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Genome-wide association study identifies eight loci associated with blood pressure

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Elevated blood pressure is a common, heritable cause of cardiovascular disease worldwide. To date, identification of common genetic variants influencing blood pressure has proven challenging. We tested 2.5 million genotyped and imputed SNPs for association with systolic and diastolic blood pressure in 34,433 subjects of European ancestry from the Global BPgen consortium and followed up findings with direct genotyping ($N \leq 71{,}225$ European ancestry, $N \leq 12{,}889$ Indian Asian ancestry) and *in silico* comparison (CHARGE consortium, $N = 29{,}136$). We identified association between systolic or diastolic blood pressure and common variants in eight regions near the *CYP17A1* ($P = 7 \times 10^{-24}$), *CYP1A2* ($P = 1 \times 10^{-23}$), *FGF5* ($P = 1 \times 10^{-21}$), *SH2B3* ($P = 3 \times 10^{-18}$), *MTHFR* ($P = 2 \times 10^{-13}$), *c10orf107* ($P = 1 \times 10^{-9}$), *ZNF652* ($P = 5 \times 10^{-9}$) and *PLCD3* ($P = 1 \times 10^{-8}$) genes. All variants associated with continuous blood pressure were associated with dichotomous hypertension. These associations between common variants and blood pressure and hypertension offer mechanistic insights into the regulation of blood pressure and may point to novel targets for interventions to prevent cardiovascular disease.

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The World Health Organization estimated that, in 2005, the annual death toll from cardiovascular disease reached 17.5 million worldwide[1–3]. Increases in systolic and diastolic blood pressure (SBP, DBP), even within the normal range, have a continuous and graded impact on cardiovascular disease risk and are major contributors in half of all cardiovascular deaths[2,3]. Lifestyle influences, including dietary sodium intake, alcohol excess, elevated body mass index and lack of exercise, are known to increase blood pressure[4]. Studies of familial aggregation suggest that there is also a substantial heritable component to blood pressure[5]. Studies of rare mendelian disorders of hypertension and hypotension have produced the most notable progress toward understanding the heritable basis of blood pressure, showing that mutations in genes influencing renal salt handling can have a severe effect on blood pressure[6]. Detailed study of these genes has identified rare variants (minor allele frequency (MAF) $< 0.1\%$) that influence blood pressure in the general population[7] and evolving evidence suggests a potential role for common variation in some of the same genes[8–10].

The identification of common variants affecting blood pressure using genome-wide association studies (GWAS) has proven challenging, compared to the success of GWAS of other common complex disorders[11,12]. However, meta-analysis of multiple studies with large total sample sizes has the potential to facilitate detection of variants with modest effects. We therefore formed the Global Blood Pressure Genetics (Global BPgen) consortium and conducted meta-analysis of GWAS in 34,433 individuals of European ancestry with SBP and DBP measurements (stage 1), followed by direct genotyping (stage 2a) and *in silico* (stage 2b) analyses (**Supplementary Fig. 1** online). Our analyses identified eight loci showing genome-wide significant association with systolic or diastolic blood pressure, each of which was also associated with hypertension.

RESULTS

Genome-wide association for blood pressure

Global BPgen includes 17 cohorts of European ancestry ascertained through population-based sampling or case-control studies. In our primary analysis (stage 1), we examined individuals aged ≤ 70 years from 13 population-based studies and from control groups from four case-control studies (**Table 1**). Individuals treated for hypertension were imputed to have 15 mm Hg higher SBP and 10 mm Hg higher DBP than the observed measurements, as this has been shown to reduce bias and improve statistical power[13]. SBP and (separately) DBP measures were each adjusted for age, age^2, body mass index and any study-specific geographic covariates within cohort- and sex-specific regression analyses. Genome-wide SNP genotyping was done on a variety of platforms and subjected to standard quality control measures (Methods and **Supplementary Table 1** online). Genotypes for ~ 2.5 million autosomal SNPs in the HapMap CEU sample were then imputed in each study and tested for association with SBP and DBP separately under an additive genetic model. Test statistics from association analysis of SBP and DBP from each cohort were adjusted using genomic control[14] to avoid inflation of results due to inter-individual relatedness or residual population stratification, and to ensure good calibration of test statistics. Meta-analysis of results was carried out using inverse variance weights. Test statistic inflation post-meta-analysis was modest ($\lambda_{GC} = 1.08$ SBP; $\lambda_{GC} = 1.07$ DBP); genomic control correction was applied again. The plots of test statistics against expectations under the null suggest an excess of extreme values (cohort-specific and meta-analysis quantile-quantile plots are presented in **Supplementary Fig. 2a** online).

On meta-analysis of results from 34,433 individuals in stage 1, we observed 11 independent signals with $P < 10^{-5}$ for SBP and 15 for DBP, with two results attaining $P < 5 \times 10^{-8}$, corresponding to genome-wide significance when adjusting for the ~ 1 million independent common variant tests estimated for samples of European ancestry (**Supplementary Fig. 2b**)[15].

Joint analysis of SBP and DBP signals with additional samples

To strengthen support for association, we undertook two analyses. First, we selected 12 SNPs for follow-up genotyping in up to 71,225 individuals drawn from 13 cohorts of European ancestry and up to 12,889 individuals of Indian Asian ancestry from one cohort (stage 2a, **Table 1**, **Supplementary Fig. 1** and **Supplementary Table 2** online). Second, we carried out a reciprocal exchange of association results for ten independent signals each for SBP and DBP (stage 2b, **Supplementary Fig. 1** and **Supplementary Table 3** online) with colleagues from the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) blood pressure consortium who had recently meta-analyzed GWAS data for SBP and DBP in 29,136 individuals, independent of Global BPgen (**Table 1**)[16]. Meta-analysis of the stage 1 Global BPgen GWAS and stage 2a direct and stage 2b *in-silico* association results identified genome-wide significant ($P < 5 \times 10^{-8}$) associations at eight loci: 1p36 in *MTHFR*, 10q24 near *CYP17A1* and 17q21 in *PLCD3* with SBP, 4q21 near *FGF5*, 10q21 in *C10orf107*, 12q24 near *SH2B3*, 15q24 near *CYP1A2* and 17q21 near *ZNF652* with DBP (**Table 2**, **Fig. 1**, **Supplementary Table 2**, **Supplementary Table 3** and **Supplementary Fig. 2b**). Three of these loci overlap with genome-wide significant loci identified in the CHARGE analyses (10q24 for SBP and 12q24 and 15q24 for DBP).

For SBP, the strongest evidence for association was at 10q24 (rs11191548, MAF $= 0.09$, 1.16 mm Hg higher per major allele, $P = 7 \times 10^{-24}$, **Table 2** and **Fig. 1b**). This SNP is part of a large cluster of associated SNPs spanning a ~ 430-kb region at 10q24 showing association in our GWAS meta-analysis. The locus includes six genes, most notably *CYP17A1*, which encodes the cytochrome P450 enzyme CYP17A1 (also known as *P450c17*) that mediates steroid 17α-hydroxylase and 17,20-lyase activity. The first enzymatic action is a key step in the biosynthesis of mineralocorticoids and glucocorticoids that affect sodium handling in the kidney and the second is involved in sex-steroid biosynthesis. Missense mutations in *CYP17A1* cause one form of adrenal hyperplasia characterized by hypertension, hypokalemia and reduced plasma renin activity[17,18]. None of the five other genes or transcripts in the region (**Fig. 1b**) is an obvious candidate for blood pressure regulation.

The second locus associated with SBP was at 1p36 (rs17367504, MAF 0.14, 0.85 mm Hg lower SBP per minor allele, $P = 2 \times 10^{-13}$, **Table 2** and **Fig. 1a**). This SNP is located in an intron of the *MTHFR* (methylenetetrahydrofolate reductase) gene in a region with many plausible candidate genes, including *MTHFR*, *CLCN6*, *NPPA*, *NPPB* and *AGTRAP*. The strongest signal in the locus is 6.4 kb away from and uncorrelated with rs1801133 (C677T, A222V, r^2 CEU $= 0.06$), a coding variant that has been related to higher plasma homocysteine concentration[19], pre-eclampsia[20], and variably hypertension[21]. In Global BPgen rs1801133 was associated with 0.08 mm Hg higher SBP per T allele ($P = 0.56$), 0.24 mm Hg higher DBP ($P = 0.01$) and an odds ratio for hypertension of 1.00 (95% CI $= 0.94$–1.05, $P = 0.90$).

The natriuretic peptides encoded by *NPPA* and *NPPB*, also located within the 1p36-associated interval, have vasodilatory and natriuretic properties and the *NPPA* knockout mouse has salt-sensitive hypertension[22]. A recent study found that the minor allele of rs5068 (43 kb from rs17367504, r^2 CEU $= 0.26$), in the 3′ untranslated region of *NPPA*, is associated with higher plasma atrial and B-type natriuretic peptide, as

well as lower SBP, DBP and odds of hypertension[23]. In the Global BPgen stage 1 meta-analysis we replicated association of the minor allele of rs5068 with 0.97 mm Hg lower SBP ($P = 3 \times 10^{-4}$), 0.60 mm Hg lower DBP ($P = 1 \times 10^{-3}$) and 10% lower odds of hypertension ($P = 0.04$). Whether the associations of rs5068 and rs17367504 reflect the same or different underlying signals remains to be established. The less well-characterized gene *CLCN6*, also at the 1p36 locus, encodes a neuronally expressed chloride channel that has not previously been implicated in blood pressure physiology, although rare mutations in other renally expressed chloride channels are associated with extremes of blood

Table 1 Study sample characteristics

Study	*N*	Women (%)	Age, years (s.d.)	SBP, mm Hg (s.d.)	DBP, mm Hg (s.d.)	BMI, kg/m^2 (s.d.)	HTN (%)[a]	Antihypertensive therapy (%)
Stage 1: GWAS								
Population-based cohorts								
BLSA	708	44	42.4 (13.2)	119.5 (15.0)	77.3 (10.2)	24.5 (3.6)	23.2	5.2
B58C – T1DGC[b]	2,580	51	44.3 (0.3)	121.7 (15.3)	79.4 (10.5)	27.4 (4.9)	20.5	4.7
B58C – WTCCC[b]	1,473	50	44.9 (0.4)	126.7 (15.2)	79.1 (10.2)	27.4 (4.7)	17.4	4.2
CoLaus	4,969	53	51.7 (9.5)	127.3 (17.4)	79.4 (10.8)	25.8 (4.6)	33.9	16
EPIC- Norfolk - GWAS	2,100	54	57.2 (7.8)	136.7 (19.1)	83.9 (11.9)	26.3 (3.9)	45.6	16
Fenland	1,401	56	45.0 (7.3)	122.8 (16.3)	75.5 (10.7)	27.1 (4.9)	18.8	5.5
InCHIANTI	562	55	56.9 (14.5)	138.4 (20.1)	81.4 (10.1)	27.1 (4.2)	59.6	23.7
KORA	1,644	51	52.5 (10.1)	133.4 (18.5)	81.8 (10.9)	27.3 (4.1)	20.9	17
NFBC1966[b]	4,761	52	31*	125.2 (13.8)	77.5 (11.7)	24.6 (4.2)	21.7	2
SardiNIA	3,998	57	40.8 (15.3)	128.7 (28.4)	79.7 (17.3)	25.1 (4.6)	29.5	10
SHIP	3,310	53	45.0 (13.9)	133.1 (20.2)	83.5 (11.3)	26.9 (4.7)	40.9	16.3
SUVIMAX	1,823	60	50.5 (6.2)	120.9 (12.3)	78.0 (8.1)	23.5 (3.3)	19.0	0
TwinsUK	873	100	45.8 (11.9)	122.9 (15.4)	78.2 (10.3)	24.8 (4.6)	27.3	22
Controls from case-control studies								
DGI controls	1,277	51	56.1 (8.7)	133.3 (18.4)	80.1 (10.0)	26.7 (3.8)	41.4	18
FUSION NGT controls	1,038	49	58.2 (10.7)	139.4 (19.3)	81.5 (10.3)	27.1 (4.0)	51.8	21
MIGen controls	1,121	38	48.9 (8.3)	127.1 (17.8)	80.2 (11.6)	27.1 (5.2)	36.4	13.4
PROCARDIS controls	795	37	58.9 (6.9)	134.7 (18.6)	82.8 (10.0)	25.9 (3.70)	15.0	2
Stage 2: follow-up								
2a. Cohorts with direct genotyping data								
ARYA	736	52	27.9 (0.9)	125.0 (13.0)	72.0 (8.0)	25.0 (4.0)	15.8	1
BRIGHT-HTN	2,445	59	57.1 (10.8)	153.9 (20.8)	94.0 (11.0)	27.4 (3.8)	100	91.2
BRIGHT-NT	673	77	55.5 (8.5)	111.1 (6.9)	71.2 (6.6)	24.4 (3.2)	0	0
EPIC-Italy	3,909	37	49.0 (7.6)	132.5 (15.5)	83.7 (9.0)	26.0 (3.6)	43.1	12.7
EPIC-Norfolk-REP	15,858	48	56.2 (7.6)	133.8 (17.5)	82.3 (11.0)	26.3 (3.8)	44	15
Finrisk97	7,023	51	47.1 (12.4)	134.9 (19.4)	82.3 (11.3)	26.6 (4.5)	45.5	12.4
FUSION2	1,162	37	57.5 (6.8)	138.2 (19.5)	83.9 (10.1)	26.8 (3.8)	8.9	1
Lolipop (Europeans)	6,006	35	51.2 (10.3)	130.4 (19.1)	79.6 (10.6)	27.5 (5.1)	39.9	20
Lolipop (Indian Asians)	12,823	36	48.8 (9.9)	129.9 (19.1)	80.8 (10.8)	27.4 (4.5)	42.9	25
MDC-CC	5,330	58	57.4 (5.9)	141.0 (19.0)	87.0 (9.5)	25.7 (4.0)	63.8	17
METSIM	5,934	0	58.1 (6.0)	142.0 (17.9)	89.8 (10.2)	27.3 (4.2)	69.6	40.5
MPP[c]	14,249	34	45.3 (7.1)	125.0 (14.0)	83.0 (9.1)	24.4 (3.4)	34.8	4
PREVEND	7,272	51	47.5 (11.4)	127.7 (19.3)	73.6 (9.7)	25.9 (4.2)	22.0	13.7
Prospect-EPIC	1,680	100	57.0 (6.0)	133.0 (20.0)	79.0 (11.0)	26.0 (4.0)	42.4	NA
Utrecht Health Project	2,829	52	40.0 (12)	128.0 (19.0)	79.0 (11.0)	25.0 (4.0)	32.9	NA
2b. Cohorts with *in silico* data								
CHARGE[d]	29,136							

Study characteristics are shown for cohort samples examined in stage 1 meta-analysis (population-based and controls from case-control studies), stage 2a (direct genotyping follow-up) and stage 2b (*in silico* follow-up with the CHARGE consortium). Population cohorts: The Baltimore Longitudinal Study of Aging (BLSA), British 1958 Birth Cohort-Wellcome Trust Case Control Consortium (B58C-WTCCC), British 1958 Birth Cohort–Type 1 Diabetes Genetics Consortium (B58C- T1DGC), Cohorte Lausannoise (CoLaus), European Prospective Investigation of Cancer-Norfolk-Genome Wide Association Study (EPIC-Norfolk-GWAS), Fenland Study (Fenland), Invecchiare in Chianti (InCHIANTI), Kooperative Gesundheitsforschung in der Region Augsburg (KORA), Northern Finland Birth Cohort of 1966 (NFBC1966), SardiNIA, Study of Health in Pomerania (SHIP), Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) and TwinsUK. Controls from case-control studies: Diabetes Genetics Initiative (DGI), Finland-United States Investigation of NIDDM Genetics (FUSION), the Myocardial Infarction Genetics Consortium (MIGen), the Precocious Coronary Artery Disease (PROCARDIS) study. Direct genotyping: The Utrecht Atherosclerosis Risk in Young Adults (AYRA), British Genetics of Hypertension study–hypertension cases (BRIGHT-HTN), BRIGHT study normotensive controls (BRIGHT-NT), EPIC-Italy, EPIC-Norfolk-Replication cohort (EPIC-Norfolk-REP), Finrisk97, FUSION stage 2 controls (FUSION2), London Life Sciences Population (LOLIPOP), Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC), Malmö Preventive Project (MPP), Prevention of REnal and Vascular ENd stage Disease (PREVEND), Metabolic Syndrome in Men Study (METSIM), Prospect-EPIC cohort, Utrecht Health Project (UHP). NA, not available; HTN, hypertension.

[a]Global BPgen definition of hypertension is SBP ≥ 140mm Hg or DBP ≥ 90mm Hg or taking antihypertensive medication. [b]Subjects from the Northern Finland Birth Cohort 1966 were examined at age 31; the British 1958 Birth Cohort samples were examined at ages 44–45. [c]The Malmö Preventive Project sample excludes all individuals who contributed to the Malmö Diet and Cancer Cardiovascular Arm (MDC-CC) [d]Full characteristics of CHARGE constituent cohorts are presented in the CHARGE paper[16].

pressure[24,25]. Lastly, *AGTRAP* (encoding angiotensin II receptor-associated protein) negatively regulates angiotensin II signaling by interacting with the angiotensin II type 1 receptor, a critical component of the renin-angiotensin-aldosterone system[26].

The third locus associated with SBP was at 17q21 (rs12946454, MAF 0.28, 0.57 mm Hg higher SBP per minor allele, $P = 1 \times 10^{-8}$, **Table 2** and **Fig. 1c**). This SNP is located in an intron in *PLCD3* (phospholipase C-delta isoform), and is part of a cluster of associated SNPs. PLCD3 is a member of the phospholipase C family of enzymes, important in vascular smooth muscle signaling and activated by the vasoactive peptides angiotensin II and endothelin[27].

The DBP SNP with the strongest association evidence on joint analysis is rs1378942 (MAF = 0.36, 0.43 mm Hg higher per minor allele, $P = 1 \times 10^{-23}$, **Table 2** and **Fig. 1g**), which is in an intron

Table 2 Loci associated with blood pressure

Chromosome	Genes nearby	BP Trait	SNP ID (pos NCBI35) function	Coded allele	Stage	Coded allele freq	*N*	Beta (s.e.) mm Hg	*P*	Beta (s.e.)	*P*	*N* total
										Joint analysis stages 1+2a+2b		
1p36	*MTHFR*	**SBP**	**rs17367504**	G	1	0.14	34,158	−0.79 (0.17)	1×10^{-5}			
	CLCN6		(11,797,044)		2a	0.16	19,751	−0.93 (0.22)	2×10^{-5}		$r^2 = 0.07\%$	
	NPPA		Intron *MTHFR*		2b	0.16	29,064	−0.85 (0.20)	3×10^{-5}	−0.85 (0.11)	2×10^{-13}	82,973
	NPPB											
	AGTRAP											
10q24	*CYP17A1*	**SBP**	**rs11191548**	T	1	0.91	33,123	1.17 (0.23)	3×10^{-7}			
	AS3MT		(104,836,168)		2a	0.91	71,225	1.19 (0.15)	9×10^{-15}		$r^2 = 0.08\%$	
	CNNM2		Intergenic *CNNM2/NT5C2*		2b	0.92	28,204	1.05 (0.27)	9×10^{-5}	1.16 (0.12)	7×10^{-24}	132,552
	NT5C2											
17q21	*PLCD3*	**SBP**	**rs12946454**	T	1	0.28	32,120	0.68 (0.15)	4×10^{-6}			
	ACBD4		(40,563,647)		2a	0.25	17,877	0.43 (0.21)	0.045		$r^2 = 0.04\%$	
	HEXIM1		Intron *PLCD3*		2b	0.27	27,693	0.50 (0.17)	0.004	0.57 (0.10)	1×10^{-8}	77,690
	HEXIM2											
3q26	*MDS1*	DBP	rs1918974	T	1	0.54	32,674	−0.28 (0.09)	1×10^{-3}			
			(170,648,590)		2a	0.55	26,910	−0.18 (0.08)	0.04		$r^2 = 0.03\%$	
			Intron		2b	0.53	28,307	−0.35 (0.09)	8×10^{-5}	−0.27 (0.05)	8×10^{-8}	87,891
4q21	*PRDM8*	**DBP**	**rs16998073**	T	1	0.21	26,106	0.65 (0.11)	7×10^{-9}			
	FGF5		(81,541,520)		2a	0.29	53,508	0.50 (0.07)	6×10^{-13}		$r^2 = 0.09\%$	
	c4orf22		Upstream *FGF5*		2b	0.24	22,009	0.36 (0.12)	0.003	0.50 (0.05)	1×10^{-21}	101,623
10q21	*c10orf107*	**DBP**	**rs1530440**	T	1	0.19	32,718	−0.51 (0.11)	3×10^{-6}			
	TMEM26		(63,194,597)		2a	0.18	19,884	−0.21 (0.11)	0.05		$r^2 = 0.04\%$	
	RTKN2		Intron *c10orf107*		2b	0.19	27,651	−0.44 (0.12)	1×10^{-4}	−0.39 (0.06)	1×10^{-9}	87,273
	RHOBTB1											
	ARID5B											
12q24	*SH2B3*	**DBP**	**rs653178**	T	1	0.53	30,853	−0.46 (0.09)	1×10^{-7}			
	ATXN2		(110,470,476)		2a	0.54	19,689	−0.40 (0.10)	3×10^{-5}		$r^2 = 0.09\%$	
			Intron *ATXN2*		2b	0.52	29,119	−0.50 (0.09)	2×10^{-8}	−0.46 (0.05)	3×10^{-18}	79,661
15q24	*CYP1A1*	**DBP**	**rs1378942**	C	1	0.36	34,126	0.48 (0.09)	6×10^{-8}			
	CYP1A2		(72,864,420)		2a	0.35	71,086	0.41 (0.06)	2×10^{-12}		$r^2 = 0.07\%$	
	CSK		Intron *CSK*		2b	0.33	29,046	0.43 (0.09)	3×10^{-6}	0.43 (0.04)	1×10^{-23}	134,258
	LMAN1L											
	CPLX3											
	ARID3B											
17q21	*ZNF652*	**DBP**	**rs16948048**	G	1	0.39	34,052	0.40 (0.09)	5×10^{-6}			
	PHB		(44,795,465)		2a	0.37	19,752	0.23 (0.10)	0.02		$r^2 = 0.04\%$	
			Upstream *ZNF652*		2b	0.37	28,637	0.29 (0.09)	0.002	0.31 (0.05)	5×10^{-9}	82,441

Shown is the top SNP for each independent locus associated with systolic or diastolic blood pressure ($P < 5 \times 10^{-7}$) on joint analysis in up to 134,258 individuals of European ancestry from Global BPgen GWAS (stage 1), follow-up genotyping (stage 2a) and *in silico* exchange with the CHARGE consortium (stage 2b). The eight genome-wide significant loci ($P < 5 \times 10^{-8}$) are shown in boldface. For stage 1 and 2b results based on imputed genotypes, an effective sample size is estimated to be the sum of the cohort-specific products of the imputation quality metric and the sample size. The total sample size is the sum of the effective sample sizes and the direct genotyping sample size. Effect sizes are on the mm Hg scale for increasing copy of the coded (alphabetically higher) allele as estimated by the beta coefficient in linear regression. The proportion of variance explained by each SNP is shown (r^2). Meta-analysis was conducted using inverse variance weighting. Note that loci 10q21 and 15q24 show results for two SNPs selected for validation genotyping in an interim analysis (rs1530440, rs1378942) that were genome-wide significant on joint analysis of stage 1+2a+2b. These two SNPs are highly correlated with alternate SNPs at the locus (rs4590817, rs4886606, respectively) with slightly stronger significance in the final stage 1 meta-analysis. The originally selected SNPs are shown throughout the text for consistency.

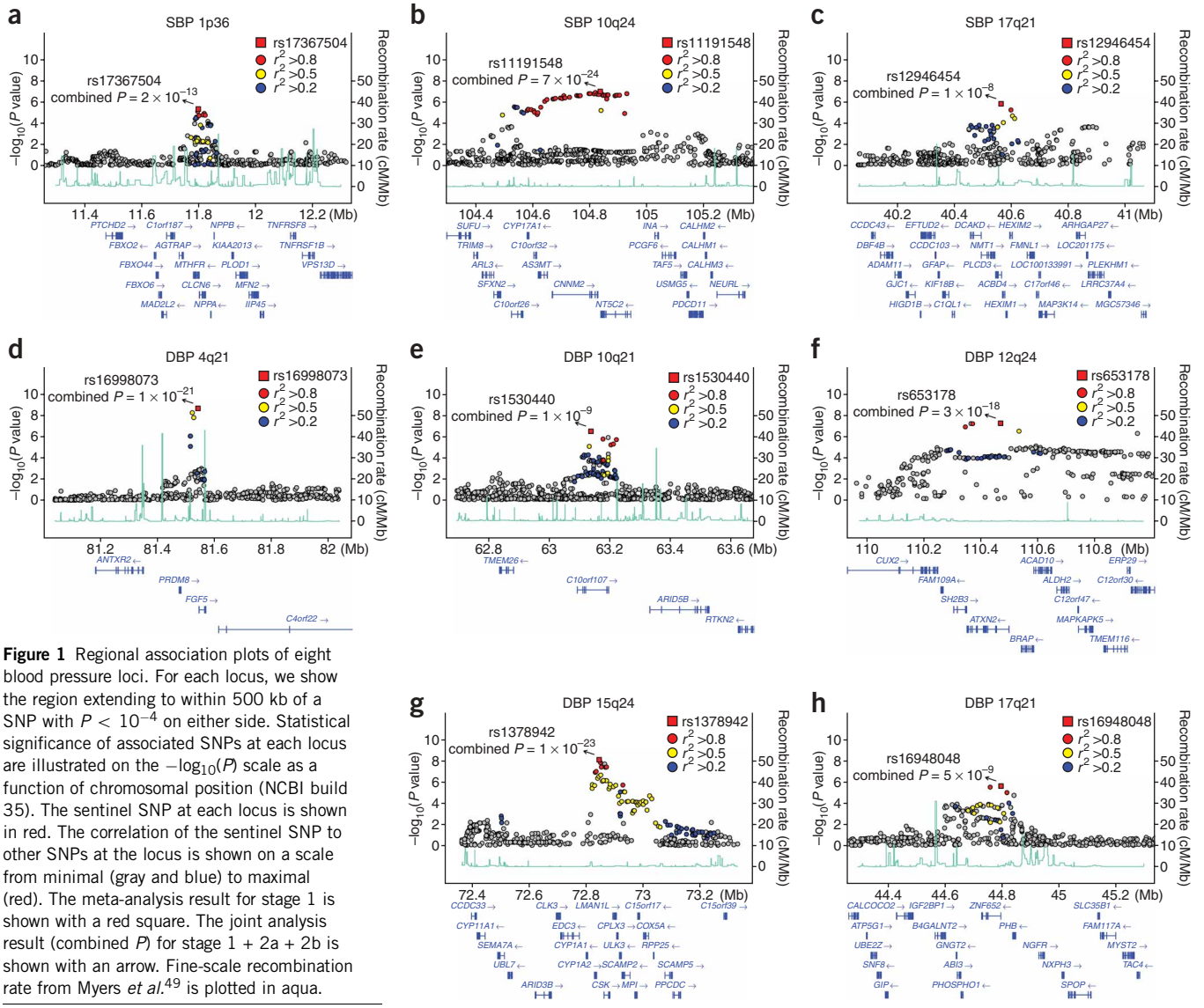

Figure 1 Regional association plots of eight blood pressure loci. For each locus, we show the region extending to within 500 kb of a SNP with $P < 10^{-4}$ on either side. Statistical significance of associated SNPs at each locus are illustrated on the $-\log_{10}(P)$ scale as a function of chromosomal position (NCBI build 35). The sentinel SNP at each locus is shown in red. The correlation of the sentinel SNP to other SNPs at the locus is shown on a scale from minimal (gray and blue) to maximal (red). The meta-analysis result for stage 1 is shown with a red square. The joint analysis result (combined *P*) for stage 1 + 2a + 2b is shown with an arrow. Fine-scale recombination rate from Myers *et al.*[49] is plotted in aqua.

of *CSK* at 15q24 and is one of a cluster of associated SNPs spanning ~72 kb. Genes in the region include *CYP1A2* (cytochrome P450 enzyme), *CSK* (c-src tyrosine kinase), *LMAN1L* (lectin mannose-binding1 like) and *ARID3B* (encoding AT-rich interacting domain protein). Other nearby genes include *CYP1A1* (~60 kb) and *CYP11A1* (~418 kb). Cytochrome P450 enzymes are responsible for drug and xenobiotic chemical metabolism in the liver and cellular metabolism of arachidonic acid derivatives[28], some of which influence renal function, peripheral vascular tone and blood pressure. *CYP1A2* is widely expressed, representing 15% of CYP450 enzymes produced in the liver and mediating the metabolism of multiple medications. A correlated SNP, rs762551 (MAF = 0.31, $r^2 = 0.63$, HapMap CEU) in an intron of *CYP1A2* has been found to influence caffeine metabolism[29]. The *ARID3B* gene is embryonic lethal when knocked out in mouse, with branchial arch and vascular developmental abnormalities[30], but is potentially interesting because of the presence of *ARID5B* at the 10q21 locus described below.

The second DBP SNP is rs16998073 (MAF = 0.21, 0.50 mm Hg higher per minor allele, $P = 1 \times 10^{-21}$, **Table 2** and **Fig. 1d**), which lies 3.4 kb upstream of *FGF5* (fibroblast growth factor 5) on 4q21. The FGF5 protein is a member of the fibroblast growth factor (FGF) family that stimulates cell growth and proliferation in multiple cell types, including cardiac myocytes, and has been associated with angiogenesis in the heart[31].

The third DBP SNP, rs653178 (MAF = 0.47, 0.46 mm Hg lower DBP per major allele, $P = 3 \times 10^{-18}$, **Table 2** and **Fig. 1f**) at 12q24 is in an intron of the *ATXN2* gene. This SNP is perfectly correlated with a missense SNP in exon 3 of *SH2B3* (rs3184504, R262W). The minor allele of rs3184504, which is associated with higher DBP, has recently been associated with increased odds of type 1 diabetes[32], celiac disease[33], myocardial infarction, hypertension and higher eosinophil and other blood cell counts[34]. We did not find that other SNPs previously reported to be associated with type 1 diabetes, celiac disease or myocardial infarction were associated with blood pressure (data not shown). *SH2B3* is expressed in hematopoietic precursor cells and in endothelial cells[35]. Murine knockout of the *SH2B3* gene (also known as lymphocyte-specific adaptor protein, *LNK*) is associated with increased hematopoietic progenitors of several lineages[36],

Table 3 Relationship of SNPs at 8 genome-wide significant loci to both blood pressure traits

SNP ID	Chr.	Position (NCBI35)	Coded allele	Noncoded allele	Coded allele frequency	*N* (effective)	Trait	Beta mm Hg	s.e.	*P*
rs17367504	1	11,797,044	G	A	0.14	34,158	SBP	−0.79	0.18	1×10^{-5}
							DBP	**−0.50**	**0.12**	$\mathbf{3 \times 10^{-5}}$
rs11191548	10	104,836,168	T	C	0.91	33,123	SBP	1.17	0.22	3×10^{-7}
							DBP	**0.56**	**0.15**	$\mathbf{2 \times 10^{-4}}$
rs12946454	17	40,563,647	T	A	0.28	32,120	SBP	0.68	0.15	4×10^{-6}
							DBP	**0.34**	**0.09**	$\mathbf{6 \times 10^{-4}}$
rs16998073	4	81,541,520	T	A	0.21	26,106	DBP	0.65	0.11	7×10^{-9}
							SBP	**0.74**	**0.17**	$\mathbf{1 \times 10^{-5}}$
rs1530440	10	63,194,597	T	C	0.19	32,718	DBP	−0.51	0.11	3×10^{-6}
							SBP	**−0.43**	**0.16**	$\mathbf{7 \times 10^{-3}}$
rs653178	12	110,470,476	T	C	0.53	30,853	DBP	−0.46	0.09	1×10^{-7}
							SBP	**−0.47**	**0.13**	$\mathbf{3 \times 10^{-4}}$
rs1378942	15	72,864,420	C	A	0.36	34,126	DBP	0.48	0.09	6×10^{-8}
							SBP	**0.62**	**0.13**	$\mathbf{2 \times 10^{-6}}$
rs16948048	17	44,795,465	G	A	0.39	34,052	DBP	0.40	0.09	5×10^{-6}
							SBP	**0.41**	**0.13**	$\mathbf{2 \times 10^{-3}}$

For each of eight SNPs, the upper row shows association statistics for the blood pressure trait used for the analysis in which they were selected (SBP or DBP). The lower row (in boldface) shows the equivalent association statistics for the alternate blood pressure trait. Results are shown for the 34,433 individuals in the stage 1 Global BPgen GWAS samples.

suggesting that the minor allele of the missense SNP in humans results in a loss of *SH2B3* function. In response to inflammatory stimuli, LNK seems to be a negative regulator of inflammatory signaling pathways in the endothelial cell, a cell type central to both blood pressure regulation and the process of atherosclerosis[35].

Noticing that the minor T allele of rs3184504 associated with higher DBP is common in HapMap CEU (frequency 0.45) and absent in HapMap YRI, JPT and CHB samples, we sought evidence for recent positive selection. The derived T allele occurs on a long-range haplotype ~1.5 Mb; relative to the haplotypes tagged by the ancestral allele, this is an unusual genomic feature (SNP-wise standardized integrated extended haplotype homozygosity [iHS] of −2.76, gene-based empirical *P* value <0.006)[37]. In addition, measures of population differentiation provide evidence of a local selective sweep in HapMap CEU (Wright's $F_{ST} = 0.26$ for CEU-YRI comparison and 0.29 for CEU-JPT/CHB). Finally, an ascertainment-adjusted Fay and Wu's H statistic of −35.7 supports the presence of an excess of high frequency–derived alleles at the locus. In sum, these measures support the hypothesis that the minor (derived) allele rose quickly to intermediate frequency in European-derived populations, possibly owing to some selective advantage of immune response to infectious pathogens. Although enhancing *SH2B3* activity might seem attractive to reduce risk for multiple diseases, the evidence for positive selection of an apparent loss-of-function allele and pleiotropic consequences suggest that enhancing *SH2B3* activity could have unintended consequences.

The fourth DBP SNP, rs1530440 (MAF = 0.19, 0.39 mm Hg lower per minor allele, $P = 1 \times 10^{-9}$, **Table 2** and **Fig. 1e**) at 10q21 is intronic and one of a cluster of SNPs in *C10orf107*, an open reading frame of unknown function. Nearby genes include *ARID5B* (A- rich interactive domain 5B (MRF1 like)), *TMEM26* (transmembrane protein 26), *RTKN2* (RhoA GTPase effector, rhotekin-2) and *RHOBTB1* (RhoBTB GTPase). The Rho family of GTPases converts guanine triphosphate to inactive guanine diphosphate. The actions relating to other GTP-modulating enzymes may modulate salt-sensitive hypertension[38,39]. The *ARID5B* gene is a member of the AT-rich interaction domain family of transcription factors and is highly expressed in cardiovascular tissue and involved in smooth muscle cell differentiation[40].

The fifth DBP SNP, rs16948048 (MAF 0.39, 0.34 mm Hg higher DBP per minor allele, $P = 5 \times 10^{-9}$, **Table 2** and **Fig. 1h**) at 17q21 is upstream of *ZNF652* (zinc finger protein 652) and *PHB* (prohibitin). Neither gene has previously been implicated in hypertension or other cardiovascular phenotypes.

We observed no significant interaction between the eight genome-wide significant SNPs and sex ($P > 0.01$, **Supplementary Table 4** online). There was also no evidence of heterogeneity of effect across the samples examined for the eight SNPs (Q-statistic $P > 0.05$).

Although we describe here promising candidates at each locus identified, the causal gene could be any of the genes around the association signal in each locus (**Fig. 1**). Fine mapping and resequencing will be required to refine each association signal and to identify likely causal genetic variants that could be studied further in humans and in animal models.

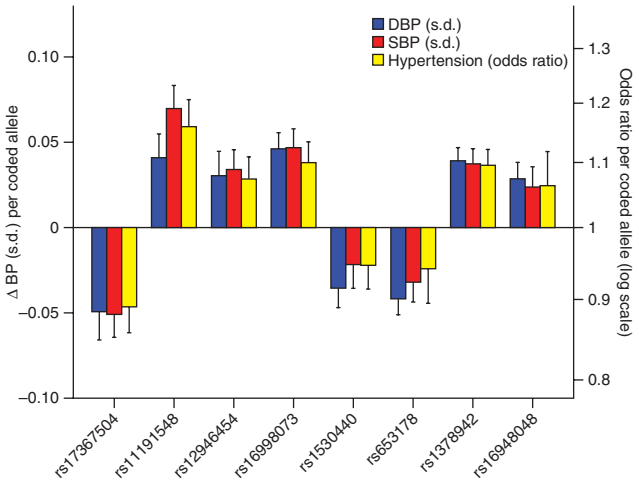

Figure 2 Relationship of genome-wide significant loci to SBP, DBP and hypertension. Shown are the effects of each variant on continuous SBP and DBP and on the odds ratio for dichotomous hypertension compared to normotension (see Methods). For comparability, SBP and DBP effects are shown on the s.d. scale (SBP s.d. = 16.6 mm Hg, DBP s.d. = 10.9 mm Hg). Alleles are coded as shown in **Table 2**.

Table 4 Association of eight SBP- and DBP-associated loci with hypertension

SNP ID	Chr	Position (NCBI35)	Continuous Trait	Coded allele	Coded allele frequency	Continuous BP effect	HTN OR	HTN 95% CI	HTN *P*	*N*
rs17367504	1	11,797,044	SBP	G	0.14	↓	0.89	0.86–0.93	2×10^{-9}	62,803
rs11191548	10	104,836,168	SBP	T	0.91	↑	1.16	1.11–1.21	3×10^{-13}	99,153
rs12946454	17	40,563,647	SBP	T	0.28	↑	1.07	1.04–1.11	2×10^{-5}	57,410
rs16998073	4	81,541,520	DBP	T	0.19	↑	1.10	1.07–1.13	7×10^{-10}	73,756
rs1530440	10	63,194,597	DBP	T	0.19	↓	0.95	0.91–0.98	2×10^{-3}	83,156
rs653178	12	110,470,476	DBP	T	0.53	↓	0.93	0.91–0.96	8×10^{-7}	60,030
rs1378942	15	72,864,420	DBP	C	0.37	↑	1.10	1.07–1.12	2×10^{-14}	99,802
rs16948048	17	44,795,465	DBP	G	0.39	↑	1.06	1.03–1.09	1×10^{-4}	62,411

Shown are the results for the top SNP from each genome-wide significant SBP or DBP locus from a logistic regression analysis of the odds of hypertension compared to normotension (see Methods). For comparison, the effect of the coded allele on the continuous blood pressure trait is shown. The inverse-variance-weighted meta-analysis results are shown. BP, blood pressure; OR, odds ratio.

All variants are related to both blood pressure traits

It remains to be clarified whether SBP or DBP is the better target for genetic investigation of blood pressure. The two traits are correlated and heritable, and both show strong increases with age, with DBP starting to plateau and in some individuals fall at ages above 60–65 years. Some have advocated the study of pulse pressure (SBP – DBP), which increases with advancing age, and is correlated positively with SBP and negatively with DBP and also shows evidence of heritability. In our GWAS and follow-up, we chose a priori to consider SBP and DBP as separate traits. Thus, validation was only attempted for either SBP or DBP, according to the trait for which the stage 1 *P* value was lowest. Because SBP and DBP are correlated ($r \sim 0.50$–0.70), it is perhaps not surprising to see that all eight genome-wide significant SNPs are associated with both SBP and DBP with the same directions of effect (**Table 3** and **Fig. 2**). Thus, our presentation of results as SBP- or DBP-associated is somewhat arbitrary. The observation that each SNP shows stronger association with one trait or the other (typically by 1–2 orders of magnitude) could reflect sampling variation, small effect sizes or true differences in the underlying biologic basis of one trait or the other. A study designed to examine pulse pressure would be expected to show weaker (if any) association signals for the variants identified, which all showed concordant effects on SBP and DBP.

All variants are related to hypertension

We did not carry out a global GWAS of hypertension, which is expected to be underpowered to detect common variants of modest incremental effects on continuous blood pressure. For the eight SNPs that were genome-wide significant in continuous trait analysis, we examined the association with hypertension (SBP $\geq$ 140 mm Hg or DBP $\geq$ 90 mm Hg or antihypertensive medication use) compared to normotension (SBP $\leq$ 120 mm Hg and DBP $\leq$ 85 mm Hg and no antihypertensive medication use) in planned secondary analyses (*N* range = 57,410–99,802). All alleles associated with continuous blood pressure were also associated with odds of hypertension in directions consistent with the continuous trait effect (**Table 4** and **Fig. 2**). The relative yields of the two approaches remain to be fully evaluated and will only become clearer upon completion of large ongoing GWA studies of dichotomous hypertension case-control collections. However, we examined the hypertension association of each of the eight SNPs genome-wide significantly associated with continuous SBP or DBP in the stage 1+2 meta-analysis. In the stage 1 Global BPgen samples alone, four of the eight SNPs had $0.01 < P \leq 0.10$. These SNPs would not have been selected for follow-up genotyping had these tests been conducted as part of a hypertension GWAS. Thus, the study of continuous blood pressure allowed us to identify effects on risk of hypertension that would not have been readily discovered in a GWAS of hypertension drawn from these samples.

Extension to non-European samples

To date, the majority of complex disease association signals reaching genome-wide significance have been concentrated in populations of European ancestry, and it remains unclear whether these findings will transfer to individuals with other genetic backgrounds. We genotyped all stage 2a SNPs (four of which were not confirmed in the European ancestry analyses) in a separate Indian Asian sample of up to 12,889 individuals. We replicated the association of the SNP at 4q21 near *FGF5* (rs16998073, $P = 5 \times 10^{-4}$, **Supplementary Table 2**) and the SNP at 10q24 near *CYP17A1* (rs11191548, $P = 0.008$, **Supplementary Table 2**). We did not replicate association of the SNP rs1378942 at *CYP1A2* ($P = 0.17$, same direction), which could reflect limited power to detect the modest effect size, differences in linkage disequilibrium patterns in Indian Asians compared to Europeans, or simply lack of association in individuals of Indian Asian ancestry. The marked allele frequency differences between the European samples (C allele frequency ~0.35), the Indian Asian samples (0.77) and HapMap YRI (1.00) suggest distinct patterns of genetic variation at this locus across populations. A signal of positive selection has been suggested at the locus[37], raising the potential functional importance of genetic variation in the region.

DISCUSSION

The eight loci described here and the additional loci reported by our colleagues in the CHARGE consortium are among the first confirmed associations between common genetic variants and blood pressure. Each association explains only a very small proportion of the total variation in SBP or DBP (~0.05–0.10%, approximately 1 mm Hg per allele SBP or 0.5 mm Hg per allele DBP, **Table 2**). However, the variants identified here have an aggregate effect on blood pressure, acting throughout the range of values (not just hypertensive), which has been shown to produce meaningful population changes in cardiovascular and stroke risk. For example, 2 mm Hg lower SBP, across the range of observed values, has been estimated to translate into 6% less stroke and 5% less coronary heart disease[2,41].

Given the modest effects observed here and the limited power of this study to detect such effects, it is likely that many more common variants exist with weak effects upon blood pressure. This study illustrates the value of well-powered meta-analysis and follow-up

genotyping, accompanied by *in silico* analysis, to establish definitively the relationship of these loci with blood pressure regulation in the general population.

In a companion paper, the CHARGE consortium reports as genome-wide significant three of the eight loci that reached genome-wide significance in our Global BPgen joint analysis of stages 1 and 2. CHARGE also reports common variants at five additional genome-wide significant loci at 11p15 (Global BPgen $P = 0.009$), 3p22 ($P = 0.01$), 12q21 ($P = 0.008$), 12q24 ($P = 0.05$), and 10p12 ($P = 0.004$, see companion CHARGE paper)[16]. Although these SNPs were not among our top ten SNPs for either blood pressure trait, the Global BPgen results from *in silico* exchange and for the same alleles are clearly consistent with the conclusions of the CHARGE investigators. Among the ten SBP and ten DBP loci at the top of the Global BPgen results, five loci were represented in the CHARGE top ten results (**Supplementary Table 3**). With the modest effect sizes we observed, it is not surprising that the top ten loci for each blood pressure trait would show only partial overlap.

We acknowledge that some limitations apply to our study. The participants in the individual studies comprising Global BPgen and our follow-up cohorts were ascertained using diverse criteria, had their blood pressure measured in a variety of ways and showed a broad range of age and treatment profiles. Even small differences in these factors could reduce power to detect the association of genetic variants with modest effect, although such heterogeneity should not increase the false-positive rate. Even though SBP and DBP are dynamic phenotypes resulting from multiple competing influences, estimates of the test-retest reliability of blood pressure measurements are approximately 0.65–0.75 in studies focused on blood pressure[2,42,43]. Moreover, a graded relationship between blood pressure measures and cardiovascular risk has been consistently observed, despite variability in blood pressure measures[2]. At the individual level, genetically determined alteration of 1 mm Hg SBP or 0.5 mm Hg DBP would be difficult to detect in the clinic, but large sample sizes use group-level differences in means to detect small genetic effects.

We chose a priori to adjust for body mass index (BMI), which explains ~6–8% of the total variation in SBP and DBP, with the goal of reducing potential nongenetic contributions to blood pressure variability. Genetic variants could influence blood pressure acting through BMI as an intermediate, but such variants are best identified through BMI GWA studies such as those recently reported by Loos *et al.*[44] and Willer *et al.*[45].

Exposures such as dietary sodium and potassium intake or excessive alcohol use also contribute to interindividual differences in blood pressure. These were measured in a minority of our samples and thus we could not meaningfully adjust for them in our study. Under the assumption that these do not alter blood pressure systematically by genotype, we would expect this omission to reduce power only slightly.

We adjusted for use of antihypertensive therapy by adding 15 mm Hg and 10 mm Hg to SBP and DBP, respectively. This approach has been shown to be superior to ignoring antihypertensive treatment or to excluding individuals on therapy[13]. However, it is clear that factors such as medication number and dosage and variation in prescription patterns in different countries and time periods make this adjustment scheme an oversimplification. Again, such effects should generally bias our findings toward the null.

There are many classes of widely used therapies with strong antihypertensive effects. We examined the association of common variants at the loci extending 100 kb on either side of the genes encoding the targets for thiazide diuretics (*SLC12A3*), loop diuretics (*SLC12A1*), ACE inhibitors (*ACE*), angiotensin II receptor type 1 blockers (*AGTR1*), beta adrenoreceptor blockers (*ADRB1*, *ADRB2*), alpha adrenoreceptor antagonists (*ADRA1A*, *ADRA1B*, *ADRA1D*), calcium channel blockers (*CACNA1S*, *CACNA1C*, *CACNA1D*, *CACNA1F*) and aldosterone antagonists (*CYP11B2*). No results exceeded chance expectations. This does not exclude the existence of variants of weaker effects or variants that were missed because they were not covered by existing arrays.

Moreover, the strength of association of variation in a gene with a trait (or lack thereof) says nothing about the potential strength of a drug designed to agonize or antagonize the product of that gene. For example, a common variant in *HMGCR* has only a modest effect on fasting lipids[46], yet statin therapy, which inhibits the HMGCR enzyme to lower LDL cholesterol, substantially lowers risk of cardiovascular disease. Thus, the implication of modest common variant genetic effects is not just a function of the ability to identify tendency toward higher or lower blood pressure in carriers of alternate alleles, but also the ability to recognize relevant targets for therapy that have defined *in vivo* relevance in humans.

Although targeted pharmacotherapy has theoretical appeal, clinical trials to demonstrate the utility and cost-effectiveness of such approaches will be required before such personalized medicine can be endorsed. The association signals identified here will need to be refined through fine mapping, and resequencing will be needed to define more fully the allelic spectrum of variants at each locus that contributes to interindividual differences in blood pressure. Our findings offer initial insights into the genetic basis of a problem of global proportions and the potential for an improved understanding of blood pressure regulation. These loci may point to new targets for blood pressure reduction and ultimately additional opportunities to prevent the growing public health burden of cardiovascular disease.

METHODS

Overall study design. An expanded description of the methods is provided in the **Supplementary Methods** online. The study comprised two-staged analyses carried out separately for SBP and DBP. Stage 1 was a meta-analysis of directly genotyped and imputed SNPs from individuals of European descent in 17 samples drawn from population-based or control samples in case-control studies in the Global BPgen consortium. In stage 2a, we selected 12 SNPs for genotyping in up to 71,225 individuals of European descent from 13 studies and up to 12,889 individuals of Indian Asian ancestry from one study. In stage 2b, we selected 20 SNPs (10 SBP, 10 DBP) for *in silico* analysis in 29,136 individuals of European descent from the CHARGE consortium (stage 2b, see **Supplementary Fig. 1**).

Stage 1 samples. The Global BPgen consortium comprises 17 GWAS studies: the Baltimore Longitudinal Study of Aging (BLSA), British 1958 Birth Cohort (B58C-T1DGC and B58C-WTCCC), Cohorte Lausannoise (CoLaus), Diabetes Genetics Initiative (DGI), European Prospective Investigation of Cancer-Norfolk-Genome Wide Association Study (EPIC-Norfolk-GWAS), Fenland Study, Finland-United States Investigation of NIDDM Genetics (FUSION) study, Invecchiare in Chianti (InCHIANTI), Kooperative Gesundheitsforschung in der Region Augsburg (KORA), the Myocardial Infarction Genetics Consortium (MIGen), Northern Finland Birth Cohort of 1966 (NFBC1966), SardiNIA, Study of Health in Pomerania (SHIP), the Precocious Coronary Artery Disease (PROCARDIS), Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) and TwinsUK. We excluded individuals >70 years of age and individuals ascertained on case status for type 1 or 2 diabetes (DGI, FUSION), coronary artery disease (MIgen, PROCARDIS) or hypertension (BRIGHT), leaving 34,433 individuals for analysis (**Table 1**). A detailed description of the study design and phenotype measurement for all cohorts can be found in the **Supplementary Methods**.

Genome-wide genotyping. Genotyping arrays and quality control filters are provided in **Supplementary Table 1**.

Imputation. Imputation of allele dosage of ungenotyped SNPs in HapMap CEU v21a or v22 was carried out using MACH[47] or IMPUTE[48] with parameters and preimputation filters as specified in **Supplementary Table 1**. SNPs were excluded from analysis if the cohort-specific imputation quality as assessed by r2.hat (MACH) or .info (IMPUTE) metrics was <0.30. In total, up to 2,497,993 genotyped or imputed autosomal SNPs were analyzed.

Phenotype modeling. In individuals taking antihypertensive therapies, blood pressure was imputed by adding 15 mm Hg and 10 mm Hg for SBP and DBP, respectively[13]. Continuous SBP and DBP were adjusted for age, age^2, body mass index and any study-specific geographic covariates in sex-specific linear regression models. In FUSION and SardiNIA, which included family-based samples, sex-pooled linear regression was carried out with the addition of sex as a covariate. Residuals on the mm Hg scale were used as univariate traits in genotype–phenotype analysis.

In secondary analyses, hypertension was defined by the presence of SBP $\geq$140 mm Hg or diastolic blood pressure $\geq$90 mm Hg or self-report of taking a medication for the treatment of hypertension. Normotensive controls were defined as individuals not taking any antihypertensives and having a SBP $\leq$120 mm Hg and a DBP $\leq$85 mm Hg.

Genotype–phenotype association analysis. Genotype–phenotype association of SBP and DBP residuals was carried out under an additive model using software as specified in **Supplementary Table 1**. Analysis of hypertension for eight genome-wide significant continuous blood pressure loci was done using logistic regression to adjust for age, age^2, sex and body mass index.

Meta-analysis of stage 1 samples. All cohort-specific effect estimates and coded alleles were oriented to the forward strand of the NCBI35 reference sequence of the human genome, using the alphabetically higher allele as the coded allele. For example, for a G/T SNP coded GG = 0, GT = 1, TT = 2, the coded allele would be T. To capture the power loss due to imperfect imputation, we estimated 'N effective', which was the sum of the cohort-specific products of the imputation quality metric and the sample size. No filtering on minor allele frequency was used. Genomic control[14] was carried out on cohort- and sex-specific test statistics. Lambda estimates are given in **Supplementary Table 1**; quantile-quantile plots are shown in **Supplementary Figure 2a**. Meta-analysis in stage 1 was conducted using inverse variance weights. Stage 1 meta-analysis results were subject to genomic control.

Selection of SNPs for stage 2. Twelve SNPs were selected for follow-up in stage 2a from among the results with $P < 10^{-5}$ during interim analyses. For *in silico* exchange with the CHARGE consortium (stage 2b), we identified the top independent loci to select ten SBP and ten DBP SNPs. If a SNP in one top ten list was also among the top ten for the alternate blood pressure trait, we kept the locus with the lower *P* value and went to the next locus on the list for the alternate blood pressure trait. Because a SNP at the 3q26 locus (*MDS1*) was selected in an interim analysis for direct genotyping, it was retained as the tenth locus for DBP even though its significance was reduced in the final stage 1 DBP GWAS analysis.

Stage 2a samples. We genotyped 12 SNPs in up to 71,225 individuals of European descent from 13 studies—Utrecht Atherosclerosis Risk in Young Adults (ARYA), British Genetics of Hypertension (BRIGHT), EPIC-Italy, EPIC-Norfolk-REP, Finrisk97, FUSION2, London Life Sciences Population (LOLI-POP), Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC), Metabolic Syndrome in Men (METSIM), Malmo Preventive Project (MPP), The Prevention of REnal and Vascular ENd stage Disease (PREVEND), Prospect-EPIC and the Utrecht Health Project (UHP)—and in up to 12,889 individuals of Indian Asian ancestry from the LOLIPOP study. Summary demographics are shown in **Table 1** and cohort information in the **Supplementary Methods**.

Stage 2a follow-up genotyping. For genotyping methods and platforms see **Supplementary Methods**.

Stage 2b *in silico* samples. We obtained results based on the analysis of the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortium, which comprises 29,136 samples from five population-based cohorts.

Pooled analysis of first and second stage samples. Meta-analysis of stage 1, 2a and 2b results was conducted using inverse variance weighting. Standard errors were multiplied by the square root of the lambda estimate for genomic control and are presented throughout the text. Nominal *P* values after genomic control[14] are presented. We considered associations genome-wide significant if they exceeded $P = 5 \times 10^{-8}$, a Bonferroni correction for the estimated 1 million independent common variant tests in the human genome of European-derived individuals[14,15].

Note: Supplementary information is available on the Nature Genetics website.

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The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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