Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk

LETTER

The International Consortium for Blood Pressure Genome-Wide Association Studies

Blood pressure is a heritable trait influenced by several biological pathways and responsive to environmental stimuli. Over one billion people worldwide have hypertension (≥140 mm Hg systolic blood pressure or ≥90 mm Hg diastolic blood pressure). Even small increments in blood pressure are associated with an increased risk of cardiovascular events. This genome-wide association study of systolic and diastolic blood pressure, which used a multi-stage design in 200,000 individuals of European descent, identified sixteen novel loci: six of these loci contain genes previously known or suspected to regulate blood pressure (GUCY1A3–GUCY1B3, NPR3–C5orf23, ADM, FURIN–FES, GOSR2, GNAS–EDN3); the other ten provide new clues to blood pressure physiology. A genetic risk score based on 29 genome-wide significant variants was associated with hypertension, left ventricular wall thickness, stroke and coronary artery disease, but not kidney disease or kidney function. We also observed associations with blood pressure in East Asian, South Asian and African ancestry individuals. Our findings provide new insights into the genetics and biology of blood pressure, and suggest potential novel therapeutic pathways for cardiovascular disease prevention.

Genetic approaches have advanced the understanding of biological pathways underlying inter-individual variation in blood pressure. For example, studies of rare Mendelian blood pressure disorders have identified multiple defects in renal sodium handling pathways. More recently two genome-wide association studies (GWAS), each of >25,000 individuals of European ancestry, identified 13 loci associated with systolic blood pressure (SBP), diastolic blood pressure (DBP) and hypertension. We now report results of a new meta-analysis for rs1813353 (pairwise $r^2 = 0.015$ in HapMap CEU). Of our 13 previously reported associations, only the association at PLCD3 was not supported by the current results (Supplementary Table 4). Some of the associations are in or near genes involved in pathways known to influence blood pressure (NPR3, GUCY1A3–GUCY1B3, ADM, GNAS–EDN3, NPPA–NPPB and CYP17A1; Supplementary Fig. 4). Twenty-two of the 28 loci did not contain genes that were a priori strong biological candidates.

As expected from prior blood pressure GWAS results, the effects of the novel variants on SBP and DBP were small (Fig. 1 and Table 1). For all variants, the observed directions of effects were concordant for SBP, DBP and hypertension (Fig. 1, Table 1 and Supplementary Fig. 3). Among the genes at the genome-wide significant loci, only CYP17A1, previously implicated in Mendelian congenital adrenal hyperplasia and hypertension, is known to harbour rare variants that have large effects on blood pressure.

We performed several analyses to identify potential causal alleles and mechanisms. First, we looked up the 29 genome-wide significant index SNPs and their close proxies ($r^2 > 0.8$) among cis-acting expression SNP (eSNP) results from multiple tissues (Supplementary Materials section 5). For 13/29 index SNPs, we found an association between nearby eSNP variants and the expression levels of at least one gene transcript ($10^{-4} > P > 10^{-3}$; Supplementary Table 6). In five cases, the index blood pressure SNP and the best eSNP from a genome-wide survey were identical, highlighting potential mediators of the SBP–blood pressure associations.

Second, because changes in protein sequence are a priori strong functional candidates, we sought non-synonymous coding SNPs that were in high linkage disequilibrium ($r^2 > 0.8$) with the 29 index SNPs. We identified such SNPs at eight loci (Table 1, Supplementary Materials section 6 and Supplementary Table 7). In addition we performed analyses testing for differences in genetic effect according to body mass index (BMI) or sex, and analyses of copy number variants, pathway enrichment and metabolomic data, but we did not find any statistically significant results (Supplementary Materials sections 7–9 and Supplementary Tables 8–10).

We evaluated whether the blood pressure variants we identified in individuals of European ancestry were associated with blood pressure in individuals of East Asian (N = 29,719), South Asian (N = 23,977) and African (N = 19,775) ancestries (Table 1 and Supplementary Tables 11–13). We found significant associations in individuals of East Asian ancestry for SNPs at nine loci and in individuals of South Asian ancestry for SNPs at six loci; some have been reported previously (Supplementary Tables 12 and 15). The lack of significant association for individual SNPs may reflect small sample sizes, differences in allele frequencies or linkage disequilibrium patterns, imprecise imputation from some ancestries using existing reference samples, or a genuinely different underlying genetic architecture. Because of limited power to detect effects of individual variants in the smaller non-European samples, we created genetic risk scores for SBP and DBP incorporating all 29 blood pressure variants weighted according to effect sizes observed in the European samples. In each non-European ancestry group, risk scores were strongly associated with SBP ($P = 1.1 \times 10^{-10}$ in East Asian, $P = 2.9 \times 10^{-13}$ in South Asian, $P = 9.8 \times 10^{-4}$ in African
are associated with an increase in cardiovascular disease risk. Epidemiological data have shown that differences in SBP and DBP of this magnitude, across the population range of blood pressure, are consistent with findings from randomized trials of blood-pressure-lowering medication in hypertensive patients, the genetic risk score was positively associated with left ventricular wall thickness, and measures of kidney function, using results from other GWAS consortia (Table 2, Supplementary Materials sections 10, 11 and 12). We also created a genetic risk score to assess association of the genetic risk score was positively associated with left ventricular wall thickness, and measures of kidney function, even though these renal outcomes were available in a similar sample size as for the other outcomes (Table 2). The absence of association with kidney phenotypes could be explained by a weaker causal relationship between blood pressure and kidney phenotypes than with CAD and stroke. This finding is consistent with the mismatch between observational data that show a positive association of blood pressure with kidney disease, and clinical trial data that show inconsistent evidence of a benefit from blood pressure lowering on kidney disease prevention in patients with hypertension. Thus, several lines of evidence converge to indicate that blood pressure variants with effect sizes similar to those arising from common blood pressure variants of small effect. By dividing our principal GWAS data set into non-overlapping discovery (N ≈ 56,000) and validation (N ≈ 14,000) subsets, we found robust evidence for the existence of such undetected common variants (Supplementary Fig. 5 and Supplementary Materials section 12). We estimate that there are 116 (95% confidence interval 57–174) independent blood pressure variants with effect sizes similar to those.
report end here, which collectively can explain ~2.2% of the phenotypic variance for SBP and DBP, compared with 0.9% explained by the 29 associations discovered thus far (Supplementary Fig. 6 and Supplementary Materials section 13).

Most of the 28 blood pressure loci harbor multiple genes (Supplementary Table 15 and Supplementary Fig. 4), and although substantial research is required to identify the specific genes and variants responsible for these associations, several loci contain highly plausible biological candidates. The NPPA and NPPB genes at the MTHFR–NPPB locus encode precursors for atrial- and B-type natriuretic peptides (ANP, BNP), and previous work has identified SNPs—moderately correlated with our index SNP at this locus—which are associated with plasma ANP, BNP and blood pressure9,20. We found the index SNP at this locus was associated with opposite effects on blood pressure and on ANP/BNP levels, consistent with a model in which the variants act through increased ANP/BNP production to lower blood pressure9 (Supplementary Materials section 14).

Two other loci identified in the current study harbour genes involved in natriuretic peptide and related nitric oxide signalling pathways17,18, both of which act to regulate cyclic guanosine monophosphate. The first locus contains NRP3, which encodes the natriuretic peptide clearance receptor (NPR-C). NRP3 knockout mice exhibit reduced clearance of circulating natriuretic peptides and lower blood pressure9. The second locus includes GUCY1A3 and GUCY1B3, encoding the α and β subunits of soluble guanylate cyclase; knockout of either gene in murine models results in hypertension20.

Another locus contains ADM—encoding adrenomedullin—which has natriuretic, vasodilatory and blood-pressure-lowering properties21. At the GNAS–EDN3 locus, ZNF831 is closest to the index SNP, but GNAS and EDN3 are two nearby compelling biological candidates (Supplementary Fig. 4 and Supplementary Table 15).

We identified two loci with plausible connections to blood pressure via genes implicated in renal physiology or kidney disease. At the first locus, SLC4A7 is an electro-neutral sodium bicarbonate co-transporter expressed in the nephron and in vascular smooth muscle22. At the second locus, PLCE1 (phospholipase C-epsilon 1 isoform) is important for normal podocyte development in the glomerulus; sequence variation in PLCE1 has been implicated in familial nephrotic syndromes and end-stage kidney disease19.

Missense variants in two genes involved in metal ion transport were associated with blood pressure in our study. The first encodes a His/Asp change at amino acid 63 (H63D) in HFE and is a low-penetration allele for hereditary hemochromatosis23. The second is an Ala/Thr polymorphism located in exon 7 of SLC39A8, which encodes a zinc transporter that also transports cadmium and manganese24. The same allele of SLC39A8 associated with blood pressure in our study has recently been associated with high-density lipoprotein cholesterol levels25 and BMI26 (Supplementary Table 15).

We have shown that 29 independent genetic variants influence blood pressure in people of European ancestry. The variants reside in 28 loci, 16 of which were novel, and we confirmed association of several of them in individuals of non-European ancestry. A risk score
derived from the 29 variants was significantly associated with blood-pressure-related organ damage and clinical cardiovascular disease, but not kidney disease. These loci improve our understanding of the genetic architecture of blood pressure, provide new biological insights into blood pressure control and may identify novel targets for the treatment of hypertension and the prevention of cardiovascular disease.

Note added in proof: Since this manuscript was submitted, Kato et al. published a blood pressure GWAS in East Asians that identified a SNP highly correlated to the SNP we report at the NPR3/Csurf23 locus.28

METHODS SUMMARY
Supplementary Materials provide complete methods and include the following sections: study recruitment and phenotyping, adjustment for antihypertensive medications, genotyping, data quality control, genotypic imputation, within-cohort association analyses, meta-analyses of discovery and validation stages, stratified analyses by sex and BMI, identification of eSNPs and non-synonymous SNPs, metabolomic and lipidomic analyses, CNV analyses, pathway analyses, stratified analyses by sex and BMI, identification of eSNPs and non-synonymous SNPs, metabolomic and lipidomic analyses, CNV analyses, pathway analyses, stratified analyses by sex and BMI, identification of eSNPs and non-synonymous SNPs, metabolomic and lipidomic analyses, CNV analyses, pathway analyses, stratified analyses by sex and BMI, identification of eSNPs and non-synonymous SNPs, metabolomic and lipidomic analyses, CNV analyses, pathway analyses, stratified analyses by sex and BMI, identification of eSNPs and non-synonymous 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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions Full author contributions and roles are listed in Supplementary Materials section 19.

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