



# Vascular Endothelial Growth Factor and Ischemic Stroke Risk: A Mendelian Randomization Study

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## ABSTRACT

**Introduction:** Previous studies have reported controversial relationships between circulating vascular endothelial growth factors (VEGF) and ischemic stroke (IS). This study aims to demonstrate the causal effect between VEGF and IS using Mendelian randomization (MR).

Xiao Zhang, Xinzhi Hu, and Shiyuan Fang contributed equally to this work and share first authorship.

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**Methods:** Summary statistics data from two large-scale genome-wide association studies (GWAS) for 16,112 patients with measured VEGF levels and 40,585 patients with IS were downloaded from public databases and included in this study. A published calculator was adopted for MR power calculation. The primary outcome was any ischemic stroke, and the secondary outcomes were large-artery stroke, cardioembolic stroke, and small-vessel stroke. We used the inverse variance-weighted (IVW) method for primary analysis, supplemented by MR-Egger regression and the weighted median method.

**Results:** Nine SNPs were included to represent serum VEGF levels. The IVW method revealed no strong causal association between VEGF and any ischemic stroke (odds ratio [OR] 1.01, 95% CI 0.99–1.04,  $p = 0.39$ ), cardioembolic

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stroke (OR 1.04, 95% CI 0.97–1.12,  $p = 0.28$ ), large-artery stroke (OR 1.02, 95% CI 0.95–1.09,  $p = 0.62$ ), and small-vessel stroke (OR 0.98, 95% CI 0.91–1.04,  $p = 0.46$ ). These findings remained robust in sensitivity analyses. MR-Egger regression suggested no horizontal pleiotropy.

**Conclusions:** This Mendelian randomization study found no relationship between genetically predisposed serum VEGF levels and risks of IS or its subtypes.

**Keywords:** Vascular endothelial growth factor; Ischemic stroke; Causal association; Mendelian randomization; Stroke prevention; Causal link

### Key Summary Points

#### *Why carry out this study?*

Currently, the causality relationship between vascular endothelial growth factors (VEGF) and ischemic stroke (IS) remains unclear because of the inherited risk of confounding of observational studies.

Therefore, we aim to use Mendelian randomization (MR) analyses to explore whether genetically predisposed circulating VEGF levels increase the risk of IS, and specifically, three subtypes of IS, in the absence of confounders and reverse causations.

#### *What was learned from this study?*

Our study provides genetic evidence that high serum VEGF levels do not increase risks of IS. Therefore, high VEGF levels may not predict the risk of stroke under clinical circumstances.

Future studies should investigate the differences between lacunar and non-lacunar strokes since they require different treatment intensities and have diverged prognosis. Secondly, the specific link between VEGFA and IS remains to be elucidated in future studies once such variables are recognized.

## INTRODUCTION

Vascular endothelial growth factors (VEGF) promote angiogenesis and nurture neurons [1, 2]. VEGF have been implicated in multiple stroke-related pathophysiological pathways, including atherosclerosis, promoting collateral circulation, and increasing vascular permeability [2]. Stroke is the second leading cause of death and loss of disability-adjusted life years worldwide [3]. Serum VEGF level is considered as a potential biomarker of stroke [4], because high VEGF levels are often observed concurrently with ischemic attacks. However, the causal relationship between VEGF and ischemic stroke (IS) has never been fully understood. VEGFA is known as the most effective factor to promote neovascularization [5] and therefore a high-level VEGFA expression within atherosclerotic plaques is believed to lead to plaque revascularization and eventually cause large-artery strokes. Compared to VEGFA, other subtypes of VEGF have received far less research attention. VEGFB is noted as a “survival” factor rather than a potent angiogenic factor, because it protects brain microvasculature stability in injured regions, rather than producing unstable neovascularization [6]. Observational studies from clinical practice have been giving controversial results in the past few years, and

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an important cause was the mixed measurement of VEGF. Most studies used the enzyme-linked immunosorbent assay (ELISA) technique to detect circulating VEGF; however, different designs of the primary antibodies would bind different epitopes of VEGF, thus generating mixed results [4]. Furthermore, a recent Mendelian randomization study aimed to explore whether high serum VEGF levels cause ischemic heart disease, and reported negative results [7]. In summary, whether high serum VEGF levels would cause ischemic stroke remains unknown.

Mendelian randomization (MR) uses genetic variants to represent a certain exposure. Such genetic variants are used as instrumental variables, which are randomly distributed in the general population by nature [8, 9]. A major advantage of using MR is that one can infer causal relationships between risk factors (exposure) and diseases (outcome) without environmental or reverse confounders [10]. In this study, we aim to use MR analyses to explore whether genetically predisposed circulating VEGF levels increase the risk of IS, and specifically, three subtypes of IS, in the absence of confounders and reverse causations. Depicting this causality would provide insights into using VEGF neutralizing antibodies as a primary prevention of IS.

## METHODS

### Study Design

We carried out MR analysis with a two-sample design, employing summary statistics from two extensive genome-wide association studies (GWAS) [11, 12]. Each of the individual studies obtained approval from the respective local institutional review board or ethics committee. In this current study, we exclusively extracted summarized data from consortia. Ethics approval was deemed unnecessary, given that all analyses were conducted utilizing publicly accessible databases [13]. A genetic variant serves as a valid instrument when it met the main assumption of MR: (1) it should be strongly associated with the risk factor, (2) it must not be tied to any confounding factors,

and (3) its impact on the outcome should come only through its effect on the exposure.

### Genetic Instruments for Circulating VEGF Levels

Genetic association estimates of genome-wide significant single nucleotide polymorphisms (SNPs) ( $p < 5 \times 10^{-8}$ ) with predicted serum VEGF levels (per log-transformed pg/mL) were obtained from a GWAS including 16,112 samples of European ancestry based on the 1000 Genomes reference data [11]. It is the most comprehensive GWAS of circulating VEGF and the instruments have been widely used in multiple MR studies confounding different outcomes, including ischemic heart disease [7, 14]. The serum VEGF levels were measured using commercial ELISA assays. Participants' age ranged from 30.4 to 76.2 years old. All SNPs were clumped with a 10,000-kB window to a threshold of  $r^2 < 0.1$  to ascertain independence between genetic variants according to the 1000 Genomes (EUR) reference data from SNP Annotation and Proxy Search (<http://www.broad.mit.edu/mpg/snap/ldsearchpw.php>). We utilized the online database Phenoscanner to investigate potential pleiotropy of the instruments concerning confounding traits. This included traits such as hypertension medical history, patient's blood lipid status, and diabetes status [15]. Proxies (with  $r^2 > 0.80$ ) for SNPs not present in the outcome dataset were determined utilizing the LDproxy tool on the National Cancer Institute LDlink platform (<https://ldlink.nih.gov/?tab=ldproxy>). The robustness of the instrument was indicated by the F-statistic. All SNPs exhibited F-statistic values exceeding 10, indicating a low likelihood of introducing substantial weak instrument bias in the MR analyses. Any paralimbic SNPs were excluded.

### Summary Statistics Data for Ischemic Stroke

A multi-ancestry GWAS conducted by the GIGASTROKE consortium, encompassing 62,100 and 1,234,808 cases of European

ancestry and controls, was used to derive the evaluation of genetic relationship between SNP and outcome [16]. This is the latest and largest comprehensive GWAS, which has been applied in most MR studies [17]. IS cases were diagnosed on the basis of clinical and imaging criteria. In accordance with the Acute Stroke Treatment (TOAST) criteria, IS was categorized into the following subtypes: large-artery stroke (LAS,  $n = 6399$ ), cardioembolic stroke (CES,  $n = 10,804$ ), and small-vessel ischemic stroke (SVS,  $n = 6811$ ). Replication analyses were conducted within the MEGASTROKE Consortium, comprising 40,585 cases and 406,111 controls. All cases of IS were categorized into LAS ( $n = 4373$ ), CES ( $n = 7193$ ), and SVS ( $n = 5369$ ) [12].

### Compliance with Ethics Guidelines

The summary-level datasets utilized in our study were retrieved from de-identified public datasets or studies. All included studies had received prior approval from the appropriate ethics committee, and informed consent was obtained from all participants involved in the original studies [11, 12, 16]. Ethical approval was not required for our study as we exclusively utilized summary-level data. The study complied with the Declaration of Helsinki.

### Mendelian Randomization Power Calculation

To assess the adequacy of the participant size for conducting MR analyses, we performed power calculations using a published calculator. A total of 10 SNPs of VEGF levels explained the variance which ranged from 19% to 52%. Rs34528081 was clumped to ascertain independence and excluded from the instruments. Under the assumption that the remaining nine SNPs contributed only 19% of the variance, we performed power calculations using an online tool (<https://shiny.cnsgenomics.com/mRnd/>).

### Statistical Analysis

The primary outcome was defined as any IS, with secondary outcomes including LAS, CES, and SVS. With regards to individual SNPs, MR estimates were computed using the ratio of coefficients method, and their standard errors were derived using the delta method. Primary analysis involved amalgamating the ratio estimates under a random-effects model inverse-variance weighting (IVW) approach. Additionally, the findings were complemented by employing MR-Egger regression and a penalized weighted median for a comprehensive assessment [18–20].

The MR-Egger intercept test was used to estimate the horizontal pleiotropic effects. The MR-Egger estimates were calculated to adjust for pleiotropy, which is the phenomenon where a single genetic variant affects multiple traits or outcomes. In this test, if the intercept value for the MR-Egger regression significantly deviates from zero ( $P < 0.05$ ), it suggests the presence of horizontal pleiotropy, which indicates that the genetic variant is influencing the outcome through pathways unrelated to the main exposure of interest [18]. In addition to other analyses, we utilized the penalized weighted median estimates to enhance robustness. This method penalizes the weights of candidate instruments in the weighted regression model, taking into account heterogeneous ratio estimates. Funnel plots were utilized for a visual inspection of heterogeneity, with any observable asymmetry around the vertical line raising the possibility of directional pleiotropy. This graphical approach provided an additional layer of scrutiny to detect potential biases or anomalies in the MR estimates. Moreover, we applied the MR pleiotropy residual sum and outlier (MR-PRESSO) method as an additional step to corroborate the presence of potential outliers in our MR analyses [21]. This method enhanced the robustness of our findings by identifying and addressing any influential data points that might impact the validity of the results.

To communicate the outcomes effectively, we exponentiated all results to present odds ratios (ORs) and their accompanying 95% confidence intervals (CIs) for stroke. These values

reflect the association per logarithmic increase in circulating VEGF levels, offering a comprehensive perspective on the impact of VEGF in the context of stroke risk. To account for multiple testing, we applied a Bonferroni correction and set a significance threshold of  $\alpha = 0.05/4$  (one exposure and four outcomes). *P* values ranging from 0.05 to 0.0125 indicate potential causal significance between the exposure and outcome. All statistical analyses were performed using R version 4.0.0, with the utilization of the R packages TwoSampleMR and MR-PRESSO.

## RESULTS

Nine SNPs were performed as instruments for analyzing ischemic stroke (Supplementary Table 1). The power calculation indicated a statistical power ranging from 85% to 100% for detecting an OR of 0.9 or 1.1. Each of the instrumental variables displayed significant associations with circulating VEGF levels and demonstrated independence from the majority of confounding factors. All instruments were also independent of the outcomes. Figure 1a illustrates MR causal effect estimates for the risk of stroke per log-transformed increase in circulating VEGF levels. In summary, the application of the IVW method did not reveal a substantial causal link between VEGF levels and the occurrence of IS (OR 1.01, 95% CI 0.99–1.04, *P* = 0.39), CES (OR 1.04, 95% CI 0.97–1.12, *P* = 0.28), LAS (OR 1.02, 95% CI 0.95–1.09, *P* = 0.62), and SVS (OR 0.98, 95% CI 0.91–1.04, *P* = 0.46). Replication analysis using MEGA-STROKE exhibited consistent results with that of GIGASTROKE (Fig. 1b). The causal effects of single instrument are described in Supplementary Table 3.

The conclusions drawn from the penalized weighted median method align with those derived from our analyses using the same method. Moreover, the MR-Egger regression intercept provided no substantial evidence of horizontal pleiotropy (*P* > 0.05), reinforcing the robustness of our findings (Table 1). There were no outliers detected in the analyses of funnel plot (shown as Fig. 2a, b), and this observation

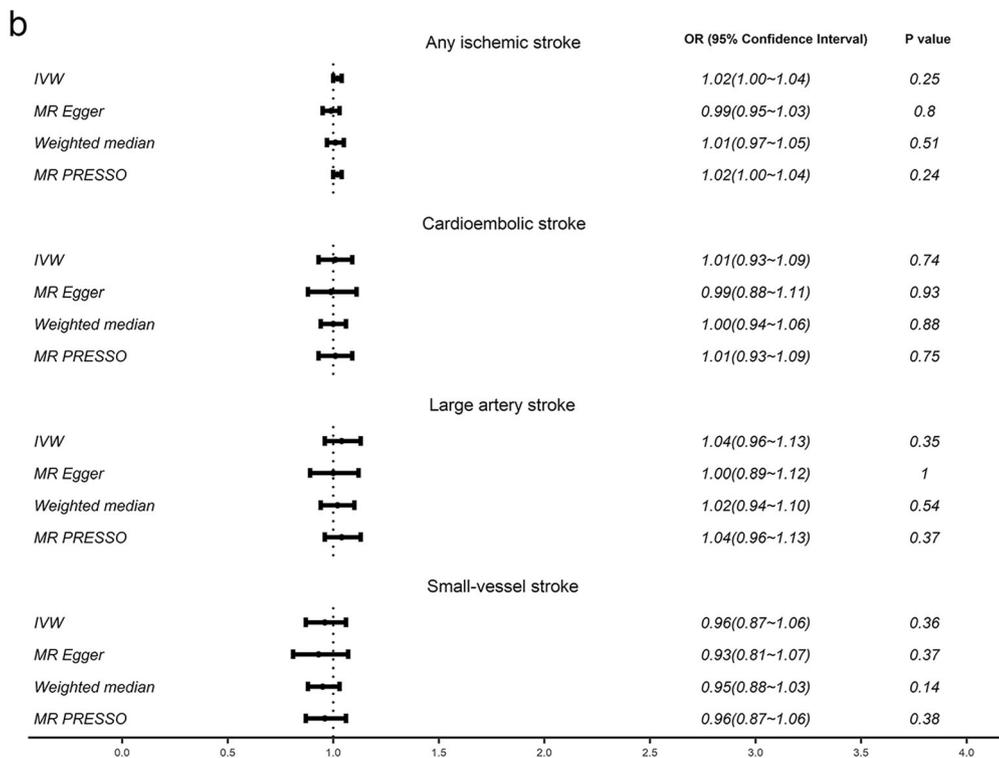
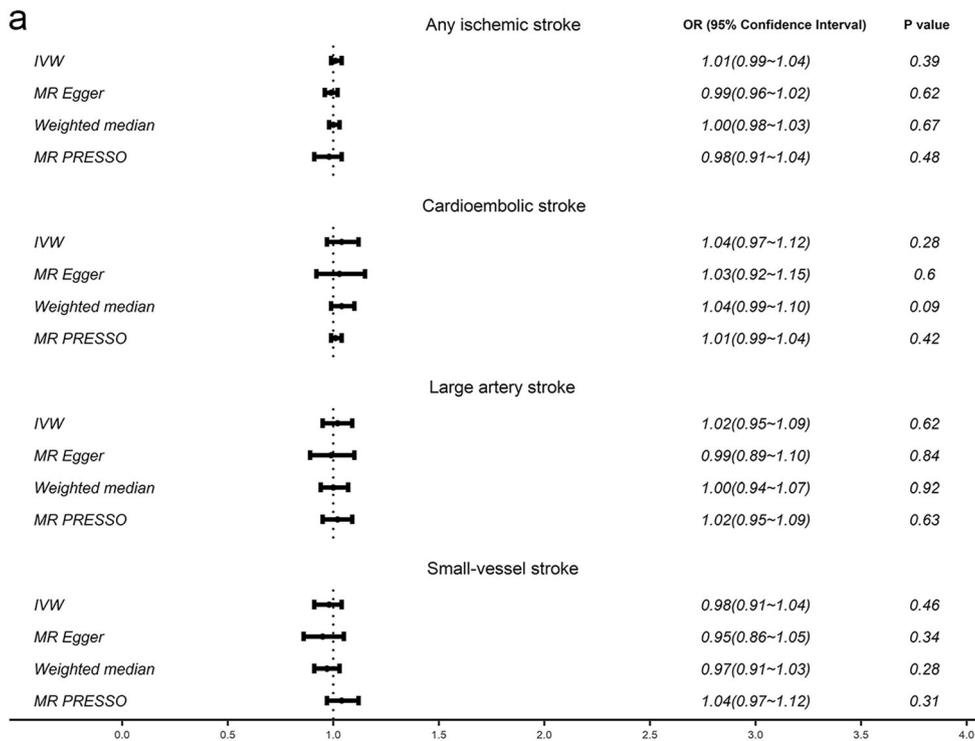
was corroborated by the results of the MR-PRESSO outlier test.

## DISCUSSION

In this two-sample MR study, we explored the causal relationship between circulating VEGF levels and risks of IS using genetic instrumental variables. Our findings suggest that a high level circulating VEGF is unlikely to result in any IS or its subtypes, specifically LAS, CES, and SVS. The findings remained robust in sensitivity analyses with different MR methods. In clinical practice, high circulating VEGF levels may not be a predictor of ischemic stroke.

The hypothesis of VEGF causing atherosclerotic stroke is deduced from its role in atherogenesis. An atherosclerotic plaque is a hypoxic and inflammatory environment. Both features upregulate VEGF expression to promote angiogenesis and relieve ischemia, but the newly generated vessels could lead to intraplaque hemorrhage and eventually plaque rupture [2]. However in real-world experiences, a prospective community-based study including 3041 participants reported a complex, inverted U-shaped relation between serum VEGF level and incidence of cardiovascular diseases, as the incidence increases with serum VEGF going up but then drops at the highest quartile of VEGF levels [22]. Such population-based studies were inevitably to suffer from epidemiological confounders, including patients' age, gender, comorbidities of coronary artery diseases [22], myocardial infarction [23], and diabetes mellitus [24], which are all known to influence VEGF levels.

Using MR analyses that eliminate the aforementioned confounders and reverse causations, the largely null results of our study provided evidence that high circulating VEGF does not cause IS or its subtypes. This result is consistent with a previous MR study focusing on VEGF's relationship with ischemic heart disease (IHD) [7]. That MR study enrolled 60,801 patients with IHD and 123,504 controls, only to find no positive effect of VEGF on IHD risks [7]. Another recent MR study involving 41 cytokines and growth factors identified VEGF as a possible



◀**Fig. 1** Causal effect of VEGF on the risk of ischemic stroke and its subtypes estimated using four MR methods. **a** GIGASTROKE; **b** MEGASTROKE. The causal effect from VEGF to outcomes is expressed as OR per unit. Error bars represent the 95% CIs of the estimates. The odds ratios (OR) are per genetically predicted 1 SD increase in genetically predicted per standard deviation (SD) increase in log odds of the circulating VEGF. *SNP* single nucleotide polymorphism, *GWAS* genome-wide association study, *VEGF* vascular endothelial growth factor, *IVW* inverse-variance weighted, *OR* odds ratio, *CIs* confidence intervals, *MR-PRESSO* MR pleiotropy residual sum and outlier

cause of CES ( $p < 0.05$ ), but its  $p$  value did not meet the Bonferroni correction threshold [25]. It also reported no causality between VEGF and any IS, LAS, or SVS [25].

The biggest strength of this study lies within the rigorous assumptions of MR analyses. We satisfied all three key assumptions that validate an MR study [26]. First, for the relevance assumption, the adopted instrumental variables in our study were associated with circulating VEGF levels and incidence of IS in two large GWAS [11, 12]. The large sample size substantially compensated for the potential weak instrumental bias. Second, in terms of the independence assumption, it is established that genetically predisposed serum VEGF accounts for the majority of serum VEGF levels. Acute-onset conditions, including acute coronary syndrome and stroke, may cause temporal fluctuations in VEGF levels, but could not be held accountable for the chronic and slow progression of atherosclerotic plaques. Last, regarding the exclusion restriction, MR-Egger

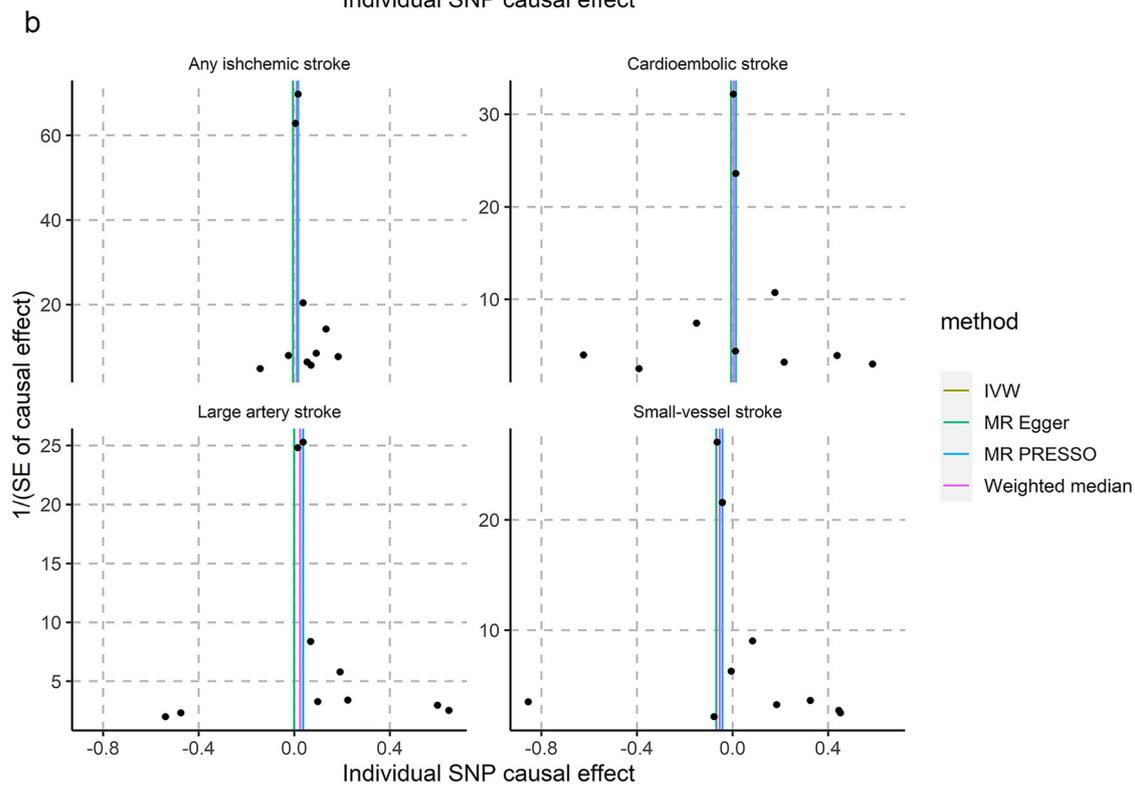
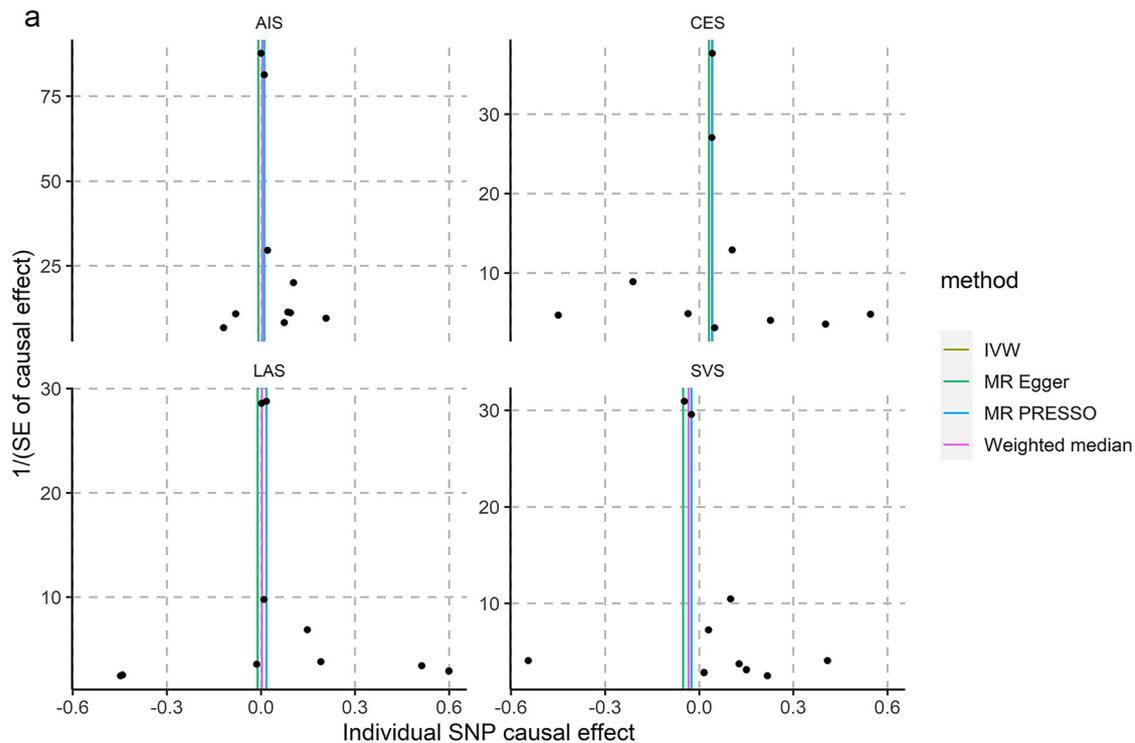
test suggested no evidence of heterogeneity, and the MR-PRESSO method found no outliers, suggesting that the genetic variants under discussion only affect the outcome through their effects on the VEGF levels. Another strength is the robustness of our findings, as we applied four methods and all gave negative results. We also distinguished between different subtypes of IS, especially LAS, which theoretically could be caused by elevated VEGF. Though our largely null finding negated the causal relationship between VEGF and IS, the biomarker role of VEGF in IS diagnosis should not be repudiated. The upregulation of VEGF is likely to be the outcome instead of the cause of ischemia.

A notable limitation of this study is that we failed to distinguish subgroups of VEGFs. VEGFs are a group of growth factors that primarily include VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor (PlGF) [2]. Among them, VEGFA is considered as the most relevant, as it originates from almost all vascularized tissues in humans, and loss of a single VEGF allele results in embryonic death in mice [5]. However, no strong genetic variables have been identified to predict serum VEGFA levels. Another limitation is that all participants were of European descent. Different populations have different genetic characteristics, and some genetic variations may be prevalent in one population but scarce or absent in another as a result of recent emergence and limited dissemination [27, 28]. Hence, the generalizability of our results to other ethnic groups may be potentially limited, and this is also a common limitation in MR studies [29, 30].

**Table 1** Results of MR-Egger intercept analyses

MEGASTROKE				GIGASTROKE		
Outcome	MR-Egger intercept	SE	$p$ value	MR-Egger intercept	SE	$p$ value
AIS	0.008	0.006	0.189	0.007	0.005	0.151
SVS	0.010	0.020	0.638			
LAS	0.014	0.016	0.399	0.011	0.015	0.487
CES	0.008	0.018	0.685	0.003	0.016	0.839

*AIS* all ischemic stroke, *CES* cardioembolic stroke, *LAS* large-artery stroke, *SVS* small-vessel stroke, *SE* standard error



◀**Fig. 2** Funnel plot of four MR methods. **a** GIGASTROKE; **b** MEGASTROKE. *SNP* single nucleotide polymorphism, *GWAS* genome-wide association study, *VEGF* vascular endothelial growth factor, *IVW* inverse-variance weighted, *MR-PRESSO* MR pleiotropy residual sum and outlier, *SE* standard error

Future studies should investigate the differences between lacunar and non-lacunar strokes, since they require different treatment intensities and have diverged prognosis [31]. Secondly, the specific link between VEGFA and IS remains to be elucidated in future studies, once such variables are recognized.

## CONCLUSION

Our two-sample MR study provides genetic evidence that high serum VEGF levels do not increase the risk of IS. Therefore, high VEGF levels may not predict the risk of stroke under clinical circumstances. Future studies should explore the relationship between VEGFA and IS.

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**Author Contributions.** Tao Wang and Liqun Jiao developed the initial ideas for this study and formulated the study designs. Xiao Zhang, Xinzhi Hu, and Shiyuan Fang performed data analysis and original drafts. Liqun Jiao, Tao Wang, Ran Xu, Adam A. Dmytriw, and Yan Ma were consulted about clinical issues. Xiao Zhang, Xinzhi Hu, Shiyuan Fang, Jiayao Li, Zhichao Liu, Weidun Xie, Ran Xu, Adam A. Dmytriw, Kun Yang, Yan Ma, Liqun Jiao, and Tao Wang were responsible for the revision of the draft. All authors read and approved the

final version of the manuscript prior to submission.

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**Data Availability.** All data pertinent to this study are comprehensively detailed within this paper, and the origin of publicly accessible data is explicitly disclosed.

## Declarations

**Conflict of Interest.** Shiyuan Fang relocated to a new institution following her graduation and the completion of this paper; her new affiliation is Department of Neurology, Peking Union Medical College Hospital, Beijing, China. We have included her both primary and updated affiliation addresses in this manuscript. Other authors have nothing to disclose. The authors declare no competing interests.

**Ethical Approval.** The summary-level datasets utilized in our study were retrieved from de-identified public datasets or studies. All included studies had received prior approval from the appropriate ethics committee, and informed consent was obtained from all participants involved in the original studies [11, 12, 16]. Ethical approval was not required for our study as we exclusively utilized summary-level data. The study complied with the Declaration of Helsinki.

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## REFERENCES

- Mackenzie F, Ruhrberg C. Diverse roles for VEGF-A in the nervous system. *Development*. 2012;139:1371–80.
- Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. *Cell Mol Life Sci*. 2013;70:1753–61.
- GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a 399 systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396:1204–22.
- Seidkhani-Nahal A, Khosravi A, Mirzaei A, Basati G, Abbasi M, Noori-Zadeh A. Serum vascular endothelial growth factor (VEGF) levels in ischemic stroke patients: a systematic review and meta-analysis of case-control studies. *Neurol Sci*. 2021;42:1811–20.
- Ylä-Herttuala S, Rissanen TT, Vajanto I, Hartikainen J. Vascular endothelial growth factors: biology and current status of clinical applications in cardiovascular medicine. *J Am Coll Cardiol*. 2007;49:1015–26.
- Jean LeBlanc N, Guruswamy R, ElAli A. Vascular endothelial growth factor isoform-B stimulates neurovascular repair after ischemic stroke by promoting the function of pericytes via vascular endothelial growth factor receptor-1. *Mol Neurobiol*. 2018;55:3611–26.
- Au Yeung SL, Lam H, Schooling CM. Vascular endothelial growth factor and ischemic heart disease risk: a Mendelian randomization study. *J Am Heart Assoc*. 2017. <https://doi.org/10.1161/JAHA.117.005619>.
- Luo J, le Cessie S, van Heemst D, Noordam R. Diet-derived circulating antioxidants and risk of coronary heart disease: a Mendelian randomization study. *J Am Coll Cardiol*. 2021;77:45–54.
- Martens LG, Luo J, Willems van Dijk K, Jukema JW, Noordam R, van Heemst D. Diet-derived antioxidants do not decrease risk of ischemic stroke: a Mendelian randomization study in 1 million people. *J Am Heart Assoc*. 2021;10:e022567.
- Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–63.
- Choi SH, Ruggiero D, Sorice R, et al. Six novel loci associated with circulating VEGF levels identified by a meta-analysis of genome-wide association studies. *PLoS Genet*. 2016;12: e1005874.
- Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524–37.
- Tan JS, Ren JM, Fan L, et al. Genetic predisposition of anti-cytomegalovirus immunoglobulin G levels and the risk of 9 cardiovascular diseases. *Front Cell Infect Microbiol*. 2022;12: 884298.
- Keller-Baruch J, Forgetta V, Manousaki D, Zhou S, Richards JB. Genetically decreased circulating vascular endothelial growth factor and osteoporosis outcomes: a Mendelian randomization study. *J Bone Miner Res*. 2020;35:649–56.
- Staley JR, Blackshaw J, Kamat MA, et al. PhenoScanner: a database of human genotype-phenotype associations. *Bioinformatics*. 2016;32:3207–9.
- Mishra A, Malik R, Hachiya T, et al. Stroke genetics informs drug discovery and risk prediction across ancestries. *Nature*. 2022;611:115–23.
- Georgakis MK, Gill D. Mendelian randomization studies in stroke: exploration of risk factors and drug targets with human genetic data. *Stroke*. 2021;52:2992–3003.
- Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44:512–25.
- Burgess S, Bowden JJ. Integrating summarized data from multiple genetic variants in Mendelian randomization: bias and coverage properties of inverse-variance weighted methods. 2015. p. [arXiv:1512.04486](https://arxiv.org/abs/1512.04486).

20. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* 2016;40:304–14.
21. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* 2018;50:693–8.
22. Kaess BM, Preis SR, Beiser A, et al. Circulating vascular endothelial growth factor and the risk of cardiovascular events. *Heart.* 2016;102:1898–901.
23. Hojo Y, Ikeda U, Zhu Y, et al. Expression of vascular endothelial growth factor in patients with acute myocardial infarction. *J Am Coll Cardiol.* 2000;35:968–73.
24. Matsuo R, Ago T, Kamouchi M, et al. Clinical significance of plasma VEGF value in ischemic stroke - research for biomarkers in ischemic stroke (REBIOS) study. *BMC Neurol.* 2013;13:32.
25. Georgakis MK, Gill D, Rannikmäe K, et al. Genetically determined levels of circulating cytokines and risk of stroke. *Circulation.* 2019;139:256–68.
26. Davies NM, Holmes MV, Davey SG. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ.* 2018;362:k601.
27. Rotimi CN, Jorde LB. Ancestry and disease in the age of genomic medicine. *N Engl J Med.* 2010;363:1551–8.
28. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. *J Med Genet.* 1997;34:275–8.
29. Shu MJ, Li JR, Zhu YC, Shen H. Migraine and ischemic stroke: a Mendelian randomization study. *Neurol Ther.* 2022;11:237–46.
30. Ma C, Wu M, Gao J, et al. Periodontitis and stroke: a Mendelian randomization study. *Brain Behav.* 2023;13:e2888.
31. Arboix A, Massons J, García-Eroles L, et al. Nineteen-year trends in risk factors, clinical characteristics and prognosis in lacunar infarcts. *Neuroepidemiology.* 2010;35:231–6.