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Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension

High blood pressure is a major risk factor for cardiovascular disease and premature death. However, there is limited knowledge on specific causal genes and pathways. To better understand the genetics of blood pressure, we genotyped 242,296 rare, low-frequency and common genetic variants in up to 192,763 individuals and used ~155,063 samples for independent replication. We identified 30 new blood pressure– or hypertension-associated genetic regions in the general population, including 3 rare missense variants in *RBM47*, *COL21A1* and *RRAS* with larger effects (>1.5 mm Hg/allele) than common variants. Multiple rare nonsense and missense variant associations were found in *A2ML1*, and a low-frequency nonsense variant in *ENPEP* was identified. Our data extend the spectrum of allelic variation underlying blood pressure traits and hypertension, provide new insights into the pathophysiology of hypertension and indicate new targets for clinical intervention.

High blood pressure, or hypertension (HTN), is a highly prevalent chronic disorder. It is estimated to be responsible for a larger proportion of global disease burden and premature mortality than any other disease risk factor[1]. Elevated systolic (SBP) and/or diastolic (DBP) blood pressure increases the risk of several cardiovascular disorders, including stroke, coronary heart disease (CHD), heart failure, peripheral arterial disease and abdominal aortic aneurysms[2]. Blood pressure is a complex heritable, polygenic phenotype, and genome-wide association studies (GWAS) have identified over 67 genetic regions associated with blood pressure and/or HTN thus far[3–11]. These variants are common (minor allele frequency (MAF) ≥ 0.05); mostly map to intronic or intergenic regions, with the causal alleles and genes not readily identified owing to linkage disequilibrium (LD)[4,5]; and explain only ~2% of trait variance[12]. Low-frequency (0.01 < MAF < 0.05) and rare (MAF ≤ 0.01) single-nucleotide variants (SNVs), predominantly unexplored by GWAS, may have larger phenotypic effects than common SNVs[13] and may help to explain the missing heritability and identify causative genes, as demonstrated previously[14].

To identify new coding variants and loci influencing blood pressure traits and HTN, we performed the largest meta-analysis thus far that included a total of ~350,000 individuals, directly genotyped with the Exome chip. The Exome chip contains ~240,000 mostly rare and low-frequency variants (Online Methods). A single-variant discovery analysis was performed, and candidate SNVs were taken forward for validation using independent replication samples. Gene-based tests were used to identify blood pressure–associated genes harboring multiple rare variant associations. We next assessed whether the newly identified blood pressure–associated SNVs were associated with expression levels of nearby genes and tested these variants in aggregate for a causal association of blood pressure with other cardiovascular traits and risk factors. Our findings highlight the contribution of rare variants in the etiology of blood pressure in the general population and provide new insights into the pathophysiology of HTN.

RESULTS

Discovery of single-variant blood pressure associations

We genotyped 192,763 individuals from 51 studies and assessed association of 242,296 SNVs with DBP, SBP, pulse pressure (PP) and HTN (Online Methods and **Supplementary Tables 1–3**). An overview of the SNV discovery study design is given in **Figure 1**. A fixed-effects meta-analysis for each trait was performed using study-level association summary statistics from (i) samples of European (EUR) ancestry (up to 165,276 individuals) and (ii) a trans-ancestry meta-analysis of the EUR and additional South Asian (SAS) ancestry samples (EUR_SAS; up to 192,763 individuals). Two analyses of DBP, SBP and PP were performed, one in which the trait was inverse normal transformed and a second in which the raw phenotype was analyzed. These sets of results were consistent (Online Methods); therefore, to minimize sensitivity to deviations from normality in the analysis of rare variants, the results from analyses of the transformed trait were used for discovery. Strong correlations between the blood pressure traits were observed across studies (Online Methods); hence, no adjustment of significance thresholds for independent trait testing was applied.

The discovery meta-analyses identified 51 genomic regions with genome-wide significant evidence of association with at least one of the four blood pressure traits tested ($P < 5 \times 10^{-8}$; **Supplementary Table 4**). There were 46 regions associated in the EUR_SAS samples, of which 14 were new (**Supplementary Fig. 1**). An additional five regions were genome-wide significant in the EUR-only meta-analyses, of which three were new (**Supplementary Fig. 2**). In total, 17 genomic regions were identified that were genome-wide significant for at least one blood pressure trait that have not been previously reported.

Replication of single-variant blood pressure associations

Next, we sought support for our findings, in an independent replication data set comprising 18 studies, 15 of which were from the

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Cohorts for Heart and Aging Research in Genomic Epidemiology+ (CHARGE+) exome chip blood pressure consortium (**Fig. 1** and ref. 15). Variants were selected for replication first using the larger (transformed) EUR_SAS data set, with additional variants from the (transformed) EUR data also selected. SNVs were selected if they mapped outside of known blood pressure genomic regions and had MAF ≥ 0.05 and $P \leq 1 \times 10^{-5}$ or MAF < 0.05 and $P \leq 1 \times 10^{-4}$ with at least one blood pressure trait, that is, choosing a lower significance threshold for the selection of rare variants (full details of the selection criteria are provided in the Online Methods). In total, 81 candidate SNVs were selected for replication (**Supplementary Table 5**). Eighty variants were selected from EUR_SAS (transformed) results and one SNV at the *ZNF101* locus was selected from the EUR (transformed) analyses. The results for EUR_SAS and EUR were consistent (association statistics were correlated, $\rho = 0.9$ across ancestries for each of the traits). Of the 81 variants, 30 SNVs were selected for association with DBP as the primary trait, 26 were selected for SBP, 19 were selected for PP and 6 were selected for HTN, with the primary trait defined as the blood pressure trait with the smallest association *P* value in the EUR_SAS discovery analyses.

Meta-analyses were performed on results from analyses of untransformed DBP, SBP, PP and HTN (as only results of untransformed traits were available from CHARGE+) in (i) up to 125,713 individuals of EUR descent and (ii) up to 155,063 individuals of multiple ancestries (4,632 of Hispanic descent, 22,077 of African-American descent and 2,641 SAS samples with the remainder EUR; **Fig. 1**). Given that a large proportion of the ancestries in the trans-ancestry meta-analyses were not included in our discovery samples, we used the EUR meta-analyses as the main data set for replication, but we also report any additional associations identified within the larger trans-ancestry data set.

New blood pressure–SNV associations were identified on the basis of two criteria (**Fig. 1** and Online Methods). First, replication of the primary blood pressure trait–SNV association was sought at a Bonferroni-adjusted *P*-value threshold in the replication data ($P \leq 6.17 \times 10^{-4}$, assuming $\alpha = 0.05$ for 81 SNVs tested and the same direction of effect; Online Methods) without the need for genome-wide significance. Second, meta-analyses of discovery and replication results across all four (untransformed) blood pressure traits were performed to assess the overall level of support across all samples for the 81 candidate SNVs; those blood pressure–SNV associations that were genome-wide significant (with statistical support in the replication studies; $P < 0.05$ and the same direction of effect as in the discovery results) were also declared as new.

Seventeen SNV–blood pressure associations formally replicated with concordant direction of effect at a Bonferroni-adjusted significance level for the primary trait. Fourteen were in the EUR meta-analyses, and among these was a rare nonsynonymous SNV mapping to *COL21A1* (**Table 1** and **Supplementary Table 6**). Three associations were in the trans-ancestry meta-analyses: these included two rare nonsynonymous SNVs in *RBM47* and *RRAS* (**Table 1**, Online Methods and **Supplementary Table 7**).

In addition to the 17 SNV–blood pressure trait associations that formally replicated, we identified 13 further SNV associations that were genome-wide significant in the combined (discovery and replication) meta-analyses. Ten of these were genome-wide significant in the combined EUR analyses (**Table 2** and **Supplementary Tables 6** and **8a**), and three were genome-wide significant in the combined trans-ancestry meta-analyses (**Table 2** and **Supplementary Tables 7** and **8b**).

This gives a total of 30 new SNV–blood pressure associations (15 SNV–DBP, 9 SNV–SBP and 6 SNV–PP; **Tables 1** and **2**, and

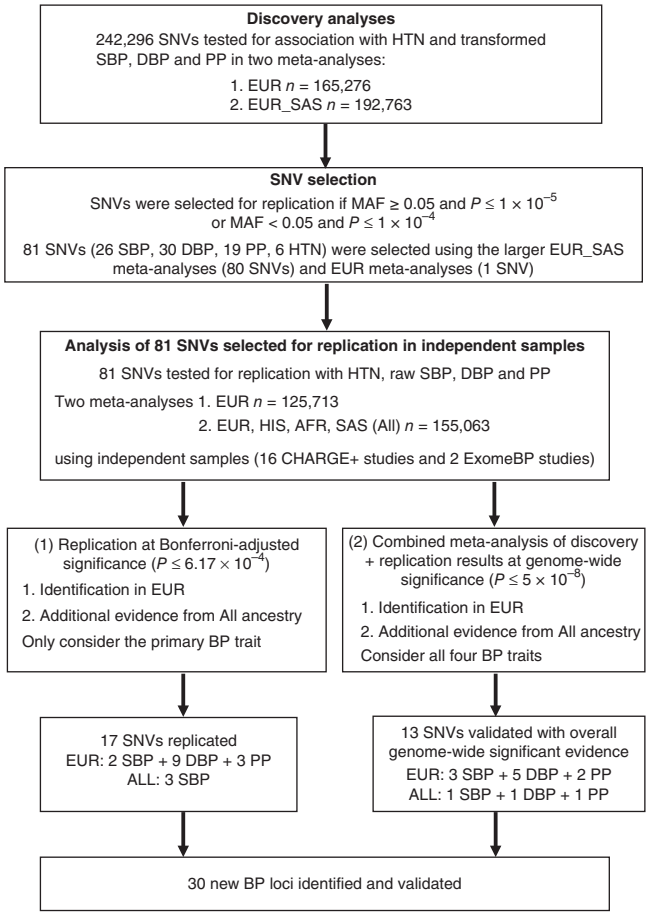

Figure 1 Study design and workflow diagram for single-variant discovery analyses. EUR, European; SAS, South Asian; HIS, Hispanic; AFR, African; HTN, hypertension; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; *n*, sample size; MAF, minor allele frequency; SNV, single-nucleotide variant. Further details of the SNV selection criteria are provided in the Online Methods.

Supplementary Figs. 3 and **4**). Five of the SNVs were genome-wide significant with more than one blood pressure trait (**Fig. 2**, **Tables 1** and **2**, and **Supplementary Table 8**). Four loci (*CERS5*, *TBX2*, *RGL3* and *OBFC1*) had genome-wide significant associations with HTN in addition to genome-wide significant associations with DBP and SBP. The *PRKAG1* locus had genome-wide significant associations with both SBP and PP.

Conditional analyses were performed to identify secondary signals of association within the new blood pressure–associated loci. The RAREMETALWORKER (RMW) package (Online Methods)[16] allows conditional analyses to be performed using summary-level data. Hence, analyses of the transformed primary traits and HTN were rerun in RMW across the discovery studies (**Fig. 3**). The results of the RMW single-variant tests were consistent with the initial discovery analyses (**Supplementary Note**). Given that the RMW analyses were based on our discovery samples, the larger EUR_SAS data set was used for the main analysis to increase power, but we also report any additional associations with evidence in the EUR cohort.

We identified secondary independent signals of association in four loci—*PREX1*, *PRKAG1* and *RRP1B* within the EUR_SAS analyses and *COL21A1* in the EUR analyses ($P_{conditional} \leq 1 \times 10^{-4}$, Bonferroni adjusted for ~500 variants within each region; Online Methods and

Table 1 New blood pressure–associated loci: variants with formal replication

Variant information				Discovery		Replication			Combined		
Locus	rsID	Chr:position (EA, EAF)	Trait	P_T	P_U	*n*	β	*P*	*n*	β	*P*
EUR											
RNF207	rs709209	1:6.28 (A, 0.655)	PP	4.57×10^{-6}	1.60×10^{-6}	122,780	0.17	5.83×10^{-4}	284,683	0.20	9.62×10^{-9}
C5orf56	rs12521868	5:131.78 (T, 0.373)	DBP	1.59×10^{-6}	3.03×10^{-7}	122,795	−0.18	2.29×10^{-5}	282,023	−0.19	6.12×10^{-11}
PHACTR1	rs9349379	6:12.90 (A, 0.566)	SBP	2.11×10^{-8}	1.78×10^{-7}	122,809	0.24	4.06×10^{-4}	284,673	0.29	8.84×10^{-10}
COL21A1	rs200999181[a]	6:55.94 (A, 0.002)	PP	3.08×10^{-8}	2.46×10^{-7}	121,487	2.70	1.90×10^{-4}	242,486	3.25	6.27×10^{-10}
ABO	rs687621	9:136.14 (A, 0.615)	DBP	8.80×10^{-8}	2.55×10^{-7}	122,798	0.16	1.96×10^{-4}	276,014	0.19	5.45×10^{-10}
ADO	rs10995311	10:64.56 (C, 0.567)	DBP	1.86×10^{-6}	1.14×10^{-6}	122,798	0.23	8.47×10^{-8}	266,456	0.21	1.12×10^{-12}
LMO1	rs110419	11:8.25 (A, 0.481)	DBP	9.41×10^{-6}	2.22×10^{-5}	122,798	0.16	1.81×10^{-4}	279,935	0.16	3.04×10^{-8}
OR5B12	rs11229457	11:58.21 (T, 0.236)	SBP	1.58×10^{-6}	4.62×10^{-5}	122,809	−0.32	7.53×10^{-5}	284,680	−0.31	2.70×10^{-8}
CERS5	rs7302981	12:50.54 (A, 0.361)	DBP	1.35×10^{-13}	4.60×10^{-11}	122,798	0.24	2.64×10^{-8}	284,718	0.25	1.38×10^{-17}
MYH6	rs452036	14:23.87 (A, 0.327)	PP	4.59×10^{-11}	2.80×10^{-13}	122,780	−0.21	1.81×10^{-5}	284,672	−0.28	2.96×10^{-16}
DPEP1	rs1126464	16:89.70 (C, 0.256)	DBP	1.19×10^{-9}	4.35×10^{-11}	118,677	0.24	1.68×10^{-6}	261,564	0.28	1.02×10^{-15}
TBX2	rs8068318[a]	17:59.48 (T, 0.698)	DBP	7.46×10^{-13}	5.71×10^{-10}	122,798	0.26	3.23×10^{-8}	281,978	0.26	1.95×10^{-16}
RGL3	rs167479	19:11.53 (T, 0.486)	DBP	2.22×10^{-23}	1.97×10^{-22}	122,797	−0.29	3.01×10^{-11}	283,332	−0.33	1.99×10^{-31}
PREX1	rs6095241	20:47.31 (A, 0.452)	DBP	5.65×10^{-6}	2.29×10^{-5}	122,798	−0.18	2.56×10^{-5}	281,322	−0.17	4.75×10^{-9}
All ancestry											
RBM47	rs35529250[a]	4:40.43 (T, 0.010)	SBP	6.56×10^{-7}	6.15×10^{-6}	148,878	−1.43	5.02×10^{-4}	306,352	−1.55	2.42×10^{-8}
OBFC1	rs4387287	10:105.68 (A, 0.157)	SBP	2.23×10^{-8}	1.32×10^{-7}	147,791	0.28	3.37×10^{-4}	320,494	0.36	9.12×10^{-10}
RRAS	rs61760904[a]	19:50.14 (T, 0.008)	SBP	1.96×10^{-6}	1.90×10^{-5}	148,878	1.38	5.70×10^{-4}	322,664	1.50	8.45×10^{-8}

SNV–blood pressure associations are reported for the newly identified blood pressure loci that replicated at $P \leq 6.17 \times 10^{-4}$ (Bonferroni correction for the 81 variants selected for replication for a primary blood pressure trait; Online Methods). Loci are categorized into EUR and all-ancestry groups on the basis of the meta-analysis used to replicate the variant for the primary blood pressure trait listed in the "Trait" column. For discovery meta-analysis results, P_T represents the *P* value for association of the variant with the transformed primary blood pressure trait in the EUR_SAS discovery meta-analyses (which was also used to select the variant for replication) and P_U represents the *P* value for association with the untransformed primary blood pressure trait in the ancestry in which the variant replicated. *n*, β and *P*, which denote the number of samples, estimated allelic effect and *P* value, respectively, are provided for the untransformed primary blood pressure trait in the replication data and also for the combined (discovery and replication) meta-analyses. Note that "All ancestry" corresponds to all ancestries in the combined (discovery and replication) meta-analyses. Locus, gene or region containing the SNV; rsID, dbSNP rsID; chr:position (EA, EAF), chromosome:NCBI Build 37 position in megabases (effect allele, effect allele frequency); trait, primary blood pressure trait for which the variant was also replicated; β, effect estimate; *n*, sample size; EUR, European.

[a]Nonsynonymous SNV or SNV in LD (r^2 >0.8) with a nonsynonymous SNV that is predicted to be damaging.

Supplementary Tables 9 and **10**). Three independent association signals were identified in the *MYH6* locus in the EUR_SAS analyses (**Supplementary Table 11**).

Gene-based blood pressure associations

To improve statistical power to detect associations in genes harboring rare variants, analytical methods that combine effects of variants across a gene into a single test have been devised and are implemented in the RMW package[16]. We applied the gene-based sequence kernel association test (SKAT)[17] and burden tests[18] to the RMW data set (MAF < 0.05 or MAF < 0.01; **Fig. 3** and Online Methods). One previously unidentified blood pressure–associated gene (*A2ML1*) was associated with HTN ($P = 7.73 \times 10^{-7}$) in the EUR_SAS studies and also in the EUR studies (Bonferroni-corrected threshold of significance $P < 2.8 \times 10^{-6}$, after adjusting for 17,996 genes tested; Online Methods and **Supplementary Table 12**). The gene showed residual association with the primary blood pressure trait after conditioning on the most associated SNV in the gene ($P_{conditional} = 5.00 \times 10^{-4}$; **Supplementary Table 12**), suggesting that the association is due to multiple rare variants in the gene. One nonsense variant (rs199651558, p.Arg893*, MAF = 3.5×10^{-4}) was observed, and there were multiple missense variants (**Fig. 4**). *A2ML1* encodes α2-macroglobulin-like 1 protein and is a member of the α-macroglobulin superfamily, which comprises protease inhibitors targeting a wide range of substrates. Mutations in this gene are associated with a disorder clinically related to Noonan syndrome, a developmental disorder that involves cardiac abnormalities[19]. We sought replication in the CHARGE+ studies for this gene; however, there was no evidence of association with HTN ($P = 0.45$). Given the very low frequencies of the variants involved, however, studies in which the variants are polymorphic will be required to replicate the association with HTN. The DBH gene was found to be associated with DBP using the SKAT test ($P = 2.88 \times 10^{-6}$). However, this was not due to multiple rare variants as the association was driven by rs77273740 (**Supplementary Table 5**) and the SNV was not validated in the replication samples.

Rare and common variant associations in established blood pressure loci

Of the 67 established blood pressure loci, 35 loci were on the Exome chip (*n* = 43 SNVs or close proxies, r^2 >0.7). All 43 SNVs had at least nominal evidence of association with blood pressure in our discovery samples ($P < 0.01$; **Supplementary Table 13**). We also assessed whether any of the established blood pressure loci contained coding variants that are associated with blood pressure traits and in LD (r^2 >0.2) with the known blood pressure variants on the Exome chip (**Supplementary Table 13**), using the 1000 Genomes Project phase 3 release for LD calculations. Focusing on SNVs that were genome-wide significant for any blood pressure trait from our transformed discovery data for either ancestry, there were 25 coding variants, of which 6 were predicted to be damaging at loci labeled *CDC25A*, *SLC39A8*, *HFE*, *ULK4*, *ST7L–CAPZA1–MOV10* and *CYP1A1–ULK3*. Three of these are published variants at loci labeled *SLC39A8*, *HFE* and *ST7–CAPZA1–MOV10*. At *CYP1A1–ULK3*, the coding variant was in moderate LD with the reported variant but was less significantly associated with DBP in our EUR_SAS data set ($P = 2.24 \times 10^{-8}$ as compared to $P = 1.68 \times 10^{-15}$ for the published variant). At the *ULK4* locus, the predicted damaging coding variant had similar association as the published coding variant (predicted to be benign), and previous work has already indicated several associated nonsynonymous SNVs in strong LD in *ULK4* (ref. 4). The nonsynonymous SNV within

Table 2 New blood pressure–associated loci: variants with genome-wide significant evidence of association in combined meta-analyses

Variant information				Discovery		Replication			Combined		
Locus	rsID	Chr:position (EA, EAF)	Trait	P_T	P_U	*n*	β	*P*	*n*	β	*P*
EUR											
2q36.3	rs2972146	2:227.10 (T, 0.652)	DBP[b] (HTN)	1.51×10^{-9}	2.47×10^{-7}	122,798	0.13	2.20×10^{-3}	275,610	0.17	8.40×10^{-9}
ZBTB38	rs16851397	3:141.13 (A, 0.953)	DBP[b] (SBP)	6.87×10^{-6}	3.20×10^{-5}	122,798	−0.38	1.20×10^{-4}	284,717	−0.38	3.01×10^{-8}
PRDM6	rs1008058	5:122.44 (A, 0.135)	SBP	5.09×10^{-7}	1.01×10^{-8}	43,109	0.46	3.61×10^{-3}	176,362	0.55	2.99×10^{-10}
GPR20	rs34591516	8:142.37 (T, 0.055)	SBP[b] (DBP)	1.54×10^{-6}	1.01×10^{-7}	122,807	0.51	4.20×10^{-4}	282,009	0.64	6.10×10^{-10}
HOXB7	rs7406910	17:46.69 (T, 0.118)	SBP	6.07×10^{-10}	2.74×10^{-9}	122,809	−0.20	4.89×10^{-2}	284,690	−0.46	3.80×10^{-8}
AMH	rs10407022[a]	19:2.25 (T, 0.822)	PP	1.63×10^{-7}	1.73×10^{-7}	118,656	−0.19	1.62×10^{-3}	252,525	−0.26	5.94×10^{-9}
ZNF101	rs2304130	19:19.79 (A, 0.914)	DBP	1.66×10^{-8}	1.92×10^{-8}	122,798	−0.17	1.71×10^{-2}	284,705	−0.29	1.53×10^{-8}
PROCR	rs867186	20:33.76 (A, 0.873)	DBP	1.44×10^{-6}	4.15×10^{-7}	122,798	0.21	2.48×10^{-3}	284,722	0.26	1.19×10^{-8}
RRP1B	rs9306160	21:45.11 (T, 0.374)	DBP[b] (SBP)	1.04×10^{-8}	1.90×10^{-6}	100,489	−0.16	4.30×10^{-4}	249,817	−0.18	6.80×10^{-9}
TNRC6B	rs470113	22:40.73 (A, 0.804)	PP	1.48×10^{-10}	1.31×10^{-9}	122,780	−0.14	1.37×10^{-2}	284,683	−0.25	1.67×10^{-9}
ALL ancestry											
7q32.1	rs4728142	7:128.57 (A, 0.433)	SBP	8.10×10^{-6}	4.21×10^{-6}	150,542	−0.21	8.62×10^{-4}	338,338	−0.24	3.45×10^{-8}
PRKAG1	rs1126930[a]	12:49.40 (C, 0.036)	PP	2.12×10^{-6}	4.62×10^{-7}	151,481	0.36	3.74×10^{-3}	314,894	0.50	3.34×10^{-8}
SBNO1	rs1060105	12:123.81 (T, 0.209)	DBP	6.66×10^{-7}	1.09×10^{-6}	150,532	−0.15	2.67×10^{-3}	336,413	−0.18	3.07×10^{-8}

SNV–blood pressure associations are reported for the newly identified blood pressure loci that showed genome-wide significant association ($P \leq 5 \times 10^{-8}$) in the combined discovery and replication meta-analyses. For discovery meta-analysis results, P_T represents the *P* value for association of the variant with the transformed primary blood pressure trait in the EUR_SAS discovery meta-analyses (used to select the variant for replication) and P_U represents the *P* value for association with the untransformed blood pressure trait in the ancestry in which the variant was validated. Loci are categorized into EUR and all-ancestry groups on the basis of the ancestry group in which the variant showed association with a blood pressure trait at $P \leq 5 \times 10^{-8}$. *n*, β and *P*, which denote the number of samples, estimated allelic effect and *P* value for association with the validated blood pressure trait, respectively, are provided for the untransformed blood pressure trait in the replication data and also for the combined (discovery and replication) meta-analyses. Note that "All ancestry" corresponds to all ancestry groups in the combined (discovery and replication) meta-analyses. Locus, gene or region containing the SNV; rsID, dbSNP rsID; chr:position (EA, EAF), chromosome:NCBI Build 37 position in megabases (effect allele, effect allele frequency); trait, blood pressure trait for which association is reported; EUR, European.

[a]Nonsynonymous SNV or SNV in LD (r^2 >0.8) with a nonsynonymous SNV that is predicted to be damaging. [b]At four loci (2q36.3, *ZBTB38*, *GPR20* and *RRP1B*), the primary trait used to select the variants for replication is given in parentheses because the variant associations were validated in the combined meta-analysis for the listed secondary trait. For these variants, P_T denotes the *P* value for association with the primary trait; the other *P* values provided are for the secondary trait.

the *CDC25A* locus (rs11718350 in *SPINK8*) had similar association with DBP as the intergenic published SNV in our EUR_SAS data set ($P = 2.00 \times 10^{-8}$ as compared to $P = 2.27 \times 10^{-8}$ for the published variant). Overall at least five of the known loci are consistent with having a coding causal variant.

Gene-based SKAT tests of all genes that map within 1 Mb of a previously reported SNV association (**Supplementary Table 14**) indicated no genes with multiple rare or low-frequency variant associations. Single-variant conditional analyses showed that rs33966350, a rare nonsense variant in *ENPEP* (MAF = 0.01), was associated with SBP ($P_{conditional} = 1.61 \times 10^{-5}$) in the EUR_SAS samples (Online Methods and **Supplementary Tables 14** and **15**) independently of the known SNV (rs6825911). *ENPEP* encodes aminopeptidase A (APA), an enzyme of the renin–angiotensin–aldosterone system (RAAS) that converts angiotensin II (AngII) to AngIII.

There were no other established loci with convincing low-frequency or rare SNV associations in the EUR_SAS samples. However, *HOXC4* had evidence of a second independent signal with a rare missense SNV in EUR samples (rs78731604; MAF = 0.005, $P_{conditional} = 5.76 \times 10^{-5}$; **Supplementary Table 15**). The secondary signal in the *HOXC4* region mapped to *CALCOCO1*, ~300 kb from the known SNV. The gene association (MAF ≤ 0.01, $P = 2.37 \times 10^{-5}$) was below the required significance threshold and attributable to rs78731604, which is not predicted to have detrimental effects on protein structure. Therefore, replication of this association is required. Three loci (*ST7L–CAPZA1–MOV10*, *FIGN–GRB14* and *TBX5–TBX3*) had evidence of a second independent signal in the region in EUR_SAS samples with a common variant ($P_{conditional} < 1 \times 10^{-4}$; **Supplementary Table 15**) that has not been previously reported.

Having identified 30 new loci associated with blood pressure traits, as well as additional new independent SNVs at 4 new loci and 5 known loci, we calculated the percentage of the trait variance explained (Online Methods). This was 2.08%/2.11%/1.15% for SBP/DBP/PP for the 43 previously reported blood pressure–SNV associations covered in our data set, increasing to 3.38%/3.41%/2.08%, respectively, with the inclusion of the 30 lead SNVs from newly identified loci, plus new independent SNV–blood pressure associations identified from new and known loci.

Effect of blood pressure SNVs on cardiovascular traits and risk factors

Among our new blood pressure–SNV associations, some have previously been reported to be associated with other cardiovascular traits and risk factors (**Supplementary Table 16**); these include CHD (*PHACTR1* and *ABO*)[20,21], QT interval (*RNF207*)[22], heart rate (*MYH6*)[23] and cholesterol levels (2q36.3, *ABO* and *ZNF101*)[24].

To test the impact of blood pressure variants on cardiovascular endpoints and risk factors, we created three weighted genetic risk scores (GRSs) according to SBP/DBP/PP on the basis of the newly identified and previously published blood pressure variants (up to $n = 125$; Online Methods). The GRS models were used to test the causal effect of blood pressure on the following traits: ischemic stroke (including the subtypes cardiometabolic, large vessel and small vessel[25]), CHD, heart failure[26], left ventricular mass[27], left ventricular wall thickness[27], high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, total cholesterol, body mass index (BMI), waist–hip ratio–adjusted BMI, height and estimated glomerular filtration rate (eGFR) (Online Methods). As expected, blood pressure was positively associated with increased CHD risk (odds ratio (95% confidence interval) = 1.39 (1.22–1.59) per increase of 10 mm Hg in SBP, $P = 6.07 \times 10^{-7}$; 1.62 (1.28–2.05) per increase of 10 mm Hg in DBP, $P = 5.99 \times 10^{-5}$; 1.70 (1.34–2.16) per increase of 10 mm Hg in PP, $P = 1.20 \times 10^{-5}$; **Table 3**) and increased risk of ischemic stroke (OR (95% CI) = 1.93 (1.47–2.55) per increase of 10 mm Hg in DBP, $P = 2.81 \times 10^{-6}$; 1.57 (1.35–1.84) per increase of 10 mm Hg in SBP, $P = 1.16 \times 10^{-8}$; 2.12 (1.58–2.84) per

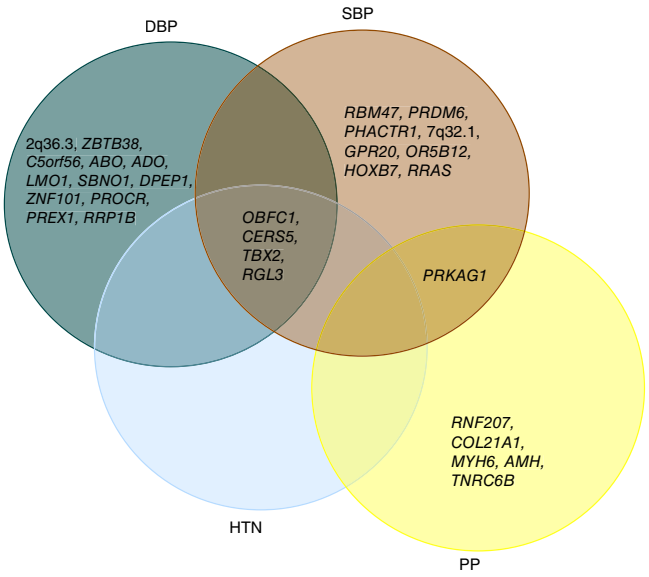

Figure 2 Overlap of the 30 new blood pressure–associated loci across SBP, DBP, PP and HTN. The Venn diagram shows which of the 30 newly identified blood pressure loci are associated with multiple blood pressure traits. Only SNV–blood pressure associations that were genome-wide significant ($P \leq 5 \times 10^{-8}$) in the combined discovery and replication meta-analyses are listed for any given blood pressure trait, within the corresponding ancestry data set that the given locus was validated (**Tables 1** and **2**). Association of the *RRAS* variant with SBP was replicated in the independent samples but did not achieve genome-wide significance in the combined discovery and replication meta-analysis and is therefore included for SBP.

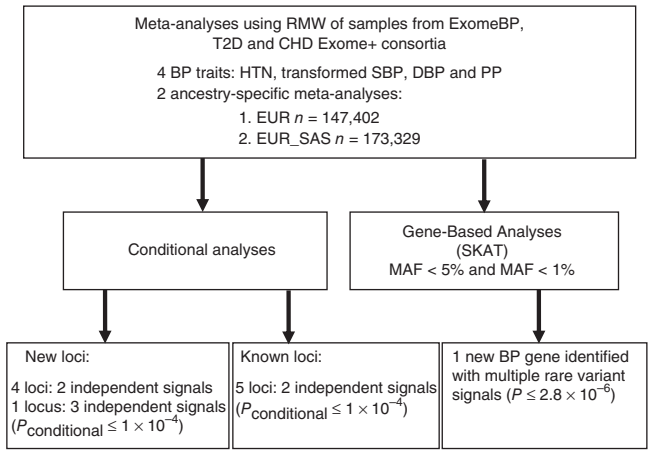

Figure 3 Study design for conditional analyses and rare variant gene-based discovery analyses. RMW, RareMetalWorker, $P_{conditional}$, conditional *P*-value significance threshold.

increase of 10 mm Hg in PP, $P = 5.35 \times 10^{-7}$). The positive association with ischemic stroke was primarily due to large vessel stroke (**Table 3**). DBP and SBP were also positively associated with left ventricular mass (9.57 (increase of 3.98–15.17) g per increase of 10 mm Hg in DBP, $P = 8.02 \times 10^{-4}$ and increase of 5.13 (1.77–8.48) g per increase of 10 mm Hg in SBP, $P = 0.0027$) and left ventricular wall thickness (increase of 0.10 (0.06–0.13) cm per increase of 10 mm Hg in DBP, $P = 1.88 \times 10^{-8}$ and increase of 0.05 (0.03–0.07) cm per increase of 10 mm Hg in SBP, $P = 5.52 \times 10^{-6}$; **Table 3**). There was no convincing evidence to support the blood pressure–associated variants having an effect on lipid levels ($P > 0.1$), BMI ($P > 0.005$), waist–hip ratio–adjusted BMI ($P > 0.1$), height ($P > 0.06$), eGFR ($P > 0.02$) or heart failure ($P > 0.04$). The causal associations with CHD, stroke and left ventricular measures augment the results from a previous association analysis using 29 blood pressure–associated variants[4]. Our analyses strongly support the previous observations of no causal relationship between blood pressure and eGFR. Lack of evidence for a blood pressure effect with heart failure may be due to lack of power, as the association was in the expected direction.

Possible functional variants at blood pressure loci and candidate genes

Twenty-six of our newly discovered blood pressure–associated SNVs had MAF ≥ 0.05; therefore, because of extensive LD with other SNVs not genotyped on the Exome array, identifying the causal genes requires additional information. If an SNV is associated with increased or decreased expression of a particular gene, that is, it is an expression quantitative trait locus (eQTL), this suggests that the gene on which the SNV acts could be in the causal pathway. To help identify potential candidate causal genes in the new blood pressure loci (**Supplementary Table 9**), information from publicly available eQTL databases was investigated (MuTHER for lymphoblastoid cell lines (LCLs), adipose tissue and skin and GTEx for nine tissues including the heart and tibial artery; Online Methods).

The DBP-increasing (A) allele of the nonsynonymous SNV rs7302981 was associated with increased expression of *CERS5* in: LCLs ($P_{MuTHER} = 3.13 \times 10^{-72}$), skin ($P_{MuTHER} = 2.40 \times 10^{-58}$), adipose ($P_{MuTHER} = 2.87 \times 10^{-54}$) and nerve tissue ($P_{GTEx} = 4.5 \times 10^{-12}$) (**Supplementary Fig. 5**). Additional testing (Online Methods) provided no evidence against colocalization of the eQTL and DBP association signals, implicating *CERS5* as a candidate causal gene for this DBP locus. *CERS5* (*LAG1* homolog; ceramide synthase 5) is involved in the synthesis of ceramide, a lipid molecule involved in several cellular signaling pathways. *Cers5* knockdown has been shown to reduce cardiomyocyte hypertrophy in mouse models[28]. However, it is unclear whether the blood pressure–raising effects at this locus are the cause or result of any potential effects on cardiac hypertrophy. Future studies investigating this locus in relation to parameters of cardiac hypertrophy and function (for example, ventricular wall thickness) should help address this question.

The DBP-increasing allele of the nonsynonymous SNV (rs867186[A]) was associated with increased expression of *PROCR* in adipose tissue ($P_{MuTHER} = 3.24 \times 10^{-15}$) and skin ($P_{MuTHER} = 1.01 \times 10^{-11}$) (**Supplementary Fig. 5**). There was no evidence against colocalization of the eQTL and DBP association, thus supporting *PROCR* as a candidate causal gene. *PROCR* encodes the endothelial protein C receptor, a serine protease involved in the blood coagulation pathway, and rs867186 has previously been associated with coagulation and hematological factors[29,30]. The PP-decreasing (T) allele of rs10407022, which is predicted to have detrimental effects on protein structure (Online Methods), was associated with increased expression of *AMH* in muscle ($P_{GTEx} = 9.95 \times 10^{-15}$), thyroid ($P_{GTEx} = 8.54 \times 10^{-7}$), nerve tissue ($P_{GTEx} = 7.15 \times 10^{-8}$), tibial artery ($P_{GTEx} = 6.46 \times 10^{-9}$), adipose tissue ($P_{GTEx} = 4.69 \times 10^{-7}$) and skin ($P_{GTEx} = 5.88 \times 10^{-8}$) (**Supplementary Fig. 5**). There was no evidence against colocalization of the eQTL and PP association, which supports *AMH* as a candidate causal gene for PP. Low AMH levels have previously been associated with hypertensive status in women, with the protein acting as a marker of ovarian reserve[31]. The intergenic SBP-increasing (A) allele of rs4728142 was associated with reduced expression of *IRF5* in skin

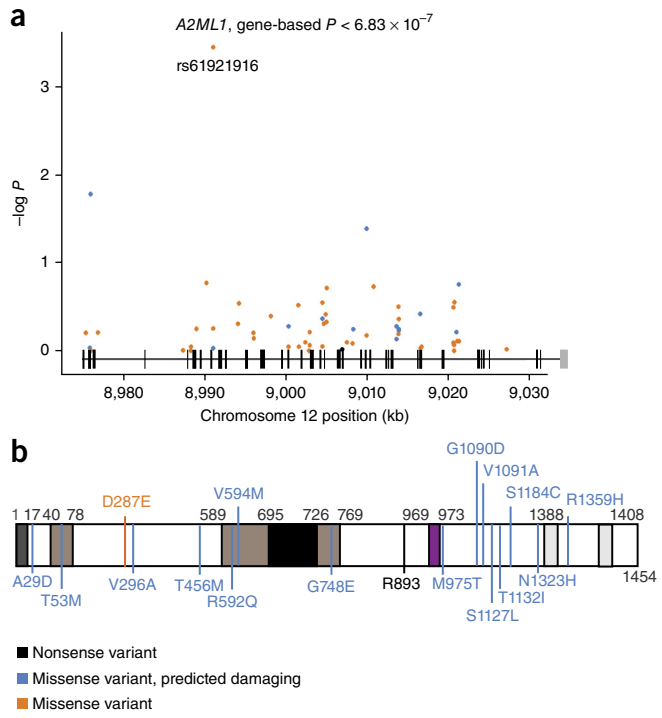

Figure 4 Locus plot for *A2ML1* and secondary amino acid structure of the gene product. (**a**) Locus plot for *A2ML1* association with HTN identified through gene-based tests. The positions of variants along the gene (*x* axis; based on human genome Build 37) and $-\log_{10}$ *P* of association (*y* axis) are shown. The schematic above the *x* axis represents the exon–intron structure; UTRs are shown as gray vertical bars. (**b**) The white box shows the full-length amino acid sequence for each of the two gene products. Black numbers correspond to amino acid positions of note. Colored vertical lines indicate the amino acid substitutions corresponding to the variants depicted in **a**. p.Asp287Glu is the SNV with the smallest *P* value. Colored boxes depict putative functional domains: dark gray, signal peptide sequence; brown, regions of intramolecular disulfide bond formation; black, bait region described to interact with proteases; purple, thiol ester sequence aiding in interaction with proteases; light gray, α-helical regions thought to mediate A2ML1 interaction with LRP1, facilitating receptor-mediated endocytosis. For simplicity, only regions coinciding with variants described are indicated.

($P_{\text{MuTHER}} = 5.24 \times 10^{-31}$) and LCLs ($P_{\text{MuTHER}} = 1.39 \times 10^{-34}$), whole blood ($P_{\text{GTEx}} = 3.12 \times 10^{-7}$) and tibial artery ($P_{\text{GTEx}} = 1.71 \times 10^{-7}$).

Three new rare nonsynonymous SNVs were identified that map to *RBM47* and *RRAS* (both associated with SBP) and *COL21A1* (associated with PP). They had larger effect sizes than common variant associations (>1.5 mm Hg per allele; **Supplementary Fig. 6**) and were predicted to have detrimental effects on protein structure (Online Methods and **Supplementary Table 16**). In *RBM47*, rs35529250 (p.Gly538Arg) is located in a highly conserved region of the gene and was most strongly associated with SBP (MAF = 0.008; increase of 1.59 mm Hg per T allele; $P = 5.90 \times 10^{-9}$). *RBM47* encodes RNA binding motif protein 47 and is responsible for post-transcriptional regulation of RNA, through direct and selective binding with the molecule[32]. In *RRAS*, rs61760904 (p.Asp133Asn) was most strongly associated with SBP (MAF = 0.007; increase of 1.51 mm Hg per T allele; $P = 8.45 \times 10^{-8}$). *RRAS* encodes a small GTPase belonging to the Ras subfamily of proteins (H-RAS, N-RAS and K-RAS) and has been implicated in actin cytoskeleton remodeling and control of cell proliferation, migration and cell cycle processes[33]. The nonsynonymous SNV in *COL21A1* (rs200999181, p.Gly665Val) was most strongly associated with PP (MAF = 0.001; increase of 3.14 mm Hg per A allele; $P = 1.93 \times 10^{-9}$). *COL21A1* encodes the collagen α1 chain precursor of type XXI collagen, a member of the FACIT (fibril-associated collagens with an interrupted triple helix) family of proteins[34]. The gene is expressed in many tissues, including the heart and aorta. On the basis of our results, these three genes represent good candidates for functional follow-up. However, because of the incomplete coverage of all SNVs across the region on the Exome chip, it is possible that other non-genotyped SNVs may better explain some of these associations. We therefore checked for variants in LD (r^2 >0.3) with these three rare nonsynonymous SNVs in the UK10K + 1000 Genomes Project data set[35] to ascertain whether there are other candidate SNVs at these loci (**Supplementary Table 17**). There were no SNVs within 1 Mb of the *RBM47* locus in LD with the blood pressure–associated SNV. At the *COL21A*1 locus, there were only SNVs in moderate LD, and these were annotated as intronic, intergenic or in the 5′ UTR. At the *RRAS* locus, there were two SNVs in strong LD with the blood pressure–associated SNV, which both mapped to introns of *SCAF1* and are not predicted to be damaging. All SNVs in LD at both loci were rare, as expected (**Supplementary Table 17**), supporting a role for rare variants. Hence, the rare blood pressure–associated nonsynonymous SNVs at *RBM47*, *COL21A1* and *RRAS* remain the best causal candidates.

Pathway and network analyses

To identify connected gene sets and pathways implicated by the blood pressure–associated genes, we used Meta-Analysis Gene-set Enrichment of variant Associations (MAGENTA)[36] and GeneGO MetaCore (Thomson Reuters). MAGENTA tests for over-representation of blood pressure–associated genes in preannotated pathways (gene sets) (Online Methods and **Supplementary Table 18a**). GeneGO MetaCore identifies potential gene networks. The MAGENTA analysis was used for hypothesis generation, and results were compared with the GeneGO MetaCore outputs to cross-validate findings.

Using MAGENTA, there was an enrichment ($P < 0.01$ and false discovery rate (FDR) < 5% in either the EUR_SAS or EUR participants) of six gene sets with DBP, three gene sets with HTN and two gene sets with SBP (**Supplementary Table 18b**). The RNA polymerase I promoter clearance (chromatin modification) pathway showed the most evidence of enrichment with genes associated with DBP ($P_{\text{REACTOME}} = 8.4 \times 10^{-5}$, FDR = 2.48%). NOTCH signaling was the pathway most associated with SBP ($P_{\text{REACTOME}} = 3.00 \times 10^{-4}$, FDR = 5%) driven by associations at the *FURIN* gene. The inorganic cation anion solute carrier (SLC) transporter pathway had the most evidence of enrichment by HTN-associated genes ($P_{\text{REACTOME}} = 8.00 \times 10^{-6}$, FDR = 2.13%).

Using GeneGO MetaCore, five network processes were enriched (FDR < 5%; Online Methods and **Supplementary Tables 19** and **20**). These included several networks with genes known to influence vascular tone and blood pressure: inflammation signaling, $P = 1.14 \times 10^{-4}$ and blood vessel development $P = 2.34 \times 10^{-4}$. The transcription and chromatin modification network ($P = 2.85 \times 10^{-4}$) was also enriched, a pathway that was also highlighted in the MAGENTA analysis, with overlap of the same histone genes (*HIST1H4C*, *HIST1H2AC*, *HIST1H2BC* and *HIST1H1T*) and has also recently been reported in an integrative network analysis of published blood pressure loci and whole-blood expression profiling[37]. Two cardiac development pathways were enriched: the oxidative stress driven (ROS/NADPH) ($P = 4.12 \times 10^{-4}$) and the Wnt/β-catenin/integrin driven ($P = 0.0010$). Both these cardiac development pathways include the *MYH6*, *MYH7* and *TBX2* genes, identifying a potential overlap

Table 3 Results of the genetic risk score analyses across cardiovascular disease traits and risk factors

Outcome	Units	*n*	DBP (per increase of 10 mm Hg)		SBP (per increase of 10 mm Hg)		PP (per increase of 10 mm Hg)	
			Effect (95% CI)	*P*	Effect (95% CI)	*P*	Effect (95% CI)	*P*
CHD	OR	82,056	1.62 (1.28, 2.05)	5.99×10^{-5}	1.39 (1.22, 1.59)	6.07×10^{-7}	1.70 (1.34, 2.16)	1.20×10^{-5}
Ischemic stroke	OR	25,799	1.93 (1.47, 2.55)	2.81×10^{-6}	1.57 (1.35, 1.84)	1.16×10^{-8}	2.12 (1.58, 2.84)	5.35×10^{-7}
Cardioembolic stroke	OR	16,113	1.43 (0.86, 2.39)	0.1683	1.33 (0.99, 1.80)	0.0584	1.73 (1.00, 3.02)	0.0518
Large vessel stroke	OR	13,903	2.26 (1.25, 4.08)	0.0068	1.85 (1.32, 2.59)	3.61×10^{-4}	3.05 (1.64, 5.68)	4.37×10^{-4}
Small vessel stroke	OR	15,617	1.96 (1.13, 3.41)	0.0168	1.56 (1.13, 2.16)	0.0064	1.98 (1.09, 3.61)	0.0248
Heart failure	OR	13,282	1.48 (1.02, 2.17)	0.0409	1.25 (1.00, 1.57)	0.0512	1.33 (0.88, 2.02)	0.1757
Left ventricular mass	g	11,273	9.57 (3.98, 15.17)	8.02×10^{-4}	5.13 (1.77, 8.48)	0.0027	5.97 (–0.38, 12.31)	0.0653
Left ventricular wall thickness	cm	11,311	0.10 (0.06, 0.13)	1.88×10^{-8}	0.05 (0.03, 0.07)	5.52×10^{-6}	0.05 (0.01, 0.09)	0.0187
HDL-C	mg/dl	80,395	0.25 (–1.00, 1.51)	0.6930	0.21 (–0.50, 0.92)	0.5622	0.47 (–0.79, 1.73)	0.4668
LDL-C	mg/dl	77,021	–1.57 (–5.20, 2.06)	0.3972	0.07 (–2.03, 2.16)	0.9498	1.87 (–1.86, 5.59)	0.3255
Total cholesterol	mg/dl	80,455	–1.34 (–5.90, 3.22)	0.5639	0.70 (–1.93, 3.32)	0.6029	3.68 (–0.97, 8.33)	0.1209
Triglycerides	mg/dl	77,779	0.02 (–0.03, 0.08)	0.3859	0.02 (–0.01, 0.05)	0.2697	0.03 (–0.03, 0.08)	0.3025
BMI	INVT	526,508	–0.10 (–0.18, –0.01)	0.0342	–0.07 (–0.13, –0.02)	0.0058	–0.12 (–0.23, –0.02)	0.0165
WHRadjBMI	INVT	344,369	0.03 (–0.04, 0.11)	0.4025	0.03 (–0.02, 0.08)	0.2170	0.06 (–0.03, 0.15)	0.1885
Height	INVT	458,927	0.02 (–0.15, 0.18)	0.8592	–0.04 (–0.15, 0.06)	0.4170	–0.18 (–0.37, 0.01)	0.0683
eGFR	INVT	51,039	–0.02 (–0.15, 0.11)	0.7810	–0.03 (–0.10, 0.04)	0.4080	–0.07 (–0.20, 0.06)	0.2741

CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure; WHRadjBMI, waist–hip ratio–adjusted BMI; OR, odds ratio; INVT, inverse normally transformed (hence, no units); *n*, sample size; *P*, *P* value for association of blood pressure with the trait listed; CI, confidence interval. Results are considered significant if $P \leq 0.0038$, which corresponds to Bonferroni correction for 13 phenotypes tested.

with cardiomyopathies and HTN, and suggesting some similarity in the underlying biological mechanisms.

DISCUSSION

By conducting the largest ever genetic study of blood pressure, we identified further new common variants with small effects on blood pressure traits, similar to what has been observed for obesity and height[38,39]. More notably, our study identified some of the first rare coding variants of strong effect (>1.5 mm Hg) that are robustly associated with blood pressure traits in the general population, complementing and extending the previous discovery and characterization of variants underlying rare Mendelian disorders of blood pressure regulation[40]. Using SNV associations in 17 genes reported to be associated with monogenic disorders of blood pressure (Online Methods), we found no convincing evidence of enrichment ($P_{enrichment} = 0.044$). This suggests that blood pressure control in the general population may occur through different pathways to monogenic disorders of blood pressure, reinforcing the relevance of our study findings. The identification of 30 new blood pressure loci plus further new independent secondary signals within 4 new and 5 known loci (Online Methods) has augmented the trait variance explained by 1.3%, 1.2% and 0.93% for SBP, DBP and PP, respectively, within our data set. This suggests that, with substantially larger sample sizes, for example through UK Biobank[41], we expect to identify many more loci associated with blood pressure traits and replicate more of our discovery SNV associations that are not yet validated in the current report.

The discovery of rare missense variants has implicated several interesting candidate genes, which are often difficult to identify from common variant GWAS and should therefore lead to more rapidly actionable biology. *A2ML1*, *COL21A1*, *RRAS* and *RBM47* all warrant further follow-up studies to define the role of these genes in regulation of blood pressure traits, as well as functional studies to understand their mechanisms of action. *COL21A1* and *RRAS* warrant particular interest because both are involved in blood vessel remodeling, a pathway of known etiological relevance to HTN.

We observed a rare nonsense SBP-associated variant in *ENPEP* (rs33966350; p.Trp317*): this overlaps a highly conserved region of both the gene and protein and is predicted to result in either a truncated protein with reduced catalytic function or is subject to nonsense-mediated RNA decay. ENPEP converts AngII to AngIII. AngII activates the angiotensin 1 (AT1) receptor, resulting in vasoconstriction, while AngIII activates the angiotensin 2 (AT2) receptor that promotes vasodilation and protects against hypertension[42]. The predicted truncated protein may lead to predominant AngII signaling in the body and increases in blood pressure. This new observation could potentially inform therapeutic strategies. Of note, angiotensin-converting enzyme (ACE) inhibitors are commonly used in the treatment of HTN. However, patients who suffer from adverse reactions to ACE inhibitors, such as dry cough and skin rash, could benefit from alternative drugs that target RAAS. Murine studies have shown that, in the brain, AngIII is the preferred AT1 agonist that promotes vasoconstriction and increases blood pressure, as opposed to AngII in the peripheral system. These results have motivated the development of brain-specific APA inhibitors to treat HTN[43]. Our results indicate that APAs, such as ENPEP, could be valid targets to modify blood pressure but suggest that long-term systemic reduction in APA activity may lead to an increase in blood pressure. Future studies are needed to examine the effects of the p.Trp317* variant on the RAAS system, specifically in the brain and peripheral vasculature, to test the benefits of the proposed therapeutic strategy in humans.

In addition to highlighting new genes in pathways of established relevance to blood pressure and HTN, and identifying new pathways, we have also identified multiple signals at new loci. For example, there are three distinct signals at the locus containing the *MYH6*/*MYH7* genes, and we note that *TBX2* maps to one of the newly associated regions. These genes are related to cardiac development and/or cardiomyopathies and provide an insight into the shared inheritance of multiple complex traits. Unraveling the causal networks within these polygenic pathways may provide opportunities for novel therapies to treat or prevent both HTN and cardiomyopathies.

URLs. Exome chip design information, http://genome.sph.umich.edu/wiki/Exome_Chip_Design; RareMetalWorker information, http://genome.sph.umich.edu/wiki/RAREMETALWORKER;

summary SNV association results, http://www.phenoscanner.medschl.cam.ac.uk/; databases used for variant annotation, http://www.ncbi.nlm.nih.gov/SNP/ (dbSNP), http://www.ensembl.org/info/docs/tools/index.html (Ensembl tools) and http://evs.gs.washington.edu/EVS/ (NHLBI Exome Sequencing Project); UCSC reference file used for annotation of variants with gene and exon information, http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/refFlat.txt.gz; MAGENTA, https://www.broadinstitute.org/mpg/magenta/; Thomson Reuters MetaCore Single-Experiment Analysis workflow tool, http://thomsonreuters.com/en/products-services/pharma-life-sciences/pharmaceutical-research/metacore.html.

METHODS

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

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

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AUTHOR CONTRIBUTIONS

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

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ONLINE METHODS

Overview of discovery studies. The cohorts contributing to the discovery meta-analyses comprise studies from three consortia (CHD Exome+, ExomeBP and GoT2D/T2D-GENES) with a total number of 192,763 unique samples. All participants provided written informed consent, and the studies were approved by their local research ethics committees and/or institutional review boards.

The CHD Exome+ consortium comprised 77,385 samples: eight studies (49,898 samples) of European (EUR) ancestry and two studies (27,487 samples) of South Asian (SAS) ancestry (**Supplementary Table 1**). The ExomeBP consortium included 25 studies (75,620 samples) of EUR ancestry (**Supplementary Table 1**). The GoT2D consortium comprised 14 studies (39,758 samples) of northern European ancestry from Denmark, Finland and Sweden (**Supplementary Table 1**). The participating studies and their characteristics including blood pressure phenotypes are detailed in **Supplementary Tables 1** and **2**. Note that any studies contributing to multiple consortia were only included once in all meta-analyses.

Phenotypes. Four blood pressure traits were analyzed: SBP, DBP, PP and HTN. For individuals known to be taking blood pressure–lowering medication, 15 and 10 mm Hg were added to the raw SBP and DBP values, respectively, to obtain medication-adjusted values[44]. PP was defined as SBP minus DBP, after adjustment. For HTN, individuals were classified as hypertensive cases if they satisfied at least one of the following criteria: (i) SBP ≥ 140 mm Hg, (ii) DBP ≥ 90 mm Hg, or (iii) taking antihypertensive or blood pressure–lowering medication. All other individuals were included as controls. The four blood pressure traits were correlated (SBP:DBP correlations were between 0.6 and 0.8, and SBP:PP correlations were ~0.8). However, they measure partly distinct physiological features including, cardiac output, vascular resistance and arterial stiffness, all measures for determining a cardiovascular risk profile. Therefore, the genetic architecture of the individual phenotypes is of interest, and a multiple-phenotype mapping approach was not adopted.

Genotyping. All samples were genotyped using one of the Illumina HumanExome BeadChip arrays (**Supplementary Table 3**). An Exome chip quality control standard operating procedure (SOP) developed by A. Mahajan, N.R.R. and N.W.R. at the Wellcome Trust Centre for Human Genetics, University of Oxford was used by most studies for genotype calling and quality control[45] (**Supplementary Table 3**). All genotypes were aligned to the plus strand of the human genome reference sequence (Build 37) before any analyses and any unresolved mappings were removed. Genotype cluster plots were reviewed for all the novel rare variants (both lead and secondary signals) and for rare variants that contributed to the gene-based testing.

Meta-analyses. Meta-analyses were performed using METAL[46], for both discovery and replication analyses, using inverse-variance-weighted fixed-effect meta-analysis for the continuous traits (SBP, DBP and PP) and sample-size-weighted meta-analysis for the binary trait (HTN).

Discovery SNV analyses. Analyses of both untransformed and inverse normal transformed SBP, DBP and PP were conducted within each contributing study. The analyses of the transformed traits were performed to minimize sensitivity to deviations from normality in the analysis of rare variants and for discovery of new SNV–blood pressure associations. The residuals from the null model obtained after regressing the medication-adjusted trait on the covariates (age, age^2, sex, BMI and disease status for CHD) within a linear regression model, were ranked and inverse normalized. These normalized residuals were used to test trait–SNV associations. All SNVs that passed quality control were analyzed for association, without any further filtering by MAF, but a minor allele count of 10 was used for the analysis of HTN. An additive allelic effects model was assumed.

Two meta-analyses were performed for each trait, one with EUR and SAS ancestries combined (EUR_SAS) and another for EUR ancestry alone. Contributing studies used principal components to adjust for population stratification. Consequently, minimal inflation in the association test statistic, λ, was observed (λ = 1.07 for SBP, 1.10 for DBP, 1.04 for PP and <1 for HTN in the transformed discovery meta-analysis in EUR_SAS; λ = 1.06 for SBP, 1.09 for DBP, 1.05 for PP and <1 for HTN in the transformed discovery meta-analysis in EUR; **Supplementary Fig. 7**). The meta-analyses were performed independently at two centers, and results were found to be concordant between the centers. Given that the studies contributing to the discovery analyses were ascertained on CHD or T2D, we tested potential systematic bias in calculated effect estimates among these studies. No evidence of bias in the overall effect estimates was obtained.

The results for the transformed traits were taken forward and used to select candidate SNVs for replication. Results (*P* values) from the transformed and untransformed analyses were strongly correlated (r^2 >0.9).

Replication SNV analyses. SNVs associated with any of the transformed traits (SBP, DBP, PP) or HTN were annotated using the Illumina SNV annotation file, humanexome-12v1_a_gene_annotation.txt, independently across two centers. Given the difference in power to detect common versus low-frequency and rare variant associations, two different significance thresholds were chosen for SNV selection. For SNVs with MAF ≥ 0.05, $P \le 1 \times 10^{-5}$ was selected, while, $P \le 1 \times 10^{-4}$ was used for SNVs with MAF < 0.05. By choosing a significance threshold of $P \le 1 \times 10^{-4}$, we maximized the opportunity to follow up rare variants (making the assumption that any true signals at this threshold could replicate at Bonferroni-adjusted significance, $P \le 6.17 \times 10^{-4}$, assuming α = 0.05 for 81 SNVs). All previously published blood pressure–associated SNVs and any variants in LD with them (r^2 >0.2) were removed from the list of associated SNVs as we aimed to replicate new findings only. SNVs for which only one study contributed to the association result or showed evidence of heterogeneity ($P_{het} \le 0.0001$) were removed from the list as they were likely to be an artifact. Where SNVs were associated with multiple traits, to minimize the number of tests performed, only the trait with the smallest *P* value was selected as the primary trait in which replication was sought. Where multiple SNVs fitted these selection criteria for a single region, only the SNV with the smallest *P* value was selected. In total, 81 SNVs were selected for validation in independent samples. These 81 SNVs had concordant association results for both transformed and non-transformed traits. Eighty SNVs were selected from EUR_SAS results (with consistent support in EUR), and one SNV was selected from EUR results only. In the next step, we looked up the 81 SNV–blood pressure associations using data from a separate consortium, the CHARGE+ exome chip blood pressure consortium (who had analyzed untransformed SBP, DBP, PP and HTN), and UHP and Lolipop (ExomeBP consortium; **Supplementary Tables 2** and **3**). The analyzed residuals from CHARGE+ were approximately normally distributed in their largest studies (**Supplementary Fig. 8**).

Two meta-analyses of the replication data sets were performed: one of EUR samples, and a second of EUR, African-American, Hispanics and SAS ancestries ("all"). Replication was confirmed if *P* (one-tailed) ≤ 0.05/81 = 6.17×10^{-4} and the effect (β) was in the direction observed in discovery meta-analyses for the selected trait. A combined meta-analysis was performed of discovery (untransformed results as only untransformed data were available from CHARGE+ exome chip blood pressure consortium) and replication results across the four traits to assess the overall support for each locus. For the combined meta-analyses, a genome-wide significance threshold of, $P \le 5 \times 10^{-8}$ was used to declare an SNV as novel rather than a less stringent experiment wide threshold, as genome-wide significance is used to declare significance in GWAS and we wish to minimize the possibility of false positive associations. (Note that genome-wide significance is equivalent to an exome-wide threshold of $P \le 2 \times 10^{-7}$ adjusted for four traits).

Note that all validated blood pressure–associated variants were associated at $P < 1 \times 10^{-5}$ in the discovery data set (for the primary trait). Hence, we could have used the same inclusion criteria for both common and rare SNVs. Therefore, the optimal threshold to choose for future experiments may need further consideration.

Conditional analyses and gene-based tests. The RMW tool[16] (version 4.13.3) that does not require individual-level data to perform conditional analyses and gene-based tests was used for conditional analyses. All studies that contributed to the SNV discovery analyses were recontacted and asked to run RMW. Only FENLAND, GoDARTS, HELIC-MANOLIS, UKHLS and EPIC-InterAct were unable to run RMW, while two new studies were included, INCIPE and NFBC1966 (**Supplementary Tables 1** and **2**). In total, 43 studies

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(147,402 samples) were included in the EUR analyses and 45 studies (173,329 samples) were included in the EUR_SAS analyses (**Supplementary Tables 2** and **3**). Comparison of discovery and RMW study-level results were made (**Supplementary Note**).

For each new locus, the genomic coordinates and size of the region were defined according to recombination rates (**Supplementary Table 9**) around the lead variant. For known loci, a 1-Mb window was used (**Supplementary Table 14**). Conditional analyses were performed across each region, in both EUR and EUR_SAS samples, for the transformed phenotype corresponding to the validated blood pressure trait for new loci and the published blood pressure trait for known loci.

Gene-based tests were performed in both the EUR and EUR_SAS data sets using the SKAT[17] method implemented in RMW, as it allows for the SNVs to have different directions and magnitudes of effect. Burden tests were also performed but are not presented as only SKAT provided significant results. The variants in the gene-based tests using SKAT were weighted using the default settings, that is, a β distribution density function to upweight rare variants, β(MAFj,1,25) where MAFj represents the pooled MAF for variant j across all studies. Analyses were restricted to coding SNVs with MAF <5% and <1%. Genes were deemed to be associated if $P \leq 2.8 \times 10^{-6}$ (Bonferroni adjusted for 17,996 genes). To confirm that the gene associations were not attributable to a solitary SNV, a gene-based test conditional on the most associated SNV was performed ($P_{\text{conditional}} \leq 0.001$). The quality control for all SNVs contributing to the gene-based tests including the number of samples and studies were checked before claiming association. We sought replication of associated genes in the CHARGE+ exome chip blood pressure consortium.

Pathway analyses with MAGENTA. We tested seven databases in MAGENTA[36] (BioCarta, Kyoto Encyclopedia of Genes and Genomes, Ingenuity, Panther, Panther Biological Processes, Panther Molecular Functions and Reactome) for over-representation of the SNV discovery results from both EUR and EUR_SAS ancestries. Each of the four blood pressure phenotypes were tested. Pathways exhibiting $P < 0.01$ and FDR < 5% were considered statistically significant.

GeneGO MetaCore network analyses. A set of blood pressure–associated genes selected on the basis of previously published studies and our current results (locus defined by r^2 >0.4 and 500 kb on either side of the lead SNV; **Supplementary Table 19**) were tested for enrichment using the Thomson Reuters MetaCore Single Experiment Analysis workflow tool. The data were mapped onto selected MetaCore ontology databases: pathway maps, process networks, GO processes and diseases/biomarkers, for which functional information is derived from experimental literature. Outputs were sorted on the basis of P value and FDR. A gene set was considered enriched for a particular process if $P < 0.05$ and FDR < 5%.

Genetic risk score. To assess the effect of blood pressure on CHD, ischemic stroke (and subtypes: large vessel, small vessel and cardioembolic stroke) left ventricular mass, left ventricular wall thickness, heart failure, HDL-C, LDL-C, total cholesterol, triglycerides and eGFR, we performed a weighted generalized linear regression of the genetic associations with each outcome variable on the genetic associations with blood pressure.

When genetic variants are uncorrelated, the estimates from such a weighted linear regression analysis using summarized data, and a GRS analysis using individual-level data, are equal[47]. We refer to the analysis as a GRS (also known as a polygenic risk score) analysis as this is likely to be more familiar to applied readers. As some of the genetic variants in our analysis are correlated, a generalized weighted linear regression model is fitted that accounts for the correlations between variants, as follows.

If β_X is the genetic association (β coefficient) with the risk factor (here, blood pressure) and β_Y is the genetic associations with the outcome, then the causal estimate from a weighted generalized linear regression is $(\beta_X{}^{T\prime}\Omega^{-1}\beta_X)^{-1}\beta_X{}^{T\prime}\Omega^{-1}\beta_Y$, with standard error,

$$\hat{\sigma}\sqrt{(\beta_X^T\Omega^{-1}\beta_X)^{-1}}$$

where T is a matrix transpose, $\hat{\sigma}$ is the estimate of the residual standard error from the regression model and the weighting matrix $'\Omega$ has terms

$$\Omega_{j_1j_2} = \sigma_{Yj_1}\sigma_{Yj_2}\rho_{j_1j_2}$$

where σ_{Yj} is the standard error of the genetic association with the outcome for the jth SNV and $\rho_{j_1j_2}$ is the correlation between SNVs j_1 and j_2. The presence of the estimated residual standard error allows for heterogeneity between the causal estimates from the individual SNVs as overdispersion in the regression model (in the case of underdispersion, the residual standard error estimate is set to unity). This is equivalent to combining the causal estimates from each SNV using a multiplicative random-effects model[48].

For each of SBP, DBP and PP, the score was created using both the new and known blood pressure–associated SNVs or a close proxy ($r^2 > 0.8$). Both the sentinel SNV association and any secondary SNV associations that remained after adjusting for the sentinel SNV were included in the GRS. For the 30 validated new SNV–blood pressure associations, β values were taken from the independent replication analyses (**Tables 1** and **2**) to weight the SNV in the GRS. For the secondary SNVs from the five new loci and five known loci, β values were taken from the discovery analyses (**Supplementary Tables 10** and **15**). For the 82 known SNVs, 43 were either genotyped or had proxies on the Exome chip and the β values were taken from discovery results (**Supplementary Table 13**), the remaining β values were taken from published effect estimates. This strategy for selecting β values for use in the GRS was taken to minimize the influence of winner's curse. The associations between the blood pressure variants with CHD, HDL-C, LDL-C, total cholesterol, log(triglycerides) and log(eGFR) were obtained using the CHD Exome+ Consortium studies, the associations with BMI were from the GIANT Consortium (A.E.J., H.M.H., M. Graff, T. Karaderi and K. Young *et al.*, unpublished data), waist–hip ratio–adjusted BMI were from the GIANT Consortium (V. Turcot, H.M.H., Y. Lu, C. Schurmann and M. Graff *et al.*, unpublished data), height were from the GIANT Consortium (E. Marouli, M. Graff, C. Medina-Gomez, K.S. Lo and A.R. Wood *et al.*, unpublished data), ischemic stroke were from METASTROKE[25], and left ventricular mass, left ventricular wall thickness and heart failure were from EchoGen[27] and CHARGE-HF[26]. A causal interpretation of the association of GRS with the outcome as the effect of blood pressure on the outcome assumes that the effects of genetic variants on the outcome are mediated via blood pressure and not via alternate causal pathways, for example via left ventricular thickness. There are also limitations of the Mendelian randomization approach in distinguishing between the causal effects of different measures of blood pressure, owing to the paucity of genetic variants associated with only one measure of blood pressure.

eQTL analyses. The MuTHER data set contains gene expression data from 850 UK twins for 23,596 probes and 2,029,988 (HapMap 2–imputed) SNVs. All *cis*-associated SNVs with FDR < 1%, within each of the 30 newly associated regions (IMPUTE info score >0.8), were extracted from the MuTHER project data set for, LCLs (n = 777), adipose tissue (n = 776) and skin (n = 667)[49]. The pilot phase of the GTEx Project (dbGaP phs000424.v3.p1) provides expression data from up to 156 individuals for 52,576 genes and 6,820,472 genotyped SNVs (imputed to 1000 Genomes Project, MAF ≥ 5%)[50]. The eQTL analysis was focused on subcutaneous adipose tissue (n = 94), tibial artery (n = 112), heart (left ventricle) (n = 83), lung (n = 119), skeletal muscle (n = 138), tibial nerve (n = 88), skin (sun exposed, lower leg) (n = 96), thyroid (n = 105) and whole blood (n = 156), which have >80 samples and genes expressed at least 0.1 RPKM in ten or more individuals in a given tissue. All transcripts with a transcription start site (TSS) within one of the 30 new blood pressure loci and for which there was a *cis*-associated SNV (IMPUTE info score > 0.4) within 1 Mb of the TSS at FDR < 5% were identified. Kidney was not evaluated because the sample size was too small (n = 8). From each resource, we report eQTL signals, which reach the resource-specific thresholds for significance described above, for SNVs that are in LD (r^2 >0.8) with our sentinel SNV.

For identified eQTLs, we tested whether they colocalized with the blood pressure–associated SNV[51]. Colocalization analyses were considered to be significant if the posterior probability of colocalization was greater than 0.95.

Annotation of variants. *In silico* prediction of the functional effect of associated variants was based on the annotation from dbSNP, the Ensembl Variant Effect Predictor tool and the Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP).

Trait variance explained. The percentage of trait variance explained for SBP, DBP and PP was assessed with 5,861 individuals with complete information for all phenotypes and covariates from the population-based cohort, 1958BC.

Two genetic models were investigated: one containing the 43 previously known blood pressure–associated SNVs covered on the Exome chip and the other additionally including the 30 new lead SNVs and 9 conditionally independent SNVs from both new and known loci. These nine conditionally independent SNVs were taken from the EUR results, as 1958BC is EUR. They included four from new loci (*PREX1*, *COL21A1*, *PRKAG1* and *MYH6* (there was only one in EUR); **Supplementary Table 10**) and five from known loci (*ST7L–CAPZA1–MOV10*, *FIGN–GRB14*, *ENPEP*, *TBX5–TBX3* and *HOXC4*; **Supplementary Table 15**).

The residual trait was obtained by adjusting each of the blood pressure traits in a regression model with sex and BMI variables (not age or age^2 as all 1958BC individuals were aged 44 years). The residual trait was regressed on all SNVs within the corresponding model and adjusted for the first ten principal components. The R^2 value calculated from this regression model was used as the percentage trait variance explained.

Monogenic enrichment analyses. To determine whether subsignificant signals of association were present in a set of genes associated with monogenic forms of disease, we performed an enrichment analysis of the discovery single-variant meta-analyses association results for all four traits, for both the EUR and EUR_SAS data sets.

The monogenic gene set included: *WNK1*, *WNK4*, *KLHL3*, *CUL3*, *PPARG*, *NR3C2*, *CYP11B1*, *CYP11B2*, *CYP17A1*, *HSD11B2*, *SCNN1A*, *SCNN1B*, *SCNN1G*, *CLCNKB*, *KCNJ1*, *SLC12A1* and *SLC12A3* (ref. 3). The association results of coding SNVs in these genes were extracted, and the number of tests with $P < 0.001$ was observed. To determine how often such an observation would be observed by chance, we constructed 1,000 matched gene sets. The matching criterion for each monogenic gene was the intersection of all genes in the same exon-length quintile and all genes in the same coding-variant-count decile. Within the matched sets, the number of variants with $P < 0.001$ was observed. The empirical *P* value was calculated as the fraction of matched sets with an equal or larger number of variants less than 0.001.

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