Original Article

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Causal relationship between hypertension and ischemic stroke: A two-sample Mendelian randomization study

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Abstract:

BACKGROUND: Ischemic stroke (IS) is a well-recognized risk factor for human health and has become a major cause of the global burden of disease over the past decades. Determining the correlation between hypertension and IS is important for the prevention of IS. In epidemiologic studies, researches have reported a strong association between hypertension and IS. However, there is a great deal of heterogeneity between these findings, and the strength of the two associations shows very different results in international studies. Here, we used genetic data to methodically assess the association between hypertension and the risk of IS using a Mendelian randomization (MR) framework. This study may provide a more comprehensive theoretical basis for the link between hypertension and IS.

METHODS: We studied three hypertension traits including essential hypertension, gestational hypertension, and preexisting hypertension, in a two-sample MR method. Genetic susceptibility to each type of hypertension was explored for the association with the risk of small-vessel IS in data from the IEU-POENGWAS.

RESULTS: We observed a strong association between essential hypertension with small-vessel IS. Our evidence from data-driven analyses further suggests that genetic susceptibility to gestational hypertension and preexisting hypertension are associated with the development of small-vessel IS. However, in multivariate analyses, these associations would be explained by congenital hypertension.

CONCLUSIONS: Through our study, we further validated that hypertension is an individual risk factor for IS, with the risk of small-vessel IS increasing approximately 6-fold for every one standard deviation increase in essential hypertension.

Keywords:

Hypertension, ischemic stroke, Mendelian randomization

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Introduction

Context

Hypertension is a widely recognized risk factor for human health and has become a leading contributor to the burden of disease worldwide over the past decades, increased risk of heart disease, strokes, kidney disease, and many other health problems.[1-5] Ischemic stroke (IS) is a disorder of local blood supply and blood flow to brain tissue caused by various cerebrovascular lesions and is the important cause of death worldwide.[6-9] Epidemiologic studies and basic medical research have reported a strong association between hypertension and IS.[10-13] However, there is significant heterogeneity between the findings, with markedly different results for the strength of the two links in international studies.[14] A number of studies have stratified and synthesized their analyses by type of IS, and the results suggest that the relationship

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between different types of hypertension and IS varies. This is often due to the difficulty of observational research methods in better controlling for the effects of important confounding factors such as age, gender, and grouping patterns on the results of the study, and thus tends to bias the results.[15-17]

Aims

The Mendelian randomization (MR) method is an epidemiologic practice that uses genetic variance in nonexperimental data to estimate the causal relationship between exposures and outcomes, which can overcome the effects of reverse causation and confounding in traditional methods.[18-25] In MR, the use of genes as instrumental variables (IV) is according to Mendel's second law, which states that the transmission of a trait is independent of the inheritance of other traits and exhibits stochasticity so that the genotypes of the offspring are unlikely to be related to environmental confounding factors in the population, thus avoiding reverse causation.[26-29] Therefore, here we used a two-sample MR method to analyze the etiological relationship between small-vessel IS and three different types of hypertension to provide a more adequate theoretical basis for the relationship between hypertension and the risk of various subtypes of IS.[30]

Settings and design

This study followed the transparency and openness promotion guidelines. Data supporting the results of this study were publicly available from genome wide association studies (GWAS) and supplemental materials.[31] All included studies were given institutional review board approval, and all participants provided written informed consent. GWAS data of essential hypertension (ukb-b-14057) and small-vessel IS (ebi-a-GCST005841) were obtained through the open GWAS program website, the visit time of the website is March 18, 2023. The specific ethical approval has been stated in the original GWAS article. In the GWAS data of small-vessel IS, it includes 198,048 samples (including 5,386 cases and 192,662 controls); whereas in the data of essential hypertension, it includes 462,933 samples (including 119,731 cases and 343,202 controls). There are no gender restrictions in this study.

We used a two-sample MR approach to investigate the potential causal relationship between three different types of hypertension and small vessel IS.[32] MR methods utilize genetic variation in nonexperimental data to estimate the causal relationship between exposure and outcome.[33-35] Here, we use the term "exposure" to refer to hypothesized causal risk factors. In general, the observed relationship between exposure and outcome is uncertain due to confounding effects, and the correlation between the two cannot be used as reliable evidence to explain causation. At the same time, reverse causality may also produce observational associations. Therefore, we conducted an MR study to overcome these shortcomings.[36] The idea of MR is to find genetic variants (or multiple variants) that are correlated with exposure, but not with other risk factors that affect outcome and are not directly related to outcomes. This means that any association between the genetic variant and the outcome must be made through the correlation between the variant and the exposure, so it implies that there is a causal relation between the exposure and the outcomes.[16] This genetic variation would be consistent with the assumption of IV.[37] In MR, genetic variation is used as IV to estimate the causal effectiveness of exposures on outcomes. There are three basic assumptions underlying MR studies:

1. The genetic variation must be related to the exposure
2. This genetic variant was not associated with any of the exposure-outcome-related confounders
3. This genetic variation does not affect the outcome unless it is possible to achieve this outcome by associating it with exposure [Figure 1].

Filtering of instrumental variables

Select meaningful single nucleotide polymorphism (SNPs) from the GWAS data of essential hypertension ($P < 5 \times 10^{-8}$). The linkage disequilibrium r^2 was then set to 0.001 and the width of the linked disequilibrium region was set to 10,000 kb to assure that each SNP was independent of each other and to eliminate the effect of gene polymorphisms on the results. We used the F-statistic to assess the impact of weak IV.[38] The formula for calculating the value of F is F = beta^2/se^2.[39] Weak instrumental variable bias is considered to exist when the F-value is <10, and the results of the F-statistics are shown in Table 1.

The causal relationship between hypertension and small-vessel IS was analyzed using five different MR methods: Inverse variance weighted (IVW), MR-Egger, weighted median, simple mode, and weighted mode. Among them, we mainly focus on the results of MR-Egger

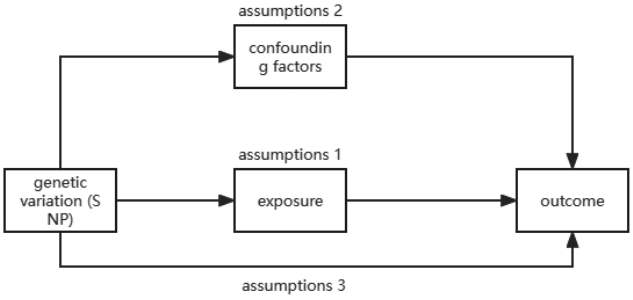

Figure 1: The basic three assumptions for genetic variation to meet the instrumental variable

and IVW [Table 1].[40-42] Using a multivariate MR approach, we adjusted for potential multiple effects associated with essential hypertension, gestational hypertension, and preexisting hypertension.

Sensitivity analysis

Sensitivity analyses were conducted mainly in three ways: heterogeneity test, multiple validity test, and reject-by-exclusion test.

Heterogeneity test

The heterogeneity test was used to identify the heterogeneity of the IVW model. When the heterogeneity is strong, the random-effects model of IVW is used for causal inference. Here, we used MR-Egger regression analysis to test for genetic heterogeneity. Finally, a leave-one-out sensitivity test was conducted to assess whether the combined IVW estimate would be affected by any single SNP [Figure 2].

We also used the same methods to conduct a MR analysis on the relationship between two other types of IS (cardioembolic and large artery atherosclerosis) and essential hypertension.

Clinical trial registry

This study obtained information through public databases and did not require clinical trial registration information.

Results

In the case of IS (small-vessel), after screening, the essential hypertension dataset remains 225 SNPs from 9,851,867 SNPs, excluding SNPs directly related to small-vessel IS, and final remains 202 SNPs.

All five MR methods demonstrated that essential hypertension is the etiology of small-vessel IS and that the risk of small-vessel IS increases approximately 6-fold for every 1 standard deviation increase in essential hypertension [Table 1]. IVW analysis showed a statistically significant correlation between essential hypertension and small-vessel IS risk (odds ratio [OR] = 6.03, P = 5.59e-17, standard error = 0.214).

To test for differences between different IVs, we performed a heterogeneity test. If the differences between the IVs are large, then there is a great deal of heterogeneity in those IVs. In the heterogeneity test, MR-Egger regression results showed that the statistic Q = 278.3778 (P < 0.001), means that there was a strong heterogeneity between IV [Table 2]. Therefore, we use the IVW to estimate the MR effect amount, the result suggests a cause-and-effect relationship between essential hypertension and small-vessel IS (P < 0.05) [Table 3].

The pleiotropy test is primarily used to test whether multiple IVs are horizontally multidirectional and is usually represented by the interception of the MR-Egger method. If the intercept term is significantly distinct from 0, it suggests the presence of horizontal pleiotropy. It can be seen from the results that there is no horizontal pleiotropy (P > 0.05) [Table 4].

The leave-one-out sensitivity test is used mainly to calculate the MR results for the leftover IVs after each IV

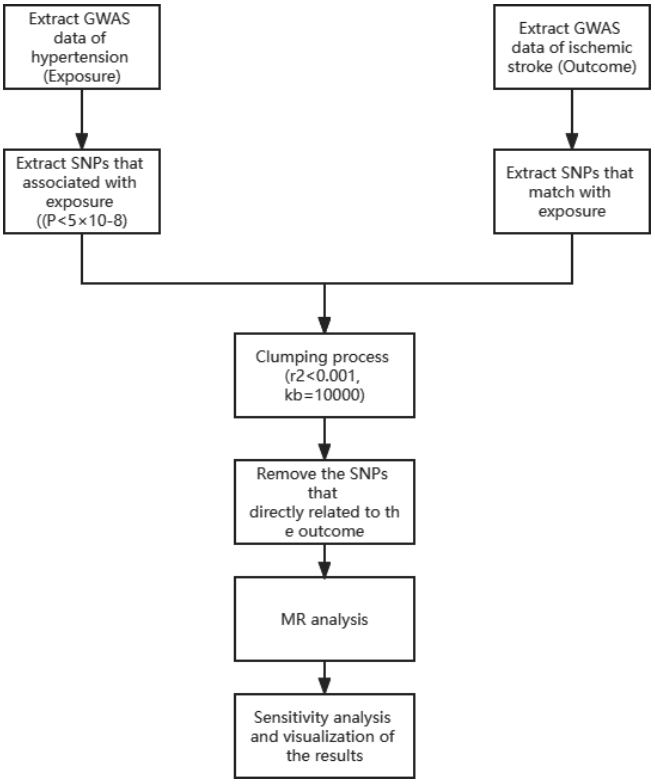

Figure 2: The process of Mendelian randomization analysis. MR: Mendelian randomization, GWAS: genome wide association studies, SNPs: single nucleotide polymorphism

Table 1: The result of five Mendelian randomization analyses

Method	*B*	SE	*P*	or	or_lci95	or_uci95	*F*
MR Eg ger	1.90694764	0.629780643	0.002807893	6.732507368	1.959281021	23.1343309	9.168513339
Weighted median	2.129193178	0.303367435	2.24E-12	8.408080251	4.639436922	15.23801588	49.25975114
Inverse variance weighted	1.797063302	0.21461438	5.60E-17	6.031907536	3.960693426	9.186247105	70.11470583
Simple mode	2.82187977	0.709923797	0.000100129	16.80841695	4.18049277	67.5812388	15.79987109
Weighted mode	2.730849249	0.567669132	3.07E-06	15.34591399	5.044092473	46.68770002	23.14221345

is rejected. If after excluding a particular IV, the estimate of MR differs significantly from the total results of the other IVs, then the MR results are sensitive to that IV. In our study, the leave-one-out plot shows no matter which SNP is culled, it does not fundamentally affect the results that indicate the MR result is reliable [Figure 3].

The scatter plot shows that the incidence rate of small-vessel IS increases significantly with the increase of essential hypertension [Figure 4].

In the forest plot, each of the horizontal solid lines reflects the results of a singular SNP estimated with the world's ratio method. If the solid line is entirely to its left of 0, the SNP estimate is positive; if the solid line is entirely to its right of 0, the SNP estimate is negative. Results beyond 0 are not significant. Our results are the red line at the bottom, which reflects the fact that the rise in essential hypertension increases the risk of small-vessel IS under the IVW approach [Figure 5].

The funnel diagram shows that all SNPs included are basically symmetrical [Figure 6], indicating that the difference between tool variables is small.

Our data-driven analysis further suggests that genetic susceptibility to gestational hypertension and preexisting hypertension is associated with small vessel IS. However, in multivariable analyses, these associations were explained by essential hypertension ($P > 0.05$) [Table 5].

Then, we analyzed the relationship between IS (cardioembolic), IS (large artery atherosclerosis), and essential hypertension using the same methods. The results show that there is also a causal relationship between them [Tables 6 and 7].

Discussion

Stroke is a devastating cerebrovascular event caused by a blockage or bursting of cerebral blood vessels, which includes IS and hemorrhagic stroke. IS is a cerebral ischemic necrosis due to vascular obstruction caused by atherosclerosis, and its incidence is significantly higher than that of hemorrhagic stroke, accounting for more than half of the total incidence. Stroke has become a growing public health burden as a cause of long-term

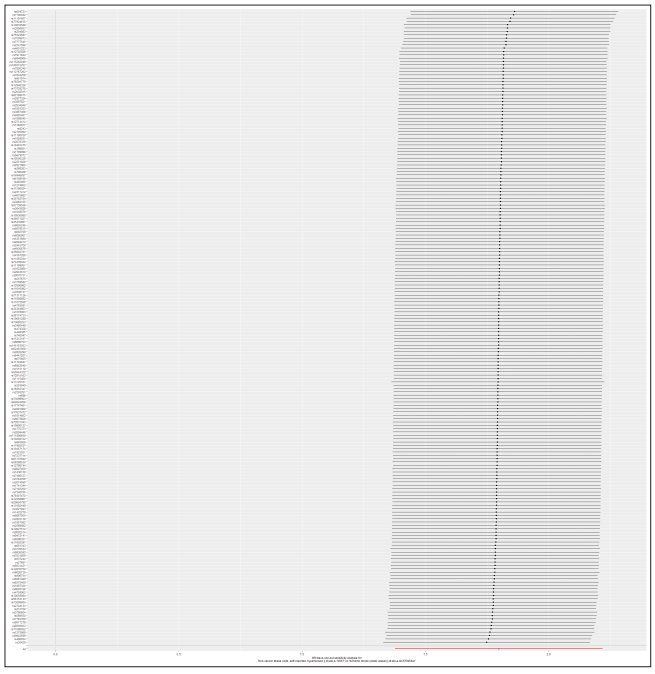

Figure 3: The leave-one-out result. MR: Mendelian randomization

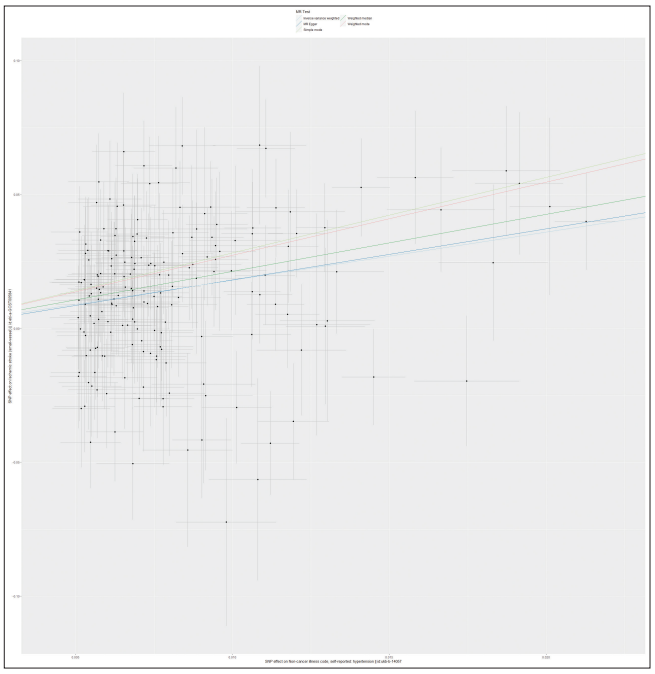

Figure 4: The scatter plot of results. MR: Mendelian randomization

Table 2: The result of heterogeneity test

Outcome	Exposure	Method	Q	*P*
Ischemic stroke (small vessel type)	essential hypertension	MR Eg ger	278.3778338	2.02E-05
Ischemic stroke (small vessel type)	essential hypertension	IVW	278.4288695	2.46E-05

Table 3: Results estimated by the inverse variance weighted method

Outcome	Exposure	Method	*B*	SE	*P*
Ischemic stroke (small vessel type)	Essential hypertension	IVW (multivariate analysis)	1.797063302	0.21461438	5.60E-17

Table 4: Results of the multidirectionality test

Outcome	Exposure	MR Egger intercept term	*P*
Ischemic stroke (small vessel type)	Essential hypertension	-0.000954257	0.852918206

Table 5: The result of multivariable analyses

Exposure	Result outcome	Result number of SNPS	Result *B*	Result SE	Result *P*
Pre-existing hypertension	Ischemic stroke (small-vessel)	1	-0.009748315	0.040386879	0.80926579
Gestational hypertension	Ischemic stroke (small-vessel)	0	-20.87239844	13.07998364	0.110544372
Essential hypertension	Ischemic stroke (small-vessel)	143	2.09343057	0.36737787	1.21E-08

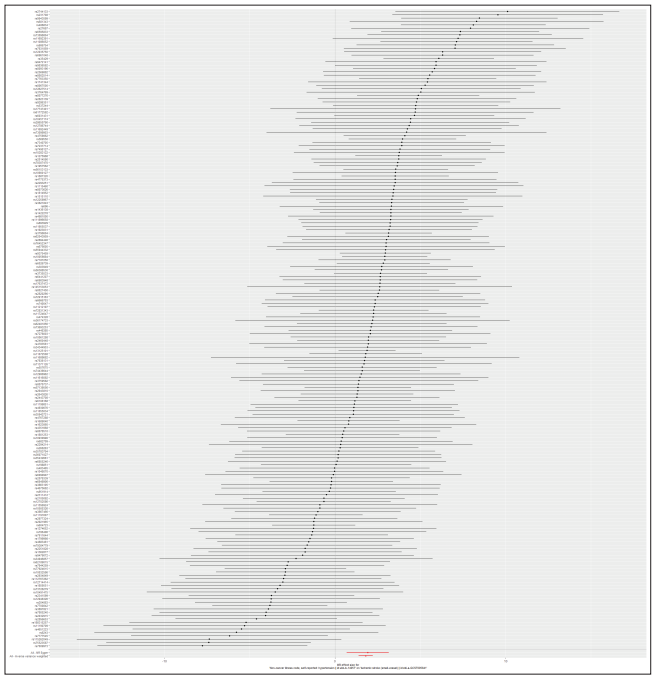

Figure 5: The forest map of the result. MR: Mendelian randomization

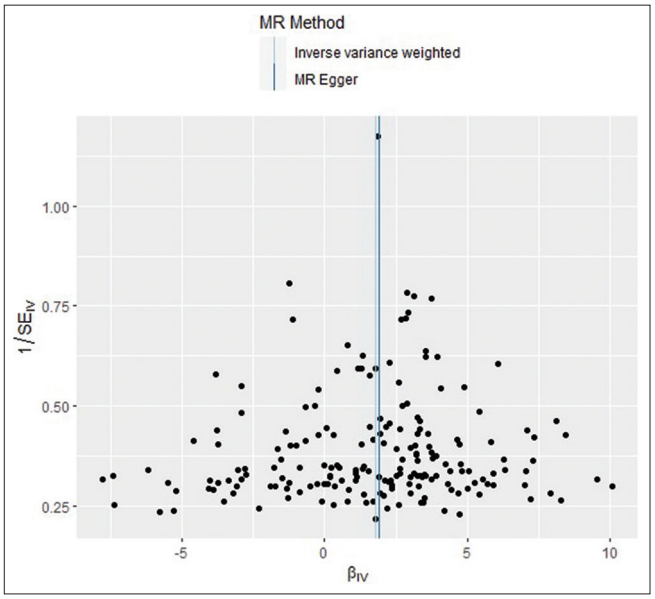

Figure 6: Funnel plot of results. MR: Mendelian randomization

disability. Therefore, identifying factors that prevent stroke is essential for planning appropriate health-care services and developing preventive treatment strategies. Although the benefits of blood pressure control for long-term stroke prevention have been largely established in previous epidemiologic studies, the results of these studies are susceptible to interference from confounding factors, resulting in a failure to establish causality.[15,16,43-46] In addition, it has been difficult for previous epidemiologic studies to avoid interference from reverse causation.[47,48] Therefore, more robust methods are needed to assess the causal relationships using observational data.

Our study further investigated the causal relationship between hypertension and stroke by means of MR and utilized the advantages of MR to complement previous observational studies.[49-51] The strength of this study is that it can test the association between exposures and outcome diseases in the presence of unknown confounders and avoid reverse causal interference, this is superior to previous observational studies and can complement previous epidemiologic studies.[52] Our genetic findings add to the complex literature on essential hypertension as potential causes of small-vessel IS, cardioembolic IS, and large artery atherosclerosis IS. In addition, our analysis incorporated the most recent and largest GWAS d hypertension and IS, and the outcomes were more reliable.

This study used MR methods to investigate the association between hypertension and IS, all of these were obtained from the large GWAS database. All SNPs in this study reached genome-level significance and were strong IV. Our study used the IVW method to generate the primary results, supplemented by the MR-Egger method, weighted medium, simple mode, and weighted mode methods. The supplementary results were consistent with the primary results. It suggests that the previously observed correlation between hypertension and stroke is unlikely to be confounded by residual confounders or reverse causation. Our results suggest a causal relationship between hypertension and increased risk of IS, suggesting that long-term reduction of hypertension is potentially beneficial for stroke prevention. Our results show that the risk of IS increased by about 6 times for every 1 standard deviation of essential hypertension.

Table 6: Results of Mendelian randomization analysis between essential hypertension and cardioembolic ischemic stroke

Outcome	Exposure	Method	*B*	SE	*P*
Ischemic stroke (cardioembolic type)	essential hypertension	MR Eg ger	1.075732994	0.618737845	0.083551419
Ischemic stroke (cardioembolic type)	essential hypertension	Weighted median	1.287526675	0.302355295	2.06E-05
Ischemic stroke (cardioembolic type)	essential hypertension	IVW	1.212364378	0.216004183	1.99E-08
Ischemic stroke (cardioembolic type)	essential hypertension	Simple mode	3.134924574	1.006708341	0.002097871
Ischemic stroke (cardioembolic type)	essential hypertension	Weighted mode	2.36304845	0.892520619	0.008709559

Table 7: Results of Mendelian randomization analysis between essential hypertension and atherosclerotic ischemic stroke of large arteries

Outcome	Exposure	Method	*B*	SE	*P*
Ischemic stroke (atherosclerotic type)	essential hypertension	MR Eg ger	1.322176995	0.588897948	0.025799219
Ischemic stroke (atherosclerotic type)	essential hypertension	Weighted median	1.852054649	0.32731154	1.53E-08
Ischemic stroke (atherosclerotic type)	essential hypertension	IVW	2.076244787	0.205512377	5.37E-24
Ischemic stroke (atherosclerotic type)	essential hypertension	Simple mode	1.677248888	0.929047535	0.072446563
Ischemic stroke (atherosclerotic type)	essential hypertension	Weighted mode	1.677248888	0.753826678	0.027141433

This provides a better basis for confirming the causal relationship between hypertension and stroke and for developing rational stroke prevention measures.

Our ongoing study focuses on the etiologic relationship between hypertension and IS. However, the association between hypertension and other types of stroke remains to be further explored in subsequent articles. In addition, the present study has some limitations: The MR assumed a direct linear relationship between exposure and outcome, but this may not apply if the relationship is nonlinear.[53,54] Therefore, the exact causal relationship between hypertension and IS needs to be added to the relevant literature. In our future studies, we will further explore the relationship between hypertension and IS using other methods to compensate for the shortcomings of the MR approach. In addition, the samples of this study were drawn from European population groups, so the findings may not generalize to other ethnicities. In our future studies, we will also include Asian populations to broaden the applicability of this study.

Conclusions

Through MR analysis between hypertension and IS, we further demonstrated that hypertension is a high-risk factor for stroke (especially for small vessel IS). The OR suggests that for every 1 standard deviation increase in essential hypertension, the risk of small-vessel IS increases approximately 6-fold. The results for IS (small vessels) were quite robust, whereas in the other two types of IS, although the results of some MR methods for IS (cardioembolic) and IS (large artery atherosclerosis) showed *P* values of slightly more than 0.05 (which may be due to insufficient sample sizes), the other four methods still showed a causal relationship with hypertension. This study used MR methods to study the association between hypertension and stroke. This article could serve as a complement to randomized controlled trials that have examined the causal relationship between hypertension and IS.[36]

Key messages

Stroke is a devastating cerebrovascular event caused by a blockage or bursting of cerebral blood vessels, which includes IS and hemorrhagic stroke. IS is a cerebral ischemic necrosis due to vascular obstruction caused by atherosclerosis, and its incidence is significantly higher than that of hemorrhagic stroke, accounting for more than half of the total incidence. Stroke has become a growing public health burden as a cause of long-term disability. Therefore, identifying factors that prevent stroke is essential for planning appropriate health-care services and developing preventive treatment strategies. Although the benefits of blood pressure control for long-term stroke prevention have been largely established in previous epidemiologic studies, the results of these studies are susceptible to interference from confounding factors, resulting in a failure to establish causality. Our study further investigated the causal relationship between hypertension and stroke by means of MR and utilized the advantages of MR to complement previous observational studies. The strength of this study is that it can test the association between exposures and outcome diseases in the presence of unknown confounders and avoid reverse causal interference, this is superior to previous observational studies and can complement previous epidemiologic studies.

Author contributions

Wenhao Zhang was responsible for developing the search strategy, data extraction and statistical analysis, as well as data verification and thesis writing, literature reading and article polishing. Yuhua Li, Mengying Pang were responsible for assisting and guiding the selection of topics. Xuejing Yue was responsible for the overall idea

and professional technical guidance as well as evaluating the quality of the literature.

Declarations

Ethics statement for the use of human and animal subjects (may require consent to participate and consent to publish for human subjects): Not applicable.

Data availability statement

The datasets generated and analyzed during the current study are available in the (IEU Open GWAS) repository (https://gwas.mrcieu.ac.uk/).

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Conflicts of interest

There are no conflicts of interest.

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