Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure

Numerous genetic loci have been associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) in Europeans. We now report genome-wide association studies of pulse pressure (PP) and mean arterial pressure (MAP). In discovery (N=74,064) and follow-up studies (N=48,607), we identified at genome-wide significance (P=2.7 x 10^{-8} to P=2.3 x 10^{-13}) four new PP loci (at 4q12 near CHIC2, 7q22.3 near PIK3CG, 8q24.12 in NOV and 11q24.3 near ADAMTS8), two new MAP loci (3p21.31 in MAP4 and 10q25.3 near ADRB1) and one locus associated with both of these traits (2q24.3 near FIGN) that has also recently been associated with SBP in east Asians. For three of these new PP loci, the estimated effect on SBP was opposite that for DBP, in contrast to the majority of common SBP- and DBP-associated variants, which show concordant effects on both traits. These findings suggest new genetic pathways underlying blood pressure variation, some of which may differentially influence SBP and DBP.

High blood pressure is a major risk factor for coronary heart disease and stroke. Large genome-wide association studies in Europeans have reported 29 new loci for SBP and DBP, in which alleles have effect sizes of up to 0.5–1.0 mm Hg. Even small increments in blood pressure levels have important effects on cardiovascular morbidity and mortality at the population level. We undertook a genome-wide association study of two further blood pressure phenotypes, PP (the difference between SBP and DBP), a measure of stiffness of the main arteries and MAP, a weighted average of SBP and DBP. Both PP and MAP are predictive of hypertension and cardiovascular disease.

This study was undertaken by the International Consortium of Blood Pressure Genome-Wide Association Studies (ICBP-GWAS), which aims to further the understanding of the genetic architecture underlying blood pressure. A concurrent publication by this consortium studied SBP and DBP with discovery GWAS among 69,395 individuals and a combined sample of ~200,000 Europeans. All but one study that was included in the discovery GWAS of SBP and DBP were included in the discovery GWAS stage of this study. In addition, we included here a further six studies added subsequent to the analyses of SBP and DBP, bringing our discovery GWAS sample size to 74,064.

We first conducted a genome-wide association meta-analysis of PP and MAP in these 74,064 individuals of European ancestry from 35 studies (Supplementary Table 1a). We imputed the genotypes using HapMap. To account for effects of anti-hypertensive treatments, we imputed underlying SBP and DBP by adding a constant to each. We adjusted the associations for age, age^2, sex and body mass index. We combined the results across studies using an inverse-variance–weighted meta-analysis and, to correct for residual test statistic inflation, applied genomic control (GC) both to study-level association statistics and to the meta-analysis (genomic control inflation factor, \( \hat{\lambda}_{GC} = 1.08 \) for PP and \( \hat{\lambda}_{GC} = 1.12 \) for MAP). The quantile-quantile plots show an excess of extreme values largely accounted for by a modest number of genomic regions (Supplementary Fig. 1a,b).

We performed independent follow-up analyses in 48,607 individuals of European ancestry (Online Methods and Supplementary Note). SNPs in 12 regions showed genome-wide significant association (P<5 x 10^{-8}) with either PP or MAP in our discovery data (stage 1) (Supplementary Fig. 1c,d), including two previously unidentifed regions for PP (7q22.3 near PIK3CG, P = 1.2 x 10^{-10} and 11q24.3 near ADAMTS8, P = 8.5 x 10^{-11}; Table 1) and 10 regions previously associated with SBP and DBP (see Supplementary Table 2a for PP and Supplementary Table 2b for MAP). For follow-up in a series of independent cohorts, we selected 99 SNPs comprising those with P<1 x 10^{-5} for either PP or MAP and SNPs reported in recent large genome-wide association studies of SBP and DBP (see Supplementary Note).

After the meta-analysis of the stage 1 and 2 data together (Supplementary Table 2c), the two new regions showing genome-wide association with PP after stage 1 (near PIK3CG and near ADAMTS8) remained genome-wide significant. In addition, we found genome-wide significant associations for SNPs at two further new loci for PP (at 4q12 near CHIC2 and 8q24.12 in NOV), two new loci for MAP (3p21.31 in MAP4 and 10q25.3 near ADRB1) and one locus for both traits (2q24.3 near FIGN) (Table 1 and Fig. 1). A locus which has not previously shown an association with SBP or DBP in Europeans but which has recently been associated with SBP in east Asians (Supplementary Note). Forest plots of the stage 1 effect sizes and standard errors are shown in Supplementary Figure 2. The new signals for MAP were strongly associated with both SBP and DBP (P = 7.7 x 10^{-7} to P = 1.8 x 10^{-12}), reflecting the high inter-correlations among these three blood pressure traits. For the sentinel SNPs in three of the new PP loci, the estimated effects on SBP were in the opposite direction to the effects on DBP (Table 1, Fig. 2 and Supplementary Table 2d.e). Our findings show that analyses of PP and MAP identify loci influencing blood pressure phenotypes that may not be detectable by studying SBP and DBP separately.
Identification of new genetic associations could help inform understanding of possible distinct mechanisms underlying relationships of PP with vascular risk.\textsuperscript{14,15}

Five additional loci for PP and 19 loci for MAP reaching genome-wide significance (P < 5 × 10^{-8} for stage 1 and 2 combined) were recently shown to be associated with SBP and/or DBP\textsuperscript{1-3} (Supplementary Table 2a,b). We used sentinel SNPs from both the new and known regions showing genome-wide significant associations with PP or MAP in the combined stage 1 and 2 data to create weighted risk scores for PP (10 independent SNPs) and MAP (22 SNPs) (Supplementary Table 2f). We studied the associations of both risk scores with hypertension and blood pressure–related outcomes including coronary heart disease, heart failure, stroke, echocardiographic measures of left ventricular structure, pulse wave velocity, renal function and renal failure. Adjusting for multiple testing for the 12 traits evaluated (P = 0.05/12 = 4.1 × 10^{-3}), the PP SNP risk score was associated with prevalent hypertension (P = 7.9 × 10^{-6}), incident stroke (P = 4.9 × 10^{-6}) and coronary heart disease (P = 4.3 × 10^{-4}), and the MAP SNP risk score was associated with hypertension (P = 5.1 × 10^{-16}), coronary heart disease (P = 4.0 × 10^{-20}), stroke (P = 0.0019) and left ventricular wall thickness (P = 2.1 × 10^{-8}) (Supplementary Table 3a), confirming the clinical relevance of these measures of blood pressure phenotype.\textsuperscript{8,9} For a range of blood pressure–related outcomes (Supplementary Note), we compared P values for the PP risk score and a series of 1,000 permutations of SBP risk scores, each based on 10 of the 26 blood pressure SNPs associated with SBP but not with PP, and constraining the selection of SNPs to have similar sized effects for SBP as the 10 SNPs for PP. The PP risk score had a significantly (P < 0.05) greater association with risk of ischemic stroke than the SBP risk score (Supplementary Note and Supplementary Table 3b).

None of the genes in the identified newly associated regions is a strong candidate for blood pressure regulation, although several of them are implicated in mechanisms that may influence blood pressure. The most significant association with PP is within a putative mRNA clone (AF086203) spanning 13.7 kb at 7q22.3, 94 kb upstream of PIK3CG (rs17477177, P = 2.3 × 10^{-12}; Table 1 and Fig. 1a). PIK3CG encodes the phosphoinositide-3-kinase, catalytic, γ polypeptide protein (PI3Kγ), which phosphorylates phosphoinositides and modulates extracellular signals. This region was earlier associated with mean platelet volume, platelet count and platelet aggregation\textsuperscript{16-18}, but the sentinel SNPs reported in those previous studies are independent of the SNP reported here, rs17477177 (r^2 < 0.01). Mice lacking the catalytic subunit of PI3Kγ have shown resistance to the SBP-lowering effects of β-adrenergic receptor agonists\textsuperscript{19}, PI3Kγ activity is increased in the failing human heart and is associated with downregulation of β-adrenergic receptors in the plasma membrane\textsuperscript{20}. The second locus for PP, located at 11q24.3, spans 35.5 kb, with the top-ranking SNP (rs11222084, P = 1.9 × 10^{-11}; Fig. 1b) lying 1.6 kb downstream of ADAMTS8. This gene is highly expressed in macrophage-rich areas of human atherosclerotic plaques and may affect extracellular matrix remodeling\textsuperscript{21}. The third locus for PP spans 28.5 kb at 8q24.12, with the sentinel SNP (rs2071518, P = 3.7 × 10^{-5}; Fig. 1c) located in the 3′ untranslated region of NOV, encoding the nephroblastoma over-expressed (CCN3) protein, which is associated with angiogenesis, proliferation and inhibition of vascular smooth muscle cell growth and migration\textsuperscript{22} and with reduced neointimal thickening in mice null for CCN3\textsuperscript{23}. Mice with mutations in Nov that truncate the NOV protein show abnormal cardiac development\textsuperscript{24}. Of the genes evaluated for expression in human aortic samples at the new PP loci, NOV showed by far the highest expression levels (Supplementary Note and Supplementary Fig. 3). The fourth locus for PP is 4q12, with the top-ranking SNP (rs871606, P = 1.3 × 10^{-8}; Fig. 1d) located 76.7 kb downstream of CHIC2, encoding a cysteine-rich hydrophobic domain–containing protein that is associated with acute myeloid leukemia\textsuperscript{25}. This SNP is located 296 kb upstream of PDGFRA, which encodes platelet-derived growth factor receptor α, a cell surface receptor for members of the platelet-derived growth factor family involved in kidney development. Variants in PDGFRA have
been associated with red blood cell count and other haematological indices\textsuperscript{26} but are independent ($r^2 < 0.3$) of rs871606.

For MAP, we identified two newly associated loci. The first locus for MAP is at 10q25.3, 22.3 kb upstream of ADRB1 (rs2782980, $P = 2.5 \times 10^{-8}$; Fig. 1e). ADRB1 encodes the $\beta$-1-adrenergic receptor, which mediates the effects of the stimulatory G protein and cAMP/protein kinase A pathway to increase heart rate and myocardial contraction. Polymorphisms in this gene have been associated with resting heart rate, response to beta blockers\textsuperscript{27} and hypertension\textsuperscript{28}. ADRB1 knockout mice have no difference in heart rate or blood pressure compared with wild type but do have a significant reduction in the response of both phenotypes to catecholamines\textsuperscript{29}, rs2782980 is associated with expression of an ADRB1 transcript in brain tissue (Supplementary Note and Supplementary Fig. 4a). The second locus for MAP spans over 300 kb at 3p21.31, with the top-ranking SNP (rs319690, $P = 2.7 \times 10^{-8}$; Fig. 1f) lying within an intron of MAP4, encoding microtubule-associated protein 4. Coating of microtubules by MAP4 may inhibit $\beta$-adrenergic-receptor recycling and number, as seen in cardiac hypertrophy and failure\textsuperscript{30}. MAP4 was detectably expressed in human aortic samples (Supplementary Note and Supplementary Fig. 3).

The locus associated both with PP (rs13002573, $P = 1.8 \times 10^{-5}$; Fig. 1g) and MAP (rs1446468, $P = 6.5 \times 10^{-12}$; Fig. 1h) is in an intergenic region spanning ~280 kb at 2q24.3. Although the two signals are
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Methods

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics.

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Competing financial interests

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Abnormal skeletal and cardiac development, cardiomyopathy, Dynamic regulation of phosphoinositide 3-kinase-

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ONLINE METHODS

Phenotypes. PP was defined as systolic minus diastolic pressure, and MAP was defined as 2/3 diastolic plus 1/3 systolic pressure. A two-staged analysis was used to discover genes associated with PP and MAP.

Stage 1 samples and analyses. Stage 1 was a meta-analysis of directly genotyped and imputed SNPs from population-based or control samples from case-control studies in the International Consortium of Blood Pressure Genome-wide Association Studies (ICBP-GWAS). The characteristics of the 35 studies, including demographics, genotyping arrays, quality control filters and statistical analysis methods used, are listed in Supplementary Table 1a,b. Imputation of the allele dosage of ungenotyped SNPs in HapMap CEU v21a or v22 was carried out by each of the studies using MACH31, IMPUTE32 or BIMBAM33 with parameters and pre-imputation filters as specified in Supplementary Table 1b. SNPs were excluded from analysis if the study-specific imputation quality (r²,hat in MACH or .info in IMPUTE) was <0.3. In total, up to 2,652,054 genotyped or imputed autosomal SNPs were analyzed. Full details of the models, methods and corrections for antihypertensive treatment are provided in the Supplementary Note. All analyses assumed an additive genetic model and were adjusted for sex, age, age², body mass index and ancestry principal components. In related individuals, regression methods that account for relatedness were applied. All study-specific effect estimates and coded alleles were oriented to the forward strand of the HapMap release 22, with the alphabetically higher allele as the coded allele. To capture loss of power caused by imperfect imputation, we estimated ‘N effective’ as the sum of the study-specific products of the imputation quality metric and the sample size. No filtering on minor allele frequency was done. Genomic control was carried out on study-level data, and inverse-variance weighting was used for the meta-analysis of stage 1. The meta-analysis results were subject to genomic control. Genomic control inflation factor, λGC, estimates are given in Supplementary Table 1a.

Selection of SNPs for stage 2. We aimed in stage 2 to follow up SNPs which had evidence of association with PP or MAP and, for completeness, to evaluate the effects on PP and MAP of SNPs reported in recent large genome-wide association studies of SBP and DBP1–3. All SNPs with \( P < 1 \times 10^{-5} \) for association with either PP or MAP (or both) were divided into independent regions based on linkage disequilibrium, and the most significant SNP was selected from each region. Within the FIGN region, different SNPs were associated with PP and with MAP, and both of these SNPs were followed up in stage 2. For SNPs with an \( N \) effective of \(<75\%\) of the total \( N \), a proxy was also included if it had \( P < 1 \times 10^{-5} \) and \( r^2 > 0.6 \) with the top SNP (this occurred for one SNP). For all regions that had previously shown association with SBP or DBP1–3, the sentinel SNP for PP and MAP and the previously reported SNP for SBP and DBP were followed up. In all, 99 SNPs were followed up in stage 2 (Supplementary Note) comprising: 44 SNPs from 22 loci with PP or MAP associations (\( P < 1 \times 10^{-5} \)) in stage 1 data and with previously reported SBP or DBP associations; 47 SNPs from 45 loci with PP or MAP associations (\( P < 1 \times 10^{-5} \)) in stage 1 data only and; 8 SNPs from 7 loci with previously reported SBP or DBP associations and no association (\( P < 1 \times 10^{-5} \)) with PP or MAP in stage 1 data.

Stage 2. The characteristics of the stage 2 studies, including the genotyping and imputation approaches, are described in Supplementary Table 1a,b and the details of the corrections for treatment are described in the Supplementary Note. For the 99 SNPs selected for follow-up, the stage 2 studies followed the analysis approach adopted in the stage 1 analyses. The meta-analysis was done using the inverse-variance weights method.

Pooled analysis of first- and second-stage samples. The meta-analysis from stages 1 and 2 was conducted using inverse-variance weighting, and genomic control was applied. A threshold of \( P = 5 \times 10^{-8} \) was taken for genome-wide significance.

Calculation of risk scores. We calculated risk scores based on the most significantly associated SNP from all regions that were genome-wide significant after the meta-analysis of stages 1 and 2 for PP (10 SNPs) and MAP (22 SNPs) (Supplementary Table 2f). Each risk score was constructed using an approach described in the Supplementary Note and was tested for association with hypertension, coronary artery disease, stroke, hypertension, chronic kidney disease, heart failure and microalbuminuria and with the continuous traits of left ventricular mass, left ventricular wall thickness, pulse wave velocity, serum creatinine, eGFR and urinary albumin:creatinine ratio (Supplementary Table 3).

Additional analyses. Identification of potentially functional SNPs in linkage disequilibrium with the reported sentinel SNPs, expression quantitative trait loci analyses and expression analyses in human aortic samples were also carried out as discussed in the Supplementary Note and Supplementary Figures 3 and 4.