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Combined admixture mapping and association analysis identifies a novel blood pressure genetic locus on 5p13: contributions from the CARe consortium

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Admixture mapping based on recently admixed populations is a powerful method to detect disease variants with substantial allele frequency differences in ancestral populations. We performed admixture mapping analysis for systolic blood pressure (SBP) and diastolic blood pressure (DBP), followed by trait-marker association analysis, in 6303 unrelated African-American participants of the Candidate Gene Association Resource (CARe) consortium. We identified five genomic regions ($P < 0.001$) harboring genetic variants contributing to inter-individual BP variation. In follow-up association analyses, correcting for all tests performed in this study, three loci were significantly associated with SBP and one significantly associated with DBP ($P < 10^{-5}$). Further analyses suggested that six independent single-nucleotide polymorphisms (SNPs) contributed to the phenotypic variation observed in the admixture mapping analysis. These six SNPs were examined for replication in multiple, large, independent studies of African-Americans [Women's Health Initiative (WHI), Maywood, Genetic Epidemiology Network of Arteriopathy (GENOA) and Howard University Family Study (HUFS)] as well as one native African sample (Nigerian study), with a total replication sample size of 11 882. Meta-analysis of the replication set identified a novel variant (rs7726475) on chromosome 5 between the *SUB1* and *NPR3* genes, as being associated with SBP and DBP ($P < 0.0015$ for both); in meta-analyses combining the CARe samples with the replication data, we observed *P*-values of 4.45×10^{-7} for SBP and 7.52×10^{-7} for DBP for rs7726475 that were significant after accounting for all the tests performed. Our study highlights that admixture mapping analysis can help identify genetic variants missed by genome-wide association studies because of drastically reduced number of tests in the whole genome.

INTRODUCTION

High blood pressure (BP) is common worldwide and is a major risk factor for cardiovascular disease and mortality (1). Its burden, however, is not equally shared across ethnically diverse populations. In the USA, for example, the prevalence of hypertension varies widely across ethnic populations, between 27% in individuals of European ancestry to 40% among people of African ancestry (2). Furthermore, the death rate attributed to hypertension in 2004 was three times higher in African-Americans than in European-Americans (3,4).

BP variation results from the combined effect of a complex set of genetic and environmental influences, with genes accounting for 30–55% of the inter-individual variance (5). Recently, several genome-wide association studies (GWASs) of BP traits have reported new loci in samples from Asians, Europeans and African-Americans (6–9). However, genetic studies of BP in these populations have not identified loci common to both ancestry groups, which may be attributable to population-specific genetic variants, variation in allele frequencies, different patterns of linkage disequilibrium (LD) across populations or low statistical power due to limited sample size particularly in African-Americans. Furthermore, no variants identified in the previous GWAS of BP in African-Americans (6) were replicated in a GWAS of BP in African-Americans in the Candidate Gene Association Resource (CARe) study, highlighting the challenges in searching for genetic variants in African ancestry populations (10). Theoretical genetic studies suggest that admixture mapping can be a powerful method to detect disease variants in recently admixed populations, such as African-Americans, when the parental populations show substantial differences in disease prevalence (11–18). Admixture mapping has identified loci or regions contributing to complex traits such as prostate cancer, multiple sclerosis, obesity, focal segmental glomerulosclerosis and blood lipids (19–24). To date, several such studies have been conducted for hypertension (25–27) and 6q24 and 21q21 have been reported to be associated with hypertension in both the Family Blood Pressure Program study (27) and the Dallas Heart Study (26). However, only weak association evidence was observed on 6q24 in an admixture mapping study by Deo *et al*. (25), suggesting that further analysis is necessary. Here, we report the findings of admixture mapping analysis for systolic BP (SBP) and diastolic BP (DBP), followed by association analysis in the regions identified using samples from the CARe consortium, the largest African-American GWAS performed to date, and provide the replication results of the most promising loci in independent individuals of African descent.

RESULTS

Our discovery sample for admixture mapping analysis included up to 6303 unrelated African-Americans from five of the nine CARe participating cohorts: Atherosclerosis Risk In Communities (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA), Cleveland Family Study (CFS), Jackson Heart Study (JHS) and Multi-Ethnic Study of Atherosclerosis (MESA), which were genotyped using the Affymetrix 6.0 array. The cohort-specific sample characteristics are presented in Supplementary Material, Table S1. For CFS and JHS, if more than one founder was available in a family, we included the founders. Otherwise, only one randomly selected offspring in each family was included in the analysis. The correlation between SBP and DBP was 0.71. We analyzed the data using ADMIXPROGRAM (18) first. Using 3230 ancestry informative markers (AIMs; see Materials and Methods), we estimated that the number of generations since the occurrence of population admixture was 10 in this African-American sample, consistent with other admixture estimates in African-Americans (26–28). The estimated average European ancestry in this sample was $18.9 \pm 11.6\%$.

Table 1. The most significant chromosome regions showing the association of SBP and DBP with African Ancestry

	Region (Mb)[a]	The most significant SNP	BETA[b]	SE	*P*-value[c]	Number of SNPs with $\delta > 0.2$[d]	Number of independent SNPs[e]
SBP							
1q41-42	217–229	rs6686694	3.7	1.06	4.6×10^{-4}	1218	581
2q21-24	132.6–164.9	rs2166488	−3.86	1.04	2.1×10^{-4}	2994[f]	1362
21q21	26.7–36.0	rs2833563	3.82	1.08	4.0×10^{-4}	1181	591
DBP							
2q22-24	143.4–163.3	rs2166488	2.16	0.60	2.8×10^{-4}	2994[f]	1362
5p13-11	31.9–51.2	rs35389	−2.21	0.60	2.2×10^{-4}	1535	742
17q11	14.8–28.7	rs8066682	−1.97	0.59	8.2×10^{-4}	851	462

[a]Region is defined as the 1-unit drop region from the $-\log_{10}(P)$ value of the most significant SNP.
[b]Positive BETA value indicates that African ancestry increase the BP level and negative BETA indicates that European ancestry increase the BP level.
[c]*P*-value is two-sided.
[d]The SNPs with $\delta > 0.2$ were selected for follow-up association tests and these SNPs were available in Affymetrix 6.0 array in each region.
[e]The number of independent tests calculated by the method of Li and Ji (30).
[f]The number of SNPs was calculated for the region 132.6–163.4 Mb on chromosome 2.

The correlations between average African ancestry and SBP and DBP were 0.056 and 0.08 ($P < 0.0001$ for both), respectively. Using the estimated marker-specific ancestry, we performed quantitative admixture analysis for SBP and DBP. Supplementary Material, Figure S1 presents the $-\log_{10}(P)$ values testing for the association of local African ancestry with SBP and DBP at the 3230 AIMs after adjusting for global ancestry. We did not observe any region reaching genome-wide significance. For follow-up studies, we chose three top peaks of association between local ancestry and SBP and three top peaks of association of local ancestry and DBP with $P < 0.001$ (Table 1). At each peak, we defined the target region as comprising the locus that is within 1 unit drop of $-\log_{10}(P)$ from the peak signal. The region on chromosome 2 showed overlap for SBP and DBP, with the maximum $-\log_{10}(P)$ occurring at the same marker (Table 1). When the same data were analyzed using STRUCTURE (28,29), we obtained almost identical results (Supplementary Material, Fig. S2).

We then searched the variants associated with SBP and DBP in the five uniquely identified regions using the single-nucleotide polymorphisms (SNPs) genotyped on the Affymetrix 6.0 array. Since only variants with substantial allele frequency differences in ancestral populations can be detected by admixture mapping, we sought SNPs with allele frequency differences larger than 0.2 between HapMap the Yoruba people of Ibadan, Nigeria (YRI) and the centre d'Etude du polymorphisme humain from Utah (CEU) samples. The number of SNPs with a δ-value of >0.2 was listed in Table 1. We then tested the strength of association between these SNPs and both SBP and DBP. The Q–Q plots for association for SBP and DBP (Fig. 1A and B) demonstrated substantial deviation from the null hypothesis that none of these variants are associated with BP after controlling for population stratification. To determine which SNPs were statistically significant in our analysis, we estimated the total number of independent tests in both the admixture mapping and the SNP association tests. Since the locus-specific ancestries are dependent and the strength of dependence is determined by the population admixture history, we estimated the total number of tests we performed in this study. It was reported that the total number of independent tests in the genome for testing local ancestry in the African-American population is ∼1000 (15,18). Since the SNPs selected in association tests are also dependent, we then calculated the number of independent tests in each region using the method of Li and Ji (30) and these numbers are presented in Table 1. Thus, the total number of tests in admixture mapping and association tests together was 4738 and the corresponding *P*-value to claim statistical significance was set at 1.06×10^{-5}. After adjusting for the number of SNPs tested, we identified two different loci (on 2q21-24 and 21q21) significantly associated with SBP and one locus (on 5p13-11) associated with DBP (Table 2 and Supplementary Material).

We next tested whether the most significant SNP in each region accounted for the observed effect in admixture mapping by adjusting for the most significant SNP in each region in the linear regression equation for modeling local ancestry association (see Materials and Methods). For DBP, after adjusting for the most significant SNP (rs4957217 in the DAB2 region on 5p13-11), we observed a substantial reduction in the association evidence between local European ancestry and DBP, although it remained significant ($P = 0.001$; Fig. 2). To search for additional variant(s) contributing to these effects, we performed conditional association analyses for 1535 SNPs in the region of 1 unit drop from the peak signal (see Table 1 for the defined region) while adding rs4957217 as a covariate in the regression model. We ranked the *P*-values and identified two additional SNPs, rs7726475 and rs7737481, as the most significant ($P \leq 0.0003$). After adjusting for all three SNPs, the association between local African ancestry and DBP in this region was no longer significant ($P = 0.14$, Fig. 2), suggesting that these three SNPs adequately account for the observed association. Notably, these three SNPs are not in LD ($r^2 \leq 0.006$) with each other in the sample. Similar analysis was performed on the chromosome regions of interest for SBP. We identified three independent SNPs on chromosomes 2 and 21 that contribute to the observed association with SBP (Table 2 and Supplementary Material, Figs S3 and S4). We did not observe any

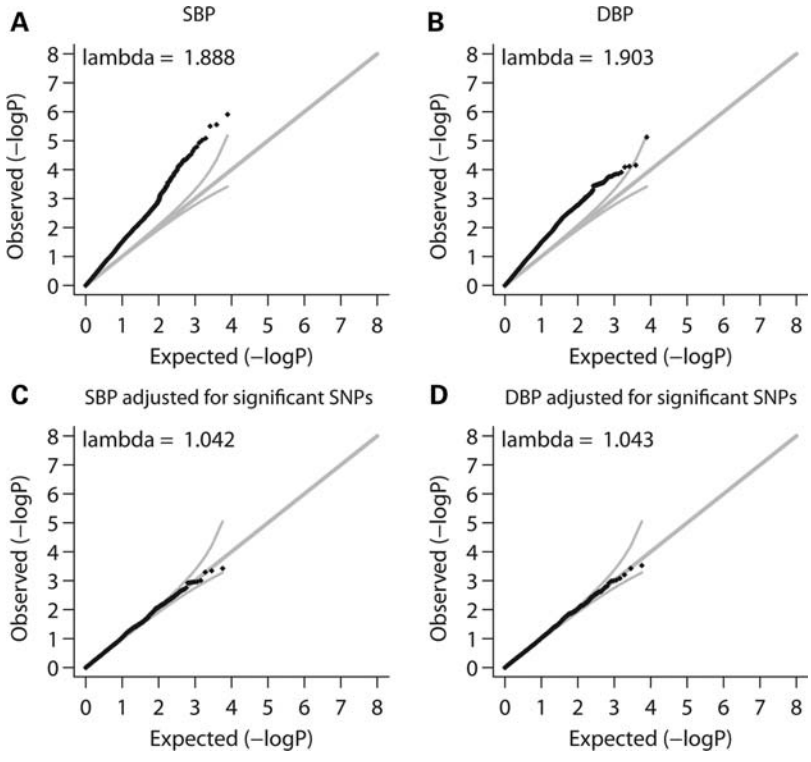

Figure 1. Q–Q plots for SNPs in the five genomic regions with allele frequency difference larger than 0.2 between YRI and CEU HapMap samples. (**A** and **B**) SBP and DBP without adjusting for the associated SNPs in the linear regression analysis in each region, respectively; (**C** and **D**) SBP and DBP after adjusting for the associated SNPs on chromosomes 2, 5 and 21 in the linear regression analysis in each region, respectively. After adjusting for associated SNPs, we did not observe any significant departure of the Q–Q plot from expectations. The two gray lines along the straight diagonal line refer the 95% confidence band.

SNP on the regions within chromosomes 1 and 17 with a *P*-value of $<1.06 \times 10^{-5}$.

We next calculated how much of BP variation could be explained by these six SNPs (starred in Table 2) using an additive genetic model. Indeed, 1.21 and 1.16% of inter-individual variation in SBP and DBP, respectively, can be explained by the six SNPs in aggregate. Furthermore, we repeated the single SNP association analysis by adding these six SNPs as covariates in the regression models demonstrating no substantial deviation from expectations as observed in the Q–Q plots of SBP and DBP in the three regions (Fig. 1C and D). Upon doing so, the genome inflation factor (lambda, λ) was close to 1.0.

Independent replication of identified SNPs

We next sought to replicate the CARe African-American BP associations at the six SNPs in five independent cohorts: Maywood, Howard University Family Study (HUFS), GENOA, Women's Health Initiative (WHI) and Nigeria (see Supplementary Material for study details). All these cohorts recruited African-American except the Nigeria cohort, which was comprised of native Africans from Nigeria. All the five cohorts have been genotyped with the Affymetrix 6.0 array.

In cohort-specific analysis, only SNP rs7726475 was significantly associated with both SBP and DBP in WHI ($P < 0.002$) after adjusting for 12 tests (six SNPs and two traits, Table 3). Table 4 presents the meta-analyses for two sets of populations, one that includes the replication cohorts only and the other also including the CARe derivation set. Again, rs7726475 was significant in the meta-analysis of replication cohorts for both SBP and DBP ($P = 0.0015$). If only the four African-American replication cohorts were included, the *P*-values were further improved to 0.0008 for SBP and 0.0013 for DBP, respectively. The *P*-value of this SNP in the meta-analysis including CARe and all replication cohorts was 8.85×10^{-7} for SBP and 3.63×10^{-6} for DBP, respectively, which remained significant after adjusting for multiple comparisons.

In the above admixture mapping and association analyses, we only included unrelated individuals and removed 1170 related individuals in CARe JHS and CFS cohorts (see Materials and Methods). After including these 1170 individuals, the *P*-values for rs7726475 in CARe improved to 3.16×10^{-5} and 4.62×10^{-5}, with final *P*-values from combining CARe and replication samples of 4.45×10^{-7} and 7.52×10^{-7}, for SBP and DBP, respectively. The *P*-values were further improved to 2.17×10^{-7} for SBP and 6.28×10^{-7} for DBP when meta-analysis was restricted in African-American samples. We did not observe any other SNPs reaching significance upon combining all cohorts. Our meta-analysis did not provide evidence of heterogeneity across the replication cohorts for rs7726475 ($P > 0.30$, Table 4). We did observe evidence of heterogeneity for SNPs other than rs7726475, however, when combining all CARe and replication cohorts together (Table 4).

Table 2. Significant SNPs in regions detected by admixture mapping for SBP and DBP

CHR	SNP	Base pair position	Nearby genes	SBP A1	A2	A1_FREQ	BETA	SE	SBP_P	DBP BETA	SE	DBP_P
2	rs2450[a]	153292667	*FMNL2*, *ARL6IP6*, *PRPF40A*	G	T	0.06253	3.117	0.7497	3.26×10^{-5}	1.607	0.4283	1.77×10^{-4}
2	rs295796	157461617		T	A	0.4579	1.579	0.3566	$\mathbf{9.65 \times 10^{-6}}$	0.6791	0.2035	8.53×10^{-4}
2	rs295813[a]	157472109	*GLANT5*	G	A	0.4045	1.807	0.3723	$\mathbf{1.24 \times 10^{-6}}$	0.7997	0.2124	1.68×10^{-4}
2	rs1033225	157514397		T	C	0.3989	1.731	0.3712	$\mathbf{3.16 \times 10^{-6}}$	0.7588	0.2116	3.39×10^{-4}
2	rs1033224	157514699		A	T	0.4849	1.6	0.3586	$\mathbf{8.29 \times 10^{-6}}$	0.6965	0.2049	6.82×10^{-4}
5	rs7726475[a]	32611671	*SUB1*, *NPR3*, *C5orf23*	A	G	0.06633	2.965	0.726	4.49×10^{-5}	1.503	0.4148	2.93×10^{-4}
5	rs7737481[a]	37818160	*C9*	G	A	0.3048	−0.5672	0.3911	1.54×10^{-1}	−0.8779	0.2228	8.23×10^{-5}
5	rs4957217[a]	39712034	*DAB2*	T	C	0.205	−1.455	0.4411	9.78×10^{-4}	−1.125	0.251	$\mathbf{7.59 \times 10^{-6}}$
21	rs2236611[a]	32607151	*URB1*, *MRAP*	T	C	0.1687	−2.302	0.4909	$\mathbf{2.79 \times 10^{-6}}$	−1.011	0.2806	3.18×10^{-4}

The number of multiple comparisons was determined by the number of SNPs tested in each region. The effect size is presented in terms of the reference allele A1. The four SNPs at 157 Mb on chromosome 2 are in strong LD with one another. *P*-values in bold are significant after adjusting for multiple tests.
[a]The independent SNPs that can explain the association evidence in admixture mapping analysis.

DISCUSSION

Admixture mapping can supplement traditional genome-wide association analyses in the search for genetic variants underlying complex traits. We performed admixture mapping analysis using the largest African-American GWAS for SBP and DBP performed to date, followed by association analysis, and identified six independent SNPs accounting for the BP signals observed in admixture mapping analysis. Replication analysis in four independent African-American cohorts and a Nigerian cohort, with a total replication sample size of 11 882, identified rs7726475 as associated with both SBP and DBP. The combined *P*-values in meta-analysis including both CARe and replication cohorts were 4.45×10^{-7} for SBP and 7.52×10^{-7} for DBP, reaching the statistical significance level after adjusting for all tests performed ($P < 1.06 \times 10^{-5}$). The similar *P*-values reflect the strong correlation between SBP and DBP ($r^2 = 0.594$). However, only WHI was individually able to replicate the association evidence for rs7726475. The inflation factor for WHI is 1.018 for both SBP and DBP, in which suggests that the association in WHI is not due to population structure. We then calculated the power given the replication sample sizes. We assumed the variances attributed to rs7726475 for SBP and DBP to be 0.24 and 0.19%, respectively. These variances were estimated from CARe discovery data, which may be overestimated in the general population. Except for the WHI, the power to replicate rs7726475 ranged from 22.1 to 39.3% in the remaining four replication cohorts (Supplementary Material, Table S3), suggesting that the sample sizes of these four cohorts were underpowered. Although the effect sizes were smaller than estimated in CARe, three of the four African American cohorts (Maywood, HUFS and WHI) demonstrated a direction of the effect that was consistent with CARe, (Table 3). GENOA had opposite direction of effect for both SBP and DBP but the estimates were not statistically significant. Using the effective sizes obtained from CARe, we had less than 30% power to detect rs7726475 in GENOA and the power was reduced to less than 14% when using the effective sizes from WHI. The effect size direction in the Nigeria cohort was also opposite in direction, but the estimate was not statistically significant. The risk allele frequency of rs7726475 was 2% in the Nigeria sample, 6% in African-American and 34.5% in HapMap CEU samples, suggesting that the European allele was responsible for the association evidence. This is consistent with our observation that European ancestry at the locus on 5p13-11 increases both SBP and DBP (Table 1). Thus, the non-significant opposite direction of rs7726475 in Nigeria is quite possible. Our results suggest that SNP rs7726475 is in LD with a causal variant. Therefore, different LD patterns may explain the different directional effects in African-Americans and native Africans. When we dropped the Nigeria cohort in the meta-analysis of replication cohorts, the *P*-values for SNP rs7726475 improved to 0.0008 and 0.0013 for SBP and DBP, respectively, and the combined CARe and replication African-American cohorts *P*-values improved to 2.17×10^{-7} for SBP and 6.28×10^{-7} for DBP.

Our results indicate that the findings for rs7726475 are replicated in African-Americans. The annotated genes near the significant SNP rs7726475 include natriuretic peptide receptor c (*NPR3*), chromosome 5 open reading frame 23 (C5orF23) and activated RNA polymerase II transcription cofactor (*SUB1*). *NPR3* belongs to the family of natriuretic peptides, which are known to elicit a number of vascular, renal and endocrine effects that are important in the maintenance of BP and extracellular fluid volume. These effects are mediated by a specific binding of peptides to cell surface receptors in the vasculature, kidney, adrenal and brain (31). *NPR3* has also been reported to have an important role in BP regulation in human and animal models (32–35). Thus, *NPR3* is an excellent candidate gene for knockout studies and follow-up resequencing studies to identify functional variants with potentially large effects on BP.

In the CARe admixture mapping analysis, we replicated the 21q21 region reported in previous admixture mapping studies of hypertension in African-American populations (26,27). We observed three additional regions (1q41-42, 2q21-24, 5p13-11) showing suggestive evidence. In the follow-up association analysis, we observed significant evidence of genetic influences on SBP and DBP for three SNPs on chromosomes 2q21-24 and 21q21. Replication analyses, however, failed to

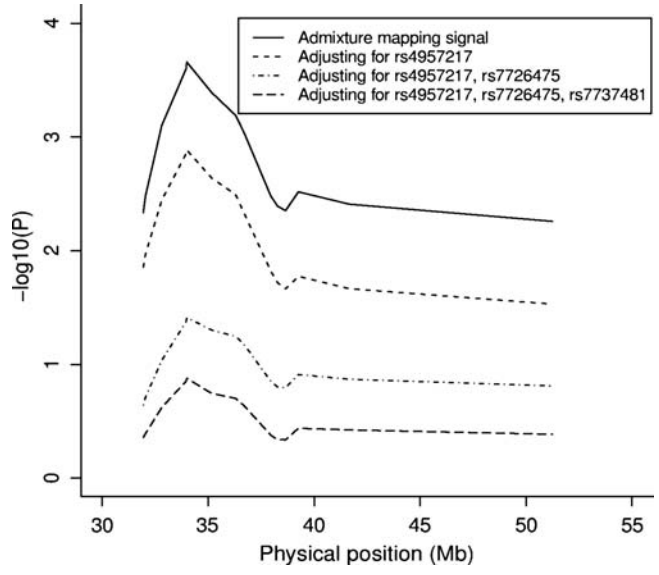

Figure 2. Change in DBP association evidence in admixture mapping analysis on chromosome 5 with, and without, adding associated SNPs. Addition of SNP rs7726475 results in a substantial drop off in the admixture signal.

confirm these associations. Replication was accomplished mostly by the WHI associations, which had large sample size of 8300 with enough power to identify associations of small magnitude as expected for common variants. As WHI includes women only, we performed sex-specific analysis in the CARe samples. These results did not reveal sex-specific association for these SNPs (Supplementary Material, Table S4).

With a replication sample size of 11 882 individuals, we had 93% power to detect a quantitative trait locus accounting for 0.1% of the trait variance at a two-tailed significance level of 0.05. Thus, our combined replication sample size should have adequate power to replicate the evidence observed in CARe. As such, the SNPs identified on chromosomes 2q21-24 and 21q21 may represent false-positive associations. However, several factors may limit our capacity to replicate true associations. First, we assumed that both SBP and DBP can be accurately measured. Second, we assumed that there were no heterogeneities across samples, although several sources of heterogeneity exist. For example, our samples were recruited from different geographical regions or countries and the environmental factors contributing to BP variation can be substantially different, as indicated in Supplementary Material, Table S1. In particular, the Nigerian cohort may have substantially different environmental influences relevant to BP compared with the African-American cohorts. Furthermore, the prevalence of antihypertensive medication use in the Maywood cohort was only 1%, which is substantially lower than that in the general African-American population. In addition, WHI includes African-American post-menopausal women only. In the WHI data, global African ancestry was not correlated with either SBP or DBP [$P > 0.37$ for the first principal component (PC) and >0.15 for the first 10 PCs]. The phenotypic heterogeneity may reduce the power to replicate findings substantially. Third, our reported association variants in CARe are for ancestry informative SNPs that are likely in LD with true unknown

Table 3. Replication results in four independent cohorts

Chr	SNP	A1	Maywood (743)				Nigeria (1188)				HUFS (1016)				GENOA (845)				WHI (8090)			
			FREQ	BETA	SE	*P*-value	FREQ	BETA	SE	*P*-value	FREQ	BETA	SE	*P*-value	FREQ	BETA	SE	*P*-value	FREQ	BETA	SE	*P*-value
SBP																						
2	rs2450	G	0.06	−0.59	2.14	0.78	0.01	−6.17	4.74	0.19	0.06	1.81	1.93	0.35	0.06	0.39	2.25	0.86	0.08	0.28	0.55	0.61
2	rs295813	G	0.39	1.09	1.04	0.29	0.32	−0.29	1.03	0.78	0.4	−0.63	1.0	0.53	0.41	0.28	1.1	0.8	—	—	—	—
5	rs7726475	A	0.06	1.65	2.2	0.46	0.02	−3.97	3.79	0.3	0.07	1.87	1.9	0.32	0.06	−1.47	2.32	0.53	0.08	1.80	0.54	**0.0009**
5	rs7737481	G	0.32	1.16	1.07	0.28	0.35	−0.59	1.01	0.56	0.29	−0.3	1.06	0.78	0.3	−0.03	1.32	0.99	0.30	−0.29	0.32	0.36
5	rs4957217	T	0.22	0.13	1.26	0.92	0.23	1.48	1.2	0.22	0.22	−0.11	1.15	0.92	0.22	1.05	1.3	0.42	0.20	0.45	0.37	0.23
21	rs2236611	T	0.16	1.35	1.39	0.33	0.04	−0.1	2.67	0.97	0.17	−1.36	1.3	0.3	0.15	−0.59	1.51	0.7	0.19	0.12	0.37	0.75
DBP																						
2	rs2450	G	0.06	0.7	1.45	0.63	0.01	−3.27	4.74	0.22	0.06	1.39	1.2	0.25	0.06	0.07	1.31	0.96	0.08	0.03	0.29	0.91
2	rs295813	G	0.39	0.32	0.7	0.66	0.32	−0.68	1.03	0.26	0.4	−1.48	0.62	0.02	0.41	−0.72	0.64	0.26	—	—	—	—
5	rs7726475	A	0.06	0.35	1.49	0.81	0.02	−1.76	3.79	0.35	0.07	1.68	1.18	0.16	0.06	−0.04	1.36	0.98	0.08	0.88	0.29	**0.002**
5	rs7737481	G	0.32	0.63	0.72	0.39	0.35	−0.56	1.01	0.51	0.29	0.14	0.66	0.83	0.3	0.47	0.77	0.54	0.30	−0.08	0.17	0.62
5	rs4957217	T	0.22	−0.79	0.86	0.36	0.23	0.79	1.2	0.08	0.22	0.04	0.72	0.96	0.22	0.04	0.76	0.96	0.20	0.22	0.20	0.28
21	rs2236611	T	0.16	1.436	0.942	0.13	0.04	−0.55	2.672	0.98	0.17	−0.302	0.809	0.71	0.15	−1.305	0.877	0.14	0.19	−0.10	0.20	0.62

Only the SNPs that can explain association evidence in Table 2 were carried forward for replication analysis. Significant results are in bold. All the *P*-values are two-side. The numbers in parentheses are cohort sample sizes.

Table 4. Meta-analysis of replication cohort data with and without including CARe unrelated samples

SNP	SBP without CARe				SBP with CARe				DBP without CARe				DBP with CARe				
	Effect	SE	*P*-value	Direction	Het *P*-value	Effect	SE	*P*-value	Direction	Het *P*-value	Effect	SE	*P*-value	Direction	Het *P*-value	Effect	SE	*P*-value	Direction	Het *P*-value
rs2450	−0.27	0.50	5.84×10^{-1}	++−−−	0.62	−1.15	0.42	5.79×10^{-3}	−++−−−	2.72E−02	−0.12	0.27	6.69×10^{-1}	−−+−−−	0.76	−0.55	0.23	1.74×10^{-2}	−−+−−−	6.25×10^{-2}
rs295813	−0.09	0.52	8.63×10^{-1}	−++−?	0.66	−1.23	0.30	5.18×10^{-5}	−−++−?	6.56E−02	0.70	0.35	4.87×10^{-2}	−+++?	0.30	−0.40	0.18	2.68×10^{-2}	−−+++?	2.09×10^{-3}
rs7726475	**1.56**	**0.49**	$\mathbf{1.51 \times 10^{-3}}$	**+−+−+**	**0.40**	**2.00**	**0.41**	$\mathbf{8.85 \times 10^{-7}}$	**++−+−+**	**2.49E−01**	**0.85**	**0.27**	$\mathbf{1.52 \times 10^{-3}}$	**+−+−+**	**0.82**	**1.04**	**0.23**	$\mathbf{3.64 \times 10^{-6}}$	**++−+−+**	$\mathbf{6.62 \times 10^{-1}}$
rs7737481	0.21	0.28	4.55×10^{-1}	−++++	0.76	0.33	0.23	1.48×10^{-1}	+−++++	7.82E−01	0.03	0.16	8.65 × 10^−1	−+−−+	0.79	0.30	0.13	1.68×10^{-2}	+−+−−+	4.17×10^{-2}
rs4957217	0.49	0.32	1.19×10^{-1}	++−++	0.87	−0.17	0.26	5.08×10^{-1}	−++−++	1.49E−02	0.16	0.18	3.61×10^{-1}	−++++	0.80	−0.27	0.15	6.41×10^{-2}	−−++++	1.82×10^{-3}
rs2236611	0.05	0.34	8.74×10^{-1}	+−−−+	0.69	−0.70	0.28	1.20×10^{-2}	−+−−−+	2.99E−03	−0.10	0.18	5.71×10^{-1}	+−−−−	0.33	−0.38	0.15	1.44×10^{-2}	−+−−−−	3.55×10^{-2}

genetic variants. Fourth, it is possible that there may be more than one functional variant underlying BP variation in these regions, as suggested by our finding that more than one SNP was required to account for the association evidence in a number of loci for our admixture mapping analysis. Thus, multiple variants can make replication studies more challenging (36). Fifth, due to the evolution process, it is also possible that there are many population-specific rare variants in these regions contributing to BP variation, reflecting allelic heterogeneity. In this case, replication studies may fail because of differences in the influence of rare variants. A resequencing analysis aimed at detecting the population-specific rare variants in these regions and a follow-up association analysis in a large cohort may be needed to detect the true variants underlying BP variation. Lastly, the effective sizes observed in CARe for these SNPs may have been overestimated because of a 'winner's curse'; consequently, our power calculation may be still overly optimistic. Thus, the association evidence on chromosomes 2 and 21 need further studies.

Our failure to replicate the loci on chromosomes 2 and 21 emphasizes the challenges to the identification of genetic variants underlying BP. Compared with the recent GWAS of BP in populations of European descent (7,8), our discovery and replication sample sizes are still relatively small. The genetic variation covered by the current commercial chips is still lower in non-European populations such as African-Americans. Therefore, many factors contribute to the difficulty identifying consistent evidence of association between genetic variants and BP. We believe that it is worthwhile to conduct GWAS of BP variation including admixture mapping in larger samples in African-Americans.

In summary, we have performed the largest admixture mapping and association study of BP in African-Americans to date. Our study reveals that admixture mapping analysis can help identify genetic variants with substantial allele frequency differences in ancestral populations. Specifically, we have shown that SNP rs7726475, located near *SUB1* and *NPR3*, is significantly associated with both SBP and DBP in African-Americans. This region deserves investigation, including additional replication studies.

MATERIALS AND METHODS

Study samples

The study was approved by the Institutional Review Board (IRB) at Case Western Reserve University. The CARe Study is described in detail elsewhere (37). CARe includes nine cohorts, five of which contributed African-American samples for this analysis. The five African-American cohorts are: ARIC, CARDIA, CFS, JHS and the MESA. The unrelated samples from these five cohorts were used in the admixture mapping analysis and the follow-up association analysis. For CFS and JHS, if more than one of the founders were available in a family, we included the founders. Otherwise, only one offspring was randomly selected in each family. As a result, we excluded 1170 related individuals in the admixture mapping and the follow-up association analysis. However, these individuals were eventually added back for association analysis. The replication cohorts included Maywood, Nigerian, HUSF,

GENOA and WHI cohorts. A detailed description of each cohort can be found in the Supplementary Material.

Adjusting for SBP and DBP

For individuals reporting the use of antihypertensive medications, BP was imputed by adding 10 and 5 mmHg for SBP and DBP (38), respectively. This imputation method has been used widely (7,8). Continuously measured values of DBP and SBP were adjusted for age, age^2, body mass index (BMI) and gender in generalized linear models. Residuals were calculated in each cohort separately and were then combined for admixture mapping analysis as well as genotype–phenotype association analysis.

Admixture mapping analysis

We calculated the δ values for the SNPs available in the Affymetrix 6.0 array. The δ-value is defined as the absolute difference of an allele frequency between HapMap CEU and YRI samples. We divided the 23 autosomes into 1 Mb bins and then selected ancestry informative SNPs as the SNPs with the largest δ in each bin. We selected an AIM panel that included 3230 markers from the 854 893 SNPs passing quality controls (QCs). We examined Hardy–Weinberg equilibrium (HWE) of these 3230 SNPs for possible genotyping errors and background LD which may violate the assumption in admixture mapping analysis (18). All the 3230 SNPs are in HWE and not in LD in HapMap CEU and YRI data. Among the AIMs, more than 98.6% markers have a δ-value of >0.4 (Supplementary Material, Table S5). The average Shannon information content (SIC) for the AIMs we selected was 0.849, suggesting that we generally have very good coverage using these AIMs. We plotted the SIC across the genome (Supplementary Material, Fig. S5).

These SNPs were entered in the hidden Markov model to estimate the marker locus-specific ancestry in African-Americans based on the expectation-maximization (EM) algorithm using the software ADMIXPROGRAM (18). This method directly maximizes the likelihood function through an EM iterative algorithm and allows consideration of uncertainty of marker allele frequencies in the parental populations. We assumed that there were two parental populations for the African-Americans. To examine whether our method would yield reliable parameter estimations, we ran ADMIXPROGRAM twice, one without using any ancestral population information and the other using the allele frequencies of HapMap CEU and YRI as the initial values. We found both methods generate the same parameter estimate values. Furthermore, we compared the estimated allele frequencies in ancestral African and European populations to those in HapMap YRI and CEU data and found high correlation
(Supplementary Material, Figs S6 and S7), indicating that our results are robust and reliable. We further re-estimated marker-specific ancestry using STRUCTURE (28,29), a Bayesian approach, and compared the results based on ADMIXPROGRAM and STRUCTURE. STRUCTURE was run under the linkage model without haplotype phase information, with 10 000 burn-in iterations followed by an additional 10 000 iterations.

We then performed linear regression analysis similar to Basu *et al*. (23). Specifically, let y_i be the residual of individual i after adjusting for age, age^2, BMI and gender. Let A_{ij} be the African ancestry at the jth AIM and $\bar{A}_i$ be the average African ancestry of individual i. A_{ij} was estimated by ADMIXPROGRAM and $\bar{A}_i$was calculated as the mean of A_{ij} for all AIMs. We performed the linear regression analysis as $y_i = \beta_0 + \beta_1\bar{A}_i + \beta_2(A_{ij} - \bar{A}_i) + \varepsilon_i$ and test the null hypothesis: $\beta_2 = 0$, which was used to assess statistical significance in admixture mapping. Here, y_i is the residual of SBP or DBP for the ith individual. We considered a P-value of <0.001 for testing the null hypothesis: $\beta_2 = 0$ as suggestive evidence of association in admixture mapping analysis, and we further defined a region of admixture mapping as comprising the locus that is within the 1 unit drop of $-\log_{10}(P)$ from the peak signal.

Single SNP association and testing SNPs accounting for admixture mapping evidence

To identify SNPs contributing to the association evidence observed in the admixture mapping analysis, we next performed SNP association analysis assuming an additive model in linear regression using PLINK (39) in regions with P-values of <0.001. In addition, we added each individual's average European ancestry $\bar{A}_i$ into the regression model to account for the effect due to population structure. Since only the SNPs with substantial allele frequency differences in ancestral populations can possibly contribute to the association evidence in admixture mapping analysis, we tested only the SNPs with a δ-value of >0.2 in these regions. To determine whether an SNP is significant, we calculated the total number of tests in our study by adding the number of independent tests in admixture mapping and the number of independent tests in the regions we identified. Since the ancestries across the genome are dependent, the total number of independent tests in admixture mapping analysis is suggested to be 1000 (15) for African Americans, which is also consistent with a simulation study (18). Since the SNPs with a δ-value of >0.2 are also correlated, we calculate the number of independent tests in each region separately using the method by Li and Ji (30). The significant SNPs were defined as those survived after the Bonferroni correction of the total number of tests in both admixture mapping and SNP analysis.

To examine whether one or several SNPs were able to account for the evidence observed in admixture mapping analysis, we adjusted for these SNPs in the regression model and evaluated the significance of testing $\beta_2 = 0$. No significance suggests that the SNPs are able to account for the evidence observed in admixture mapping analysis.

Analysis of adding back 1170 related individuals

In admixture mapping and follow-up association analysis, we removed 1170 related individuals from CFS and JHS cohorts. We added them back in our final analysis for SNP rs7726475. In this analysis, the first 10 PCs were calculated and included in the model testing genotype–phenotype association in each cohort separately. This procedure may control for any population structure due to factors other than African-European

admixture. The PCs were calculated based on selected AIMs. We tested the association of rs7726475 and SBP and DBP by linear regression with an additive genetic model using PLINK for all the cohorts except for CFS, in which association was tested using a linear mixed-effect (LME) model that accounted for family structure (40). The results for JHS for applying PLINK and LME were almost identical (10). Meta-analysis of the results was then carried out using the inverse-variance weighting method in METAL (41).

Replication analysis

In each replication cohorts, SBP and DBP were imputed in the same way as in CARe data, in which 10/5 units was added to SBP/DBP if a subject was under medication treatments. In each cohort, SNP association analysis was performed assuming an additive model in linear regression using PLINK (39) for the SNPs carried forward to the replication analysis, adjusting for age, age^2, BMI and gender. Since all the replication cohorts have been genotyped with the Affymetrix 6.0 array, we calculated the first 10 PCs using all the SNPs after QCs. These 10 PCs were added into the linear regression model to control the effect by population structure. Meta-analysis of results was then carried out using the inverse-variance weighting method in METAL (41).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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