to the nearest 0.1 cm at the narrowest part of the torso as seen from the anterior aspect. BP was measured in the sitting position using an oscillometric device (Omron Healthcare Inc, Vernon Hills, Illinois, USA). Three BP readings were taken with a 10-min interval between readings. The reported SBP and DBP readings were the average of the

second and third readings. Pulse pressure was calculated as the difference between the SBP and DBP. Hypertension status was defined as SBP at least 140 mmHg and/or DBP at least 90 mmHg and/or treatment with antihypertensive medication.

Single nucleotide polymorphism selection and genotyping

To ensure optimal coverage of variations, SNPs in the three genes were selected using a haplotype tagging strategy. Using the International HapMap Project YRI data as a reference, tag SNPs at a pairwise r^2 value at least 0.8 and with a minimum minor allele frequency (MAF) of 0.02 were selected for genotyping. We supplemented this set of SNPs with all known nonsynonymous coding SNP in the genes. This strategy yielded a total number of 87 SNPs from the three genes. Out of these, 29 SNPs were in the *ATP1B1* gene, 33 SNPs in the *RGS5* gene and 25 SNPs in the *SELE* gene (see Table, Supplemental Digital Content 1, <http://links.lww.com/HJH/A117>). Average intermarker distances were 992 bp for *ATP1B1*, 2017 bp for *RGS5* and 828 bp for *SELE* gene. Genotyping was done as part of a 768-SNP custom panel on the Illumina GoldenGate assay. Four of the 87 SNPs had a MAF less than 0.01 and one SNP had a locus success rate of less than 90%. These five SNPs were excluded from further analyses. The remaining 82 SNPs were all in Hardy–Weinberg equilibrium ($P > 0.001$). For the overall experiment, genotype call rate was 99.32% and the concordance rate for blind duplicates was 99.98%.

Statistical analyses

Genotype and allele frequencies were estimated by gene counting. Fisher's exact test was performed to determine deviations of genotype distributions from the expected Hardy–Weinberg equilibrium. Linkage disequilibrium was visualized using Haploview [21]. Hypertension was analyzed as a binary trait (cases versus controls) using logistic regression under an additive genetic model with adjustment for age, sex, BMI and the first principal component of the genotypes derived from a set of 142 ancestry informative SNPs. Association with SBP and DBP was tested under an additive linear model with adjustment for age, sex, BMI, treatment and the first principal component of the genotypes. Given the strong prior information about the role of these genes in hypertension, we considered this a replication study and nominal P values of less than 0.05 were considered significant. Haplotype blocks were constructed using the method of Gabriel *et al.* [22] and haplotype association tests were conducted using the resulting haplotype blocks with the same phenotypes, covariates and models as for the single-locus analyses. We first performed an overall omnibus test followed by tests for each haplotype against the others within a block. All association analyses were performed using the PLINK software package, v1.06 [23]. Multiple testing was controlled for by adjusting for the number of SNPs within each gene using the Bonferroni method. Bonferroni-corrected P value thresholds were 1.7×10^{-3} for *ATP1B1*, 1.5×10^{-3} for *RGS5* and 2×10^{-3} for *SELE*.

Results

The characteristics of the hypertensive ($n = 532$) and normotensive ($n = 436$) individuals are shown in Table 1. Mean weight, waist-to-hip ratio and DBP were higher in men compared with their female counterparts in both groups. Conversely, women had higher BMI and waist circumference than the men.

Significantly associated SNPs at a nominal replication P value of 0.05 or less from all three genes are shown in Table 2. Three SNPs from the *RGS5* gene were associated with hypertension: rs2815272, rs2815271 and rs2999967 (Fig. 1). The strongest association with hypertension was for rs2815272 ($P = 9.3 \times 10^{-3}$); this SNP also showed significant nominal association with both SBP and DBP (Fig. 1). When SBP was examined as the phenotype, six

intronic SNPs from *RGS5* showed association with a *P* value less than 0.05 (Table 2, Fig. 1), rs3917420 being the strongest ($P = 9.0 \times 10^{-4}$). Five SNPs were associated with DBP (Table 2, Fig. 1), four of which are intronic (rs10917690, rs2841997, rs2815287 and rs2456899) and one (rs2815272) is located in the upstream of the *RGS5* gene.

No SNP in the *ATP1B1* or the *SELE* gene was significantly associated with hypertension in our study. One intronic SNP, rs2901029 in the *ATP1B1* gene, showed association with SBP (Fig. 1, IIB). Two SNPs in the *SELE* gene appeared associated with SBP (Fig. 1, IIIB), rs3917420 bearing the stronger signal ($P = 9.0 \times 10^{-4}$). The second one, rs5361, is a nonsynonymous coding SNP (S149R) and was associated with both SBP ($P = 0.0398$) and DBP ($P = 0.0276$).

The effect sizes of SBP-associated and DBP-associated alleles adjusted for age, sex, BMI and ancestry are shown in Table 3. The effect sizes ranged from -2.244 [standard error of the mean (SEM) 0.866; confidence interval (CI) -3.94 to -0.547] to 7.233 mmHg (SEM 2.175; CI 2.971–11.5) for SBP and from -2.015 (SEM 0.804; CI -3.591 to -0.439) to 2.773 mmHg (SEM 1.256; CI 0.31–5.235) for DBP, for individual SNPs. The largest effect size for SBP was 7.233 mmHg for rs3917420, located in intron 4 of the *SELE* gene ($P = 9 \times 10^{-4}$).

Linkage disequilibrium plots for the three genes in the study population are shown in Fig. 2. Two haplotype blocks could be defined in each of the *RGS5* and the *SELE* gene (data not shown). One haplotype CCAGC (rs4656701–rs4363475–rs12038818–rs4786–rs5368) in the *SELE* gene showed a significant association with SBP ($P = 6.5 \times 10^{-3}$). The association remained significant after correction for multiple testing ($P < 0.05/5$ haplotypes in the block, $P < 0.01$).

Discussion

Identifying genes underlying human essential hypertension has proven challenging. The recent publications of several GWAS have shed insight into some of the genes that are important at the population level [10–12]. Although the list of genetic variants associated with hypertension has increased over the past few years, few have been replicated in independent studies. This is probably due to genetic heterogeneity as well as modulation of hypertension by low-risk variants with small effect and low penetrance. Additionally, there is the possibility of interpopulation variation given that several susceptibility variants for hypertension vary across geographic regions and/or populations. In the present study, we focused on three chromosome 1q positional candidate genes: *ATP1B1*, *RGS5* and *SELE* in a sample of African-Americans. To maximize the utility of the study, we carefully characterized each study participant, adjusted for covariates in analysis, selected tag SNPs that covered the entire span of each gene and adjusted for ancestry (admixture), which could produce spurious association in population-based case-control study designs [24].

We found one *ATP1B1* variant, rs2901029, to be significantly associated with SBP. Two recent reports investigated the relationship of BP with *ATP1B1* genetic polymorphisms and haplotypes [17,18], but neither of these studies included this SNP. Among three SNPs (rs3766031, rs12079745 and rs1138486) reported to be associated with SBP previously [17], the only one in our SNP set – rs3766031 – did not show a significant association (data not shown). Our observed SBP effect size for rs2901029 was 1.698 mmHg, which is somewhat less than previously reported for *ATP1B1* SNPs (3.5–5.1 mmHg) [17]. The *ATP1B1* gene encodes for the β_1 subunit of the Na,K-ATPase (the sodium pump), a plasma membrane bound oligomeric enzyme that catalyses one molecule of ATP to exchange three Na^+ ions for two K^+ ions across the cell membrane by a coupled active transport, thereby maintaining

their normal physiological gradient [25]. Na,K-ATPase influences BP by regulating sodium reabsorption in renal tubules. In rats of the Milan hypertensive strain (MHS), a primary increase of renal tubular Na⁺ reabsorption is involved in the development of hypertension [26]. Particularly, the MHS rat showed an increased activity and expression of Na-K pump units per cell compared with their Milan normotensive strain controls [27]. Thus, the finding of association between *ATP1B1* variants and hypertension in this study is consistent with the gene's biological function.

For the *RGS5* gene, one SNP, rs2815272, was significantly associated with hypertension, SBP and DBP, whereas two SNPs (rs2815287 and rs2841997) were associated with both SBP and DBP. These three SNPs have been reported to be associated with SBP and/or DBP [17]. The estimated effect sizes on SBP in our study ranged from 2.2 to 3.6 mmHg, similar to the range of effect sizes (1.5–3.6 mmHg) reported in the literature [17]. *RGS5* is one of the intracellular regulators of G protein signaling (RGS) proteins [28]. It is expressed in most major organs, including heart, lungs and kidneys, as well as in highly specialized cell types such as vascular smooth muscle cells and cardiac myocytes [29]. Recently, two independent studies demonstrated that *Rgs5*-deficient mice were hypotensive relative to the wild-type controls [30,31], further lending credence to its potential role in BP regulation.

E-selectin (SELE) is a member of the selectin family and is specifically expressed on the surface of stimulated endothelial cells [32]. Endothelium plays an important role in the regulation of BP, and endothelial dysfunction, defined as reduced vasodilating response to endothelial stimuli, is a hallmark of hypertensive patients [33,34]. Soluble E-selectin (sE-selectin) is released by the endothelial cells and serves as a plasma marker of endothelial dysfunction or damage [35]. Increased serum levels of sE-selectin have been reported in essential hypertension [36,37]. Two SNPs (rs3917420 and rs5361) in the *SELE* gene were associated with SBP in this study. We also found rs3917420 in complete linkage disequilibrium ($r^2 = 1$) with a nonsynonymous coding SNP, rs5366. A G→C change at this SNP leads to a Glu→Gln change in position 421 of the protein. However, this mutation is classified as 'possibly damaging' by PolyPhen and 'tolerated' by the SIFT prediction tool, making it less likely to be the functional variant at this locus. We did not observe any association of rs5368 and rs2076059 with SBP in our study sample, as previously described [17].

An important issue in many association studies including ours is that significantly associated variants are often located in noncoding regions, raising the question of their functionality and possible role in disease predisposition. An intronic SNP may alter the function of a nearby regulatory element or be in linkage disequilibrium with another causative variant that is directly involved in hypertension susceptibility. Introns also contain several short sequences of cis-splicing motifs that are important for efficient splicing, such as acceptor and donor sites at either end of the intron, and a branch point site, which are required for proper splicing by the spliceosome. Additionally, 25% or more of the miRNAs genes that are short noncoding RNAs and known to regulate gene expression by binding to sequences on the target mRNA [38] and are often embedded within introns [39,40]. Similarly, upstream sequences as well as introns are known to contain promoters that control gene transcription.

Appropriate correction of statistical significance for multiple testing is required in genetic association studies to reduce false-positive findings. These corrections range from the most conservative Bonferroni correction, to False Discovery Rate, to weighted correction of combined data, to no correction at all [41,42]. We have reported our findings as replication of previous reports and, thus, relied on a significance threshold with a *P* value of 0.05. We also tested for Bonferroni correction of our positive findings. One intronic SNP rs3917420,

and a five SNP haplotype, CCAGC, in the *SELE* gene (rs4656701–rs4363475–rs12038818–rs4786–rs5368) showed association with SBP after Bonferroni correction for multiple testing.

Consistency and accuracy of phenotypic characterization are essential for genetic association studies. BP readings show heterogeneity depending on the method, environment and time of measurement that may increase type II error and, thus, reduce the chance of identifying all the genetic loci-influencing BP [43]. In one such study, a majority of the best associated signals were seen with ambulatory SBP and DBP compared to BP measured at home or clinic [43]. Therefore, the reliance on clinic measurements in the present study is a potential limitation. Also, although we adjusted for prior treatment in our analyses, not being able to measure ‘true’ SBP and DBP values may have weakened potential associations.

In conclusion, we have systematically investigated variants in the *ATP1B1*, *RGS5* and *SELE* genes on chromosome 1q for their association with hypertension and related traits. We have extended previous reports of association of these genes in an independent African–Americans population from Washington, District of Columbia metropolitan area. Although we have replicated some of the previous findings, we are also reporting new BP-associated genetic variants. On the basis of the physiological roles of these three genes in BP homeostasis, follow-up functional studies of these variants may decipher their underlying pathophysiological implications. The current wave of sequencing studies will facilitate the identification of rare and less common hypertension susceptibility variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ATP1B1	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide (gene)
DBP	diastolic blood pressure
RGS5	regulator of G-protein signaling 5 (gene)
SBP	systolic blood pressure
SELE	selectin E (gene)
SNP	single nucleotide polymorphism

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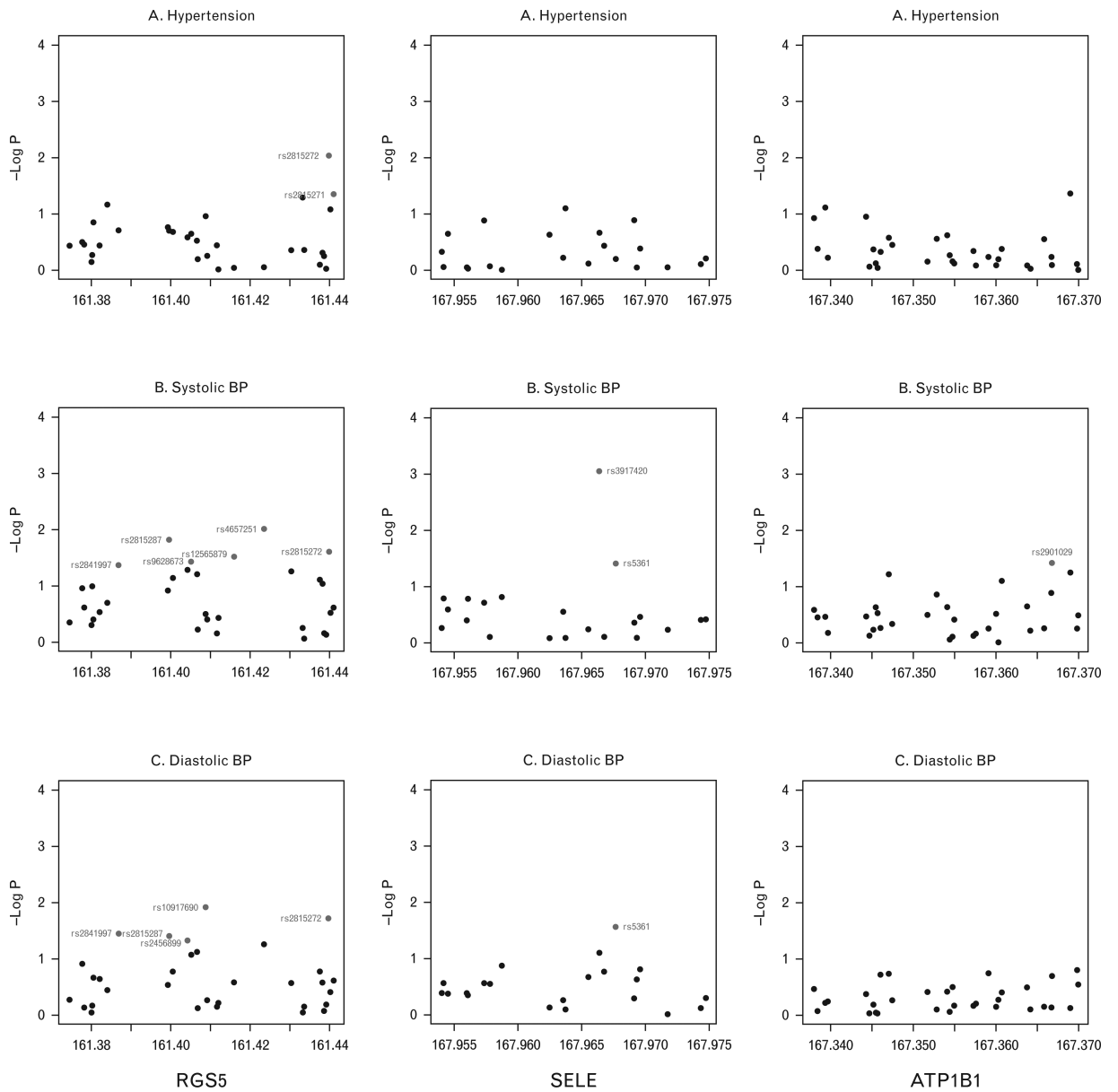


Fig. 1. Association plots of single nucleotide polymorphisms in the three genes with hypertension, SBP and DBP plotted as $-\log_{10}(P)$ against the SNPs tested with corresponding physical position (Mb). Red circles represent SNPs with $P < 0.05$.

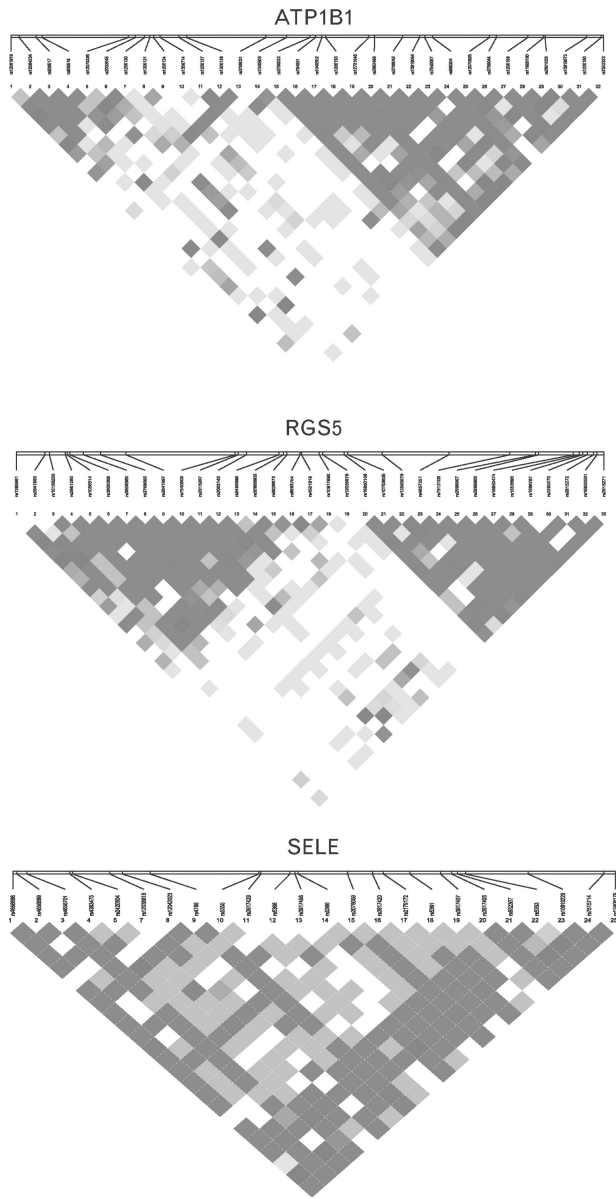


Fig. 2. Patterns of linkage disequilibrium in the three genes in our study samples. Red squares for strong linkage disequilibrium (LD), blue squares for nonsignificant LD and white squares for little or no LD.

Table 1Characteristics of the study population ($n = 968$)

Characteristic	<u>Hypertensive individuals ($n = 532$)</u>		<u>Normotensive individuals ($n = 436$)</u>	
	Male	Female	Male	Female
Number of participants	217	315	184	252
Age (years)	52.8 (10.8)	54.4 (12.2)	44.6 (9.1)	43.2 (9.9)
Weight (kg)	92.5 (26.7)	87.3 (24.0)	84.6 (20.0)	83.9 (24.0)
BMI (kg/m^2)	30.2 (8.2)	33.2 (8.8)	27.2 (6.4)	31.2 (8.7)
Waist circumference (cm)	99.8 (18.2)	99.9 (16.3)	92.1 (14.7)	94.8 (17.60)
Waist-to-hip ratio	0.92 (0.07)	0.86 (0.09)	0.89 (0.06)	0.84 (0.07)
SBP (mmHg)	143.4 (21.3)	144.1 (21.9)	120.1 (10.5)	117.7 (11.1)
DBP (mmHg)	91.1 (14.0)	86.0 (13.1)	75.6 (8.5)	74.8 (7.7)

Data are mean (SD).

Table 2Single nucleotide polymorphisms showing nominal evidence of association ($P < 0.05$) in the study

SNP	Coordinate	Type	Gene	A1	MAF	P value
HTN						
rs2815272 ^a	163173247	Upstream	<i>RGS5</i>	G	0.1872	9.3×10^{-3}
rs2815271	163174426	Upstream	<i>RGS5</i>	G	0.3340	0.0425
rs2999967	163166676	Intronic	<i>RGS5</i>	C	0.3183	0.0500
SBP						
rs3917420	169699779	Intronic	<i>SELE</i>	C	0.0357	9.0×10^{-4}
rs4657251	163156864	Intronic	<i>RGS5</i>	C	0.3107	9.7×10^{-3}
rs2815287 ^b	163132965	Intronic	<i>RGS5</i>	A	0.3005	0.0150
rs2815272 ^a	163173247	Upstream	<i>RGS5</i>	G	0.1872	0.0246
rs12565879	163149312	Intronic	<i>RGS5</i>	A	0.2919	0.0304
rs9628673	163138501	Intronic	<i>RGS5</i>	A	3446	0.0369
rs2901029	169100169	Intronic	<i>ATPIB1</i>	A	0.4013	0.0388
rs5361	169701060	Coding	<i>SELE</i>	C	0.0429	0.0398
rs2841997 ^b	163120239	Intronic	<i>RGS5</i>	A	0.0517	0.0423
DBP						
rs10917690	163142168	Intronic	<i>RGS5</i>	G	0.1096	0.0123
rs2815272 ^a	163173247	Upstream	<i>RGS5</i>	G	0.1872	0.0194
rs5361	169701060	Coding	<i>SELE</i>	C	0.0429	0.0276
rs2841997 ^b	163120239	Intronic	<i>RGS5</i>	A	0.0517	0.0366
rs2815287 ^a	163132965	Intronic	<i>RGS5</i>	A	0.3005	0.0401
rs2456899	163137589	Intronic	<i>RGS5</i>	G	0.0703	0.0483

Coordinate based on NCBI human assembly 37.1. A1, associated allele; HTN, hypertension; MAF, minor allele frequency.

^aShows association with three phenotypes.^bShows association with two phenotypes.

Table 3

Effect sizes of associated variants

SNP	Gene	Phenotype	Effect size	SEM (95% CI)	P value
rs3917420	<i>SELE</i>	SBP	7.233	2.175 (2.971–11.5)	0.0009
rs4657251	<i>RGS5</i>	SBP	-2.244	0.866 (-3.94 to -0.547)	0.0097
rs2815287	<i>RGS5</i>	SBP	2.152	0.883 (0.42–3.883)	0.0150
rs2815287	<i>RGS5</i>	DBP	1.169	0.569 (0.054–2.284)	0.0402
rs2815272	<i>RGS5</i>	SBP	2.297	1.02 (0.297–4.297)	0.0246
rs2815272	<i>RGS5</i>	DBP	1.547	0.66 (0.253–2.841)	0.0194
rs12565879	<i>RGS5</i>	SBP	-1.894	0.874 (-3.606 to 0.181)	0.0304
rs9628673	<i>RGS5</i>	SBP	1.789	0.857 (0.111–3.468)	0.0369
rs2901029	<i>ATP1B1</i>	SBP	1.698	0.821 (0.089–3.306)	0.0388
rs5361	<i>SELE</i>	SBP	4.018	1.952 (0.192–7.845)	0.0398
rs5361	<i>SELE</i>	DBP	2.773	1.256 (0.31–5.235)	0.0275
rs2841997	<i>RGS5</i>	SBP	3.614	1.777 (0.131–7.097)	0.0423
rs2841997	<i>RGS5</i>	DBP	2.396	1.144 (0.153–4.639)	0.0366
rs10917690	<i>RGS5</i>	DBP	-2.015	0.804 (3.591 to -0.439)	0.0124
rs2456899	<i>RGS5</i>	DBP	1.955	0.989 (0.0173–3.893)	0.0483

Effect sizes in mmHg. Data were analyzed under an additive model. CI, confidence interval; SEM, standard error of the mean; SNP, single nucleotide polymorphism.