Genome Wide Pleiotropic Analysis to Identify Novel Variants and Improve Genetic Risk Score Construction

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Article

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Abstract

Systolic and diastolic blood pressure (S/DBP) are highly correlated and modifiable risk factors for cardiovascular disease (CVD). We report here a bidirectional Mendelian Randomization (MR) and GWAS pleiotropy analysis of S/DBP summary statistics from large published BP GWAS and construct a composite genetic risk score (GRS), capturing respectively 21%, 11%, and 227% more of SBP, DBP and PP heritability than achieved with the traditional GRS. The composite GRS improves the prediction of hypertension and CVD in persons of European as well as African and Asian descent. We identified and confirmed 120 novel BP pleiotropic variants that are not in linkage disequilibrium with known variants, including 17 novel BP loci. We further observed significant age-modulated genetic effects on BP hypertension and CVD in both Europeans and Asians. Our study provides further insight into BP regulation and provides a novel way to construct a GRS for correlated traits.

Introduction

Poorly controlled blood pressure (BP) accounts for a large portion of the excess risk for cardiovascular disease (CVD), stroke, and heart failure\(^1\). Understanding biological mechanisms for BP regulation could potentially help improve BP control and lead to a reduction in the burden of CVD. BP is characterized as systolic and diastolic blood pressure (SBP/DBP) and these measurements are long-standing risk predictors for CVD. Genome-wide association studies (GWAS) have to date been performed on BP traits separately by focusing on main effects. In studies that included subjects of diverse ancestry over 1,000 BP-associated loci have been identified\(^2\)\(^4\). Genome-wide search of gene-environment interactions on BP traits have been recently conducted, however limited new associations have been identified, in part because of low statistical power\(^13\)\(^14\). Although many variants are shared among SBP and DBP as correlated traits, a portion of the variants are associated only with SBP or DBP separately, suggesting among the true positive associations there may be evidence of trait-specific biological mechanisms. It has also been reported that a joint analysis of SBP and DBP leads to the identification of BP variants that are missed by analyzing SBP or DBP separately\(^9\). However prior studies have not addressed the mechanism underlying the relationship of SBP and DBP, which may reflect arterial stiffness or arterial compliance \(^15\). Dissecting the causal relationships of BP variants on SBP and DBP, in particular, whether the variants affect SBP and DBP through the same (mediation) or different (pleiotropic) paths, and how many pleiotropic variants contribute jointly to these highly correlated traits, may advance our understanding of the biology of BP regulation.

Genetic risk scores (GRS) are usually constructed as linear combinations of individual variant effects estimated from GWAS to predict individual-level risk of common disease. For BP, a final GRS is the average of SBP- and DBP-specific GRS\(^3\)\(^6\) and the GRS predicts the risk of CVD. However, published BP GRS’s have explained no more than 6% of the heritability, and have limited predictive power for HTN and CVD. GWAS of gene-age interaction analysis have identified genetic variants with age-dependent effect sizes, including BP\(^16\)\(^17\), lipid levels\(^18\) and BMI\(^19\). A recent study based on a proportional hazards model reported age-varying risk profiles in nine diseases, including HTN\(^20\). However, these studies were under powered because the interactive contribution of a variant by age is often weak.

In this study we address the underlying mechanistic relationship between SBP and DBP by performing a bidirectional Mendelian Randomization (MR) \(^21\) and GWAS pleiotropy analysis using the summary statistics from >750,000 subjects of European ancestry from the UKB and ICBP consortium\(^6\), followed by the summary statistics of 318,891 subjects of European ancestry from the Million Veteran Program (MVP) \(^12\). We searched for novel BP variants with pleiotropic effects and constructed a composite GRS using variants with and without pleiotropic effects, and studied the age-varying effects of GRS on prediction of BP, HTN and CVD in groups of European, African and Asian descent.

Results

We present a bi-directional MR analysis of SBP and DBP using the 1,125 and 1,183 independent genome-wide significant variants for SBP and DBP (P< 5x10^-8) as genetic instrumental variables (IVs) obtained from the UKB-ICBP GWAS\(^6\) and software IMRP\(^22\) and MRmix\(^23\) (Online Methods). We standardized SBP and DBP and obtained an identical causal effect of SBP on DBP and DBP on SBP (0.864 ± 0.005 and 0.862 ± 0.005 by IMRP, respectively, Supplementary Table 1), which is significantly larger than the observed trait correlation 0.738 between SBP and DBP in UKB European descents, with estimated 74.8% of variation is the shared causal contribution between SBP and DBP. The causal estimates by MRmix were concordant (0.89 ± 0.012 and 0.90 ± 0.01, respectively, Supplementary Table 1). Among the genetic IVs, 43% of the variants had pleiotropic effects on SBP and DBP.

We next extended the pleiotropic effect analysis to search for variants with evidence of pleiotropy for SBP and DBP across the whole genome (OnlineMethods). The Manhattan and QQ plots for testing pleiotropy are presented in Fig 1. LD score regression analysis\(^24\) estimated that 8.7% of the heritability was due to pleiotropic variants. We observed 906 independent variants (r^2 <0.05) reaching genome-wide significance level in pleiotropy tests (P<5x10^-8), 234 of which had not been detected by the univariate GWAS analysis of SBP, DBP or PP in the original UK Biobank+ICBP consortium\(^6\) (Supplementary Table 2). Among the 234 variants, 201 variants were novel and were not reported in any previous BP GWAS. In the set of associations, 163 variants in 124 loci were within a 1Mb region of previous reported known loci but were not in LD with known BP variants (r^2 <0.05); the remaining 38 variants were 1Mb away from the previous reported known BP loci, and resided in 35 loci; the corresponding locus zoom plots are presented in Supplementary Figure 1. We evaluated the associations of our sentinel SNPs at the 35 novel loci with other traits and disease using GWAS Catalog\(^25\) and FUMA\(^26\). The GWAS Catalog and FUMA search of published GWAS showed that 29 of the 35 novel loci are also significantly associated with other traits, including lipid levels, cardiovascular-related outcomes, anthropometric traits, sleep traits, educational attainment, smoking, blood protein level and Schizophrenia (Supplementary Table 3).

Replication of novel signals in Million Veteran Program (MVP). Using the causal effect estimate between SBP and DBP from the UKB-ICBP summary statistics, we performed pleiotropy tests among the 201 novel variants. Eighteen variants in the 35 novel loci (Table 1), and 102 of 163 novel variants on known 124 loci
could be replicated in MVP at $P<0.05$ (Supplementary Table 2), suggesting the identified novel pleiotropic signals are not false positives.

**Functional analysis.** We performed expression quantitative trait locus (eQTL) analysis using GTEx data. Among the 35 novel loci, we identified 26 novel loci with expression quantitative trait locus (eQTL) (Supplementary Table 4) and 11 novel loci with Splicing Quantitative Trait Loci (sQTLs) (Supplementary Table 5). The eQTLs were most often enriched in arterial tissues, followed by adipose, heart and nerve tibial tissues. SNP rs17713879 is an eQTL affecting expression of the SH3YL1 and ACP7 genes in 34 tissues and is also a sQTL affecting splicing of these two genes in 50 tissues. SNP rs112500920 is an eQTL affecting expression of several genes, including EFL1 and AB3B2, in multiple tissues, notably adipose and arterial tissues. SNP rs12478520 is an eQTL affecting expression of multiple genes, including C2orf72, HTR2B, ARM C9 and PSMD1.

We defined mediation variants as those variants associated with SBP or DBP with $P<5 \times 10^{-8}$ but not significant in a test for pleiotropy, and pleiotropic variants as the variants reaching genome wide significance ($P<5 \times 10^{-8}$) in a pleiotropy test using the UKB-ICBP data. We assessed tissue enrichment of BP loci using DEPICT at a false discovery rate (FDR) < 5% but separated mediation from pleiotropic variants in the analysis. There were 1,324 independent mediation variants and 906 independent pleiotropic variants. DEPICT analysis identified enrichment across 42 and 72 tissues and cells using mediation and pleiotropic variants, respectively (Supplementary Table 6). The enriched tissues are highly similar (correlation=0.78) but notable differences were also observed (Supplementary Figure 2a). Enrichment was greatest for arteries in the cardiovascular system for both mediation and pleiotropic variants ($P=2.8 \times 10^{-6}$ and $2.41 \times 10^{-11}$, respectively). In general, enrichments observed for mediation variants were also observed for pleiotropic variants, but not vice versa. For example, heart ventricle, heart valves, heart atria and the atrial appendage were enriched for pleiotropic variants ($P=1.23 \times 10^{-5}$) but not for mediation variants (Supplementary Table 6). Pathway enrichments for mediation variants and pleiotropic variants were less well correlated (correlation=0.478, Supplementary Figure 2b). Pleiotropic variants were enriched in many molecular pathways that were missed by mediation variants, including oxygen levels, basement membrane, and renal system development ($P<2.23 \times 10^{-7}$ for pleiotropic variants, Supplementary Table 7). Evaluation of enriched mouse knockout phenotype terms pointed to the importance of abnormal kidney morphology, impaired wound healing and increased body weight, etc ($P<3.28 \times 10^{-5}$) for pleiotropic variants, although these were absent for mediation variants. Protein–protein interaction subnetwork enrichments were also substantially different; using the pleiotropic variants we identified the HSPG2, DDR1, MMP9, COL4A4, TGM2 and COL4A5 subnetworks ($P<1.90 \times 10^{-5}$) which were missed relying solely on mediation variants (Supplementary Table 7).

**Improved prediction of BP, hypertension and CVD by including pleiotropic variants.** It is known that polygenic scores derived from multiple related traits can improve prediction of outcomes but these approaches do not use the pleiotropic variants directly. Here we constructed a traditional BP GRS using all independent 1,616 BP variants from UKB-ICBP. We further constructed a pleiotropic genetic risk score (PGRS) using the 906 pleiotropic variants (Online Methods). We jointly modeled GR S and PGRS adjusting for age, gender, BMI and 10 principal components, and observed PGRS significantly predicted BP traits, as well as risk of HTN and CVD conditional on GR S in all the models (Tables 2A and 2B) and Figure 2) in UKB European ancestry subjects. The variance explained by GR S only was 5.91%, 6.09% and 2.23% for SBP, DBP and PP, increased to 7.13%, 6.75% and 7.27% when including PGRS, respectively, suggesting independent additive predictive power for the PGRS. Interestingly the PGRS had opposite directional effects for SBP and DBP. We observed odds ratios (ORs) of 1.64 and 1.15 for individual GR S and PGRS on risk of HTN ($P<1 \times 10^{-300}$), respectively. The observed ORs of individual GR S and PGRS for CVD were 1.21 and 1.06 ($P=1.71 \times 10^{-231}$ and $P=2.74 \times 10^{-25}$), respectively, and increased to 1.66 and 1.19 ($P = 9.83 \times 10^{-67}$ and $P = 1.28 \times 10^{-13}$) when comparing between the upper and lower quantiles of the GR S and PGRS, respectively. However, the odds ratios were further increased to 6.78 and 2.44 ($P<1 \times 10^{-300}$ and $P=1.15 \times 10^{-39}$) for HTN and CAD, respectively, when comparing the top decile and quintile with bottom decile and quintile of GR S and PGRS (Figure 2). We observed a clear advantage of including PGRS over GR S only (Figure 2). After including the interactions of age and GR S and PGRS the main effects for GR S and PGRS on BP, HTN and CVD were unchanged. However, we observed a significant interaction effect of age and GR S for all BP traits and HTN, but not for CVD. The interaction of age and PGRS significantly contributed to all BP traits, HTN and CVD ($P$ value between $3.0 \times 10^{-2}$ and $7.74 \times 10^{-29}$, Table 2A).

**Extension to other ancestries.** We examined associations with BP and CVD of the above defined GR S and PGRS in unrelated African (N=7,904) and South Asian (N=8,509) subjects in the UKB (Table 2, B, C). Although sample sizes were much smaller than among UKB European subjects, the GR S was significantly associated with SBP, DBP, PP, HTN and CVD in both UKB African- and Asian-descent subjects. PGRS was associated with a 1.21, -0.35 and 1.56 mmHg higher for SBP, DBP and PP ($P=8.34 \times 10^{-6}$, $1.09 \times 10^{-2}$ and $2.05 \times 10^{-27}$) in Africans, a 1.25, -0.64 and 1.89 mmHg higher ($P=3.21 \times 10^{-10}$, $1.69 \times 10^{-8}$ and $1.50 \times 10^{-48}$) in Asians, respectively. The ORs of PGRS for HTN and CVD were significant ($P=9.27 \times 10^{-5}$ and $P=2.81 \times 10^{-5}$) in Asians but not in Africans. The effects of PGRS on DBP were also negative as observed in UKB Europeans. Significant interactions of age and PGRS were again observed for BP traits and CVD in UKB Asians (Table 2C).

**Mendelian Randomization of BP on CAD, MI and stroke.** We downloaded the published GWAS summary statistics for CAD, MI and stroke and performed MR analysis of BP on CAD, MI and Stroke, using mediation and pleiotropic variants as IVs separately (Online Methods). Both SBP and DBP causally contributed to CAD, MI and stroke using mediation as the IVs. The new trait - BPpleio - also causally contributed to CAD, MI and stroke using pleiotropic variants (Online Methods, Table 3). The estimated ORs ranged from 1.66 to 1.85 per SD unit increase in BP on the three outcomes using mediation variants, and ranged from 1.13 to 1.48 using pleiotropic variants. Our analysis identified 10 to 22% IVs demonstrating pleiotropic effects for BP and the three clinical outcomes (Table 3).

**Discussion**

Our analysis of the summary statistics derived from over 1 million subjects describe important aspects of the genetic architecture of the two principle highly correlated BP traits. The bi-directional MR analysis of SBP and DBP demonstrated that 1 SD unit increase of SBP leads to 0.86 SD unit increase of DBP and vice versa, indicating SBP and DBP share 74.8% of the variation, presumably attributing to common factors and common biological mechanisms. This shared causal contribution is substantially higher than 55.6% estimated by phenotype correlation analysis between SBP and DBP. Most of the BP variants identified through SBP or DBP univariate association were mediation variants and these variants contribute to the shared causal contribution and would be expected to
be found in either the SBP or DBP GWAS. The pleiotropic variants are associated with the rest of the BP variation, which is conferred through different biological pathways for SBP and DBP. The pleiotropic variants often demonstrated an effect size that was opposite in direction for SBP and DBP and contributed 8.71% heritability of the new BP trait. The pleiotropy defined trait BP\textsubscript{pleio} was highly correlated with PP (p ≥0.66) in UKB whites, taken as an indicator of arterial stiffness and considered as an independent risk factor for CVD\textsuperscript{32}. Thus, the BP\textsubscript{pleio} can be an independent risk factor for CVD. This is consistent with the finding that SBP DBP and BP\textsubscript{pleio} all contributed to risk estimation of CAD, Stroke and MI in the MR analysis (Table 3).

In addition to a large number of known genetic variants that contribute to SBP and DBP reported by the original UK Biobank+ICBP consortium\textsuperscript{6}, we identified 906 independent variants demonstrating pleiotropy evidence (P<5×10\textsuperscript{-6}), 201 were undetected by the univariate GWAS analysis of SBP, DBP or PP. Replication analysis in the MVP confirmed 120 of the 201 novel variants, including the 17 novel loci.

In addition to the traditional BP GRS calculated in literature\textsuperscript{3,6}, we defined a composite genetic risk score consisting of GRS and PGRS. The joint model of GRS and PGRS suggested both genetic risk scores independently predict BP, HTN and CVD outcomes (Table 2) in the UK Biobank European ancestry subjects. Additionally, including the PGRS led to substantial increments in heritability for BP traits (Table 2 and Figure 2). Although we observed consistent opposite directional effects of PGRS for SBP and DBP in UK Biobank Europeans, Africans and Asians, the prediction of HTN and CVD risk was significantly improved by including PGRS (Figure 2 C and D). The GRS and PGRS defined on European participants both consistently and significantly predict BP, HTN and CVD in UK Biobank Africans and Asians, suggesting that PGRS is able to improve prediction accuracy across ethnic populations. Recent studies have suggested that current GRS models alone have modest improvement of predictive accuracy for CAD\textsuperscript{33-35}. The principle outcome of this set of analyses, therefore, demonstrated that adding PGRS significantly improves the prediction model over the GRS alone. This approach of constructing polygenic risk score is conceptually different from the existing approaches using multiple related traits\textsuperscript{28-31} and could be generalized to other diseases by incorporating multiple disease-related traits through pleiotropy analysis.

Our analysis avoided an examination of the interaction of individual variants and age because of insufficient power. We were able to observe the interaction effects of both GRS and PGRS with age in UKB Europeans for SBP, DBP, PP and HTN although the interaction for CVD was only significant for PGRS (Table 2). The age-PGRS interactions were also replicated in Asians despite a substantially smaller sample size. We observed that the interaction contribution to phenotype variation was consistently small (range from 0.1% to 0.3% BP heritability in both UKB Europeans and Asians). The negative interaction contributions of both GRS and PGRS on DBP may partially explain the decline of DBP after 60 years older\textsuperscript{36}. In comparison, the interaction of age and GRS was positive for SBP, suggesting genetic effect on SBP increases in older individuals. As noted, the GRS interaction effects in UKB Europeans could not be observed in UKB Africans or Asians, likely as a result of small sample sizes. In comparison, the age-modulated interaction of PGRS were observed in both UKB Europeans and Asians, indicating stronger PGRS interaction effects than for the GRS. In our functional analysis, we observed a wider range of BP related tissues and biological pathways for the BP pleiotropic variants than mediation variants, which may imply that the pleiotropic variants are influenced by a wider range of environmental factors and therefore continue to make an evolving genetic contribution over the age span. Our study also supports an omnigenic model for complex traits\textsuperscript{37-39}. In fact, it could be inferred that a pleiotropic variant acts on multiple peripheral genes to impact the expression of a core gene in different biological pathways. As a result, the pleiotropic variants have weak effects on a phenotype and are more difficult to detect in a traditional BP GWAS that focuses on single trait analysis, as we observed in the current analysis. In comparison, mediation variants may be more likely to occur in core genes, although it is clearly important to detect the putative set of core genes as well. We acknowledge that the data presented here can only provide suggestive evidence for that hypothesis.

In conclusion, the new findings we bring forward here include the 906 independent BP pleiotropic variants - 201 of which were previously not identified in traditional BP GWAS - and a novel way to construct polygenic risk score represent a substantial advance in understanding the genetic architecture of highly correlated SBP and DBP.

**Online Methods**

**Summary statistics of UK Biobank (UKB) and International Consortium for Blood Pressure (ICBP).** UKB consists of 458,577 UK European and ICBP consists of 299,024 European decent subjects. GWAS of BP traits were conducted in UKB and ICBP separately and results were meta-analyzed\textsuperscript{3,6}. Our analysis was based on the summary results from the UKB and ICBP GWAS that were calculated based on up to 757,601 participants and ~7.1 M genotyped and imputed SNPs with MAF ≥1% for variants present in both the UKB data and ICBP meta-analysis for SBP, DBP and PP.

**Summary statistics of the Million Veteran Program (MVP).** The summary statistics of the Million Veteran Program (MVP) consists of 318,891 European ancestry participants and 18.2M genotyped and imputed SNPs for SBP, DBP and PP\textsuperscript{12}. The MVP summary statistics were used for replication analysis.

**UKB phenotypic data.** (There was an inadvertent mistake in the last version). We analyzed three BP traits in UKB: SBP, DBP and pulse pressure (PP) (SBP-DBP). We calculated the mean SBP and DBP values from two baseline BP measurements. We added 15 and 10 mmHg to SBP and DBP for individuals who took antihypertensive medications. Hypertensive cases were defined as either SBP≥140 or DBP≥90 or taking antihypertensive medications. CVD cases in UKB were defined using self-reported baseline information on CVD prevalence and the ICD9 and ICD10 diagnostic codes on hospital admissions. The CVD took antihypertensive medications. Hypertensive cases were defined as either SBP≥140 or DBP≥90 or taking antihypertensive medications. CVD cases in UKB phenotypic data includes ICD10 codes (I210, I211, I212, I213, I214, I219, I21X, I220, I221, I228, I229, I230, I231, I232, I233, I234, I235, I236, I238, I240, I241, I248, I249, I250, I251, I252, I253, I254, I255, I256, I258, I259), and ICD9 codes ("4109", "4119","4219", "4139", "4140", "4141", "4148", "4149"). This procedure resulted 35,968 CVD cases in European, African and Asian ancestries.

**Mendelian Randomization analysis.** We applied the iterative Mendelian randomization and pleiotropy analysis (IMRP)\textsuperscript{22} and MR mixture model (MRmix)\textsuperscript{23} for bi-directional MR analysis of SBP and DBP, as well as to estimate the causal contributions of BP on CAD, MI and STROK. IMRP is an iterative approach by
combining the pleiotropy test and the MR analysis. The iteration starts by performing MR-Egger analysis\(^40\) to estimate causal effect of an exposure to outcome, following by inverse variance weighted (IVW)\(^41,\)\(^42\) analysis until the causal effect estimation converges. At each iteration step, IMRP performs pleiotropy test to update which genetic instrument variants showing pleiotropy evidence (P < 0.05) by performing the test \(T_{\text{MVP}} = \frac{1}{\sqrt{1 - \rho^2}} \rho\), where \(\hat{f}\) and \(\hat{f}_c\) are the estimated effect sizes of an IV on the exposure and the outcome, respectively, and \(\hat{\rho}\) is the causal estimate which is updated at each iterative step. IMRP takes the advantages of MR-Egger, which is less bias, and IVW, which is efficient. IMRP can be applied to GWAS summary statistics of an exposure and an outcome obtained with overlapped or non-overlapped samples. To ensure the causal estimate is robust, we also applied a substantially different MR approach MRmix\(^23\), which is an estimating equation approach that assumes \(\hat{f} - \hat{f}_c\) follow a normal mixture model. MRmix usually shows a good trade-off between bias and variance even with more than 50% invalid IVs\(^23\).

### GWAS of pleiotropy analysis for SBP and DBP
We first perform a bi-directional MR analysis by IMRP to estimate the causal effect using genome-wide significant independent variants associated with SBP and DBP separately in UKB-ICBP summary statistics. We then perform the pleiotropy test \(T_{\text{MVP}}\) to all the 7.1M SNPs by fixing the causal effect estimated in the IMRP analysis. This is equivalent to performing GWASs for two new traits: \(BP_{\text{pleio}} = \hat{f} \cdot DBP\) and \(BP_{\text{pleio}} = \hat{f}_c \cdot DBP\), where \(\hat{f}\) and \(\hat{f}_c\) are the estimated causal effects of SBP on DBP and DBP on SBP, respectively. We noted \(BP_{\text{pleio}}\) and \(BP_{\text{pleio}}\) were highly correlated and represented essentially one phenotype \(BP_{\text{pleio}}\). We declared a variant is significant when its \(P\)-value is less than \(5 \times 10^{-8}\). In the replication analysis in using MVP summary statistics, we applied the causal effect estimate obtained in UKB-ICBP data. Thus, the replication analysis had the same analytic model as in the UKB-ICBP.

### Genomic inflation and confounding
We applied the LD score regression method\(^24\) to test for genomic inflation in the GWAS pleiotropy analysis. It is expected that \(BP_{\text{pleio}}\) will have large genomic control inflation coefficient because of large sample sizes and dense genetic variants in high LD\(^43\). The GC lambda was 1.533 and LDRS intercept was 1.057 (0.013), with inflation ratio 4.23%, suggesting little inflation in the pleiotropic analysis.

### Novel locus definition
Novel loci were defined as the variants reaching genome wide significance and are 1Mb away from known BP variants as well as LD \(\hat{r}^2 < 0.05\) with any known BP variants. Novel signals at known loci are variants within 1Mb region of known BP variants and reach genome-wide significance level, as well as not being in LD with any known BP variants \((\hat{r}^2 < 0.05)\). The 1000G European ancestry data was used as the reference genetic data for LD calculation.

### Functional analyses
We evaluated all sentinel SNPs at novel loci for evidence of mediation of expression quantitative trait loci (eQTL) and alternative splicing quantitative trait loci (sQTL) in all 44 tissues using the Genotype-Tissue Expression (GTEx) database. Following the method in Evangelou et al.\(^6\), a locus is annotated with a given eGene(sGene) only if the most significant eQTL(sQTL) SNP for the given eGene(sGene) is in high LD \((\hat{r}^2 \geq 0.8)\) with the sentinel SNP. We performed overall enrichment testing but using the mediation and pleiotropic variants separately. We used DEPICT\(^27\) (Data-driven Expression Prioritized Integration for Complex Traits) to identify tissues and cells that are highly expressed at genes within the BP mediation loci, as well as BP pleiotropic loci. We also used DEPICT to test for enrichment in gene sets associated with biological annotations including GO ontology, mouse knockout phenotype studies and protein-protein interaction subnetworks. We reported significant enrichments with a false discovery rate < 0.05. Analysis was done using the platform: Complex-Traits Genetics Virtual Lab\(^44\).

### Genetic risk score (GRS) and pleiotropic Genetic risk scores (PGRS)
We constructed a traditional genetic risk score using independent 1,616 genome-wide significant BP variants from UKB-ICBP. We first constructed SBP and DBP weighted GRSs and then derived a single BP GRS as the average of SBP and DBP GRSs. This approach was used in the literature\(^6\) to estimate the combined effect of the BP variants on BP and risks of hypertension and CVD. In addition, we constructed a pleiotropic genetic risk score PGRS using the 906 pleiotropic variants in a similar way. We first constructed \(BP_{\text{pleio1}}\) and \(BP_{\text{pleio2}}\) weighted PGRSs and next derived a single PGRS as the difference of SBP and DBP GRSs. The composite GRS was constructed by joint model of GRS and PGRS in a linear regression. We also performed a linear regression by jointly modeling GRS and PGRS, as well as the interaction of age*GRS and age*PGRS on blood pressure baseline in the UKB data. Similarly, we performed logistic regression of GRS, PGRS, age*GRS and age*PGRS on risk of hypertension and cardiovascular events at baseline in the UKB data. We examined whether PGRS is able to predict additional variations of BP, hypertension and CVD after accounting for GRS. We also examined the age-varying effects of GRS and PGRS by testing the interaction effects. Our analysis included 386,752 unrelated individuals of European ancestry with phenotypes measured at baseline, respectively. To assess the association of GRS, PGRS and their interactions with age on BP, risk of hypertension and CVD, we performed the regression analysis, with adjustment for sex, age, BMI, geographical region and 10 genetic principal components. CVD was defined in unrelated participants in UKB data on the basis of self-reported medical history and linkage to hospitalization and mortality data\(^6\).

We assessed the association of the GRS, PGRS and their interactions with age on blood pressure in unrelated Africans \((n = 7,904)\) and South Asians \((n = 8,509)\) from the UKB to see whether blood pressure–associated SNPs identified from GWAS predominantly in Europeans are also associated with blood pressure in populations of non-European ancestry. All the analyses were performed using the residuals after adjusting for sex, age, BMI, geographical region and 10 genetic principal components.

### Cross-trait lookups of novel loci
We supplied the index SNPs at the novel loci observed in UK Biobank-ICBP pleiotropic analyses to FUMA\(^26\) and GWAS catalog\(^25\) to investigate the trait pleiotropy with traits other than BP, extracting all association results with \(P < 5 \times 10^{-8}\), for all SNPs in high LD \((r^2 \geq 0.8)\).

### Declarations

#### URLs

CTG-View: [https://view.genoma.io/](https://view.genoma.io/)
Data Availability

Full summary statistics related to UKB-ICBP were obtained through request to the authors of UKB-ICBP Paul Elliott or Mark Caulfield. Summary statistics relating to the Million Veteran Program (MVP) are publically available with the accession code phs001672.v1.p1: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001672.v1.p1. The UK Biobank data are available upon application to the UKBiobank (https://www.ukbiobank.ac.uk). The coronary artery disease and myocardial infarction summary statistics can be downloaded at http://www.cardiogramplusc4d.org/data-downloads/; Stroke summary statistics can be downloaded at: http://megastroke.org/privacy.html.

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Data on coronary artery disease / myocardial infarction have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from www.CARDIOGRAMPLUSC4D.ORG'. For the Exome chip study please acknowledge the source of the data as follows: 'Data on coronary artery disease / myocardial infarction have been contributed by the Myocardial Infarction Genetics and CARDIoGRAM Exome investigators and have been downloaded from www.CARDIOGRAMPLUSC4D.ORG

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Author contributions

X.Z conceived and designed the study. X.Z, L.Z. and H.W. performed analysis. X.Z. drafted the initial manuscript. All authors critically revised and approved the manuscript.

Competing interests

There is no competing interest.

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### Tables

**Table 1. The 17 novel BP loci identified by pleiotropic analysis**

| SNP          | CHR  | BP   | \(P_{SBP}\) | \(P_{DBP}\) | \(P_{PP}\) | \(BP_{pleio}\) | MVP \(P_{pleio}\) | Annotated genes   |
|--------------|------|------|-------------|-------------|-------------|-----------------|-------------------|------------------|
| rs11162906   | 1    | 80500974 | 3.19×10⁻²   | 1.75×10⁻²   | 1.78×10⁻⁶   | 2.41×10⁻⁹     | 1.43×10⁻³       | AC989857.2       |
| rs17713879   | 2    | 234215 | 5.19×10⁻⁴   | 2.66×10⁻⁴   | 2.05×10⁻⁰   | 3.75×10⁻⁸     | 7.97×10⁻⁴       | SH3YL1           |
| rs8735304    | 2    | 101617631 | 4.18×10⁻³   | 2.38×10⁻³   | 1.43×10⁻⁶   | 1.47×10⁻⁷     | 9.31×10⁻⁴       | RPL31/TBC1D8     |
| rs12470661   | 2    | 232060050 | 5.09×10⁻⁶   | 3.07×10⁻⁶   | 6.68×10⁻⁸   | 5.33×10⁻¹⁰    | 1.25×10⁻²       | HTGRB/ARMC9      |
| rs10947978   | 6    | 41471608 | 7.01×10⁻⁵   | 9.88×10⁻⁵   | 1.44×10⁻⁵   | 2.65×10⁻¹⁰    | 1.81×10⁻²       | LIN01276         |
| rs56908119   | 6    | 90296727 | 7.63×10⁻²   | 1.38×10⁻²   | 6.81×10⁻⁶   | 1.69×10⁻⁸     | 3.18×10⁻²       | ANKRBD6          |
| rs150953973  | 6    | 120780033 | 2.98×10⁻⁴   | 9.7×10⁻⁴   | 4.01×10⁻⁶   | 3.54×10⁻⁸     | 2.07×10⁻²       | RN16-214P        |
| rs180271     | 7    | 93539479 | 6.11×10⁻³   | 1.72×10⁻⁴   | 2.63×10⁻³   | 3.69×10⁻⁹     | 5.64×10⁻⁵       | GNT1             |
| rs11989271   | 8    | 122632611 | 1.51×10⁻³   | 8.47×10⁻⁴   | 7.80×10⁻⁷   | 1.14×10⁻⁰     | 7.38×10⁻²       | HAS2             |
| rs10868842   | 9    | 73119085 | 1.57×10⁻³   | 3.58×10⁻⁴   | 9.71×10⁻⁶   | 1.37×10⁻¹     | 2.16×10⁻²       | LINC00583        |
| rs12708143   | 10   | 22808844 | 3.92×10⁻²   | 5.03×10⁻³   | 8.61×10⁻⁸   | 9.18×10⁻¹⁵    | 1.61×10⁻²       | PIPK4A           |
| rs134367     | 12   | 33537387 | 7.19×10⁻⁴   | 2.03×10⁻⁷   | 8.65×10⁻⁵   | 9.56×10⁻¹⁵    | 1.26×10⁻²       | SYT10            |
| rs7322054    | 13   | 38246708 | 6.71×10⁻¹   | 1.99×10⁻⁷   | 7.76×10⁻⁴   | 1.04×10⁻¹⁵    | 8.10×10⁻⁵       | TRPC4            |
| rs61972411   | 13   | 100602630 | 2.82×10⁻⁴   | 6.59×10⁻⁷   | 1.87×10⁻¹⁷  | 1.45×10⁻⁸     | 2.29×10⁻²       | LOC101927437     |
| rs62621400   | 15   | 101718239 | 2.25×10⁻⁵   | 1.70×10⁻⁵   | 1.94×10⁻⁵   | 3.76×10⁻⁵     | 2.12×10⁻²       | CHSY1            |
| rs116643984  | 15   | 101791212 | 1.64×10⁻³   | 7.73×10⁻⁵   | 5.14×10⁻⁷   | 4.04×10⁻¹⁵    | 1.04×10⁻²       | CHSY1            |
| rs73957040   | 18   | 32587133 | 7.08×10⁻⁵   | 2.07×10⁻⁵   | 7.77×10⁻⁸   | 4.67×10⁻⁷     | 2.01×10⁻⁶       | MHLI12A/MHLI12B  |
| rs146822716  | 20   | 35169916 | 6.48×10⁻⁵   | 3.10×10⁻⁵   | 1.61×10⁻⁸   | 2.13×10⁻³     | 9.76×10⁻³       | LOC101926987/MHL9 |

**Table 2: Associations of the BP GRS, PGRS and their interactions with age on blood pressure traits, hypertension and cardiovascular events in unrelated populations in UK Biobank European, African and Asian descents.**
The effect represents regression coefficient for SBP, DBP and PP and represents odd ratio for HTN and CVD.

c The effect for CVD represents the odd ratio when comparing top quantile with bottom quantile of GRS and PGRS.

Table 3. Using mediation variants and pleiotropy variants separately in MR analysis with outcomes: CAD, MI and STROKE

| Outcome | IV type | # of IVs | Causal effect | 95% CI | P | # of PPs | Causal effect | 95% CI | P |
|---------|---------|----------|---------------|--------|---|----------|---------------|--------|---|
| Exposure: SBP | CAD | mediation | 758 | 1.85 | (1.73;1.97) | 3.73×10⁻¹⁰ | 103 | 1.80 | (1.56;2.09) | 2.68×10⁻¹⁰ |
| | MI | mediation | 756 | 1.77 | (1.65;1.90) | 1.60×10⁻⁶ | 89 | 1.72 | (1.42;2.08) | 3.57×10⁻⁶ |
| | stroke | mediation | 758 | 1.66 | (1.55;1.76) | 1.37×10⁻⁹ | 77 | 1.70 | (1.29;2.24) | 1.80×10⁻⁸ |
| | CAD | pleiotropy | 730 | 1.48 | (1.34;1.63) | 2.64×10⁻¹³ | 150 | 1.49 | (1.06;2.10) | 9.02×10⁻⁹ |
| | MI | Pleiotropy | 729 | 1.32 | (1.18;1.47) | 7.11×10⁻⁹ | 125 | 1.43 | (1.09;1.88) | 9.41×10⁻⁹ |
| | stroke | Pleiotropy | 730 | 1.42 | (1.30;1.55) | 1.98×10⁻¹⁴ | 92 | 1.16 | (0.81;1.67) | 6.41×10⁻² |
| Exposure: DBP | CAD | mediation | 774 | 1.85 | (1.74;1.97) | 1.16×10⁻⁸ | 105 | 1.86 | (1.60;2.16) | 5.39×10⁻⁹ |
| | MI | mediation | 771 | 1.85 | (1.73;1.99) | 3.22×10⁻⁹ | 91 | 1.86 | (1.51;2.32) | 5.65×10⁻⁹ |
| | stroke | MI | 773 | 1.67 | (1.57;1.77) | 2.77×10⁻⁸ | 79 | 1.86 | (1.51;2.33) | 8.84×10⁻⁸ |
| | CAD | stroke | 730 | 1.37 | (1.24;1.50) | 4.63×10⁻¹¹ | 157 | 1.34 | (1.02;1.75) | 0.037 |
| | MI | STROKE | 729 | 1.13 | (1.02;1.25) | 0.017 | 134 | 1.31 | (1.05;1.63) | 0.527 |
| | stroke | STROKE | 730 | 1.28 | (1.18;1.39) | 5.07×10⁻⁹ | 90 | 1.13 | (0.80;1.59) | 0.491 |

- # of IVs: number of genetic instrumental variables
- b causal effect represents odd ratio
- c # of PPs: number of pleiotropic variants detected by IMRP.
- d Exposure: SBP: The exposure was SBP for mediation variants as instrumental variables, and the exposure was BP<sub>pleio1</sub> for pleiotropy variants as instrumental variables. (see the definition of BP<sub>pleio1</sub> in Online Methods)
- e Exposure: DBP: The exposure was DBP for mediation variants as instrumental variables, and the exposure was BP<sub>pleio2</sub> for pleiotropv variants as instrumental variables. (see the definition of BP<sub>pleio2</sub> in Online Methods)
- CAD: coronary artery disease
- MI: myocardial infarction

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