Mendelian Randomization

Association of Cardiovascular Risk Factors and Lifestyle Behaviors With Hypertension
A Mendelian Randomization Study

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Abstract—Hypertension is a major risk factor for cardiovascular disease and mortality. To identify targets for the prevention of hypertension and its associated disease burden, we used the 2-sample Mendelian randomization method to investigate the causal associations of 18 cardiovascular risk factors and lifestyle behaviors with hypertension. From European-descent genome-wide association studies, we selected genetic variants (\(P<5\times10^{-8}\)) for type 2 diabetes, fasting glucose, lipids, body mass index, smoking, alcohol and coffee consumption, physical activity, sleep duration, insomnia, and educational level. We extracted the genetic associations with hypertension from 2 European cohorts: the FinnGen Study (15 870 cases and 74 345 controls) and UK Biobank (54 358 cases and 408 652 controls). The inverse-variance weighted method was used as main analysis method. Genetically predicted triglycerides (pooled odds ratio [OR] per 1 SD, 1.17 [1.10–1.25]), body mass index (OR per 1 SD, 1.42 [1.37–1.48]), alcohol dependence (OR, 1.10 [1.06–1.13]), and insomnia (OR, 1.17 [1.13–1.20]) were associated with a higher odds of hypertension. Higher genetically predicted high-density lipoprotein cholesterol (OR per 1 SD, 0.88 [0.83–0.94]) and educational level (OR per 1 SD, 0.56 [0.54–0.59]) were associated with a lower odds of hypertension. Suggestive evidence was obtained for type 2 diabetes, smoking initiation and alcohol consumption with a higher hypertension odds, and longer sleep duration with a lower hypertension odds. This Mendelian randomization study identified high-density lipoprotein cholesterol, triglycerides, body mass index, alcohol dependence, insomnia, and educational level as causal risk factors for hypertension. This implicates that these modifiable risk factors are important targets in the prevention of hypertension. (\textit{Hypertension}, 2020;76:1971-1979. DOI: 10.1161/HYPERTENSIONAHA.120.15761.) \textbf{Data Supplement}

Key Words: education, hypertension, lifestyle, risk factors

The prevalence of high blood pressure has increased over the past decades.\textsuperscript{1} Hypertension is a major risk factor for cardiovascular disease (CVD)\textsuperscript{2} and typically coexists with other CVD risk factors and unhealthy lifestyle behaviors, such as smoking, diabetes, dyslipidemia, overweight, physical inactivity, and unhealthy diet.\textsuperscript{3} From randomized controlled trials, we have learned that weight reduction,\textsuperscript{4} reduction of alcohol intake,\textsuperscript{4} and exercise training\textsuperscript{5} lower blood pressure and are causally related to hypertension. For other risk factors, it remains unclear whether they have a causal role in the pathophysiology of hypertension or merely are a consequence of a shared risk factor profile, because the majority of evidence originates from observational studies,\textsuperscript{6–21} which cannot be used to infer causality because of confounding and reverse causation bias. A better understanding of which risk factors play a causal role will contribute to the identification of potential additional targets in the prevention of hypertension and eventually CVD and mortality.

The Mendelian randomization (MR) design is a genetic instrumental variable analysis with observational data that can be used to address causal hypotheses because this design is less vulnerable to confounding and reverse causation bias.\textsuperscript{22} The MR design has been previously used to study the association of certain CVD risk factors and lifestyle behaviors, including lipids,\textsuperscript{23} body mass index (BMI),\textsuperscript{24–26} smoking,\textsuperscript{27} alcohol consumption,\textsuperscript{28,29} physical activity,\textsuperscript{30} sleep duration,\textsuperscript{13,31} and educational level,\textsuperscript{32} with risk of hypertension. For the majority of these risk factors, larger genome-wide association studies...
Methods

Two-Sample MR Design

In this study, we used the 2-sample MR design.22 MR is a genetic instrumental variable analysis that uses single nucleotide polymorphisms (SNPs) as instrumental variables for the risk factors of interest. SNPs are randomly allocated at meiosis and can therefore not be influenced by reverse causation bias. By exclusion of SNPs (1) associated with confounders of the risk factor-outcome association or (2) that influence the outcome via another pathway than through the exposure, confounding bias can be limited. This makes it possible to test causal hypotheses using the MR design. All data used in this MR study are publicly available. Information on how to access these data can be found in the below cited GWAS articles.

Data Sources for and Selection of Genetic Instruments

We used GWASs conducted primarily among individuals of European ancestry as data sources for the genetic instruments of the modifiable risk factors. An overview of these data sources has been provided in the Table. We selected SNPs as instrument if associated with the risk factors at the genome-wide significance level ($P<5\times10^{-8}$). If SNPs were in linkage disequilibrium ($r^2>0.1$), we included the SNP with the strongest correlation with the risk factor. Finally, we excluded SNPs that were not available in the hypertension GWASs. This led to an inclusion of 278,292 SNPs (Finngen/UK Biobank) for type 2 diabetes,37 35/35 SNPs for fasting glucose,39 54/54 SNPs for low-density lipoprotein cholesterol,45 65/67 SNPs for high-density lipoprotein cholesterol (HDL-C),40 35/36 SNPs for triglycerides,41 812/832 SNPs for BMI,43 339/357 SNPs for smoking initiation,44 2/3 SNPs for BMI,45 21/23 SNPs for insomnia,46 7/7 SNPs for overall sleep duration,43 26/27 SNPs for short sleep duration (<7 versus 7–8 hours/day),43 7/7 SNPs for long sleep duration (≥9 versus 7–8 hours/day),43 and 1084/1138 SNPs for educational level11 (Figure 1). The phenotypic variance explained by the genetic instruments varied from 0.073% for moderate-to-vigorous physical activity to 16.3% for type 2 diabetes (Table).

Data Sources for Hypertension

We extracted the genetic associations of the instrumental variables with hypertension from 2 European cohorts: the FinnGen Study (second release) and UK Biobank.

The FinnGen Study is a Finnish, nationwide GWAS meta-analysis of 13 cohorts and biobanks,44 which has no to very limited overlap with the exposure GWASs (Table). The cohorts have been linked with longitudinal digital health record data from nationwide health registries. The FinnGen Study included 15870 individuals with hypertension, defined as the presence of essential (primary) hypertension and 74,345 individuals without essential hypertension or any other hypertensive diseases. The International Classification of Diseases diagnosis codes used to define essential hypertension have been provided in Table S1 in the Data Supplement. The GWAS was adjusted for age, sex, 10 principal components, and genotyping batch.

UK Biobank is a cohort study that included over 500,000 men and women from the UK general population between 2006 and 2010.46 Information on primary hypertension was obtained from discharge registries using the secondary International Classification of Diseases 10 diagnosis code 110—Essential (primary) hypertension. UK Biobank included 54,358 cases with primary hypertension and 408,652 controls. We accessed the data through the MR-Base platform (UKB-b:12493).46 Several exposure GWASs had substantial or full overlap with UK Biobank (Table). In one of the sensitivity analyses, we used data on self-reported hypertension (199,731 cases and 343,202 controls) from UK Biobank (UKB-b:14057).46

Statistical Analyses

For the principal analyses, the inverse-variance weighted (IVW) method under a multiplicative random-effects model was used in each cohort separately.22 This method combines the Wald ratio estimates of each individual SNP ($\beta_{SNP-outcome} / \beta_{SNP-risk~factor}$) into one causal estimate for each risk factor. The causal estimates from both cohorts were pooled using fixed-effect meta-analysis.

Since the IVW estimates might be affected by invalid instrument bias or pleiotropy, we tested the validity and robustness of the results by conducting several sensitivity analyses. The sensitivity analyses were performed in each cohort separately. First, the weighted median method was used to check invalid instrument bias.23 This method provides a consistent estimate if over 50% of the weight in the meta-analysis has been derived from valid SNPs. Second, the MR pleiotropy residual sum and outlier method was used to identify outlying SNPs that are potentially horizontally pleiotropic and to check whether exclusion of the outlying SNPs changed the causal estimate. Third, MR-Egger was used to identify potential directional pleiotropy. Fourth, as there is genetic correlation between the different lipid traits, we used multivariable MR analyses to adjust for the genetic correlation with the other 2 lipid traits. Fifth, we explored the role of BMI in the association between genetically predicted type 2 diabetes and hypertension using the genetic association estimates from the genome-wide significant SNPs in the GWAS on type 2 diabetes adjusted for BMI (175 SNPs in FinnGen and 181 SNPs in UK Biobank).47 Finally, for the complementary analyses, the IVW analyses were repeated with self-reported hypertension data from UK Biobank.

The statistical analyses were conducted using RStudio (Version 1.2.5019) with R packages MendelianRandomization, MR pleiotropy residual sum and outlier, TwoSampleMR, and metafor. Results were reported as odds ratio (OR) with corresponding 95% CIs. To account for multiple testing in our principal analyses, we used a Bonferroni-corrected significance level of $P<2.78\times10^{-3}$ (0.05 divided by 18 risk factors). $P$ value between 2.78$x10^{-3}$ and 0.05 were considered as potential associations.

Results

Cardiovascular and Lifestyle-Related Risk Factors and Hypertension: Principal Results

A significantly higher OR of essential hypertension was observed for the following 6 modifiable risk factors in the pooled analysis (Figure 2): genetically predicted type 2 diabetes (pooled OR, 1.12 [95% CI, 1.09–1.14]), triglycerides (OR per 1 SD increase, 1.17 [1.10–1.25]), BMI (OR per 1 SD increase, 1.42 [1.37–1.48]), smoking initiation (OR, 1.24 [1.18–1.31]), alcohol dependence (OR, 1.10 [1.06–1.13]), and insomnia (OR, 1.17 [1.13–1.20]). A higher genetically predicted HDL-C (OR per 1 SD increase, 0.88 [0.83–0.94]) and educational level (OR per 1 SD increase, 0.56 [0.54–0.59]) were associated with a lower odds of hypertension. Potential evidence was obtained for higher genetically predicted alcohol consumption (OR per 1 SD increase in log-transformed alcoholic drinks/week, 1.28 [1.07–1.52]) and short sleep duration (OR, <7 versus 7–8 hours/day, 1.17 [1.03–1.34]) with a higher odds of hypertension and genetically predicted longer sleep duration with...
a lower odds of hypertension (OR per 1 hour/day increase, 0.84 [0.72–0.98]). No significant associations were observed for genetically higher fasting glucose, low-density lipoprotein cholesterol, smoking heaviness, coffee consumption, moderate-to-vigorous physical activity, and sedentary behavior (Figure 2). Significant heterogeneity in the results from FinnGen and UK Biobank was only present in the meta-analysis on insomnia ($I^2=80.6\%$; $P=0.023$).
Robustness of the Results

The MR-Egger regression findings for type 2 diabetes and BMI indicated potential pleiotropy, as the causal estimates attenuated compared with the IVW analyses and the intercepts were significantly larger than zero (Table S2). However, the results of the other sensitivity analyses to investigate potential pleiotropy were consistent, except that the association between type 2 diabetes and hypertension attenuated in the weighted median analysis in both FinnGen and UK Biobank. This association between genetic liability to type 2 diabetes and hypertension also partly attenuated compared with the IVW analysis when SNPs for type 2 diabetes were adjusted for BMI (FinnGen: OR, 1.06 [1.02–1.10], \( P: 5.44 \times 10^{-3} \) and UK Biobank: OR, 1.09 [1.07–1.12], \( P: 1.10 \times 10^{-12} \)). Although the pooled analysis showed no significant association between smoking heaviness and hypertension, the IVW and MR pleiotropy residual sum and outlier analysis revealed a potential association between higher genetically predicted number of cigarettes and a higher odds of hypertension in UK Biobank, while the MR-Egger regression in
FinnGen revealed a potential protective association (Table S2). The findings for the other modifiable risk factors were robust in all sensitivity analyses (Table S2). After adjustment for the genetic overlap in the lipid traits, the association between HDL-C and hypertension attenuated (OR per 1 SD increase, 0.99 [0.88–1.11] in FinnGen and 0.93 [0.85–1.01] in UK Biobank), but the findings for triglycerides and low-density lipoprotein cholesterol remained (Table S3). In the sensitivity analysis with self-reported hypertension in UK Biobank as outcome, similar risk factors were significantly associated as in our main analyses (Table S4).

Discussion

In this MR study on modifiable cardiovascular and lifestyle-related risk factors of hypertension, we found that genetically predicted type 2 diabetes, triglycerides, BMI, smoking initiation, alcohol dependence, and insomnia were associated with a higher hypertension risk, and that higher genetically predicted HDL-C and educational level were associated with a lower hypertension risk. Furthermore, we found potential associations of genetically predicted higher alcohol consumption with increased risk of hypertension and longer sleep duration with a lower risk of hypertension. For the other modifiable risk factors, there was insufficient evidence for a causal relationship with hypertension.

To draw conclusions about causality from MR studies, it is important to determine whether potential violations of the MR assumptions might have introduced bias and to assess whether the MR results are similar to the observational literature.48 Our observed associations of HDL-C, triglycerides, BMI, and educational level with hypertension are likely to be causal considering the robust results in a wide variety of sensitivity analyses to test the MR assumptions. Moreover, our findings are consistent with previous prospective observational studies7,12–15,49 and MR studies.23–26,32 Likewise, our observed association between insomnia and hypertension is probably causal, as most sensitivity analyses were consistent and showed no indication of a violation of the MR assumptions. In addition, the findings are in line with results from prospective observational studies.19

The observed associations of genetic predisposition to alcohol dependence and higher alcohol consumption with an increased hypertension risk are in line with previous randomized intervention studies4 and MR studies.50 Previous prospective observational studies have suggested a potential nonlinear association between alcohol consumption and...
hypertension risk in women, with moderate drinkers having no increased risk compared with abstainers. As our MR study was not designed to reveal nonlinear associations, we can only conclude that excessive alcohol consumption is likely to be a causal risk factor for hypertension.

The observed association between genetic liability to type 2 diabetes and hypertension risk was potentially biased by pleiotropy according to the sensitivity analyses. In addition, BMI partly explained this association. It is not completely clear whether the association that remained after adjustment for BMI represents an independent causal effect of type 2 diabetes on hypertension risk. However, previous prospective observational studies\(^5\) and a previous MR study\(^6\) found similar evidence for a harmful effect.

The observational literature has been inconsistent about the association between smoking and incident hypertension. Two previous prospective observational studies reported an increased hypertension risk in current smokers but not in former smokers. Another observational study found an increase in blood pressure following smoking cessation,\(^5\) but this association might be driven by residual confounding related to weight gain. In our MR study, we found an association between smoking initiation (ever smoked regularly) and increased hypertension risk, a finding that is in line with a previous MR study on smoking and arterial hypertension in UK Biobank,\(^4\) but not with another MR study that did not observe an association between 2 smoking-increasing alleles and hypertension.\(^6\) Despite the uncertainty about the evidence for smoking and hypertension, smoking should still be discouraged as the total body of evidence robustly indicates that smoking is a strong risk factor for CVD.\(^4\)

The potential association between longer sleep duration and lower hypertension risk is supported by previous prospective observational studies.\(^6\) However, this association was only observed in UK Biobank. UK Biobank had a larger sample size than FinnGen but also had a full sample overlap with the sleep duration GWAS. The latter might have increased the type I error rate.\(^5\) Thus, it is not possible to draw conclusions on the causal role of sleep duration in the development of hypertension.

The null findings for fasting glucose, low-density lipoprotein cholesterol, coffee consumption, and physical activity might suggest that the associations found in observational studies\(^11,14,17,18,56\) reflect confounding or reverse causation bias. This could especially be the case for coffee consumption, which has less biological plausibility for a causal association with hypertension. On the other hand, it is possible that our null findings were a consequence of insufficient power because of the relatively low variance explained for some of these risk factors. The latter explanation is supported by the lack of significant association with physical activity, for which randomized intervention studies have shown that different types of exercise training programs lower blood pressure compared with sedentary controls\(^3\) and for which the prospective observational literature consistently supports this association.\(^14\) Taken together, it is not possible to make any firm conclusions on the causal role of these modifiable risk factors in hypertension based on our MR analysis.

Underlying Mechanisms
Hypertension is characterized by a multifactorial and complex pathophysiology. Factors contributing to high blood pressure vary from obesity, high-salt intake, and insulin resistance to disparities in the renin-angiotensin and the sympathetic nervous system. The modifiable risk factors we identified as causal factors for hypertension in this MR study might interfere with variety of these pathophysiological processes. For example, obesity is thought to increase the activity of the sympathetic nervous system and to dysregulate the renin-angiotensin system, which both have an important role in blood pressure regulation.\(^8\) High triglyceride levels have been associated with endothelial dysfunction and increased arterial stiffness,\(^58,60\) whereas high HDL-C levels have been inversely related to arterial stiffness.\(^8\) Furthermore, there are several factors that link insomnia to hypertension, such as increased sympathetic nervous system activity, elevated heart rate, and abnormal hormone secretion.\(^9\) The beneficial effect of higher educational level on hypertension risk could be because of, among others, more resources to maintain a healthy lifestyle and to access health care.

Clinical Implications
Modification of the cardiovascular and lifestyle-related risk factors we identified in this study will contribute to the prevention of hypertension and, eventually, of the hypertension-associated disease burden and mortality. We now have an improved understanding of which risk factors play a causal role. This should be used to inform cardiovascular prevention guidelines and governmental policy makers. For example, our MR study further strengthens the current evidence for a causal role of insomnia in the pathophysiology of hypertension, which implicates that sleep should get a more prominent role as lifestyle factor affecting CVD risk in current guidelines.\(^62\)

Strengths and Limitations
A strength of this study was the use of the MR design, which minimizes residual confounding and reverse causation bias, to investigate the associations of a variety of modifiable risk factors with hypertension risk. Furthermore, there was no or very limited overlap in the exposure GWASs and FinnGen to keep the type I error rate as low as possible (Table), while UK Biobank has a large sample size to maximize statistical power. The hypertension diagnosis in the outcome GWASs was obtained through linkage with high-quality, national health registry data, which reduced selection bias because of loss to follow-up.

It is important to acknowledge that we were not able to draw conclusions on causality for certain modifiable risk factors because of limited precision as a result of the small variance explained by the genetic instruments (Table). Moreover, as people had to survive to a certain age to be included in the GWASs, the effect estimates might be distorted in either direction by survivor bias. Yet, we think that this potential survivor bias did not lead to a reversal of the effects, because the FinnGen GWAS included participants from different age groups and our major findings had relatively large effect sizes. Finally, although our results were robust in 2 different European populations, the results are less generalizable to populations of non-European ancestry. However, this restriction reduced bias from population stratification.
Conclusions
This MR study identified HDL-C, triglycerides, BMI, alcohol dependence, insomnia, and educational level as causal risk factors for hypertension. This implicates that these modifiable risk factors are important targets in the prevention of hypertension and its associated disease burden. For other modifiable risk factors, the evidence is insufficient to draw conclusions on causality.

Perspectives
Cardiovascular risk factors and lifestyle behaviors are amenable to modification and may therefore be relevant targets in the prevention of hypertension. For some modifiable risk factors, it is unclear whether they are causally related to hypertension. This MR study found that HDL-C, triglycerides, BMI, alcohol dependence, insomnia, and educational level are causal risk factors of hypertension. This improved understanding of the pathophysiology of hypertension can be used to identify additional targets for the prevention of hypertension and its association diseases.

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Disclosures
None.

References

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**Novelty and Significance**

**What Is New?**

- Cardiovascular risk factors and lifestyle behaviors are amenable to modification and may therefore be relevant targets in the prevention of hypertension.
- This Mendelian randomization study investigated the causal role of 18 cardiovascular risk factors and lifestyle behaviors in primary hypertension risk.

**What Is Relevant?**

- This study improved the understanding of the pathophysiology of hypertension.

**Summary**

- The findings can be used to identify additional targets for the prevention of hypertension and its association diseases.

- High-density lipoprotein cholesterol, triglycerides, body mass index, alcohol dependence, insomnia, and educational level were identified as causal risk factors for hypertension.
- The potential causal role of type 2 diabetes, smoking, alcohol consumption, and sleep duration requires further study in future large Mendelian randomization analyses.