Arteriosclerosis, Thrombosis, and Vascular Biology

TRANSLATIONAL SCIENCES

Association Between Genetic Variation in Blood Pressure and Increased Lifetime Risk of Peripheral Artery Disease

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OBJECTIVE: We aimed to estimate the effect of blood pressure (BP) traits and BP-lowering medications (via genetic proxies) on peripheral artery disease.

APPROACH AND RESULTS: Genome-wide association studies summary statistics were obtained for BP, peripheral artery disease (PAD), and coronary artery disease. Causal effects of BP on PAD were estimated by 2-sample Mendelian randomization using a range of pleiotropy-robust methods. Increased systolic BP (SBP), diastolic BP, mean arterial pressure (MAP), and pulse pressure each significantly increased risk of PAD (SBP odds ratio [OR], 1.20 [1.16–1.25] per 10 mm Hg increase, $P=1\times10^{-24}$; diastolic BP OR, 1.27 [1.18–1.35], $P=4\times10^{-11}$; MAP OR, 1.26 [1.19–1.33], $P=6\times10^{-16}$; pulse pressure OR, 1.31 [1.24–1.39], $P=9\times10^{-23}$). The effects of SBP, diastolic BP, and MAP were greater for coronary artery disease than PAD (SBP ratio of OR [ROR], 1.06 [1.0–1.12], $P=0.04$; MAP ratio of OR, 1.15 [1.06–1.26], $P=8.6\times10^{-4}$; diastolic BP ratio of OR, 1.21 [1.08–1.35], $P=6.9\times10^{-4}$). Considered jointly, both pulse pressure and MAP directly increased risk of PAD (pulse pressure OR, 1.26 [1.17–1.35], $P=3\times10^{-10}$; MAP OR, 1.14 [1.06–1.23], $P=2\times10^{-4}$). The effects of antihypertensive medications were estimated using genetic instruments. SBP-lowering via β-blocker (OR, 0.74 per 10 mm Hg decrease in SBP [95% CI, 0.65–0.84]; $P=5\times10^{-6}$), loop diuretic (OR, 0.66 [0.48–0.91], $P=0.01$), and thiazide diuretic (OR, 0.57 [0.41–0.79], $P=6\times10^{-4}$) associated variants were protective of PAD.

CONCLUSIONS: Higher BP is likely to cause PAD. BP-lowering through β blockers, loop diuretics, and thiazide diuretics (as proxied by genetic variants) was associated with decreased risk of PAD. Future study is needed to clarify the specific mechanisms by which BP influences PAD.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: atherosclerosis ■ blood pressure ■ coronary artery disease ■ peripheral artery disease ■ risk factors

Peripheral artery disease (PAD) is a common manifestation of atherosclerotic cardiovascular disease (ASCVD), estimated to affect >12 million individuals in the United States and >120 million individuals worldwide.[1,2] PAD shares a number of risk factors with other forms ASCVD like coronary artery disease (CAD) and ischemic stroke.[3] These risk factors include smoking, diabetes, hypertension, hyperlipidemia, and obesity.[2–4] Observational studies have identified hypertension as one of the strongest risk factors for incident and prevalent PAD,[5–11] although these studies may be limited by residual environmental confounding or reverse-causality. While randomized controlled trials of antihypertensive medications have demonstrated broad protection from CAD and death from cardiovascular causes, whether lower blood pressure (BP) reduces risk of PAD specifically has not

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Nonstandard Abbreviations and Acronyms

ASCVD	atherosclerotic cardiovascular disease
BP	blood pressure
CAD	coronary artery disease
DBP	diastolic BP
GWAS	genome-wide association studies
MAP	mean arterial pressure
MR	Mendelian randomization
OR	odds ratio
PAD	peripheral artery disease
PP	pulse pressure
SBP	systolic BP

Highlights

- Although peripheral artery disease (PAD) is a common manifestation of atherosclerotic cardiovascular disease, the causal impact of blood pressure on risk of PAD has remained uncertain.
- In this Mendelian randomization study, increases in blood pressure were robustly associated with increased risk of PAD.
- Genetic proxies of several antihypertensive medication classes were associated with decreased risk of PAD.
- Overall, this study provides evidence consistent with a causal association between blood pressure and PAD and prioritizes medications for future studies that consider PAD-specific outcomes.

been reliably established. Similarly, the relative effect of BP on PAD has not been fully investigated.

Recent genome-wide association studies (GWAS) of PAD and BP including >700 000 individuals have identified hundreds of genetic variants associated with these traits.[12,13] The Mendelian randomization (MR) framework (under certain assumptions) can leverage this genetic variation (which is randomly assorted during meiosis, mimicking a randomized trial), to provide unconfounded causal estimates of the relationship between traits.[14] MR assumes that genetic variants are likely to be independent of many confounders of the exposure-outcome relationship. This assumption is plausible because genetic variants are randomly inherited by offspring from parents during meiosis and conception, analogous to treatment allocation in a randomized trial. Because large, randomized trials evaluating the relationship between treatment of hypertension and PAD outcomes may be unfeasible, other study designs are needed to fill this evidence gap. Here, we leverage population-scale genetic variation within the MR framework to (1) establish the relationship between BP and risk of PAD, (2) quantify differences in the effect of BP on CAD and PAD risk, and (3) estimate the effect of BP lowering (using genetic proxies of antihypertensive medications) on PAD risk.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Exposures

Trans-ancestry BP GWAS of systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), and pulse pressure (PP) were obtained from the Pan UK Biobank resource (https://pan.ukbb.broadinstitute.org/). These studies included up to 483 729 participants (262 223 male and 221 506 female; 420 136 European, 8842 Central/South Asian, 6614 African, 2705 East Asian, 1590 Middle Eastern, and 980 American [Hispanic/Latino]). BP measurements included both automated and manual measurements and were adjusted for antihypertensive medication use. Details of genotyping and quality control, as well as links to download summary statistics can be found at https://pan.ukbb.broadinstitute.org/.

Ancestry-specific BP effect estimates were obtained by first identifying genetic variants associated with each BP trait in the trans-ancestry Pan UK Biobank BP GWAS studies, and then extracting the effect estimates and standard errors for the corresponding variants in European and African ancestry-specific analyses. European ancestry-specific BP effect estimates were obtained by first identifying from the 2018 Evangelou et al.[15] International Consortium for Blood Pressure+UK Biobank GWAS, which included measurements of SBP, DBP, and PP in up to 757 601 individuals. Full GWAS summary statistics for the European ancestry BP genome-wide association study are publicly available and may be downloaded from the National Heart, Lung, and Blood Institute GRASP catalog (https://grasp.nhlbi.nih.gov/FullResults.aspx). African ancestry-specific BP effect estimates were obtained from BioVU (Vanderbilt University) and UK Biobank and combined using fixed-effects inverse variance-weighted meta-analysis, including up to 16 784 individuals across both studies (Methods in the Data Supplement).

Study Outcomes

The 2019 Million Veteran Program genome-wide association study of PAD by Klarin et al[12] identified 31 307 PAD cases (24 009 European; 7373 African; and 1925 Hispanic) and 211 753 PAD-free controls. This study defined cases and controls based on electronic health record phenotyping within the Veterans Affairs Healthcare System and was validated against ankle brachial index measurement and manual chart review. The current analyses included trans-ancestry, European, and African-specific GWAS results. Million Veteran Program PAD genome-wide association study summary statistics are available on dbGAP (Accession phs001672.v2.p1).

We considered CAD (as another manifestation of atherosclerosis traditionally associated with elevations in BP) for comparison. GWAS summary statistics for CAD were obtained from the Nikpay et al[13] 2015 CARDIoGRAMplusC4D 1000 genomes-based GWAS. This study was a meta-analysis

including 60 801 CAD cases and 123 504 controls, with genotypes imputed using the 1000 genomes phase 1 version 3 reference. Summary statistics were downloaded from www.cardiogramplusc4d.org/data-downloads/.

Mendelian Randomization

Two-sample MR analyses were performed in R using the *TwoSampleMR* package (https://github.com/MRCIEU/TwoSampleMR).[16] Genetic instruments for BP traits were constructed using variants that were in linkage equilibrium, physically separate (r^2<0.001, distance=10 000 kb; 1000 genomes reference panel), and associated with each trait at genome-wide significance ($P<5\times10^{-8}$). For bidirectional MR analysis, additional instruments were constructed for CAD and PAD using the same procedure. F statistics were calculated for each variant using the formula F=β2/SE^2.The primary MR analyses used inverse-variance weighting with random effects. The MR-Egger intercept test was used to evaluate for evidence of horizontal pleiotropy. Leave-one-out, single-SNP, and funnel-plot diagnostic MR analyses were performed. Sensitivity analyses were performed using MR methods that make different assumptions about the presence of pleiotropy (weighted median, penalized weighted median, and weighted mode).[17] Multivariable MR was used in additional sensitivity analyses to jointly estimate the direct effects of BP traits, again using genetic instruments based on variants that were in linkage equilibrium, physically separate (r^2<0.001, distance=10 000 kb), and associated with any exposure at genome-wide significance ($P<5\times10^{-8}$), weighted by the effect of each SNP on each exposure.[18] MR-Steiger was performed to test the correct direction of effect.[19] Effect estimates were scaled to correspond to a 10 mm Hg change in BP.

Antihypertensive Drug MR

MR analyses were performed to estimate the effect of 10 mm Hg lowering of BP by antihypertensive drugs. Genetic instruments consisted of variants that were associated with each BP trait at genome-wide significance and located near (±200 kb) or within genes encoding protein targets of 12 antihypertensive medication classes, with effect estimates for each genetic variant derived for each BP trait from the trans-ancestry BP GWAS.[20,21] The primary analysis focused on the SBP-lowering effect, with sensitivity analyses considering the remaining BP traits (DBP, PP, MAP). Inverse-variance weighted, weighted median, penalized weighted median, and weighted mode 2-sample MR was performed, with MR-Egger intercept test used to assess for horizontal pleiotropy. For instruments with only 1 variant, Wald-ratio MR was performed.

Statistical Analysis

The primary analysis of the effect of BP on PAD was performed using 2-sample MR considering trans-ancestry BP exposures and trans-ancestry outcomes. We performed 2 additional ancestry-specific sensitivity analyses. First, we performed an analysis considering trans-ancestry BP exposures and ancestry-specific PAD outcomes. Second, due to the lack of genetic variants associated with BP traits as genome-wide significance in African-specific BP GWAS, we also performed a 3-sample MR analysis. Here, genetic variants associated with BP were obtained from the trans-ancestry BP GWAS, with corresponding effect estimates and standard errors obtained from the European- and African-specific BP GWAS. Instruments were then filtered to include only those with F statistic >10 to minimize weak instrument bias.[14] Heterogeneity across ancestries was assessed using I^2 and Cochran Q. The ratio of odds ratios (ROR) was used to compare effects of each BP trait on PAD and CAD.[22] For all analyses we used Bonferroni adjustment for 4 BP traits, with P values <0.05/4=0.0125 considered significant. All statistical analyses were performed using R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Effects of Genetic Variation in BP on PAD: MR

We performed 2-sample MR using summary statistics from trans-ancestry GWAS to estimate the effect of genetic variation in BP traits on PAD. Genetic instruments for BP contained between 259 and 333 independent genetic variants, with F statistics ranging from 29 to 938 (consistent with low risk of weak-instrument bias; Tables I and II in the Data Supplement).

In inverse-variance weighted analyses, each genetically proxied 10 mm Hg increase in SBP, DBP, MAP, and PP significantly increased the risk of PAD (SBP OR, 1.20 [1.16–1.25] per 10 mm Hg increase, $P=1\times10^{-24}$; DBP OR, 1.27 [1.18–1.35], $P=4\times10^{-11}$; MAP OR, 1.26 [1.19–1.33], $P=6\times10^{-16}$; PP OR, 1.31 [1.24–1.39], $P=9\times10^{-23}$; Figure 1 and Table III in the Data Supplement). The MR-Egger bias intercept term was P>0.05 for all trait-outcome pairs (Table III in the Data Supplement). The results remained robust in sensitivity analyses using MR methods that make different assumptions about the presence of pleiotropy (Table III in the Data Supplement). MR-Steiger confirmed the directionality of all associations.

When considering trans-ancestry genetic instruments for BP and ancestry-specific PAD outcomes, SBP, DBP, MAP, and PP were significantly associated with PAD in a European-specific population, while only SBP and PP were associated with PAD in an African-specific population (Figure IA and IB and Tables IV and V in the Data Supplement). In these ancestry-specific analyses, we detected heterogenous effects of BP on PAD across all BP measures (I^2 ranging from 87% to 90%, Cochran P<0.05). Effects remained heterogeneous when considering both ancestry-specific BP effects and ancestry-specific PAD outcomes (Figure 2 and Tables VI and VII in the Data Supplement).

For comparison, we estimated the effects of SBP, DBP, MAP, and PP on CAD. As with PAD, each BP trait was significantly associated with CAD (Tables I through III in the Data Supplement). The effects of SBP, DBP, and MAP were greater for CAD than PAD (SBP ratio of ORs, 1.06 [1.0–1.12], P=0.04; MAP ROR, 1.15 [1.06–1.26], $P=8.6\times10^{-4}$; DBP ROR, 1.21 [1.08–1.35], $P=6.9\times10^{-4}$; Figure 2).

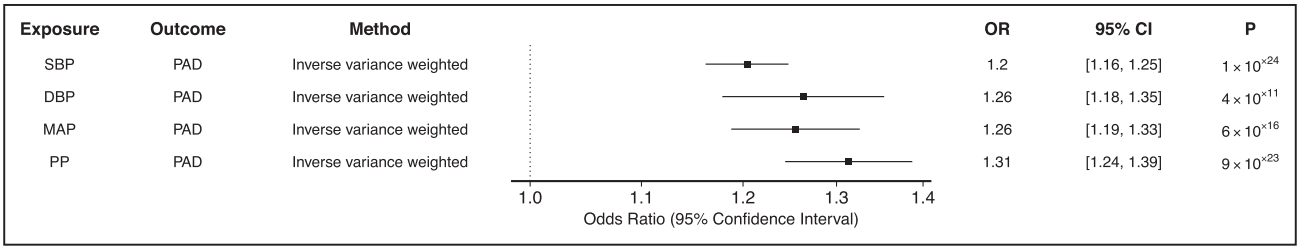

Figure 1. Effect of blood pressure (BP) traits on peripheral artery disease (PAD).
In inverse variance-weighted Mendelian randomization analyses, elevations in each BP trait increased risk of PAD. Results scaled to reflect odds of outcome per 10 mm Hg increase in BP. DBP indicates diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic BP.

Effects of Genetic Liability to PAD on BP: MR

Because stiffening of peripheral vessels may affect BP, the possibility of reverse-causation exists in assessment of the relationship between BP and PAD. To test for the presence of reverse-causation, we next performed bidirectional MR analyses. Genetic instruments for PAD were selected and used to estimate the effect of genetic liability to PAD on BP traits (Figure 3; Tables VIII and IX in the Data Supplement). In inverse-variance weighted analysis, genetic liability to PAD increased SBP (β=0.28 mm Hg per 1 log-odds increase in risk of PAD [0.16–0.41], $P=8\times10^{-4}$), MAP (β=0.13 [0.027–0.24], $P=0.01$), and PP (β=0.22 [0.13–0.32], $P=7\times10^{-6}$). The MR-Egger bias intercept term was $P>0.05$ for all analyses, indicating no positive evidence for bias. Results were consistent in sensitivity analysis applying MR methods making different assumptions about the presence of pleiotropy (Table IX in the Data Supplement). For comparison, genetic liability CAD was not associated with BP traits after accounting for multiple testing (Figure 2 and Tables VIII and IX in the Data Supplement). MR-Steiger confirmed the direction of effect for all associations.

Multivariable MR

Because BP traits are highly correlated and unlikely to affect cardiovascular outcomes in isolation, we performed multivariable MR to jointly estimate the direct effects of BP (as reflected by MAP), and arterial stiffness (as reflected by PP) trait on PAD. Considered jointly, each 10 mm Hg increase in both PP and MAP increased risk of PAD (PP OR, 1.26 [1.17–1.35], $P=3\times10^{-10}$; MAP OR, 1.14 [1.06–1.23], $P=2\times10^{-4}$; Figure III in the Data Supplement).

Antihypertensive Drug MR

In MR analyses designed to proxy the BP-lowering effects of antihypertensive medications, we identified several medications with protective effects on PAD. Genetically proxied SBP-lowering via β-blockers (OR,

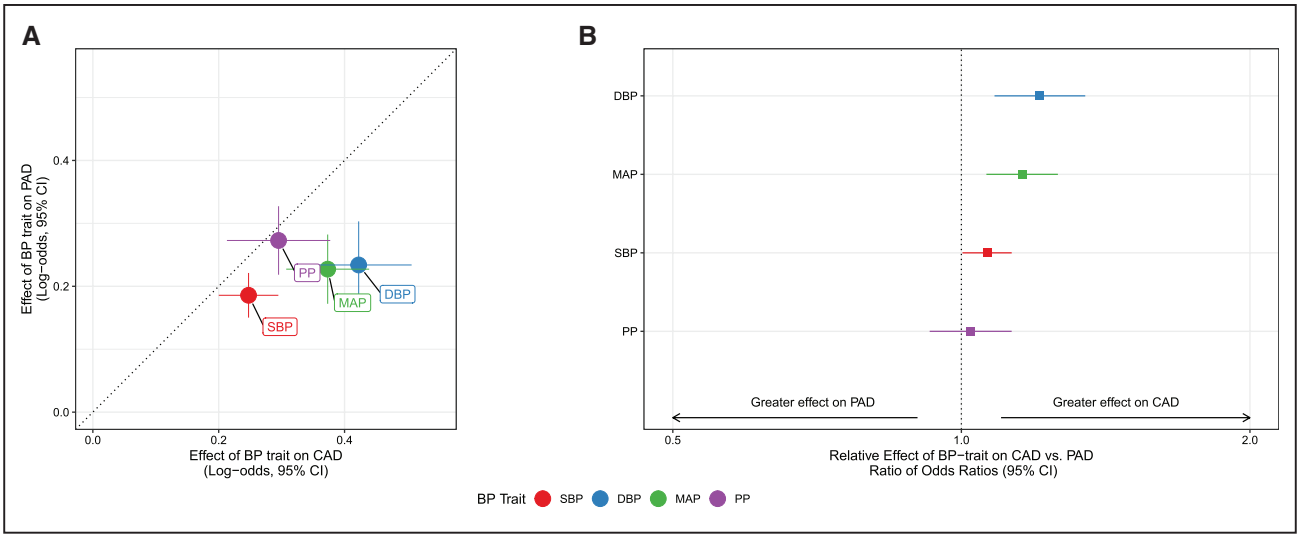

Figure 2. Effects of blood pressure (BP) traits on peripheral artery disease (PAD) vs coronary artery disease (CAD).
Comparison of the effects of BP traits on CAD and PAD. **A**, Log-odds effect estimates for CAD and PAD, with dotted line representing equal effects on both atherosclerotic cardiovascular disease outcomes, and crosshairs representing 95% CIs for each effect estimate. **B**, Comparison of effect of each BP trait on PAD vs CAD using the ratio of odds ratios test, with ratio of odds ratio (ROR) >1 representing greater effect on CAD and ROR <1 representing greater effect on PAD. DBP indicates diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic BP.

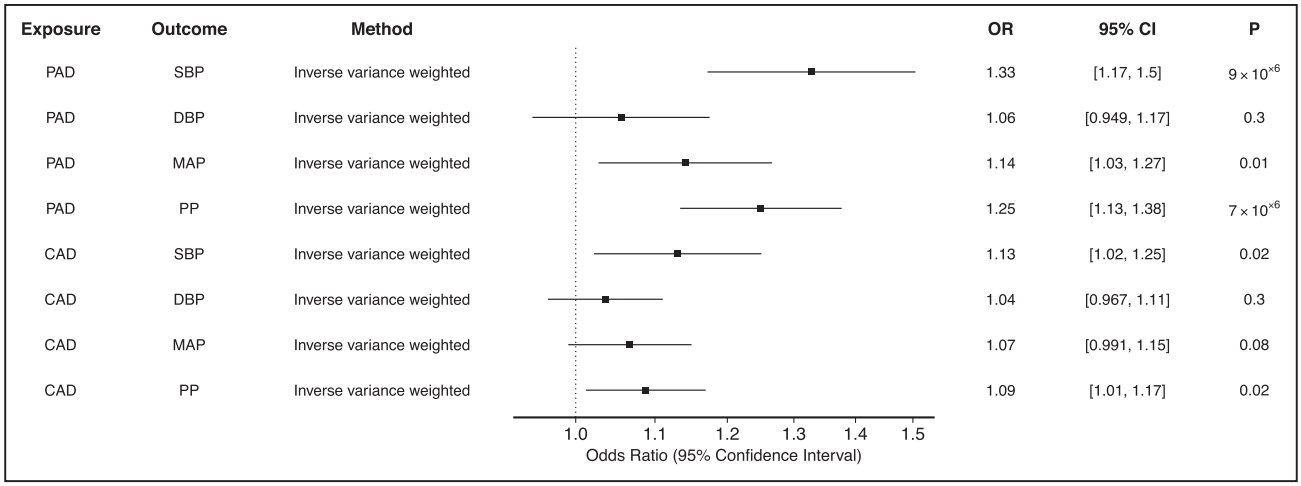

Figure 3. Effects of peripheral artery disease (PAD) and coronary artery disease (CAD) on blood pressure (BP) traits.
The effect of liability to PAD and CAD on each BP trait was estimated using inverse variance-weighted 2-sample Mendelian randomization. DBP indicates diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic BP.

0.74 per 10 mm Hg decrease in SBP; [95% CI, 0.65–0.84]; $P=5\times10^{-6}$), loop diuretics (OR, 0.66 [0.48–0.91], $P=0.01$), and thiazide diuretics (OR, 0.57 [0.41–0.79], $P=6\times10^{-4}$) were associated with decreased risk of PAD (Figure 4; Tables X and XI in the Data Supplement). The MR-Egger bias intercept term was $P>0.05$ for all analyses, indicating no positive evidence for bias. Results were similar using MR methods making different assumptions about the presence of pleiotropy (Tables X and XI in the Data Supplement). When considering genetically proxied DBP and MAP-lowering effects we detected an additional protective association for renin-inhibitors (DBP OR, 0.34 [0.17–0.67], $P=0.001$; MAP OR, 0.52 [0.29–0.91], $P=0.02$; Tables X and XI in the Data Supplement). We did not detect any significant associations between genetically proxied drug effects on PP-lowering and PAD.

DISCUSSION

This MR study leveraged natural genetic variation to examine the relationship between BP and both PAD. The principal findings were (1) lifetime exposure to elevated SBP, DBP, MAP, and PP all increased risk of PAD; (2) elevated BP more strongly increased risk of CAD compared with PAD; (3) PAD led to small but significant increases in SBP, MAP, and PP; and (4) based on genetic proxies, β-blockers, loop diuretics, thiazide diuretics, and renin-inhibitors were associated with decreased PAD risk. There are several implications from the results of this study.

First, this study supports observational findings that elevated BP is associated with increased risk of PAD. Multiple observational studies have identified elevated SBP and clinical diagnosis of hypertension as strong risk factors for PAD, while the relationship between DBP and PAD has remained less clear.[5–11,23–25] Unlike other observational studies, our MR study leveraged genetic variants as instrumental variables for SBP, DBP, MAP, and PP. Because genetic variants are randomly inherited by offspring from their parents, mimicking a trial randomizing individuals to a lifetime of increased BP, the MR framework is less susceptible to residual environmental confounding than traditional observational studies.[14] The

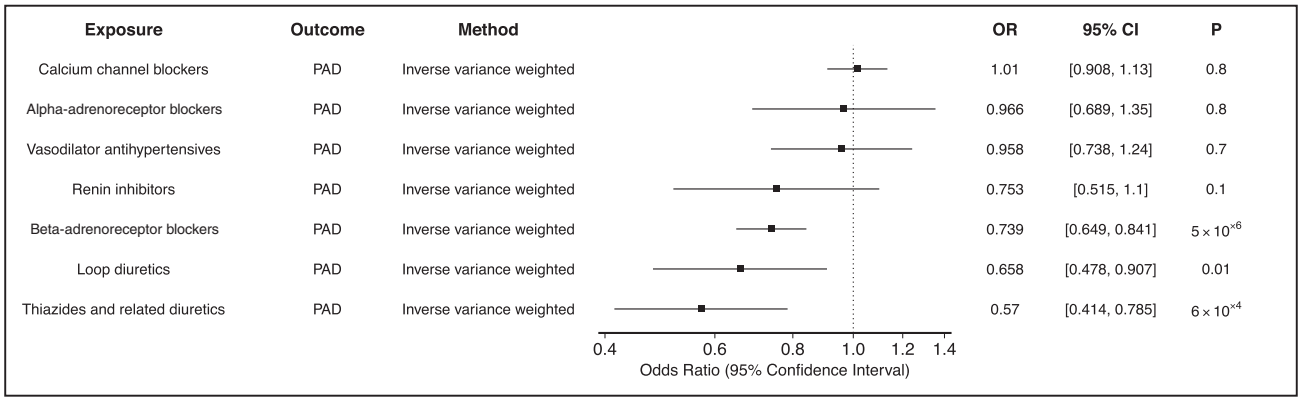

Figure 4. Effect of systolic blood pressure (SBP)-lowering via genetic proxies of antihypertensive medications.
Inverse variance weighted Mendelian randomization was performed to estimate the SBP-lowering effect of genetic proxies of antihypertensive medications on peripheral artery disease (PAD).

finding of our MR analysis that elevated SBP increases risk of both PAD and CAD is consistent with prior studies. We also find a strong effect of DBP on both PAD and CAD, clarifying discrepant findings in prior observational studies. Similarly, our multivariable MR findings demonstrate that both MAP and PP, reflecting pressure and pulsatility/stiffness respectively, both influence PAD risk. Overall, the MR findings of our study are consistent with a causal relationship between BP traits and PAD.

Next, we found that elevated SBP, DBP, and MAP each increased risk of CAD more than PAD. These findings are in contrast to a prior observational analysis that found that SBP or DBP had similar effects on CAD and PAD.[7] While broad recommendations for lifestyle modification and treatment of ASCVD risk factors are clearly important at both the population level and individual level, understanding the impact of interventions on specific ASCVD outcomes may further inform treatment and prevention guidelines and discussions with patients. Particularly in light of our recent finding that smoking more strongly increases risk of PAD in comparison to CAD or ischemic stroke,[26] this study adds further nuance to the relationship between traditional ASCVD risk factors and specific ASCVD outcomes.

Our finding that increased PP increases PAD risk is consistent with findings from multiple prior observational studies.[27–30] Because increased PP is a marker of increased arterial stiffness and may be caused by PAD, the observational studies investigating the relationship between these traits may have been limited by the possibility of reverse causality. Using bidirectional MR, we were able to overcome this limitation, finding elevated PP to be a risk factor for PAD, and PAD to be a risk factor for increased PP. Similarly, when jointly considering MAP and PP we detected direct effects of both traits on PAD, suggesting that both arterial pressure (as measured by MAP) and arterial stiffness (as reflected by PP) directly influence development of PAD. Further study is warranted to determine the specific mechanisms by which these traits impact PAD, though our findings suggest interventions targeting both traits may be useful in reducing the burden of PAD.

Finally, we used antihypertensive drug MR to estimate the effect of 10 mm Hg lowering of BP by different classes of medication. In this analysis, we identified protective effects of several antihypertensive medications. A Cochrane Review found poor evidence for the use of antihypertensive medications specifically for PAD, though recognized the large benefit of these medications for prevention of cardiovascular events and mortality more broadly.[31] Our results provide a genetic basis for considering future trials focused on β-blockers, loop diuretics, and thiazide diuretics. While small beneficial genetic effects may compound over a lifetime leading to protection from ASCVD, the effects of antihypertensive medications occur on a much shorter timescale. Our findings do not exclude meaningful beneficial effects of other potent antihypertensive medications on risk of PAD, particularly given the strong overall causal effects of each BP trait on PAD. The optimal antihypertensive regimens for prevention/treatment of PAD remains unclear and may represent a focus for future effectiveness studies.

The overall findings of our study have implications for PAD prevention and treatment guidelines. The current 2016 American Heart Association/American College of Cardiology and 2017 European Society of Cardiology PAD guidelines make strong recommendations for the treatment of hypertension to prevent cardiovascular events.[3,4] The trials cited to support these recommendations focused on cardiovascular events broadly, or differences in safety and efficacy between different antihypertensive classes, rather than PAD-specific outcomes.[32–40] Our MR study provides strong evidence consistent with a casual effect of increased BP on PAD. In the absence of large, randomized trials of antihypertensive medications focused on PAD-specific outcomes, these results add support for current guideline recommendations, and suggest possible medication classes that warrant further study specifically for PAD. Recent studies like SPRINT and multi-society BP guidelines have suggested that aggressive BP-lowering may be associated with improved outcomes in individuals at high ASCVD risk.[41,42] While our current analyses do not provide a specific BP-lowering target that minimizes risk of PAD, future analyses leveraging participant-level data may help identify treatment thresholds. Our current results may help calibrate the overall expected benefit that programs to treat hypertension may have on the global burden of PAD.

This study has several limitations. Although we considered trans-ancestry studies of BP and PAD, the underlying populations were primarily composed of individuals of European ancestry. We performed extensive ancestry-specific analyses, identifying heterogenous effects of BP on PAD. Whether these findings reflect biological differences in the pathogenesis of elevated BP and PAD that vary by ancestry or reflect limitations of our current understanding of the genetic basis of these traits remains uncertain. Further study of BP and ASCVD genetics in diverse ancestral populations is necessary to improve the generalizability of our findings. Similarly, the stronger associations between BP traits and CAD in comparison to PAD may reflect pathophysiological differences in the risk factors for atherosclerosis across diverse vascular beds or may be due to differences in sampling or ascertainment of the underlying PAD and CAD GWAS studies. MR relies on a number of assumptions in order for causal estimates to be valid.[14] While we have employed multiple MR methods and sensitivity analyses to assess for and address potential violations of these assumptions, we cannot completely exclude the

possibility of confounding. Future study on the role of hypertension treatment in the prevention and treatment of PAD focused on PAD-specific outcomes is warranted.

Overall, we find strong evidence consistent with a causal effect of BP traits on PAD, although find a stronger effect of SBP, DBP, and MAP on CAD in comparison to PAD. We identify genetic proxies of antihypertensive medications associated with decreased PAD risk, which may be prioritized for future study.

ARTICLE INFORMATION

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Disclosures

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