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 factor1. 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 aneurysms2. 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 far3–11. These variants are common (minor allele frequency (MAF) ≥ 0.05); mostly map to intrinsic 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 variance12. 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 SNVs13 and may help to explain the missing heritability and identify causative genes, as demonstrated previously14.

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 × 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
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 ≤ 1 × 10^{-5} or MAF < 0.05 and P ≤ 1 × 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, θ = 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 ≤ 6.17 × 10^{-4}, assuming α = 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 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 RRPI8 within the EUR_SAS analyses and COL21A1 in the EUR analyses (P_{conditional} ≤ 1 × 10^{-4}, Bonferroni adjusted for ~500 variants within each region; Online Methods and 17 SNV’s replicated EUR: 2 SBP + 9 DBP + 3 PP ALL: 3 SBP
13 SNVs validated with overall genome-wide significant evidence EUR: 3 SBP + 5 DBP + 2 PP ALL: 1 SBP + 1 DBP + 1 PP
30 new BP loci identified and validated

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

| Locus   | Variant information | Discovery | Replication | Combined |
|---------|---------------------|-----------|-------------|----------|
| EUR     | rs7007909           | PP        | 122,780     | 284,683  |
| C5orf56 | rs12521868          | DBP       | N/A         | N/A      |
| PHACTR1 | rs9349973           | SBP       | 122,795     | 282,023  |
| COL21A1 | rs2009991181        | PP        | 122,809     | 284,673  |
| ABO     | rs668671             | PP        | 122,798     | 276,019  |
| LMO1    | rs110419             | PP        | 122,798     | 264,465  |
| OR5B12  | rs11229457           | PP        | 122,809     | 279,935  |
| CER5    | rs7302981            | PP        | 122,780     | 284,718  |
| MYH6    | rs452036             | PP        | 122,809     | 264,564  |
| DPEP1   | rs1126464            | PP        | 122,798     | 281,978  |
| TBX2    | rs8068318            | PP        | 122,798     | 282,978  |
| RGL3    | rs1676479            | PP        | 122,797     | 283,332  |
| PREX1   | rs6095241            | PP        | 122,798     | 281,322  |

All ancestry

| Locus   | Variant information | Discovery | Replication | Combined |
|---------|---------------------|-----------|-------------|----------|
| RMB47   | rs35529250a          | SBP       | N/A         | N/A      |
| OBFC1   | rs4387287            | SBP       | N/A         | N/A      |
| RRAS    | rs61760904a          | SBP       | N/A         | N/A      |

SNV–blood pressure associations are reported for the newly identified blood pressure loci that replicated at P ≤ 6.17 × 10⁻⁸ (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 variants. The primary blood pressure trait listed in the “Trait” column. For discovery meta-analysis results, P1 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 P2 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 associated; associated with HTN (P < 0.01); Supplementary Table 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 package16. We applied the gene-based sequence kernel association test (SKAT)17 and burden tests18 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 × 10⁻⁷) in the EUR_SAS studies and also in the EUR studies (Bonferroni-corrected threshold of significance P < 2.8 × 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 × 10⁻⁷; Supplementary Table 12), suggesting that the association is due to multiple rare variants in the gene. One nonsense variant (rs199651558, p.Arg983*, 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 abnormalities19. 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 × 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, r² > 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 (r² > 0.2) with the known blood pressure variants on the Exome chip (Supplementary Table 13), using the 10,000 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, ST7–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 × 10⁻⁸ as compared to P = 1.68 × 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

| Locus   | rsID       | Chr:position (EA, EAF) | Trait          | Discovery | Replication | Combined |
|---------|------------|-------------------------|----------------|-----------|-------------|----------|
|         |            |                        |                |            | P₁          | n  β     | P      |          |
| EUR     | rs2972146  | 2:227.10 (T, 0.652) DBP<sup>+</sup> (HTN) | 1.51 × 10<sup>−9</sup> | 2.47 × 10<sup>−7</sup> | 122,798 | 0.13 | 2.20 × 10<sup>−3</sup> | 275,610 | 0.17 | 8.40 × 10<sup>−9</sup> |
| ZBTB38  | rs16851397 | 3:141.13 (A, 0.953) DBP<sup>+</sup> (SBP) | 6.87 × 10<sup>−6</sup> | 3.20 × 10<sup>−5</sup> | 122,798 | 0.08 | 1.20 × 10<sup>−4</sup> | 284,717 | 0.30 | 3.01 × 10<sup>−8</sup> |
| PRDM6   | rs1000058  | 5:122.44 (A, 0.135) SBP | 5.09 × 10<sup>−7</sup> | 1.01 × 10<sup>−8</sup> | 43,109 | 0.46 | 3.61 × 10<sup>−3</sup> | 176,362 | 0.55 | 2.99 × 10<sup>−10</sup> |
| GPR20   | rs34591516 | 8:142.37 (T, 0.055) DBP<sup>+</sup> (DBP) | 1.54 × 10<sup>−6</sup> | 1.01 × 10<sup>−8</sup> | 122,807 | 0.51 | 4.20 × 10<sup>−4</sup> | 282,009 | 0.64 | 6.10 × 10<sup>−10</sup> |
| HOXB7   | rs7409610  | 7:46.69 (T, 0.118) SBP | 6.07 × 10<sup>−10</sup> | 2.74 × 10<sup>−9</sup> | 122,809 | 0.02 | 4.89 × 10<sup>−2</sup> | 284,690 | 0.46 | 3.80 × 10<sup>−8</sup> |
| AMH     | rs10407722 | 9a:21.92 (T, 0.822) PP | 1.63 × 10<sup>−7</sup> | 1.73 × 10<sup>−9</sup> | 118,656 | 0.19 | 1.62 × 10<sup>−2</sup> | 252,525 | 0.26 | 5.94 × 10<sup>−9</sup> |
| ZNF101  | rs2304130   | 21.19.19 (A, 0.914) DBP | 1.66 × 10<sup>−6</sup> | 1.92 × 10<sup>−8</sup> | 122,798 | 0.17 | 1.71 × 10<sup>−2</sup> | 284,705 | 0.29 | 1.53 × 10<sup>−8</sup> |
| PROCOR  | rs867186   | 20.33.76 (A, 0.873) SBP | 1.44 × 10<sup>−6</sup> | 4.15 × 10<sup>−7</sup> | 122,798 | 0.21 | 2.48 × 10<sup>−3</sup> | 284,722 | 0.26 | 1.19 × 10<sup>−8</sup> |
| RRP18   | rs9306110  | 21.45.11 (T, 0.374) DBP<sup>+</sup> (DBP) | 1.04 × 10<sup>−9</sup> | 1.90 × 10<sup>−6</sup> | 100,489 | 0.16 | 4.30 × 10<sup>−4</sup> | 249,817 | 0.18 | 6.80 × 10<sup>−9</sup> |
| TNRC6B  | rs470113   | 22.40.73 (A, 0.804) PP | 1.48 × 10<sup>−9</sup> | 1.31 × 10<sup>−9</sup> | 122,780 | 0.14 | 1.37 × 10<sup>−2</sup> | 284,683 | 0.25 | 1.67 × 10<sup>−9</sup> |

SNV–blood pressure associations are reported for the newly identified blood pressure loci that showed genome-wide significant association (P < 5 × 10<sup>−8</sup>) in the combined discovery and replication meta-analyses. For discovery meta-analysis results, P₁ 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<sub>replication</sub> 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 ≤ 5 × 10<sup>−8</sup>, 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.

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<sup>20,21</sup>), QT interval (RN207)<sup>22</sup>, heart rate (MYH6)<sup>23</sup> and cholesterol levels (2q36.3, ABO and ZNF101)<sup>24</sup>.

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 cardioembolic, large vessel and small vessel[32]), CHD, heart failure[26], left ventricular mass[37], left ventricular wall thickness[37], 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 × 10<sup>−7</sup>; 1.62 (1.28–2.05) per increase of 10 mm Hg in DBP, P = 5.99 × 10<sup>−5</sup>; 1.70 (1.34–2.16) per increase of 10 mm Hg in PP, P = 1.20 × 10<sup>−5</sup>; 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 × 10<sup>−5</sup>; 1.57 (1.35–1.84) per increase of 10 mm Hg in SBP, P = 1.16 × 10<sup>−8</sup>; 2.12 (1.58–2.84) per...
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 \( \geq 0.05 \); 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 (\( P_{\text{MuTHER}} = 3.13 \times 10^{-7} \), skin (\( P_{\text{MuTHER}} = 2.40 \times 10^{-38} \)) and nerve tissue (\( P_{\text{GTEx}} = 4.5 \times 10^{-12} \)) (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 (\( P_{\text{MuTHER}} = 3.24 \times 10^{-15} \)) and skin (\( P_{\text{MuTHER}} = 1.01 \times 10^{-11} \)) (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 (\( P_{\text{GTEx}} = 9.95 \times 10^{-15} \)), thyroid (\( P_{\text{GTEx}} = 8.54 \times 10^{-7} \)), nerve tissue (\( P_{\text{GTEx}} = 7.15 \times 10^{-8} \)), tibial artery (\( P_{\text{GTEx}} = 6.46 \times 10^{-9} \)), adipose tissue (\( P_{\text{GTEx}} = 4.69 \times 10^{-7} \)) and skin (\( P_{\text{GTEx}} = 5.88 \times 10^{-8} \)) (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.
COL21A1 (rs200999181, p.Gly665Val) was most strongly associated with PP (MAF = 0.001; increase of 3.14 mm Hg per A allele; \( P = 1.93 \times 10^{-8} \)). COL21A1 encodes the collagen \( \alpha 1 \) chain precursor of type XXI collagen, a member of the FACIT (fibril-associated collagens with an interrupted triple helix) family of proteins34. 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 set35 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 COL21A1 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 (\( P_{\text{REACTOME}} = 8.4 \times 10^{-4}, \text{FDR} = 2.48\% \)). NOTCH signaling was the pathway most associated with SBP (\( P_{\text{REACTOME}} = 3.00 \times 10^{-4}, \text{FDR} = 5\% \)) 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 (\( P_{\text{REACTOME}} = 8.00 \times 10^{-4}, \text{FDR} = 2.13\% \)).

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 profiling37. 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.

**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.

(\( P_{\text{MATHER}} = 5.24 \times 10^{-31} \)) and LCLs (\( P_{\text{MATHER}} = 1.39 \times 10^{-34} \)), 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 molecule32. In \( RRAS \), rs61760904 (p.Asp133Glu) 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 processes33.
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 (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. \(A2ML1\), \(COL21A1\), \(RRA5\) 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 \(RRA5\) 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.phenoscan.med-schl.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/ (NHHLI 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 Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

Full acknowledgments appear in the Supplementary Note.

AUTHOR CONTRIBUTIONS

Supervision and management of the project: J.M.H. and P.B.M. The following authors contributed to the drafting of the manuscript: J.M.H., P.B.M., P. Surendran, H.W., A.S.B., D.P., J.D., D.R.B., K.W., M. Tomaszewski, F.W.A., L.W.V., N.S.D., J.D., A.K.M., H.Y., N.G., X.S., T. Tukiainen, D.F.F., O.G., T.F. and V.T. All authors critically reviewed and approved the final version of the manuscript. Statistical analysis review: J.M.H., P. Surendran, F.D., D.R.B., K.W., M. Tomaszewski, F.W.A., L.W.V., N.S.D., J.D., A.K.M., H.Y., N.G., X.S., T. Tukiainen, D.F.F., O.G., T.F. and V.T. All authors critically reviewed and approved the final version of the manuscript. Statistical analysis review: J.M.H., P. Surendran, F.D., D.R.B., K.W., M. Tomaszewski, F.W.A., L.W.V., N.S.D., J.D., A.K.M., H.Y., N.G., X.S., T. Tukiainen, D.F.F., O.G., T.F. and V.T. All authors critically reviewed and approved the final version of the manuscript.

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

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Lim, S. S. et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2223–2260 (2012).
2. Rapsomanikis, E. et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1.25 million people. Lancet 383, 1899–1911 (2014).
3. Munroe, P. B., Barnes, M. R. & Caulfield, M. J. Advances in blood pressure genomics. Circ. Res. 112, 1365–1373 (2013).
4. Ehret, G. B. et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 478, 103–109 (2011).
5. Wain, L. V. et al. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat. Genet. 43, 1005–1011 (2011).
6. Johnson, T. et al. Blood pressure loci identified with a gene-centric array. Am. J. Hum. Genet. 89, 688–700 (2011).
7. Tomaszewski, M. et al. Genetic architecture of ambulatory blood pressure in the general population: insights from cardiovascular gene-centric array. Hypertension 56, 1069–1076 (2010).
8. Tragante, V. et al. Gene-centric meta-analysis in 87,736 individuals of European ancestry identifies multiple blood-pressure-related loci. Am. J. Hum. Genet. 94, 363–380 (2014).
9. Ganesh, S. K. et al. Loci influencing blood pressure identified using a cardiovascular gene-centric array. Hum. Mol. Genet. 22, 1663–1678 (2013).
10. Simino, J. et al. Gene–age interactions in blood pressure regulation: a large-scale investigation with the CHARGE, Global BPgen, and ICBP Consortia. Am. J. Hum. Genet. 95, 24–38 (2014).
11. Zhu, X. et al. Meta-analysis of correlated traits via summary statistics from GWASs with an application in hypertension. Am. J. Hum. Genet. 95, 21–36 (2015).
12. Saftai, E., Morrison, A. C., Boerwinkle, E. & Dhakravarti, A. Direct Estimates of the Genomic Contributions to Blood Pressure Heritability within a Population-Based Cohort (ARIC). PLoS One 10, e0133031 (2015).
13. Schork, N. J., Murray, S. S., Frazer, K. A. & Topol, E. J. Common vs. rare allele hypotheses for complex diseases. Curr. Opin. Genet. Dev. 19, 212–219 (2009).
14. Nejentsev, S., Walker, N., Riches, D., Egholm, M. & Todd, J. A. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science 342, 387–395 (2013).
15. Liu, C. et al. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat. Genet. http://dx.doi.org/10.1038/ng.3660 (2016).
16. Liu, D. J. et al. Meta-analysis of gene–gene studies to test for frequent variant association. Nat. Genet. 46, 200–204 (2014).
17. Wu, M. C. et al. Rare-variant association testing for sequencing data with the sequence kernel association test. Am. J. Hum. Genet. 89, 82–93 (2011).
18. Li, B. & Leal, S. M. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. Am. J. Hum. Genet. 83, 311–321 (2008).
19. Vissers, L. E. et al. Heterozygous germline mutations in A2ML1 are associated with a disorder clinically related to Noonan syndrome. Eur. J. Hum. Genet. 23, 317–324 (2015).
20. Coronary Artery Disease (CAD) Genetics Consortium. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat. Genet. 43, 339–344 (2011).
21. Schunkert, H. et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat. Genet. 43, 333–338 (2011).
22. Arking, D. E. et al. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. Nat. Genet. 46, 826–836 (2014).
23. de los Heros, M. et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat. Genet. 45, 621–631 (2013).
24. Willer, C. J. et al. Discovery and refinement of loci associated with lipid levels. Nat. Genet. 45, 1274–1283 (2013).
25. Taylor, M. et al. Genetic risk factors for ischaemic stroke and their subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. Lancet Neurol. 11, 951–962 (2012).
26. Smith, N.L. et al. Association of genome-wide variation with the risk of incident heart failure in adults of European and African ancestry: a prospective meta-analysis from the cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium. Circ Cardiovasc Genet 3, 256–266 (2010).

27. Vasan, R.S. et al. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. J. Am. Med. Assoc. 302, 168–178 (2009).

28. Smith, N.L. et al. Novel associations of multiple genetic loci with plasma levels of natural anticoagulant inhibitors and protein C anticoagulant pathway: the MLTHA project. Br. J. Haematol. 157, 230–239 (2011).

29. Smith, N.L. et al. Novel associations of multiple genetic loci with plasma levels of factor V, factor VIII, and von Willebrand factor: the CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. Circulation 121, 1382–1392 (2010).

30. Blei, M.E., Gregorich, S.E., McConnell, D., Rosen, M.P. & Cedars, M.I. Does accelerated reproductive aging underlie premature atherosclerotic disease? Menopause 20, 1139–1146 (2013).

31. Guan, R. et al. rmb47, a novel RNA binding protein, regulates zebrafish head development. Dev. Dyn. 242, 1395–1404 (2013).

32. Bleil, M.E., Gregorich, S.E., McConnell, D., Rosen, M.P. & Cedars, M.I. Does accelerated reproductive aging underlie premature atherosclerotic disease? Menopause 20, 1139–1146 (2013).

33. Wozniak, M.A., Kwong, L., Chodniewicz, D., Klemke, R.L. & Keely, P.J. R-Ras controls membrane protrusion and cell migration through the spatial regulation of Rac and Rho. Mol. Biol. Cell 16, 84–96 (2005).

34. Tuckwell, D. Identification and analysis of collagen alpha 10(XXI), a novel member of the FACIT collagen family. Matrix Biol. 21, 63–66 (2002).

35. Huang, J. et al. Improved imputation of low-frequency and rare variants using the UK10K haplotype reference panel. Nat. Commun. 6, 8111 (2015).

36. Segé, A.V., Groop, L., Mostha, V.K., Daly, M.J. & Altshuler, D. Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycaemic traits. PLoS Genet. 6, e1001058 (2010).

37. Huan, T. et al. Integrative network analysis reveals molecular mechanisms of blood pressure regulation. Mol. Syst. Biol. 11, 799 (2015).

38. Locke, A.E. et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 518, 197–206 (2015).

39. Wood, A.R. et al. Defining the role of common variation in the genomic and biological architecture of adult human height. Nat. Genet. 46, 1173–1186 (2014).

40. Park, H.W. et al. Serine-threonine kinase with no-lysine 4 (WNK4) controls blood pressure via transient receptor potential canonical 3 (TRPC3) in the vasculature. Proc. Natl. Acad. Sci. USA 108, 10750–10755 (2011).

41. Sudlow, C. et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 12, e1001779 (2015).

42. Te Riet, L., van Esch, J.H., Roks, A.J., van den Meiracker, A.H. & Danser, A.H. Hypertension: renin-angiotensin-aldosterone system alterations. Circ. Res. 115, 960–975 (2015).

43. Gao, J. et al. A new strategy for treating hypertension by blocking the activity of the brain renin-angiotensin system with aninopeptidase A inhibitors. Clin. Sci. (Lond.) 127, 135–148 (2014).

Praveen Surendran1,166, Fotios Drenos2,3,166, Robin Young1,166, Helen Warren4,5,166, James P Cook6,7,166, Alisa K Manni8–10,166, Niels Grarup11,166, Xueling Sim12–14,166, Daniel R Barnes1, Kate Witkowska4,5, James R Stailey1, Vinicius Tragante15, Taru Tukiainen8,9,16, Hanieh Yaghoobtkar17, Nicholas Masca18,19, Daniel F Freitag3,166, John Connell1,166, Miki Moriguchi20, Olga Giannakopoulou21, Andrew Tinker3,21, Magdalena Harakalova15, Evelin Mihaliov22, Chunyu Liu23, Aldi T Kraja24,25, Sune Fallgaard Nielsen26, Asif Rasheed27, Maria Samuel27, Wei Zhao28, Lori L Bonnycastle29, Anne U Jackson12,13, Narisu Narisu29, Amy J Swift29, Lorraine Southam20,30, Jonathan Marten31, Jeroen R Huyge12,13, Alena Stančáková32, Cristiano Fava33,34, Therese Ohlsson33, Angela Matchan30, Kathleen E Stirrups21,35, Jette Bork-Jensen11, Anette P Gjesing31, Jukka Kontto36, Markus Perola22,36,37, Susan Shaw-Hawkins4, Aki S Havelin36, He Zhang38, Louise A Donnelly39, Christopher J Groves40, N. William Rayner20,30,40, Matt J Neville40,41, Neil R Robertson20,40, Andriasos M Yiorkas42,43, Karl-Heinz Herzig44,45, Eero Kajantie36,46,47, Weihua Zhang48,49, Sara M Willems50, Lars Lannfelt51, Giovanni Malerba6, Nicoletta Ramacci43,53,54, Elisabetta Trabetti55,56, Evangelos Evangelou48,57, Alireza Moayyeri48,58, Anne-Claire Vergnaud59,60, Christopher P Nelson4,8,19, Alaitz Poveda59,60, Tibor V Varga59, Muriel Caslake61, Anton J M de Craen62,165, Stella Trompet62,63, Jianfan Lan50, Robert A Scott50, Sarah E Harris64,65, David C M Liewald66,64, Riccardo Marioni64,65,66, Cristina Manni68, Aliki-Eleni Farmaki69, Göran Hallmans70, Frida Renström79,70, Jennifer E Huffman31,23, Maija Hassinen71, Stephen Burgess71, Ramachandran S Vasan72,73, Janine F Felix74,75,76, Charles Y Hyeyoul77, Maria Uria-Nickelsen76, Anders Malarstig77, Dermot F Reilly78, Maarten Hoek79, Thomas F Vogt79,80, Honghuang Lin23,81, Wolfgang Lieb82, EchoGen Consortium75, Matthew Traynor83, Hugh S Markus84, METASTROKE Consortium75, Heather M Highland84, Anne E Justice84, Eirini Marouli77, GIANT Consortium75, Jaana Linndahl36, Matti Uusitupa85,86, Pirjo Komulainen71, Timo A Lakkas71,87,88, Rainer Rauramaa71,88, Ozren Polasek89,90, Igor Rudan89, Olov Rolandsson90, Paul W Franks90,91,92, George Dedoussis93, Timothy D Spector68, EPIC-InterAct Consortium75, Pekka Jousilahti86, Satu Mannistö86, Ian J Deary86,66, John M Starb64,93, Claudia Langenberg50, Nick J Wareham50, Morris J Brown4, Anna Dominicza94, John M Connell95, J Wouter Jukema63,95, Naveed Sattar94, Ian Ford96, Chris J Packard96, Tönö Esko97,9,22,96, Reedik Mägi22, Andreas Metspalu22,97, Rudolf A de Boer55, Peter van der Meer55, Pim van der Harst55,98,99, Lifelines Cohort Study75, Giovanni Gambaro100, Erik Ingelsson101,102, Lars Lind101, Paul I W de Bakker103,104, Mattiis E Numans104,105, Ivan Brandslund106,107, Cramer Christensen108, Eva R B Petersen109, Eeva Korpi-Höyvölti110, Heikki Oksa111, John C Chambers48,49,112, Jaspal S Koonen112,113, Alexandra I F Blakemore42,43, Steve Franks114, Marjo-Riitta Jarvelin115,118, Lisa L Husemoen119,120, Allan Linneberg119–121, Tea Skaaby119, Bettina Thuesen119, Fredrik Karpe40,41, Jaakko Tuomilehto36,122–124, Alex S F Doney39, Andrew D Morris125, Colin N A Palmer39, Oddgeir Lingaas Holmen126,127, Kristian Hveem126,128, Cristen J Willer38,129,130, Tiina-Maija Tuomia131–133, Leif Groon133,134, Anne-Mari Käärämäki135,136, Aarno Palotie16,133,137, Samuli Ripatti30,133,138, Veikko Salomaa36, Dewan S Alam139, Abdulla al Shafi Majumder140, Emanuele Di Angelantonio1,54, Rajiv Chowdhury1,
Cambridge Biomedical Campus, Cambridge, UK. 84Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.
85Department of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland. 86Research Unit, Kuopio University Hospital, Kuopio, Finland.
87Institute of Biomedicine/Physiology, University of Eastern Finland, Kuopio Campus, Kuopio, Finland. 88Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland. 89Centre for Global Health Research, Usher Institute for Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, UK. 90Faculty of Medicine, University of Split, Split, Croatia. 91Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden. 92Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, USA. 93Alzheimer Scotland Dementia Research Centre, University of Edinburgh, Edinburgh, UK. 94Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK. 95Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands. 96Division of Endocrinology, Boston Children's Hospital, Boston, Massachusetts, USA. 97Institute of Molecular and Cell Biology, Tartu, Estonia. 98Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands. 99Durrer Center for Cardiogenetic Research, ICIN-Netherlands Heart Institute, Utrecht, the Netherlands. 100Division of Nephrology, Department of Internal Medicine and Medical Specialties, Columbus-Gemelli University Hospital, Catholic University, Rome, Italy. 101Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden. 102Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, California, USA. 103Department of Medical Genetics, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, the Netherlands. 104Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands. 105Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, the Netherlands. 106Department of Clinical Biochemistry, Lillebaelt Hospital, Vejle, Denmark. 107Institute of Regional Health Research, University of Southern Denmark, Odense, Denmark. 108Medical Department, Lillebaelt Hospital, Vejle, Denmark. 109Department of Clinical Immunology and Biochemistry, Lillebaelt Hospital, Vejle, Denmark. 110Department of Internal Medicine, South Ostrobothnia Central Hospital, Seinäjoki, Finland. 111Department of Internal Medicine, Tampere University Hospital, Tampere, Finland. 112Imperial College Healthcare NHS Trust, London, UK. 113National Heart and Lung Institute, Imperial College London, London, UK. 114Institute of Reproductive and Developmental Biology, Imperial College London, London, UK. 115Department of Epidemiology and Biostatistics, Medical Research Council Public Health England Centre for Environment and Health, School of Public Health, Faculty of Medicine, Imperial College London, St. Mary's Campus, London, UK. 116Centre for Life Course Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland. 117Biocenter Oulu, University of Oulu, Oulu, Finland. 118Unit of Primary Care, Oulu University Hospital, Oulu, Finland. 119Research Centre for Prevention and Health, Capital Region of Denmark, Copenhagen, Denmark. 120Department of Clinical Experimental Research, Glostrup University Hospital, Glostrup, Denmark. 121Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. 122Dasman Diabetes Institute, Dasman, Kuwait. 123Centre for Vascular Prevention, Danube University Krems, Krems, Austria. 124Diabetes Research Group, King Abdulaziz University, Jeddah, Saudi Arabia. 125School of Molecular, Genetic and Population Health Sciences, University of Edinburgh, Medical School, Teviot Place, Edinburgh, UK. 126HUNT Research Centre, Department of Public Health and General Practice, Norwegian University of Science and Technology, Levanger, Norway. 127St. Olav Hospital, Trondheim University Hospital, Trondheim, Norway. 128Department of Medicine, Levanger Hospital, Nord-Trøndelag Health Trust, Levanger, Norway. 129Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA. 130Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA. 131Folkhälsan Research Centre, Helsinki, Finland. 132Department of Endocrinology, Helsinki University Central Hospital, Helsinki, Finland. 133Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland. 134Department of Clinical Sciences, Diabetes and Endocrinology, Lund University Diabetes Centre, Malmö, Sweden. 135Department of Primary Health Care, Vaasa Central Hospital, Vaasa, Finland. 136Diabetes Center, Vaasa Health Care Center, Vaasa, Finland. 137Psychiatric and Neurodevelopmental Genetics Unit, Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts, USA. 138Department of Public Health, University of Helsinki, Helsinki, Finland. 139ICDDR, B, Dhaka, Bangladesh. 140National Institute of Cardiovascular Diseases, Sher-e-Bangla Nagar, Dhaka, Bangladesh. 141School of Public Health, Imperial College London, London, UK. 142Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland. 143University of Lille, UMR 1167, Risk Factors and Molecular Determinants of Aging-Related Diseases, Lille, France. 144INSERM, Lille, France. 145CHU Lille, Public Health, Lille, France. 146Institut Pasteur de Lille, Lille, France. 147Department of Epidemiology and Public Health, EA 3430, University of Strasbourg, Strasbourg, France. 148Department of General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany. 149University Medical Center Hamburg-Eppendorf, Hamburg, Germany. 150Department of Epidemiology, UMR 1027, INSERM, Toulouse University, CHU Toulouse, Toulouse, France. 151UKCRC Centre of Excellence for Public Health, Queens University, Belfast, UK. 152Institute of Genetic Epidemiology, Helmholtz Zentrum München -German Research Center for Environmental Health, Neuherberg, Germany. 153Department of Medicine I, University Hospital Grosshadern, Ludwig Maximilians Universität, Munich, Germany. 154DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany. 155Research Center in Epidemiology and Preventive Medicine, Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy. 156Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders (PACER-HD), King Abdulaziz University, Jeddah, Saudi Arabia. 157Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts, USA. 158Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA. 159A collaboration between the University Medical Schools and NHS, Aberdeen, Dundee, Edinburgh and Glasgow, UK. 160Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. 161Division of Cardiovascular Sciences, University of Manchester, Manchester, UK. 162Faculty of Population Health Sciences, Institute of Cardiovascular Science, University College London, London, UK. 163Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK. 164Welcome Trust Sanger Institute, Hinxton, UK. 165Deceased. 166These authors contributed equally to this work. 167These authors jointly directed this work. Correspondence should be addressed to J.M.M.H. (jmmh2@medschl.cam.ac.uk) or P.B.M. (p.b.munroe@qmul.ac.uk).
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 values44. 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 distinctive 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 control45 (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 METAL46, 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<sup>2</sup>, 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, \( \lambda \), was observed (\( \lambda = 1.07 \) for SBP, 1.10 for DBP, 1.04 for PP and <1 for HTN in the transformed discovery meta-analysis in EUR_SAS; \( \lambda = 1.06 \) 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, \( P ≤ 1 \times 10^{-5} \) was selected, while, \( P ≤ 1 \times 10^{-3} \) was used for SNVs with MAF < 0.05. By choosing a significance threshold of \( P ≤ 1 \times 10^{-4} \), 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, \( P ≤ 6.17 \times 10^{-5} \), assuming \( \alpha = 0.05 \) 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 (\( P_{het} ≤ 0.0001 \)) 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 Lilopip (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 (\( \beta \)) 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, \( P ≤ 5 \times 10^{-8} \) 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 \( P ≤ 2 \times 10^{-7} \) adjusted for four traits).

Note that all validated blood pressure–associated variants were associated at \( P < 1 \times 10^{-4} \) 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 tool16 (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
(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 SKAT17 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 function to weight up to five rare variants, β(MAF)j,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 ≤ 2.8 × 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 (Pr(conditional) < 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 MAGENTA16 (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 r2 >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 equal17. 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 βj is the genetic association (β coefficient) with the risk factor (here, blood pressure) and β0 is the genetic associations with the outcome, then the causal estimate from a weighted generalized linear regression is:

\[ \hat{\beta}_j = \left( \sum_{j=1}^{n} \sigma_j^2 \beta_j \right)^{-1} \left( \sum_{j=1}^{n} \sigma_j^2 \beta_j Y \right) \]

where T is a matrix transpose, \( \hat{\sigma}_j^2 \) is the estimate of the residual standard error from the regression model and the weighting matrix \( \Omega \) has terms

\[ \Omega_{jj} = \hat{\sigma}_j^2 \]

where \( \sigma_j^2 \) is the standard error of the genetic association with the outcome for the jth SNV and \( \beta_{ij} \) is the correlation between SNVs j and j′. 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 model48.

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 (r2 > 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., M.H.H., M. Graff, T Karaderi and K. Young et al., unpublished data), waist–hip ratio–adjusted BMI were from the GIANT Consortium (V. Turcot, M.H.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 METASTROKE25, and left ventricular mass, left ventricular wall thickness and heart failure were from EchoGen27 and CHARGE-HF28. 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). 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%)30. The eQTL analysis was focused on subcutaneous adipose tissue (n = 94), bilateral artery (n = 112), heart (left ventricle) (n = 83), lung (n = 119), skeletal muscle (n = 138), bilateral 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 (r2 >0.8) with our sentinel SNV.

For identified eQTLs, we tested whether they colocalized with the blood pressure–associated SNV31. 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 age2 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*, *HSV11B2*, *SCN1A*, *SCN1B*, *SCN1G*, *CLCNK1*, *KCNJ11*, *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.