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Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci

Meta-analyses of association results for blood pressure using exome-centric single-variant and gene-based tests identified 31 new loci in a discovery stage among 146,562 individuals, with follow-up and meta-analysis in 180,726 additional individuals (total *n* = 327,288). These blood pressure–associated loci are enriched for known variants for cardiometabolic traits. Associations were also observed for the aggregation of rare and low-frequency missense variants in three genes, *NPR1*, *DBH*, and *PTPMT1*. In addition, blood pressure associations at 39 previously reported loci were confirmed. The identified variants implicate biological pathways related to cardiometabolic traits, vascular function, and development. Several new variants are inferred to have roles in transcription or as hubs in protein–protein interaction networks. Genetic risk scores constructed from the identified variants were strongly associated with coronary disease and myocardial infarction. This large collection of blood pressure–associated loci suggests new therapeutic strategies for hypertension, emphasizing a link with cardiometabolic risk.

Hypertension (HTN), or high blood pressure, is a major risk factor for cardiovascular disease, chronic kidney disease, and mortality[1]. Thus far, in addition to identifying rare mutations that cause monogenic disorders with high or low blood pressure[2–4], candidate gene studies, genome-wide association studies (GWAS), and admixture mapping approaches[5–15] have identified variants at more than 60 genetic loci that are associated with blood pressure or HTN. Most of the known blood pressure loci identified in large population-based studies are common noncoding variants with small effects on blood pressure.

The Human Exome BeadChip (Exome Chip; Illumina) was designed to facilitate identification of functional variants that contribute to human traits, by focusing on variants that alter amino acid sequence. The Exome Chip includes 247,039 markers, of which >90% are nonsynonymous or splice-modulating exonic variants that were not covered by previous genotyping arrays. Whereas variants on previous GWAS arrays are largely common (minor allele frequency (MAF) ≥ 0.05), 83% of the Exome Chip variants are rare (MAF < 0.01) and another 6% are low frequency (MAF = 0.01–0.05). Only 11% of the Exome Chip variants are common, including a set of 5,542 common variants (approximately 2% of overall array content) that were drawn from the associations reported in the National Human Genome Research Institute (NHGRI) GWAS catalog[16].

To identify functional coding variation associated with blood pressure, we conducted a two-stage study in up to 327,288 individuals who were genotyped with the Exome Chip (**Fig. 1**) for systolic and diastolic blood pressure (SBP and DBP), pulse pressure (PP), mean arterial pressure (MAP), and HTN. We identified single-variant associations at 31 new loci and gene-based associations for three new genes (two of which overlapped with the single-variant loci) with blood pressure phenotypes. About half of the new blood pressure–associated variants identified in this study reside in loci that were previously reported in GWAS to be associated with circulating lipid levels, immunological diseases, and metabolic phenotypes, suggesting common etiologies of blood pressure and metabolic risk factors and an opportunity to identify therapies that more broadly influence HTN in the context of cardiometabolic risk.

RESULTS

New loci associated with blood pressure by single-variant analyses

In the discovery stage (stage 1), a total of 15 distinct new candidate loci were associated ($P < 3.4 \times 10^{-7}$) with at least one blood pressure trait in a primary meta-analysis among samples of all ancestries and secondary meta-analyses among samples of exclusively European (EA) or African (AA) ancestry (**Supplementary Fig. 1** and **Supplementary Table 1**). Meta-analysis using individuals from all ancestry groups identified 22 new associations at 13 loci that reached experiment-wide significance (**Supplementary Table 1**). All associations with $P < 1 \times 10^{-4}$ for at least one trait in the primary analysis are listed in **Supplementary Table 2**. The sole locus that was associated in the EA analysis but not in the all-ancestry analysis was a rare missense variant, rs3025380, in *DBH* (MAF = 0.005, 0.001, and 0.003 in EA, AA, and Hispanic-ancestry (HA) samples, respectively). Meta-analysis of AA individuals identified association at a common missense variant, rs12941884, in *SEZ6* (MAF = 0.21 and 0.12, respectively, in AA and EA individuals) that was not identified in EA or all-ancestry samples.

The Exome Chip contains 43 SNPs from loci previously identified in GWAS of blood pressure[5–15]. Of these 43 loci, 39 were associated

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with at least one blood pressure trait in stage 1 analyses ($P < 0.05/43 \sim 0.001$) (**Supplementary Table 3**). Twenty-six of these SNPs reached experiment-wide significance ($P < 3.4 \times 10^{-7}$). Conditional analysis did not identify any new independent variants at any of these previously identified loci[5–15].

The 15 newly associated variants ($P < 3.4 \times 10^{-7}$; **Supplementary Table 1**) and 62 additional variants ($P < 1 \times 10^{-5}$ for at least one blood pressure phenotype; **Supplementary Table 2**) from stage 1 were selected for follow-up in 180,726 independent individuals (**Supplementary Note**). Of the 15 newly identified variants, 11 replicated ($P < 0.05/15 \sim 0.0033$) in the follow-up samples (**Supplementary Tables 4** and **5**). In stage 2 analyses (joint meta-analysis of results from the stage 1 and follow-up samples), we identified 48 new blood pressure variants at 31 loci (including the 11 replicated loci) associated with SBP, DBP, PP, or HTN at $P < 3.4 \times 10^{-7}$ (MAP analysis was not possible in the follow-up analyses; **Supplementary Tables 4** and **5**). Among the top variants at the 31 loci, 13 were missense (**Table 1**). In stage 2 analyses restricted to EA samples (**Supplementary Table 4**), all newly identified associations in EA samples meeting the significance threshold were also statistically significant in meta-analysis combining samples from all ancestry groups (**Supplementary Table 5**), with the exception of rs1925153 in *COL21A1*. In addition, all of the variants except for the four that were nominated for follow-up on the basis of PP (SBP minus DBP) showed concordant directions of effect for SBP and DBP (**Supplementary Table 6**).

Three of the 31 new significant SNPs were low frequency (MAF = 0.01–0.05). These SNPs encode nonsynonymous substitutions in the genes *NPR1* (rs35479618), *SVEP1* (rs111245230), and *PTPMT1* (rs11537751). *NPR1* encodes natriuretic peptide receptor 1 and has been reported to be associated with blood pressure regulation in animal models[17,18] but not previously in humans; *SVEP1* and *PTPMT1* are new blood pressure–associated genes. The minor alleles of all three SNPs were associated with increased blood pressure and had larger absolute effects on blood pressure than the alleles of any of the newly identified common variants. For example, each minor allele of rs35479618 was associated with an increase of 0.85 mm Hg in SBP in the follow-up samples as compared with a maximum absolute difference (per minor allele) among the new common variants of 0.43 mm Hg in SBP (for rs8068318 in *TBX2*; **Supplementary Table 5**).

Of the 28 newly identified common variants for blood pressure, 14 were genome-wide significant in previous GWAS of lipid levels[19], immunological disease[20–22], diabetes[23–25], kidney function[26], age at menarche[27], resting heart rate[28], waist–hip ratio[29], and homocysteine concentration[30] but not blood pressure (**Table 2** and **Supplementary Table 7**). Six additional variants were reported for several phenotypes (**Table 2**) in previous candidate gene studies, patent filings, or GWAS, but their *P* values were not specified or did not reach the genome-wide significance level[31–35]. By contrast, the remaining eight variants were missense SNPs that have not been reported in the NHGRI GWAS catalog for any trait (**Table 2**). Several genes in **Table 2** contain multiple variants showing distinct allelic roles. *HOXA3* and *NOS3* harbor variants rs17428471 (*HOXA3*)[12] and rs3918226 (*NOS3*)[10] with genome-wide significant blood pressure associations that are independent of the Exome Chip variants ($r^2 = 0.007$ for rs17428471 and rs6969780 and $r^2 = 0.007$ for rs3918226 and rs891511 in 1000 Genomes Project data). Variant rs2651899 in *PRDM16* has been reported to be associated with migraine[36], but this variant is not in linkage disequilibrium (LD) with the new blood pressure variant, rs2493292 ($r^2 = 0.01$ in 1000 Genomes Project data), suggesting predisposition to distinct vascular consequences for different variants in this locus. In addition, *PRDM16* has been shown to have a critical role in vascular development[37], adipocyte function in subcutaneous fat, and development of diabetes[38]. Finally, several variants in *DOT1L* were reported to be associated with cartilage thickness and hip osteoarthritis[39]. The new blood pressure variant rs2302061, however, was not in LD with any of the previously identified signals at this locus[39].

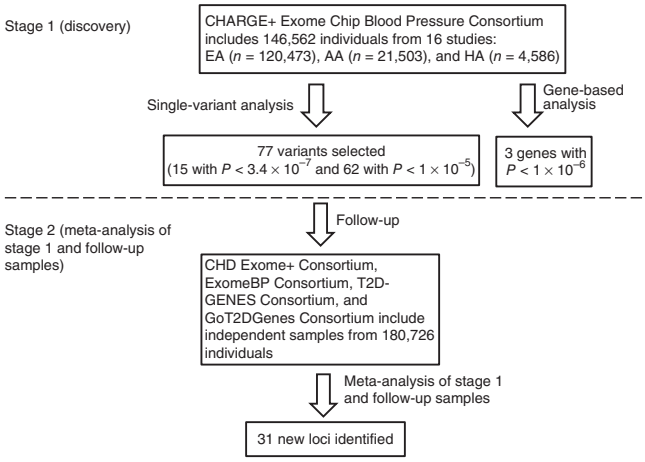

Figure 1 Overall study design. In the discovery phase, single-variant and gene-based analyses were performed for SBP, DBP, PP, MAP, and HTN among 146,562 individuals from the CHARGE+ Exome Chip BP Consortium. Association for 15 variants was significant ($P < 3.4 \times 10^{-7}$), and 62 variants displayed association at $P < 1 \times 10^{-5}$. In the follow-up phase, meta-analysis was performed for 77 variants on results from 180,726 individuals from the CHD Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium, and T2D-GENES Consortium.

Together, the 31 newly identified single variants explain 0.7% and 1.3% of between-individual variation in SBP and DBP, respectively. The previously established and newly identified variants together explain 2.8% and 2.9% of phenotypic variation in SBP and DBP.

Gene-level analyses

We considered the possibility that an aggregation of rare or low-frequency coding alleles in individual genes contributes to variation in blood pressure and specifically tested for effects of nonsynonymous, stop codon, and splicing coding variants with MAF <0.05 (T5 test) or MAF <0.01 (T1 test) using the seqMeta package. The standard burden test[40,41], which is sensitive in detecting association when all variants have effects on blood pressure in a concordant direction, identified an aggregation of rare and low-frequency coding alleles in *PTPMT1* that contribute to higher odds of HTN (experiment-wide significance threshold $P < 1 \times 10^{-6}$; **Table 3** and **Supplementary Table 8a**). Sequence kernel association testing (SKAT)[42], which is designed to detect the effects of alleles that collectively contribute to higher and lower blood pressure, identified significant blood pressure associations for *DBH* (T1) and *NPR1* (T5; **Table 3** and **Supplementary Table 8a**). Among additional EA individuals (up to 154,543 individuals) who were used for follow-up analysis, gene-based SKAT (with the RAREMETAL package) was performed for inverse-normal-transformed DBP, SBP, PP, and HTN (Online Methods). The gene-based associations replicated in the follow-up samples at $P < 0.05/3 \sim 0.017$ for *NPR1* ($P = 4.4 \times 10^{-5}$ for SBP) and were marginally significant for *PTPMT1* ($P = 0.019$ for HTN) and *DBH* ($P = 0.053$ for DBP) (**Supplementary Table 8b**).

Twenty-eight genes previously reported to be associated with monogenic blood pressure disorders[3] contained at least two nonsynonymous, stop codon, or splice-site coding variants with MAF <0.05 for gene-based testing on the Exome Chip. Burden testing of these

Table 1 Significant blood pressure loci ($P < 3.4 \times 10^{-7}$) newly identified in meta-analysis of discovery and follow-up samples

								Discovery (n = 146,562)		Follow-up (n = 180,726)		Combined (n = 327,288)		ICBP discovery (n = 69,395)	
Trait	Locus[a]	dbSNP ID	Chr.	Position (bp)	CA/NCA	CAF	Function[b]	β (SE) or z score[c]	P value	β (SE) or z score[c]	P value	β (SE) or z score[c]	P value	IQ[e] or r^2/IQ	P value SBP, DBP
Low-frequency variants (0.01 < MAF < 0.05)															
SBP	*NPR1*	rs35479618	1	153,662,423	A/G	0.014	Glu967Lys	1.34 (0.28)	2.1×10^{-6}	0.85 (0.30)	3.9×10^{-3}	1.11 (0.20)	5.7×10^{-8}	NA	NA
SBP	*SVEP1*	rs111245230	9	113,169,775	C/T	0.032	Asp2702Gly	0.94 (0.18)	2.9×10^{-7}	0.44 (0.19)	2.2×10^{-2}	0.70 (0.13)	1.2×10^{-7}	1/0.91	0.009, 0.003
HTN	*PTPMT1*	rs11537751	11	47,587,452	T/C	0.048	Ser93Leu	5.09	3.6×10^{-7}	2.72	0.006	5.40	6.9×10^{-8}	1/0.97	0.13, 0.11
Common variants (MAF > 0.05)															
SBP	*PRDM16*	rs2493292	1	3,328,659	T/C	0.151	Pro633Leu	0.42 (0.09)	4.0×10^{-6}	0.32 (0.09)	7.2×10^{-4}	0.37 (0.07)	1.4×10^{-8}	NA	NA
DBP	*PABPC4*	rs4660293	1	40,028,180	G/A	0.208	IN	0.27 (0.05)	1.1×10^{-7}	0.11 (0.04)	0.016	0.18 (0.03)	9.6×10^{-8}	1	0.0030, 0.0018
SBP	*SULT1C3*	rs6722745	2	108,875,244	C/T	0.338	Met194Thr	0.28 (0.08)	3.3×10^{-4}	0.26 (0.07)	9.0×10^{-5}	0.27 (0.05)	1.1×10^{-7}	0.99	0.37, 0.37
PP	*CSNK1G3*	rs4530754	5	122,855,416	G/A	0.411	IN	0.22 (0.05)	4.5×10^{-6}	0.13 (0.04)	2.5×10^{-3}	0.17 (0.03)	9.9×10^{-8}	1	0.03, 0.46
DBP	*C5orf56*	rs2188962	5	131,770,805	T/C	0.366	ncRNA_IN	–0.2 (0.04)	4.2×10^{-6}	–0.19 (0.04)	1.6×10^{-6}	–0.20 (0.03)	3.0×10^{-11}	1	0.86, 0.05
DBP	*SNORD32B*	rs926552	6	29,548,089	T/C	0.111	ITG	–0.31 (0.07)	8.5×10^{-6}	–0.22 (0.07)	1.6×10^{-3}	–0.26 (0.05)	7.2×10^{-8}	0.88	0.44, 0.45
PP	*MSH5-SAPCD1*	rs409558	6	31,708,147	G/A	0.176	ncRNA_IN	–0.22 (0.06)	3.7×10^{-4}	–0.29 (0.06)	1.4×10^{-6}	–0.26 (0.04)	2.7×10^{-9}	1/0.98	0.0019, 0.10
SBP	*SLC22A7*	rs2270860	6	43,270,151	T/C	0.367	SYN, splicing	0.33 (0.07)	2.6×10^{-6}	0.31 (0.07)	2.4×10^{-6}	0.32 (0.05)	2.9×10^{-11}	0.9	0.00013, 0.037
PP	*COL21A1*	rs1925153[d]	6	56,102,780	T/C	0.445	IN	–0.21 (0.05)	1.9×10^{-5}	–0.17 (0.05)	5.9×10^{-4}	–0.19 (0.04)	4.9×10^{-8}	0.71	0.16, 0.42
DBP	*PHIP*	rs10943605	6	79,655,477	A/G	0.462	IN	0.18 (0.04)	1.2×10^{-5}	0.15 (0.04)	5.4×10^{-5}	0.16 (0.03)	3.3×10^{-9}	1	0.05, 0.01
DBP	*HOXA3*	rs6969780	7	27,159,136	C/G	0.125	5′UTR, splicing	0.32 (0.06)	7.8×10^{-7}	0.21 (0.07)	2.0×10^{-3}	0.26 (0.05)	1.1×10^{-8}	0.98	0.02, 0.1
PP	*IGFBP3*	rs11977526	7	46,008,110	A/G	0.397	ITG	–0.41 (0.05)	3.8×10^{-18}	–0.32 0.04)	3.9×10^{-13}	–0.36 (0.03)	2.9×10^{-29}	0.87	0.62, 0.004
DBP	*NOS3*	rs891511	7	150,704,843	A/G	0.373	IN	–0.25 (0.04)	1.8×10^{-8}	–0.26 (0.04)	2.0×10^{-9}	–0.26 (0.03)	2.0×10^{-16}	NA	NA
DBP	*HRCT1*	rs76452347	9	35,906,471	T/C	0.191	Arg63Trp	–0.25 (0.05)	1.1×10^{-6}	–0.20 (0.05)	1.1×10^{-4}	–0.23 (0.04)	6.8×10^{-10}	NA	NA
PP	*PHF19*	rs1953126	9	123,640,500	T/C	0.331	ITG	0.27 (0.05)	6.3×10^{-8}	0.10 (0.05)	0.035	0.17 (0.03)	1.8×10^{-7}	0.99	0.11, 0.86
DBP	*ADO*	rs10995311	10	64,564,934	G/C	0.381	Pro39Ala	–0.20 (0.04)	2.4×10^{-6}	–0.20 (0.04)	1.9×10^{-6}	–0.20 (0.03)	2.1×10^{-11}	NA	NA
DBP	*CYP2C19*	rs4494250	10	96,563,757	A/G	0.319	IN	0.21 (0.05)	5.2×10^{-6}	0.11 (0.04)	5.1×10^{-3}	0.15 (0.03)	3.4×10^{-7}	0.93/0.98	0.017, 0.0030
DBP	*ARNTL*	rs900145	11	13,293,905	G/A	0.336	ITG	–0.25 (0.05)	9.1×10^{-7}	–0.15 (0.05)	0.002	–0.20 (0.03)	1.8×10^{-8}	1	0.0041, 0.00087
SBP	*KCNJ11*	rs5219	11	17,409,572	T/C	0.320	Lys23Glu	0.48 (0.07)	1.8×10^{-11}	0.21 (0.06)	9.4×10^{-4}	0.32 (0.05)	4.9×10^{-12}	0.94/1	0.00018, 0.0023
DBP	*CERS5*	rs7302981	12	50,537,815	A/G	0.338	Cys75Arg	0.23 (0.04)	1.8×10^{-7}	0.27 (0.04)	6.5×10^{-13}	0.25 (0.03)	9.4×10^{-19}	1	7.7×10^{-5}, 0.0053
PP	*MYH6*	rs452036	14	23,865,885	A/G	0.400	IN	–0.23 (0.05)	1.6×10^{-6}	–0.31 (0.05)	1.4×10^{-11}	–0.27 (0.03)	2.4×10^{-16}	0.89	0.64, 0.094
SBP	*TNRC6A*	rs11639856	16	24,788,645	A/T	0.193	Asn185Lys	–0.37 (0.08)	7.7×10^{-6}	–0.30 (0.08)	3.6×10^{-4}	–0.34 (0.06)	1.3×10^{-8}	0.99	0.068, 0.54
DBP	*DPEP1*	rs1126464	16	89,704,365	C/G	0.215	Glu351Gln	0.23 (0.05)	6.4×10^{-6}	0.26 (0.04)	7.0×10^{-9}	0.24 (0.03)	2.4×10^{-13}	1/0.39	0.050, 0.077
DBP	*TBX2*	rs8068318	17	59,483,766	C/T	0.350	IN	–0.23 (0.05)	2.2×10^{-7}	–0.28 (0.04)	1.8×10^{-12}	–0.26 (0.03)	3.0×10^{-18}	1	$0.00080, 9.0 \times 10^{-6}$
PP	*DOT1L*	rs2302061	19	2,226,772	C/G	0.163	Val1418Leu	0.30 (0.07)	5.1×10^{-6}	0.28 (0.06)	1.0×10^{-5}	0.29 (0.05)	2.2×10^{-10}	0.64	0.019, 0.88
PP	*INSR*	rs7248104	19	7,224,431	A/G	0.395	IN	–0.20 (0.05)	1.8×10^{-5}	–0.20 (0.04)	3.3×10^{-6}	–0.20 (0.03)	2.6×10^{-10}	1	0.16, 0.43
DBP	*RGL3*	rs167479	19	11,526,765	T/G	0.448	Pro162His	–0.26 (0.04)	6.4×10^{-10}	–0.33 (0.04)	3.8×10^{-20}	–0.30 (0.03)	4.2×10^{-28}	NA	NA
SBP	*ZNRF3*	rs4823006	22	29,451,671	G/A	0.424	3′UTR	–0.33 (0.07)	8.7×10^{-7}	–0.20 (0.06)	9.2×10^{-4}	–0.26 (0.05)	7.9×10^{-9}	0.98	0.29, 0.093

The discovery meta-analysis was performed in CHARGE+ Exome Chip BP Consortium samples (n = 146,562). The follow-up meta-analysis was performed with samples from the CHD Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium and T2D-GENES Consortium (n = 180,726). The 'combined', or joint, meta-analysis was performed with both discovery and follow-up samples (n = 327,288). Chr., chromosome; CA, coded allele; NCA, non-coded allele; CAF, coded allele frequency; SE, standard error; SYN, synonymous; IN, intronic; ITG, intergenic; ICBP discovery, the discovery sample for the International Consortium for Blood Pressure; NA, not available; IQ, imputation quality; r^2/IQ, LD between the best proxy in ICBP and the one in the "dbSNP ID" column and imputation quality for the best proxy.

[a]Loci are named according to the closest gene, determined on the basis of the position of the lead SNP. [b]Amino acid substitutions are given for missense variants. [c]Meta-analysis used the inverse-variance-weighted method for DBP, PP, and SBP and the optimal z-score method for HTN. [d]rs1925153 was significant in joint meta-analysis of EA-only samples; the remaining SNPs were significant in meta-analysis of samples from all ancestry groups. [e]The same variants in the "dbSNP ID" column were analyzed in the ICBP cohort.

Table 2 New common blood pressure SNPs associated with non–blood pressure traits

Locus[a] (function)	dbSNP ID	Chr:position	CA/NCA	CAF	GWAS trait[b]	Amino acid substitution	Literature Lab term(s)[c]
SNPs not previously reported in GWAS							
PRDM16 (NS)	rs2493292	1:3,328,659	T/C	0.15	NA	Pro633Leu	
SULT1C3 (NS)	rs6722745	2:108,875,244	C/T	0.34	NA	Met194Thr	
HRCT1 (NS)	rs76452347	9:35,906,471	T/C	0.19	NA	Arg63Trp	
ADO (NS)	rs10995311	10:64,564,934	G/C	0.38	NA	Pro39Ala	
CERS5 (NS)	rs7302981	12:50,537,815	A/G	0.34	NA	Cys75Arg	
TNRC6A (NS)	rs11639856	16:24,788,645	A/T	0.19	NA	Asn185Lys	
DOT1L (NS)	rs2302061	19:2,226,772	C/G	0.16	NA	Val1418Leu	
RGL3 (NS)	rs167479	19:11,526,765	T/G	0.448	NA	Pro162His	
SNPs previously reported to be significant in GWAS of other traits[d]							
PABPC4 (IN)	rs4660293	1:40,028,180	G/A	0.21	HDL		
CSNK1G3 (IN)	rs4530754	5:122,855,416	G/A	0.41	LDL and TC		
C5orf56 (IN)	rs2188962	5:131,770,805	T/C	0.35	Crohn's disease		
	rs926552	6:29,548,089	T/C	0.11	T1D		
MSH5–SAPCD1 (IN)	rs409558	6:31,708,147	G/A	0.18	SLE		
IGFBP3	rs11977526	7:46,008,110	A/G	0.40	IGFBP3		Insulin, 9%; IGF-1 signaling, 55%
PHF19 (5′ near gene)	rs1953126	9:123,640,500	T/C	0.33	RA		
	rs900145	11:13,293,905	G/A	0.34	Age at menarche		
KCNJ11 (NS)	rs5219	11:17,409,572	T/C	0.32	T2D	Lys23Glu	Insulin, 0.6%; T2D, 2.5%
MYH6 (IN)	rs452036	14:23,865,885	A/G	0.40	Resting heart rate		Heart development, 73%; hypertrophy model, 83%; cardiac muscle contraction, 84%
DPEP1 (NS)	rs1126464	16:89,704,365	C/G	0.22	Homocysteine concentration	Glu351Gln	
TBX2 (IN)	rs8068318	17:59,483,766	C/T	0.35	Creatinine and eGFR		Heart development, 17.5%
INSR (IN)	rs7248104	19:7,224,431	A/G	0.395	TG		Insulin, 90%; IGF-1 signaling, 45%; T2D, 93%; hypertrophy model, 5.4%
ZNRF3 (3′ UTR)	rs4823006	22:29,451,671	G/A	0.424	WHR		
SNPs previously reported in patent filing, candidate gene study, or GWAS[e]							
SLC22A7 (SYN)	rs2270860	6:43,270,151	T/C	0.37	HTN (patent filing)		
COL21A1 (IN)	rs1925153	6:56,102,780	T/C	0.45	Bipolar disease traits		
PHIP (IN)	rs10943605	6:79,655,477	A/G	0.46	Colon cancer (patent filing)		
HOXA3 (5′ UTR)	rs6969780	7:27,159,136	C/G	0.13	Hypospadias		
NOS3 (IN)	rs891511	7:150,704,843	A/G	0.37	Endothelium-dependent vasodilation		Heart development, 6.7%; T2D, 3.9%; cardiac muscle contraction, 14.5%
CYP2C19 (IN)	rs4494250	10:96,563,757	A/G	0.32	Breast cancer		

The SNPs included in this table are the common SNPs in **Table 1**. CA, coded allele; NCA, non-coded allele; CAF, coded allele frequency; IN, intron; NS, nonsynonymous; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; T1D, type 1 diabetes; T2D, type 2 diabetes; SLE, systemic lupus erythematosus; IGFBP3, insulin-like growth factor–binding protein 3; RA, rheumatoid arthritis; TG, triglycerides; WHR, waist–hip ratio; NA, not available.
[a]Loci are named according to the closest gene, as determined on the basis of the position of the index SNP. [b]Trait for which a SNP was reported in previous GWAS. [c]Literature Lab terms that were strongly associated with corresponding blood pressure candidate genes. The relative contribution of a blood pressure candidate gene to a Literature Lab term is indicated by a percentage (**Supplementary Table 10** and ref. 44). [d]Reported to be significant in GWAS using $P < 5 \times 10^{-8}$ or a prespecified significance level in the reported study. Details of association direction are included in **Supplementary Table 7**. [e]*P* values were not mentioned or did not reach the specified significance level.

28 genes identified a statistically significant association of *SLC12A1* (26 variants all having MAF <0.005) with SBP ($P = 0.0006 < 0.05/28$, T1 test; **Supplementary Table 9**). Mutations in *SLC12A1*, encoding the Na-K-2Cl co-transporter, cause Bartter's syndrome, a Mendelian salt-wasting condition associated with hypotension[43]. The 26 variants in *SLC12A1*, however, did not overlap with the previously reported variants for Bartter's syndrome[43]. The 27 other genes associated with monogenic blood pressure disorders did not reach statistical significance in standard burden testing. Additionally, none of the 28 genes showed significant association with blood pressure using SKAT[42] (all $P > 0.0006$; **Supplementary Table 9**).

Inferred function of the identified blood pressure loci

We applied several computational strategies and conducted *cis* expression quantitative trait locus (*cis*-eQTL) analysis to infer biological functions associated with genes at the 31 significant single-variant blood pressure loci (see details in the **Supplementary Note**).

Disease and pathway enrichment analysis. We examined functional annotations derived from precompiled gene sets in GeneGo and literature-based inference in Literature Lab[44]. In GeneGo biological processes, the 31 new loci were enriched for cell signaling and development functions (for example, 'regulation of signaling' and 'regulation of growth') as compared with the largely cardiovascular functions (for example, 'negative regulation of (smooth) muscle contraction' and 'blood circulation') found for the 39 validated blood pressure loci (**Supplementary Table 10**). The new loci were also enriched for several conditions related to cardiovascular and metabolic disease (for example, 'myocardial ischemia', 'congenital hyperinsulinism', and 'acid–base imbalance'), whereas the validated loci were enriched for conditions more directly related to blood pressure or cardiovascular conditions

Table 3 CHARGE+ Exome Chip BP Consortium: significant genes in burden tests and SKAT

Gene	Chr.	Test[a]	T1 or T5[b]	Phenotype	β (SE) or Q_{meta}[c]	*P* value[d]	Variants[e]	CAF
PTPMT1	11	Burden	T5	HTN	0.05 (0.01)	3.5×10^{-7}	4	0.053
NPR1	1	SKAT	T5	MAP	270678.8	4.4×10^{-8}	14	0.025
DBH	9	SKAT	T1	MAP	145331.4	9.2×10^{-7}	27	0.028

The experiment-wide significance level for gene-based tests is $P < 1 \times 10^{-6}$. Chr., chromosome; CAF, coded allele frequency.
[a]The standard burden test collapses rare variants into a single variable and tests the association between this variable and blood pressure; SKAT was designed to detect the effects of alleles that collectively contribute to higher and lower blood pressure. [b]Meta-analysis was conducted at the gene level to evaluate aggregate effects from multiple nonsynonymous or splicing variants with MAF <0.01 (T1) or <0.05 (T5). [c]The burden test yields β (s.e.m.) values, and SKAT provides Q_{meta} values. [d]*P* value in pooled samples from all ancestry groups. [e]Number of variants used in analysis.

(for example, 'arrhythmias, cardiac', 'hypertension', and 'hypotension'). Significant Literature Lab[44] (**Supplementary Table 11**) pathways and disease MeSH headings were enriched for insulin-related terms (for example, 'IGF-1', 'type 2 diabetes', and 'hyperinsulinism') for the new loci as compared to blood pressure–related terms (for example, 'cardiac muscle contraction') and cardiovascular electrophysiology (for example, 'antiarrhythmics') for the validated loci; both sets of loci were significant for 'heart development'. In the Literature Lab[44] anatomical annotations, the cardiovascular system (for example, 'myocardium' and 'heart ventricles') was highlighted for both the new and validated SNPs, while the validated SNPs were also associated with the renal system (for example, 'nephron' and 'urinary tract'). Almost no annotations for either GeneGo or Literature Lab[44] were unique to the set of combined new and validated loci with the exception of a few terms predominantly related to blood pressure or the renal system.

Protein–protein interaction analysis. Using NCBI protein–protein interaction (PPI) network resources (**Supplementary Note**), a total of 399 genes were found to be connected to at least one of the 31 new blood pressure–associated genes (**Supplementary Fig. 2**). Ordered on the basis of connectivity ('degree'; **Supplementary Table 12**), a measure that signifies a hub disposition in the PPI network, the top five blood pressure candidate genes were *INSR*, *PABPC4*, *NOS3*, *IGFBP3*, and *DOT1L*. On the basis of Google page rank, a connectivity measure that recognizes degree of connectivity while also emphasizing connections between highly connected nodes, the five top genes differed only by the replacement of *IGFBP3* by *PTPMT1* (**Supplementary Table 12**).

ENCODE and Roadmap Epigenomics analyses. RegulomeDB[45] and HaploReg[46] evaluations of potential *cis*-regulatory functions identified rs8068318 (intronic to *TBX2*) as having the highest score among loci (or their LD proxies) that showed relatively strong evidence for a role in transcription (**Supplementary Table 13**). This SNP maps to an active *TBX2* promoter histone mark in lung fibroblasts and DNase I hypersensitivity marks in seven cell types, while overlapping with five transcriptional regulatory motifs. *TBX2* is a member of a highly conserved T-box family of transcription factors and has been implicated in cardiac developmental abnormalities[47,48] and kidney function[26].

Cis-*eQTL analysis.* The 31 newly identified blood pressure variants were queried for *cis*-eQTL association (**Supplementary Table 14**) in over 5,000 participants from the Framingham Heart Study (FHS), using microarray-based transcriptomic profiling of RNA from whole blood. A total of 720 SNP–transcript pairs were tested. Forty-three pairs (representing 17 variants) were significant at FDR < 10%, among which 8 variants were *cis*-eQTLs for multiple gene transcripts. For example, rs1953126 (near the 5′ UTR of *PHF19*) is a *cis*-eQTL for *PHF19* and for multiple nearby genes, including *C5*, *GSN*, *PSMD5*, *RAB14*, *FBXW2*, and *TRAF1*. Query of publicly available eQTL databases via GRASP[49] and recent publications[50,51] based on profiling of whole blood or other tissue types[50–57] yielded eQTL assignments that were concordant with the FHS findings for most variants listed in **Supplementary Table 14**.

Effects of blood pressure–associated variants on clinical outcomes

We considered the aggregate effects of the blood pressure loci on blood pressure–related clinical outcomes using new Exome Chip–based results for coronary artery disease (CAD) and myocardial infarction, including 42,335 cases and 78,239 controls[58], and for renal function measured by glomerular filtration rate (GFR) in up to 111,655 individuals. For 59 of the 70 (31 new and 39 validated) blood pressure–associated SNPs, the alleles that were associated with higher blood pressure were also associated with increased odds of CAD and myocardial infarction (**Supplementary Tables 15** and **16**), a highly significant concordance with the known influence of blood pressure on CAD and myocardial infarction (sign test, binomial $P = 4.5 \times 10^{-9}$). Similarly, genetic risk scores (GRSs) constructed from the 70 blood pressure SNPs using weights derived from their effects on SBP, DBP, and MAP were highly significantly associated with CAD and myocardial infarction with odds ratios (per 1 mm Hg of SNP-based blood pressure) of 1.05 ($P = 8.6 \times 10^{-44}$), 1.08 ($P = 1.9 \times 10^{-41}$), and 1.06 ($P = 1.1 \times 10^{-45}$), respectively (**Supplementary Table 17** and **Supplementary Note**). Notably, the blood pressure–raising allele of one of the new low-frequency SNPs from single-variant analysis, rs111245230 in *SVEP1*, was by itself associated with increased CAD at genome-wide significance[58]. GRSs constructed solely from the rare and low-frequency variants at the three loci with significant gene-based tests (*DBH*, *NPR1*, and *PTPMT1*) were significant for CAD and myocardial infarction using MAP-based weightings for *DBH* ($P = 0.026$) and HTN-based weightings for *PTPMT1* ($P = 0.003$) with a non-significant concordant trend using MAP-based weightings for *NPR1* ($P = 0.13$; **Supplementary Table 18**). By contrast, the blood pressure–raising alleles for only 39 of the 70 blood pressure–associated SNPs were associated with diminished kidney function

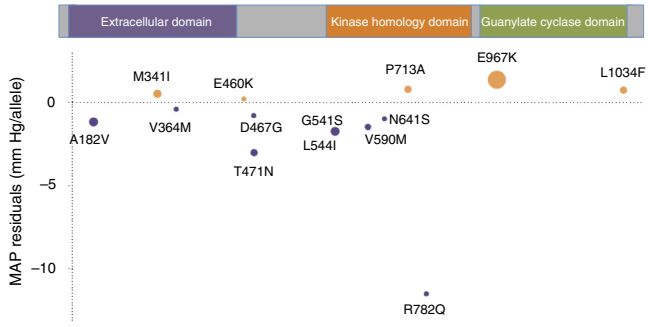

Figure 2 *NPR1* gene: low-frequency and rare variants associated in aggregate with mean arterial pressure. The NPR1 protein (1,061 amino acids) comprises three domains: an extracellular domain, a kinase homology domain, and a guanylate cyclase domain. The effects of the 14 low-frequency and rare variants after adjustment for age, age^2, sex, and body mass index on MAP are shown as higher (orange) or lower (purple) values; dot area is proportional to the number of carriers of the minor allele. The minor allele of rs35479618 (MAF ~0.012; p.Glu967Lys) was carried by 3,164 participants. The minor allele of rs201787421 (MAF ~2.6×10^{-5}; p.Arg782Gln) was carried by five participants.

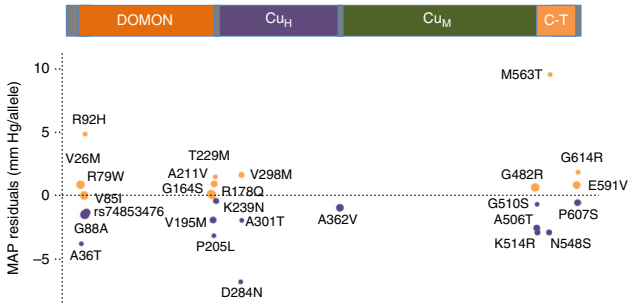

Figure 3 *DBH* gene: rare variants associated in aggregate with mean arterial pressure. The DBH protein (617 amino acids) contains a dopamine β-monooxygenase N-terminal (DOMON) domain, a catalytic core (Cu_H and Cu_M domains), and a C-terminal (C-T) domain. The effects of the 27 rare variants after adjustment for age, age^2, sex, and body mass index on MAP are shown as higher (orange) or lower (purple) values. The minor allele of rs74853476 (MAF ~0.0015), a splicing variant, was carried by 291 participants. The minor allele of rs201681337 (MAF ~7.9×10^{-5}; p.Ala301Thr) was carried by four participants.

(CKD) as reflected by lower GFR, a degree of concordance that was not significant (sign test, binomial $P = 0.40$). A similar lack of association was observed for the blood pressure GRS associations with GFR using weights for SBP ($P = 0.18$), DBP ($P = 0.63$), and MAP ($P = 0.31$).

DISCUSSION

Through a two-stage study design of discovery ($n = 146{,}562$) followed by external lookups ($n = 180{,}726$) and joint analysis ($n = 327{,}288$), we identified single-variant associations at 31 new loci and gene-based associations for three new genes (two of which overlapped with the single-variant loci) with blood pressure phenotypes. We also confirmed common variants at 39 previously reported blood pressure–associated loci, increasing the number of statistically significant loci in our study to 71 and extending the number of non-monogenic blood pressure–associated loci[5–15] to over 90. The sample size for the joint analysis in this study is far larger than that for any previous genetic study of blood pressure[5–15]. This large increase in sample size is an important reason for the discovery of many new blood pressure loci and likely explains why some of the newly identified common loci were not discovered in previous blood pressure GWAS. In addition, direct genotyping of coding variants likely added incremental power over imputed genotypes and tagging SNPs that were the basis of previous GWAS, suggesting that new common variants will continue to be identified for blood pressure phenotypes using the same set or similar sets of samples with exome sequencing and whole-genome sequencing. Furthermore, phenotypic and possibly genetic heterogeneity (due to additional samples in this study), differences in analysis plans, and the play of chance may be additional explanations of why some of the common variants identified in this study were not identified in previous blood pressure GWAS.

Fourteen of the new blood pressure variants identified in the present study reside in loci that were previously reported in GWAS to be associated with lipid levels[19], immunological diseases[20–22], and metabolic phenotypes[23–25,29] (**Table 2** and **Supplementary Table 7**). Thirteen of the previously identified blood pressure variants were also linked to non–blood pressure traits or diseases (**Supplementary Table 19**). Considerable evidence has accumulated linking high blood pressure to insulin resistance, altered lipid levels, inflammation, and other features of the metabolic syndrome[59–64]. Gene set enrichment, regulatory sequence variation, and PPI annotations of the new blood pressure loci implicate genes that contribute to cardiac structure and function as well as insulin signaling and type 2 diabetes. In addition, among the previously reported blood pressure–associated genes that were confirmed in our study, *ATXN2*, *GRB14*, *HECTD4*, *PTPN11*, and *SLC39A8* (**Supplementary Table 3**) have been proposed as candidate genes for metabolic syndrome on the basis of their associations with metabolic traits and inflammatory biomarkers[64].

The *NPR1* gene was associated with blood pressure in both single-variant and gene-based tests. This gene encodes the receptor for atrial and B-type natriuretic peptides, which regulate blood volume and pressure[17,18]. The functional consequences of the p.Glu967Lys amino acid substitution that is encoded by rs35479618 (the significant *NPR1* SNP in single-variant analysis) are unknown, but the change results in opposite charge and a large difference in side chain volume, and it is predicted to be possibly damaging (score = 0.513) by PolyPhen-2 (ref. 65). The effects of the 13 rare variants and 1 low-frequency variant in *NPR1* varied in direction, explaining why gene-based testing was significant using SKAT[42], which is sensitive to effects raising and lowering blood pressure, but not in burden testing[40,41], which requires a consistent direction of effect on blood pressure (**Fig. 2** and **Supplementary Fig. 3**). Of note, *Npr1*-knockout mice have HTN, cardiac hypertrophy, and sudden death phenotypes[17,18,66], and mice with only one copy of the *Npr1* gene have salt-sensitive HTN as compared to wild-type mice[17]. Future studies are warranted to determine whether humans carrying the rare blood pressure–increasing alleles of *NPR1* also have salt-sensitive HTN. We have previously demonstrated that common variation that raises atrial natriuretic peptide levels lowers blood pressure[13], suggesting the potential for blood pressure–lowering strategies that target the interaction of natriuretic peptide with natriuretic peptide receptors. Similarly, molecular mimicking of the action of blood pressure–lowering alleles in *NPR1* may be worth exploring as a novel blood pressure treatment.

Both single-variant and gene-based (T1) analyses in stage 1 identified *DBH* as a blood pressure–associated gene (**Fig. 3**). *DBH* encodes the enzyme dopamine β-hydroxylase, which catalyzes the transformation of dopamine into norepinephrine. Both dopamine and norepinephrine act on the sympathetic nervous system, influencing a variety of complex traits, including blood pressure. Impaired dopamine β-hydroxylase activity has been identified in individuals with severe autonomic failure, including orthostatic hypotension[67,68], and mutation of *DBH* has been identified in two individuals with autonomic dysfunction[69]. The rare minor allele of rs3025380, encoding the p.Gly88Ala nonsynonymous substitution, was associated with a comparatively large reduction of 1.81 mm Hg in MAP even though the amino acid change is predicted to be remote from the active site[70]. Inhibition of dopamine β-hydroxylase has long been considered a potential target for antihypertensive therapy[71], but these efforts have been undermined because of the broad involvement of catecholamines in a variety of critical biological processes[72,73] and the potential for undesirable side effects.

The remaining significant gene in gene-based testing was *PTPMT1*, which encodes mitochondrial protein tyrosine phosphatase 1. Knockdown of *Ptpmt1* expression in a rat pancreatic insulinoma cell line was found to enhance ATP production and insulin secretion[74], an observation that is closely aligned with the insulin and cardiometabolic regulatory features of many of the new blood pressure loci identified in this study. In addition, targeted burden testing of uncommon and rare variants in genes that underlie monogenic blood pressure disorders identified a significant blood pressure association with *SLC12A1*, the Na-K-2Cl co-transporter that is well established to harbor rare mutations that cause Bartter's syndrome, a salt-wasting condition associated with hypotension[43].

The Exome Chip array was designed to aid in the search for rare functional variants with large effect sizes. This study did not, however, identify any rare variants associated with blood pressure phenotypes through single-variant analyses, suggesting that rare variants with large effects on blood pressure are an uncommon occurrence. Even with the current sample size, this study was not adequately powered to identify rare variants with only modest effect sizes (Online Methods). Within the predominant class of variants studied (low-frequency and rare nonsynonymous SNPs), there may not be a large enough number of variants or effects of sufficient size to account for a substantial proportion of the remaining missing heritability of blood pressure. Nevertheless, this study greatly extends the number of known blood pressure–associated loci and moreover demonstrates their potential relevance to cardiovascular disease. The discovery of a total of 32 new blood pressure–associated loci (31 from single-variant tests, 1 from gene-based tests) and their overlap with other disease-related phenotypes suggest common etiologies of blood pressure and metabolic risk factors and an opportunity to identify therapies that more broadly influence HTN in the context of cardiometabolic risk.

URLs. BIND, http://thebiogrid.org/; BioGRID, http://thebiogrid.org/; CHARGE+ Exome Chip, http://www.chargeconsortium.com/main/exomechip; EcoCys, http://www.ecocyc.org/; GeneGo, http://lsresearch.thomsonreuters.com/; Literature Lab, http://www.acumenta.com/acumenta/overview/index.php; HaploReg, http://www.broadinstitute.org/mammals/haploreg/haploreg_v3.php; Human Protein Reference Database (HPRD), http://www.hprd.org/; NCBI Protein–Protein Interaction (PPI) database, ftp://ftp.ncbi.nih.gov/gene/GeneRIF/; National Human Genome Research Institute (NHGRI) GWAS catalog, http://www.genome.gov/gwastudies/; PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/; RAREMETAL, http://genome.sph.umich.edu/wiki/RAREMETAL_Documentation; RegulomeDB, http://regulomedb.org/; Recode alleles, http://www.chargeconsortium.com/main/exomechip; Roadmap Epigenomics, http://www.roadmapepigenomics.org/; seqMeta package, http://cran.r-project.org/web/packages/seqMeta/index.html.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. The meta-analysis results at the single-variant level for SBP, DBP, MAP, PP, and HTN can be downloaded from the database of Genotypes and Phenotypes (dbGaP) CHARGE Summary site under accession phs000930.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Study participants. A total of 146,562 individuals comprising EA (n = 120,473), AA (n = 21,503), and HA (n = 4,586) individuals contributed from 16 studies (**Supplementary Table 20** and **Supplementary Note**) were included in the discovery-stage association analyses. The entire discovery sample was also included in the meta-analyses of results from the discovery and follow-up stages (**Fig. 1**). All study participants provided written informed consent for genetic research, with the exception of the BioVU biorepository, in which DNA was extracted from discarded blood collected during routine clinical testing and was linked to deidentified medical records. All studies received approval to conduct this research from their respective institutional review boards. Studies contributing to the discovery analyses included a wide range of mean measured blood pressure values (110–142 mm Hg for SBP and 69–84 mm Hg for DBP), HTN prevalence (2–77%), and proportion of individuals taking antihypertensive medications (0.6–63%) (**Supplementary Table 20**).

Genotyping and quality control. All samples were genotyped on the Illumina Infinium Human Exome Array v1.0 or v1.1 (**Supplementary Table 21**). Ten studies (51,106 individuals) were jointly called at the Human Genetics Center of the University of Texas Health Science Center in Houston[76]. Six additional studies followed genotype calling protocols from Illumina or from the CHARGE Consortium and strand assignment for allele coding specified by the CHARGE Consortium[76]. All studies followed quality control guidelines recommended by the CHARGE analysis committee. Quality control procedures were further applied at the cohort level (**Supplementary Table 21**). Variants were removed for having a genotype call rate less than 95%, Hardy–Weinberg equilibrium P value less than 1×10^{-6}, and concordance rate (between overlapping variants from previous GWAS and the Exome Chip) less than 95%; individual samples were removed for having a call rate less than 95%, having a concordance rate less than 95% with GWAS data, or in the event of a suspected sample swap, sex mismatch, or heterozygosity F value greater than 10.

Blood pressure phenotypes. In the discovery stage, the blood pressure phenotypes included were SBP, DBP, PP (SBP minus DBP), and MAP (1/3 SBP + 2/3 DBP). A participant was classified as having HTN if she/he had SBP ≥140 mm Hg or DBP ≥90 mm Hg or was taking antihypertensive medication. SBP and DBP values were obtained from the first examination attended for longitudinal studies; when available, the average of two single-occasion measurements was used for SBP and DBP. To account for the reduction in blood pressure due to medication use, all individuals taking blood pressure–lowering medication had 15 mm Hg added to the measured SBP and 10 mm Hg added to the measured DBP[15]. The four continuous blood pressure traits are moderately or highly correlated, such that among the larger contributing cohorts the ranges of correlation were 0.70–0.82 (SBP–DBP), 0.92–0.95 (SBP–MAP), 0.73–0.89 (SBP–PP), 0.92–0.99 (DBP–MAP), 0.20–0.45 (DBP–PP), and 0.43–0.68 (MAP–PP). Such correlations appeared to be consistent across different ancestry groups within these same studies.

Association analyses and meta-analyses. *Power estimation*. Nearly 90% of the markers on the Exome Chip are low-frequency (MAF = 0.01–0.05) or rare (MAF < 0.01) variants. Power for association was evaluated for MAP assuming a mean of 100 mm Hg with standard deviation of 10 mm Hg using QUANTO[77] for sample size n = 150,000 at the significance level of 3.4×10^{-7} for a variant with MAF of 0.0005, 0.001, 0.005, or 0.01. To reach 80% power, an effect size of 5, 3.5, 1.6, or 1.1 mm Hg is needed, respectively, for a variant with MAF = 0.0005, 0.001, 0.005, or 0.01.

Fraction of the common variants tagged by the Exome Chip. We downloaded the phase 3 genotype data for EA individuals from the HapMap Project. The phase 3 file hapmap3_r2_b36_fwd.CEU.qc.poly includes 1,416,121 variants (1,352,770 with MAF >0.01 and 1,223,919 with MAF >0.05). We used the PLINK command 'show-tags' to estimate the number of common (MAF >0.05) variants that can be tagged by Exome Chip variants. We estimated that 172,220 (LD r^2 ≥0.5) and 88,186 (LD r^2 ≥0.8) common SNPs (MAF >0.05) can be tagged by the Exome Chip variants. In comparison to the number of variants tagged by a GWAS chip (for example, Affymetrix 500K), the Exome Chip tags many fewer common variants.

Cohort-specific analysis. Gene-based (or region-based) testing was performed using the seqMeta package. Covariates included age, age^2, sex, body mass index, and principal components (if applicable) to account for population structure. All variants were recoded to conform to the alleles specified in a 'recode' file distributed to each study. In all analyses, variant effects were modeled additively. Conditional analysis was performed to identify independent blood pressure signals at previously reported blood pressure loci[5–15] using the seqMeta package by adjusting at the cohort level for the previously reported GWAS SNP with the smallest P value in association analysis. Similarly, for any newly identified locus with multiple variants, conditional analysis was performed by adjusting for the most significant variant in the region to identify non-redundant signals.

Meta-analysis at the single-variant level. Meta-analysis of single-variant associations from discovery and follow-up results was performed using the inverse-variance-weighted fixed-effects method[77] implemented in the seqMeta package. In the discovery stage, the primary meta-analysis was performed in all samples to identify variants showing consistent effects with blood pressure traits across multiple ancestry groups. Secondary analysis was performed in each of the three ancestries separately to identify new variants with different ancestral origin. Meta-analysis was also performed on results from conditional analysis and compared with the original meta-analysis to identify non-redundant signals. Although we performed association and meta-analysis on all genotyped variants that passed quality control, we only report results from about 147,000 variants that had minor allele counts (MACs) ≥30 in meta-analyses of all samples. Because the blood pressure traits are highly correlated, we used an array-wide Bonferroni-corrected significance threshold of 3.4×10^{-7} (= 0.05/147,000). The Exome Chip array contains numerous previously published variants or their LD proxies, mostly from GWAS using imputed genotype information for a variety of human traits. Using exome chip experimental genotypes, associations from previous blood pressure GWAS[5–15] were considered significant with $P \leq 0.05/n$, where n is the number of previously identified SNPs or SNPs that showed at least moderate LD (r^2 ≥0.3) on the Exome Chip.

Meta-analysis at the gene level. Meta-analysis was also conducted at the gene level to evaluate aggregate effects from multiple nonsynonymous and splicing variants with MAFs ≤0.01 (T1) and ≤0.05 (T5) in a gene using both SKAT[42] and the standard burden test[40,41] implemented in the seqMeta package. The standard burden test collapses the rare variants and has optimal properties when these variants all have the same directionality and magnitude of effect on phenotype. In contrast, SKAT aggregates individual variant score test statistics and offers better power than the burden test when there are a variety of effect sizes and directions, for example, when there are both protective and deleterious effects in a gene[42]. Approximately 17,000 genes included two or more nonsynonymous variants in the primary meta-analysis of all study samples. An association was deemed to be significant at $P < 1 \times 10^{-6}$ for gene-based tests. Among up to 154,543 EA individuals from the CHD Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium, and T2D-GENES Consortium (**Supplementary Note**), gene-based SKAT was applied to HTN and inverse-normal-transformed DBP, SBP, and PP using the RAREMETAL software package[78]. We performed lookups in their SKAT results for the genes that reached $P < 1 \times 10^{-6}$ in stage 1 analysis of this study.

Follow-up study at the single-variant level. The follow-up study was performed in external samples (follow-up samples) including a total of 180,726 individuals from the CHD Exome+ Consortium, ExomeBP Consortium, GoT2DGenes Consortium, and T2D-GENES Consortium (**Supplementary Note**). Summary information from participants, genotyping, and quality control in the follow-up samples are presented in the **Supplementary Note**. The follow-up samples provided SNP association statistics for DBP, PP, SBP, and HTN but not MAP for a total of 180,726 individuals. Significant variants ($P \leq 3.4 \times 10^{-7}$) in the discovery samples were considered replicated in the follow-up samples with $P \leq 0.05/n$ with their prespecified blood pressure trait in the follow-up sample alone, where n was the number of variants tested in the follow-up samples. From the discovery stage, the significant variants and additional variants with $P \leq 1 \times 10^{-5}$ were selected for joint meta-analysis with the follow-up samples. The primary meta-analysis of the discovery and follow-up results was performed in individuals of all ancestries. The secondary

meta-analysis was conducted in EA-only samples. The inverse-variance-weighted method was used in meta-analysis of the discovery and follow-up results for DBP, PP, and SBP. Because the follow-up samples provided only *z* scores and sample sizes for HTN, the optimally weighted *z*-score method[79] was used in meta-analysis of HTN. The threshold of $P \leq 3.4 \times 10^{-7}$ was required for significance in meta-analyses of the discovery and follow-up samples.

Functional inference. We applied several computational strategies to infer biological functions associated with candidate genes from the 31 new loci reaching $P < 3.4 \times 10^{-7}$ (**Table 1**) and 39 validated loci (**Supplementary Table 3**). (i) To test whether the SNPs in **Table 1** and **Supplementary Table 3** were significantly enriched among prespecified gene sets defined in pathways or by shared roles in particular diseases or biological processes, we performed gene pathway, disease, and Gene Ontology (GO) enrichment analysis using GeneGo software and Literature Lab[44] data mining of the literature (**Supplementary Note**). (ii) To investigate whether the coding and noncoding variants listed in **Table 1** might influence transcriptional regulation, we compared blood pressure candidate SNPs with ENCODE and Roadmap Epigenomics regulome features summarized for mainly *cis*-regulatory function in HaploReg[46] and RegulomeDB[45]. The inclusion of coding variants in this analysis was justified by previous research showing that transcriptional regulation can be influenced by both noncoding and coding variations; a recent publication has shown that ~15% of human codons simultaneously specify both amino acids and transcription factor recognition sites[80]. (iii) To identify genes that encode proteins especially connected with other proteins and therefore inferred to be important, we performed PPI network analysis on the SNPs in **Table 1**. The PPI network was constructed using NCBI PPI database information, which sources information from the HPRD, BIND, BioGRID, and EcoCys databases. By design, 2% of the Exome Chip variants were identified from previous GWAS. To investigate whether these previous GWAS SNPs might artificially increase the extent of GeneGo enrichment in known functional classes, we performed GeneGo enrichment analysis on ten randomly selected sets of genes from the Exome Chip (with replacement) with the size of new and previous blood pressure candidates discovered. None of these random sets showed gene set enrichment with significance comparable to the enrichment for the blood pressure SNPs.

To further assess putative functionality for the new loci, we performed *cis*-eQTL analysis between each of the newly identified variants and gene expression within 1 Mb flanking that variant in peripheral whole-blood samples from ~5,000 individuals from FHS. Statistical significance in the FHS expression data was evaluated at FDR < 10% for newly identified variants[81]. We also searched for *cis* associations between new variants and gene transcripts within 1 Mb flanking the lead SNP based on databases of previously published eQTL analyses at FDR < 10% (refs. 50,82).

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