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# Discovery and replication of novel blood pressure genetic loci in the Women's Genome Health Study

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## Abstract

**Objectives—**Genome-wide association meta-analyses have recently identified multiple loci associated with blood pressure. We sought to validate previously identified blood pressure loci by replication in a single large homogenous population-based cohort, and to identify new genome-wide significant loci using both conventional and expression-guided approaches.

**Methods—**We examined the associations of 18 SNPs with genome-wide significance ($P < 5.0 \times 10^{-8}$, 'primary'), and 13 suggestive SNPs ($5.0 \times 10^{-8} < P < 2.6 \times 10^{-5}$, 'secondary'), all from previously established genome-wide association studies, with self-reported blood pressure in 23,019 women from the Women's Genome Health Study. We then targeted for replication 12 gene expression-associated SNPs (eSNPs) that were also previously associated with blood pressure phenotypes.

**Results—**Using these replication strategies, we found confirmatory evidence for 13/18 primary SNPs, 3/13 secondary SNPs, and 4/12 eSNPs in the Women's Genome Health Study. Meta-analysis combining the Women's Genome Health Study results with prior study results revealed one previously unrecognized blood pressure locus with genome-wide significance: a *BLK-GATA4*-adjacent region ($P = 3.2 \times 10^{-8}$).

**Conclusion—**In this analysis, conventional and eSNP-guided strategies were complementary and illustrate two ways for extending initial genome-wide association results for discovery of new genes involved in human disease. Using this strategy, we report a newly identified blood pressure locus, *BLK-GATA4*, that may further understanding of the complex genetic pathways regulating blood pressure.

## Keywords

hypertension; genetics; blood pressure; women

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## Introduction

Hypertension (HTN) affects one in four adults worldwide, a proportion that is expected to increase to one in three by 2025 [1]. Due to the substantial heritability (30–60%) of blood pressure (BP) [2] and the dire clinical consequences of HTN, understanding the genetic basis of BP regulation is of great interest. Rare mutations in renal salt handling genes have been shown to be associated with substantial BP effects [3] but they are too rare to explain a substantial proportion of inter-individual BP variation within the population. Similarly, candidate gene studies to date have only explained a small fraction of the population-wide variation in BP, and data have been inconsistent [4,5,6].

Because BP is influenced by multiple common genetic variants, each with small independent effects, the identification of common polymorphisms associated with HTN susceptibility has been challenging. Recently, two large-scale genome-wide association studies (GWAS), both conducted by meta-analysis, identified common genetic variants associated with BP in individuals of European ancestry. The Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) and Global BPgen consortia each identified 8 BP loci with P-values $< 5.0 \times 10^{-8}$ [7,8]. We sought to validate previously identified BP loci by *in silico* replication, and to identify new genome-wide significant loci associated with BP in a large and homogeneous prospective cohort study, the Women's Genome Health Study (WGHS) [9]. We used two complementary strategies to identify truly associated BP loci that might not satisfy conventional thresholds for genome-wide significance ($P < 5 \times 10^{-8}$), which is expected to minimize false positive results but may be too restrictive. First, SNPs were prioritized for replication solely based on association p-value in previous GWAS efforts. Second, the selection procedure was guided by an independent source of biological function through targeting SNPs that had not only prior evidence of some association with BP but also were associated with gene expression patterns in liver or lymphocyte derived cell-lines.

## Methods

### Participants

Analysis was conducted in the WGHS, a prospective cohort of participants in the Women's Health Study (WHS) [10], which enrolled female health professionals from North America, ≥ 45 years of age without cardiovascular disease or other major chronic illnesses [11]. The WGHS is the subset of 28,345 (70.6%) of the original WHS participants, who provided a sample at baseline for blood-based analysis. The primary aim of the WGHS was to create a comprehensive genome-wide database of > 360 000 SNPs among initially healthy WHS participants, and to link genome-wide data to the existing epidemiologic databank of the parent WGHS.

### Determination of BP phenotype

Baseline BP was self-reported in categories by the female health professionals, a group in which self-reported BP has been highly accurate when compared to chart review [12,13,14]. BP was broken into 9 categories for systolic BP (SBP) (< 110, 110–119, 120–129, 130–139, 140–149, 150–159, 160–169, 170–179, ≥ 180 mmHg), and 7 for diastolic BP (DBP) (<65, 65–74, 75–84, 85–89, 90–94, 95–104, ≥ 105 mmHg). The midpoint of each category was used for analysis. The use of self-reported BP in categories was acknowledged to be a limitation in our study from the inception, as it would predominantly limit our power to detect smaller associations, and underestimate the strength of any positive associations found. Prevalent HTN was defined as a history of physician-diagnosed HTN and ongoing HTN treatment, or SBP ≥ 140 or DBP ≥ 90 mmHg. To account for treatment effect, 10 and

5 mmHg were added to the measured SBP and DBP, respectively, if a participant was taking antihypertensive medication [15].

### Genotyping

Detailed methods regarding genotyping have been previously described [9]. In brief, SNP genotyping was performed using the Illumina Infinium II assay [16] applied to the HumanHap300 Duo "+" platform, including a genome-wide set of haplotype-tagging SNP markers suitable for populations with European ancestry [17]. A focused panel of 45,882 missense and haplotype-tagging SNPs was included (the "+" content) to enhance coverage of genomic regions believed to be of significance in cardiovascular diseases. In the final experimental data, all samples had complete genotype information for 98% of the SNPs; all SNPs met Hardy-Weinberg equilibrium using an exact test ($P > 1.0 \times 10^{-6}$) and had successful genotyping for at least 90% of the samples. Among participants of European ancestry, imputation using Mach v. 1.0.16 (http://www.sph.umich.edu/csg/abecasis/mach/) and linkage disequilibrium relationships from the HapMap CEU population allowed estimation of dose of the coded allele at SNPs not determined experimentally or missing from samples which nonetheless met quality standards. The allele dose estimates were used in all regression analysis to evaluate SNP associations with BP phenotypes.

### Selection of candidate SNPs for replication

We used two complementary methods to validate previous findings and identify novel associations with BP. First, on the basis of previously published p-value alone, candidate SNPs were selected from BP-related loci identified in previous studies, and classified as primary when the prior association was genome-wide significant ($P < 5.0 \times 10^{-8}$), or secondary when it was suggestive ($5.0 \times 10^{-8} < P < 5.6 \times 10^{-5}$). For rs5761405, imputation was unavailable in Global BPgen, and a proxy rs2092201 within the same locus ($r^2 = 1.0$) was selected for joint analysis with CHARGE and external validation in the WGHS. Similarly, rs880315 was used as a proxy for rs12046278 ($r^2 = 0.86$). Two additional loci implicated in prior association studies (*STK39* [18] and *CDH13* [19]) were also included among the secondary SNPs for a total of 13.

Second, we selected an additional set of candidate SNPs for replication by targeting SNPs that were both associated with *cis*-gene expression levels in liver (n=3,322) [20] or immortalized lymphocyte (n=10,823) [21] derived cell lines and also associated with BP in previous analysis from CHARGE [7] The threshold for significance of association with BP in CHARGE was defined as $P < 1/n$, for n equal to the number of tissue specific SNPs interrogated, a threshold that was expected to generate one false-positive result per tissue examined. For liver expression, this threshold was $P < 3.0 \times 10^{-4}$; for lymphoblastoid cell lines the threshold was $P < 9.2 \times 10^{-5}$. A total of 24 eSNPs meeting these criteria from 12 loci were identified in CHARGE. Of these, one eSNP per locus was selected for a total of 12 eSNPs for analysis in the WGHS.

### Statistical analysis

Association of SNP genotypes with BP in the WGHS was evaluated with cross-sectional analyses using an additive genetic model. Multiple linear regression was used for BP, and logistic regression for dichotomous HTN outcomes. We addressed the multiple testing as follows: a total of 43 SNPs were tested (18 primary SNPs, 13 secondary SNPs, and 12 eSNPs), thus significant replication for the primary trait was considered at $P = 0.05/43 = 1.2 \times 10^{-3}$. The primary trait was defined as either SBP or DBP, whichever association was more significant in prior studies. For non-primary traits, significant replication was considered at $P = 0.05/(43\times2) = 5.8 \times 10^{-4}$. One-tailed P-values were used if the beta coefficients for the allele were in the same direction as in prior studies.



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Using meta-analysis with inverse variance weighting and fixed effects, the WGHS results were combined with findings from prior studies. Of note, *CDH13* was not included in the meta-analysis, as the previous report used a dominant, rather than additive genetic model [18]. Similarly, *STK39* was not included, because the beta-coefficients and standard errors were not available from the previous study [19]. Neither locus met replication criteria within WGHS.

A trait-specific genetic BP risk score was developed using loci that had been identified as associated with BP at a $P < 5.0 \times 10^{-8}$ in prior or present analyses. For a locus with more than one candidate SNP, the SNP with the largest absolute beta-coefficient was selected. A risk score was constructed for each individual in the WGHS by summing the weighted dose of the risk alleles at the chosen SNPs, with weights equal in magnitude to the beta-coefficients in previous reports [7,8] and risk allele chosen to be consistently associated with higher BP across all score SNPs. Both SBP and DBP risk scores were used to calculate odds ratios of HTN, with the reference group being those with median scores (SBP risk score = 8, DBP risk score = 4.5).

## Results

The WGHS sample with BP data and genome-wide genotyping that was eligible for this investigation included 23,019 women of confirmed, self-reported European descent with mean age of 54 ± 7 years. The mean SBP was 125 ± 15 mmHg, DBP was 77 ± 10 mmHg, and 5,699 (25%) had a physician diagnosis of HTN at baseline. Thirteen percent were taking anti-hypertensive medications, 12% were smokers, and 44% were using hormone replacement therapy at baseline.

### Replication of GWA-derived SNPs

Replication in the WGHS supported associations with 5 of 7 primary SNPs for SBP (5 of 6 SBP loci due to 2 SNPs within the same locus: *SH2B3*, *ATP2B1*, *MTHFR*, *CYP17A1* [rs11191548 and rs1004467, pairwise $r^2 = 0.42$], *PLEKHA7*) at the pre-specified P-value < $1.2 \times 10^{-3}$. Similarly, 8 of 11 primary SNPs were associated with DBP in the WGHS (6 of 9 DBP loci, *CACNB2*, *ATP2B1*, *CYP1A2-CSK-ULK3* [rs1378942 and rs6495122, $r^2 = 0.59$], *c10orf107*, *SH2B3* [rs653178 and rs3184504, $r^2 = 1.0$], *ZNF652*) (Table 1). Among secondary SNPs, 2 of 8 SNPs were associated with SBP (*CACNB2*, *CASZ1*), and 1 of 5 with DBP (*PLEKHA7*) in the WGHS (Table 2). For beta coefficients, please see Supplemental Digital Content, Tables 1 and 2.

The association between candidate SNPs and BP was robust across different BP traits, as loci replicating with SBP in the WGHS also showed evidence of association with DBP, and vice versa. There was also significant association between 3 of 12 replicated primary, and 1 of 3 secondary SNPs with hypertension (Supplemental Digital Content, Table 3). Overall, the directional association (sign of beta) in the WGHS matched CHARGE or Global BPgen for 27 of 29 SNPs. Two secondary SNPs that were not associated with BP in WGHS had discordant beta coefficients compared with previous studies (rs13423988, P=0.39, and rs13401889, P=0.40).

### Replication of expression-associated SNPs

Four of 12 loci selected for potential functional effects through their association with gene expression met replication thresholds for BP phenotypes within WGHS (Table 3). The validated eSNPs were rs7537765 (*CLCN6* near *MTHFR*, *NPPA*; expressed gene *CLCN6*), rs6495126 (*MPI*, *SCAMP2*, *ULK3*; expressed genes *ULK3*, *AK001918*, *RPP25*), rs6601414

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(*MSRA*; expressed gene *C8orf5*), and rs2898290 (*BLK*, *GATA4*; expressed genes C8orf5, FAM167A, BLK).

### Meta-analysis of WGHS results and prior studies

Table 4 provides meta-analysis results combining WGHS data with prior study results (for beta coefficients please see Supplemental Digital Content, Table 4). For GWA-derived candidate SNPs, one new genome-wide association emerged for SBP (rs880315; *CASZ1*), a locus that recently replicated with genome-wide significance in the Japanese population.[22] One additional association was suggestive with $P = 9.1 \times 10^{-8}$ (rs448378; *MDS1*). One locus previously associated with DBP emerged as newly genome-wide significant in association with SBP (*CACNB2*), and one locus previously associated with SBP emerged as newly significant in association with DBP (*PLEKHA7*). In meta-analysis of eSNP results, there were 3 loci that were associated with BP at $P < 5.0 \times 10^{-8}$: *CLCN6-MTHFR-NPPA* and *ULK3* met genome-wide significance in prior studies, whereas the association at *BLK-GATA4* was newly significant. Of note, the eSNP near *ULK3* (rs6495126), and the GWA-derived SNP at *ULK3* (rs6495122) are in modest linkage disequilibrium ($r^2 = 0.49$), suggesting that SNP-SNP correlation may not fully explain this finding, and that rs6495126 may potentially represent a separate locus of interest. One additional locus was suggestive with $P = 2.5 \times 10^{-7}$ (*SEC31A-SCD5*).

### Blood pressure risk score

Weighted genetic risk scores were evaluated for the association with the deviation of SBP and DBP from the mean, and the odds ratio for prevalent HTN (Figure 1). As expected, there was a dose-response relationship between increasing risk score and higher BP: the range of the SBP risk score was associated with a 6 mmHg spread in SBP, and the odds ratio of HTN ranged from 0.7 for individuals with a risk score < 5 (95% CI 0.4–1.0, P=0.03), to an odds ratio of 1.9 for a risk score > 11 (95% CI 1.4–2.4, P<0.0001) when compared to individuals with a median risk score of 8. The proportion of total variance in BP (increment in $r^2$) explained in the WGHS by the SBP risk score was 0.3%, and that of the DBP risk score was 0.4%, after accounting for age, age$^2$, and BMI. The relatively minor contribution of aggregate genetic loci to overall variation in BP is consistent with prior studies [7,8].

## Discussion

While demonstrating the utility of a strategy for functional enrichment based on gene expression, the major new finding from this work is the successful identification of novel genome-wide significant BP loci. Our eSNP analysis identified rs2898290 as a novel locus associated with BP on a genome-wide level, an eSNP that is in linkage disequilibrium with a SNP located within *GATA4* (rs13259242, $r^2 = 0.56$, distance 159,124 bp, based on HapMap CEU population, release 22). *GATA4* is known to be involved in the regulation of brain natriuretic peptide [23], and has been linked to congenital heart disease and the development of cardiac hypertrophy in response to pressure overload [24]. The latter pathway may be mediated via atrial natriuretic peptide, which recently has been shown to reduce *GATA4* phosphorylation and *GATA4*-dependent transcriptional activity, resulting in inhibition of endothelin-1 gene expression in cardiac fibroblasts [25]. While *GATA4* has not previously been linked to hypertension, its regulation of endothelin-1 gene expression, a potent vasoconstrictor, is a biologically plausible mechanism by which common variants at the *GATA4* locus may influence BP phenotypes.

Furthermore, rs880315 located in an intronic region of *CASZ1*, a zinc finger transcription factor, was associated with SBP after meta-analysis of WGHS and CHARGE results. While this association is newly genome-wide significant in a population of European descent, it

appeared to have a stronger association with BP in the Japanese population, where the association was genome-wide significant for both SBP and DBP.[22] The gene encodes castor homolog-1, a zinc finger transcription factor that is thought to be involved in cell cycle signaling and the regulation of apoptosis. It resides on chromosome 1p36.22, and is expressed in a variety of tissues, including cardiac myocytes. Loss of the chromosomal region around *CASZ1* is frequently seen in neuroectodermal tumors [26,27].

These findings support the concept that targeted searches for variants associated with gene expression may be useful in the identification of significant loci associated with clinical phenotypes in the absence of genome-wide significance. Previous studies have provided a basis for this concept by linking expression-associated SNPs to BP [7] and other phenotypes, such as dyslipidemia [28]. To our knowledge, this is the first study to replicate successfully and by design both conventional and gene expression-associated loci. We provide initial evidence that our eSNP-based approach may be a rapid and cost-effective strategy for identifying relevant loci now that databases of eSNPs are increasingly available. Nevertheless, broader replication efforts are needed to corroborate this approach.

Two recent studies have replicated results from CHARGE and Global BPgen in Asian populations: the Korean Association Resource (KARE) study showed replication for 4 BP loci (*ATP2B1, CSK, CYP17A1,* and *PLEKHA7*) [29], and 7 additional loci (*CASZ1, MTHFR, ITGA9, FGF5, CYP17A1-CNNM2, ATP2B1,* and *CSK-ULK3*) were replicated in a Japanese study combining 3 separate cohorts [22]. The WGHS is unique in exceeding the sample size of any other single cohort study used in genome-wide association analysis examining BP, and it alone approaches the size of prior consortia. Our study results extend previous findings of CHARGE and Global BPgen by providing external validation of a number of loci including *CYP17A1* (a gene associated with a rare Mendelian form of HTN [30]) and *ATP2B1* (encoding a plasma membrane calcium/calmodulin-dependent ATPase known to regulate BP in animal models [31]). For functions of other BP-related loci, please see Supplemental Digital Content, Table 5. Unlike prior GWAS, ours was conducted exclusively in women. Despite known sex differences in the natural history of BP [32], it is notable that previous BP genetic loci from cohorts including both men and women could be confirmed in this large cohort of women. Further studies are needed to explore the potential for gene-sex interactions. At the same time, the lack of heterogeneity with respect to sex in the WGHS might have improved our ability to validate loci.

Of the primary BP loci we studied, 4 were not supported by highly significant association within WGHS (*PLCD3*, *TBX3-TBX5*, *FGF5*, and *ULK4*), but all had the same direction of effects as prior studies (sign of beta). The non-replicating SNPs may reflect associations that are intrinsically weaker in women than men, or may be due to variable SNP imputation quality, complex underlying haplotype structures not accounted for in the analysis, or limitations in power for the given sample size in WGHS.

While our results indicate a strong correlation between a BP genetic risk score and BP traits in WGHS, the aggregate of the effect of the validated loci in the genetic risk score did not explain a large proportion of the inter-individual variability in BP, a finding that is consistent with prior studies [7,8]. Many BP-related loci, including loci harboring rare variants, remain unidentified, and complex gene-gene and gene-environment interactions are unaccounted for, rendering our risk score incomplete. The role, if any, for the genetic risk score in the prediction of incident HTN and long-term BP-related outcomes is unknown.

Aside from the restriction of the WGHS to the socio-economic group defined by white, female, health care professionals, the major potential limitation of our study is the use of self-reported estimates of BP. However, this limitation would be expected to bias our



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findings toward the null, limiting the ability to detect associations, and thus is in sharp contrast with the high rate of validation of candidate associations in the current analysis using the WGHS. Prior evidence suggests that within a population of health professionals, self-reported BP appears extremely accurate when validated against directly measured blood pressure [12]. Furthermore, the validity of this approach has been examined in the Nurses' Health Study, in which 99% of women who reported high blood pressure had their diagnosis confirmed by chart review [13]. Previous analyses from the WHS have shown self-reported BP to be a strong predictor of cardiovascular risk, with relative risks similar in magnitude to other major studies, and SBP is in fact the strongest cardiovascular risk factor after age in WHS, which strengthens the validity of self-reported BP [33,34]. In a prior report from the WGHS, self-reported BP progression was also associated with *NPPA* gene polymorphisms, and provides evidence that despite these limitations in outcome assessment, a significant genetic association could be detected [35]. We relied on assigning midpoints from each BP category (spanning up to 10 mmHg for SBP), which may have limited precision of the primary outcome measurement, given that the effect sizes of each locus we detected were < 1 mmHg/allele. Furthermore, we adjusted for antihypertensive treatment effect by adding 10 and 5 mmHg to SBP and DBP, as was done in CHARGE[7]. However, adjustment in Global BPgen was done using 15 and 10 mmHg, respectively, and thus may have decreased our power to validate results specifically driven by Global BPgen[8]. Again, these limitations in assessment of BP would be expected to bias our results toward the null. However, given the limited precision of self-reported BP assessment in categories, we cannot exclude potential small effects and false negative findings of genetic loci evaluated in our study. Finally, validation of our findings in an additional independent sample would be an important consideration in the future.

## Conclusion

Replication of several previously identified genetic loci and putative functional variants associated with BP was successful in an independent large cohort of women health professionals of European ancestry. Using a novel approach to select candidate SNPs by incorporating both GWAS-derived loci, as well as a strategy for functional enrichment based on a gene expression, we report a new genetic locus associated with SBP at a genome-wide significance level: *GATA4*, a gene thought to regulate brain natriuretic peptide and myocyte hypertrophy. Functional characterization of this locus may further understanding of BP regulation and HTN pathophysiology, improve early risk detection, and spawn new therapeutic interventions. In addition, the bioinformatics analysis strategy implemented here to identify new loci is broadly applicable to areas beyond HTN and may hasten a better understanding of the genetic underpinnings of other complex human diseases.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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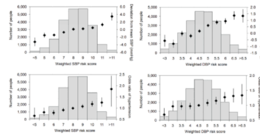

**Figure 1. SBP and DBP risk scores**

Aggregate effects of risk alleles on blood pressure phenotypes are summarized in a weighted risk score for SBP and DBP, respectively. The relationship between risk score and deviation from mean SBP or DBP are shown in the top panels. Black diamonds indicate the mean BP deviation for each risk score grouping, with black whiskers indicating the standard errors. The bottom panels show black diamonds to indicate the relationship between SBP (left) and DBP (right) risk score and odds ratios of HTN, with black whiskers representing 95% CIs. The p-values for slopes across risk score groups were all highly significant ($P < 0.0001$ for all 4 comparisons)

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**Table 1**

Replication of primary SNPs in WGHS by BP trait

| Trait | SNP | Chromosome (Position, NCBI 36.3) | Nearest Gene(s) | Allele (coded/other) | Freq. coded allele | CHARGE/Global BPgen p-value | WGHS p-value* | WGHS (non-primary trait)† p-value* |
|---|---|---|---|---|---|---|---|---|
| SBP | **rs3184504** | 12 (110,368,991) | ***SH2B3*** | C/T | 0.51 | 5.7E-07 | **5.0E-06** | **2.3E-04** |
| | **rs2681492** | 12 (88,537,220) | ***ATP2B1*** | C/T | 0.19 | 3.0E-11 | **6.0E-06** | **5.0E-06** |
| | **rs17367504** | 1 (11,785,365) | ***MTHFR*** | A/G | 0.84 | 1.0E-05 | **4.0E-05** | **4.0E-05** |
| | **rs11191548** | 10 (104,836,168) | ***NT5C2; CYP17A1*** | C/T | 0.09 | 3.0E-07 | **2.1E-04** | 1.1E-03 |
| | **rs381815** | 11 (16,858,844) | ***PLEKHA7*** | C/T | 0.73 | 5.8E-07 | **1.1E-03** | **7.5E-05** |
| | rs1004467 | 10 (104,584,497) | *CYP17A1* | A/G | 0.90 | 2.0E-06 | 2.8E-03 | 2.1E-03 |
| | rs12946454 | 17 (40,563,647) | *ACBD4* | A/T | 0.74 | 4.0E-06 | 3.2E-01 | 2.8E-01 |
| DBP | **rs11014166** | 10 (18,748,804) | ***CACNB2*** | A/T | 0.65 | 8.7E-07 | **2.2E-06** | **3.2E-05** |
| | **rs2681472** | 12 (88,533,090) | ***ATP2B1*** | A/G | 0.83 | 3.7E-08 | **3.2E-06** | **1.6E-06** |
| | **rs1378942** | 15 (72,864,420) | ***CSK*** | A/C | 0.66 | 6.0E-08 | **2.8E-05** | **4.6E-05** |
| | **rs1530440** | 10 (63,194,597) | ***c10orf107*** | C/T | 0.81 | 3.0E-06 | **3.1E-05** | **2.3E-06** |
| | **rs653178** | 12 (110,492,139) | ***ATXN2; SH2B*** | C/T | 0.49 | 1.0E-07 | **2.1E-04** | **5.0E-06** |
| | **rs3184504** | 12 (110,368,991) | ***SH2B3*** | C/T | 0.51 | 1.7E-08 | **2.3E-04** | **5.0E-06** |
| | **rs6495122** | 15 (72,912,698) | ***CSK-ULK3*** | A/C | 0.43 | 8.1E-07 | **3.0E-04** | 9.5E-04 |
| | **rs16948048** | 17 (44,795,465) | ***ZNF652*** | A/G | 0.63 | 5.0E-06 | **4.9E-04** | 3.8E-03 |
| | rs2384550 | 12 (113,837,114) | *TBX3-TBX5* | A/G | 0.35 | 1.3E-07 | 1.9E-02 | 3.6E-03 |
| | rs16998073 | 4 (81,403,365) | *FGF5* | A/T | 0.76 | 7.0E-09 | 8.0E-02 | **5.5E-04** |
| | rs9815354 | 3 (41,887,655) | *ULK4* | A/G | 0.17 | 7.8E-07 | 2.2E-01 | 9.5E-02 |

*One-tailed p-value, *a priori* significance at $P < 1.2 \times 10^{-3}$ for primary trait, and $P < 5.8 \times 10^{-4}$ for the non-primary trait (met when indicated in bold face font)

†Non-primary trait is DBP if the association was first reported with SBP in previous studies, and SBP if previous association was with DBP.

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## Table 2

Replication of secondary SNPs in WGHS by BP trait

| | | | | | | Prior study* | WGHS | WGHS (non-primary trait)‡ |
|---|---|---|---|---|---|---|---|---|
| **Trait** | **SNP** | **Chromosome (Position, NCBI 36.3)** | **Nearest Gene** | **Allele (coded/other)** | **Freq. coded allele** | **p-value** | **p-value†** | **p-value†** |
| SBP | **rs11014166** | **10 (18,748,804)** | ***CACNB2*** | A/T | 0.65 | 2.1E-06 | **3.2E-05** | **2.2E-06** |
| | **rs880315** | **1 (10,719,453)** | ***CASZ1*** | C/T | 0.35 | 2.1E-07 | **7.0E-04** | 7.0E-03 |
| | rs448378 | 3 (170,583,593) | *MDS1* | A/G | 0.53 | 1.3E-06 | 3.4E-03 | 3.7E-02 |
| | rs1910252 | 8 (49,569,915) | *EFCAB1* | C/T | 0.83 | 1.7E-06 | 3.0E-02 | 2.4E-01 |
| | rs2736376 | 8 (11,155,175) | *MTMR9* | C/G | 0.13 | 1.9E-06 | 2.2E-01 | 3.3E-01 |
| | rs2092201 | 22 (24,910,475) | *SEZ6L* | C/T | 0.96 | 2.6E-05 | 2.3E-01 | 4.2E-01 |
| | rs7571613 | 2 (190,513,907) | *PMS1* | A/G | 0.82 | 7.2E-07 | 3.4E-01 | 4.4E-01 |
| | rs6749447 | 2 (168,749,632) | *STK39* | G/T | 0.27 | 1.6E-07 | 3.8E-01 | 1.5E-01 |
| DBP | **rs11024074** | **11 (16,873,795)** | ***PLEKHA7*** | C/T | 0.30 | 2.8E-07 | **3.4E-04** | 4.4E-03 |
| | rs7016759 | 8 (49,574,969) | *EFCAB1* | C/T | 0.17 | 1.9E-06 | 2.5E-01 | 3.9E-02 |
| | rs13423988 | 2 (68,764,770) | *GPR73-ARGHGAP25* | C/T | 0.83 | 1.1E-06 | 3.9E-01 | 4.2E-01 |
| | rs13401889 | 2 (190,618,804) | *MSTN* | C/T | 0.22 | 9.7E-07 | 4.0E-01 | 4.1E-01 |
| | rs11646213 | 16 (66,940,701) | *CDH13* | A/T | 0.41 | 5.6E-05 | 3.2E-01 | 2.8E-01 |

*Prior study is CHARGE (Levy et al [7]), except for *STK39* (Wang et al [18]) and *CDH13* (Org et al [19])

†One-tailed p-value, *a priori* significance at $P < 1.2 \times 10^{-3}$ for primary trait, and $P < 5.8 \times 10^{-4}$ for the non-primary trait (met when indicated in bold face font)

‡Non-primary trait is DBP if the association was first reported with SBP in previous studies, and SBP if previous association was with DBP.

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## Table 3

Association of CHARGE eSNPS with blood pressure phenotypes in the WGHS

| SNP | Chromosome (Position, NCBI 36.3) | Nearest Gene(s) | Expressed gene | Allele (coded/other) | Freq. coded allele | CHARGE p-values* | | WGHS p-values† | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | SBP | DBP | SBP | DBP |
| Liver eSNPs | | | | | | | | | |
| rs7537765 | 1 (11,809,890) | *CLCN6; MTHFR;NPPA* | *CLCN6* | A/G | 0.84 | **1.8E-05** | 9.2E-04 | **5.2E-05** | **1.1E-04** |
| rs249209 | 5 (79,902,965) | *ANKRD34B* | *HSS00169533* | G/T | 0.41 | **8.5E-05** | 0.07 | 0.85 | 0.73 |
| rs525381 | 12 (255,053) | *KDM5A; SLC6A13* | *CCDC77; SLC6A12* | A/G | 0.28 | **9.3E-05** | 0.14 | 0.04 | 0.06 |
| rs739496 | 12 (110,372,042) | *SH2B3; ATXN2* | *HSS00340376* | A/G | 0.79 | **2.9E-04** | **1.3E-05** | 0.01 | 0.02 |
| rs6495126 | 15 (72,962,079) | *MPI;SCAMP2; ULK3* | *ULK3;RPP25; AK001918;* | A/G | 0.30 | **3.0E-04** | **3.6E-05** | **1.1E-04** | **3.7E-05** |
| Lymphoblastoid cell line eSNPs | | | | | | | | | |
| rs1384883 | 1 (74,274,065) | *LRRIQ3* | *LRRC44;BC042056* | C/T | 0.46 | 9.9E-03 | **7.2E-05** | 0.51 | 0.56 |
| rs12466395 | 2 (190,488,943) | *PMS1* | *ORMDL1* | A/G | 0.22 | 8.8E-04 | **5.3E-05** | 0.37 | 0.28 |
| rs7571613 | 2 (190,513,907) | *MSTN* | *ORMDL1;PMS1* | A/G | 0.82 | **7.3E-07** | **2.2E-06** | 0.67 | 0.88 |
| rs2272007 | 3 (41,971,140) | *ULK4* | *ULK4* | C/T | 0.83 | 0.87 | **1.5E-06** | 0.22 | 0.37 |
| rs4572871 | 4 (83,979,911) | *SEC31A; SCD5* | *SCD5* | A/G | 0.21 | **2.3E-05** | 9.7E-03 | 2.4E-03 | 3.1E-03 |
| rs6601414 | 8 (10,014,158) | *MSRA* | *C8orf5* | A/G | 0.46 | 3.0E-04 | **3.4E-05** | **6.9E-04** | 0.01 |
| rs2898290 | 8 (11,471,318) | *BLK;GATA4* | *C8orf5;BLK; FAM167A;* | C/T | 0.53 | **2.3E-05** | 7.0E-03 | **4.1E-04** | 0.02 |

*Bold face font indicates statistical significance met in CHARGE at P = 1/n (n = number of eSNPs interrogated in CHARGE)

†Bold face font indicates replication threshold met in WGHS at $P < 1.2 \times 10^{-3}$ for primary traits, and $P < 5.8 \times 10^{-4}$ for non-primary traits

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## Table 4

Meta-analysis of WGHS and CHARGE or Global BPgen results*

| | Trait | SNP | Nearest gene(s) | Allele (coded/other) | Freq. coded allele | p-value* |
|---|---|---|---|---|---|---|
| Primary SNPs | SBP | **rs3184504** | ***SH2B3*** | C/T | 0.51 | **1.9E-11** |
| | | **rs2681492** | ***ATP2B1*** | C/T | 0.19 | **4.9E-15** |
| | | **rs17367504** | ***MTHFR*** | A/G | 0.84 | **1.2E-09**† |
| | | **rs11191548** | ***NT5C2;CYP17A1*** | C/T | 0.09 | **1.1E-09**† |
| | | **rs381815** | ***PLEKHA7*** | C/T | 0.73 | **1.1E-08** |
| | | rs1004467 | *CYP17A1* | A/G | 0.90 | 1.1E-07 |
| | | rs12946454 | *ACBD4* | A/T | 0.74 | 4.4E-04† |
| | DBP | **rs11014166** | ***CACNB2*** | A/T | 0.65 | **9.7E-12** |
| | | **rs2681472** | ***ATP2B1*** | A/G | 0.83 | **7.0E-13** |
| | | **rs1378942** | ***CSK;CYP1A2*** | A/C | 0.66 | **3.5E-11**† |
| | | **rs1530440** | ***c10orf107*** | C/T | 0.81 | **9.6E-10**† |
| | | **rs653178** | ***ATXN2;SH2B3*** | C/T | 0.49 | **1.2E-09**† |
| | | **rs3184504** | ***SH2B3*** | C/T | 0.51 | **5.6E-11** |
| | | **rs6495122** | ***CSK-ULK3*** | A/C | 0.43 | **2.2E-09** |
| | | **rs16948048** | ***ZNF652*** | A/G | 0.63 | **4.5E-08**† |
| | | rs2384550 | *TBX3-TBX5* | A/G | 0.35 | 1.3E-07 |
| | | rs16998073 | *FGF5* | A/T | 0.76 | 2.1E-07† |
| | | rs9815354 | *ULK4* | A/G | 0.17 | 3.7E-05 |
| Secondary SNPs | SBP | **rs11014166** | ***CACNB2*** | A/T | 0.65 | **4.6E-10** |
| | | **rs880315** | ***CASZ1*** | C/T | 0.35 | **5.2E-09** |
| | | rs448378 | *MDS1* | A/G | 0.53 | 9.1E-08 |
| | | rs1910252 | *EFCAB1* | C/T | 0.83 | 2.6E-06 |
| | | rs2736376 | *MTMR9* | C/G | 0.13 | 1.2E-04 |
| | | rs2092201 | *SEZ6L* | C/T | 0.96 | 6.7E-04 |
| | | rs7571613 | *PMS1* | A/G | 0.82 | 2.3E-04 |
| | DBP | **rs11024074** | ***PLEKHA7*** | C/T | 0.30 | **1.1E-09** |
| | | rs7016759 | *EFCAB1* | C/T | 0.17 | 8.9E-05 |

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| | Trait | SNP | Nearest gene(s) | Allele (coded/other) | Freq. coded allele | p-value* |
|---|---|---|---|---|---|---|
| | | rs13423988 | *GPR73-ARGHGAP25* | C/T | 0.83 | 8.1E-04 |
| | | rs13401889 | *MSTN* | C/T | 0.22 | 8.7E-04 |
| eSNPs | SBP | **rs7537765** | ***CLCN6;MTHFR*** | A/G | 0.84 | **2.6E-09** |
| | | rs249209 | *ANKRD34B* | G/T | 0.41 | 5.0E-03 |
| | | rs525381 | *KDM5A;SLC6A13* | A/G | 0.28 | 2.5E-05 |
| | | rs7571613 | *MSTN* | A/G | 0.82 | 2.3E-04 |
| | | rs4572871 | *SEC31A;*near *SCD5* | A/G | 0.21 | 2.5E-07 |
| | | **rs2898290** | ***BLK;GATA4*** | C/T | 0.53 | **3.2E-08** |
| | DBP | rs739496 | *SH2B3;ATXN2* | A/G | 0.79 | 1.2E-06 |
| | | **rs6495126** | ***MPI;SCAMP2;ULK3*** | A/G | 0.30 | **3.4E-09** |
| | | rs1384883 | *LRRIQ3* | C/T | 0.46 | 1.2E-03 |
| | | rs12466395 | *PMS1* | A/G | 0.22 | 2.0E-04 |
| | | rs2272007 | *ULK4* | C/T | 0.83 | 3.7E-05 |
| | | rs6601414 | *MSRA* | A/G | 0.46 | 2.0E-06 |

Bold face font indicates genome-wide significance met at $P < 5.0 \times 10^{-8}$

*Of note, *CDH13* was not included in the meta-analysis, as the previous report used a dominant, rather than additive genetic model [18]. Similarly, *STK39* was not included, because the beta-coefficients and standard errors were not available from the previous study [19]

†Meta-analysis combines CHARGE and WGHS, unless indicated by footnote, in which case the analysis combines Global BPgen and WGHS results