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Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in East Asians

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Abstract

We conducted a meta-analysis of genome-wide association studies of systolic (SBP) and diastolic (DBP) blood pressure in 19,608 subjects of East Asian ancestry from the AGEN-BP consortium followed by *de novo* genotyping in 2 stages of replication involving 10,518 and 20,247 East Asian samples. We identified novel genome-wide significant ($P < 5 \times 10^{-8}$) associations between SBP or DBP and variants at four novel loci: *ST7L-CAPZA1*, *FIGN-GRB14*, *ENPEP*, and *NPR3*, as well as a novel variant near *TBX3*. Except for *NPR3*, all novel findings were significantly replicated for SBP or DBP in independent samples. Seven loci previously reported in populations of European descent were confirmed. On 12q24.13, we observed an ethnic specific association (implicating rs671 at the *ALDH2* locus as the causal variant) that affected SBP, DBP and multiple traits related to coronary artery disease. These findings provide novel insights into blood pressure regulation and potential targets for intervention.

Hypertension is a leading risk factor of cardiovascular disease and premature deaths globally.¹⁻⁴ It is especially common in Asian populations, contributing to a high incidence and mortality from stroke.^{5,6} Genetic and environmental factors and their interaction determine an individual's risk for hypertension. Considerable efforts to elucidate the genetic determinants of hypertension, or elevated blood pressure (BP) levels,^{7,8} yielded little success until 2009 when two large-scale meta-analyses of genome-wide association studies (GWAS) from the Global BP Genetics (Global BPgen) and Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortia identified a total of 13 independent loci significantly associated with BP.^{9,10} The results from the 2 consortia represented an important advance in hypertension genomic research.¹¹ However, these studies were conducted almost exclusively in populations of European descent. Studies in non-European populations will allow us to assess the relevance of these findings to other ethnic groups and potentially discover novel variants. The latter is important because some variants may be more common in specific ethnic groups, thereby providing greater power, or the effects of genetic variants on BP may be enlarged in specific ethnic groups.

The Asian Genetic Epidemiology Network BP (AGEN-BP) work group was established to facilitate the identification of genetic variants influencing BP among populations of East Asian ancestry. AGEN-BP includes 19,608 East Asian participants from 8 population- and family-based GWAS with standardized BP measurements. Here, we report the findings from our three-stage study which included stage 1, meta-analysis of BP GWAS from AGEN-BP ($N=19,608$); stage 2, *de novo* genotyping of top loci in additional individuals ($N=10,518$); and stage 3, replication of findings in independent East Asian samples ($N=20,247$) (Supplementary Fig. 1).

RESULTS

Meta-analysis and follow-up of novel association signals

The stage-1 meta-analysis included 19,608 individuals from 8 GWAS in East Asian populations (Table 1, and Supplementary Table 1). The $-\log_{10} P$ -values by chromosome location for SBP are shown in Fig. 1a and DBP in Fig. 1b. In addition, quantile-quantile plots for SBP and DBP are presented in Supplementary Fig. 2. The overall inflation post-meta-analysis was modest ($\lambda_{GC}=1.03$, SBP; $\lambda_{GC}=1.02$, DBP). The meta-analysis revealed two independent association signals reaching genome-wide significance (defined as $P < 5 \times 10^{-8}$). These included 1 locus known to harbor variants associated with BP—*ATP2B1*—and 1 novel locus—*FIGN-GRB14*. Additional 11 variants that have not been previously implicated in the pathogenesis of hypertension were associated with SBP and/or DBP at a significance level of $P < 1 \times 10^{-5}$ (Supplementary Table 2). To increase the statistical power and strengthen support for the stage-1 findings, we undertook stage-2 follow-up *de novo* genotyping for 13 SNPs in an additional 10,518 Japanese individuals. These included 12 SNPs that showed associations with $P < 1 \times 10^{-5}$ and one additional SNP with $P < 1 \times 10^{-3}$, which was close to a biological candidate gene, *NPR3*.¹² In the joint analysis of stage 1+2, one additional SNP (rs11066280 at the *RPL6-PTPN11* locus) reached genome-wide significance. This SNP, and additional 5 SNPs attaining borderline significance (defined as $P < 5 \times 10^{-6}$) in our joint analysis of stage 1+2 were carried forward for stage-3 replication study involving 20,247 individuals. All 6 SNPs showed some evidence of replication in the stage-3 study, although the SBP association for rs1173766 ($P = 0.016$) did not reach a significance level after adjustment for multiple testing, i.e., $P = 0.05/6 \approx 0.008$. All reached genome-wide significance in the joint analysis of stage 1+2+3 (Table 2 and Fig. 2). Moreover, 2 additional variants (rs880315 at the *CASZ1* locus and rs155524 at the *ITGA9* locus) had previously been genotyped in our stage 2 and stage 3 samples.¹³ When combined with data from stage 1, we found that rs880315 at *CASZ1* showed an association with DBP that reached genome-wide significance as previously reported.¹³

Finally, on 12q24.21 near *TBX3*, we detected an association between BP and rs35444, which is approximately 200-kb apart from the reported lead SNP (rs2384550) identified in populations of European descent and its proxies. Since there is no linkage disequilibrium (LD) between the two clusters of SNPs on 12q24.21 in both ethnic groups ($r^2 = 0.000$, $D' = 0.018$ in HapMap JPT/CHB; $r^2 = 0.003$, $D' = 0.063$ in HapMap CEU between rs35444 and rs2384550), we consider the BP association at rs35444 independent and novel, presumably indicating the presence of allelic heterogeneity between the ethnic groups.

East Asian-specific associations on 12q24.13

One of the most prominent BP associations was detected on 12q24.13 [rs11066280, effect allele frequency = 0.75, per-allele effect = 1.56 mm Hg for SBP ($P = 7.9 \times 10^{-31}$) and 1.01 mm Hg for DBP ($P = 1.3 \times 10^{-35}$) in the joint analysis of stage 1+2+3, Table 2]. This association is likely to derive from a known functional variant rs671 at *ALDH2*, which is common in East Asians and has been reported to associate with hypertension principally through modification of alcohol consumption.^{14–17} Among the proxy SNPs in the surrounding region, a strong LD ($r^2 = 0.87$) was found between rs11066280 and rs671 (Supplementary Table 3). In addition, both SNPs showed an association with BP in the CAGE-Amagasaki, Fukuoka, and KING Studies ($N=21,875$; Supplementary Table 4). When both SNPs were simultaneously included in the regression model, statistical significance remained at rs671 ($P = 0.018$ for SBP and $P = 8.9 \times 10^{-4}$ for DBP) but not at rs11066280 ($P > 0.05$). However, conclusive evidence for the primary source of association on 12q24.13 remains to be provided. The two SNPs are close to *SH2B3* (Fig. 3a), one of the BP loci identified in populations of European-descent.^{9,10}

The SNP associated with BP in populations of European descent (rs3184504 at the *SH2B3* locus) is not polymorphic in East Asians and the SNP associated with BP in our study (rs671 at the *ALDH2* locus) is not polymorphic in Europeans. Also, we found modest signatures of recent selection, which was supported by several types of population genetic evidence, e.g., reduction of haplotype diversity in East Asians, and suggestive evidence of a selective sweep for East Asians at 110.7–111.4 Mb (empirical p-values < 0.01) by two haplotype-based tests—iHS scores¹⁸ and XP-EHH¹⁹ (Fig. 3a and 3b and Supplementary Fig. 3). These could be further supported by data from the HapMap Project (release 22) [<http://www.hapmap.org>] and 1000 Genomes Project (pilot 1) [<http://www.1000genomes.org/>]; i.e., 8 common SNPs (minor allele frequency [MAF] = 0.23–0.24 in the Japanese) appeared to identify a common haplotype (H5), which arose on a haplotype (H4) more common in East Asians but absent in Europeans and rare in Africans (Supplementary Fig. 4), spanning almost the same length of interval (0.7 Mb) as that of a suggestive selective sweep. Moreover, in the haplotype analysis involving 5 (of 8) SNPs genotyped on 12q24.13, rs671 and rs11066132 ($r^2 = 0.99$ between the 2 SNPs) clearly differentiated a group of BP-increasing haplotypes—H1 to H3—from that of BP-decreasing haplotypes H4 to H6 (Supplementary Fig. 5).

These SNPs showed substantial pleiotropic effects on risk factors of cardiovascular disease¹⁷ as well as on susceptibility to coronary artery disease (CAD) in the Japanese (a paper submitted, F.T., *et al.*) with the strongest association being detected at rs671 for several traits besides SBP/DBP in the Japanese population studied (Fig. 3c and Supplementary Table 4). Two additional observations are of note. Firstly, the alleles associated with increased BP were associated with relatively small elevations in fasting glucose and body mass index (BMI), which would be expected to result in elevated risk of CAD [OR = 1.69 for A-allele vs. G-allele (95% CI 1.50–1.91), $P = 7.7 \times 10^{-18}$; Supplementary Table 4]. However, the effects of rs671 on LDL cholesterol and HDL cholesterol were larger than those on BP, glucose and BMI, and in a direction that would be expected to decrease CAD risk. Indeed, overall, rs671 was associated with reduced risk of CAD. Here, a previous European GWAS claimed significant evidence supporting CAD association of a haplotype involving rs3184504 (at *SH2B3*) identical to that for BP association and signatures of natural selection in an overlapping region on 12q24.13.²⁰ By inspecting the phylogeny of haplotypes in the relevant region, we confirmed that these haplotypes arose independently and each of the haplotypes was responsible for independent association signals in the 2 populations (Supplementary Fig. 4). Secondly, given the biological role of *ALDH2* in the propensity for alcohol consumption, we further examined the following issue: to what extent alcohol intake could mediate the effects of rs671 on various cardiovascular risk factors. We showed that most of the associations between rs671 and each of the cardiovascular risk factors were substantially attenuated after adjustment for alcohol intake (Fig. 3d and Supplementary Table 4).

Associations at BP loci identified in populations of European descent

We performed replication study of the genome-wide significant and suggestive associations previously identified in populations of European descent^{9,10} in the AGEN-BP samples (Fig. 4 and Supplementary Table 5). We had genotyped variants at 7 loci—*CASZ1*, *MTHFR*, *ITGA9*, *FGF5*, *CNNM2-NT5C2*, *ATP2B1* and *CSK-ULK3*—in the CAGE-Amagasaki, CAGE-Fukuoka and CAGE-KING samples and 2 loci—*CACNB2* and *PLEKHA7*—in the CAGE-Amagasaki samples as part of the previous replication study.¹³ In the present study, we additionally genotyped in the CAGE Amagasaki samples 3 (in *c10orf107*, *PLCD3*, and *ZNF652*) of 7 variants that showed directionally consistent and nominally significant BP associations in the stage 1 study; the remaining 4 (of 7) variants were overlapped with those previously genotyped in the CAGE study samples (Supplementary Table 2). When these

data were combined with our stage 1 data, 6 of the 7 previously replicated loci,¹³ and 1 additional locus (*ZNF652*) still showed directionally consistent associations with BP with p-values < 0.05.

In the 6 variants that we did not detect BP associations in the East Asian populations (Supplementary Fig. 6), we compared the effects in our study to those in the follow-up panels in Europeans (to exclude the possible winner's curse effect²¹). Cross-population heterogeneity in effect size was detected for *c10orf107* and *PLEKHA7* (Supplementary Table 5). One possibility for our failure to detect an association between these 6 variants and BP in our population is the lack of power. To claim replication of BP associations previously identified in populations of European descent with p-values < 0.05 in East Asians, sample size was not sufficient for 3 (of the 6) variants—*CACNB2*, *c10orf107*, and *TBX3-TBX5* (Supplementary Table 6). Another possibility could relate to differences in LD patterns between the ethnic groups. We examined LD differences between Europeans (HapMap CEU) and East Asians (HapMap JPT+CHB) at these loci by using the varLD program²² (Supplementary Fig. 7). For the 7 reported variants that showed an association with BP in East Asians, there was limited evidence of LD differences between European and East Asian populations at 5 (of 7) loci that harbor these variants. On the other hand, for the 6 reported variants for which we did not detect an association with BP in East Asians, we found significant differences in LD between the ethnic groups at 4 (of 6) loci harbouring these variants—*CACNB2*, *c10orf107*, *PLEKHA7*, and *TBX3-TBX5*.

Cumulative impact of risk alleles and genetic association with dichotomous hypertension

In total, 10 variants showed associations that reached genome-wide significance after all stages of our study. These 10 variants had a cumulative impact on SBP and DBP (Supplementary Note and Supplementary Fig. 8) and were also associated with odds of hypertension (as a dichotomous trait) in directions consistent with the continuous trait effect (Supplementary Note, Supplementary Fig. 9 and Supplementary Tables 7 and 8).

eQTL analysis

In search of putative functional variation at the newly identified loci, we found that among the 6 genes on 1p13.2, expression of *ST7L* was associated with expression-associated SNP (eSNP) rs17030613 ($P = 9.4 \times 10^{-15}$ and 1.4×10^{-8} , reported in 2 independent studies)^{23,24} in lymphoblastoid cell lines (LCLs) derived from samples of European and East Asian ancestry (Supplementary Fig. 10). In the current study, the rs17030613 A-allele associated with lower *ST7L* transcript levels is also associated with lower BP. No other eSNPs or non-synonymous SNPs, which were in LD with BP-associated variants, were identified for the 5 novel association signals in the public databases currently available.

DISCUSSION

By using a three-stage GWAS meta-analysis of up to 50,373 individuals of East Asian ancestry, we identified a total of 5 novel BP association signals with genome-wide significance. These include: 1p13.2 in *ST7L-CAPZA1* ($P = 1.2 \times 10^{-8}$ for DBP), 2q24.3 in *FIGN-GRB14* ($P = 3.5 \times 10^{-11}$ for SBP), 4q25 in *ENPEP* ($P = 9.0 \times 10^{-9}$ for DBP), and 5p13.3 near *NPR3* ($P = 1.9 \times 10^{-8}$ for SBP). On 12q24.21, near *TBX3* (a known BP-associated locus in populations of European descent), we also identified a novel BP-associated variant, illustrating the possible presence of allelic heterogeneity at this locus in relation to BP regulation.

The glutamyl aminopeptidase encoded by *ENPEP* plays a pivotal role in BP regulation by facilitating conversion of angiotensin II, the main effector protein of the renin-angiotensin-

aldosterone system, to angiotensin III and the knock out mouse develops hypertension.²⁵ The natriuretic peptide receptor C/guanylate cyclase C encoded by *NPR3* is one of three receptor subtypes that mediate specific binding of the natriuretic peptides, which are important in the maintenance of BP and extracellular fluid volume, to the cell surface.¹² On 2q24.3, the lead SNP (rs16849225) is located in between the *FIGN* and *GRB14* genes. *FIGN* encodes fidgetin, a member of ATPases associated with diverse cellular activities.²⁶ *GRB14* encodes the growth factor receptor-bound protein 14 that interacts with insulin receptors and insulin-like growth factor receptors, suggesting the role of GRB14 in signaling pathways that regulate growth and metabolism.²⁷ A variant close to the *GRB14* locus (rs10195252) has been identified in association with a waist-to-hip ratio (WHR) in populations of European descent.²⁸ However, the variant we identified for the BP association in this study (rs16849225) is distant (>600 kb) from that identified for the WHR association with the two SNPs being in linkage equilibrium. While the novel variant on 1p13.2 is in close proximity to 6 annotated genes, it is an eSNP for *ST7L*, with the consistent results supporting an association between rs17030613 and *ST7L* transcript levels in the LCLs being demonstrated in two independent studies^{23,24} in three ethnic groups (HapMap CEU, YRI, and JPT+CHB, $P > 0.05$ for testing inter-population per-allele effect difference).²³ *ST7L* (suppression of tumorigenicity 7 like) has been identified by its similarity to the *ST7* tumor suppressor gene found in the 7q31 region known to be deleted and rearranged in a variety of cancers,²⁹ although the function of this gene remains to be determined.

Our study provides important information on the genetic architecture of BP and CAD in relation with known loci. In this study, we provided evidence suggesting that the association between variants on 12q24.13 and BP is likely to relate to a non-synonymous SNP (rs671, E504K) in the *ALDH2* gene. Furthermore, the associations seem to be largely mediated by alcohol intake, a finding that is supported by pre-existing studies showing that this variant determines an individual's tolerance of alcohol intake by altering ALDH2 enzymatic activity.^{30,31} Variants at this locus also appear to have undergone natural selection, while phylogenetic analysis suggests that rs671 is not responsible for the BP association identified in the overlapping chromosomal region (near *SH2B3*) in populations of European descent.^{9,10} Thus, the associations described in our study appear to be East Asian specific. Moreover, our study highlights the importance of extending studies examining associations with surrogate markers of cardiovascular risk factors (such as BP) to include clinically relevant events (such as CAD) when trying to identify therapeutic targets, especially if the variant of interest could have pleiotropic effects. In this instance, the allele (of rs671) associated with elevated BP was associated with reduced risk of CAD, a finding that is counter-intuitive based on what is known about the relationship between BP and CAD. Further analysis suggests that the deleterious effect of the variant on BP was balanced by protective effects on HDL cholesterol and LDL cholesterol, resulting in a net reduction in CAD risk. This is reminiscent of the effects of the CETP inhibitor, torcetrapib, which increased HDL cholesterol and decreased LDL cholesterol. Despite these cardioprotective effects, its use was associated with increased risk of CAD and mortality, which may relate to an off-target effect causing BP elevation.³² Secondly, on examining BP associations for 13 variants identified in European GWAS meta-analyses (Supplementary Table 5), we found 7 of the 13 loci (54%) to show nominally significant associations of the reported lead SNPs in East Asians. This is consistent with previous studies showing that findings from populations of European ancestry may be relevant to other ethnic groups.^{13,33,34} We also found another locus (*TBX3-TBX5*) to show some evidence of allelic heterogeneity in relation to BP. These data suggest that although some inter-population differences may exist in the pathways involved in the pathogenesis of BP elevation (or hypertension) between Europeans and East Asians, the majority of pathways are common.

A number of factors are considered to influence our ability to detect associations at the remaining 6 loci in the East Asian populations. Firstly, the sample size for genome-wide exploration (stage 1) was smaller in AGEN-BP ($N = 19,608$) than two previous consortia of European-descent populations, the Global BPgen ($N = 34,433$) and CHARGE ($N = 29,136$),^{9,10} and this provided limited power to detect several of these loci, even using a p -value threshold of <0.05 (Supplementary Table 6). This limited power also means that additional novel loci could be identified by enlarging the sample size in the discovery stage, providing motivation for larger meta-analyses in the future. Secondly, we found some evidence of LD differences between European and East Asian populations at 2 of 7 loci (29%) for which we did detect an association in our study, versus at 4 of 6 loci (67%) for which we did not detect an association (Supplementary Fig. 7). This could also contribute to our failure to detect associations for some of the known variants in the present study.

In conclusion, even though variants identified in European populations are often relevant to other ethnic groups, by conducting a large-scale GWAS meta-analysis in East Asians, we have identified previously unreported association signals for BP—at *ST7L-CAPZA1*, *FIGN-GRB14*, *ENPEP*, and *NPR3*—that reach genome-wide significance. Near *TBX3*, we have identified some evidence for allelic heterogeneity in East Asians compared to Europeans in relation to BP associations. Further, our data have provided evidence of East Asian-specific BP association at *ALDH2* which has pleiotropic effects on other metabolic traits and CAD, highlighting the importance of fine mapping efforts to pinpoint causal variants and causal genes, thereby providing new insights into the physiology of complex diseases.

METHODS

Stage 1 samples

The Asian Genetic Epidemiology Network (AGEN) is a consortium of genetic epidemiology studies of cardiovascular disease related phenotypes, such as BP (or hypertension), diabetes, and obesity, conducted among Asian populations. AGEN-BP consists of 19,608 East Asian participants who underwent standardized collection of BP measurements in eight population- and family-based GWAS, including: the Cardio-metabolic Genome Epidemiology (CAGE) Network, Genetic Epidemiology Network of Salt-Sensitivity (GenSalt), Korean Association Resource (KARE) Project, Shanghai Hypertension Study, Singapore Malay Eye Survey (SiMES), Singapore Prospective Study (SP2) Program, Suita Study, and Taiwan Super Control Study. Each study established a consensus on phenotype harmonization and analytical plan for within-study GWAS and meta-analysis of results across studies. Each study received an approval from the institutional review board and all participants in each study provided written informed consent for participation in the study. A detailed description of the study design and phenotype measurement for each study (or cohort) is provided in the Supplementary Note and Supplementary Table 1 online. Overall design of the three-staged GWAS meta-analysis is depicted in Supplementary Fig. 1. Stage 1 was a meta-analysis of directly genotyped and imputed SNPs from individuals of East Asian descent, drawn from population-based or control samples in case-control studies in AGEN-BP.

Genome-wide genotyping and quality control

Genotyping arrays and quality control filters applied to the individual studies are provided in Supplementary Table 1.

Genotype imputation

Imputation of genotypes to the HapMap Phase 2 (JPT+CHB) except for SiMES, which used HapMap JPT+CHB+CEU+YRI) set was carried out using MACH,³⁷ IMPUTE,³⁸ or

BEAGLE³⁹ with preimputation filters as specified in Supplementary Table 1. Imputation results are summarized as an ‘allele dosage’ defined as the expected number of copies of the coded allele at that SNP (a fractional value between 0 to 2) for each genotype. In total, up to 2.4 million genotyped or imputed autosomal SNPs were analyzed.

Phenotype modeling and SNP association analysis

For participants taking antihypertensive therapies, BP was imputed by adding 10 mm Hg and 5 mm Hg to SBP and DBP values, respectively.⁴⁰ Within each study, continuous SBP and DBP were adjusted for age, age², sex, BMI, and any study-specific covariates in linear regression models.

In secondary and confirmatory analyses of hypertension, hypertensive cases were defined as follows: (i) SBP \geq 160 mm Hg and/or DBP \geq 100 mm Hg for untreated subjects; (ii) patients receiving chronic antihypertensive treatments; and (iii) age of onset \leq 65 years.

Normotensive controls were defined as follows: (i) SBP $<$ 130 mm Hg and DBP $<$ 85 mm Hg without antihypertensive treatments and (ii) age \geq 50 years. Within each study, a dichotomous trait of hypertension status was adjusted for age, age², sex, and BMI in logistic regression models, except for the CAGE samples used for follow-up and replication (3,294 cases and 6,831 controls; Supplementary Table 7), in which age was not adjusted for due to its relation to case-control status; i.e., the mean age was younger in cases than in controls.

Stage 1 meta-analysis

All study-specific effect estimates and coded alleles were oriented to the forward strand of the NCBI36 reference sequence of the human genome. If an SNP from a study did not meet quality standards, it was reported as missing from that study for the purpose of meta-analysis. Results for this SNP were pooled among the other contributing studies. SNPs were excluded if they had study-specific imputation quality $R^2 < 0.5$. Genomic control⁴¹ was carried out on study-specific test statistics: λ_{GC} estimates are given in Supplementary Table 1. We used inverse variance-weighted meta-analysis to combine association results for stage 1 with METAL software (<http://www.sph.umich.edu/csg/abecasis/Metal>). Evidence for heterogeneity of effect sizes was investigated using Cochran’s Q statistic.⁴² We retained 1.7 million SNPs with minor allele frequency (MAF) of >0.05 and an effective sample size of $>10,000$; in Fig. 1, we show the Manhattan plots drawn using the WGAViewer software⁴³ and the quantile-quantile plots.

SNP prioritization for stage 2

We selected 13 SNPs for follow-up in stage 2 using two methods in parallel. Using the first method, the most strongly associated SNP was chosen from each of 12 distinct regions containing ≥ 1 SNPs with $P < 1 \times 10^{-5}$ for SBP and/or DBP based on the stage-1 data, in which samples from ≥ 7 (of 8) GWAS were available for the meta-analysis, in principle, to decrease the potential noise of false positive associations due to the small sample size. We excluded SNPs within 50 kb of the lead SNPs for BP associations previously identified in Europeans at *CNNM2-NT5C2*, *ATP2B1*, and *CSK-ULK3*.^{9,10} Although attaining a significance level of $P < 1 \times 10^{-5}$ for SBP, we did not subject 2 SNPs (rs4671977 and rs12547784) to follow-up in stage 2, because their association with the DBP trait was not significant ($P > 0.05$) in stage 1. Using the second method, we generated a list of the next most significant SNPs (21 unique loci showed $P \leq 1 \times 10^{-3}$ for both SBP and DBP and consistent association signals across the studies). We selected a SNP at 5p13.3 *ad hoc*, due to physiological candidacy of the nearby gene, *NPR3*.¹²

Stage 2 samples and genotyping

We genotyped 13 SNPs in 10,518 individuals of Japanese descent from 3 studies—CAGE-Amagasaki Study, Suita (2) Study, and Ehime Study. Summary characteristics are shown in Table 1. Study information and genotyping methods are provided in the Supplementary Note.

Stage 3 samples, genotyping and joint analysis

For SNP selection in the stage-3 study, we set a threshold of $P < 5 \times 10^{-6}$ for joint analysis of stage 1+2. Six of 13 SNPs exceeded this significance level and were followed up in 20,247 individuals of East Asian descent from 3 studies by *de novo* genotyping (for Fukuoka Study and Kita-Nagoya Genomic Epidemiology [KING] Study of the CAGE Network) and *in silico* replication (for HEXA-shared control).

We carried out meta-analysis of stage 1+2+3 results and considered associations genome-wide significant if they attained $P < 5 \times 10^{-8}$. Association results for multiple stages (1+2 or 1+2+3) were combined using inverse variance weighting with the *rmeta* package of the R software (<http://www.r-project.org>).

Replication of previously reported loci

Along with genome-wide exploration of novel loci, we examined the BP associations at 27 loci reported by the European GWAS meta-analyses:^{9,10} 13 genome-wide significant loci and 14 loci with suggestive association (Supplementary Table 5). A one-tailed $P < 0.05$ (two-tailed $P < 0.1$) was considered statistically significant for the 13 loci previously shown to have genome-wide significant ($P < 5 \times 10^{-8}$) associations in Europeans; for an association to be considered significant, it had to involve the same risk allele as that reported in Europeans, and was accordingly assessed with the one-tailed test. However, two-tailed P values are presented throughout the text for readability. For the 14 loci with suggestive association in the original European studies,^{9,10} a one-tailed $P < 0.05/14 = 0.0036$ was considered statistically significant after Bonferroni correction.

Haplotype phylogeny and positive selection on 12q24.13

Previous studies in population of European descent claimed significant evidence supporting BP associations^{9,10} and signatures of natural selection²⁰ in a 1.6 Mb interval on 12q24.13, near which the present East Asian study also identified strong BP associations. To clarify phylogenetic differences in susceptibility variants between the 2 populations, we inferred the haplotypes and constructed their phylogeny across 3 HapMap panels—JPT, CEU, and YRI—in the 12q24.13 region of interest. Further, using haplotype-based tests, *iHS*¹⁸ and *XP-EHH*,¹⁹ we tested the hypothesis that a long-range, evolutionarily derived haplotype, upon which BP-decreasing alleles could lie, arose from a positive selection in East Asians independently of Europeans.

Pleiotropic effects on cardio-metabolic traits

We tested SNP—trait associations of 5 SNPs located in the target interval on 12q24.13 for risk factors of cardiovascular disease and CAD. The associations with SBP/DBP, BMI, fasting plasma glucose, and lipids were analyzed in CAGE-Amagasaki Study (for 5 SNPs) and CAGE-Fukuoka Study (for rs11066280 and rs671 alone) samples with and without adjustment for alcohol intake; where the amount of alcohol consumed was denoted in terms of servings of sake (1 *gou* [180 ml] of Japanese rice wine is considered equal to 22 g of ethanol).¹⁷ The association with CAD was analyzed in the CAD case-control panel (1,347 CAD cases and 1,337 controls) derived from the CAGE Network (a paper submitted, F.T., *et al.*).

eQTL analysis

Using publicly available data [GTEx (Genotype-Tissue Expression) eQTL Browser (<http://www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi>); <http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/>; <http://www.sph.umich.edu/csg/liang/imputation/>; SNPExpress (<http://people.genome.duke.edu/~dg48/SNPExpress/>)], we examined the *cis*-associations (defined as genes within 1 Mb) between each of the 5 novel BP SNPs and expression of nearby genes in a variety of cells/tissues; e.g., LCLs, monocytes, fibroblasts, liver and brain tissues. After initial screening, we narrowed the targets of eQTLs down to LCLs in two databases.^{23,24} Briefly, LCLs, derived from peripheral blood lymphocytes, were available for 209 HapMap samples (comprising 60 CEU, 60 YRI, and 89 JPT+CHB individuals)²³ and 378 individuals of white European descent²⁴. The expression of 47,294 (for 209 HapMap samples) and 54,675 (for 378 Europeans) transcripts was assessed for each individual, whose genotypes were also imputed using the corresponding HapMap dataset. SNPs were tested for *cis* associations, assuming an additive genetic model, adjusting for non-genetic effects in the gene expression value.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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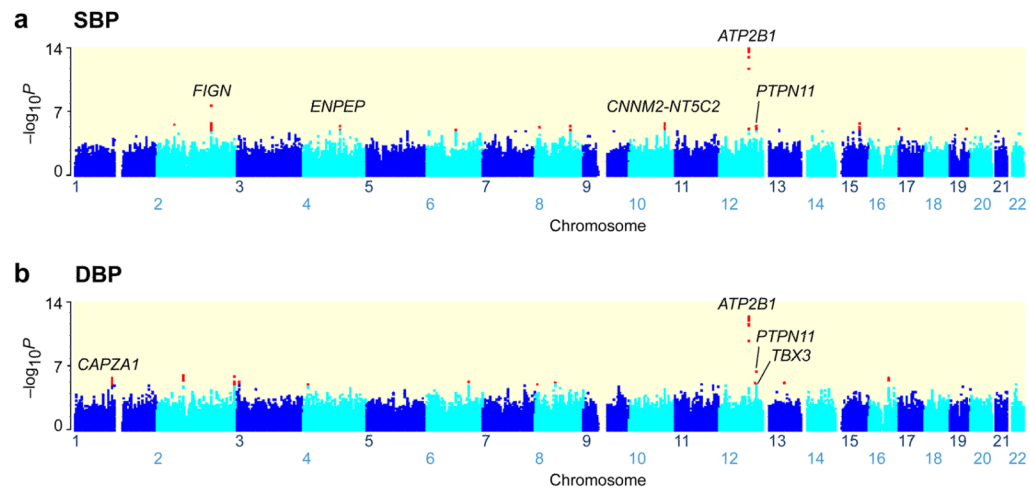


Fig. 1.

Genome-wide association results for the AGEN-BP meta-analysis for blood pressure. Manhattan plots show the significance of association between all SNPs and SBP (**a**) and DBP (**b**) in the stage 1 meta-analysis, highlighting signals with suggestive levels of significance ($P < 10^{-5}$) in red. Seven named loci showed genome-wide significance ($P < 5 \times 10^{-8}$) in the joint analysis (stage 1 + 2 + 3; 2 reported loci—*CNNM2-NT5C2* and *ATP2B1*—were followed up in part of stage 2 and stage 3). The genes used to name signals have been chosen on the basis of proximity to the lead SNP and should not be presumed to indicate causality.

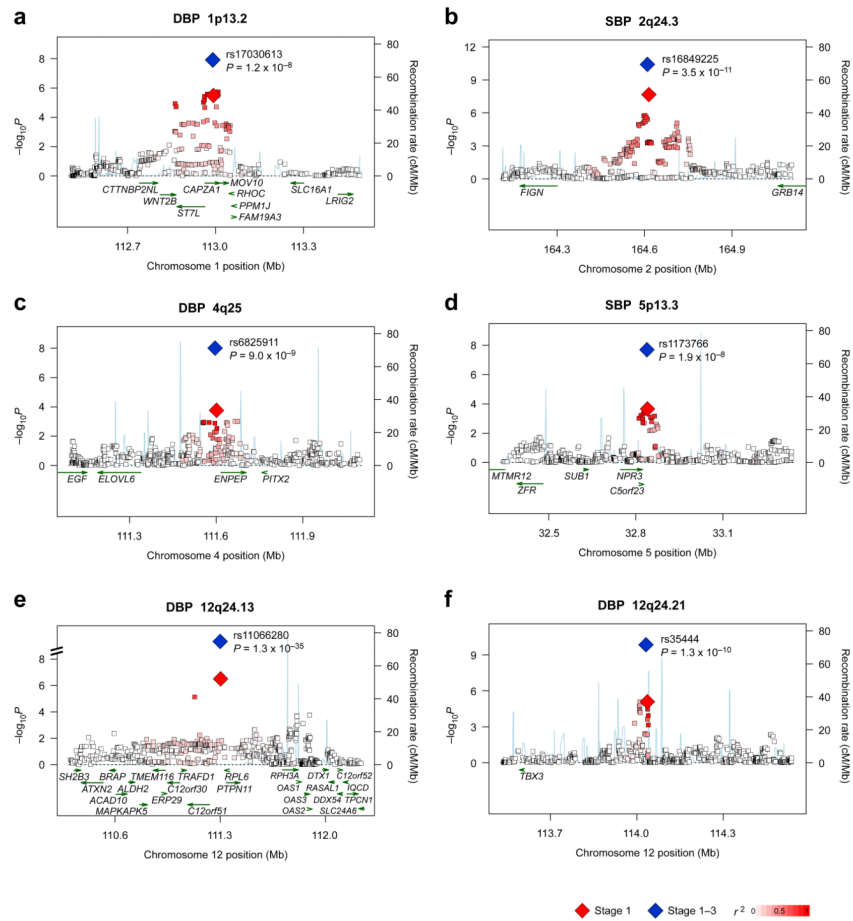


Fig. 2. Regional association plots of six blood pressure loci. Genotyped and imputed SNPs passing quality control measures in stage 1 are plotted with their meta-analysis P values (as $-\log_{10}$ values) as a function of genomic position (build 36). In each panel, the lead SNP is represented by a diamond, with stage 1 meta-analysis results denoted by a red diamond and the joint analysis (combined P) results denoted by a blue diamond. The correlation of the lead SNP to other SNPs at the locus is shown on a scale from minimal (white) to maximal (red). Superimposed on the plot are gene locations (green) and recombination rates (blue). The regional plots were drawn using the SNAP software.³⁵ On 12q24.13, rs671 was not included in the construction of regional association plots (Fig. 2e) because the genotype data for this SNP were unavailable from two GWAS in stage 1, KARE and Suita (1), which resulted in an effective sample size of 9,828.

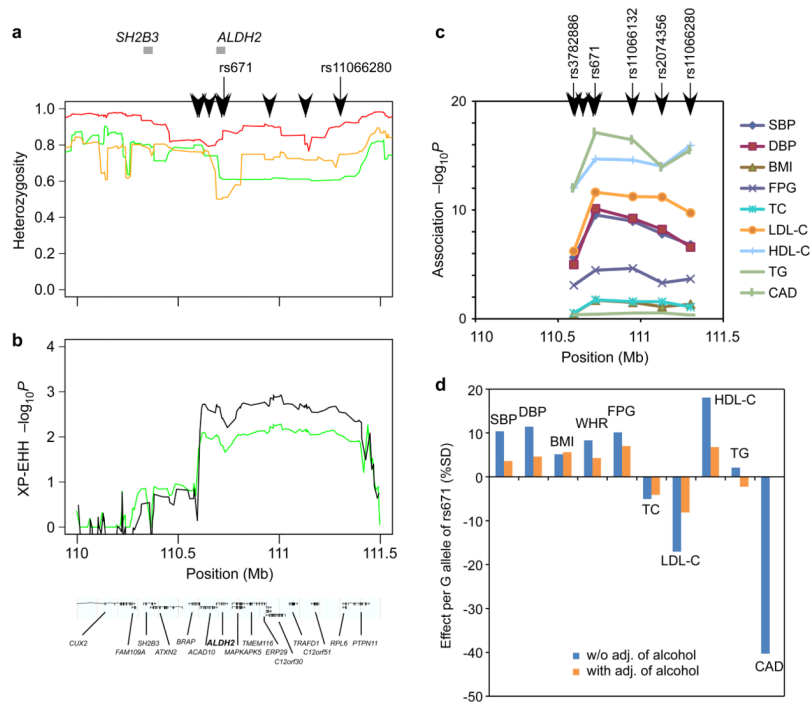
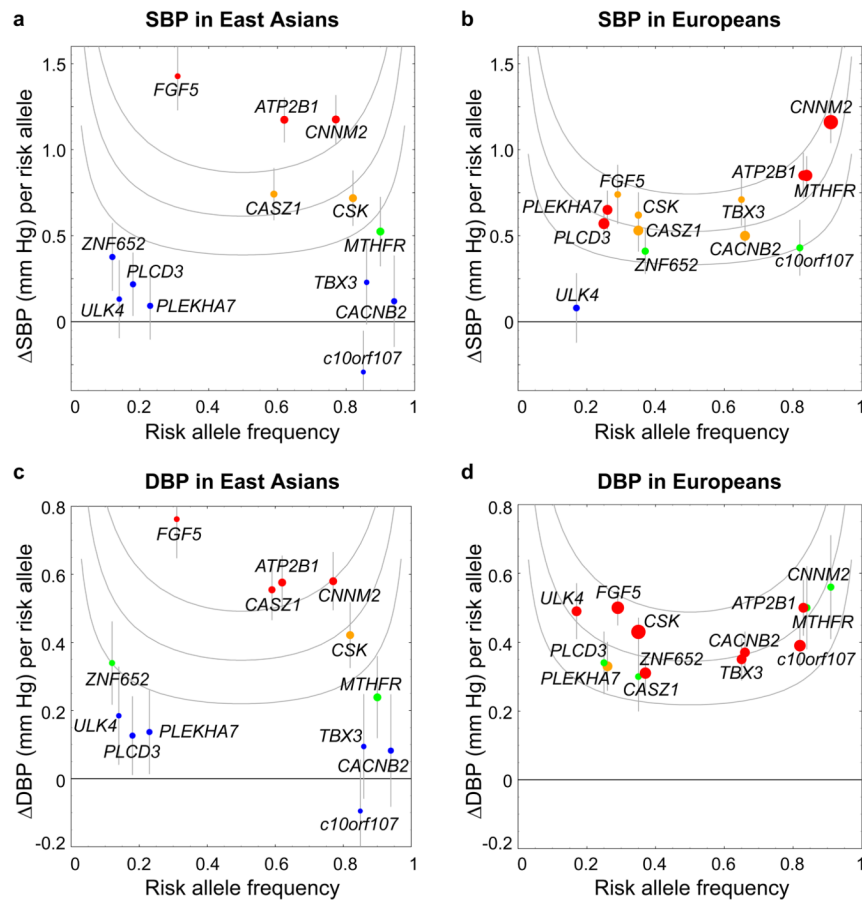


Fig. 3. Evidence for positive selection and pleiotropic effects on 12q24.23. **a.** Lines show heterozygosity calculated in a sliding window of 20 SNPs in the East Asian (green), European (orange), and African (red) populations of the Human Genome Diversity Panel.³⁶ Heterozygosity in East Asians dropped to 0.61 in a range from 110.7 to 111.4 Mb. Black arrowheads at the top of the plot represent the positions of SNPs forming an East Asian-specific haplotype with *ALDH2* rs671. The positions of the *SH2B3* and *ALDH2* genes are highlighted by gray bars at the top of the plot. Signals of selection detected using a haplotype-based test, XP-EHH (**b**)¹⁹, in the East Asian population. Since there are multiple SNP clusters showing high LD within a cluster but relatively modest LD between clusters and no ancestral haplotype in East Asians (Supplementary Fig. 4), XP-EHH is assumed to provide more appropriate signals of selection than iHS¹⁸ on 12q24.23. Vertical axes represent empirical P values, where a suggestive level for positive selection is set at $P < 0.01$; i.e., $-\log_{10}(P) > 2$. The results are shown for all East Asians combined (green line) and for Japanese (black line). **c.** The $-\log_{10}(P)$ for associations with cardio-metabolic traits at 5 of the 8 SNPs forming a common East Asian-specific haplotype. The position of each SNP is denoted at the top of the plot. **d.** Per-allele effects of rs671 (G vs. A) on cardio-metabolic traits with and without adjustment for alcohol intake.

**Fig. 4.**

Plots of effect size (β) versus risk-allele frequency of 13 loci previously identified in European GWAS meta-analysis. The list of 13 loci includes 1 locus (*CASZ1*) that did not reach genome-wide significance in Europeans but is found to show a significant association with BP in the present AGEN-BP meta-analysis. Plots for SBP [East Asians(**a**); Europeans(**b**)] and DBP [East Asians(**c**); Europeans(**d**)] are depicted separately. Each point refers to a single BP association signal, with colors denoting the strength of the BP association (red, $P < 5 \times 10^{-8}$; orange, $5 \times 10^{-8} \leq P < 10^{-5}$; green, $10^{-5} \leq P < 0.05$; blue, $P \geq 0.05$) and with sizes being proportional to the tested sample size. Whiskers are \pm standard errors. The gene names associated with each signal have been chosen on the basis of proximity to the lead SNP and should not be presumed to indicate causality. The gray curves represent the coefficient of determination (R^2), i.e., BP variance explained by an SNP (%); those from the top to the bottom correspond to $R^2 = 0.1, 0.05,$ and 0.02 . For estimating R^2 , the standard deviations of BP were assumed to be 19.4 mm Hg (SBP) and 11.0 mm Hg (DBP) in East Asians, and 16.6 mm Hg (SBP) and 10.9 mm Hg (DBP) in Europeans.⁹ The risk alleles were designated as previously reported in European GWAS meta-analysis. See the details in Supplementary Table 5.

Table 1

Study design and sample characteristics.

Study	N	Ancestry	BP Measurement (Device, Number of Measures)	Genotyping Platform	Women, %	Age (SD), y	SBP (SD), mm Hg	DBP (SD), mm Hg	BMI (SD), kg/m ²	HTN, % ^a	Antihypertensive Therapy, %
Stage 1: AGEN-BP GWAS meta-analysis (N=19,608)											
CAGE	1,547	Japanese	Standard mercury sphygmomanometer, 2-3/Digital, 2-3	Illumina 550/610K	42.8	66.1 (8.0)	134.1 (20.3)	76.8 (11.9)	23.5 (3.3)	56.1	37.9
GenSalt	1,881	Han Chinese	Random zero sphygmomanometer, ⁹	Affymetrix 6.0	47.2	38.7 (9.5)	116.9 (14.2)	73.7 (10.3)	23.3 (3.2)	9.8	0.37
KARE	8,842	Korean	Standard mercury sphygmomanometer, ³	Affymetrix 5.0	52.7	52.2 (8.9)	118.7 (19.4)	75.6 (12.0)	24.6 (3.1)	22.3	10.9
Shanghai-Ruijin	455	Han Chinese	Standard mercury sphygmomanometer, ²⁻³	Illumina 610K	49.5	54.2 (7.0)	109.1 (8.6)	72.7 (8.6)	21.9 (1.9)	0	0
SIMES	2,519	Malay	Digital, 2-3	Illumina 610K	50.5	59.0 (11.0)	147.9 (23.9)	80.1 (11.3)	26.4 (5.1)	66.6	22.9
SP2	2,431	Han Chinese	Digital, 2-3	Illumina 550K/610K/1M	46.5	48.1 (11.2)	129.4 (19.8)	76.9 (10.8)	22.9 (3.7)	34.6	14.3
Suita (1)	933	Japanese	Random zero sphygmomanometer, ³	Illumina 550K	56.3	59.0 (7.0)	119.7 (17.5)	75.0 (10.6)	22.7 (2.9)	14.8	0
Taiwan	1,000	Han Chinese	Digital, 2-3	Illumina 550K	49.8	51.2 (17.8)	121.9 (18.5)	76.2 (10.8)	23.8 (3.5)	10.9	6.8
Stage 2: De novo genotyping follow-up study (N=10,518)											
CAGE-Amagasaki	5,331	Japanese	Digital, 2-3	TaqMan	39.8	47.8 (12.3)	124.3 (17.3)	75.9 (11.0)	23.0 (3.2)	21.5	9.0
Ehime	2,895	Japanese	Digital, 3	TaqMan	56.6	61.1 (14.0)	137.7 (22.2)	81.0 (11.8)	23.4 (3.2)	53.4	25.7
Suita (2)	2,292	Japanese	Random zero sphygmomanometer, ³	TaqMan	53.8	67.2 (11.0)	127.4 (19.0)	76.5 (10.3)	22.9 (3.2)	48.1	36.6
Stage 3: Replication study (N=20,247)											
CAGE-Fukuoka	12,569	Japanese	Digital, 2	TaqMan	54.9	62.6 (6.8)	138.9 (21.2)	83.9 (11.7)	23.1 (3.0)	57.9	23.9
CAGE-KING	3,975	Japanese	Digital, 2	TaqMan	56.9	63.6 (6.6)	132.3 (19.8)	76.9 (11.2)	22.9 (3.0)	48.4	24.3
HEXA-shared control	3,703	Korean	Standard mercury sphygmomanometer, ³	Affymetrix 6.0	55.4	53.2 (8.3)	121.7 (14.4)	77.1 (9.9)	24.0 (2.9)	18	0

AGEN-BP, Asian Genetic Epidemiology Network Blood Pressure; CAGE, Cardio-metabolic Genome Epidemiology Network; GenSalt, Genetic Epidemiology Network of Salt-Sensitivity, KARE, Korean Association Resource Project; Shanghai-Ruijin, Shanghai Hypertension Study; SIMES, Singapore Malay Eye Survey; SP2, Singapore Prospective Study; Suita, The Suita Study, Taiwan, Taiwan Type 2 Diabetes Study; N, Sample size; SD, Standard deviation; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HTN, Hypertension; GWAS, Genome-wide association study; NA, Not applicable.

Hypertension is defined as SBP \geq 140 mm Hg and/or DBP \geq 90mm Hg or taking antihypertensive medication.

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Table 2

Top genome-wide association results for SBP and DBP.

Chr	SNP ID (pos Build 36.3)	Coded/Other allele	Nearby Gene(s)	Stage	N	Coded allele freq.	SBP		DBP	
							Beta (SE), mm Hg	P	Beta (SE), mm Hg	P
<i>LocI newly identified in or unique to Asians</i>										
1	rs17030613 (112,971,190)	C/A	ST7L CAPZA1	1	19,251	0.47	0.52 (0.17)	0.002	0.50 (0.11)	3.2E-06
				2	10,465	0.50	0.43 (0.24)	0.073	0.25 (0.14)	0.078
				1+2	29,716	0.48	0.49 (0.14)	4.0E-04	0.41 (0.09)	1.8E-06
				3	20,236	0.50	0.49 (0.18)	0.007	0.34 (0.11)	0.002
			Joint Analysis		49,952	0.49	0.49 (0.11)	8.4E-06	0.38 (0.07)	1.2E-08
2	rs16849225 (164,615,066)	C/T	FIGN GRB14	1	18,867	0.60	0.97 (0.17)	2.2E-08	0.40 (0.11)	3.4E-04
				2	10,465	0.62	0.56 (0.25)	0.022	0.26 (0.15)	0.068
				1+2	29,332	0.61	0.84 (0.14)	3.8E-09	0.35 (0.09)	7.7E-05
				3	20,179	0.62	0.60 (0.19)	0.001	0.20 (0.11)	0.076
			Joint Analysis		49,511	0.61	0.75 (0.11)	3.5E-11	0.29 (0.07)	2.7E-05
4	rs6825911 (111,601,087)	C/T	ENPEP	1	19,033	0.48	0.79 (0.17)	3.3E-06	0.40 (0.11)	1.7E-04
				2	10,463	0.54	0.66 (0.24)	0.007	0.40 (0.14)	0.005
				1+2	29,496	0.50	0.75 (0.14)	7.8E-08	0.40 (0.09)	2.5E-06
				3	20,019	0.52	0.33 (0.18)	0.070	0.36 (0.11)	9.1E-04
			Joint Analysis		49,515	0.51	0.60 (0.11)	7.3E-08	0.39 (0.07)	9.0E-09
5	rs1173766 (32,840,285)	C/T	NPR3	1	19,414	0.62	0.64 (0.17)	2.2E-04	0.38 (0.11)	6.1E-04
				2	10,461	0.58	0.94 (0.24)	1.3E-04	0.54 (0.14)	1.8E-04
				1+2	29,875	0.61	0.74 (0.14)	1.7E-07	0.44 (0.09)	5.9E-07
				3	20,095	0.58	0.45 (0.19)	0.016	0.25 (0.11)	0.026
			Joint Analysis		49,970	0.60	0.63 (0.11)	1.9E-08	0.36 (0.07)	1.2E-07
12	rs11066280 (111,302,166)	T/A	RPL6 PTPN11 ALDH2	1	16,268	0.78	1.03 (0.22)	3.8E-06	0.73 (0.14)	3.1E-07
				2	10,453	0.75	1.41 (0.28)	3.2E-07	0.83 (0.16)	3.5E-07

Chr	SNP ID (pos Build 36.3)	Coded/Other allele	Nearby Gene(s)	Stage	N	Coded allele freq.	SBP		DBP	
							Beta (SE), mm Hg	P	Beta (SE), mm Hg	P
12	rs354444 (114,036,820)	A/G	<i>TBX3</i>	1+2 3 Joint Analysis	26,721 20,236 46,957	0.77 0.74 0.75	1.18 (0.17) 2.13 (0.21) 1.56 (0.13)	1.0E-11 2.6E-23 7.9E-31	0.77 (0.11) 1.34 (0.13) 1.01 (0.08)	5.9E-13 6.5E-27 1.3E-35
12	rs354444 (114,036,820)	A/G	<i>TBX3</i>	1 2 1+2 3 Joint Analysis	19,286 10,460 29,746 20,238 49,984	0.75 0.75 0.75 0.75 0.75	0.69 (0.19) 0.49 (0.28) 0.63 (0.16) 0.64 (0.21) 0.63 (0.13)	3.7E-04 0.077 8.6E-05 0.003 7.5E-07	0.54 (0.12) 0.48 (0.16) 0.52 (0.10) 0.46 (0.13) 0.50 (0.08)	8.1E-06 0.003 9.6E-08 3.0E-04 1.3E-10
<i>Loci previously identified in Europeans</i>										
1	rs880315 (10,719,453)	C/T	<i>CASZ1</i>	1 Follow-up ^a Joint Analysis	10,765 21,846 32,611	0.61 0.67 0.65	0.29 (0.24) 1.03 (0.19) 0.74 (0.15)	0.226 8.1E-08 7.3E-07	0.26 (0.14) 0.72 (0.11) 0.56 (0.09)	0.073 5.9E-11 3.1E-10
4	rs16998073 (81,541,520)	T/A	<i>FGF5</i>	1 Follow-up ^a	N/A 21,864	N/A 0.30	N/A 1.43 (0.20)	N/A 3.9E-13	N/A 0.76 (0.11)	N/A 2.0E-11
10	rs11191548 (104,836,168)	T/C	<i>CNNM2</i> <i>NT5C2</i> <i>CYP17A1</i>	1 Follow-up ^a Joint Analysis	19,457 21,858 41,315	0.74 0.73 0.74	0.91 (0.19) 1.47 (0.20) 1.18 (0.14)	2.1E-06 4.9E-13 3.9E-17	0.49 (0.12) 0.66 (0.12) 0.58 (0.08)	5.6E-05 1.6E-08 6.6E-12
12	rs17249754 (88,584,717)	G/A	<i>ATP2B1</i>	1 Follow-up ^a Joint Analysis	18,856 21,863 40,719	0.65 0.63 0.64	1.38 (0.18) 0.94 (0.19) 1.17 (0.13)	7.6E-15 4.2E-07 7.7E-20	0.83 (0.11) 0.35 (0.11) 0.58 (0.08)	3.2E-13 1.2E-03 1.9E-13

Beta is the effect size on blood pressure in mm Hg per coded allele based on an additive genetic model.

Shown is the top SNP for each independent locus significantly ($P < 5 \times 10^{-8}$) associated with systolic and/or diastolic BP on joint analysis in up to 50,373 individuals of East Asian ancestry. Detailed results for the individual loci separately by study are presented in Supplementary Table 2.

For the Fukuoka study and KING study in stage 3, we used genotype data of rs12413409 for rs11191548 ($r^2 = 0.98$) at *CNNM2-NT5C2* and a proxy (rs2681472, $r^2 = 0.98$) for rs17249754 at *ATP2B1*; linkage disequilibrium coefficient (r^2) was estimated based on 5331 Japanese samples (CAGE-Amagasaki study).

Four loci—*CASZ1*, *FGF5*, *CNMM2-NT5C2*, and *ATP2B1*—in the table were previously reported to associate with BP in the Japanese replication study,¹³ the samples of which constitute the participants in the present GWAS meta-analysis.

^aLead SNPs at the loci previously identified in Europeans were directly genotyped for follow-up in part of stage 2 and stage 3 samples. Imputed data were unavailable for an SNP rs16998073 at *FGF5* and only the results for the follow-up analysis are demonstrated in the table.