Blood pressure and Risk of Cardiovascular disease in UK Biobank: A Mendelian randomization study

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Abstract

This study aims to evaluate the causal association of blood pressure with cardiovascular diseases (CVD). Two-sample Mendelian randomization (MR) was performed using a large genome-wide association study (n = 299,024) and the UK Biobank cohort (n = 375,256). We identified 327 and 364 single nucleotide polymorphisms (SNPs) strongly and independently associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively, as genetic instruments to assess the causal association of blood pressure with total CVD, CVD mortality and 14 cardiovascular conditions. Non-linearity was examined with non-linear instrumental variable assumptions.

Genetically predicted blood pressure was significantly positively associated with total CVD (SBP, per 10mmHg: odds ratio (OR): 1.32; 95% confidence interval (CI): 1.25-1.40; DBP, per 5mmHg: OR: 1.20; 95% CI: 1.15-1.26). Similar positive causal associations were observed for 14 cardiovascular conditions including ischemic heart disease (SBP, per 10 mmHg: OR: 1.33; 95% CI: 1.24-1.41; DBP, per 5 mmHg: odds ratio 1.20; 95% CI: 1.14-1.27) and stroke (SBP, per 10 mmHg: OR: 1.35; 95% CI: 1.24-1.48; DBP, per 5 mmHg: OR: 1.20; 95% CI: 1.12-1.28). Non-linearity MR test demonstrated linear causal association of blood pressure with these outcomes. Consistent estimates were observed in sensitivity analyses, suggesting robustness of the associations and minimal horizontal pleiotropy.

The linear positive causal association of blood pressure and CVD was consistent with previous findings that lower blood pressure is better, thus consolidating clinical knowledge on hypertension management in CVD risk reduction.

Keywords:

Mendelian randomization, systolic blood pressure, diastolic blood pressure, cardiovascular disease

Abbreviations

BP = blood pressure

CVD = cardiovascular disease

DBP = diastolic blood pressure

GWAS = genome-wide association study

IVW = inverse variance weighted

MR = Mendelian randomization

NSTEMI = non-ST-elevation myocardial infarction

SBP = systolic blood pressure

SNP = single nucleotide polymorphism

STEMI = ST-elevation myocardial infarction

Manuscript Text

Introduction

The prevalence and disease burden of hypertension are increasing globally [1]. High blood pressure (BP) is the most common modifiable risk factor for cardiovascular diseases (CVD), and thus several international medical associations and health authorities provide recommended blood pressure targets for hypertension management. The blood pressure target is however controversial given inconsistent findings from randomized controlled trials (RCTs) and observational studies.

The recent finding from the Systolic Blood Pressure Intervention Trial (SPRINT) RCT is in favor of the "lower is better" paradigm, suggesting that the intensive target (systolic blood pressure (SBP) < 120 mmHg) is superior to the current usual target (SBP < 140 mmHg) [2]. The role of diastolic blood pressure (DBP), however, remains unexplored in SPRINT. Other RCTs and observational studies have demonstrated no benefit, or even increased CVD risk in lower blood pressure targets [3-12]. For instance, some observational studies have obtained J- or U-shaped associations of SBP/ DBP with CVD [13-19]. The inconsistency in these findings has been attributed to the susceptibility to biases, such as confounding and selection bias [20-22], and heterogeneity in study design in previous studies. In addition, observational studies on BP treatments give an incomplete estimate of the effect of a long-term exposure to the risk factor, given the relatively short-term BP-lowering treatments [23]. Taken together, the question of whether lower BP is better remains an area of scientific and clinical interest.

In this study, we aim to confirm the causal association of BP with CVD using a two-sample Mendelian randomization (MR) approach. Taking advantage of nature's random assortment of genetic make-up, MR minimizes the effect of confounding and is able to assess causal

relationship. MR studies are known to be less vulnerable to bias than classical observational studies [24], given the non-modifiable and unconfounded nature of genetic variants. Genetic instruments used in MR, which typically affect usual levels of exposures on a long-term basis, also capture lifetime exposure to risk factors, thus reflecting long-term differences. Previous MR studies have explored associations of SBP with coronary heart disease [25] and valvular heart diseases [26]. However, none was on the effect of DBP nor the effects of BP on a wide range of CVD outcomes. This study extends the investigation to both SBP and DBP, with CVD and its subtypes as the outcome of interest. Non-linear MR is also performed to assess the shape of relationship between blood pressure and CVD. While the MR analysis approach might not be able to directly inform the optimal blood pressure target, the prove of causality in a large general population dataset could enhance understanding of optimal blood pressure and CVD control.

Methods

A two-sample Mendelian randomization was performed in our analysis. We obtained genetic associations of SNPs with SBP and DBP from the genome-wide association study (GWAS) by Evangelou et al. [27], and with CVD and its subtypes from the UK Biobank. This MR analysis is reported as per the STROBE-MR guidelines [28]. Data and materials in this study will be made available to other researchers on request to the corresponding author.

Study population

For a two-sample MR analysis, two non-overlapping populations, International Consortium of Blood Pressure Genome Wide Association Studies (ICBP) and the UK Biobank, were used to determine the respective genetic associations with exposure and outcomes.

The ICBP consists of 299,024 individuals of European ancestry from a total of 77 cohorts, with genetic data genotyped with various arrays and imputed to either the 1,000 Genomes Reference

Panel or the HRC platforms. Details of the included cohorts were described in the meta-analysis [29]. The UK Biobank comprises observational and genotyping data of 502,519 people aged between 40-69 years, who were recruited in 2006-2010 and are mainly of British ancestry. Details of the study protocol have been described elsewhere [30-32]. Genetic data were available for 487,409 participants. To minimize population stratification, we restricted our analysis to people of self-reported white British descent with genetically validated British white ancestry. We further excluded participants with missing genotyping rates ≥1%, who had sex aneuploidy, genetic sex disconcordance, or were related to at least one individual (kinship index >0.088) in the final database for analysis. The 3rd version of the imputed genotype data from UK Biobank were used, in which genotypes were imputed with reference to the Haplotype Reference Consortium.

Instrumental variable for BP

Genetic predictors, or single nucleotide polymorphisms (SNPs), of the BP traits were obtained from summary statistics of a large GWAS of blood pressure traits with over 1 million people of European ancestry [27]. The GWAS study included data from the UK Biobank, ICBP, and replication data from other smaller biobanks. Because of the sample overlap between the GWAS and UK Biobank, we obtained genetic associations with BP traits from ICBP meta-analysis only (n = 299,024) for the purpose of a two-sample MR analysis. We included both novel SNPs and confirmed SNPs from previous studies, resulting in a total of 362 and 405 SNPs for SBP and DBP traits respectively. SNPs were filtered for genotype missingness <0.015, minor allele frequency >0.01, and assessed for violation of Hardy-Weinberg equilibrium and linkage disequilibrium in the GWAS [27]. In the GWAS, linkage disequilibrium was estimated for all variants within a 5 mb window downstream and upstream of the reference SNP. All variants in linkage disequilibrium with the reference SNP reaching an $r^2 \geq 0.1$ threshold were identified [27].

Exposure and outcomes

The exposures were genetically predicted SBP and DBP, which were determined using genetic instrumental variables described in the previous section. Blood pressure measurement in studies included in ICBP was described elsewhere [29], whereas measurement in the UK Biobank was described in **Supplementary Methods**. The primary outcome was all cardiovascular disease (CVD) events. Disease outcome definitions were based on International Statistical Classification of Diseases and Related Health Problems (ICD) 9 and 10, and UK Biobank self-reported outcomes (**Table S1** in the **Data Supplement**). Outcomes were defined using algorithmic definitions from UK Biobank or previous studies [2, 33, 34]. We considered all reported cases as binary outcomes. Secondary outcomes were CVD-related mortality and 14 cardiovascular conditions including ischemic heart disease and its subtypes (myocardial infarction, ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), stable angina, unstable angina), stroke and its subtypes (ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage), heart failure, transient ischemic attack, peripheral arterial disease, peripheral vascular disease and arrhythmia and conduction disorders (including atrial fibrillation). Positive and negative controls were hypertension and asthma respectively. All exposure and outcome data from UK Biobank were retrieved on 14th November 2019.

Ethical approval

The UK Biobank received ethics approval from the North West Multi-centre Research Ethics Committee. All participants provided written consent to the study, and any participant who withdrew from the study were removed from our analysis.

Data analysis

Two-sample Mendelian randomization

The Mendelian randomization approach in this study was based on the following assumptions: the selected instrumental variables, i.e. SNPs were robustly associated with BP traits; the instrumental variables were not associated with confounding factors that bias associations of BP with CVD outcomes; and the instrumental variables were associated with the CVD outcomes solely via their association with the modifiable exposures (no horizontal pleiotropy).

The strength of genetic instruments was assessed by calculating the F-statistics [35], where SNPs with an F statistic of <10 were excluded. The total variance explained for all instruments is 5.7% and 5.3% respectively for SBP and DBP [27]. Direct effects of SNPs on the CVD outcomes were determined based on Phenoscanner [36, 37] and Ensembl [38], which provide curated genotype to phenotype cross-references of publicly available large-scale GWAS. Associations of SNPs with confounders age, sex, body mass index (BMI), education, alcohol, smoking, and Townsend index were assessed, and potentially invalid SNPs were excluded from the MR analysis. Sources of selection bias, including BMI and smoking, may violate the instrumental variable assumptions [39], because of the inevitable recruitment into the UK Biobank on survival of genetically predicted BP and the competing risk of the outcome [39, 40]. SNPs with incompatible alleles and palindromic SNPs with minor allele frequencies close to 0.5 were removed from the analysis.

To assess the association of each genetic instrument with CVD, we performed multivariable logistic regression, adjusting for age, sex, BMI, smoking status, genotyping array, assessment center, and the first 10 genetic principal components [27, 39, 40]. Similarly, associations of genetic instruments with other binary outcomes were obtained using multivariable logistic regression.

Two-sample Mendelian randomization using inverse variance weighted (IVW) with multiplicative random effects was performed to produce summary estimates of the effects of BP traits on CVD via BP-associated genetic instruments. IVW assumes uncorrelated and

independent effects from multiple genetic variants [41]. The IVW estimate was obtained by combining the ratio estimates of the causal effect of exposure and outcome using each genetic variant. We used the Bonferroni correction to calculate an adjusted p-value threshold to account for multiple testing. Heterogeneity of effects were assessed by scatter plots of the SNP-exposure and SNP-outcome associations and Cochran's Q test [42]. To assess the pleiotropic effect of the instrumental variables, we used MR-Egger, which gives the Egger intercept. A significant deviation of the intercept from zero indicates possible horizontal pleiotropy. Directional pleiotropy was additionally detected by asymmetry in funnel plot of the MR estimate against its precision. Leave-one-out analysis, performed by leaving each SNP out of the MR analysis in turn, was conducted to assess the sensitivity of each genetic variant [43]. Additionally, multivariable MR was performed with an aim to explore the independent effect of SBP and DBP on CVD [44].

Non-linearity Mendelian randomization

To assess the possible non-linearity of the causal association between SBP/ DBP and CVD, exposure-outcome correlations were evaluated with non-linear instrumental variable assumptions [45]. Weighted genetic risk scores were first calculated for SBP and DBP by summing up the number of effect alleles in each individual, using SNP-exposure associations from ICBP as weights [27]. Using individual data from UK Biobank, participants were stratified into quintiles by the residual SBP/ DBP conditional on the corresponding genetic risk scores. Residual SBP is the difference between SBP and the fitted values obtained from a regression of SBP on the weighted genetic risk score, and likewise for DBP. By considering the residual BP, each strata of participants should have approximately the same BP levels if they have the same genetic composition. MR in each quintile represents the localized average causal effect in each stratum of the population. Localized average causal effect should be consistent across each stratum for a linear shape of relationship. Heterogeneity test by Cochran's Q statistic and

trend test were performed to assess non-linearity of the estimates [45]. An insignificant p-value (p > 0.05) in heterogeneity or trend test suggests no evidence of non-linearity.

Sensitivity analysis

To assess the robustness of the MR results, we performed weighted median [46], MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) [47], MR using Robust Adjusted Profile Score (MR RAPS) [48], and contamination mixture method [49] as sensitivity analysis. Weighted median adopts a majority valid assumption, giving valid estimates when at least half of the weights is derived from valid SNPs. MR-PRESSO relies on the outlier robust assumption and allows the evaluation of horizontal pleiotropy, which could potentially violate the MR assumptions if present. MR RAPS relies on the assumption that pleiotropic effects, except outliers, are normally distributed about zero, whereas the contamination mixture method assumes a plurality of the genetic variants are valid instrumental variables [49].

While two-sample MR has the advantage of reduced weak instrument bias, we further conducted sensitivity analyses using the one-sample MR approach, which is less susceptible to concerns arising from differences between two sample populations. In the one-sample MR sensitivity analysis, data from UK Biobank was used to assess both SNP-exposure and SNP-outcome associations. Details of the one-sample MR analysis was described in **Supplementary Methods**. MR analyses by IVW, weighted median and Egger were performed using R package TwoSampleMR [50] in R software, version 3.6.2. MR-PRESSO, MR RAPS, and the contamination mixture method were performed using R packages MRPRESSO, mr.raps, and MendelianRandomization [51] respectively. All direct analyses on the SNPs were performed using PLINK 2.0 [52]. Considering the possible collider bias due to BMI adjustment in the GWAS by Evangelou et al., an analysis unadjusted for BMI was conducted for comparison. To increase

the validity of the results, positive and negative control outcomes analysis that hypertension and asthma as positive and negative outcomes, respectively, was conducted.

Results

Baseline characteristics of the 375,256 participants of white British ancestry with valid genetic data are displayed in **Table 1**. The mean age of the participants was 57.0 years, and 53.7% were women. Among the included participants, 22.8% reported use of antihypertensive drugs (n = 85,435), and 35.2% were classified as having hypertension. The mean SBP and DBP of the participants were 138.4 mmHg and 82.3 mmHg respectively. 45,647 (12.16%) of the participants had had a cardiovascular event, 36,748 (9.8%) had ischemic heart disease, and 10,785 (2.87%) had stroke.

The two-sample Mendelian randomization estimates for the associations of BP traits and CVD outcomes are reported in **Figure 1**. After exclusion of SNPs with direct associations with the outcomes or confounders, we obtained a final set of 327 SNPs for SBP and 364 SNPs for DBP (**Table S2** in the **Data Supplement**). All included SNPs have F statistics >10, suggesting little weak instrument bias. For SNPs that did not overlap between the exposure and outcome data sources, proxy SNPs were used, as defined by Evangelou et al. [27]. Using the final set of SNPs, we found that each genetically predicted 10 mmHg increase in SBP was associated with an odds ratio (OR) of 1.32 in CVD (95% CI, 1.25-1.40). With the same array of SNPs, we found significantly increased odds in ischemic heart disease and stroke by 1.33 folds (95% CI, 1.24-1.41) and 1.35 folds (95% CI, 1.24-1.48) respectively. We found a similar association of DBP with CVD, in which every genetically associated 5 mmHg increase in DBP was associated with a 1.20-fold increase in odds of having CVD (95% CI, 1.15-1.26). A similar trend could also be observed in the association of DBP with ischemic heart disease (OR, 1.20; 95% CI, 1.14-1.27) as well as DBP and stroke (OR, 1.20; 95% CI, 1.12-1.28). The significant associations of BP

traits with the majority of the assessed CVD-related outcomes. Non-linear MR suggested no evidence of non-linearity on the effect of SBP/ DBP on CVD, with insignificant p-values in heterogeneity tests (**Table S3**, **Figure S1** in the **Data Supplement**). The comparison between mean values of the effects across different strata showed no signs of non-linearity. As displayed, the localized average causal effects of each stratum overlaps, indicating similar exposure effect on CVD across different strata. Similarly, in other CVD subtypes, no evidence of non-linearity was observed in SBP or DBP.

Results from the MR Egger sensitivity analysis were directionally consistent with the IVW estimates, suggesting on average, there are no horizontal pleiotropy effects in our chosen genetic variants for SBP and DBP with CVD or related outcomes. MR-Egger intercepts suggested little evidence of directional pleiotropy in all analyses. Analyses using four other MR approaches, including weighted median, MR-PRESSO, MR RAPS, and contamination mixture, reported significant association of the BP traits with all major CVD outcomes (CVD, CHD, stroke), and are directionally consistent with others (**Figure S2** in the **Data Supplement**). Results from one-sample MR using UK Biobank data were in concordance with our two-sample MR conclusions (**Figure S3** in the **Data Supplement**). As a positive control, we found robust associations of BP with hypertension (SBP: OR, 2.11; 95% CI, 1.95-2.30; DBP: OR, 1.92; 95% CI, 1.82-2.03) (**Figure S4** in the **Data Supplement**). As a negative control, we found no observable association of BP with asthma in the MR analyses, suggesting no causal association. The scatter plot of SNP-exposure and SNP-outcome associations showed that heterogeneity of genetic instruments was balanced at around zero, and suggested no violation due to horizontal pleiotropy, given that the intercept from MR-Egger passes through zero (**Figure S5** in the **Data Supplement**). The MR regression funnel plots appear generally symmetrical, suggesting minimal deviation from pleiotropy (**Figure S6** in the **Data Supplement**). Leave-one-out analysis identified no outlying variants. Conditional F statistics

for multivariable MR were 0.54 for SBP and 0.49 for DBP, which was possibly attributed to the high degree of overlap between SNPs associated with SBP and DBP. The lack of independent variants associated with DBP could result in inadequate power for DBP association and hence null finding in the multivariable MR. Given that adequate joint strength of genetic instruments, as indicated by conditional F statistics > 10, is one of the criteria for reliable multivariable MR [44], caution should be paid to the interpretation of the multivariable MR results (**Table S4** in the **Data Supplement**).

Discussion

This study is the first to examine the causal relationship of genetically predicted BP traits with a wide range of CVD outcomes using MR, with a robust set of >300 SNPs as instrumental variables for each BP trait and data from two large independent cohorts. Our results showed that elevated lifelong BP is causally associated with an increased risk of CVD and related outcomes, with no evidence of non-linearity. This suggests potential benefits in lowering SBP and DBP in the long term to prevent CVD, which is supported by other studies [2, 4, 34, 53-58].

Conversely, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, with a mean follow-up of 4.5 years, showed a greater risk reduction in stroke but not in overall cardiovascular events for SBP treatment target <120 mmHg group compared to <140 mmHg in patients with diabetes [4]. In the Third Heart Outcomes Prevention Evaluation (HOPE-3) trial, cardiovascular benefits were demonstrated in patients with a BP target of <140/ <90 mmHg, but not in those with a lower target of <130/ <80 mmHg [59]. However, the RCT design usually limits sample size and the length of the follow-up period. The ACCORD trial also recognized that the test may be underpowered for detecting the effect of the lower treatment target [4]. Indeed, the findings grouping both ACCORD and SPRINT data revealed a benefit for the lower treatment target [60]. The effect of BP on CVD may also be influenced by different

antihypertensive drug choices and treatment regimens in these RCTs [2, 4, 59]. In addition, lifetime risk should be also considered when evaluating CVD risk [61]. Considering triangulation of the evidence from different designs with different underlying assumptions, our MR study with different large cohorts, analytics and SNP selection offers additional evidence that lifelong BP increases CVD risk.

The MR study design has the advantage of being able to test associations in the presence of unmeasured confounding and selection bias, which are common in observational studies. Lacking randomization, post-hoc observational analyses are open to both confounding and selection bias, hence risk factors for adverse outcomes may differ across groups. The low number of patients achieving the lower BP targets also biased the results from these analyses [13, 15]. The inclusion of only populations with poor health conditions, such as hypertensive patients [62-64] and coronary artery disease patients [19, 65], made these studies vulnerable to reverse causality and selection bias. Given the random assortment of alleles and the non-modifiable nature of genetic variants, MR has the benefit of minimizing confounding and reverse causality over observational studies. The positive causal association in our study demonstrated that the previously observed linear associations of BP with CVD outcomes are unlikely to be biased by residual confounding or reverse causality. Our results further demonstrated that the causal associations of BP with CVD are unlikely to be non-linear.

Our study adopted a two-sample MR approach, in which SNP-exposure association and SNP-outcome association were obtained from two different studies. The use of two independent sets of participants avoids bias from weak instrument, which can bias the results towards the confounded direction in one-sample MR [66]. Nonetheless, results from the one-sample MR sensitivity analysis are in concordance with our main analysis results. The use of multiple genetic variants that was strongly associated with the BP traits enhanced statistical power of the study. Despite the robust study design, the validity of the MR results depends largely on

the satisfaction of the three underlying assumptions; that is, the genetic instruments are strong predictors of the exposure, the instruments are not associated with confounders of BP on CVD, and the instruments affect the outcome solely via the exposure. Our study has addressed the above assumptions by assessing the instrument strength and removing SNPs with potential association with confounders or outcomes from the analysis. In addition to using summary results from published curated databases, we assessed SNP associations with confounders using the UK Biobank individual data. We also restricted samples to participants of white British descent only to reduce confounding due to population stratification. Moreover, we conducted sensitivity analyses using approaches with different underlying assumptions, including methods for checking pleiotropy (MR Egger, MR-PRESSO, MR RAPS), median-based (weighted median) and mode-based methods (contamination mixture method). The similar estimates across all methods indicate credibility of our causal claim [66]. Moreover, our results from the positive and negative control outcomes analyses showed certain validity of the findings.

The limitation of the MR study is that the magnitude of the associations through genetic effects may not be directly translatable to the magnitude of effect of clinical interventions. The difference in effect sizes between MR studies and RCTs is partly attributable to the difference in magnitude of genetic effects and clinical interventions. MR studies primarily serves to assess the causal relationship of exposure on outcomes [25]. While MR analyses are unconfounded for causal effects, a loss of precision in the estimate is a tradeoff for the unconfounded estimation [24]. Although we used one of the largest available sources of genetic associations with CVD, the number of cases of myocardial infarction and stroke subtypes were relatively low, which might explain the wide confidence intervals for these outcomes. Another limitation of this study is that the instruments did not predict SBP and DBP strongly enough independently for multivariable MR to be possible to distinguish between the roles of SBP and DBP. Ideally,

multivariable MR would be the most appropriate approach to explore the independent effect of SBP and DBP on cardiovascular diseases. However, low conditional F statistics suggested that the results may be unreliable due to the overlap between SNPs associated with SBP and DBP. Further study is needed to confirm the role of DBP in the increase in CVD risk independent of SBP. The generalizability of our results to other populations, such as Asians, might also need further investigation. Specifically, significant differences in blood pressure prevalence and control rates based on ethnicity have been reported [67, 68]. It might be useful to investigate the causal associations in populations from different genetic backgrounds. Finally, while our study adds evidence to the causal role of SBP and DBP on the risk of CVD and related outcomes, modification of current clinical practice in hypertension treatment will require multifaceted consideration, given the polygenic nature of hypertension.

Perspectives:

Our Mendelian randomization study demonstrated the effect of long-term differences in BP for risk of major cardiovascular events. Our study provides more evidence of the causal role of SBP and DBP in risk of CVD, CVD mortality and 14 cardiovascular conditions, concurring with the recommendations to lower BP levels in CVD prevention in the general population. While modifications to the clinical approach of the hypertensive patients is based on evidence from multiple perspectives, the prove of association emphasizes the importance of lowering blood pressure levels in CVD prevention in the general population.

Author Contributions

E.Y.F.W. and W.T.F. contributed to the study design and acquisition of data, researched the data, contributed to the statistical analysis and interpretation of the results, and wrote the manuscript. All authors contributed to the interpretation of the results, reviewed and edited

the manuscript. E.Y.F.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Novelty and Significance

What is new?

- The optimal blood pressure treatment target remains controversial, and most previous
  studies focused on evaluation of systolic blood pressure only.

- Using a two-sample Mendelian randomization approach, this analysis assessed the causal role of systolic and diastolic blood pressure on a wide range of cardiovascular outcomes.

What is relevant?

- Our results consolidated recommendations that blood pressure lowering reduces risk of cardiovascular diseases in the general population.

Summary

Positive causal effects of genetically predicted blood pressure on risk of cardiovascular disease and related outcomes were demonstrated using general population data from the UK Biobank.

Figure Legends

Figure 1. (**A**) Association of systolic blood pressure (per 10 mmHg) with cardiovascular outcomes in two-sample Mendelian randomization; (**B**) Association of diastolic blood pressure (per 5 mmHg) with cardiovascular outcomes in two-sample Mendelian randomization. CI = Confidence interval; CVD = Cardiovascular disease; NSTEMI = Non-ST-elevation myocardial infarction; OR = Odds ratio; SNP = Single nucleotide polymorphism; STEMI = ST-elevation myocardial infarction.

† A total of 362 and 405 SNPs associated with systolic and diastolic blood pressure respectively, as identified in genome-wide association study by Evangelou et al.

‡ 327 and 364 SNPs included in the final set for Mendelian randomization analysis for systolic and diastolic blood pressure respectively, after exclusion of SNPs having association with confounders or direct effect on the cardiovascular outcomes, based on public data sources and the UK Biobank data, or with strand issues.

§ Including Atrial fibrillation.

Table 1. Summary characteristics of participants included in the final analysis.

Characteristics	No. (%) All (N = 375,246)	Men (n = 173,744)	Women (n = 201,502)
Age, mean (SD), y	56.97 (7.93)	57.21 (8.03)	56.77 (7.84)
BMI, mean (SD)	27.42 (4.76)	27.85 (4.23)	27.05 (5.14)
Smoking status			
Never	204,208 (54.42)	84,733 (48.77)	119,475 (59.29)
Previous	132,194 (35.23)	68,042 (39.16)	64,152 (31.84)
Current	37,527 (10.00)	20,343 (11.71)	17,184 (8.53)
Prefer not to answer	1,317 (0.35)	626 (0.36)	691 (0.34)
SBP, mean (SD), mm Hg	138.37 (18.59)	141.40 (17.43)	135.74 (19.15)
DBP, mean (SD), mm Hg	82.33 (10.09)	84.20 (9.97)	80.71 (9.92)
Use of hypertensive drugs	85,435 (22.77)	46,235 (26.61)	39,200 (19.45)
Hypertension	131,913 (35.15)	70,037 (40.31)	61,876 (30.71)
Cardiovascular disease	45,647 (12.16)	29,542 (17.00)	16,105 (7.99)
Ischemic heart disease	36,748 (9.79)	24,722 (14.23)	12,026 (5.97)
Stroke	10,785 (2.87)	6,371 (3.67)	4,414 (2.19)

BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure;

SD = standard deviation.