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Association of Genetic Variation with Systolic and Diastolic Blood Pressure among African Americans: the Candidate Gene Association Resource (CARe) Study

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ABSTRACT

The prevalence of hypertension in African Americans (AA) is higher than in other US groups, yet few have performed genome wide association studies (GWAS) in AA. Among people of European descent, genome-wide association studies have identified genetic variants at 13 loci that are associated with blood pressure. It is unknown if these variants confer susceptibility in people of African ancestry. Here we examined genome-wide and candidate gene associations with systolic and diastolic blood pressure (SBP, DBP) using the CARe Consortium consisting of 8,591 AAs. Genotypes included genome-wide SNP data utilizing the Affymetrix 6.0 array with imputation to 2.5 million HapMap SNPs and candidate gene SNP data utilizing a 50K cardiovascular gene-centric array (ITMAT-Broad-CARe [IBC] array). For Affymetrix data, the strongest signal for DBP was rs10474346, ($P=3.6\times10^{-8}$) located near *GPR98* and *ARRDC3*. For SBP the strongest signal was rs2258119 in *C21orf91* ($P=4.7\times10^{-8}$). The top IBC association for SBP was rs2012318 ($P=6.4\times10^{-6}$) near *SLC25A42* and for DBP was rs2523586 ($P=1.3\times10^{-6}$) near *HLA-B*. None of the top variants replicated in additional AA (n = 11,882) or European American (n = 69,899) cohorts. We replicated previously reported European American blood pressure SNPs in our AA samples (*SH2B3*, P=0.009; *TBX3-TBX5*, P=0.03; and *CSK-ULK3*, P=0.0004). These genetic loci represent the best evidence of genetic influences on SBP and DBP in African Americans to date. More broadly, this work supports that notion that blood pressure among African Americans is a trait with genetic underpinnings but also with significant complexity.

INTRODUCTION

In the United States, hypertension is more common among people of African compared to European descent. According to data from the National Health and Nutrition Examination Survey (NHANES) collected between 1999 and 2004, the prevalence of hypertension in African Americans was 40%, compared to 27% in European Americans.(1,2) The risk of suffering hypertensive end-organ damage including end-stage renal disease, heart failure, and stroke is also greater among African Americans than European Americans.(1,3) Furthermore, in 2004 the death rate from hypertension was three times greater in African Americans compared to European Americans.(4,5)

A portion of the excess burden of hypertension among African Americans may be due to genetic susceptibility. Admixture mapping analysis of hypertension suggested that African ancestry is associated with hypertension.(6) Two recent genome-wide association studies (GWAS) of blood pressure, each involving about 30,000 participants of European descent, have identified common genetic variants at 13 loci that are associated with blood pressure or hypertension. It is unknown at present, however, if these variants confer susceptibility to hypertension in people of African descent. Prior investigations have reported considerable differences in genetic association patterns for blood pressure and other traits across ethnic/racial groups. These association differences may be due to differences in linkage disequilibrium (LD) patterns, allele frequencies, causal pathways, or environmental exposures. Therefore, the relations of genetic variants to blood pressure must be examined within ethnicities.

The first GWAS for blood pressure phenotypes in African Americans did not identify any SNPs reaching genome-wide significance ($p < 5 \times 10^{-8}$) with hypertension, although six were associated with blood pressure SBP in a secondary analysis in a subset of 508 normotensive individuals.(7) The present study represents the largest GWAS for blood pressure in African Americans to date. We also attempted replication of our top findings in individuals of African

ancestry and individuals of European ancestry. Understanding genetic contributions to blood pressure may provide insight into the mechanisms underlying ethnic disparities in cardiovascular disease and findings may assist in more personalized and targeted treatments to prevent target-organ damage and its associated morbidity and mortality.

RESULTS

Study sample

The analyzed study sample included individuals from five cohorts [Atherosclerosis Risk in Communities study (ARIC, n=2511); Coronary Artery Risk Development in Young Adults (CARDIA, n=833); Cleveland Family Study (CFS, n=489), Jackson Heart Study (JHS, n=2017) and Multi-Ethnic Study of Atherosclerosis (MESA, n=1623); total n=7473] for the GWAS analysis and six cohorts [ARIC (n=2692), CARDIA (n=1134), Cleveland Family Study (CFS, n=530), CHS (n=735), JHS (n=1916), and MESA (n=1584); total n=8591)] for the IBC analysis. For JHS, we excluded these individuals who were overlapped with ARIC participants. The cohort specific sample characteristics are described in **Table 1**.

Genome-wide association of CARe African American cohorts for blood pressure

Meta-analysis quantile-quantile (QQ) and Manhattan plots of genome-wide SNPs including both genotyped and imputed for the two blood pressure phenotypes are presented in **Supplementary Figure 1**. If a SNP was genotyped, we always reported the it result based on genotyped data. In the meta-analysis of GWAS data, 1 SNP for DBP and 1 for SBP attained genome-wide significance (defined as $P < 5\times10^{-8}$) (**Table 2**). The strongest signal for DBP was rs10474346 ($P=3.6\times10^{-8}$) in the inter-genic region of *GPR98* and *ARRDC3* on chromosome 5q14. This SNP is in tight LD with a missense SNP (rs4377733; pairwise $r^2 = 0.9$) in hypothetical gene *LOC729040*. For SBP, the strongest signal was for rs2258119 in *C21orf91* on chromosome 21q21 ($P=4.7\times10^{-8}$), which is in tight LD with nearby rs2824495 ($r^2 = 1.0$), which is a missense SNP in *C21orf91*. Suggestive evidence of association was detected in the

regions of *IPO13* (rs1990151, P=7.4×10^{-7}), *FMNL2* (rs13413144, P=5.6×10^{-7}) and *GPD2* (rs592582, P=4.5×10^{-7}). The regional plots of association for the genome-wide significant loci are presented in **Figure 1**.

Pooled genotype data analysis was conducted for the five cohorts with Affymetrix 6.0 genotyping data using FamCC(8) , on genotyped SNPs only. In general, the results were highly consistent with those from the meta-analysis**.**

Association of SNPs on the IBC chip with blood pressure

Meta-analysis of CARe cohorts with IBC chip data did not identify any SNPs that reached the pre-specified array-wide significance level based on the estimated effective number of independent tests (SNPs) after adjusting for multiple testing (0.05/25,000=P<2.0×10^{-6}). There was suggestive evidence of association with SBP for two genes *NUCB2* (rs214070, P=8.7×10^{-6}; in LD with missense SNP rs757081, r^2=0.7) and *SLC25A42* (rs2012318, P=6.4×10^{-6}) in LD with missense SNP rs4808907, in *SFRS14*, r^2=0.6); these results are summarized in **Table 3**. The top results for DBP were rs2523586 (near *HLA-B*, P=1.3×10^{-6}) and rs4930130 (near *KCNQ1*, P=3.2×10^{-6}). These and other SNPs with P<10^{-4} are summarized in **Supplementary Table 3**.

Independent replication of top CARe SNPs in cohorts of African and European Ancestry

Replication cohorts for the study are described in detail in **Supplementary Section II**. Nine top SNPs (six selected from the genome wide meta-analysis and two selected from the candidate gene meta-analysis, and one selected from the CARDIA GWAS) in the CARe analyses were submitted for lookup in five AA cohorts (Maywood African-American study (n=743), Howard University Family Study (HUFS, n=1016), the International Collaborative Study on Hypertension in Blacks (ICSHIB, n=1188), the Genetic Epidemiology Network of Arteriopathy (GENOA, n=845) and the Women Health Initiative (WHI, n=8090)) and in whites of European ancestry in the ICBP consortium (n=69,899). Criteria for declaring replication was either 5.0×10^{-8} for final meta-analysis of GWAS SNPs or 2.0×10^{-6} for final meta-analysis of IBC SNPs. Results of

replication for SBP and DBP by replication cohort and results of the final meta-analysis of cohorts of African ancestry are provided in **Table 4**. None of the top SNPs from the Affymetrix 6.0 or the IBC array met the *a priori* criteria for replication after correcting for multiple comparisons. Results of replication by cohort are displayed in **Supplementary Table 4.**

Lookup of published SNPs from previous studies of people with African ancestry

We examined whether published SNPs from GWAS of blood pressure in people of African ancestry(9) could be replicated in our sample (**Supplementary Table 2A**). None of the previously reported loci for SBP or DBP replicated in our study.

Lookups of published SNPs from previous studies including populations of European ancestry

Two large scale GWAS in European populations have been published and 13 independent loci have been shown to be associated with blood pressure at a genome-wide significant level(10,11). We then performed one-sided test to examine if the association evidence for these published loci can be replicated in the CARe datasets. The one-sided test ensures the effect directions are the same in both discovery and replication samples. Of the SNPs reported in these two European GWAS (**Table 5 and Supplementary Tables 2A & 2B**), we identified the following association signals in our African Americans samples: rs3184504 (*SH2B3*, one-sided P=0.009), rs2384550 (*TBX3-TBX5*, one-sided P=0.03) and rs6495122 (*CSK-ULK3*, one-sided P=0.0004).

DISCUSSION

This study represents the largest GWAS of blood pressure in African Americans to date including a total of 8591 individuals for discovery and 11,882 individuals of African descent and 69,899 of European descent for replication. In a meta-analysis across five U.S. community-based cohorts using the Affymetrix 6.0 array, we identified two novel loci, rs2258119 and rs10474346 that reached genome-wide significance, but did not replicate in independent

African-American samples. We replicated several previously reported European American blood pressure SNPs in our CARe African American samples.

Top Loci for the Affymetrix 6.0 array GWAS

We identified a locus on chromosome 5 that reached genome-wide significance for DBP in CARe African-American cohorts. The top SNP (rs10474346, $P=3.6\times10^{-8}$) is in tight LD with a non-synonymous coding SNP rs4377733. Genes in the region include G protein-coupled receptor 98 (*GPR98*) and arrestin C (*ARRDC3*). GRR98 is a very large G-protein coupled receptor expressed in the central nervous system and other tissue and implicated in Usher syndrome characterized by hearing loss and retinitis pigmentosa. SNPs in GPR98 have been associated with markers of hyperglycemia in patients taking the antipsychotic medication olanzapine.(12) Arrestin C is a peroxisome proliferator-activated receptor gamma (*PPARG*) ligand and *PPARG* activator. PPARs are a family of nuclear receptors that are activated by nutrient molecules and their derivatives.(13) PPARG activators may play a role in hypertension and atherosclerosis through modification of inflammation and the innate immunity system in vascular cells.(13,14)

Another locus that reached genome wide significance for SBP in CARe AA cohorts is on chromosome 21,where a region was previously reported in admixture mapping analysis.(15) The top SNP at this locus, rs2258119 ($P=4.7\times10^{-8}$), is in tight LD with missense variant rs2824495 in *C21orf91* (pairwise $r^2=1.0$). The minor allele frequencies of this SNP in HapMap CEU and YRI samples are 21% and 34%, respectively, which suggests that this SNP may contribute to the association signal observed in the admixture mapping analysis. (15) This region includes *CXADR (*Coxsackie and Adenovirus receptors), which encodes a tight junction protein of the intercalated disks between cardiomyocytes. This protein is an entry point for virus uptake in myocarditis and is involved in cardiac remodeling.(16) A SNP of interest, rs1990151 on chromosome 1, showed suggestive association with SBP ($p=7.4\times10^{-7}$). This is an intronic SNP in importin beta (*IPO13*). Importin beta is a nuclear transport protein that modifies nuclear

availability of glucocorticoids through nucleocytoplasmic shuttling.(17) There is a potential link proposed between early-onset glucocorticoid exposure and hypertension through changes in gene expression and function in the kidney.(18) Of note, another importin beta protein (*IPO7*) was identified by Adeyemo et al in a genome wide association analysis of a normotensive subset of African Americans.(7)

Top SNPs from the meta-analysis of the IBC array

In our IBC array analysis we identified suggestive evidence of association for rs2012318, which is an intronic SNP in SLC25A42, a carrier protein that transports cofactor coenzyme A (CoA) and adenosine 3', 5' -diphosphate into the mitochondria in exchange for intramitochondrial (deoxy)adenine nucleotides and adenosine 3', 5' -diphosphate.(19) SNPs in this region were associated with LDL cholesterol and triglyceride levels in a whole genome analysis of European populations.(20)

Two tightly linked SNPs, rs4930130 and rs1791926 (r^2=1.0) on chromosome 11, were associated with DBP with $p < 1\times10^{-5}$. They are in proximity to *KCNQ1,* which encodes a protein for a voltage-gated potassium channel required for the repolarization phase of the cardiac action potential. The gene product is associated with hereditary long QT syndrome, Romano-Ward syndrome, Jervell and Lange-Nielsen syndrome, and familial atrial fibrillation.(21) Another signal of interest was found for rs1791926, near *P2RY2 (*purinergic receptor P2Y, G coupled 2) on chromosome 11q13.5-q14.1 that mediates vasoactive and proliferative stimuli. There is evidence that the purinergic system may affect the activity of epithelial sodium channel (ENaC) in the renal collecting duct, which is responsible for reabsorption of sodium.(22,23) Genetic defects in this channel in humans have been associated with hypertension in Liddle's syndrome. P2Y2 (a homologue of P*2RY2*) knock-out mice manifest a salt resistant hypertensive phenotype.(24) A recent case control association study by Wang et al. showed an association of *P2RY2* with hypertension in Japanese men.(25)

Association evidence of SNPs with blood pressure in CARDIA

It is intriguing that we observed a strong association signal in a 1.26Mb region on chromosome 11 (smallest $P=3.95\times10^{-9}$ for rs17610514) (**Supplementary Table 1**) in African Americans in the CARDIA cohort only. Although the allele frequencies for these significant SNPs are all relatively small (<4%), the results are unlikely due to the genotyping errors given the number of SNPs reaching genome wide significance. The sentinel SNP is in tight LD with several missense variants in olfactory receptor genes. The subjects recruited in CARDIA cohort are much younger than in the other cohorts, suggesting that the association is stronger in populations composed of younger individuals.

A particularly important contribution of this study is the generalization of findings from two large meta-analyses of Europeans and European Americans (10,11) to individuals of African ancestry. The three loci, near the *SH2B3, TBX3-TBX5* and *CSK-ULK3* genes, provide evidence for common genetic variants influencing BP phenotypes in AA and also suggest that at least some loci may confer broad susceptibility to hypertension across race/ethnicies.

Limitations

Because multiple cohorts were used to maximize the sample size in the analyses, heterogeneity in blood pressure measurement across the centers may bias our findings toward the null. Additionally, a substantial proportion of individuals were on blood pressure lowering medications, which may introduce some degree of misclassification of blood pressure. In addition, participants in JHS and ARIC were older with a large number on antihypertensive medications, whereas participants in CARDIA were significantly younger than the other cohorts with only a small percentage of participants on antihypertensive medications. We observed some evidence for heterogeneity across studies, with SNPs in GPR98 region (for DBP) on the Affymetrix 6.0 array (**Table 2**) and SNPs in the *SLC25A42* region (for both DBP and SBP) on the IBC array (**Table 3**) displaying the smallest heterogeneity p-values. Heterogeneity in the association results across studies may have attenuated association p-values, but also revealed mechanisms of action of genetic variants on blood pressure.

We did not observe clear replication of our two top loci that were genome-wide significant in our CARe GWAS. Our replication cohorts were generally small thus reducing the power to replicate significant findings. We estimated the proportion of variation in blood pressure associated with the two genome-wide significant SNPs at about 0.4% in CARe samples. Because of the winner's curse and the variation in LD between a true causal SNP and our identified SNP, our effect size may be overestimated, which may contribute to failure to replicate. In addition, population admixture may result in different LD patterns for the African-American samples from different geographical regions because the LD is dependent on the admixture proportion. It has been reported that the admixture proportion rate is different across the African-American population.(26,27) Thus, replication analysis can be challenging in African-American populations.

These limitations are leveraged against the advantage of using large community-based cohorts of African Americans for this analysis and the implementation of quality control procedures in individual examination centers and the harmonization of imputation strategies and analytical methods.

Conclusions

We found evidence of genetic influences on SBP and DBP. Evidence of association in our GWAS was found for DBP (rs10474346 on chromosome 5 near *GPR98* and *ARRDC3*) and for SBP (rs2258119 on chromosome 21in *C21orf91*). Caution should be paid because the two top SNPs identified in CARe GWAS were not replicated in independent cohorts of African ancestry and further replication efforts with large sample size are warranted.

Of note, several previously reported EA blood pressure SNPs did replicate in our CARe African American samples. These SNPs are in the regions of *SH2B3*, *TBX3-TBX5*, and *CSK-ULK3*.

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Implications

We identified genetic variants that reached genome wide significance for SBP and DBP in a large number of African Americans from the CARe consortium that did not replicate in a meta-analysis of cohorts of African ancestry. To our knowledge, these genetic loci represent the best evidence of genetic influences on SBP and DBP in African Americans to date. Hypertension represents the leading cause of death from cardiovascular disease in African Americans. Our study lends support to prior admixture analyses, which indicate that blood pressure represents a complex disease trait with genetic underpinnings within the African American community. Further investigation of the genetic loci identified in our analysis including replication efforts is warranted. Identification of potential genetic loci implicated in hypertension represents a unique opportunity to introduce new treatment and management strategies for this high risk population.

MATERIALS AND METHODS

Study sample

NHLBI's Candidate-gene Association REsource (CARe) Study includes six cohort studies with African American representation: the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Coronary Artery Risk Development in Young Adults (CARDIA), the Cleveland Family Study (CFS), the Jackson Heart Study (JHS), and the Multiethnic Study of Atherosclerosis (MESA; see **Supplementary Section I** for sampling details). Each study adopted collaboration guidelines and established a consensus on phenotype harmonization, covariate selection, and an analytical plan for within-study genetic association and prospective meta-analysis of results across studies. Each study received institutional review board approval of its consent procedures, examination and surveillance components, data security measures, and DNA collection and its use for genetic research. All participants in each study gave written informed consent for participation in the study and the

conduct of genetic research. African American samples from five cohorts (ARIC, CARDIA, CFS, JHS, and MESA) had genome-wide genotyping using the Affymetrix Genome-Wide Human SNP Array 6.0 array and blood pressure data for association analysis. Six cohorts (ARIC, CARDIA, CFS, CHS, JHS, MESA) had candidate gene genotyping in African Americans using the Illumina iSelect HumanCVD bead array.(28) We excluded individuals younger than 18 years of age.

Genotyping and quality control

Quality control of genotyping data was performed using PLINK.(29) Quality control efforts were conducted at two levels: exclusion of individuals and exclusion of SNPs. Samples with genotyping success rate <95% were removed. An inbreeding coefficient was calculated and used as a measure of heterozygosity. Outliers for heterozygosity (defined as <-4 SD or > 4 SD beyond the mean) were removed because of possible DNA contamination or poor DNA quality. For population-based cohorts, pair-wise identity-by-descent score was calculated and for each pair of identical samples, the sample with the lowest genotyping success rate was removed. In addition, samples that shared 5% or more of their genome with other samples also were excluded. Multidimensional scaling (MDS) was used to estimate population substructure and the identified outliers were removed.

There were 1176 SNPs that mapped to more than one locus in the human genome that were excluded from analysis. Individual SNPs were also excluded if they had a call rate of less than 90% or were monomorphic. For family data, Mendelian inconsistency was checked using PLINK and the corresponding SNPs were removed. No SNPs were removed due to significant deviation from Hardy-Weinberg Equilibrium (HWE) because the African-American population is an admixed population, which may result in departure from HWE.

Genotype imputation.

SNP imputation was performed using MACH and the HapMap phase 2 datasets (build 36 release 22) employing a similar strategy as that used by Kang et al.(9) In order to address the admixture component of our African-American population, a reference panel consisting of equal proportions of the YRI and CEU HapMap phased haplotypes (using only SNPs found in both YRI and CEU panels, i.e. ~2.2 million SNPs) was constructed. Because the CARe project had both IBC array and Affymetrix 6.0 data genotypes on the ~8500 individuals of African ancestry it was possible to assess the quality of the imputation process. The observed concordance was 95.6%, which is comparable to previous studies.(30) Imputation was performed for the Affymetrix 6.0 data only.

Phenotype modeling

Systolic (SBP) and diastolic (DBP) blood pressures were modeled at the first examination for ARIC, CHS, MESA and JHS, and at the most recent examination for CARDIA, and CFS in order to minimize the effect of extreme age differences between the cohorts. For ARIC and JHS, seated BP was measured with a random-zero sphygmomanometer three times with the last two measurements averaged. For CARDIA, seated BP was measured on the right arm following five minutes rest using a random-zero sphygmomanometer. SBP and DBP were recorded as Phase I and Phase V Korotkoff sounds. Three measurements were taken at one-minute intervals with the average of the second and third measurements taken as the blood pressure value. For CFS, blood pressure was measured using a mercury sphygmomanometer and was the average of nine readings (three each made over three intervals in an 18 hour period). Three measures were made supine before bed, three measures were made awake supine after bed and three were measured awake while sitting. For MESA, resting seated blood pressure was measured three times at one minute intervals using an automated oscillometric sphygmomanometer (Dinamap PRO 100, Critikon); the average of the second and third blood pressure measurements was used for these analyses. For individuals taking antihypertensive medication, we added 10 mm Hg and 5 mm Hg to the measured SBP and DBP(31),

respectively, to account for treatment effect. Continuous DBP and SBP were adjusted for age, age^2, sex, and body mass index (BMI) in linear regressions. Residuals were calculated and applied within cohort for analysis of genotype-phenotype associations.

Statistical analyses

Within each cohort, the first ten main eigenvectors from principal components (PCs) were calculated and included in the model testing genotype-phenotype association. The PCs were calculated based on selected ancestry informative markers. For comparison, we also calculated the PCs using the method described in Zhu et al.(8), in which the eigenvectors were calculated based on only unrelated individuals. PCs were then calculated for all individuals, including family members. Additionally in this method, all SNPs were used to calculate PCs. The results between the two methods were consistent, except for a few individuals (**Supplementary Figure 2**). We did not find that the discrepancy affected final association results. For all datasets except CFS, which includes family datasets, association of SNPs with SBP and DBP was tested by linear regression with additive genetic model using PLINK; for CFS, association was tested using a linear mixed effect (LME) model that accounted for family structure.(32)

Meta-analysis of results was carried out using the inverse-variance weighting method in METAL (http://www.sph.umich.edu/csg/abecasis/metal/). Genomic control was carried out on cohort-specific test statistics and used to adjust results within each study.

For comparison, analysis of pooled raw data from the five cohorts genotyped with the Affymetrix 6.0 array was carried out with FamCC (8). Cohort specific genotypes and standardized DBP or SBP residuals were pooled together. Principal components were calculated for all unrelated individuals and predicted for related individuals. Genotype-phenotype association was tested using a linear regression model with adjustment for the first ten PCs.

Previously published genome-wide significant SNP associations with blood pressure 7, 9, 10 were examined. If the published SNPs were not available in either genotyped SNPs or imputed SNPs in the current study, we used SNPs in strong LD with the sentinel SNPs as proxies.

Loci with a p value less than 1×10^{-6} for the GWAS data and less than 1×10^{-5} for IBC data were selected for replication analysis in independent cohorts of African and European ancestry. SNPs in LD ($r^2 \geq 0.5$) were considered to represent the same signal; consequently, the SNP with the smallest p value at a locus was selected for replication analysis.

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Conflict of Interest

The Authors declare no conflict of interests

Appendix

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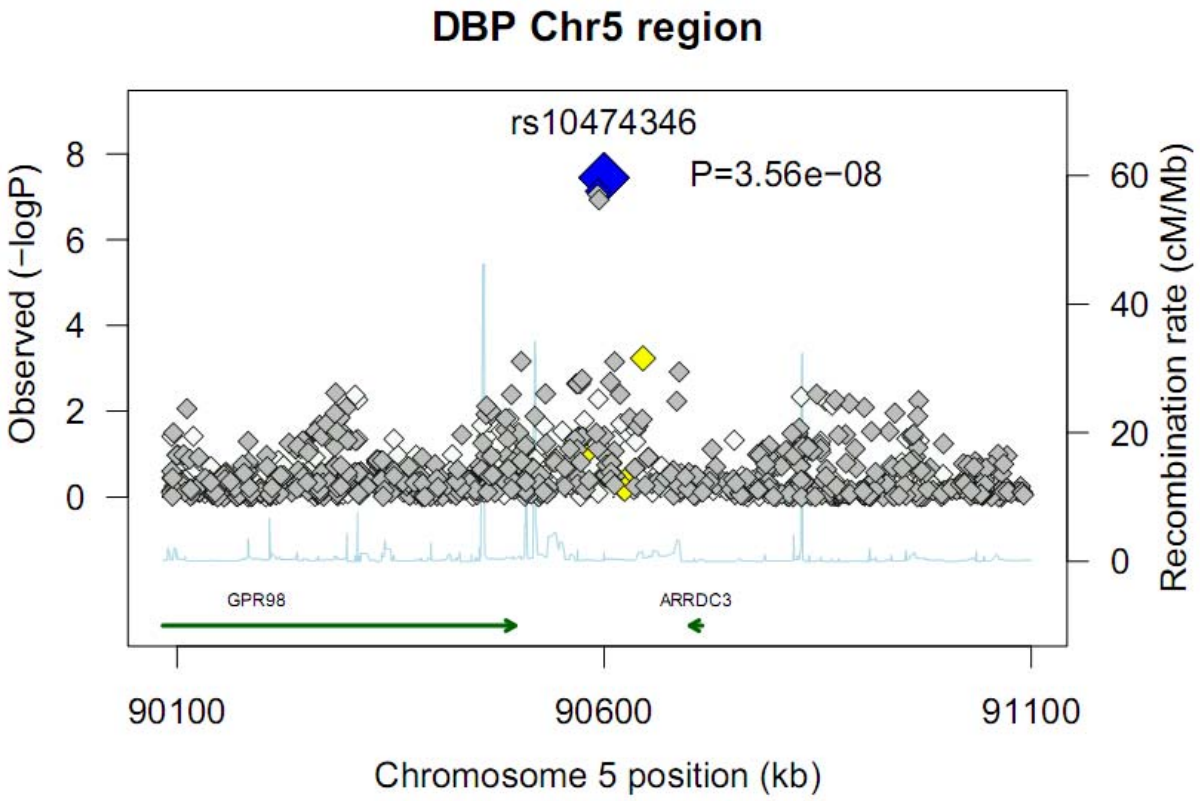
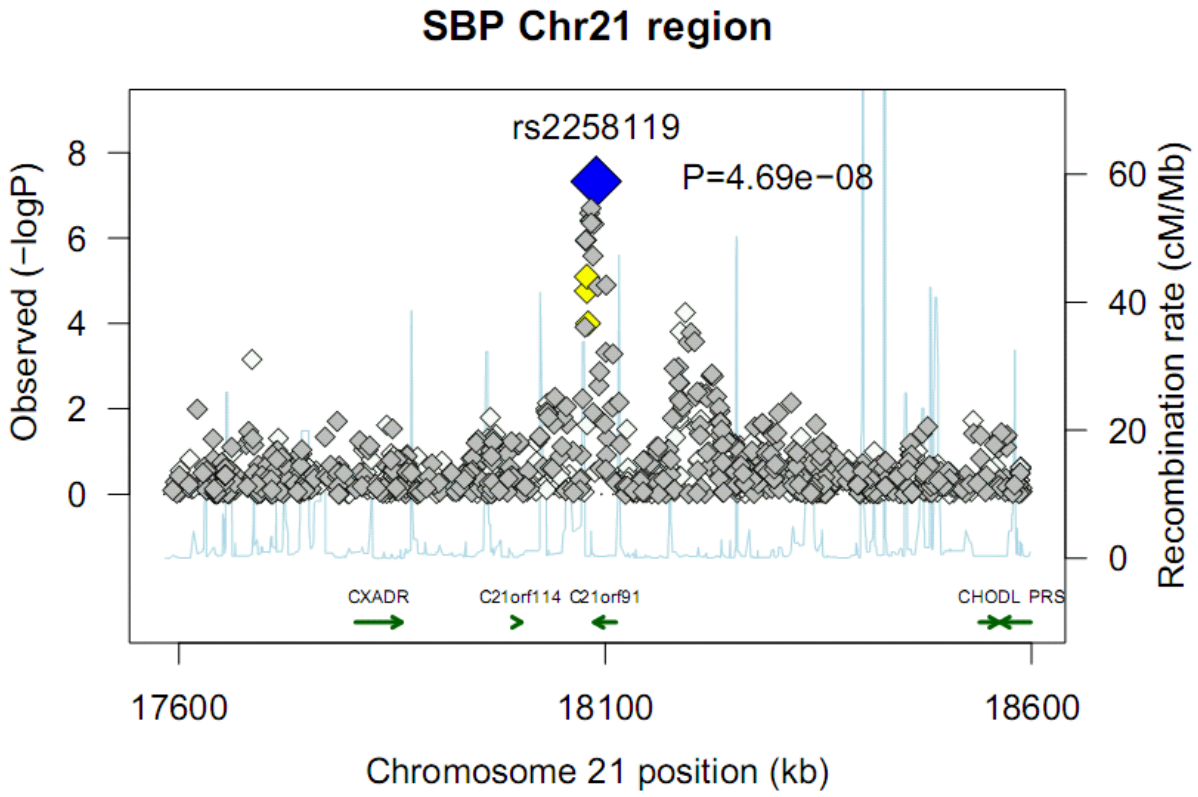
Figure 1. Regional plots of two blood pressure loci in African Americans from meta-analysis of Affymetrix 6.0 arrays. For each locus, we show the region extending to within 500kb of the associated SNP on either side. Statistical significance of SNPs around each locus are plotted as $-\log_{10}(P)$ against chromosomal position. For each locus, the most significant SNP is shown in blue. If the most significant SNP of a locus is imputed SNP (as in a.), then the most significant genotype SNP is shown in blue too. Among genotyped SNPs, SNPs in yellow have $r^2 \geq 0.8$ with the most significant genotyped SNP. Imputed SNPs are shown in grey. Superimposed on the plot are gene locations (green0 and recombination rate (blue). Chromosome positions are based on HapMap release 22 build 36.





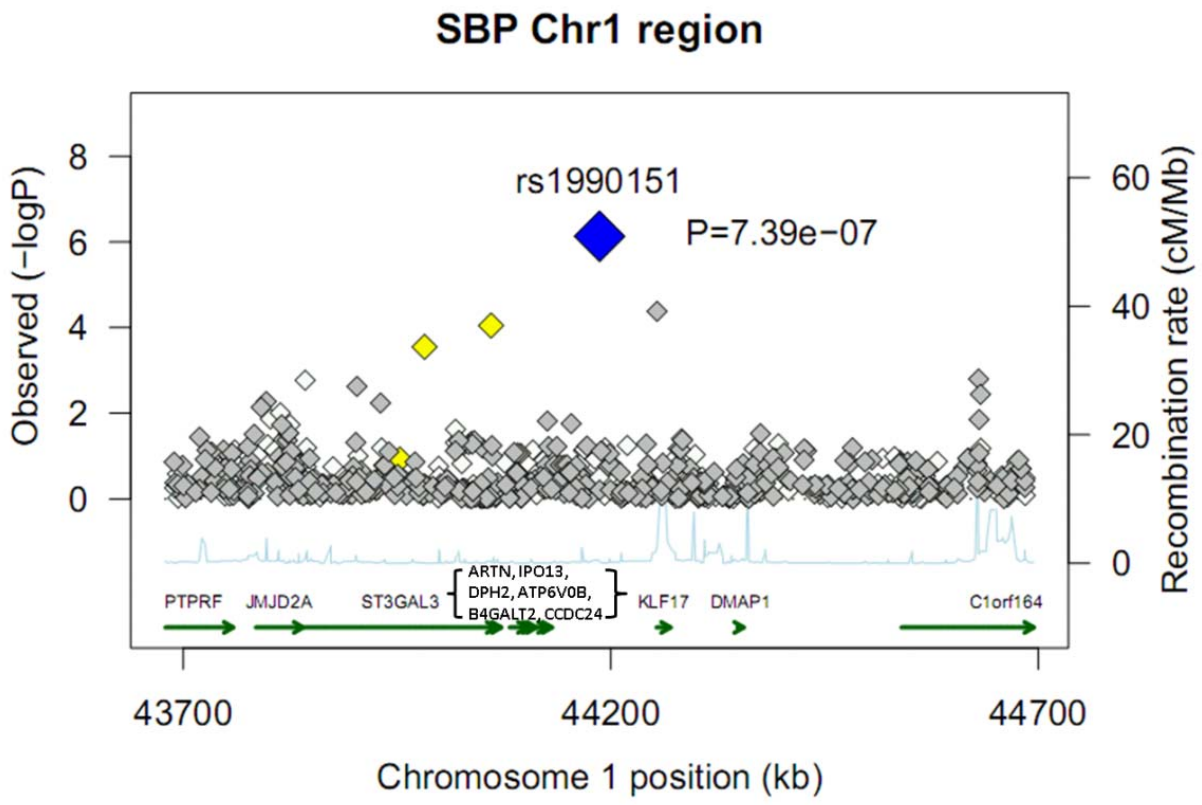


Table 1. Study sample characteristics

Study	N	Male (%)	Antihypertensive medication (%)	Age (years)		BMI (kg/m^2)		DBP (mm Hg)		SBP (mm Hg)	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
Affymetrix 6.0 genotyping											
ARIC	2511	37.1	44.0	53.3	5.8	29.6	6.0	79.7	12.1	128.3	20.8
CARDIA	833	38.1	13.0	39.5	3.9	30.8	7.5	76.9	12.1	116.9	16.4
CFS	489	40.7	38.9	45.7	16.2	34.6	9.6	76.5	10.7	128.2	16.0
JHS	2017	38.7	46.3	49.9	11.9	32.3	7.8	80.0	10.6	124.9	18.0
MESA	1623	45.7	50.5	62.2	10.1	30.2	5.9	74.5	10.2	131.4	21.7
IBC genotyping											
ARIC	2692	36.9	44.1	53.2	5.8	29.7	6.1	79.4	12.0	128.0	20.7
CARDIA	1134	40.5	13.8	39.6	3.8	30.7	7.5	77.0	12.4	117.1	16.3
CFS	530	42.1	37.7	45.2	16.1	34.2	9.6	76.4	10.8	127.7	15.8
CHS	735	37.6	51.8	73.0	5.7	28.5	5.6	75.1	11.3	141.8	22.7
JHS	1916	39.2	46.0	49.9	12.0	32.2	7.8	79.9	10.6	124.9	18.0
MESA	1584	46.1	50.9	62.2	10.1	30.2	5.9	74.6	10.3	131.7	21.6

Study characteristics are shown for cohort samples examined in meta-analysis. N, sample size - the number of individuals with genotype and phenotype data available.

Table 2. Top associated SNPs for blood pressure in African Americans from meta-analysis of Affymetrix 6.0 arrays

BP Trait	SNP ID	Chr	Position	Type	Nearest Gene	Effect allele	Effect allele freq	Other allele	CARe meta-analysis , DBP				CARe meta-analysis , SBP			
									Beta	s.e.	P	Heterogeneity P	Beta	s.e.	P	Heterogeneity P
SBP	rs1990151	1	44186879	Genotyped	IPO13	A	0.05	G	1.21	0.41	3.39×10^{-3}	0.56	3.48	0.70	7.39×10^{-7}	0.41
SBP	rs13413144	2	153183499	Imputed	FMNL2	T	0.05	A	1.75	0.50	4.77×10^{-4}	0.67	4.28	0.86	5.55×10^{-7}	0.08
SBP	rs592582	2	157481632	Imputed	GPD2	G	0.41	T	0.75	0.20	1.20×10^{-4}	0.94	1.66	0.33	4.46×10^{-7}	0.91
DBP	rs1858309	5	90592314	Imputed	GPR98	C	0.29	T	1.09	0.20	8.76×10^{-8}	0.04	1.35	0.35	1.03×10^{-4}	0.25
DBP	rs7709572	5	90592789	Genotyped	GPR98	G	0.30	C	1.10	0.20	7.41×10^{-8}	0.04	1.34	0.35	1.05×10^{-4}	0.29
DBP	rs7724489	5	90594122	Imputed	GPR98	A	0.29	T	1.08	0.21	1.17×10^{-7}	0.04	1.35	0.35	1.07×10^{-4}	0.25
DBP	**rs10474346**	**5**	**90599895**	**Imputed**	**GPR98/ ARRDC3**	**C**	**0.33**	**T**	**1.10**	**0.20**	$\mathbf{3.56\times10^{-8}}$	**0.11**	**1.40**	**0.34**	$\mathbf{3.73\times10^{-5}}$	**0.42**
SBP	rs243601	21	18081637	Imputed	C21orf91	G	0.49	A	0.63	0.21	2.26×10^{-3}	0.28	1.80	0.35	2.61×10^{-7}	0.36
SBP	rs243603	21	18082171	Imputed	C21orf91	C	0.45	T	-0.59	0.20	4.06×10^{-3}	0.29	-1.75	0.35	3.91×10^{-7}	0.52
SBP	rs243605	21	18082991	Imputed	C21orf91	C	0.46	G	-0.60	0.20	3.62×10^{-3}	0.29	-1.76	0.35	3.83×10^{-7}	0.40
SBP	rs243607	21	18083386	Imputed	C21orf91	G	0.44	A	-0.59	0.20	3.84×10^{-3}	0.24	-1.76	0.35	1.99×10^{-7}	0.46
SBP	rs243609	21	18083560	Imputed	C21orf91	T	0.40	C	-0.56	0.22	1.01×10^{-2}	0.18	-1.87	0.37	4.41×10^{-7}	0.48
SBP	rs2220511	21	18086782	Imputed	C21orf91	C	0.50	T	-0.52	0.21	1.17×10^{-2}	0.58	-1.75	0.35	4.68×10^{-7}	0.47
SBP	**rs2258119**	**21**	**18089350**	**Genotyped**	**C21orf91**	**C**	**0.32**	**T**	**0.79**	**0.20**	$\mathbf{6.90\times10^{-5}}$	**0.75**	**1.84**	**0.34**	$\mathbf{4.69\times10^{-8}}$	**0.70**

Beta: the effect size on blood pressure in mmHg, per effect allele based on the additive genetic model. Results of the two SNPs with genome-wide significance ($P<5\times10^{-8}$) are shown in bold.

Table 3. Top associated SNPs for blood pressure in African Americans from meta-analysis of IBC arrays

BP Trait	SNP ID	Chr	Position	Type	Nearest Gene	Effect allele	Effect allele freq	Other allele	CARe meta-analysis , DBP				CARe meta-analysis , SBP			
									Beta	s.e.	P	Heterogeneity P	Beta	s.e.	P	Heterogeneity P
African Americans																
SBP	rs12408339	1	154622134	Imputed	RHBG	A	0.08	G	-1.50	0.44	6.55×10^{-4}	0.27	-3.32	0.75	8.64×10^{-6}	0.53
SBP	rs214070	11	17261893	Genotyped	NUCB2	A	0.06	T	0.83	0.39	3.42×10^{-2}	0.16	2.97	0.67	8.65×10^{-6}	0.24
SBP	rs6511018	19	19047705	Imputed	SLC25A42	G	0.35	A	0.59	0.19	1.69×10^{-3}	0.08	1.43	0.32	5.83×10^{-6}	0.03
SBP	rs12985799	19	19048575	Imputed	SLC25A42	C	0.33	T	0.61	0.19	1.29×10^{-3}	0.15	1.48	0.32	3.24×10^{-6}	0.09
SBP	rs2012318	19	19069240	Genotyped	SLC25A42	C	0.35	T	0.58	0.19	1.90×10^{-3}	0.09	1.42	0.31	6.42×10^{-6}	0.03
SBP	rs11666627	19	19072238	Imputed	SLC25A42	C	0.35	T	0.59	0.19	1.55×10^{-3}	0.09	1.47	0.32	3.00×10^{-6}	0.03
SBP	rs10417974	19	19083070	Imputed	SLC25A42	C	0.35	T	0.59	0.19	1.57×10^{-3}	0.06	1.46	0.32	3.71×10^{-6}	0.03

MAF: minor allele frequency. Beta: the effect size on blood pressure in mmHg, per allele based on the additive genetic model.

Table 4. Meta-Analysis of CARe and additional African-origin cohorts, as well as the p-values in ICBP

					SBP		DBP	
					Meta	ICBP	Meta	ICBP
SNP ID	Chr	Position	Type	Nearest Gene	P	P	P	P
rs10474346	5	90599895	Affy6 Imputed	GPR98/ ARRDC3	9.96×10^{-3}	5.19×10^{-1}	1.02×10^{-3}	5.65×10^{-1}
rs13413144	2	153183499	Affy6 Imputed	FMNL2	9.28×10^{-4}	4.22×10^{-1}	1.06×10^{-1}	8.00×10^{-1}
rs17610514#	11	55652374	CARDIA	Olfactory	8.50×10^{-5}	2.59×10^{-1}	3.90×10^{-5}	2.20×10^{-1}
rs1990151	1	44186879	Affy6 Genotyped	IPO13	1.14×10^{-3}	8.20×10^{-1}	8.63×10^{-3}	8.14×10^{-1}
rs2012318	19	19069240	IBC Genotyped	SLC25A42	5.68×10^{-6}	5.92×10^{-1}	6.21×10^{-3}	6.04×10^{-1}
rs214070*	11	17261893	IBC Genotyped	NUCB2	NA	3.23×10^{-2}	NA	1.67×10^{-2}
rs2258119	21	18089350	Affy6 Genotyped	C21orf91	5.00×10^{-4}	5.28×10^{-1}	1.41×10^{-2}	6.54×10^{-1}
rs592582	2	157481632	Affy6 Imputed	GPD2	9.15×10^{-3}	9.16×10^{-1}	1.22×10^{-1}	6.59×10^{-1}
rs7709572	5	90592789	Affy6 Genotyped	GPR98	1.38×10^{-2}	4.56×10^{-1}	6.49×10^{-4}	5.68×10^{-1}

META_P: p-value by combining all cohorts of African ancestry.

ICBP_P: One sided p value in ICBP data.

For SNPs genotyped in Affy 6.0, p value < 5×10^{-8} is considered as statistically significant. For SNPs genotyped in IBC chip, p value < 2×10^{-6} is considered as statistically significant.

*: SNP rs214070 was not genotyped in the cohorts of African ancestry except CARe.

#: SNP rs17610514, failed WHI QC due to low concordance rate among duplicates (<98%) and/or low call rate (<95%) and thus was not included in the meta-analysis.

Table 5. Look-up of top SNPs for SBP and DBP from the meta-analysis of CHARGE and Global BPgen. Results of SNPs names labeled with * are from imputed SNPs.

SNP identifier	Chr	Position	Nearest gene	Alleles (coded/ other)	CHARGE + Global BPgen meta-analysis			CARe meta-analysis , DBP				CARe meta-analysis , SBP			
					Beta	s.e.	*P* value	Effect allele	Other allele	Effect	P	Effect allele	Other allele	Effect	P
SNPs in boldface attained $P < 5\times10^{-8}$ in meta-analysis of CHARGE and Global BPgen.															
Systolic blood pressure															
rs12046278*	1	10,722,164	*CASZ1*	T/C	-0.53	0.12	4.77×10^{-6}	C	T	0.40	0.23	C	T	0.06	0.92
rs7571613*	2	190,513,907	*PMS1*	A/G	-0.54	0.13	1.90×10^{-5}	G	A	-0.16	0.46	G	A	-0.28	0.45
rs448378	3	170,583,593	MDS1	A/G	-0.51	0.10	1.18×10^{-7}	A	G	0.15	0.42	A	G	0.13	0.68
rs2736376*	8	11,155,175	MTMR9	C/G	-0.48	0.15	9.15×10^{-4}	C	G	-0.31	0.13	C	G	-0.47	0.18
rs1910252*	8	49,569,915	*EFCAB1*	T/C	-0.43	0.13	6.13×10^{-4}	T	C	0.03	0.90	T	C	-0.23	0.51
rs11014166*	10	18,748,804	*CACNB2*	A/T	0.50	0.10	7.03×10^{-7}	T	A	-0.20	0.44	T	A	-0.30	0.50
rs1004467*	**10**	**104,584,497**	***CYP17A1***	**A/G**	**1.05**	**0.16**	$\mathbf{1.28\times10^{-10}}$	G	A	0.01	0.97	G	A	-0.31	0.48
rs381815*	**11**	**16,858,844**	***PLEKHA7***	**T/C**	**0.65**	**0.11**	$\mathbf{1.89\times10^{-9}}$	T	C	0.41	0.07	T	C	0.62	0.10
rs2681492 ***	**12**	**88,537,220**	***ATP2B1***	**T/C**	**0.85**	**0.13**	$\mathbf{3.76\times10^{-11}}$	C	T	-0.18	0.54	C	T	-0.22	0.65
rs3184504*	**12**	**110,368,991**	***SH2B3***	**T/C**	**0.58**	**0.10**	$\mathbf{4.52\times10^{-9}}$	T	C	0.53	0.14	T	C	1.61	9.13×10^{-3}
Diastolic blood pressure															
rs13423988*	2	68,764,770	*GPR73-*	T/C	0.33	0.08	5.00×10^{-5}	T	C	-0.10	0.62	T	C	0.08	0.81
rs13401889*	2	190,618,804	*MSTN*	T/C	-0.31	0.08	4.82×10^{-5}	T	C	-0.01	0.94	T	C	0.16	0.63
rs9815354*	**3**	**41,887,655**	***ULK4***	**A/G**	**0.49**	**0.08**	$\mathbf{2.54\times10^{-9}}$	A	G	0.16	0.49	A	G	0.26	0.40
rs7016759	8	49,574,969	*EFCAB1*	T/C	0.30	0.08	2.29×10^{-4}	C	T	-0.27	0.50	C	T	-0.64	0.44
rs11014166*	**10**	**18,748,804**	***CACNB2***	**A/T**	**0.37**	**0.06**	$\mathbf{1.24\times10^{-8}}$	T	A	-0.20	0.44	T	A	-0.30	0.50
rs11024074	11	16,873,795	*PLEKHA7*	T/C	-0.33	0.07	1.20×10^{-6}	C	T	0.45	0.20	C	T	0.52	0.13
rs2681472*	**12**	**88,533,090**	***ATP2B1***	**A/G**	**0.50**	**0.08**	$\mathbf{1.47\times10^{-9}}$	G	A	-0.40	0.20	G	A	-0.61	0.25
rs3184504*	**12**	**110,368,991**	***SH2B3***	**T/C**	**0.48**	**0.06**	$\mathbf{2.58\times10^{-14}}$	T	C	0.53	0.14	T	C	1.61	9.13×10^{-3}
rs2384550	**12**	**113,837,114**	***TBX3-TBX5***	**A/G**	**-0.35**	**0.06**	$\mathbf{3.75\times10^{-8}}$	A	G	-0.35	0.07	A	G	-0.75	0.03
rs6495122*	**15**	**72,912,698**	***CSK-ULK3***	**A/C**	**0.40**	**0.06**	$\mathbf{1.84\times10^{-10}}$	C	A	-0.51	0.02	C	A	-1.32	3.74×10^{-4}

Abreviations:
Candidate-gene Association REsource (CARe)
African Americans (AA)
genome wide association studies (GWAS)
systolic blood pressure (SBP)
diastolic blood pressure (DBP)
cardiovascular gene-centric array (ITMAT-Broad-CARe [IBC] array)
National Health and Nutrition Examination Survey (NHANES)
linkage disequilibrium (LD)
Single nucleotide polymorphism (SNP)
Atherosclerosis Risk in Communities study (ARIC)
Coronary Artery Risk Development in Young Adults (CARDIA)
Cleveland Family Study (CFS)
Jackson Heart Study (JHS)
Multi-Ethnic Study of Atherosclerosis (MESA)
quantile-quantile (QQ)
Howard University Family Study (HUFS)
the International Collaborative Study on Hypertension in Blacks (ICSHIB)
the Genetic Epidemiology Network of Arteriopathy (GENOA)
Women Health Initiative (WHI)
The International Consortium for Blood Pressure Genome-wide Association Studies (ICBP)
Hardy-Weinberg Equilibrium (HWE)
Multidimensional scaling (MDS)
body mass index (BMI)
linear mixed effect (LME)