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Hyperhomocysteinemia and intracranial aneurysm: A mendelian randomization study

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Objective: To investigate the link between genetic variants associated with plasma homocysteine levels and risk of intracranial aneurysm (IA) using two-sample Mendelian randomization.

Methods: We used single-nucleotide polymorphisms associated with human plasma homocysteine levels as instrumental variables for the primary analysis in a genome-wide association study of 44,147 subjects of European ancestry. Summary-level statistics were obtained for 79,429 individuals, including 7,495 IA cases and 71,934 controls. To enhance validity, five different Mendelian randomization methods (MR-Egger, weighted median, inverse variance weighted, simple mode, and weighted mode) were used for the analyses.

Results: The inverse variance weighted analysis method produced *P*-values of 0.398 for aneurysmal subarachnoid hemorrhage [odds ratio (OR): 1.104; 95% confidence interval (CI): 0.878–1.387], 0.246 for IA (OR: 1.124; 95% CI: 0.923–1.368), and 0.644 for unruptured IA (OR: 1.126; 95% CI: 0.682–1.858). The MR-Egger analysis showed no association between IAs and homocysteine, with all *P* > 0.05.

Conclusion: Using gene-related instrumental variables, the Mendelian randomization analyses demonstrated a lack of an association between plasma homocysteine levels and IAs or aneurysmal subarachnoid hemorrhage.

KEYWORDS

Mendelian randomization, intracranial aneurysm, hyperhomocysteinemia, causality, cerebrovascular disease

Introduction

Intracranial aneurysm (IA) is confined, pathological dilatations of the walls of intracranial arteries that are at risk of rupture. About 85% of spontaneous subarachnoid hemorrhage (SAH) is due to ruptured IA (1). The incidence of IA was reported to be about 3.2% in a worldwide study with a mean age of 50 years (2). Aneurysmal SAH (aSAH) often has a poor prognosis, with high disability and mortality rates (3, 4). However, the etiopathology of IAs remains unclear.

Hyperhomocysteinemia has been widely reported to be associated with the development of cerebrovascular disease (5–8). Excessive homocysteine levels lead to inflammation of the vessel wall, atherosclerotic plaque formation, endothelial cell damage, smooth muscle cell proliferation, and altered oxidative stress response (9–11). These pathological changes play a critical role in the formation and rupture of IAs (12, 13). We, therefore, speculated that the formation and rupture of IAs may be associated with homocysteine.

Recent studies have shown an association between IAs and hyperhomocysteinemia in the Chinese Han population (14, 15). In 2011, a study reported that hyperhomocysteinemia in a rat model accelerated IA formation (16). However, it has been reported that homocysteine is not associated with the IAs in other races (17). Therefore, the association between IA formation and homocysteine remains unresolved.

Mendelian randomization (MR) is the use of genetic variation in non-experimental data to estimate the causal link between exposure and outcome, and it can reduce the impact of behavioral, social, psychological, and other factors (18). And in recent years, many MR studies have emerged to provide clinical evidence (19–21). This proves that MR is a reliable research method to solve some problems. Using recently published summary data for plasma homocysteine levels and summary data for IA in a genome-wide association study (GWAS), we aimed to analyze the causal connection between homocysteine and IA using two-sample MR.

Materials and methods

Genetic instruments and data sources

We used single-nucleotide polymorphisms (SNPs) associated with human plasma homocysteine levels as instrumental variables (IVs) for the primary analysis in a GWAS of 44,147 subjects of European ancestry (22).

We extracted SNPs associated with IA from a large GWAS involving 7,495 IA cases and 71,934 controls (23). The MR analysis was performed on three summary datasets from this GWAS. The three pooled datasets were GWAS of IA (ruptured, unruptured, and unknown rupture status) ($n = 7,495$), UIA-only ($n = 2,070$), and aSAH-only ($n = 5,140$) vs. controls ($n = 71,934$) in individuals of European ancestry.

The following steps were applied to select the best IVs to guarantee the accuracy and validity of the inferences on the causal relationship between the risk of IA and plasma homocysteine. The first step was to select SNPs with thresholds of significant association with the plasma homocysteine levels as IVs. A set of genome-wide statistically significant ($P < 5 \times 10^{-8}$) SNPs were used as IVs. Second, linkage disequilibrium (LD) must not exist between the selected IVs, because it can lead to interpretation bias. Among the selected SNPs, we performed

a clumping step (clumping distance = 10,000 kb, $R^2 < 0.001$) to reduce the LD during our MR analysis. Third, guaranteeing that the impact of SNPs on outcome and exposure is related to only one allele during MR analysis is an important condition, and in accordance, SNPs with a palindromic structure were removed.

Standard protocol approval, registration, and patient consent

All the data used in this MR analysis were based on summary data publicly available from the GWASs. Ethical approval and participant consent were not needed as they were previously obtained for each of the original GWASs.

The assumptions of MR

To investigate the causal impact of the plasma homocysteine on IA, genetic variation was used as an IV in MR. To serve as an IV, the following criteria must be met: the variation must be related to the plasma homocysteine; it must not be related to any confounding factor related to the plasma homocysteine or IA; it must not affect the outcome, except possibly through association with exposure (24). The F -statistic, whose formula is $F = \frac{R^2(n-k-1)}{k(1-R^2)}$, is commonly used to evaluate the strength of the correlation between exposure and IVs. Here, n represents the number of samples in the GWAS related to exposure, k represents the number of IVs, and R^2 is the extent to which IVs explain exposure. When the F -statistic is < 10 , we usually consider the IVs as weak, which may bias the results somewhat.

Statistical analysis

We used the inverse variance weighted (IVW), MR-Egger, weighted median, simple mode, and weighted mode methods to evaluate the causal link between IAs and plasma homocysteine. The IVW method is characterized by an analysis that does not take into account the presence of an intercept term and uses the inverse of the outcome variance (quadratic of the standard error) as a weight to provide a comprehensive estimate of the impact of the plasma homocysteine on the incidence of IA. Ensuring these SNPs are not pleiotropic when using the IVW method is important, otherwise, the results will be highly biased. The MR-Egger method can provide causal estimates that are unaffected by breaches of standard IV assumptions and can detect whether standard IV assumptions are violated (25). The weighted median method combines information from various hereditary variations into a solitary causal gauge, and that gauge is predictable even when half of the IVs are null (26).

To test whether horizontal pleiotropy was present among the included SNPs, we performed MR-Egger regression. To

examine for a potentially strong impact of an SNP and whether causal effect estimates were reliable, a leave-one-out analysis was performed. In addition, Cochran's Q-statistic was applied to examine whether heterogeneity was present among the selected SNPs. We calculated MR power through a web-based tool (<https://shiny.cnsgenomics.com/mRnd/>) (27). The statistical power under each odds ratio (OR) value was calculated by combining the proportion of cases with IA GWAS, the variance jointly explained by the instrumental variable single nucleotide polymorphisms (SNPs), and the sample size together (Supplementary Table 1). For the primary analysis using serum homocysteine, a relative difference of 21.2% was detected with 80% power (OR: 1.212/0.795) and an alpha value of 5% (Supplementary Table 1). The MR analyses were performed utilizing the TwoSampleMR package for R (version 4.1.2).

Results

First, we screened 18 SNPs as IVs (genome-wide statistical significance threshold, $P < 5 \times 10^{-8}$) from a GWAS of plasma homocysteine levels (22). After the removal of SNPs with LD, 13 SNPs remained as IVs ($P < 5 \times 10^{-8}$) (rs7422339, rs12134663, rs957140, rs12921383, and rs2851391 were removed). When homocysteine was analyzed against IAs and aneurysmal subarachnoid hemorrhage, two SNPs (rs838133 and rs548987) were found to be absent in the IA and aneurysmal subarachnoid hemorrhage datasets, and when homocysteine was analyzed with unruptured aneurysms, four SNPs (rs838133, rs548987, rs234709, and rs1801133) were absent in the unruptured aneurysm dataset. None of these SNPs have a proxy SNP. The SNPs we used and their association with IAs are shown in Table 1.

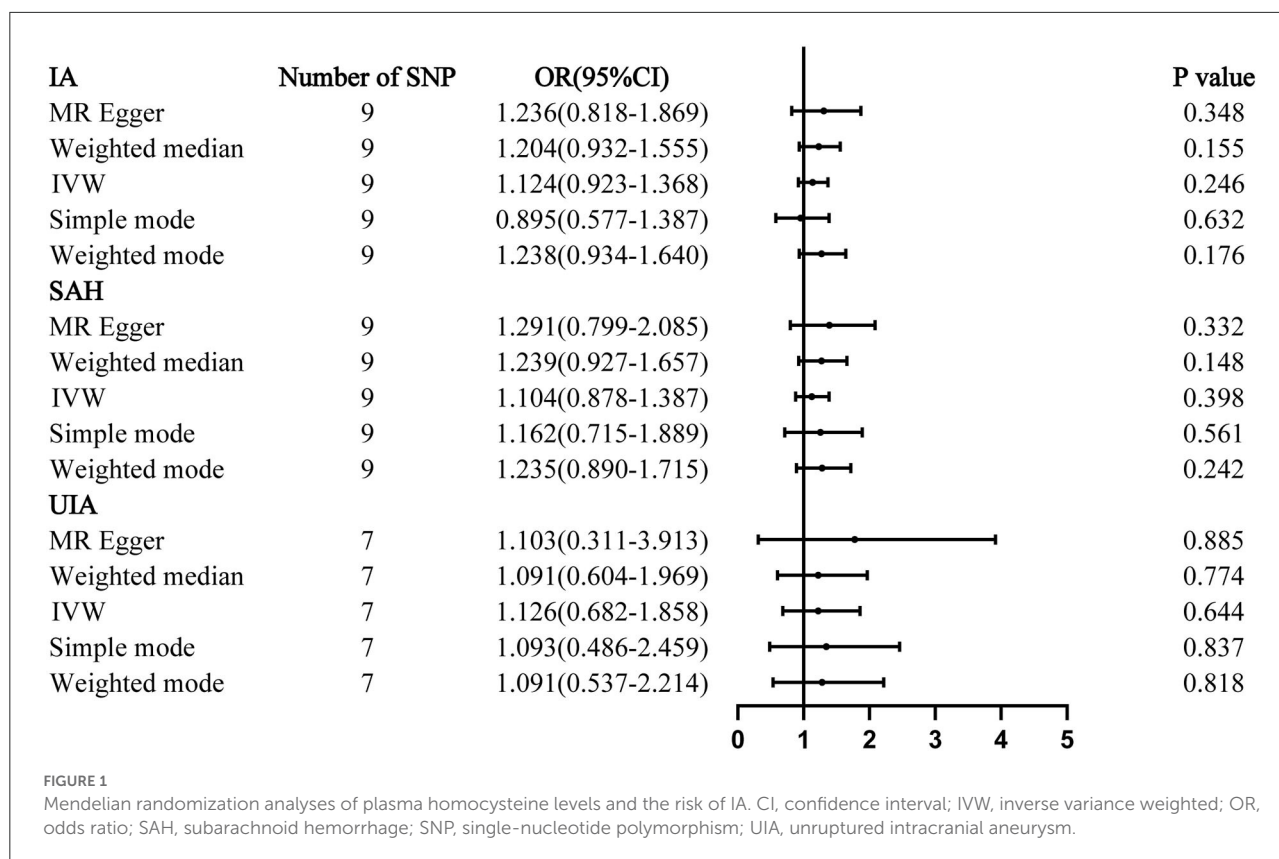
The MR-Egger regression indicated no horizontal pleiotropy in the analysis of the relationship between homocysteine and aneurysms ($P = 0.622$ for IA, $P = 0.491$ for aSAH, $P = 0.975$ for UIA). Furthermore, there were no weak instrumental variables (F -statistic: 100.340 for IA and aSAH, and 47.203 for UIA [all >10]). The Cochran's Q-statistics showed no significant heterogeneity ($P = 0.849$ for IA, $P = 0.943$ for aSAH, $P = 0.998$ for UIA). The limited number of SNPs included prevented examination of horizontal pleiotropy and heterogeneity.

The results of all MR analyses showed no association between IAs and homocysteine, with all $P > 0.05$ (Figure 1). The results of the IVW analysis for aSAH [OR: 1.104; 95% confidence interval (CI): 0.878–1.387, $P = 0.398$], IA (OR: 1.124; 95% CI: 0.923–1.368, $P = 0.246$), and UIA (OR: 1.126; 95% CI: 0.682–1.858, $P = 0.644$) showed no association between IAs and homocysteine.

TABLE 1 Characteristics of the single nucleotide polymorphisms used as instrumental variables for plasma homocysteine and their association with intracranial aneurysm.

rsID	Gene	plasma Hcy level		IA (unruptured and ruptured)		Unruptured IA		Ruptured IA (aSAH)					
		β	SE	p -value	β	SE	p -value	β	SE	p -value			
rs9369898	MUT	0.045	0.007	2.17E-10	-0.010	0.023	0.669	0.004	0.040	0.93	-0.016	0.027	0.56
rs7130284	NOX4	-0.124	0.013	1.88E-20	-0.020	0.040	0.609	-0.018	0.070	0.80	-0.017	0.046	0.72
rs4660306	MMACHC	0.044	0.007	2.33E-09	-0.004	0.023	0.868	-0.011	0.041	0.78	0.001	0.028	0.99
rs42648	GTPB10	-0.040	0.007	1.97E-08	-0.002	0.023	0.918	-0.004	0.040	0.91	-0.002	0.027	0.94
rs234709	CBS	-0.072	0.007	3.90E-24	-0.030	0.023	0.190	N/A	N/A	N/A	-0.022	0.026	0.39
rs2275565	MTR	-0.054	0.009	1.96E-10	-0.066	0.026	0.010	-0.058	0.047	0.22	-0.062	0.030	0.04
rs2251468	HNF1A	-0.051	0.007	1.28E-12	-0.019	0.022	0.390	-0.003	0.040	0.95	-0.021	0.025	0.39
rs1801222	CUBN	0.045	0.007	8.43E-10	0.017	0.022	0.431	0.029	0.041	0.48	0.010	0.025	0.70
rs1801133	MTHFR	0.158	0.007	4.34E-104	0.034	0.024	0.165	N/A	N/A	N/A	0.035	0.028	0.21
rs154657	DPEP1	0.096	0.007	1.74E-43	-0.018	0.023	0.434	0.008	0.040	0.83	-0.016	0.027	0.56
rs12780845	CUBN	0.053	0.009	7.80E-10	-0.007	0.024	0.780	0.005	0.042	0.91	-0.015	0.029	0.59

aSAH, aneurysmal subarachnoid hemorrhage; IA, intracranial aneurysm; Hcy, homocysteine.



Discussion

This MR study provides evidence that IAs are not associated with homocysteine in Europeans. To our knowledge, this is the first MR study on the association between plasma homocysteine levels and IAs.

Based on the data from the Global Burden of Disease Study 2019 (<https://www.healthdata.org>), stroke is the second leading cause of disability and mortality worldwide (28). Hyperhomocysteinemia has long been recognized as an independent risk factor for stroke (29). Hyperhomocysteinemia is common in the Chinese population (30). Hyperhomocysteinemia can lead to elevated inflammatory factors in blood vessels, damage to the vascular endothelium, and proliferation of vascular smooth muscle cells (31, 32). High homocysteine has been reported to promote atherosclerosis and increase the risk of ischemic strokes (11, 33, 34). Because mechanisms such as inflammation are involved in the formation and rupture of IAs, pathological changes caused by homocysteine may contribute to their formation and rupture. Xu et al. found accelerated IA formation in rats with methionine diet-induced hyperhomocysteinemia (16). Another study showed that methionine-induced hyperhomocysteinemia from excessive methionine intake promotes aneurysmal rupture in orchietomized rats (35). However, such studies are

lacking in humans, and therefore, the relationship between homocysteine and IAs has remained unknown. While some observational studies have reported that IAs are associated with hyperhomocysteinemia in the Chinese population, there is no evidence of a causal link (14, 15). In a Brazilian case-control study, IAs were reported to occur independently of hyperhomocysteinemia, and another study reported that hyperhomocysteinemia is not associated with abdominal aortic aneurysms (17, 36). Thus, the association between IAs and homocysteine remains questionable.

Elevated levels of serum homocysteine mainly cause a decrease in the antithrombotic effect of the vessel wall, increasing the risk of stroke (37). In contrast, aneurysm formation and rupture are mainly considered to be related to damage to the vessel wall and the release of inflammatory factors, and may not be related to the level of homocysteine. Serum levels of homocysteine can be elevated by nutritional deficiencies of folic acid, vitamin B6, and vitamin B12 in the diet. Dietary effects have not been considered in most studies of intracranial aneurysms and homocysteine. Elevated homocysteine levels may also be the result of a ruptured aneurysm; therefore, large prospective studies are still needed to confirm the relationship between aneurysms and homocysteine.

The fundamental benefit of this MR analysis is that estimates of the causal effect of MR were not affected by

confounding factors or reverse causal associations found in traditional epidemiological studies. Therefore, compared with observational studies, our current findings may be more reliable. Yet, several limitations remain. First, Genotypic variants in enzymes associated with blood homocysteine levels increase the risk of unprovoked pulmonary embolism (38). Due to the differences in genetic characteristics among different populations, our results may only apply to European populations because all participants in the GWAS were of European origin. Second, not all SNPs were examined, as some were removed because of LD (and no proxy SNPs were found), which may have impacted the results.

At the genetic level, the present MR study suggests that there is no causal relationship between hyperhomocysteinemia and IA or IA rupture. However, further studies are needed to more comprehensively assess the relationship between homocysteine and IAs.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

CM and HL conceived the study. LM, GZ, and YS performed the analyses and manuscript writing. HC and ZL were involved

in the acquisition of data. WZ and XX were involved in the interpretation of data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2022.948989/full#supplementary-material>

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