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

Changwei Li, MD, PhD*; Yun Kyoung Kim, PhD*; Rajkumar Dorajoo, PhD*; Huaixing Li, PhD*; I-Te Lee, MD, PhD*; Ching-Yu Cheng, MD, PhD*; Meian He, MD, PhD*; Wayne H-h Sheu, MD, PhD*; Xiuqing Guo, PhD*; Santhi K. Ganesh, MD*; Jiang He, MD, PhD; Juyoung Lee, PhD; Jianjun Liu, PhD; Yao Hu, PhD; Dabeeru C. Rao, PhD; Fuu-Jen Tsai, MD, PhD; Jia Yu Koh, MS; Hua Hu, PhD; Kae-Woei Liang, MD; Walter Palmas, MD; James E. Hiscox, PhD; Sohee Han, PhD; Yik-Ying Teo, PhD; Yiqin Wang, MD; Jing Chen, MD; Chieh Hsiang Lu, MD, PhD; Yingfeng Zheng, MD, PhD; Lixuan Gui, MS; Wen-Jane Lee, PhD; Jie Yao, MD; Dongfeng Gu, MD, PhD; Bok-Ghee Han, PhD; Xueling Sim, PhD; Liang Sun, PhD; Jinying Zhao, MD, PhD; Chien-Hsiun Chen, PhD; Neelam Kumari, PhD; Yunfeng He, PhD; Kent D. Taylor, PhD; Leslie J. Raffel, MD; Sanghoon Moon, PhD; Jerome I. Rotter, MD†; Yii-der Ida Chen, PhDb†; Tangchun Wu, MD, PhDb†; Tien Yin Wong, MD, PhDb†; Jer-Yuwn Wu, PhDb†; Xu Lin, MD, PhDb†; E-Shyong Tai, MD, PhDb†; Bong-Jo Kim, PhDb†; Tanika N. Kelly, PhDb†

Background—Genome-wide single marker and genome-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 ≤46,629 participants of European ancestry (stage 2). Significant single-nucleotide polymorphisms and genes were determined by a P=5.0×10^{-8} and 2.5×10^{-8}, respectively, in joint analyses of stage-1 and stage-2 data. We identified 1 novel ARL3 variant, rs4919669 at 10q24.32, influencing LTA systolic BP (stage-1 P=5.03×10^{-8}, stage-2 P=8.64×10^{-9}, joint P=2.63×10^{-8} and mean arterial pressure (stage-1 P=3.59×10^{-9}, stage-2 P=2.35×10^{-9}, joint P=2.64×10^{-9}). 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 KCNN11 with LTA systolic BP (stage-1 P=8.55×10^{-8}, stage-2 P=1.62×10^{-5}, joint P=3.28×10^{-8}) and mean arterial pressure (stage-1 P=9.19×10^{-8}, stage-2 P=6.99×10^{-9}, joint P=2.15×10^{-8}) phenotypes. Fourteen genes (TMEM180, ACTRIA1, SUFU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, ASMT, 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 transethnically replicated associations of the KCNK3 marker rs1275988 at 20p23.3 with LTA systolic BP and mean arterial pressure phenotypes (P=1.27×10^{-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. (Circ Cardiovasc Genet. 2017;10:e001527. DOI: 10.1161/CIRCGENETICS.116.001527.)

Key Words: arterial pressure ■ blood pressure ■ epidemiology ■ genome-wide association study

See Clinical Perspective

Elevated blood pressure (BP) is a major public health challenge because of its high prevalence and association with increased risk of cardiovascular disease and premature death.1–4 BP has been long established as an inheritable trait, with heritability estimates ranging from 30% to 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 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 transethnic 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 DFTJ-Cohort study (Dongfeng-Tongji cohort), the GenSalt (Genetic Epidemiology Network of Salt-Sensitivity), KARE (Korean Association Resource Project), Chinese participants of MES (Multi-Ethnic Study of Atherosclerosis), NHAPC (Nutrition and Health of Aging Population in China), SiMES (Singapore Malay Eye Survey), SP2 (Singapore Prospective Study Program), TWTD2S (Taiwan Type 2 Diabetes Study), and the TUDR study (Taiwan-US Diabetic Retinopathy). All study participants provided written informed consent, and approval was obtained from the institutional review board from the respective local institutions. Detailed descriptions of these 9 studies are presented in the Data Supplement.

Genotype Data and Quality Control
All studies imputed ~2.4 million single-nucleotide polymorphisms (SNP) from the HapMap release 22 build 36 CHB+JPT samples. SNPs with minor allele frequency <0.05, Hardy–Weinberg value < 1×10−6, call rate <95%, or imputation quality score r²<0.5 were excluded before performing genome-wide analysis. Detailed information on genotyping, imputation, quality control measures before and after imputation, and genonic control before meta-analysis according to AGEN studies are shown in Table I Data Supplement.

Phenotype Harmonization
Each AGEN study collected at least 2 measurements of SBP and DBP collected at least 1 year apart in a clinical setting using standard methods as described previously.11 At each study visit, for those taking antihypertensive medication, BP was imputed by adding 10 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+2×DBP/3 and PP as PP=SBP–DBP. For each follow-up visit, the 4 BP phenotypes were first Winsorized at 4 SDs in both tails.12 Specifically, if a BP value was >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 BP variables. BP residuals were then calculated for each study visit by performing linear regression controlling for age, age², sex, body mass index, enrollment site, and study-specific covariates (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 were 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 <10000 or showed evidence of heterogeneity across studies (P for Cochrane’s Q test <1×10⁻⁸). Genomic control was applied in each study before 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 kbp flanking regions of a gene were assigned to the gene. SNPs in the overlapping region of ≥2 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 (GATES) procedure 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<1×10⁻⁶ 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.19 CHARGE consists of 46,629 participants of European ancestry from 8 longitudinal population studies, including the ARIC study (Atherosclerosis Risk in Communities), the CARDIA study (Coronary Artery Risk Development in Young Adults), the CHS (Cardiovascular Health Study), the FHS (Framingham Heart Study), the AGES Reykjavik study (Age, Gene/Environment Susceptibility), MESA, the RS (Rotterdam Study), and the WGHS (Women’s Genome Health Study). Detailed descriptions of these studies are presented in the Data Supplement. The GWAS meta-analysis of LTA BP from the CHARGE consortium used an analysis protocol identical to that of the current study. Briefly, in CHARIE, 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<5.0×10⁻⁸) 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=1.67×10⁻² 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<1×10⁻⁶ were further evaluated for replication in the CHARIE 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 CHARIE were used to generate gene-based P values using GATES.16 The Fisher 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<2.5×10⁻⁴ (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)*β², where β refers to the
discovery stage meta-analysis association effect size and EAF refers to coded allele frequency.\textsuperscript{19} We obtained the variance of SBP (18.32 mm Hg\textsuperscript{2}) and variance of DBP (10.31 mm Hg\textsuperscript{2}) by taking the weighted average of SDs of SBP and DBP.\textsuperscript{11} 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, body mass index, 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 (\(P < 5.0 \times 10^{-8}\)) was achieved for 7 SNP–BP associations at 4 loci. Borderline significance (\(5.0 \times 10^{-8} < P < 1.0 \times 10^{-6}\)) was achieved for 10 SNP–BP associations at 5 loci (Figures I through IV in the Data Supplement). No sex-based or racial/ethnic-based differences were present.

The 17 independent SNP–BP associations at 8 loci (\(r^2 < 0.3\)) that achieved \(P < 1.0 \times 10^{-6}\) in the stage-1 GWAS meta-analysis (Figures I through IV in the Data Supplement) 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 variances 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 \(P = 5.03 \times 10^{-8}\), stage-2 \(P = 8.64 \times 10^{-7}\), joint \(P = 2.63 \times 10^{-8}\)) and LTA MAP (stage-1 \(P = 3.59 \times 10^{-8}\), stage-2 \(P = 2.35 \times 10^{-5}\), joint \(P = 2.64 \times 10^{-8}\)). 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 discovered 18 genes at 9 loci (\(r^2 < 0.3\) for at least 1 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 (\(P < 2.5 \times 10^{-8}\)) 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 (\(P = 3.26 \times 10^{-7}\) for PP and 3.03\(\times 10^{-6}\) for SBP) in KCNJ11 showed that the gene was still significantly associated with SBP (\(P = 2.55 \times 10^{-7}\)) and PP (4.92\(\times 10^{-5}\)). The remaining 14 genes have been implicated by previous BP GWAS meta-analyses of single markers and include genes TMEM180, ACTRIA1, SUXU, 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 NCR3L1G1 at 11p15.1 (which associated with LTA SBP and LTA MAP in the current study).

In addition, as presented in Table 4, we transethnically replicated 2 associations at the 2p23.3 KCNK3 locus (rs1275988; \(P = 1.27 \times 10^{-4}\) and 3.30\(\times 10^{-4}\) 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.\textsuperscript{8} The other 2 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 AGENT 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 transethnic replication of CHARGE findings,\textsuperscript{9} 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 GWAS meta-analyses. Single-marker analysis verified 9 associations at 3 previously identified BP loci (WBP1L,\textsuperscript{20} NT5C2\textsuperscript{11,20} and ATP2B1\textsuperscript{11}), whereas gene-based analysis identified 14 genes (implicated previously by single-marker analyses) including TMEH180, ACTRIA1, SUXU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, AS3MT, CNNM2, and NT5C2 at 10q24.32; gene ATP2B1 at 12q21.33; and gene NCR3L1G1 at 11p15.1. These findings contribute further information toward 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.\textsuperscript{7,11,20–23} The 3 SNPs were all associated with BP phenotypes in the current discovery stage analysis with \(P\) values ranging from \(1.53 \times 10^{-4}\) to \(4.81 \times 10^{-9}\). The variant rs4919669 identified in our study is not in linkage disequilibrium with any of these 3 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.\textsuperscript{24} The ARL3 gene encodes ADP-ribosylation factor-like 3, a member of the ADP-ribosylation factor family of GTP-binding proteins.\textsuperscript{25} Mutations of the ARL3 gene cause Bardet–Beidl syndrome, a heterogeneous disorder that increases the risk of hypertension and diabetes mellitus.\textsuperscript{26} Physiological support for a role of ARL3 in BP is evidenced by animal experiments showing that ARL3 gene knockout mice develop elevated BP.\textsuperscript{27} Although 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 studied the potential functional impact of rs4919669, we calculated its Genome Wide Annotation of Variant (GWA) score.\textsuperscript{28} With a region score of 0.37, transcription start site score of 0.34, and unmatched score of 0.56, this SNP is unlikely to
GWAS meta-analysis of LTA BP in a predominantly European locus, which were identified in a previous study. The causal variants underlying this gene-based signal. LTA MAP, indicating that multiple causal variants may attribute with both LTA SBP and KCNJ11 showed significant gene-based analysis. In addition, sensitivity analysis excluding analysis, highlighting the gain of power and potential utility of death. The developed hypertension and were vulnerable to heart failure and diabetes mellitus. Interestingly, an association between this gene and BP was suggested previously in a candidate gene study by Sakamoto et al. However, the association was not significant after adjustment for multiple testing, and no evidence of be pathogenic. However, 3 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 mellitus. Interestingly, an association between this gene and BP was suggested previously in a candidate gene study by Sakamoto et al. However, the association was not significant after adjustment for multiple testing, and no evidence of replication data was available in the study by Sakamoto et al. In addition to previous studies in human populations, animal experiments provide further support of a KCNJ11–BP association. Kane et al reported that KCNJ11 gene knockout mice developed hypertension and were vulnerable to heart failure and death. 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 transethnically replicated 2 associations at the KCNK3 locus, which were identified in a previous GWAS meta-analysis of LTA BP in a predominantly European population by Ganesh et al. With follow-up analysis in an Asian population, Ganesh et al showed an association of this locus with single-visit BP. 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. Furthermore, functional studies demonstrated that Task1-null littermate mice (kcnk3 knockouts) had significantly lower MAP ($\simeq 9$ mm Hg) compared with wild-type littermate mice.

In addition to identifying novel variants and genes in East Asian populations, both single-marker and gene-based analyses confirmed signals reported by previous 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 that were implicated previously by single-marker analyses, including TMEM180, ACTRIA, 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. Although 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. 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 analytic protocol and stringent genotyping and imputation quality control at the study and meta-analysis levels. Although the current analysis had fewer participants compared with the previously conducted GWAS meta-analysis of single-visit BP in the AGENT 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. Because only transethnic replication could be conducted, the current study is not able to robustly identify such loci. However, gene-based analyses were used, which may be more consistent across populations because these methods are based on the entire functional unit.

### Table 1. Characteristics of Discovery Stage Cohorts

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>Visits/Follow-Up Years</th>
<th>Age, Y (Mean, SD)</th>
<th>BMI, kg/m² (Mean, SD)</th>
<th>SBP, mm Hg (Mean, SD)</th>
<th>DBP, mm Hg (Mean, SD)</th>
<th>Anti-HTN Medication, %</th>
<th>BMI, kg/m² (Mean, SD)</th>
<th>SBP, mm Hg (Mean, SD)</th>
<th>DBP, mm Hg (Mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFTJ</td>
<td>1155</td>
<td>2/5</td>
<td>62.5 (7.7)</td>
<td>24.8 (3.3)</td>
<td>132.5 (20.4)</td>
<td>79.7 (11.7)</td>
<td>29.7</td>
<td>24.4 (3.3)</td>
<td>146.7 (23.8)</td>
<td>83.3 (12.8)</td>
</tr>
<tr>
<td>GenSalt</td>
<td>1767</td>
<td>3/8</td>
<td>39.0 (9.2)</td>
<td>23.4 (3.1)</td>
<td>117.0 (14.1)</td>
<td>73.8 (10.2)</td>
<td>0.4</td>
<td>24.2 (3.2)</td>
<td>123.4 (15.0)</td>
<td>78.6 (9.7)</td>
</tr>
<tr>
<td>KARE</td>
<td>6447</td>
<td>3/7</td>
<td>52.2 (8.8)</td>
<td>24.6 (3.1)</td>
<td>122.6 (19.3)</td>
<td>80.9 (11.7)</td>
<td>10.6</td>
<td>24.6 (3.0)</td>
<td>120.0 (16.4)</td>
<td>97.4 (9.7)</td>
</tr>
<tr>
<td>MESA (Chinese subjects)</td>
<td>775</td>
<td>4/6</td>
<td>62.4 (10.4)</td>
<td>24.0 (3.3)</td>
<td>127.4 (23.7)</td>
<td>73.3 (10.9)</td>
<td>29.0</td>
<td>24.0 (3.2)</td>
<td>126.8 (21.2)</td>
<td>72.3 (9.6)</td>
</tr>
<tr>
<td>NHAPC</td>
<td>2004</td>
<td>2/6</td>
<td>58.2 (5.9)</td>
<td>24.5 (3.6)</td>
<td>139.6 (22.6)</td>
<td>79.9 (10.8)</td>
<td>26.4</td>
<td>24.6 (3.5)</td>
<td>137.8 (18.8)</td>
<td>80.3 (9.5)</td>
</tr>
<tr>
<td>SIMES</td>
<td>1494</td>
<td>2/6</td>
<td>57.2 (10.1)</td>
<td>26.6 (4.8)</td>
<td>148.0 (24.0)</td>
<td>81.2 (11.3)</td>
<td>29.4</td>
<td>26.7 (4.8)</td>
<td>146.9 (19.9)</td>
<td>80.3 (9.4)</td>
</tr>
<tr>
<td>SP2</td>
<td>2177</td>
<td>2/10</td>
<td>38.2 (11.1)</td>
<td>22.5 (3.5)</td>
<td>118.8 (16.8)</td>
<td>71.8 (11.8)</td>
<td>5.2</td>
<td>22.9 (3.7)</td>
<td>130.6 (21.2)</td>
<td>77.6 (11.4)</td>
</tr>
<tr>
<td>TWT2D2</td>
<td>1599</td>
<td>2/2</td>
<td>61.6 (11.8)</td>
<td>33.2 (9.0)</td>
<td>134.6 (16.7)</td>
<td>80.8 (10.5)</td>
<td>30.8</td>
<td>33.2 (9.0)</td>
<td>135.1 (16.9)</td>
<td>78.7 (11.2)</td>
</tr>
<tr>
<td>TUDR</td>
<td>1004</td>
<td>4/4</td>
<td>64.3 (11.9)</td>
<td>24.8 (4.3)</td>
<td>134.6 (17.7)</td>
<td>75.8 (10.9)</td>
<td>60.3</td>
<td>136.6 (20.7)</td>
<td>76.8 (12.1)</td>
<td>81.7 (9.3)</td>
</tr>
</tbody>
</table>

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; and TWT2DS, Taiwan Type II Diabetes Study.
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 because of differences in age and other covariables.37 In this case, we may have missed some genetic loci whose effects can be modified by such factors. Finally, because 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 transethnic replication study, we are the first to report an

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>Position (Build 36)</th>
<th>CA</th>
<th>CAF</th>
<th>Nearest Gene</th>
<th>Study</th>
<th>β, mm Hg</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4919669</td>
<td>10</td>
<td>104461965</td>
<td>A</td>
<td>0.43</td>
<td>ARL3</td>
<td>AGEN</td>
<td>−0.96</td>
<td>0.18</td>
<td>5.03E-08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>−0.41</td>
<td>0.15</td>
<td>8.64E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>−0.65</td>
<td>0.12</td>
<td>2.63E-08</td>
</tr>
<tr>
<td>rs284844</td>
<td>10</td>
<td>104544519</td>
<td>A</td>
<td>0.49</td>
<td>WBP1L</td>
<td>AGEN</td>
<td>−0.99</td>
<td>0.17</td>
<td>4.61E-09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>−0.57</td>
<td>0.15</td>
<td>8.75E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>−0.75</td>
<td>0.11</td>
<td>1.05E-11</td>
</tr>
<tr>
<td>rs11191580</td>
<td>10</td>
<td>104896201</td>
<td>T</td>
<td>0.74</td>
<td>NT5C2</td>
<td>AGEN</td>
<td>1.18</td>
<td>0.20</td>
<td>1.83E-09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>0.83</td>
<td>0.16</td>
<td>1.78E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>0.97</td>
<td>0.12</td>
<td>4.44E-15</td>
</tr>
<tr>
<td>rs12579302</td>
<td>12</td>
<td>88574634</td>
<td>A</td>
<td>0.65</td>
<td>ATP2B1</td>
<td>AGEN</td>
<td>0.96</td>
<td>0.19</td>
<td>2.50E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>0.93</td>
<td>0.12</td>
<td>5.08E-15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>0.94</td>
<td>0.10</td>
<td>&lt;1E-17</td>
</tr>
</tbody>
</table>

AGEN indicates Asian Genetic Epidemiology Network; CA, coded allele; CAF, coded allele frequency; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; LTA, long-term average; and 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Start Position (Build 36)</th>
<th>Length, bp</th>
<th>SNPs</th>
<th>Study</th>
<th>SBP</th>
<th>DBP</th>
<th>MAP</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMEM180</td>
<td>10</td>
<td>104211159</td>
<td>15634</td>
<td>26</td>
<td>AGEN</td>
<td>8.5E-06</td>
<td>1.93E-06</td>
<td>2.98E-07</td>
<td>4.79E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>1.22E-03</td>
<td>9.62E-03</td>
<td>3.48E-03</td>
<td>9.06E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>2.02E-07</td>
<td>3.49E-07</td>
<td>2.25E-08</td>
<td>2.79E-02</td>
</tr>
<tr>
<td>ACTR1A</td>
<td>10</td>
<td>104228975</td>
<td>23528</td>
<td>28</td>
<td>AGEN</td>
<td>5.76E-06</td>
<td>1.91E-06</td>
<td>2.58E-07</td>
<td>3.81E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>1.34E-03</td>
<td>8.94E-03</td>
<td>3.61E-03</td>
<td>2.11E-01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>1.52E-07</td>
<td>3.22E-07</td>
<td>2.03E-08</td>
<td>4.68E-02</td>
</tr>
<tr>
<td>SUFU</td>
<td>10</td>
<td>104253708</td>
<td>115503</td>
<td>87</td>
<td>AGEN</td>
<td>3.04E-06</td>
<td>9.00E-07</td>
<td>9.87E-08</td>
<td>2.03E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>2.99E-04</td>
<td>3.10E-03</td>
<td>7.24E-04</td>
<td>1.25E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>1.98E-08</td>
<td>5.77E-08</td>
<td>1.74E-09</td>
<td>2.35E-03</td>
</tr>
<tr>
<td>ARL3</td>
<td>10</td>
<td>104423473</td>
<td>40708</td>
<td>14</td>
<td>AGEN</td>
<td>3.06E-07</td>
<td>8.99E-07</td>
<td>2.19E-08</td>
<td>1.02E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>1.65E-02</td>
<td>3.53E-02</td>
<td>2.43E-02</td>
<td>8.51E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>1.02E-07</td>
<td>5.80E-07</td>
<td>1.19E-08</td>
<td>6.99E-03</td>
</tr>
<tr>
<td>SFXN2</td>
<td>10</td>
<td>104464287</td>
<td>24650</td>
<td>23</td>
<td>AGEN</td>
<td>4.40E-07</td>
<td>1.29E-06</td>
<td>3.14E-08</td>
<td>8.40E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>1.86E-03</td>
<td>2.05E-03</td>
<td>1.64E-03</td>
<td>6.76E-02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>1.79E-08</td>
<td>5.49E-08</td>
<td>1.27E-09</td>
<td>4.81E-03</td>
</tr>
<tr>
<td>WPB1L</td>
<td>10</td>
<td>104525877</td>
<td>40135</td>
<td>23</td>
<td>AGEN</td>
<td>7.16E-08</td>
<td>1.11E-06</td>
<td>1.79E-08</td>
<td>1.16E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>5.31E-04</td>
<td>5.25E-05</td>
<td>5.05E-05</td>
<td>3.95E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>9.50E-10</td>
<td>1.43E-09</td>
<td>2.60E-11</td>
<td>6.09E-05</td>
</tr>
<tr>
<td>CYP17A1</td>
<td>10</td>
<td>104580277</td>
<td>7004</td>
<td>13</td>
<td>AGEN</td>
<td>3.71E-06</td>
<td>1.11E-04</td>
<td>4.42E-06</td>
<td>1.00E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>2.59E-05</td>
<td>1.89E-05</td>
<td>2.40E-05</td>
<td>6.46E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>2.31E-09</td>
<td>4.40E-08</td>
<td>2.54E-09</td>
<td>9.85E-06</td>
</tr>
<tr>
<td>C10orf32</td>
<td>10</td>
<td>104603956</td>
<td>10753</td>
<td>14</td>
<td>AGEN</td>
<td>2.26E-08</td>
<td>2.96E-06</td>
<td>4.93E-08</td>
<td>5.33E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>2.85E-05</td>
<td>1.84E-06</td>
<td>3.43E-06</td>
<td>8.09E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>1.87E-11</td>
<td>1.47E-10</td>
<td>5.17E-12</td>
<td>6.75E-06</td>
</tr>
<tr>
<td>C10orf32-ASMT</td>
<td>10</td>
<td>104603956</td>
<td>47690</td>
<td>37</td>
<td>AGEN</td>
<td>3.98E-08</td>
<td>5.22E-06</td>
<td>8.67E-08</td>
<td>3.32E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>2.72E-06</td>
<td>3.27E-06</td>
<td>6.14E-06</td>
<td>5.18E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>3.34E-12</td>
<td>4.40E-10</td>
<td>1.56E-11</td>
<td>3.25E-07</td>
</tr>
<tr>
<td>AS3MT</td>
<td>10</td>
<td>104619199</td>
<td>32447</td>
<td>37</td>
<td>AGEN</td>
<td>1.01E-07</td>
<td>4.90E-05</td>
<td>6.77E-07</td>
<td>5.33E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>2.14E-06</td>
<td>3.39E-06</td>
<td>7.43E-06</td>
<td>4.08E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>3.32E-12</td>
<td>3.91E-09</td>
<td>1.36E-10</td>
<td>1.10E-07</td>
</tr>
<tr>
<td>NTSC2</td>
<td>10</td>
<td>104837763</td>
<td>105291</td>
<td>77</td>
<td>AGEN</td>
<td>2.05E-08</td>
<td>1.08E-05</td>
<td>1.21E-07</td>
<td>1.94E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>1.38E-06</td>
<td>1.66E-05</td>
<td>4.84E-05</td>
<td>2.83E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>9.11E-13</td>
<td>4.20E-09</td>
<td>1.57E-10</td>
<td>1.10E-07</td>
</tr>
<tr>
<td>NCR3LG1</td>
<td>11</td>
<td>17329884</td>
<td>25561</td>
<td>16</td>
<td>AGEN</td>
<td>5.27E-05</td>
<td>6.60E-03</td>
<td>1.93E-03</td>
<td>8.14E-06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>7.61E-04</td>
<td>2.18E-01</td>
<td>1.67E-02</td>
<td>8.26E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>7.23E-07</td>
<td>1.09E-02</td>
<td>3.66E-04</td>
<td>1.33E-07</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>11</td>
<td>17363371</td>
<td>3412</td>
<td>10</td>
<td>AGEN</td>
<td>8.55E-06</td>
<td>3.32E-03</td>
<td>5.65E-04</td>
<td>9.19E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>1.62E-05</td>
<td>2.70E-02</td>
<td>3.75E-04</td>
<td>9.69E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>3.28E-09</td>
<td>9.25E-04</td>
<td>3.47E-06</td>
<td>2.15E-09</td>
</tr>
<tr>
<td>ATP2B1</td>
<td>12</td>
<td>88505956</td>
<td>68020</td>
<td>19</td>
<td>AGEN</td>
<td>2.03E-06</td>
<td>8.94E-07</td>
<td>5.92E-07</td>
<td>9.31E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE</td>
<td>8.40E-15</td>
<td>2.91E-13</td>
<td>5.19E-17</td>
<td>4.96E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta</td>
<td>&lt;1.0E-17 &lt;1.0E-17 &lt;1.0E-17 &lt;1.0E-17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bolded values are significant gene-based associations with discovery stage \(P<1.0\times10^{-4}\), replication stage \(P<0.05\), and joint meta-analysis \(P<5.0\times10^{-6}\). AGEN indicates Asian Genetic Epidemiology Network; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic blood pressure.
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.

Acknowledgments

Detailed acknowledgment information for both discovery stage and replication stage studies is presented in the Data Supplement.

From the Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA (C.L., J.H., J.Z., T.N.K.); Center for Genome Science, Korea National Institute of Health, Osong Health Technology Administration Complex, Chungcheongbuk-do, Korea (Y.K.K., J.L., S.H., B.-G.H., S.M., B.-J.K.); Genome Institute of Singapore, Agency for Science, Technology and Research (R.D., J.L., Y.-Y.T.); Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate University of the Chinese Academy of Sciences (H.L., Y.H., Y.W., L.S., L.X.); National Yang-Ming University Hospital, Taipei, Taiwan (I-T.L.); National Yang-Ming University Hospital, Taipei, Taiwan; Singapore Eye Research Institute, Singapore National Eye Center (Y.C., Y.K., Y.Z., N.K., Y.W.); Duke-NUS Graduate Medical School, National University of Singapore, (Y.C., N.K., T.Y.W., E-S.T.); Department of Ophthalmology (Y.C., T.Y.W.), Department of Statistics and Applied Probability (Y.-Y.T.), Life Sciences Institute (Y.-Y.T.), NUS Graduate School for Integrative Science and Engineering (Y.-Y.T.), Department of Medicine, National University Health System, Singapore (E-S.T.); MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubet, China (M.H., H.H., L.G., Y.H., T.W.); Division of Endocrine and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, Taipei, Taiwan (I-T.L., W.H.-h.S); School of Medicine, National Yang-Ming University, Taipei, Taiwan (W.H.-h.S., K.-W.L.); Laboratory of Translational Genomics and Population Sciences, Department of Business Administration, National Chung Cheng University, Chia-yi, Taiwan (C.H.L.); Department of Nursing, DaYeh University, Changhua, Taiwan (C.H.L.); Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan (W.-J.L.); State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center of Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (D.G.); National Center for Genome Medicine, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan (C.-H.C., J.-Y.W.); Department of Ophthalmology and Visual Science, Khoi Teck Puat Hospital, Singapore, Singapore (N.K.); Medical Genetics Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA (L.J.R.); and Department of Epidemiology and Biostatistics, University of Georgia at Athens, Athens (C.L.); Department of Cardiovascular Center, Taichung Veterans General Hospital, Taichung, Taiwan (K.-W.L.).

Disclosures

None.

References

10. Rossignol P, Criqld J, Lehebt P, Kessler M, Zannad F. Visit-to-visit blood pressure variability is a strong predictor of cardiovascular events in...

Table 4. Transethnic Replication of Associations at 3 Novel Loci Previously Reported Among European Participants of the CHARGE Consortium

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP</th>
<th>Nearest Gene</th>
<th>Locus</th>
<th>CHARGE</th>
<th>AGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs7599598</td>
<td>FERL1</td>
<td>2q11</td>
<td>A</td>
<td>−0.31 0.05 2.91E-08</td>
</tr>
<tr>
<td>SBP</td>
<td>rs1275988</td>
<td>KCNK3</td>
<td>2p23</td>
<td>T</td>
<td>−0.60 0.09 2.61E-10</td>
</tr>
<tr>
<td>MAP</td>
<td>rs1275988</td>
<td>KCNK3</td>
<td>2p23</td>
<td>T</td>
<td>−0.60 −0.39 0.06 1.51E-09</td>
</tr>
<tr>
<td>PP</td>
<td>rs10948071</td>
<td>CRIP3</td>
<td>6p21</td>
<td>T</td>
<td>0.71 −0.38 0.07 9.06E-09</td>
</tr>
</tbody>
</table>

AGEN indicates Asian Genetic Epidemiology Network; CA, coded allele; CAF, coded allele frequency; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; and SNP, single-nucleotide polymorphism.

*CAF based on data from the HapMap project, version 2010-08_phaseIII.


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

Changwei Li, Yun Kyoung Kim, Rajkumar Dorajoo, Huaxing Li, I-Te Lee, Ching-Yu Cheng, Mei-an He, Wayne H-h Sheu, Xi-qing Guo, Santhi K. Ganesh, Jiang He, Juyoung Lee, Jianjun Liu, Yao Hu, Day-er C. Rao, Fuu-Jen Tsai, Jia Yu Koh, Hua Hu, Kae-Woei Liang, Walter Palmas, James E. Hixson, Sohee Han, Ying-Ying Teo, Yi-qin Wang, Jing Chen, Chieh Hsiang Lu, Ying-feng Zheng, Li-xuan Gui, Wen-Jane Lee, Jie Yao, Dong-feng Gu, Bo-k-Ghee Han, Xueling Sim, Liang Sun, Jinying Zhao, Chien-Hsiun Chen, Neelam Kumari, Yun-feng He, Kent D. Taylor, Leslie J. Raffel, Sang-hoon Moon, Jerome I. Rotter, Yi-der Id-a Chen, Tang-chun Wu, Tien Yin Wong, Jer-Yuan Wu, Xu Lin, E-Shyong Tai, Bong-Jo Kim and Tanika N. Kelly

Circ Cardiovasc Genet. 2017;10:e001527
doi: 10.1161/CIRCGENETICS.116.001527

Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/10/2/e001527

Data Supplement (unedited) at:
http://circgenetics.ahajournals.org/content/suppl/2017/03/27/CIRCGENETICS.116.001527.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/
Supplementary Materials

Description of AGEN studies:

**DFTJ-cohort study** includes 27,009 retired employees from a state-owned automobile enterprise in China. This study was launched in 2008 and the cohort is followed up every 5 years. In 2013, the first follow-up was conducted. By using semi-structural questionnaire and a health examination, those having cancer or severe diseases were excluded from the study. Fasting blood samples and detailed epidemiology data were collected. The main goal of the cohort study was to identify the environmental and genetic risk factors and the gene-environment interactions on chronic diseases, and to find novel biomarkers for chronic disease and mortality prediction. A total of 1,461 participants had GWAS data available for the present study. Among them, there were 1,155 subjects with BP data at baseline and the first follow up. All of the participants wrote informed consent and the ethical committees in the Tongji Medical College approved this research project. Detailed information about the study cohort has been described elsewhere [1].

**GenSalt** participants were assessed at a baseline examination in 2003 and at two follow-up visits in 2008-2009 and 2011-2012, respectively. During the baseline and each follow-up visit, a 3-day clinical examination was performed using the same standardized protocol. Of the 1,881 individuals with baseline genotype and phenotype data, 187 and 248 were lost to follow-up in visit 1 and visit 2, respectively. A total of 1,767 (89.5%) participants with BP data at more than 1 visit were included in the current analysis. During the baseline and follow-up visits, 3 morning BP measurements were obtained during each day of the 3-day observation. All BP readings were measured by trained and
certified observers using a random–zero sphygmomanometer according to a standard protocol. BP was measured with the participant in the sitting position after 5 minutes of rest. In addition, participants were advised to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 minutes prior to their BP measurements. Nine systolic and diastolic BP measures taken during each of the three 3-day visits were used in this analysis.

**KARE** was launched in 2007 to carry out a large-scale GWA analysis for Type 2 Diabetes and many other complex quantitative traits among 10,038 participants aged 40-69 of the Ansung (n=5,018) and Ansan (n=5020) population-based cohorts [2]. The two KARE cohorts were established as part of the Korean Genome Epidemiology Study (KoGES) in 2001. Both cohorts were sampled from KyungGi-Do province, close to Seoul, the capital of the Republic of Korea and adopted the same investigational strategy. The KARE participants were followed-up every 2 years [3]. The third follow-up study was conducted in 2008. In each follow-up visit, three BP measurements were obtained from each study participants using a random zero sphygmomanometer and used to calculate MAP and PP. Genotypes and LTA BP measures are available for a total of 6,447 participants, and were included in the current study.

**MESA study** is a study of the characteristics of subclinical cardiovascular diseases and their risk factors [4]. MESA recruited 6,814 men and women aged 45-85 including 38% Whites, 28% African-Americans, 22% Hispanics, and 12% Asians from 6 field centers across the United States [4]. MESA participants were free of cardiovascular disease at baseline. The baseline information was collected in 2000-2002. Since then, four follow-up exams have been conducted. Genotype data, LTA BP measures, and
covariate data were covariate for 775 Chinese participants, and were included in the current study.

**NHAPC** is a population-based cohort study of 3,210 unrelated Chinese Hans, aged 50 to 70 years, recruited from Beijing and Shanghai [5]. The participants were recruited using a multistage sampling method from 2 urban districts and 1 rural district of each city. Data on demographic variables, health status, health behavior, and physical activity was collected using a standardized questionnaire, and standard anthropometric measurements and overnight fasting blood samples were collected using a standardized protocol when the participants attended a physical examination. Blood pressures were measured three times after 5 minutes quiet rest, and the average of the last two measurements was used to calculate SBP, DBP, MAP and PP in the current analyses. Genotypic data and LTA BP measures calculated from 2 follow-up visits are available for 2,004 participants who were included in the current study.

**SiMES** is a prospective population based survey of 3,280 Malay adults. The SiMES participants were selected from Malay adults aged 40-80 years residing in 15 residential districts in the southwestern part of Singapore through age-stratified random sampling method. Of the 4,186 eligible participants invited, 3,280 participated in the study, yielding a response rate of 78.7%. Details of the study participants and methods at baseline in 2004-2006 and at 6-year follow-up visit were published previously [6, 7]. During baseline and 6-year follow-up visit, BP was taken by trained observers with the participants seated after 5 minutes of rest using a digital automatic BP monitor (Dinamap model Pro Series DP110X-RW, 100V2; GE Medical Systems Information Technologies,
SBP and DBP were measured twice, 5 minutes apart. One of two cuff sizes was chosen based on the participant’s arm circumference. If the BP measures differed by more than 10 mmHg systolic and 5 mmHg diastolic, a third measurement was taken. The mean of the two closest readings were used to estimate SBP and DBP for the calculation of MAP and PP. Genotypic data and LTA BP measures are available for 1,494 participants who were included in the current study.

**SP2** is a population-based, cross-sectional study of Singaporean Chinese, Malay and Asian Indian subjects who took part in one of four previous studies, including: Thyroid and Heart Study 1982-1984 [8], National Health Survey 1992 [9], National University of Singapore Heart Study 1993-1995 [10] or National Health Survey 1998 [11]. Disproportionate ethnicity-stratified sampling of Singapore residents aged 18 to 95 years was employed to increase the number of minority ethnic groups (Malay and Asian Indians). A total of 10,747 participants were invited to participate in the follow-up examination in 2003-2007 by linking their unique national identification numbers with national registries, of whom 5,499 Chinese, 1,405 Malays and 1,138 Asian Indian participants provided informed consent and attended both interview and clinical examination components of the study. Only Singaporean Chinese samples were genotyped in the current study and 2,177 Singaporean Chinese participants with LTA BP measures and genotype information were available for analysis. At both baseline and the follow-up visit, two BP readings were taken by trained observers after 5 min of seated rest using an automated BP monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany). SBP and DBP were measured twice, 5 minutes apart. One of two cuff size (regular, large) was chosen based on the participant’s arm circumference. If the BP
measures differed by more than 10 mmHg systolic and 5 mmHg diastolic, a third measurement was taken. The mean of the two closest readings were used to estimate SBP and DBP for the calculation of MAP and PP.

**TUDR study** is a cohort that enrolled subjects with T2DM receiving care at Taichung Veterans General Hospital, Taichung, Taiwan, and a small number of subjects were included from Tri-Service General Hospital, Taipei, Taiwan. All TUDR subjects underwent a complete fundoscopic examination to carefully document the presence and extent of retinopathy. Genome wide genotyping using Illumina OminiExpress 730k array was completed in the subjects with proliferative diabetic retinopathy (PDR) or non-proliferative diabetic retinopathy (NPDR), and the controls (longstanding T2DM for more than 8 years but no diabetic retinopathy). Blood pressure was measured once by using the standard mercury sphygmomanometer after the patients had been sitting at rest for 10 minutes. The measurement was performed and recorded by the licensed nurse in the clinic or lab. After recruitment, 4 annual follow-up examinations have been performed for each subject in the study site hospitals. For the current study, genotype and long-term average blood pressure phenotypes were available for a total of 1,004 subjects, and were included in the analysis.

**TWT2DS** is a joint effort between Academia Sinica and collaborative hospitals, and aims to identify genetic components associated with T2DM, diabetic subphenotypes and medication efficacy, which is significant for the population of non-aboriginal Taiwanese. China Medical University Hospital, Chiayi Christian Hospital, Taichung Veterans General Hospital, Mackay Memorial Hospital, Tungs’ Taichung Metro Harbor
Hospital, and Changhua Christian Hospital are the study sites and responsible for subject screening and recruitment, bio-specimen collection and packaging, as well as phenotypic information collection. Six thousands non-aboriginal Taiwanese subjects with T2DM aged ≥20 years old and signed inform consents were recruited from the designated hospitals. Blood and urine samples, questionnaires and other phenotypic information were collected. Blood chemistry profiles were assessed by an Academia Sinica designated clinical laboratory to provide high quality clinical data. DNA was used for allele frequency determination for T2DM association study, comparing patients with a matched control group selected from The Cell Bank and Genetic Database, and from Taiwan Biobank on Non-Aboriginal Taiwanese, either at the pooled level or at the individual level. The study follows participants annually for 9 years. The study was approved by the institutional review board and the ethics committee of each institution. Written informed consent was obtained from each participant in accordance with institutional requirements and the Declaration of Helsinki Principles. After recruitment, 4 annual routine examinations have been performed for each subject in the study site hospitals. For the current study, genotype and long term average blood pressure phenotypes were available for a total of 1,599 subjects, and were included in the analysis.
Description of CHARGE studies:

The CHARGE Consortium [12] includes cohort studies that completed genome-wide genotyping and had extensive data on multiple phenotypes including blood pressure. Each study adopted collaboration guidelines and established a consensus on phenotype harmonization, covariate selection and an analytical plan for within-study genome-wide association and prospective meta-analysis of results across studies. Each study received institutional review board approval of its consent procedures, examination and surveillance components, data security measures, and DNA collection and its use for genetic research. All participants provided written informed consent. In the current analysis, most of the participating cohorts were general population samples (AGES, ARIC, CHS, FHS, RS, MESA, and CARDIA). Demographic information, blood pressure, height, and weight were directly measured in all participants, except for the Women’s Genome Health Study as described. All studies with GWAS data used hidden Markov model approaches [13-15] and HapMap reference panels [16] to impute genotypes at unmeasured SNPs and excluded SNPs, so that a common set of ~2.5M HapMap SNPs were available across the discovery samples [15, 17].

AGES Reykjavik

The Age Gene/Environment Susceptibility-Reykjavik (AGES- Reykjavik) Study cohort originally comprised a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967. A total of 19,381 people attended, resulting in 71% recruitment rate. The study sample was divided into six groups by birth year and birth
examined in all stages. One group was designated for longitudinal follow up and was
excluded in examinations until 1991. Other groups were invited to participate in specific stages of
the study. Between 2002 and 2006, the GESReykjavik study re-examined 5764 survivors
of the original cohort who had participated before in the Reykjavik Study [18]. The
midlife data blood pressure measurement was taken from stage 3 of the Reykjavik Study
(1974-1979), if available. Half of the cohort attended during this period. Otherwise an
observation was selected closest in time to the stage 3 visit. Participants came in a fasting
state to the clinic. The supine blood pressure was measured twice by a nurse using a
mercury sphygmomanometer after a 5-min rest. Blood pressure was measured according
to World Health Organization recommendations. Individuals with previous MI were
excluded from the analyses (n=12). Successful genotyping was available for 3219 AGES
participants who were eligible for this study. The AGES Reykjavik Study GWAS was
approved by the National Bioethics Committee and the Data Protection Authority.

**ARIC**

The Atherosclerosis Risk in Communities Study (ARIC) is a population-based
prospective cohort study of cardiovascular disease sponsored by National Heart, Lung,
and Blood Institute (NHLBI). ARIC included 15,792 individuals aged 45-64 years at
baseline (1987-89), chosen by probability sampling from four US communities [19].
Cohort members completed four clinic examinations, conducted three years apart
between 1987 and 1998. The data used in this study are from all four visits. A detailed
study protocol is available on the ARIC study website (https://www2.cscc.unc.edu/aric/).
Clinic examinations included assessment of cardiovascular disease risk factors, a detailed
medical and psychosocial history, and measurement of various clinical and laboratory variables. The physical examination included measurements of weight and height from which the body mass index (BMI) was calculated. Blood pressure was measured using a standardized Hawksley random-zero mercury column sphygmomanometer with participants in sitting position after a resting period of 5 minutes. The size of the cuff was chosen according to the arm circumference. For the first three visits, three sequential recordings for systolic and diastolic blood pressure were obtained and the mean of the last two measurements used in this analysis. At the fourth visit, two blood pressure measurements were taken and averaged. Blood pressure lowering medication use was recorded from the medication history. Outliers (>4SD from the mean) with respect to the systolic or diastolic blood pressure distribution were excluded from the analysis. For this investigation we limited the sample to individuals of European descent by self-report and in whom GWAS was carried out.

CARDIA

The Coronary Artery Risk Development in Young Adults (CARDIA) is a prospective multicenter study with 5115 adults Caucasian and African American participants of the age group 18-30 years, recruited from four centers at the baseline examination in 1985-1986. The recruitment was done from the total community in Birmingham, AL, from selected census tracts in Chicago, IL and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. The details of the study design for the CARDIA study have been previously published [20]. Eight examinations have been completed since initiation of the study, respectively in the years 0, 2, 5, 7, 10, 15, 20 and 25. Written informed consent was obtained from participants at
each examination and all study protocols were approved by the institutional review
boards of the participating institutions. At each examination, systolic and diastolic blood
pressure was measured in triplicate on the right arm using a random-zero
sphygmomanometer with the participant seated and following a 5-min. rest. The average
of the second and third measurements was taken as the blood pressure value. Blood
pressure medication use was obtained by questionnaire. Blood pressure data measured at
year 7 through year 20 were used in this study. In addition, the sample was restricted to
individuals of European descent by self-report and principal component analysis using
genome-wide genotypes.

**CHS**

The CHS is a population-based cohort study of risk factors for cardiovascular
disease in adults 65 years of age or older conducted across four field centers. The original
predominantly white cohort of 5201 persons was recruited in 1989-1990 from random
samples of the Medicare eligibility lists and an additional 687 African-Americans were
enrolled in 1992-93 for a total sample of 5888. Details of the study design are
summarized elsewhere [21]. A total of 1908 persons were excluded from the study
sample due to prevalent coronary heart disease (n=1195), congestive heart failure (n=86),
peripheral vascular disease (n=93), valvular heart disease (n=20), stroke (n=166) or
transient ischemic attack (n=56). Participants with missing BMI (n=10) or BP
measurements (n=8) were excluded. CHS participants completed standardized clinical
examinations and questionnaires at study baseline and at nine annual follow-up visits.
Research staff who received central training in blood pressure measurement assessed
repeat right-arm seated systolic and diastolic blood pressure levels at baseline with a 92
Hawksley random-zero sphygmomanometer. Means of the repeated blood pressure measurements from the baseline examination were used for GWAS analyses. Because the other cohorts were predominantly white, African American participants were excluded from this analysis. 3,159 CHS subjects contributed to this analysis.

**FHS**

The Framingham Heart Study (FHS) began in 1948 with the recruitment of an original cohort of 5,209 men and women who were 28 to 62 years of age (mean age 44 years; 55 percent women) at entry. In 1971 enrollment of a second generation of study participants took place; this cohort consisted of 5,124 children and spouses of children of the original cohort. The mean age of the offspring cohort was 37 years; 52 percent were women. A third generation cohort began in 1948 with the recruitment of an original cohort of 5,209 men and women who were 28 to 62 years of age (mean age 44 years; 55 percent women) at entry. In 1971 enrollment of a second generation of study participants took place; this cohort consisted of 5,124 children and spouses of children of the original cohort. The mean age of the offspring cohort was 37 years; 52 percent were women [22-24]. At each clinic visit, a medical history was obtained with a focus on cardiovascular content, and participants underwent a physical examination including measurement of height and weight from which BMI was calculated. Systolic and diastolic blood pressures were measured twice by a physician on the left arm of the resting and seated participant using a mercury column sphygmomanometer. Pressures were recorded to the nearest even number. The means of two separate systolic and diastolic blood pressure readings at the first clinic examination of each cohort were used for GWAS analyses. For a subset of offspring cohort participants only one measurement
was obtained. Individuals under 20 years of age, those who had a myocardial infarction or congestive heart failure were excluded from the analyses because those conditions may affect blood pressure levels.

**MESA**

The Multi-Ethnic Study of Atherosclerosis investigation is a population-based study of 6,814 men and women age 45 to 85 years, without clinical cardiovascular disease, recruited from six United States communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and St. Paul, MN). The main objective of MESA is to determine the characteristics of subclinical cardiovascular disease and its progression. Sampling and recruitment procedures have been previously described in detail [25]. Adults with symptoms or history of medical or surgical treatment for cardiovascular disease were excluded. During the recruitment process, potential participants were asked about their race/ethnicity. Self-reported ethnicity was used to classify participants into groups [4]. After a 5-minute rest BP was measured three times at 1 minute intervals using a Dinamap PRO 100 automated oscillometric device (Critikon, Tampa, FL) with the subject in seated, and the average of the second and third BP measurements was recorded for each visit. Data from white participants, collected at MESA exams 1 through 4, was used in this analysis.

**Rotterdam Study - RS1, RS2**

The RS is a prospective population-based cohort study comprising 7,983 subjects aged 55 years or older. Participants completed an interview at home and at the research center, where participants were subsequently examined. Baseline data were collected between 1990 and 1993. In 1999, inhabitants who turned 55 years of age or moved into
the study district since the start of the study were invited to participate in an extension of the RS (RES) of whom 3011 participated (67% response rate). The rationale and design of the RS have been described in detail elsewhere [26]. At the research center, we obtained two seated blood pressure measurements in the right brachial artery with a random zero sphygmomanometer. The mean of two consecutive measurements was used in association analyses. We excluded participants who were older than 85 years of age and those who had a history myocardial infarction or congestive heart failure, because of the impact of these conditions on blood pressure levels.

WGHS

The Women’s Genome Health Study (WGHS) [27] is a prospective cohort of female North American health care professionals representing participants in the Women’s Health Study (WHS) trial who provided a blood sample at baseline and consent for blood-based analyses. Participants in the WHS were 45 years or older at enrolment and free of cardiovascular disease, cancer or other major chronic illness. For the primary WHS endpoints of cardiovascular disease, full medical records were obtained for reported endpoints and reviewed by an endpoints committee of physicians unaware of assignment. The current data are derived from 23,294 WGHS participants for whom whole genome genotype information was available at the time of analysis and for whom self-reported European ancestry could be confirmed by multidimensional scaling analysis of 1,443 ancestry informative markers in PLINK v. 1.06. Baseline BP in the WGHS was ascertained by a self-reported questionnaire, an approach which has been validated in the WGHS demographic, namely female health care professionals. Questionnaires recorded systolic blood pressure in 9 categories (<110, 110-119, 120-129, 130-139, 140-149, 150-
159, 160-169, 170-179, ≥180 mmHg), and diastolic blood pressure in 7 categories (<65, 65-74, 75-84, 85-89, 90-94, 95-104, ≥105 mmHg). The midpoint of each category was used for analysis. Hypertension was defined as a history of physician-diagnosed HTN and ongoing HTN treatment, or SBP ≥ 140 or DBP ≥ 90 mmHg. To account for treatment effects, 10 and 5 mmHg were added to the measured systolic and diastolic blood pressures respectively, if a participant was taking antihypertensive medication.
Acknowledgment:

**Discovery Stage Studies from AGEN Consortium**

**DFTJ** cohort was supported by grants from the National Basic Research Program grant (2011CB503800) and the Programme of Introducing Talents of Discipline to Universities to T. Wu and the Program for New Century Excellent Talents in University and the General Program of the National Natural Science Foundation of China (81473051) to M. He.

**GenSalt:** The Genetic Epidemiology Network of Salt Sensitivity (GenSalt) is supported by a cooperative agreement project grant (U01HL072507, R01HL087263, and R01HL090682) from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

**KARE:** The KARE study was supported by an intramural grant from the Korea National Institute of Health (2012-N73002-00) and a grant from the Korea Centers for Disease Control and Prevention (4845-301).

**MESA:** This research was supported by the Multi-Ethnic Study of Atherosclerosis (MESA) contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169 and by grants UL1-TR-000040 and UL1-TR-001079 from NCRR. Funding for MESA SHARe genotyping was provided by NHLBI Contract N02-HL-6-4278. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research
Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

**NHPAC**: This study is supported by the National High Technology Research and Development Program (863 Program 2009AA022704), the National Basic Research Program of China (973 Program 2012CB524900), the National Natural Science Foundation of China (30930081, 81170734 and 81021002), and the Chinese Academy of Sciences (KSCX2-EW-R-10 and SIBS2008006) and the natural National Natural Science Foundation of China (81170734 and 81471013). We are grateful to all participants of the Nutrition and Health of Aging Population in China. We also thank our colleagues at the laboratory and the local Centers for Disease Control staffs of Beijing and Shanghai for their assistance with data collection.

**SiMES** is funded by National Medical Research Council (grants 0796/2003, IRG07nov013, IRG09nov014, STaR/0003/2008 and CG/SERI/2010) and Biomedical Research Council (grants 09/1/35/19/616), Singapore. The Singapore Tissue Network and the Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore provided services for tissue archival and genotyping, respectively. Ching-Yu Cheng is supported by the National Medical Research Council, Singapore (CSA/033/2012).

**SP2**: The Singapore Prospective Study Program (SP2) was funded through the individual research grant scheme from the BMRC, and the individual research grant and the clinician scientist award schemes from the NMRC.
**TUDR:** This study was supported by the National Eye Institute of the National Institutes of Health (EY014684 to J.I.R.) and ARRA Supplement (EY014684-03S1, -04S1), the National Institute of Diabetes and Digestive and Kidney Disease grant DK063491 to the Southern California Diabetes Endocrinology Research Center, the Eye Birth Defects Foundation Inc., the National Science Council, Taiwan (NSC 98-2314-B-075A-002-MY3 to W.H.S.) and the Taichung Veterans General Hospital, Taichung, Taiwan (TCVGH-1003001C to W.H.S.). DNA handling and genotyping were supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124 and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. Dr. Wayne H.H. Sheu is supported by funding from National Science Council of Taiwan (NSC101-2314-075A-006-MY3) and Taichung Veterans General Hospital (TCVGH-1040101C)

**TWT2DS** was supported by the National Science Council, Taiwan (NSC101-2320-B-001-020-MY3) and research grants from Biosignature project, Academia Sinica, Taiwan.

**Replication Studies from CHARGE Consortium**

**AGES:** Age, Gene/Environment Susceptibility (AGES) Reykjavik Study is funded by NIH contract N01-AG-12100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association) and the Althingi (the Icelandic Parliament). The study is approved by the Icelandic National Bioethics Committee, VSN: 00-063.
**ARIC:** The Atherosclerosis Risk in Communities (ARIC) Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. The meta-analysis and meta-regression analyses were funded by grant R01 HL086694 from the National Heart, Lung, and Blood Institute.

**CARDIA:** The Coronary Artery Risk Development in Young Adults Study (CARDIA) is conducted and supported by the National Heart, Lung, and Blood Institute in collaboration with the University of Alabama at Birmingham (HHSN268201300025C & HHSN268201300026C), Northwestern University (HHSN268201300027C), University of Minnesota (HHSN268201300028C), Kaiser Foundation Research Institute (HHSN268201300029C), and Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging. This manuscript has been reviewed by CARDIA for scientific content. Genotyping of the CARDIA participants and statistical data analysis was supported by grants U01-HG-004729 from the National Human Genome Research Institute and R01-HL-084099 from the National Heart, Lung
and Blood Institute to MF. The authors thank the investigators and staff of the GENEVA coordinating center and genotyping center, as well as the staff and participants of the CARDIA study for their important contributions.

**Cardiovascular Health Study:** This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the National Institute on Aging (NIA). A full list of CHS investigators and institutions can be found at http://chs-nhlbi.org/. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

**FHS:** The National Heart, Lung, and Blood Institute’s Framingham Heart Study (FHS) is a joint project of the National Institutes of Health and Boston University School of Medicine and was supported by the National Heart, Lung, and Blood Institute’s Framingham Heart Study (contract No. N01-HC-25195) and its contract with Affymetrix, Inc. for genotyping services (contract No. N02-HL-6-4278). Analyses reflect the efforts and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. A portion of this research was
conducted using the Linux Cluster for Genetic Analysis (LinGAll) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center.

**MESA**: The Multi-Ethnic Study of Atheroclerosis (MESA) and the MESA SHARE project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support is provided by grants and contracts N01 HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169 and RR-024156. Funding for SHARE genotyping was provided by NHLBI Contract N02-HL-6-4278. Genotyping was performed at the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) and at Affymetrix (Santa Clara, California, USA).

**RS I and RS II**: The GWA database of the Rotterdam Study was funded through the Netherlands Organisation of Scientific Research NWO (nr. 175.010.2005.011). The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University, Rotterdam; the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Abbas Dehghan is supported by NWO grant (veni, 916.12.154) and the EUR Fellowship.
WGHS: The WGHS is funded by the Donald W. Reynolds Foundation (Las Vegas, NV), the Fondation LeDucq (Paris, France), the National Heart, Lung and Blood Institute (NHLBI; HL043851) and the National Cancer Institute (NCI; CA047988). Funding for genotyping and collaborative scientific support was provided by Amgen.

We thank Dr. Aravinda Chakravarti for his help in replication analysis.
References


<table>
<thead>
<tr>
<th>Study</th>
<th>Genotype Platform</th>
<th>Individual call rate</th>
<th>SNP call rate</th>
<th>HW-P</th>
<th>MAF</th>
<th>Other filters</th>
<th>SNPs used in Imputation</th>
<th>Imputation software</th>
<th>Imputation reference sample</th>
<th>NCBI Build</th>
<th>Genomic Control Lambda</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESA</td>
<td>Affy 6.0</td>
<td>95%</td>
<td>95%</td>
<td>≥1E-6</td>
<td>0.01</td>
<td>Ethnic outliers; duplicates; gender mismatch</td>
<td>881,666</td>
<td>IMPUTE2</td>
<td>HapMap II CHB+JPT</td>
<td>36</td>
<td>1.047  1.048  1.044  1.04</td>
</tr>
<tr>
<td>DFTJ</td>
<td>Affy 6.0</td>
<td>95%</td>
<td>95%</td>
<td>≥1E-6</td>
<td>0.01</td>
<td>-</td>
<td>820,017</td>
<td>MACH 1.0</td>
<td>HapMap Release 22 CHB+JPT</td>
<td>36</td>
<td>1.017  1.015  1.018  1.006</td>
</tr>
<tr>
<td>GenSalt</td>
<td>Affy 6.0</td>
<td>95%</td>
<td>75%</td>
<td>≥1E-6</td>
<td>0.01</td>
<td>-</td>
<td>352,228</td>
<td>IMPUTE2</td>
<td>CHB+JPT, Phase 2</td>
<td>36 v22</td>
<td>1.019  1.021  1.02  1.027</td>
</tr>
<tr>
<td>KARE</td>
<td>Affy 5.0</td>
<td>98%</td>
<td>95%</td>
<td>≥1E-6</td>
<td>0.01</td>
<td>-</td>
<td>473,679</td>
<td>IMPUTE v.2.2.2</td>
<td>CHB+JPT, Phase 2</td>
<td>36 v22</td>
<td>1.040  1.035  1.044  1.035</td>
</tr>
<tr>
<td>NHAPC</td>
<td>Illumina 660w</td>
<td>97%</td>
<td>95%</td>
<td>≥1E-4</td>
<td>0.01</td>
<td>SNPs mapping to more than 1 locus in genome</td>
<td>557,824</td>
<td>IMPUTE</td>
<td>CEU+ASIAN+YBI</td>
<td>36</td>
<td>1.004  0.998  1  1.013</td>
</tr>
<tr>
<td>SiMES</td>
<td>Illumina Quad 610</td>
<td>95%</td>
<td>95%</td>
<td>≥1E-4</td>
<td>0</td>
<td>&gt;25% IBD sharing, and non-autosomal</td>
<td>574,381</td>
<td>IMPUTE</td>
<td>CHB+JPT, Phase 2</td>
<td>36 v22</td>
<td>0.999  0.988  0.992  1.006</td>
</tr>
<tr>
<td>SP2 (912 samples)</td>
<td>Illumina 1 M duo</td>
<td>95%</td>
<td>95%</td>
<td>≥1E-6</td>
<td>0</td>
<td>Gender discrepant samples, samples with misclassified ethnicity via PCA analysis, and excluded 1 sample from each pair of 1st degree relatives</td>
<td>944,241</td>
<td>IMPUTE v.0.2.1</td>
<td>CHB+JPT, Phase 2</td>
<td>36 v22</td>
<td>1.012  1.028  1.007  1.021</td>
</tr>
<tr>
<td>SP2 (930 samples)</td>
<td>Illumina 610 quad</td>
<td>95%</td>
<td>95%</td>
<td>≥1E-6</td>
<td>0</td>
<td>Gender discrepant samples, samples with misclassified ethnicity via PCA analysis, and excluded 1 sample from each pair of 1st degree relatives</td>
<td>542,298</td>
<td>IMPUTE v.0.2.1</td>
<td>CHB+JPT, Phase 2</td>
<td>36 v22</td>
<td>1.014  1.006  1.009  1.025</td>
</tr>
<tr>
<td>SP2 (335 samples)</td>
<td>Illumina 550</td>
<td>95%</td>
<td>95%</td>
<td>≥1E-6</td>
<td>0</td>
<td>Gender discrepant samples, samples with misclassified ethnicity via PCA analysis, and excluded 1 sample from each pair of 1st degree relatives</td>
<td>504,625</td>
<td>IMPUTE v.0.2.1</td>
<td>CHB+JPT, Phase 2</td>
<td>36 v22</td>
<td>1.006  0.997  1  1.005</td>
</tr>
<tr>
<td>TWT2DS</td>
<td>Illumina 550K</td>
<td>-</td>
<td>95%</td>
<td>≥1E-4</td>
<td>-</td>
<td>Gender discrepant duplicate individuals</td>
<td>613,969</td>
<td>IMPUTE2</td>
<td>ALL_1000G_phase1 Integrated_v3_imp_20180328.macGT1</td>
<td>37</td>
<td>1.008  0.999  1.004  1.016</td>
</tr>
<tr>
<td>TUDR</td>
<td>OmniExpress beadchip</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Gender discrepant duplicate individuals</td>
<td>~700,000</td>
<td>IMPUTE2</td>
<td>CEU+CHB+JPT+YRI</td>
<td>36</td>
<td>1.008  0.999  1.004  1.016</td>
</tr>
</tbody>
</table>

AGEN=Asian Genetic Epidemiology Network; CHB=Chinese Han of Beijing; CEU=Utah residents with ancestry from northern and western Europe; DBP=Diatolic blood pressure; DFTJ=Dong Feng Tongji Cohort Study; GenSalt=Genetic Epidemiology Network of Salt-Sensitivity; HW-P=Hardy-Weinberg p-value; JPT=Japanese in Tokyo; KARE=Korean Association Resource; MAF=Minor allele frequency; MAP=Mean arterial pressure; MESA=Multi-Ethnic Study of Atherosclerosis; NHAPC=Nutrition and Health of Aging Population in China; PP=Pulse pressure; SBP=Systolic blood pressure; SiMES=Singapore Malay Eye Study; SP2=Singapore Prospective Study; SNP=Single nucleotide polymorphism; TUDR=Taiwan and US Diabetic Retinopathy Study; TWT2DS=Taiwan Type II Diabetes Study.
S1 Fig.

Manhattan Plot of Single SNP Analysis Results for LTA SBP

GLIS3

ARL3, WBP1L, NT5C2, LOC729020

GLIS3

ATP2B1

-\log_{10}(P)

Chromosome
Manhattan Plot of Single SNP Analysis Results for LTA DBP

- \(-\log_{10}(P)\) vs. Chromosome

Genes of interest:
- IGF2BP2
- SUFU, WBP1L, C10orf32-AS3MT
- ATP2B1
Manhattan Plot of Single SNP Analysis Results for LTA MAP

The plot shows the -log10(P) values across different chromosomes. Notable SNPs include:

- **ARL3**, **WBP1L**, **C10orf32-AS3MT**, **LOC729020**
- **ATP2B1**
Manhattan Plot of Single SNP Analysis Results for LTA PP

- $-\log_{10}(P)$
- Chromosome
Manhattan Plot of Gene-based Analysis Results for LTA SBP

S5 Fig.

-log_{10}(P)

Chromosome

EFNA1, SLC50A1, DPM3, GBA

RPARP-AS1, PCGF6, USMG5, MIR1307, TAF5, TMEM180, ACTR1A, CYP17A1, SUFU, SFXN2, ARL3, INA, AS3MT, WBP1L, C10orf32-ASMT, CNNM2, C10orf32, NT5C2, RPEL1

ATP2B1

CYP11B2

NCR3LG1, KCNJ11

DNAJA3
S6 Fig.

Manhattan Plot of Gene-based Analysis Results for LTA DBP

- \(-\log_{10}(P)\)

Chromosome

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

\(\text{RPARP-AS1, TMEM180, ACTR1A, SUFU, ARL3, SFXN2, WBP1L, C10orf32, C10orf32-ASMT, AS3MT, CNNM2, NT5C2, RPEL1, INA}\)

\(\text{EFNA1, SLC50A1, CRB1, IGF2BP2, SLC12A2, ATP2B1, MIR4488, LRRC10B, SYT7}\)
Manhattan Plot of Gene-based Analysis Results for LTA MAP

EFNA1, SLC50A1, DPM3, IGF2BP2, TEC, SLC12A2, IGFBP2, LOC285740, CYP11B2, ATP2B1, SLCL15A4

RPARP-AS1, TMEM180, ACTR1A, SUFU, ARL3, SFXN2, WBP1L, CYP17A1, C10orf32, C10orf32-ASMT, AS3MT, CNNM2, NT5C2, RPEL1, INA, TAF5
S8 Fig.

Manhattan Plot of Gene-based Analysis Results for LTA PP