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Genome-Wide Association Study Meta-Analysis of Long Term Average Blood Pressure in East Asians

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Abstract

Background—Genome-wide single marker and gene-based meta-analyses of long term average (LTA) blood pressure (BP) phenotypes may reveal novel findings for BP.

Methods and Results—We conducted genome-wide analysis among 18,422 East Asian participants (stage-1) followed by replication study of up to 46,629 participants of European ancestry (stage-2). Significant SNPs and genes were determined by a $P<5.0\times10^{-8}$ and 2.5×10^{-6}, respectively, in joint analyses of stage-1 and stage-2 data. We identified one novel *ARL3* variant, rs4919669 at 10q24.32, influencing LTA systolic BP (stage-1 $P=5.03\times10^{-8}$, stage-2 $P=8.64\times10^{-3}$, joint $P=2.63\times10^{-8}$) and mean arterial pressure (stage-1 $P=3.59\times10^{-9}$, stage-2 $P=2.35\times10^{-2}$, joint $P=2.64\times10^{-8}$). Three previously reported BP loci (*WBP1L, NT5C2*, and *ATP2B1*) were also identified for all BP phenotypes. Gene-based analysis provided the first robust evidence for association of *KCNJ11* with LTA SBP (stage-1 $P=8.55\times10^{-6}$, stage-2 $P=1.62\times10^{-5}$, joint $P=3.28\times10^{-9}$) and mean arterial pressure (stage-1 $P=9.19\times10^{-7}$, stage-2 $P=9.69\times10^{-5}$, joint $P=2.15\times10^{-9}$) phenotypes. Fourteen genes (*TMEM180, ACTR1A, SUFU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, AS3MT, CNNM2*, and *NT5C2* at 10q24.32; *ATP2B1* at 12q21.33; and *NCR3LG1* at 11p15.1) implicated by previous genome-wide association study meta-analyses were also identified. Among the loci identified by the previous genome-wide association study meta-analysis of LTA BP, we trans-ethnically replicated associations of the *KCNK3* marker rs1275988 at 2p23.3 with LTA systolic BP and mean arterial pressure phenotypes ($P=1.27\times10^{-4}$ and 3.30×10^{-4}, respectively).

Conclusions—We identified 1 novel variant and 1 novel gene, and present the first direct evidence of relevance of the *KCNK3* locus for LTA BP among East Asians.

Keywords

Genome Wide Association Study; blood pressure; genetic epidemiology; gene-based analysis; long-term average

Journal Subject Terms

Genetic; Association Studies; High Blood Pressure

Introduction

Elevated blood pressure (BP) is a major public health challenge due to its high prevalence and association with increased risk of cardiovascular disease (CVD) and premature death[1–4]. BP has been long established as an inheritable trait, with heritability estimates ranging from 30% – 60% in pedigree data to as high as 70% in twin studies[5, 6]. Although genome-wide association studies (GWASs) have identified many genetic loci underlying BP regulation, they together explain only a small proportion of the heritability of this complex trait[7]. A recent genome-wide association study (GWAS) meta-analysis suggested that averaging BP

measured across time could improve phenotypic accuracy and thereby increase statistical power to detect genetic associations[8]. Long-term average (LTA) BP, which has been shown to predict future cardiovascular disease events beyond a single-visit measurement of BP[9], may more accurately reflect cumulative burden of elevated BP[10]. Although GWAS meta-analyses of LTA BP is potentially useful for identifying novel genes or genetic variants underlying BP regulation, such studies have yet to be conducted in an Asian population.

In the current study, we aimed to identify novel genetic variants and genes influencing BP regulation by conducting GWAS meta-analyses of LTA systolic BP (SBP), LTA diastolic BP (DBP), LTA mean arterial pressure (MAP), and LTA pulse pressure (PP) among 18,422 participants of the Asian Genetic Epidemiology Network (AGEN) consortium. Furthermore, we attempted trans-ethnic replication of novel LTA BP loci previously identified by the predominantly European Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium.

Methods

Stage-1 GWAS meta-analysis

Study Population—The AGEN consortium was established to facilitate the identification of genetic variants influencing cardiovascular disease related traits among populations of Asian ancestry. For the current study, we included AGEN GWAS with at least 2 BP measures collected at least 1 year apart, including: Dongfeng-Tongji cohort (DFTJ-Cohort) study, the Genetic Epidemiology Network of Salt-Sensitivity (GenSalt), Korean Association Resource Project (KARE), Chinese participants of Multi-Ethnic Study of Atherosclerosis (MESA), Nutrition and Health of Aging Population in China (NHAPC), Singapore Malay Eye Survey (SiMES), Singapore Prospective Study Program (SP2), Taiwan Type 2 Diabetes Study (TWT2DS), and the Taiwan-US Diabetic Retinopathy (TUDR) study. All study participants provided written informed consent, and approval was obtained from the institutional review board (IRB) from the respective local institutions. Detailed descriptions of these 9 studies are presented in the supplementary materials.

Genotype Data and Quality Control—All studies imputed approximately 2.4 million single nucleotide polymorphisms (SNP) from the HapMap release 22 build 36 CHB+JPT samples. SNPs with minor allele frequency (MAF) <0.05, Hardy-Weinberg P value < 1×10^{-6}, call rate<95%, or imputation quality score r^2<0.5 were excluded before performing genome-wide analysis. Detailed information on genotyping, imputation, quality control measures prior and post imputation, and genomic control before meta-analysis according to AGEN studies are shown in supplementary Table S1.

Phenotype Harmonization—Each AGEN study collected at least 2 measurements of SBP and DBP collected at least one year apart in a clinical setting using standard methods as described previously[11]. At each study visit, for those taking anti-hypertensive medication, blood pressure was imputed by adding 10 mmHg and 5 mmHg to SBP and DBP, respectively. Mean MAP and PP were calculated for each participant from SBP and DBP values as MAP=SBP/3+2×DBP/3, and PP as PP=SBP-DBP. For each follow-up visit, the 4 BP phenotypes were first Winsorized at 4 standard deviations (SDs) in both tails[8, 12].

Specifically, if a blood pressure value was more than 4 SDs above or below the mean, it was set exactly at 4 SDs from the mean. This was done in both tails, and separately for each of the 4 blood pressure variables. BP residuals were then calculated for each study visit by performing linear regression controlling for age, age^2, gender, body mass index (BMI), enrollment site, and study specific covariables (the first 2 principal components in MESA, SiMES, and TUDR). LTA BP was calculated by taking the average of the BP residuals over the available follow up visits for study participants.

Single SNP Analysis—Imputed genotype data was analyzed using an additive genetic model. GWAS of adjusted LTA SBP, LTA DBP, LTA MAP, and LTA PP were conducted using linear regression models. Inverse-variance-weighted fixed effect meta-analyses of LTA BP results from the 9 GWAS were performed using METAL software[13]. SNPs were further excluded if they had sample size less than 10,000 or showed evidence of heterogeneity across studies (P for Cochrane's Q-test$<1\times10^{-6}$). Genomic control was applied in each study prior to meta-analyses and in the final meta-analyses (λ=1.007 for LTA SBP and LTA DBP, 1.006 for LTA MAP, and 1.002 for LTA PP, respectively, in the final meta-analyses).

Gene-based Analysis—SNPs within the 5 kilo bp flanking regions of a gene were assigned to the gene. SNPs in the overlapping region of two or more genes were assigned to each of the genes. SNP based analysis results were used to generate gene-based p values using the Gene-based Analysis Using Extended Simes Procedure (GATES) method implemented in KGG software[14]. The GATES method uses an extended Simes test and integrates functional information and association evidence to combine the p values from single marker analyses within a gene to generate an overall p value for the association of the entire gene. This method has been shown to be more powerful than single SNP based tests. Furthermore, the type I error rate is well controlled and independent of gene size and linkage-disequilibrium pattern among markers[14]. The GATES method has been adopted by many genome-wide gene-based studies[15–18]. To explore whether the identified gene-based signal was driven by the most significant SNP in the gene, we performed sensitivity analysis excluding the most significant SNP in the identified gene using GATES method.

Stage-2 Replication Study and Joint Analysis

Single SNP Analysis—SNPs with a stage-1 $P<1\times10^{-6}$ for LTA SBP, LTA DBP, LTA MAP or LTA PP were carried forward for stage-2 replication analyses among participants of European ancestry in the CHARGE consortium. The CHARGE consortium is the only previous GWAS meta-analysis with results on LTA BP traits[8]. CHARGE consists of 46,629 participants of European ancestry from 8 longitudinal population studies, including the Atherosclerosis Risk in Communities (ARIC) study, the Coronary Artery Risk Development in Young Adults Study (CARDIA), the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), the Age, Gene/Environment Susceptibility (AGES) Reykjavik Study, MESA, the Rotterdam Study (RS), and the Women's Genome Health Study (WGHS). Detailed descriptions of these studies are presented in the supplementary materials. The GWAS meta-analysis of LTA BP from the CHARGE consortium used an analysis protocol identical to that of the current study. Briefly, in CHARGE, BP phenotypes were harmonized using the same procedures as described in the current stage-1 analysis.

Additive associations between SNPs and adjusted LTA BP phenotypes were evaluated separately in each study. Inverse-variance-weighted meta-analysis was used to combine results across studies. Genomic control was applied to individual study results and to the final meta-analysis results to control population stratification or cryptic relatedness.

After ensuring the strand orientation and coded alleles were the same, meta-analysis was again used to combine results across stage-1 and stage-2 findings. SNPs that achieved nominal significance ($P<0.05$) in stage-2 analysis and genome-wide significance ($P<5.0\times10^{-8}$) in the joint analysis, with consistent effect directions across stages, were considered significant. For LTA BP loci previously reported in a European population, a Bonferroni $P<0.05/3=1.67\times10^{-2}$ and consistency in effect direction was considered evidence of trans-ethnic replication in the East Asian AGEN consortium.

Gene-based Analysis—Genes with gene-based $P<1\times10^{-4}$ were further evaluated for replication in the CHARGE consortium. Specifically, SNPs from promising genes in stage-1 were tested for associations with the corresponding LTA BP traits in the CHARGE consortium. SNP based analysis results in CHARGE were used to generate gene-based p values using GATES[14]. The Fisher's method was used to combine gene-based results across stage-1 and stage-2 findings. Genes with replication stage $P<0.05$ and combined $P<2.5\times10^{-6}$ (Bonferroni corrected genome-wide significance level for gene-based analysis of 20,000 genes) were considered significant.

Variance Explained by Significant SNPs

We calculated variance explained (VarExp) by each significant SNP using the formula: $VarExp=2EAF*(1-EAF)*\beta^2$, where β refers to the discovery stage meta-analysis association effect size and EAF refers to coded allele frequency[19]. We obtained the variance of SBP $(18.32\text{ mm Hg})^2$ and variance of DBP $(10.31\text{ mm Hg})^2$ by taking the weighted average of standard deviations of SBP and DBP[7], respectively, across all 9 studies.

Results

The discovery stage analyses of LTA BP phenotypes were conducted among 18,422 East Asian participants. Characteristics, including age, BMI, SBP, DBP at baseline and follow-up times are shown in Table 1. In the discovery stage GWAS meta-analysis (stage-1), genome-wide significance ($P<5.0\times10^{-8}$) was achieved for 7 SNP-BP associations at 4 loci. Borderline significance ($5.0\times10^{-8}<P<1.0\times10^{-6}$) was achieved for 10 SNP-BP associations at 5 loci (Figures S1–S4). No sex-based or racial/ethnic-based differences were present.

The 17 independent SNP-BP associations at 8 loci ($r^2<0.3$) which achieved $P<1.0\times10^{-6}$ in the stage-1 GWAS meta-analysis (Figures S1–S4) were evaluated for replication in the stage-2 study of 46,629 CHARGE consortium participants. Eleven SNP-BP associations at 4 loci were nominally significant in the stage-2 analysis and reached genome-wide significance in the joint analysis of stage-1 and stage-2 studies. Replication and joint meta-analysis results for the 11 SNP-BP associations are presented in Table 2. The total variance explained by SBP and DBP loci were 0.37%, and 0.25%, respectively. Although all of the identified loci have been reported previously, one novel variant was identified ($r^2<0.3$ with

previously reported SNPs). The novel *ARL3* rs4919669 variant was associated with both LTA SBP (stage-1 P=5.03×10^{-8}, stage-2 P=8.64×10^{-3}, joint P=2.63×10^{-8}) and LTA MAP (stage-1 P=3.59×10^{-9}, stage-2 P=2.35×10^{-2}, joint P=2.64×10^{-8}). The remaining 9 SNP-BP associations were for independent variants at 3 previously identified loci including *WBP1L, NT5C2*, and *ATP2B1*.

Genome-wide gene-based analysis discovery stage results are shown in supplementary Figures S5 – S8. A total of 18 genes reached suggestive significance (P<1.0×10^{-4}) for at least one LTA BP phenotype in the discovery stage and were evaluated for replication in stage-2 gene-based analysis. Fifteen genes were nominally significant (P<0.05) in the stage-2 gene-based analysis and reached genome-wide significance (P<2.5×10^{-6}) in the joint analysis of stage-1 and stage-2 studies. Gene-based analysis results of these 15 genes are shown in Table 3. Novel gene *KCNJ11* at 11p15.1 was replicated in stage-2 gene-based analysis, and reached genome-wide gene-based significance in the joint meta-analysis for LTA SBP and LTA MAP phenotypes. Sensitivity analysis excluding the most significant SNP, rs1002227 (p=3.26×10^{-7} for PP and 3.03×10^{-6} for SBP) in *KCNJ11* showed that the gene was still significantly associated with SBP (P=2.55×10^{-5}) and PP (4.92×10^{-6}). The remaining 14 genes have been implicated by previous BP GWAS meta-analyses of single markers and include genes *TMEM180, ACTR1A, SUFU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, AS3MT, CNNM2*, and *NT5C2* at 10q24.32 and *ATP2B1* at 12q21.33 (which associated with LTA SBP, LTA DBP and LTA MAP phenotypes in the current study); and *NCR3LG1* at 11p15.1 (which associated with LTA SBP and LTA MAP in the current study).

In addition, as presented in Table 4, we trans-ethnically replicated two associations at the 2p23.3 *KCNK3* locus (rs1275988; P=1.27×10^{-4} and 3.30×10^{-4} for LTA SBP and LTA MAP, respectively), which had been previously identified among European participants of the CHARGE consortium and verified for only single-visit BP associations in East Asians[8]. The other two variants rs7599598 at 2q11 and rs10948071 at 6p21 reached nominal significance (P=0.0236 and 0.0361, respectively) in our study, but were not significant after adjustment for multiple testing.

Discussion

GWAS meta-analysis of LTA BP traits among 18,422 AGEN participants revealed a novel *ARL3* variant, rs4919669 at 10q24.32, influencing LTA SBP and LTA MAP. In the first genome-wide gene-based analysis of LTA BP, we identified novel associations between the *KCNJ11* gene and both LTA SBP and LTA PP. Through trans-ethnic replication of CHARGE findings[8], the current analysis also provided the first direct evidence for association of variants at the *KCNK3* locus (at 2p23.3) with LTA SBP and LTA MAP among East Asians. Both single-marker and gene-based analyses confirmed signals reported by previous BP GWAS meta-analyses. Single-marker analysis verified 9 associations at 3 previously identified BP loci (*WBP1L*[20], *NT5C2*[11], and *ATP2B1*[11]), while gene-based analysis identified 14 genes (implicated previously by single-marker analyses) including *TMEM180, ACTR1A, SUFU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, AS3MT, CNNM2*, and *NT5C2* at 10q24.32; gene *ATP2B1* at 12q21.33; and gene

NCR3LG1 at 11p15.1. These findings contribute further information towards delineating the biological mechanisms underlying BP regulation.

We identified a novel *ARL3* gene variant, rs4919669 at 10q24.32, associated with LTA SBP and LTA MAP in the current study. Although the variant appears novel, this locus has been identified previously. Three highly correlated variants including rs1004467, rs11191548, and rs3824755 (r^2>0.7) were reported in previous GWAS meta-analyses of BP, including the BP GWAS meta-analysis conducted in East Asian participants[7, 11, 20–23]. The three SNPs were all associated with BP phenotypes in the current discovery stage analysis with p values ranging from 1.53×10^{-6} to 4.81×10^{-9}. The variant rs4919669 identified in our study is not in LD with any of these three SNPs in East Asians, indicating that this signal may reflect a different causal variant from that captured by rs1004467, rs11191548, and rs3824755. These data suggest that the 10q24.32 locus may harbor multiple causal variants for BP phenotypes[24]. The *ARL3* gene encodes ADP-ribosylation factor-like 3, a member of the ADP-ribosylation factor family of GTP-binding proteins[25]. Mutations of the *ARL3* gene cause Bardet-Beidl syndrome, a heterogeneous disorder which increases the risk of hypertension and diabetes[26]. Physiologic support for a role of *ARL3* in BP is evidenced by animal experiments showing that *ARL3* gene knock-out mice develop elevated blood pressure[27]. While *ARL3* represents an interesting BP candidate gene, the identified variant rs4919669 lies in an intronic region and is not in high LD with any coding variant. To assess the potential functional impact of rs4919669, we calculated its GWAVA score[28]. With a region score of 0.37, transcription start site (TSS) score of 0.34, and unmatched score of 0.56, this SNP is unlikely to be pathogenic. However, three nearby SNPs in high LD with rs4919669, including rs8354 (r^2=0.96), rs11191355 (r^2=0.59), and rs2298278 (r^2=0.79), had high GWAVA scores (GWAVA scores range from 0.51 to 0.74), indicating likely pathological consequences of these SNPs. Future sequencing and functional studies will be needed to better understand the causal mechanism underlying this association.

Gene-based analysis provided the first robust evidence of associations for the *KCNJ11* gene with LTA SBP and LTA MAP. The *KCNJ11* gene encodes member 11 of inwardly rectifying subfamily J of the potassium channel. This gene has been identified in several GWAS and a GWAS meta-analysis of type 2 diabetes[29–32]. Interestingly, an association between this gene and blood pressure was suggested previously in a candidate gene study by Sakamoto and colleagues[33]. However, the association was not significant after adjustment for multiple testing, and no evidence of replication data was available in Sakamoto and colleagues' study[33]. In addition to previous studies in human populations, animal experiments provide further support of a *KCNJ11*-BP association. Kane and colleagues reported that *KCNJ11* gene knock-out mice developed hypertension and were vulnerable to heart failure and death[34]. The *KCNJ11* gene signal was missed in the single-marker analysis, highlighting the gain of power and potential utility of gene-based analysis. In addition, sensitivity analysis excluding the most significant SNP within *KCNJ11* showed significant gene-based associations of *KCNJ11* with both LTA SBP and LTA MAP, indicating that multiple causal variants may attribute to the gene-based signal. Future studies are warranted to identify the causal variants underlying this gene-based signal.

The current analysis trans-ethnically replicated two associations at the *KCNK3* locus which were identified in a previous GWAS meta-analysis of LTA BP in a predominantly European population by Ganesh and colleagues[8]. With follow-up analysis in an Asian population, Ganesh and colleagues showed an association of this locus with single visit BP[8]. Our analysis provides the first direct evidence of replication for this locus with LTA BP. The *KCNK3* gene encodes a member of the superfamily of potassium channel proteins and is involved in metabolism of potassium, which has well-known protective effects on BP[35]. Furthermore, functional studies demonstrated that Task1-null littermate mice (kcnk3 knockouts) had significantly lower MAP (about 9 mmHg) compared to wild-type littermate mice[8, 36].

In addition to identifying novel variants and genes in East Asian populations, both single-marker and gene-based analyses confirmed signals reported by previous BP GWAS meta-analyses. Single-marker analyses verified 9 associations at 3 previously identified BP loci, including *WBP1L, NT5C2*, and *ATP2B1*. Furthermore, gene-based analysis identified 14 genes which were implicated previously by single-marker analyses, including: *TMEM180, ACTR1A, SUFU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, AS3MT, CNNM2*, and *NT5C2* at 10q24.32; gene *ATP2B1* at 12q21.33; and gene *NCR3LG1* at 11p15.1. While numerous genes at 10q24.32 were implicated by this study, it is unlikely that all are causally associated with BP given the high LD of variants in numerous genes across this region[7, 11]. Functional studies will likely be necessary to identify the causal genomic mechanisms at this gene dense locus.

Our study represents the first GWAS meta-analysis of LTA BP conducted in Asians. Additional study strengths include the adherence of all studies to a standard analytical protocol and stringent genotyping and imputation quality control at the study and meta-analysis levels. Although the current analysis had fewer participants compared to the previously conducted GWAS meta-analysis of single-visit BP in the AGEN consortium, we were able to identify a novel variant and a novel gene associated with BP, highlighting the importance of exploring novel phenotypes and gene-based analysis in the identification of BP loci. Certain limitations should be acknowledged for our study. Some novel loci identified in the discovery stage may be specific to the Asian population. Since only trans-ethnic replication could be conducted, the current study is not able to robustly identify such loci. However, gene-based analyses were employed, which may be more consistent across populations since these methods are based on the entire functional unit. In addition, we used fixed effect models to assess the additive associations between SNPs and BP, assuming that genetic variants have similar effects across studies. Although our study participants were all of East Asian ancestry, there might be heterogeneity in genetic effects due to differences in age and other covariables[37]. In this case, we may have missed some genetic loci whose effects are be modified by such factors. Finally, since we only examined common variants, future studies will be necessary to identify important low frequency and rare variants influencing LTA BP.

In conclusion, we conducted the first GWAS meta-analysis of LTA BP phenotypes in an Asian population. The current study identified a novel association of the *ARL3* gene variant rs4919669 with LTA BP phenotypes in single-marker analysis and an association of the

KCNJ11 gene in gene-based analyses. Furthermore, through trans-ethnic replication study, we are the first to report an association of the *KCNK3* gene locus with LTA BP phenotypes in East Asians. In aggregate, our findings highlight the utility of examining novel phenotypes and conducting gene-based analyses to identify novel genes and variants. Furthermore, we add to the accumulating evidence of reproducible genomic associations across populations with distinct LD structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Detailed acknowledgment information for both discovery stage and replication stage studies is presented in the supplementary materials.

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Clinical Perspective

Long term average (LTA) blood pressure (BP), which has been shown to predict future cardiovascular disease events beyond a single-visit measurement of BP, may more accurately reflect cumulative burden of elevated BP. We conducted the first GWAS meta-analysis of LTA BP phenotypes in an Asian population. The current study identified a novel association of the *ARL3* gene variant rs4919669 with LTA BP phenotypes in single-marker analysis and an association of the *KCNJ11* gene in gene-based analyses. Furthermore, through trans-ethnic replication study, we are the first to report an association of the *KCNK3* gene locus with LTA BP phenotypes in East Asians. Although the current analysis had fewer participants compared to the previously conducted GWAS meta-analysis of single-visit BP in the AGEN consortium, we were able to identify a novel variant and a novel gene associated with BP, highlighting the importance of exploring novel phenotypes and gene-based analysis in the identification of BP loci. Furthermore, we add to the accumulating evidence of reproducible genomic associations across populations with distinct LD structure. However, Future sequencing and functional studies will be needed to better understand the causal mechanism underlying the identified association and to identify the causal variants underlying the gene-based signals.

Table 1

Characteristics of Discovery Stage Cohorts

Cohort	N	Visits/ follow-up years	Baseline					Long term average		
			Age, Years Mean (SD)	BMI, kg/m^2 Mean (SD)	SBP, mmHg Mean (SD)	DBP, mmHg Mean (SD)	Anti-HTN Medication, %	BMI, kg/m^2 Mean (SD)	SBP, mmHg Mean (SD)	DBP, mmHg Mean (SD)
DFTJ	1,155	2/5	62.5 (7.7)	24.8 (3.3)	132.5 (20.4)	79.7 (11.7)	29.7	24.4 (3.3)	146.7 (23.8)	83.3 (12.8)
GenSalt	1,767	3/8	39.0 (9.2)	23.4 (3.1)	117.0 (14.1)	73.8 (10.2)	0.4	24.2 (3.2)	123.4 (15.0)	78.6 (9.7)
KARE	6,447	3/7	52.2 (8.8)	24.6 (3.1)	122.6 (19.3)	80.9 (11.7)	10.6	24.6 (3.0)	120.0 (16.4)	79.4 (9.7)
MESA (Chinese subjects)	775	4/6	62.4 (10.4)	24.0 (3.3)	127.4 (23.7)	73.3 (10.9)	29.0	24.0 (3.2)	126.8 (21.2)	72.3 (9.6)
NHAPC	2,004	2/6	58.2 (5.9)	24.5 (3.6)	139.6 (22.6)	79.9 (10.8)	26.4	24.6 (3.5)	137.8 (18.8)	80.3 (9.5)
SiMES	1,494	2/6	57.2 (10.1)	26.6 (4.8)	148.0 (24.0)	81.2 (11.3)	29.4	26.7 (4.8)	146.9 (19.9)	80.3 (9.4)
SP2	2,177	2/10	38.2 (11.1)	22.5 (3.5)	118.8 (16.8)	71.8 (11.8)	5.2	22.9 (3.7)	130.6 (21.2)	77.6 (11.4)
TWT2D2	1,599	2/2	61.6 (11.8)	33.2 (9.0)	134.6 (16.7)	80.8 (10.5)	30.8	33.2 (9.0)	135.1 (16.9)	78.7 (11.2)
TUDR	1,004	4/4	64.3 (11.9)	24.8 (4.3)	134.6 (17.7)	75.8 (10.9)	60.3	-	136.6 (20.7)	76.8 (12.1)

BMI=Body mass index; DBP=Diastolic blood pressure; DFTJ=Dong Feng Tongji Cohort Study; GenSalt=Genetic Epidemiology Network of Salt-Sensitivity; HTN=Hypertension; KARE=Korean Association Resource; MESA=Multi-Ethnic Study of Atherosclerosis; NHAPC=Nutrition and Health of Aging Population in China; SBP=Systolic blood pressure; SiMES=Singapore Malay Eye Study; SP2=Singapore Prospective Study Program; TUDR=Taiwan and US Diabetic Retinopathy Study; TWT2DS=Taiwan Type II Diabetes Study

Table 2

Loci Achieving Genome-Wide Significance ($P<5.0\times10^{-8}$) for Any Long Term Average Blood Pressure Phenotype in Meta-Analysis.

SNP	Chr	Position (Build 36)	CA	CAF	Nearest Gene	Study	Beta, mm Hg	SE	*P*
LTA Systolic Blood Pressure									
rs4919669	10	104461965	A	0.43	*ARL3*	AGEN	−0.96	0.18	5.03E-08
						CHARGE	−0.41	0.15	8.64E-03
						Meta	−0.65	0.12	2.63E-08
rs284844	10	104544519	A	0.49	*WBP1L*	AGEN	−0.99	0.17	4.61E-09
						CHARGE	−0.57	0.15	8.75E-05
						Meta	−0.75	0.11	1.05E-11
rs11191580	10	104896201	T	0.74	*NT5C2*	AGEN	1.18	0.20	1.83E-09
						CHARGE	0.83	0.16	1.78E-07
						Meta	0.97	0.12	4.44E-15
rs12579302	12	88574634	A	0.65	*ATP2B1*	AGEN	0.96	0.19	2.50E-07
						CHARGE	0.93	0.12	5.08E-15
						Meta	0.94	0.10	<1E-17
LTA Diastolic Blood Pressure									
rs284844	10	104544519	A	0.49	*WBP1L*	AGEN	−0.53	0.10	7.14E-08
						CHARGE	−0.23	0.09	5.81E-03
						Meta	−0.36	0.06	2.05E-08
rs4409766	10	104606653	T	0.72	*C10orf32*	AGEN	0.55	0.11	4.13E-07
					-AS3MT	CHARGE	0.30	0.09	8.42E-04
						Meta	0.40	0.07	7.43E-09
rs11105364	12	88593407	T	0.65	*ATP2B1*	AGEN	0.58	0.11	1.08E-07
						CHARGE	0.53	0.07	7.29E-14
						Meta	0.54	0.06	<1E-17
LTA Mean Arterial Pressure									
rs4919669	10	104461965	A	0.43	*ARL3*	AGEN	−0.72	0.12	3.59E-09
						CHARGE	−0.24	0.10	2.35E-02
						Meta	−0.44	0.08	2.64E-08
rs284844	10	104544519	A	0.49	*WBP1L*	AGEN	−0.70	0.12	1.15E-09

SNP	Chr	Position (Build 36)	CA	CAF	Nearest Gene	Study	Beta, mm Hg	SE	*P*
						CHARGE	−0.34	0.10	5.23E-04
						Meta	−0.49	0.07	4.66E-11
rs4409766	10	104606653	T	0.72	*C10orf32*	AGEN	0.74	0.13	6.87E-09
					-AS3MT	CHARGE	0.42	0.10	4.38E-05
						Meta	0.54	0.08	9.90E-12
rs12579302	12	88574634	A	0.66	*ATP2B1*	AGEN	0.68	0.13	7.27E-08
						CHARGE	0.68	0.08	3.43E-17
						Meta	0.68	0.07	<1E-17

CA=Coded allele; CAF=Coded allele frequency; LTA=Long term average; SE=standard error; SNP=single nucleotide polymorphism;

Table 3

Genes Achieving Genome-Wide Significance ($P<2.5\times10^{-6}$) for Any Long Term Average Blood Pressure Phenotype in the Meta-Analysis.

Gene	Chr	Start position (Build 36)	Length (bp)	SNPs	Study	SBP	DBP	MAP	PP
TMEM180	10	104211159	15634	26	AGEN	8.56E-06	1.93E-06	2.98E-07	4.79E-02
					CHARGE	1.22E-03	9.62E-03	3.48E-03	9.06E-02
					Meta	**2.02E-07**	**3.49E-07**	**2.25E-08**	2.79E-02
ACTR1A	10	104228975	23528	28	AGEN	5.76E-06	1.91E-06	2.58E-07	3.81E-02
					CHARGE	1.34E-03	8.94E-03	3.61E-03	2.11E-01
					Meta	**1.52E-07**	**3.22E-07**	**2.03E-08**	4.68E-02
SUFU	10	104253708	115503	87	AGEN	3.04E-06	9.00E-07	9.87E-08	2.03E-02
					CHARGE	2.99E-04	3.10E-03	7.24E-04	1.25E-02
					Meta	**1.98E-08**	**5.77E-08**	**1.74E-09**	2.35E-03
ARL3	10	104423473	40708	14	AGEN	3.06E-07	8.99E-07	2.19E-08	1.02E-02
					CHARGE	1.65E-02	3.53E-02	2.43E-02	8.51E-02
					Meta	**1.02E-07**	**5.80E-07**	**1.19E-08**	6.99E-03
SFXN2	10	104464287	24650	23	AGEN	4.40E-07	1.29E-06	3.14E-08	8.40E-03
					CHARGE	1.86E-03	2.05E-03	1.64E-03	6.76E-02
					Meta	**1.79E-08**	**5.49E-08**	**1.27E-09**	4.81E-03
WBP1L	10	104525877	40135	23	AGEN	7.16E-08	1.11E-06	1.79E-08	1.16E-03
					CHARGE	5.31E-04	5.25E-05	5.05E-05	3.95E-03
					Meta	**9.50E-10**	**1.43E-09**	**2.60E-11**	6.09E-05
CYP17A1	10	104580277	7004	13	AGEN	3.71E-06	1.11E-04	4.42E-06	1.00E-03
					CHARGE	2.59E-05	1.89E-05	2.40E-05	6.46E-04
					Meta	**2.31E-09**	**4.40E-08**	**2.54E-09**	9.85E-06
C10orf32	10	104603956	10753	14	AGEN	2.26E-08	2.96E-06	4.93E-08	5.33E-04
					CHARGE	2.85E-05	1.84E-06	3.45E-06	8.09E-04
					Meta	**1.87E-11**	**1.47E-10**	**5.17E-12**	6.75E-06
C10orf32-ASMT	10	104603956	47690	37	AGEN	3.98E-08	5.22E-06	8.67E-08	3.32E-04
					CHARGE	2.72E-06	3.27E-06	6.14E-06	5.18E-05
					Meta	**3.34E-12**	**4.40E-10**	**1.56E-11**	3.25E-07
AS3MT	10	104619199	32447	37	AGEN	1.01E-07	4.90E-05	6.77E-07	2.57E-04

Gene	Chr	Start position (Build 36)	Length (bp)	SNPs	Study	SBP	DBP	MAP	PP
					CHARGE	2.14E-06	3.39E-06	7.43E-06	4.08E-05
					Meta	**6.52E-12**	**3.91E-09**	**1.36E-10**	2.03E-07
CNNM2	10	104668064	160271	122	AGEN	3.94E-08	5.32E-06	1.50E-07	2.64E-04
					CHARGE	1.33E-06	8.92E-06	3.15E-05	2.85E-05
					Meta	**1.65E-12**	**1.18E-09**	**1.28E-10**	1.48E-07
NT5C2	10	104837763	105291	77	AGEN	2.05E-08	1.08E-05	1.21E-07	1.94E-04
					CHARGE	1.38E-06	1.66E-05	4.84E-05	2.83E-05
					Meta	**9.11E-13**	**4.20E-09**	**1.57E-10**	1.10E-07
NCR3LG1	11	17329884	25561	16	AGEN	5.27E-05	6.60E-03	1.93E-03	8.14E-06
					CHARGE	7.61E-04	2.18E-01	1.67E-02	8.26E-04
					Meta	**7.23E-07**	1.09E-02	3.66E-04	**1.33E-07**
KCNJ11	11	17363371	3412	10	AGEN	8.55E-06	3.32E-03	5.65E-04	9.19E-07
					CHARGE	1.62E-05	2.70E-02	3.75E-04	9.69E-05
					Meta	**3.28E-09**	9.25E-04	3.47E-06	**2.15E-09**
ATP2B1	12	88505956	68020	19	AGEN	2.03E-06	8.94E-07	5.92E-07	9.31E-03
					CHARGE	8.40E-15	2.91E-13	5.19E-17	4.96E-07
					Meta	**<1.0E-17**	**<1.0E-17**	**<1.0E-17**	9.32E-08

DBP=Diastolic blood pressure; MAP=Mean arterial pressure; PP=Pulse pressure; SBP=Systolic blood pressure

Note: Bolded are significant gene-based associations with discovery stage $P<1.0\times10^{-4}$, replication stage $P<0.05$, and joint meta-analysis $P<5.0\times10^{-6}$.

Table 4

Trans-ethnic Replication of Associations at 3 Novel Loci Previously Reported among European Participants of the CHARGE Consortium.

Trait	SNP	Nearest gene	Locus	CHARGE					AGEN				
				CA	CAF*	Beta, mm Hg	SE	P	CA	CAF	Beta, mm Hg	SE	P
DBP	rs7599598	*FER1L5*	2q11	A	0.57	−0.31	0.05	2.91E-08	A	0.55	−0.29	0.13	2.36E-02
SBP	rs1275988	*KCNK3*	2p23	T	0.60	−0.60	0.09	2.61E-10	T	0.24	−0.76	0.2	1.27E-04
MAP	rs1275988	*KCNK3*	2p23	T	0.60	−0.39	0.06	1.51E-09	T	0.24	−0.48	0.14	3.30E-04
PP	rs10948071	*CRIP3*	6p21	T	0.71	−0.38	0.07	9.06E-09	T	0.71	−0.26	0.12	3.61E-02

CA=coded allele; CAF=coded allele frequency; SE=standard error; SNP=single nucleotide polymorphism;

*coded allele frequency based on data from the HapMap project, version 2010-08_phaseII+III.