Title

Variants in CXADR and F2RL1 are associated with blood pressure and obesity in African-Americans in regions identified through admixture mapping

Journal

Journal of Hypertension, 30(10)

ISSN

0263-6352

Authors

Shetty, Priya B
Tang, Hua
Tayo, Bamidele O
et al.

Publication Date

2012-10-01

DOI

10.1097/hjh.0b013e3283578c80

Peer reviewed

Published in final edited form as:
J Hypertens. 2012 October ; 30(10): 1970–1976. doi:10.1097/HJH.0b013e3283578c80.

Variants in *CXADR* and *F2RL1* are associated with blood pressure and obesity in African-Americans in regions identified through admixture mapping

Priya B. SHETTY[a], **Hua TANG**[b], **Bamidele TAYO**[c], **Alanna C. MORRISON**[d], **Craig L. HANIS**[d], **D.C. RAO**[e], **J. Hunter YOUNG**[f], **Ervin R. FOX**[g], **the Candidate Gene Association Resource (CARe) Consortium**[h], **Eric BOERWINKLE**[d], **R.S. COOPER**[c], **Neil RISCH**[i], and **Xiaofeng ZHU**[a]

[a]Department of Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106

[b]Department of Genetics, Stanford University School of Medicine

[c]Department of Preventive Medicine and Epidemiology, Loyola University of Chicago Stritch School of Medicine

[d]Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas Health Science Center at Houston School of Public Health

[e]Division of Biostatistics, Washington University in St. Louis School of Medicine

[f]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

[g]Division of Cardiology, Department of Medicine, University of Mississippi Medical Center

[h]National Heart, Lung, and Blood Institute

[i]Department of Epidemiology and Biostatistics, University of California, San Francisco

Abstract

Objective—Genetic variants in 296 genes in regions identified through admixture mapping of hypertension, BMI, and lipids were assessed for association with hypertension, blood pressure, BMI, and HDL-C.

Methods—This study identified coding SNPs identified from HapMap2 data that were located in genes on chromosomes 5, 6, 8, and 21, where ancestry association evidence for hypertension, BMI or HDL-C was identified in previous admixture mapping studies. Genotyping was performed in 1,733 unrelated African-Americans from the National Heart, Lung and Blood Institute's (NHLBI) Family Blood Pressure Project, and gene-based association analyses were conducted for hypertension, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, and HDL-C. A gene score based on the number of minor alleles of each SNP in a gene was created and used for gene-based regression analyses, adjusting for age, age^2, sex, local marker ancestry, and BMI, as applicable. An individual's African ancestry estimated from 2,507 ancestry-informative markers was also adjusted for to eliminate any confounding due to population stratification.

Author Responsible for Correspondence Concerning the Manuscript and to Whom Requests for Reprints Should be made: Xiaofeng Zhu, Department of Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, Wolstein Research Building, 2103 Cornell Road, Room 1317, Cleveland, OH 44106; Xiaofeng.Zhu@case.edu; 216-368-0201.

Conflicts of Interest:
The authors declare no conflicts of interest.

Results—*CXADR* (rs437470) on chromosome 21 was associated with SBP and DBP with or without adjusting for local ancestry ($p < 0.0006$). *F2RL1* (rs631465) on chromosome 5 was associated with BMI ($p = 0.0005$). Local ancestry in these regions was associated with the respective traits as well.

Conclusions—This study suggests that *CXADR* and *F2RL1* likely play important roles in blood pressure and obesity variation, respectively; and these findings are consistent with other studies, so replication and functional analyses are necessary.

Keywords

Blood pressure; Obesity; African Americans; Genetic Association Studies

Background

More than 82.6 million adults in the United States have cardiovascular disease, which is associated with hypertension, poorly controlled cholesterol levels, and obesity [1]. Poor cardiovascular health is associated with a number of serious outcomes, including stroke and coronary heart disease, and death. It is evident that this is a substantial public health issue.

Approximately one-fourth of adults worldwide have high blood pressure with about 7.5 million deaths worldwide due to high blood pressure [2]. Some 34–67% of the inter-individual variation in blood pressure is thought to be due to genetic factors [3–7]. Similarly, approximately 2.6 million deaths worldwide are due to high cholesterol and about 2.8 million deaths worldwide are due to overweight or obese status [2]. Estimates of the genetic contribution to the phenotypic variation of these diseases are 40–69% for HDL-C, 40–66% for LDL-C, and 16–85% for obesity determined by BMI [8–14]. Genome-wide association studies (GWAS) and admixture mapping analyses in recently admixed populations are two methods that have been used to identify genetic variants associated with blood pressure, lipids, and BMI.

Common genetic variants for hypertension have been identified through GWAS, but the resulting associations are of modest effect sizes. In the CHARGE Consortium and the Global BPgen Consortium blood pressure GWAS, 13 loci were collectively associated with 1.0 mm Hg increase in SBP and 0.5 mm Hg increase in DBP [15, 16]. Recently, the largest blood pressure GWAS identified 29 independent variants at 28 loci (16 novel loci) significantly associated with blood pressure in approximately 200,000 subjects; however these variants collectively accounted for only 0.9% of the phenotypic variance [17].

In a smaller GWAS conducted in 8,591 African-Americans in the Candidate Gene Association Resource (CARe) study, Fox et al. reported two SNPs that were significantly associated with blood pressure but were not replicated [18]. Another GWAS of 1,017 African-Americans identified several SNPs that were significantly associated with SBP, DBP, and hypertension; however, none of these findings were successfully replicated in the larger CARe study [18, 19]. Difficulty in replicating significant findings illustrates the challenges in identifying genetic variants that affect blood pressure in the African-American population.

In the largest GWAS conducted for blood lipids, significant results for 95 loci (59 novel loci) were reported [20]. For HDL-C, 62 loci were significant and 57 loci were significant for LDL-C. A number of loci overlapped between the two traits. In an analysis that included secondary signals in 26 loci, the mapped variants collectively accounted for 25–30% of the genetic variance of each lipid phenotype.

For obesity, one of the earliest GWAS found a genetic variant in the *FTO* gene on chromosome 16q that was significantly associated with BMI [21]. A multi-stage obesity GWAS later reported significant results for 11 regions (7 novel regions), and the cumulative effect of the 11 loci accounted for less than 1% of the population variability of obesity [22]. Another large GWAS reported 32 loci (18 novel loci) that were associated with BMI, but cumulatively, the loci explained only 2–4% of the genetic variance of BMI [23]. Further, the authors estimated that only 6–11% of the genetic variation of BMI could be explained by almost 300 genetic variants of similar effect sizes that probably exist in a sample size of over 730,000. These analyses clearly demonstrate some of the difficulties of conducting GWAS for complex diseases.

In addition to GWAS, African-American populations and other admixed populations are well-suited for admixture mapping studies to identify genetic variants associated with blood pressure, lipids and BMI [24, 25]. Admixture mapping capitalizes on differences in disease prevalence between parental populations of an admixed population to detect genetic variants associated with the disease. Previously, results of admixture analyses for hypertension, BMI and lipids in African-Americans were reported [26–30]. Several genomic regions were identified for the phenotypes of interest, including 6q24 and 21q21 for hypertension, 5q14-5q32 for BMI, and 8q11-8q21 for high-density lipoprotein (HDL-C).

The present study follows up SNPs identified from HapMap2 data in genes in four genomic regions, 5q14-32, 6q24, 8q11-8q21, and 21q21, that were reported in previous admixture analyses [26–29]. African-American subjects from the National Heart, Lung and Blood Institute's (NHLBI) Family-Based Blood Pressure Program (FBPP) were genotyped for SNPs in the four admixture regions and for 2,507 ancestry informative markers [31]. The aim of this study was to find variants in coding regions that are associated with systolic and diastolic blood pressure, hypertension, BMI, and HDL-C in these four genomic regions that were identified in admixture mapping studies. Gene-based analyses, rather than single SNP analyses, were used to assess the variants with an emphasis on biological function. In addition, the study was conducted in the African-American population, so there are advantages in utilizing their admixture to further examine the genetic variation of hypertension and in focusing on a population that has been shown to be more likely to have poorly controlled blood pressure than non-Hispanic white hypertensive adults [32].

Methods

Sample

The NHLBI's Family-Based Blood Pressure Program is a multi-center study that examines the genetic causes of hypertension and related phenotypes in different racial and ethnic groups, African Americans, Asians and Asian Americans, European Americans, and Mexican Americans [31]. As the FBPP study was designed for linkage analysis, each of the networks in the FBPP ascertained families via probands with elevated blood pressure. This study focused on African Americans, who were recruited for three FBPP Networks. The Hypertension Genetic Epidemiology Network (HyperGEN) recruited African American subjects from Birmingham, Alabama and Forsyth County, North Carolina. The Genetic Epidemiology Network of Atherosclerosis (GENOA) included African Americans from Jackson, Mississippi, and the GenNet study recruited African Americans from Maywood, Illinois. Additional details of the FBPP Networks are described elsewhere [31].

In this study, 1,733 unrelated subjects, 18 to 70 years, were selected for analysis from the FBPP data by first selecting the control from the families, if any were available. If there were multiple controls present, the oldest control was selected. For cases, the youngest case in the family was selected. Systolic and diastolic blood pressure measurements were

obtained from Dinamap blood pressure monitors. BMI was calculated from weight (in kilograms) and height (in meters) measurements as weight/height^2.

Genotyping Methods

SNPs from HapMap2 data that were located in the exons of 296 genes in regions on chromosomes 5q14-32, 6q24, 8q11-21, and 21q21 were identified. Each of these regions was defined as the one unit drop of the −log(P) value from the peak in the admixture mapping analysis. As a result, 91 genes on chromosome 5, 117 genes on chromosome 6, 37 genes on chromosome 8, and 51 genes on chromosome 21 were examined in this study. These regions were selected for this study because they showed association evidence to hypertension, BMI, or HDL-C in previous admixture studies [26–29]. Genes on chromosome 5 were examined for association with BMI, genes on chromosome 8 were examined for association with HDL-C, and genes on chromosomes 6 and 21 were examined for association with SBP, DBP, and hypertension. In addition, 2,507 ancestry-informative markers (AIMs) across the genome were assessed to differentiate between African and European local and global ancestry and to adjust for population stratification.

Each of the four networks obtained blood samples from the subjects, and DNA was extracted by standard methods. The subjects were genotyped using the Illumina iSelect Custom Bead Chip at the University of California San Francisco. The genetic data were examined for departures from Hardy-Weinberg equilibrium (HWE) in the hypertension controls. Two SNPs were found to have departures from HWE at the threshold corrected for testing 611 coding SNPs ($p = 8 \times 10^{-5}$), and these two SNPs were removed from the analysis. In addition, the genotype call rates were all ≥ 95%. The AIMs were selected from SNPs available on the Illumina Human 1M array, the Illumina 650K array, and the Affymetrix 6.0 array.

Statistical Methods

Hypertension was defined as having SBP ≥ 140 mm Hg, DBP ≥ 90 mm Hg, and/or taking prescription medication for high blood pressure [33]. Sub-optimal HDL-C levels were < 40 mg/dL in men and < 50 mg/dL in women, and a sub-optimal low density lipoprotein cholesterol (LDL-C) level was ≥ 100 mg/dL [34]. Obesity was defined as having a body-mass index (kg/m^2) ≥ 30 [35].

First, SBP and DBP were imputed for subjects being treated for hypertension by adding 10 mm Hg to SBP and 5 mm Hg to DBP for these subjects, consistent with the CHARGE GWAS strategy [15]. Descriptive statistics were presented in Table 1. The number of minor alleles for each SNP was counted, and the SNPs in each gene were collapsed in an additive manner. Specifically, each SNP was coded as 0, 1 or 2, based on the number of minor alleles present in the SNP; then, a gene score was obtained by summing all SNP values in each gene for each person. Association tests were based on these gene scores.

For hypertension, multivariable logistic regression analysis was conducted with adjustment for age, age^2, sex, and BMI. For SBP, DBP and HDL-C, age, age^2, sex, and BMI were adjusted for in regression models, and the BMI regression models were adjusted for age, age^2, and sex. Local ancestry and global African ancestry were estimated from the 2,507 AIMs using ADMIXPROGRAM [36]. To eliminate the effect by population stratification, global ancestry was included in the regression models [30]. Analyses were performed with and without local ancestry as a covariate in the regression models [37]. Finally, multiple comparisons were corrected for by adjusting for the number of genes in all four regions using the Bonferroni correction to obtain adjusted critical p-values.

It was expected that variants with association evidence should show a substantial difference in allele frequencies between African and European populations, since this study focused on variants in regions where local marker-specific ancestry was associated with phenotypic variation. Therefore, the minor allele frequencies were compared for each variant for the presumed representative reference populations for African Americans, the Yoruba in Ibadan, Nigeria and the CEPH (Utah residents with ancestry from northern and western Europe) [38].

Replication analyses of the study findings were performed in two cohorts obtained from the NHLBI's Candidate-gene Association Resource (CARe) [39]. CARe is a multi-network study examining associations between genotypes and phenotypes that are of interest to the NHLBI, including blood pressure, lipids and blood biomarkers. There are five African American cohorts in CARe, and they were genotyped on the Affymetrix 6.0 platform. The replication analyses were conducted on 2,916 African Americans in the ARIC (Atherosclerosis Risk in Communities) cohort and 2,144 African Americans in the JHS (Jackson Heart Study) cohort, as they were most comparable to the FBPP dataset in terms of demographics. The SNPs identified in this study were not available in Affymetrix 6.0 platform; therefore, imputed SNPs in ARIC and JHS were used in the replication analysis and these SNPs had quality scores of $R^2 > 0.92$. The local ancestry estimates for the genes of interest were also used for the replication analysis.

All statistical analyses were conducted with PLINK [40] and SAS 9.2 (Cary, North Carolina).

Results

The effects of SNPs in genes in coding regions on chromosomes 5, 6, 8, and 21 on SBP, DBP, hypertension and BMI were evaluated in 1,733 unrelated African-American subjects. The descriptive statistics of the FBPP dataset were presented in Table 1.

In the analysis, 117 genes on chromosome 6 (6q24) and 51 genes on chromosome 21 (21q21) were studied. For BMI, 91 genes in the genomic region 5q14-32 were examined. For HDL-C, 37 genes in the genomic region 8q11-21 were studied. Genes were reported in Table 2 if they were statistically significant after adjusting for the total number of genes tested for each trait. Genes were reported with and without local ancestry in the models to determine if additional variants of interest were present in the same local ancestry region. The effect of local ancestry alone was also included.

The gene *CXADR* on chromosome 21 was significantly associated with increased SBP (β = 3.17, p = 0.0001) and DBP (β = 1.70, p = 0.0002) before adjusting for local ancestry. This gene was still significant after including local ancestry in the regression models (SBP:β = 2.94, p = 0.0004; DBP:β = 1.60, p = 0.0006). All of these analyses adjusted for age, age^2, sex, BMI, and global African ancestry; these results were still significant after adjusting for testing 51 genes for SBP and for DBP. Since local ancestry was significant (p = 0.011 for SBP and p = 0.001 for DBP), there may be other variants in this region that are independently associated with SBP and DBP. In this study, *CXADR* only contained the non-synonymous SNP rs437470. The minor allele was far more prevalent in the YRI population than in the CEU population (C: 40.7% vs 9.7%), and it was moderately common (C: 23.5%) in the HapMap ASW (individuals with African ancestry in Southwest USA) population.

The gene *F2RL1* on chromosome 5 was associated with obesity as measured by BMI (β = 3.71, p = 0.0005) after adjusting for age, age^2, sex, and local and global African ancestry. The association with BMI was less significant before adjusting for local ancestry (β = 3.41, p = 0.0011). Local African ancestry was not significant for BMI (p = 0.352). The

synonymous SNP rs631465 was the only SNP examined in *F2RL1* in our study. The minor allele was similarly infrequent in the YRI population as in the CEU population (A: 0.0% vs 4.1%). Despite testing 37 genes in a region on chromosome 8 that were selected based on admixture mapping results, no significant results were found for HDL-C.

Additional genes that were nominally significant at $\alpha = 0.05$ but were not significant after correction for multiple comparisons were reported in Table 4. The results in Table 4 were reported from regression models with and without local ancestry. The effect of local ancestry alone was also reported. For models that were more significant with the local ancestry adjustment than without it, the results indicated that there may be additional variants in this region associated with the phenotype.

Replication Analyses

In the replication analyses using local ancestry estimates, association evidence for *CXADR* and *F2RL1* was not seen in the combined ARIC and JHS dataset (N = 3,723). Local ancestry at *F2RL1* was marginally associated with BMI in the multivariable model ($\beta = -0.86$, $p = 0.0549$) (Table 3). For SBP and DBP, the association of local ancestry at *CXADR* was not significant, but the effects were in the same direction as in the FBPP dataset (SBP-$\beta = 1.59$, $p = 0.2483$; DBP-$\beta = 1.23$, $p = 0.1248$).

When only the genes were assessed, the statistically-significant results from the FBPP study did not replicate in the combined ARIC and JHS cohorts (N = 5,044), and the effect sizes were muted compared to the results in the primary dataset. The results for *CXADR* in the combined ARIC and JHS datasets were in the same direction as in the FBPP analysis after accounting for local ancestry (β-SBP = 0.19, $p = 0.71$ and β-DBP = 0.04, $p = 0.90$) (Table 2). The SNP in *F2RL1* was not present in the replication sets, so it was not possible to test this gene. It is important to note that the subjects in the ARIC and JHS datasets had SNPs imputed, rather than genotyped directly. Although the imputation quality for each of the SNPs was high, imputed SNPs may have reduced the study's power to replicate the FBPP findings.

Discussion

An association analysis of genetic variants in genomic coding regions that demonstrated association evidence in previous admixture mapping analyses was conducted [26–29, 31]. Coding SNPs that were identified from the HapMap project, rather than tagging SNPs, were of interest in this study. Thus, the burden due to the multiple comparisons was reduced by increasing the prior probability of the testing SNPs. To further reduce the number of comparisons, the SNPs for each gene were collapsed and a gene based analysis was performed, similar to a rare variant analysis [41]. The advantages of such an approach are a substantial reduction in the number of tests conducted and the incorporation of a biological framework into the analysis.

The gene *CXADR* on chromosome 21 was identified as being statistically-significantly associated with SBP and DBP after correcting for testing 51 genes. One non-synonymous SNP, rs437470, was identified from the HapMap data in *CXADR*. Interestingly, there was a substantial difference in the minor allele frequency of rs437470 between the HapMap YRI and CEU samples, suggesting that this result was consistent with the finding that African ancestry is associated with increased blood pressure in admixture mapping studies [28–30]. Adjustment of local African ancestry resulted in models that were still significantly associated with SBP and DBP, suggesting that additional variants in this region may play a role in blood pressure variation. The replication analyses using the local ancestry estimates were consistent with these findings. Furthermore, *CXADR* plays a role in the electrical

conduction of the heart, and it has also been reported to be associated with viral myocarditis and subsequent dilated cardiomyopathy, which is associated with high blood pressure [42, 43]. Recently, a SNP near *CXADR* was reported to have an association with ventricular fibrillation in acute myocardial infarction [44].

The gene *F2RL1* on chromosome 5 was significantly associated with BMI after correcting for 91 genes. In our analysis, this gene only contains the synonymous SNP rs631465, and the frequency of this SNP's minor allele was infrequent in both the YRI and CEU populations. Interestingly, the association evidence was improved for this gene when local ancestry was included in the regression model, suggesting that this variant was unlikely to fully explain the association evidence observed in the admixture mapping of BMI [26]. The replication analyses showed consistent results that the local ancestry was significantly associated with BMI, indicating other variants for BMI may exist in this region. The association with obesity as measured by BMI was consistent with the finding that mice lacking this gene were resistant to weight gain [45]. The gene *F2RL1* encodes the protein protease-activated receptor 2 (PAR2), and the activation of this receptor via coagulation factor VIIa is a key pathway for obesity. The association of *F2RL1* with nadir BMI was also reported recently in a study of expression SNPs (eSNPs) in Roux-en-Y gastric bypass patients [46].

Overall, the results indicated that the local ancestry association regions contain variant(s) of interest for the phenotypes. The results implied that the association between BMI and *F2RL1* may be real, but it was not known if rs631465 (*F2RL1)* is in linkage disequilibrium (LD) with a causal SNP for BMI. Local ancestry of *F2RL1* was not significantly associated with BMI in the FBPP analysis, but it was associated with BMI in the multivariable replication analysis in the CARe dataset. This indicated that other local variants of interest for BMI may be present, but rs631465 in *F2RL1* likely accounted for some of the admixture evidence in this region. As *F2RL1* is a large gene spanning over 16 Kb, different variant(s) in *F2RL1* may be associated with BMI [47].

For *CXADR*, local ancestry was significantly associated with SBP and DBP in the FBPP analysis, indicating that there may be additional variants in this region that are associated with blood pressure in this region. The local ancestry marker for *CXADR* in the CARe dataset was shifted from the admixture peak that was previously reported in this region, so this may have contributed to the lack of replication with local ancestry [29]. As with *F2RL1*, the causal SNPs could be elsewhere in the gene as *CXADR* is over 80 Kb [48]. As the results in *CXADR* and *F2RL1* were significant in this study and consistent with other studies, these genes warrant further study [42–46].

The association results did not replicate in the gene-based analysis using the CARe replication dataset. This difficulty in replicating the gene-level associations is expected *a priori* as the subjects from the FBPP dataset were ascertained based on a family or personal history of hypertension, but the CARe dataset subjects were recruited into population-based studies. As a result, the difficulty in replicating the gene-level results may also reflect the phenotypic heterogeneity among these cohorts, the possible overestimation of the FBPP effect sizes due to the "winner's curse," and the use of imputed variants, which all further reduce statistical power in a replication study.

One of this study's main strengths was that the results of related admixture mapping analyses were used to narrow down the regions in which the candidate gene analysis was performed. In conducting a gene-based analysis, the interpretations of the study's results were improved by employing a more biologically relevant framework than was possible from testing all SNPs. As the selection of candidate genes was based on previous findings,

the number of tests performed was reduced compared to the number of tests that would be necessary in a genome-wide association study.

Since the gene score method did not account for LD patterns, the effects of the correlation structure between SNPs on the estimates are unclear. This may have resulted in muted effect sizes, as this method only identified genes that contained SNPs in the dataset. Further, this study was limited by the necessity to impute blood pressures for subjects undergoing treatment for hypertension rather than obtaining pre-treatment blood pressures.

This study reported significant associations between hypertension and obesity phenotypes and genes in the African-American community. The genes *CXADR* and *F2RL1* were associated with blood pressure and BMI, and these findings are consistent with replication analyses and published literature. As a result, these genes may be important in predicting the risk of hypertension and obesity, so future replication and functional analyses including resequencing studies are warranted. Furthermore, this study described how local ancestry affected effect estimates of association and demonstrated how local ancestry may be useful in replication analyses. Consideration of such factors may be important in determining the contributions of genetic and genomic effects in disease association studies,

Acknowledgments

Source of Funding:

This work was supported by the National Institutes of Health and grant number HL086718 from the National Heart, Lung and Blood Institute.

References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, et al. Executive summary: heart disease and stroke statistics--2012 update: a report from the american heart association. Circulation. 2012; 125 (1):188–97. [PubMed: 22215894]
2. WHO. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: 2009.
3. Hottenga JJ, Boomsma DI, Kupper N, Posthuma D, Snieder H, Willemsen G, et al. Heritability and stability of resting blood pressure. Twin Res Hum Genet. 2005; 8 (5):499–508. [PubMed: 16212839]
4. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJ. Heritability of daytime ambulatory blood pressure in an extended twin design. Hypertension. 2005; 45 (1):80–5. [PubMed: 15557390]
5. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, et al. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. Hypertension. 2000; 36 (4):477–83. [PubMed: 11040222]
6. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005; 365 (9455):217–23. [PubMed: 15652604]
7. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002; 360 (9349):1903–13. [PubMed: 12493255]
8. Kathiresan S, Manning AK, Demissie S, D'Agostino RB, Surti A, Guiducci C, et al. A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. BMC Med Genet. 2007; 8 (Suppl 1):S17. [PubMed: 17903299]
9. Weiss LA, Pan L, Abney M, Ober C. The sex-specific genetic architecture of quantitative traits in humans. Nat Genet. 2006; 38 (2):218–22. [PubMed: 16429159]
10. Adeyemo A, Luke A, Cooper R, Wu X, Tayo B, Zhu X, et al. A genome-wide scan for body mass index among Nigerian families. Obes Res. 2003; 11 (2):266–73. [PubMed: 12582223]

11. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K. The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord. 1996; 20 (6):501–6. [PubMed: 8782724]
12. McQueen MB, Bertram L, Rimm EB, Blacker D, Santangelo SL. A QTL genome scan of the metabolic syndrome and its component traits. BMC Genet. 2003; 4 (Suppl 1):S96. [PubMed: 14975164]
13. Pietilainen KH, Kaprio J, Rissanen A, Winter T, Rimpela A, Viken RJ, et al. Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. Int J Obes Relat Metab Disord. 1999; 23 (2):107–15. [PubMed: 10078843]
14. Platte P, Papanicolaou GJ, Johnston J, Klein CM, Doheny KF, Pugh EW, et al. A study of linkage and association of body mass index in the Old Order Amish. Am J Med Genet C Semin Med Genet. 2003; 121C (1):71–80. [PubMed: 12888987]
15. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009; 41 (6):677–87. [PubMed: 19430479]
16. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009; 41 (6):666–76. [PubMed: 19430483]
17. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478 (7367):103–9. [PubMed: 21909115]
18. Fox ER, Young JH, Li Y, Dreisbach AW, Keating BJ, Musani SK, et al. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum Mol Genet. 2011; 20 (11):2273–84. [PubMed: 21378095]
19. Adeyemo A, Gerry N, Chen G, Herbert A, Doumatey A, Huang H, et al. A genome-wide association study of hypertension and blood pressure in African Americans. PLoS Genet. 2009; 5 (7):e1000564. [PubMed: 19609347]
20. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010; 466 (7307): 707–13. [PubMed: 20686565]
21. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variantin the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007; 316 (5826):889–94. [PubMed: 17434869]
22. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet. 2009; 41 (1):18–24. [PubMed: 19079260]
23. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010; 42 (11):937–48. [PubMed: 20935630]
24. Smith MW, O'Brien SJ. Mapping by admixture linkage disequilibrium: advances, limitations and guidelines. Nat Rev Genet. 2005; 6 (8):623–32. [PubMed: 16012528]
25. Zhu X, Tang H, Risch N. Admixturemapping and the role of population structure for localizing disease genes. Adv Genet. 2008; 60:547–69. [PubMed: 18358332]
26. Basu A, Tang H, Arnett D, Gu CC, Mosley T, Kardia S, et al. Admixture mapping of quantitative trait loci for BMI in African Americans: evidence for loci on chromosomes 3q, 5q, and 15q. Obesity (Silver Spring). 2009; 17 (6):1226–31. [PubMed: 19584881]
27. Basu A, Tang H, Lewis CE, North K, Curb JD, Quertermous T, et al. Admixture mapping of quantitative trait loci for blood lipids in African-Americans. Hum Mol Genet. 2009; 18 (11):2091–8. [PubMed: 19304782]
28. Zhu X, Cooper RS. Admixture mapping provides evidence of association of the VNN1 gene with hypertension. PLoS One. 2007; 2 (11):e1244. [PubMed: 18043751]
29. Zhu X, Luke A, Cooper RS, Quertermous T, Hanis C, Mosley T, et al. Admixture mapping for hypertension loci with genome-scan markers. Nat Genet. 2005; 37 (2):177–81. [PubMed: 15665825]

30. Zhu X, Young JH, Fox E, Keating BJ, Franceschini N, Kang S, et al. Combined admixture mapping and association analysis identifies a novel blood pressure genetic locus on 5p13: contributions from the CARe consortium. Hum Mol Genet. 2011; 20 (11):2285–95. [PubMed: 21422096]

31. FBPP. Multi-center genetic study of hypertension: The Family Blood Pressure Program (FBPP). Hypertension. 2002; 39 (1):3–9. [PubMed: 11799070]

32. Redmond N, Baer HJ, Hicks LS. Health behaviorsand racial disparity in blood pressure control in the national health and nutrition examination survey. Hypertension. 2011; 57 (3):383–9. [PubMed: 21300667]

33. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003; 42 (6):1206–52. [PubMed: 14656957]

34. Association AH. What Your Cholesterol Levels Mean. 2011.

35. James, WP.; Jackson-Leach, R.; Ni, Mhurchu C.; Kalamara, E.; Shayeghi, M.; Rigby, N., et al. Overweight and Obesity (High Body Mass Index). In: Ezzati, M.; Lopez, AD.; Rodgers, A.; Murray, CJL., editors. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. Geneva: WHO; 2004. p. 497-596.

36. Zhu X, Zhang S, Tang H, Cooper R. A classical likelihood based approach for admixture mapping using EM algorithm. Hum Genet. 2006; 120 (3):431–45. [PubMed: 16896924]

37. Qin H, Morris N, Kang SJ, Li M, Tayo B, Lyon H, et al. Interrogating local population structure for fine mapping in genome-wide association studies. Bioinformatics. 2010; 26 (23):2961–8. [PubMed: 20889494]

38. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449 (7164):851–61. [PubMed: 17943122]

39. Musunuru K, Lettre G, Young T, Farlow DN, Pirruccello JP, Ejebe KG, et al. Candidate gene association resource (CARe): design, methods, and proof of concept. Circ Cardiovasc Genet. 2010; 3 (3):267–75. [PubMed: 20400780]

40. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81 (3): 559–75. [PubMed: 17701901]

41. Zhu X, Feng T, Li Y, Lu Q, Elston RC. Detecting rare variants for complex traitsusing family and unrelated data. Genet Epidemiol. 2010; 34 (2):171–87. [PubMed: 19847924]

42. Bowles NE, Richardson PJ, Olsen EG, Archard LC. Detection of Coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. Lancet. 1986; 1 (8490):1120–3. [PubMed: 2871380]

43. Lisewski U, Shi Y, Wrackmeyer U, Fischer R, Chen C, Schirdewan A, et al. The tight junction protein CAR regulates cardiac conduction and cell-cell communication. J Exp Med. 2008; 205 (10):2369–79. [PubMed: 18794341]

44. Bezzina CR, Pazoki R, Bardai A, Marsman RF, de Jong JS, Blom MT, et al. Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation in acute myocardial infarction. Nat Genet. 2010; 42 (8):688–91. [PubMed: 20622880]

45. Badeanlou L, Furlan-Freguia C, Yang G, Ruf W, Samad F. Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. Nat Med. 2011; 17 (11):1490–7. [PubMed: 22019885]

46. Greenawalt DM, Dobrin R, Chudin E, Hatoum IJ, Suver C, Beaulaurier J, et al. A survey of the genetics of stomach, liver, and adipose gene expression from a morbidly obese cohort. Genome Res. 2011; 21 (7):1008–16. [PubMed: 21602305]

47. National Center for Biotechnology Information-National Library of Medicine. Entrez-Gene: F2RL1 coagulation factor II (thrombin) receptor-like 1. Homo sapiens. 2012

48. National Center for Biotechnology Information-National Library of Medicine. Homo sapiens. 2012. Entrez-Gene: CXADR coxsackie virus and adenovirus receptor.

Table 1

Summary Statistics

Variable	N	Mean	Median	Min, Max
Age (years)	1733	48.2	48.0	(19.0, 70.0)
BMI (kg/m^2)	1733	31.0	29.9	(13.8, 70.7)
Mean Proportion African Ancestry	1733	83.6%	85.5%	(33.5%, 98.8%)
Sex	1733			
Male	617 (35.6%)	.	.	.
Female	1116 (64.4%)	.	.	.
Taking Any Anti-Hypertension Medication	1733			
Yes	770 (44.4%)	.	.	.
No	963 (55.6%)	.	.	.
SBP, Adjusted for Treatment (mm Hg)	1730	132.9	129.0	(74.0, 237.5)
DBP, Adjusted for Treatment (mm Hg)	1730	75.9	74.7	(42.0, 136.0)

Table 2

Results of Gene-Based Multivariable Regression Models

				Without Local Ancestry			With Local Ancestry			
Dataset	**Outcome**	**Gene (Chr)**	**SNP(s)**	**β**	**SE(β)**	**P value**	**β**	**SE(β)**	**P value**	**P value of Local Ancestry**
FBPP	SBP	*CXADR* (21)	rs437470	3.17	0.830	0.0001	2.94	0.835	0.0004	0.011
FBPP	DBP	*CXADR* (21)	rs437470	1.70	0.460	0.0002	1.60	0.463	0.0006	0.001
FBPP	BMI	*F2RL1* (5)	rs631465	3.41	1.045	0.0011	3.71	1.056	0.0005	0.352
ARIC & JHS	SBP	*CXADR* (21)	rs437470	0.27	0.499	0.5918	0.19	0.504	0.7110	0.277
ARIC & JHS	DBP	*CXADR* (21)	rs437470	0.10	0.291	0.7269	0.04	0.294	0.9005	0.134

SE: Standard Error. (Chr): Chromosome number. Statistically-significant after adjusting for the total number of genes tested. 51 genes on chromosome (chr) 21 were tested for DBP and SBP (critical p-value = 0.000980392), and 91 genes on chromosome 5 were tested for BMI (critical p = 0.000549451) for the FBPP dataset analyses. All SBP and DBP multivariable models were adjusted for age, age^2, sex, BMI, and global ancestry, unless otherwise noted. All BMI multivariable models were adjusted for age, age^2, sex, and global ancestry, unless otherwise noted.

Table 3

Results of Gene-Based Multivariable Regression Models Using Local Ancestry for Replication Analysis

Dataset	Outcome	Gene (Chr)	β	SE(β)	P value
ARIC & JHS	SBP	*CXADR* (21)	1.59	1.377	0.2483
ARIC & JHS	DBP	*CXADR* (21)	1.23	0.803	0.1248
ARIC & JHS	BMI	*F2RL1* (5)	−0.86	0.447	0.0549

SE: Standard Error. (Chr): Chromosome number.

Models were testing local ancestry, instead of the gene of interest in the model. Multivariable SBP and DBP models were adjusted for age, age^2, sex, BMI, and global ancestry. Multivariable BMI model was adjusted for age, age^2, sex, and global ancestry.

Table 4

Results of Gene-Based Multivariable Regression Models that were Nominally Significant at $\alpha = 0.05$

				Without local ancestry			With local ancestry			
Phenotype	**Gene**	**Chr**	**SNPs**	**β**	**SE(β)**	**P value**	**β**	**SE(β)**	**P value**	**P Value of Local Ancestry**
BMI	ATG10	5	rs3734114, rs1864183	0.50	0.243	0.0380	0.59	0.246	0.0176	0.1929
BMI	IQGAP2	5	rs10036913, rs7722711, rs2431352, rs2910819, rs2455230, rs2431363	0.24	0.109	0.0281	0.25	0.109	0.0238	0.3301
BMI	MRPS27	5	rs17375461, rs10942927	−0.44	0.193	0.0217	−0.43	0.194	0.0279	0.6049
BMI	THBS4	5	rs1866389	0.84	0.391	0.0320	0.92	0.393	0.0195	0.2632
SBP	ENPP1	6	rs9483347, rs1804025	2.10	0.915	0.0220	2.01	0.938	0.0324	0.0310
SBP	GPR1266	6	rs11155242	2.18	0.959	0.0234	2.15	0.959	0.0252	0.0025
SBP	L3MBTL3	6	rs9388768, rs7451021	1.23	0.522	0.0185	1.55	0.563	0.0058	0.0481
SBP	NOX3	6	rs12195525	−2.82	1.242	0.0233	−2.82	1.242	0.0233	0.4296
SBP	RSPO3	6	rs1892172	1.58	0.809	0.0507	1.69	0.813	0.0380	0.0165
DBP	C6orf184	6	rs9400272, rs6927569	−1.35	0.443	0.0023	−1.37	0.451	0.0024	0.0626
DBP	ENPP1	6	rs9483347, rs1804025	1.27	0.507	0.0122	1.28	0.520	0.0135	0.0786
DBP	L3MBTL3	6	rs9388768, rs7451021	0.68	0.290	0.0192	0.82	0.313	0.0091	0.1246
DBP	TXLNB	6	rs17068451, rs9321712	0.75	0.317	0.0186	0.73	0.325	0.0255	0.0112
SBP	DOPEY2	21	rs3827183	2.97	1.248	0.0173	3.10	1.252	0.0134	0.3174
DBP	DOPEY2	21	rs3827183	1.40	0.691	0.0437	1.39	0.694	0.0454	0.2382
HTN	C6orf211	6	rs9397054	−0.20	0.102	0.0484	−0.20	0.103	0.0525	0.2965
HTN	ENPP1	6	rs9483347, rs1804025	0.23	0.091	0.0105	0.22	0.093	0.0162	0.0138
HTN	L3MBTL3	6	rs9388768, rs7451021	0.11	0.051	0.0358	0.14	0.055	0.0126	0.0368
HTN	CXADR	21	rs437470	0.20	0.082	0.0166	0.19	0.083	0.0227	0.1348
HDL-C	LOC137886	8	rs13277646	−1.99	0.796	0.0125	−1.98	0.799	0.0132	0.6924