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A Mendelian randomisation, propensity score matching study to investigate causal association between serum homocysteine and intracranial aneurysm

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ABSTRACT

Background and purpose Recent observational studies have reported that serum total homocysteine (tHcy) is associated with intracranial aneurysms (IAs). However, the causal effect of tHcy on IAs is unknown. We leveraged large-scale genetic association and real-world data to investigate the causal effect of tHcy on IA formation.

Methods We performed a two-sample Mendelian randomisation (MR) using publicly available genome-wide association studies summary statistics to investigate the causal relationship between tHcy and IAs, following the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology-MR statement. Furthermore, a propensity score matching (PSM) analysis was conducted to evaluate the detailed effects of tHcy on risk of IA formation by utilizing real-world multicentre data, including 9902 patients with and without IAs (1:1 matched). Further interaction and subgroup analyses were performed to elucidate how tHcy affects risk of IA formation.

Results MR analyses indicated that genetically determined tHcy was causally associated with IA risk (OR, 1.38, 95% CI 1.07 to 1.79; p=0.018). This is consistent with the more conservative weighted median analysis (OR, 1.41, 95% CI 1.03 to 1.93; p=0.039). Further sensitivity analyses showed no evidence of horizontal pleiotropy or heterogeneity of single nucleotide polymorphisms in causal inference. According to the PSM study, we found that, compared with low tHcy (≤15 µmol/L), moderate tHcy (>15–30 µmol/L) (OR 2.13, 95% CI 1.93 to 2.36) and high tHcy (>30 µmol/L) (OR 3.66, 95% CI 2.71 to 4.95) were associated with a higher IA risk (p trend <0.001). Subgroup analyses demonstrated significant ORs of tHcy in each subgroup when stratified by traditional cardiovascular risk factors. Furthermore, there was also a synergistic effect of tHcy and hypertension on IA risk (p interaction <0.001; the relative excess risk due to interaction=1.65, 95% CI 1.29 to 2.01).

Conclusion Both large-scale genetic evidence and multicentre real-world data support a causal association between tHcy and risk of IA formation. Serum tHcy may serve as a biomarker to identify high-risk individuals who would particularly benefit from folate supplementation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Several studies reported the association between serum total homocysteine (tHcy) and intracranial aneurysms (IAs). We leveraged large-scale genetic association and real-world data to evaluate the causal effect of tHcy on IAs.

WHAT THIS STUDY ADDS

⇒ Genetically determined tHcy is causally associated with IAs. Propensity score matching of multi-centre real-world data validated this association and assessed the effect size of tHcy on IAs, which was modified by age, sex and hypertension.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We provide evidence that serum tHcy may be a biomarker for identifying individuals at high risk of IAs; it may also be a modifiable risk factor via folate supplementation.

INTRODUCTION

Intracranial aneurysm (IA) is a major public health burden that affects 3.2%–7.0% of the adult population.[1] Subarachnoid haemorrhages resulting from a ruptured IA are associated with a 35% mortality within 3 months and 50% of survivors suffer from serious neurological dysfunction.[2] The high morbidity and mortality of this disease are partly attributable to a lack of effective preventive approaches. IA rupture often occurs suddenly and most patients are not aware of the presence of an aneurysm.[3 4] Previous studies have aimed to identify the risk factors for the formation, growth and rupture of IA, but these investigations have been limited to unmodifiable risk factors such as gender, age, menopause, family history, etc.[5] The identification of novel biologic pathways underlying IA development is important to informing the development of new preventive and therapeutic strategies.

There are few IA biomarkers; these include plasma total homocysteine (tHcy),[6] which remains controversial.[7 8] tHcy is an amino acid generated by the metabolism of dietary methionine and is sensitive to vitamin B_{12}, folate and various lifestyle factors.[9] Multiple studies demonstrate a role for tHcy in endothelial injury,[10 11] the mechanisms of which overlap with the pathogenesis of IA.[12 13] Some clinical studies have questioned the relationship between tHcy and IAs; however, their findings are limited by the utilisation of small study cohorts and the conventional observational design of the research.[7 8 14] It remains unclear whether the association between circulating tHcy levels and IA is causal.

Mendelian randomisation (MR) analysis, thus, offers the most appropriate approach for evaluating the causal association between tHcy and IA formation from a genetic perspective. In this approach, gene data are used to test causal relationships between genetic variants and outcomes while avoiding confounding biases.[15] Several published MR studies report negative results but have not rigorously excluded potential modifiers and mediators; the reported single nucleotide polymorphisms (SNPs) have included rs1801133, rs154657 and rs7422339.[16 17] However, these findings violate MR criteria because these SNPs are strongly associated with hypertension ($p<5\times10^{-8}$) according to the results of genome-wide association studies (GWAS) from the UK biobank,[18] complicating the exact causal pathophysiological pathway.[19] In short, further convincing evidence is required to properly test what role tHcy plays in the occurrence of IAs.

In this study, we used a two-sample MR analysis and a large-sample, multicentre observational analysis to explore the effect of tHcy on IA formation from the perspective of genetic variation data and real-world data. These two research methods complement each other as MR analysis provides causal evaluations that redress the limitations of conventional observational studies, while the latter can assess the real effect size of tHcy on the risk of IA formation, which verifies the results of MR and remedy the deficiency of the MR evidence in extrapolation.

METHODS

Study design

We performed a two-stage study combining genetic and observational analyses. First, we tested for a causal association between tHcy and IAs by utilising a two-sample MR analysis following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)-MR recommendations.[19] Second, we explored the real effect size of tHcy on IA formation using electronic medical record data from a real-world and multicentre cohort of mainland Chinese IA and non-IA patients.

Stage 1: MR analysis

We performed a two-sample MR analysis to test for a tHcy-IA causal association. SNPs associated with serum tHcy concentrations at a genome-wide significance level of $p<5\times10^{-8}$ were selected from the currently largest genome-wide association meta-analysis, which included 44 147 individuals of European ancestry.[20] We pruned SNPs in linkage disequilibrium by applying stringent criteria ($r^2<0.01$ in 1000G EUR population; Clumping window 10 000 kb). The remaining 14 SNPs were evaluated to determine whether they were associated with other traits related to IAs by Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk). To replace SNPs that were not available in the outcomes data set, proxy SNP was searched in LDlink ($r^2>0.99$ in 1000G EUR population, https://ldlink.nci.nih.gov).

As the outcome data set, GWAS summary statistics for IAs were acquired from the International Stroke Genetics Consortium, consisting of 7495 IA cases and 71 934 European controls.[21] We used the inverse variance weighted (IVW) method to test the causal association between tHcy and IA, followed by confirmation using a more conservative weighted median approach. As a sensitivity analysis, we calculated the Cochran Q-derived P in the IVW method to assess the heterogeneity of the SNPs. Then, we performed the MR-Egger regression and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) global tests for horizontal pleiotropy. Finally, we used leave-one-out analysis to investigate the robustness of the MR estimates by removing each SNP individually from the regression model based on the IVW method.

Stage 2: real-world multicentrer cohort

Data sources and study population

We collected retrospective data from IA and non-IA adults from electronic medical records between January 2010 and April 2022 in Zhujiang Hospital, Beijing Tiantan Hospital and Guangdong Provincial People's Hospital. Baseline characteristics were collected at the initial IA diagnosis or the last exclusionary diagnosis for non-IA patients during hospitalisation or from outpatient services. Venous blood markers were tested during fasting; 1.6% of emergency cases had an uncertain fasting state during the blood draw. Data were independently extracted from electronic medical records by two individuals and validated by two additional neurosurgeons. Included baseline characteristics were age, sex, blood pressure, dyslipidaemia, diabetes and histories of smoking and alcohol consumption. All tests of tHcy were based on an enzymatic cycling assay from three centres. For patients with IA, the inclusion criteria included: (1) IA diagnosis (ruptured or unruptured) by digital subtractive angiography (DSA); (2) aged ≥18 years; (3) tHcy, blood pressure, blood lipid and glucose levels were recorded; (4) no history of coronary heart disease (CHD), stroke, intracranial arteriovenous malformation (AVM), Moyamoya, polycystic kidney disease, Ehlers-Danlos syndrome, Gronblad-Strandberg syndrome or Marfan syndrome; (5) no family histories of stroke or IA and (6) not pregnant. For non-IA patients, in addition to meeting criteria (2)–(6) few had DSA-based exclusion without AVM, Moyamoya and other cerebrovascular diseases, so we also included patients who

underwent cranial CT angiography (CTA) or MR angiography (MRA). The presence or absence of IAs was stringently screened using formal reading reports of the DSA, MRA or CTA by two radiologists and then confirmed by two treating neurosurgeons from raw images. The neurosurgeons were blinded to the tHcy results. In addition, we excluded any individuals suspected of harbouring a dissecting or mycotic aneurysm or aneurysm-like lesions, which were indistinguishable from infundibulum, fenestration, dilation, or atherosclerotic remodelling on structural imaging scans. Any disagreement with the IA diagnosis was resolved by two chief neurosurgeons.

Propensity score matching

To control for potential non-random assignment of patients, a logistic regression model that predicts the likelihood of IAs was constructed and used as the propensity score. Patients were propensity-matched in a 1:1 ratio into the IA and non-IA groups by using the nearest neighbour matching algorithm with a calliper width equal to 0.05 of the SD of the logit of the propensity score. Variables used for matching included age, sex, blood pressure, dyslipidaemia and diabetes. No replacement was allowed, and patients were matched only once. Inverse probability of treatment weighting (IPTW) was used to verify the robustness of the analysis.

Interactive effect analyses

Within the matched IA and non-IA groups, we assessed for heterogeneity in the tHcy effects with tests of interaction among covariates used in propensity score matching (PSM) by entering multiplicative or additive interaction terms into a logistic regression model. Subgroup analyses by age (<40, ≥40–59, ≥60), sex (male/female) and hypertension status (present/absent), diabetes (present/absent), dyslipidaemia (present/absent) were performed to evaluate the interaction between tHcy with these covariates modifying the IA risk.

Statistical analysis

For the effect estimates in stage 1, we calculated ORs with 95% CIs per 1-SD tHcy increase. In stage 2, serum tHcy concentrations were subdivided into three categories: (1) low (≤15 µmol/L); (2) moderate (>15–30 µmol/L) and (3) high (>30 µmol/L) and the risk of IA formation was expressed as an OR by taking low tHcy as the reference.[22 23] The ORs were estimated by the univariate or multivariate logistic regression model. Two-tailed tests of probability (p<0.05) were used to estimate statistical significance; 95% CIs are provided for all analyses. For normally distributed variables, group differences were tested using Student's t-tests or paired t-tests; Mann-Whitney U-test or Wilcoxon test were used for non-normally distributed variables. Categorical variables and their differences were analysed with X^2 tests. A test for linear trend was conducted using the classification of tHcy as a continuous variable. MR analysis was executed using the TwoSampleR (V.0.5.6) and MRPRESSO (V.1.0) packages. PSM was performed using the MatchIt (V.4.4.0) and interaction analysis was performed using the epiR (V.2.0.52) packages for R (V.4.2.1).

RESULT

Stage 1: Mendelian randomisation

Selection process of SNPs

Nine SNPs met our screening criteria as shown in online supplemental table S1. Eighteen SNPs were associated with tHcy extracted from the GWAS meta-analysis of tHcy and 14 remained after being pruned by our stringent criterion (r^2 <0.01 in 1000G EUR population; Clumping window, 10 000 kb). Without any suitable proxy SNPs, rs548987 and rs838133 were removed as they were absent in the outcome data set. We then excluded rs1801133, rs7422339 and rs154657 due to their recognised relationships with blood pressure or hypertension according to the results of GWAS from the UK biobank.[18]

Causal effects of tHcy on IAs

Two-sample MR analysis provided evidence for a causal association between tHcy and IAs. tHcy was causally associated with a 38% increase in IAs risk (unruptured or ruptured) among the EUR population per 1-SD increase in genetically determined tHcy levels (OR, 1.38, 95% CI 1.07 to 1.79; p=0.018) (figure 1) when using the IVW method. This is consistent with the more conservative weighted-median analysis approach (OR, 1.41, 95% CI 1.03 to 1.93; p=0.039). For the sensitivity analysis, the Cochran Q statistic based on IVW (Q=6.67, p=0.57) and MR-Egger (Q=6.63, p=0.47) showed no evidence of heterogeneity of these nine SNPs. MR-Egger regression (intercept=−0.022, p=0.85) and the MR-PRESSO global test (residual sum of squares, 8.15, p=0.60) showed no evidence of horizontal pleiotropy. The leave-one-out analysis confirmed that our findings were robust and reliable as the causal estimate was not driven by any single SNP (online supplemental figure S1).

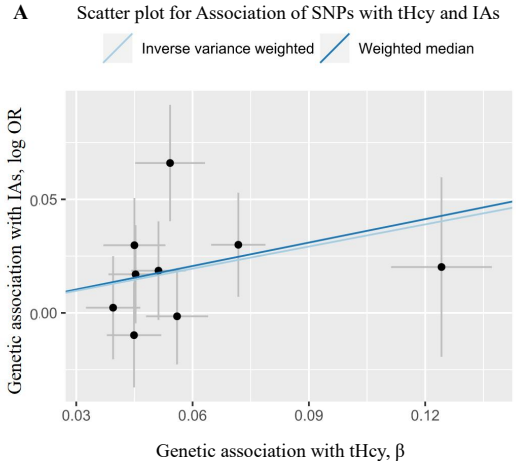
Stage 2: observation of a real-world multicentre cohort

Study population

We identified 15 327 patients who met our inclusion criteria (figure 2); 5496 (35.9%) had DSA-diagnosed IAs and 9831 (64.1%) had IAs excluded by DSA, CTA or MRA. Of all included patients, 4951 IA cases could be matched (1:1) to a non-IA control based on our criteria. There were no significant differences in the baseline demographics and clinical characteristics between the groups matched by PSM except for tHcy levels (tables 1 and 2); effective matching was also reached by utilising the IPTW approach (online supplemental table S2).

Association of tHcy with IAs

Within the PSM-matched cohort (table 2), the IA group had a higher concentration of tHcy (14.4 vs 11.7 µmol/L, p<0.001; Cohen's d=0.33, 95% CI, 0.29 to 0.37). For a per-SD increase (8.2 µmol/L) in tHcy, the OR for IA risk was 1.82 (95% CI 1.70 to 1.95) and for a per 5 µmol/L



B Results for Two-sample Mendelian Randomisation and Sensitive analyses

	P	OR (95% CI)
Two-sample MR Analyses		
IVW	.018	1.38 (1.07;1.79)
Weighted median	.039	1.41 (1.03;1.93)
Sensitive Analyses		
Test for pleiotropy		
MR Egger-Intercept	.851	
MR-PRESSO	.602	
Test for heterogeneity		
MR Egger	.468	
IVW	.572	

Figure 1 Mendelian randomisation results for genetically determined tHcy and risk of IAs. A, scatter plot for association of nine SNPs between tHcy and IAs. β, coefficient presented as the number of standard deviation of difference in homocysteine concentrations per allele; B, summary results for two-sample mendelian randomisation and sensitive analyses. MR pleiotropy residual sum and MR-PRESSO global tests indicated no sign of horizontal pleiotropy. Test for heterogeneity showed no strong heterogeneity between nine SNPs and the outcome of IAs. Abbreviations: SNPs and the outcome of IAs. Abbreviations: IAs, intracranial aneurysms; IVW, inverse variance weighted; MR-PRESSO, MR-Egger regression and MR Pleiotropy Residual Sum and Outlier; SNPs, single nucleotide polymorphisms; tHcy, total homocysteine.

increase in tHcy, the OR was 1.44 (95% CI 1.38 to 1.50) (table 3). Using the categorised values, moderate (OR 2.13, 95% CI 1.93 to 2.36) and high tHcy (OR 3.66, 95% CI 2.71 to 4.95) were associated with a higher IA risk, with a linear positive correlation (p-trend <0.001). After adjustment for age, sex, blood pressure level and smoking in the multivariate logistic regression model 1, the OR of tHcy remained significantly associated with the risk of IA formation before and after matched, with a higher OR of tHcy in the PSM-matched cohort (table 3). The adjusted ORs of tHcy were similar and remained statistically significant on the whole when estimated by model 2, which included variables from model 1 plus dyslipidaemia and diabetes. A similar effect size of tHcy was confirmed in the IPTW analysis model (online supplemental table S3).

Exploratory subgroup analyses of the PSM-matched cohort showed significant ORs of tHcy and a linear positive trend (all p trend <0.01) in each subgroup when stratified by age (<40, ≥40–59, ≥60), sex (male/female), hypertension (present/absent), diabetes (present/absent) and dyslipidaemia (present/absent). The test for interaction between tHcy and hypertension was significant (p interaction <0.001) and tHcy-hypertension synergism was detected (the relative excess risk due to interaction, RERI=1.65, 95% CI 1.29 to 2.01; the proportion attributable to interaction, AP=0.66, 95% CI 0.58 to 0.74) by the additive interaction model (online supplemental table S4). Further subgroup analysis based on hypertension (present/absent) suggested that the tHcy association with IA risk was independent of hypertension: (1) moderate tHcy, OR 3.32 (95% CI 2.80 to 3.94); (2) high tHcy, OR 8.05 (95% CI 4.65 to 13.90) in patients without hypertension (n=4344) (figure 3). Similarly, the interaction between tHcy and sex was also significant (p interaction <0.001) with a greater effect size of tHcy observed in men (high, OR 4.63, 95% CI 3.25 to 6.59) compared with women (high, OR 2.61, 95% CI 1.42 to 4.77). The subgroup analysis by age also provided evidence suggestive of effect modification by age, with a larger effect size of tHcy in patients aged ≥60 (moderate, OR 2.20 (95% CI 1.92 to 2.53); high, OR 5.34 (95% CI 3.09 to 9.24), p interaction=0.004). No significant interaction was observed between tHcy and the subgroup stratified by diabetes and dyslipidaemia status (present/absent).

DISCUSSION

In this study, we use a two-sample MR analysis of genetic variations and found evidence of a genetic-based increase in tHcy that is causally associated with the risk of IAs. This was followed up with a 1:1 propensity score-matched cohort consisting of 9902 patients, which verified that tHcy is a risk for IAs; the relationship was positively correlated with a linear trend. We found that the effects of tHcy on IA formation are dose dependent but independent of hypertension and modified by hypertension, age, and sex.

To our knowledge, this is the first MR analysis testing the association between tHcy and IAs using SNPs under the criteria of instrumental variables' assumption in MR studies,[19] and the largest multicentre observational study was conducted on this topic. Two published MR studies on tHcy and IA used three gene variants, rs1801133, rs7422339 and rs154657,[16 17] which are associated with hypertension; this approach violates the principle of MR and, thus, leads us to question the negative findings. After removing these SNPs associated with confounders, we reassess the causal relationship between tHcy and IAs by a strict two-sample MR analysis following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)-MR.[19] Further sensitivity analyses indicated

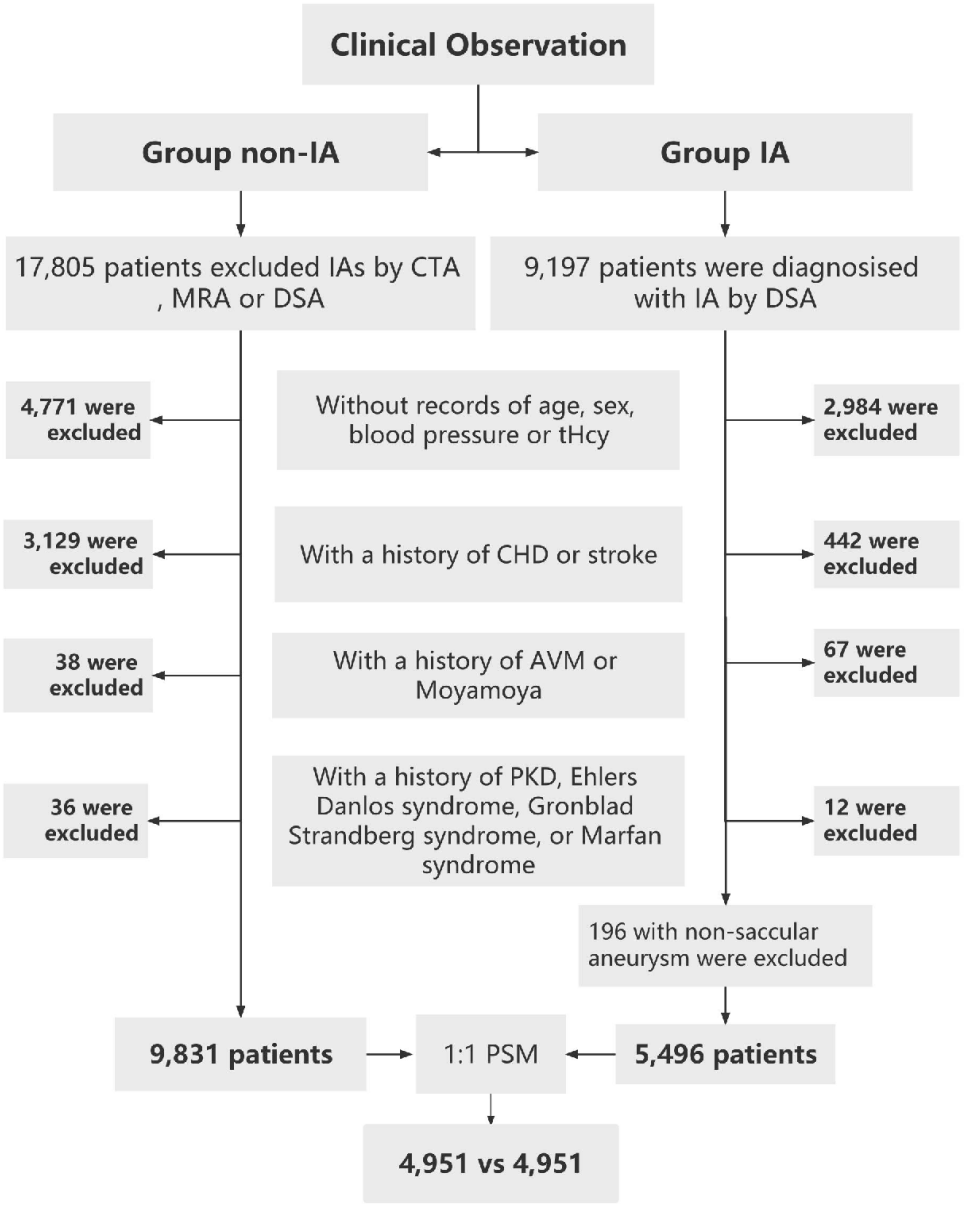


Figure 2 Flow diagram of patients inclusion. AVM, arteriovenous malformation; CHD, coronary heart disease; CTA, CT angiography; DSA, digital subtraction angiography; IAs, intracranial aneurysms; MRA,magnetic resonance angiography; PKD, polycystic kidneydisease; PSM, propensity score matching.

no evidence of horizontal pleiotropy confounding the causal inference, confirmed by the MR-Egger regression (p=0.85) and the MR-PRESSO test (p=0.60). However, the deficiency of MR evidence was that the estimation of the effect size of tHcy on IA risk was based on exposure to abnormal tHcy for a life long time, while being independent of other risk factors such as hypertension and age. This was not correspondent with clinical practice, and, therefore, we further verified the genetic effect size in a real-world cohort using multicentre data. By observing the real effect size and heterogeneity of tHcy for risk of IA formation among different subgroups, it makes such association embodied concretely in clinical settings and reveals the potential value of the application of tHcy in identifying specific population at risk of IA. This approach provides high-confidence findings that could only be surpassed by a large prospective cohort study or randomised clinical trial which, for ethical reasons, cannot be conducted. What is more, our results differed from the previously negative conclusions. [7] The large-sample, multicentre cohort could guarantee the reliability of our results, with strict inclusion and exclusion of patients and rigorous statistical methods mitigating potential distortion and bias.

The relationship between tHcy and IA occurrence was positively correlated with a linear trend. Taking low tHcy as a reference, the ORs increased with increasing tHcy (moderate tHcy, OR 2.13 (95% CI 1.93 to 2.36) and high tHcy, OR 3.66 (95% CI 2.71 to 4.95), p trend<0.001). Similar tHcy risks exist in CHD and stroke, where

Table 1 Baseline characteristics of patients before and after propensity score matching

	Before matched			After matched		
	Non-IAs	IAs	P	Non-IAs	IAs	P
	n=9831	n=5496		n=4951	n=4951	
Sex, number (%)			<0.001			0.063
Male	5693 (57.9)	2254 (41.0)		2264 (45.7)	2171 (43.8)	
Female	4138 (42.1)	3242 (59.0)		2687 (54.3)	2780 (56.2)	
Age, mean, (SD), years	63.9 (13.5)	57.8 (12.0)	<0.001	58.0 (12.9)	58.4 (12.0)	0.072
Age class, number (%)			<0.001			0.190
18–39 years	477 (4.9)	394 (7.2)		404 (8.16)	357 (7.21)	
40–59 years	2981 (30.3)	2656 (48.3)		2215 (44.7)	2258 (45.6)	
≥60 years	6373 (64.8)	2446 (44.5)		2332 (47.1)	2336 (47.2)	
BP class, mm Hg, SBP/DBP, number (%)			<0.001			0.939
<140/<90	3449 (35.1)	2434 (44.3)		2174 (43.9)	2170 (43.8)	
140~159/90~99	3404 (34.6)	1111 (20.2)		1094 (22.1)	1089 (22.0)	
160~179/100~109	2065 (21.0)	888 (16.2)		872 (17.6)	859 (17.4)	
≥180/≥110	913 (9.3)	1063 (19.3)		811 (16.4)	833 (16.8)	
Hyperlipidaemia, number (%)			<0.001			0.431
No	4726 (48.1)	3060 (55.7)		2665 (53.8)	2705 (54.6)	
Yes	5105 (51.9)	2436 (44.3)		2286 (46.2)	2246 (45.4)	
Diabetes, type 1 and 2, number (%)			<0.001			0.560
No	7098 (72.2)	4319 (78.6)		3879 (78.3)	3854 (77.8)	
Yes	2733 (27.8)	1177 (21.4)		1072 (21.7)	1097 (22.2)	
Smoke*, number (%)			<0.001			0.291
No	7684 (78.2)	4778 (86.9)		4297 (86.8)	4260 (86.0)	
Yes	2147 (21.8)	718 (13.1)		654 (13.2)	691 (14.0)	
Alcohol use*, number (%)			<0.001			0.125
No	7892 (90.0)	4775 (92.6)		4604 (93.0)	4563 (92.2)	
Yes	875 (10.0)	383 (7.4)		347 (7.0)	388 (7.8)	

*Missing data were imputed for 830 (5.4%) and 1402 (9.1%) patients about smoking and alcohol using by Multiple Imputation utilizing Mice package (3.14) for R (4.2.2).
BP, blood pressure; DBP, diastolic blood pressure; IAs, intracranial aneurysms; SBP, systolic blood pressure.

increases of 3–5 µmol/L markedly increase the risk of disease.[24 25] In these conditions, it is widely recommended that tHcy ≥15 µmol/L is classified as hyperhomocysteinemia (16 µmol/L in the American Heart Association/American Stroke Association (AHA/ASA) Guideline) to assist therapeutic decision-making.[23 26] Nevertheless, the OR of group tHcy (>13.2 µmol/L) increasing risk of IAs was also observed with a smaller CI when subdividing tHcy into three groups by tertiles (OR 3.12, 95% CI 2.82 to 3.45, taking tHcy <10 µmol/L as the reference). It is suggested that further prospective studies are warranted to establish a better cut-off value of tHcy for stratifying IA risk.

Table 2 Distribution of tHcy among patients before and after propensity score matching

	Before matched			After matched		
	Non-IAs	IAs	P	Non-IAs	IAs	P
	n=9831	n=5496		n=4951	n=4951	
tHcy* (SD), µmol/L	12.9 (6.3)	14.2 (9.8)	<0.001*	11.7 (5.4)	14.4 (10.1)	<0.001†
Group tHcy, number (%)			<0.001			<0.001
Low	7647 (77.8)	3905 (71.1)		4155 (83.9)	3464 (70.0)	
Moderate	1999 (20.3)	1405 (25.6)		739 (14.9)	1313 (26.5)	
High	185 (1.9)	186 (3.4)		57 (1.2)	174 (3.5)	
Moderate+high	2184 (22.2)	1591 (28.9)		796 (16.1)	1487 (30.0)	

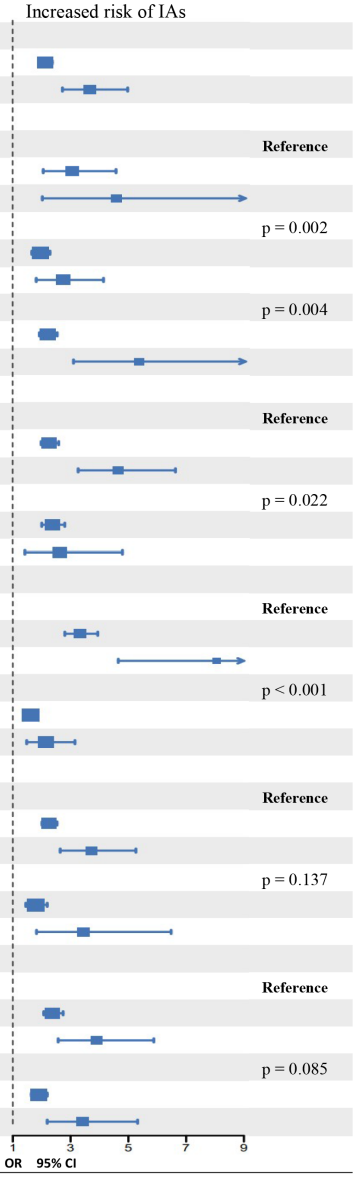
*P value based on Mann-Whitney U-test.
†P value based on Wilcoxon test.
IAs, intracranial aneurysms; tHcy, total homocysteine.

Table 3 Association between tHcy and intracranial aneurysm

	Before matched						After matched					
	Crude OR		Model 1*		Model 2†		Crude OR		Model 1*		Model 2†	
			Adjusted OR		Adjusted OR				Adjusted OR		Adjusted OR	
	95% CI	P	95% CI	P	95% CI	P	95% CI	P	95% CI	P	95% CI	P
tHcy‡ (SD), μmol/L	1.23 (1.18 to 1.28)	<0.001	1.50 (1.43 to 1.57)	<0.001	1.50 (1.43 to 1.57)	<0.001	1.82 (1.70 to 1.95)	<0.001	1.99 (1.85 to 2.13)	<0.001	1.98 (1.85 to 2.14)	<0.001
tHcy§	1.13 (1.11 to 1.16)	<0.001	1.30 (1.24 to 1.33)	<0.001	1.29 (1.25 to 1.33)	<0.001	1.44 (1.38 to 1.50)	<0.001	1.51 (1.45 to 1.59)	<0.001	1.52 (1.45 to 1.59)	<0.001
Group tHcy, number (%)								<0.001¶		<0.001¶		<0.001¶
Low	Reference.		Reference.		Reference.		Reference.		Reference.		Reference.	
Moderate	1.38 (1.27 to 1.49)	<0.001	1.72 (1.47 to 2.00)	<0.001	1.71 (1.61 to 1.82)	<0.001	2.13 (1.93 to 2.36)	<0.001	2.70 (2.19 to 3.37)	<0.001	1.80 (1.67 to 1.94)	<0.001
High	1.97 (1.60 to 2.42)	<0.001	0.94 (0.85 to 1.05)	0.281	2.06 (1.76 to 2.40)	<0.001	3.66 (2.71 to 4.95)	<0.001	0.90 (0.78 to 1.04)	0.200	2.69 (2.17 to 3.34)	<0.001
Moderate+high	1.43 (1.32 to 1.54)	<0.001	1.59 (1.50 to 1.69)	<0.001	2.20 (2.02 to 2.39)	<0.001	2.24 (2.03 to 2.47)	<0.001	1.88 (1.75 to 2.02)	<0.001	1.87 (1.74 to 2.00)	<0.001

P-value for OR (odd ratios) was based on Wald tests.
*Adjusted for age, sex, hypertension and smoking.
†Adjusted for variables from Model 1 plus dyslipidaemia and diabetes.
‡: OR was estimated by one standard difference increase of tHcy (8.2 μmol/L).
§: OR was estimated by a increase of 5 μmol/L of tHcy.
¶: P value for trend.
tHcy, total homocysteine.

Subgroups	tHcy	No. (%) IAs	No. (%) non-IAs	OR 95% CI	Interaction*
All patients		4951	4951		
	Low	3464 (70.0)	4155 (83.9)	**Reference**	
	Moderate	1313 (26.5)	739 (14.9)	2.13 (1.93;2.36)	
	High	174 (3.5)	57 (1.15)	3.66 (2.71;4.95)	
Age					
18-39 y	Low	242 (67.8)	353 (87.4)	**Reference**	**Reference**
	Moderate	90 (25.2)	43 (10.6)	3.05 (2.05;4.55)	
	High	25 (7.0)	8 (2.0)	4.56 (2.02;10.30)	
40-59 y	Low	1684 (74.6)	1896 (85.6)	**Reference**	p = 0.002
	Moderate	494 (21.9)	286 (12.9)	1.94 (1.66;2.28)	
	High	80 (3.5)	33 (1.5)	2.73 (1.81;4.12)	
≥60 y	Low	1538 (65.8)	1906 (81.7)	**Reference**	p = 0.004
	Moderate	729 (31.2)	410 (17.6)	2.20 (1.92;2.53)	
	High	69 (3.0)	16 (0.7)	5.34 (3.09;9.24)	
Sex					
Male	Low	1213 (55.9)	1710 (75.5)	**Reference**	**Reference**
	Moderate	820 (37.8)	512 (22.6)	2.26 (1.98;2.58)	
	High	138 (6.4)	42 (1.9)	4.63 (3.25;6.59)	
Female	Low	2251 (81.0)	2445 (91.0)	**Reference**	p = 0.022
	Moderate	493 (17.7)	227 (8.4)	2.36 (2.00;2.79)	
	High	36 (1.29)	15 (0.6)	2.61 (1.42;4.77)	
Hypertension†					
no	Low	1510 (69.6)	1940 (89.2)	**Reference**	**Reference**
	Moderate	566 (26.1)	219 (10.1)	3.32 (2.80;3.94)	
	High	94 (4.33)	15 (0.7)	8.05 (4.65;13.90)	
Yes	Low	1954 (70.3)	2215 (79.8)	**Reference**	p < 0.001
	Moderate	747 (26.9)	520 (18.7)	1.63 (1.43;1.85)	
	High	80 (2.9)	42 (1.5)	2.16 (1.48;3.15)	
Diabetes, type 1 and 2					
no	Low	2708 (70.3)	3290 (84.8)	**Reference**	**Reference**
	Moderate	1011 (26.2)	545 (14.1)	2.25 (2.01;2.53)	
	High	135 (3.5)	44 (1.1)	3.73 (2.64;5.26)	
Yes	Low	756 (68.9)	865 (80.7)	**Reference**	p = 0.137
	Moderate	302 (27.5)	194 (18.1)	1.78 (1.45;2.19)	
	High	39 (3.6)	13 (1.2)	3.43 (1.82;6.48)	
Dyslipidemia					
no	Low	1890 (69.9)	2272 (85.3)	**Reference**	**Reference**
	Moderate	718 (26.5)	363 (13.6)	2.38 (2.07;2.74)	
	High	97 (3.6)	30 (1.1)	3.89 (2.57;5.88)	
Yes	Low	1574 (70.1)	1883 (82.4)	**Reference**	p = 0.085
	Moderate	595 (26.5)	376 (16.4)	1.89 (1.64;2.19)	
	High	77 (3.4)	27 (1.2)	3.41 (2.19;5.32)	



*: p value for interaction was based on multiplicative interaction model by subgroup variables × tHcy.

†: Hypertension, defined as those with systolic blood pressure ⩾ 140mmHg or diastolic blood pressure ⩾ 90mmHg.

Figure 3 Forest plot for subgroup analyses. IAs, intracranial aneurysms; tHcy, total homocysteine.

Further subgroup analysis stratified by age demonstrated that higher ORs were observed in IA patients aged ≥60 years, suggesting that the detrimental effects of tHcy may develop over the lifetime. Multiple experimental studies have shown that tHcy contributes to endothelial dysfunction by inducing the inflammatory response, oxidative stress, DNA damage and cell apoptosis.[27–29] We theorise that chronically abnormal tHcy impairs the endothelial cell and smooth muscle cell remodelling function, weakening the arterial wall and thereby permitting IA formation.[13] Based on these considerations, we hypothesise that tHcy influences the formation of IAs by weakening the arterial wall over time, in a concentration-dependent manner. Further experimental validation in cell and animal models is required to confirm this hypothesis. These findings agree with the higher population-attributable risk of tHcy for stroke in the population of older than 60, which was estimated from he NHANES Epidemiologic Follow-up Study III.[26] Based on our findings and that of others, tHcy may be a potential biomarker, and individuals with higher tHcy levels should be assessed for IA risk, including early screening using MRA/CTA of older patients with a presumably prolonged exposure to higher tHcy.

Hyperhomocysteine correlates with hypertension and human and animal studies have shown that attenuation of hypertension can prevent IA rupture,[30] but no evidence directly addresses whether tHcy causes IA formation by mediating hypertension. Our MR analyses support a causal relationship between tHcy and IA risk independent

of hypertension and any other known confounders such as smoking. Further verified by observational analysis, the OR of high tHcy was 8.05 (95% CI, 4.65 to 13.90) among patients without hypertension; this risk was even higher than in those with hypertension. It needs cautious interpretation for the reason that the effect of tHcy on the risk of IA formation may be masked by hypertension to a certain degree, which is a well-established traditional risk factor for IA.[1] In general, the simultaneous presence of hyperhomocysteine and hypertension also produce a positive interaction for IA formation (RERI[31]=1.65, 95% CI 1.29 to 2.01; AP=0.66, 95% CI 0.58 to 0.74, online supplemental table S4). These findings are clinically relevant, as tHcy screening for hyperhomocysteinemia (tHcy ≥15 µmol/L) is prioritised in patients at risk of CHD and stroke widely for those with hypertension,[22 23] whereas our findings suggest that such screening should be broadened. In particular, patients with tHcy >30 µmol/L need to be identified and promptly treated.

The association between tHcy and IA formation is independent of sex. A significant interaction between sex and tHcy was tested, demonstrating that men with high tHcy were associated with greater odds of IA formation than women. For one thing, it is well established that men have a higher mean concentration of tHcy than women throughout all ages.[23] For another, men exposed to high tHcy have a higher rate of hypertension and unhealthy lifestyles such as smoking.[23 32 33] Therefore, men with high tHcy potentially suffer more from additive adverse effects, contributing to higher risks for IA formation than women.

tHcy is also a treatable risk factor as low-cost interventions are available; these include dietary changes, and supplementation with folate or vitamin B.[23] Folate or vitamin B therapy treatment of patients with increased tHcy can effectively reduce the incidence of ischaemic stroke and CHD.[34 35] Further MR and clinical evidence is warranted to test whether folate or vitamin B supplementation can decrease the risk of IA occurrence.

There are some limitations to our work. (1) The two-sample MR study used GWAS data from a European population and the real effects of tHcy were explored in a Chinese population. Despite the genetic and non-genetic population differences, the direction of elevated tHcy contributing to IAs risk remains consistent among these two populations. (2) We used a retrospective case–control study to verify tHcy effects on IA formation; this limits the level of evidence but a prospective longitudinal cohort study would be too costly to implement and a randomised control study is not feasible. (3) Balanced baseline characteristics achieved by PSM were limited to observed confounders because analyses do not address unobserved confounders associated with the formation of IAs. (4) Limited by the retrospective nature of the available data, we did not take folate and B vitamin therapy into consideration; this might influence the estimated effect size of tHcy. (5) Although the adjustment for dyslipidaemia and diabetes did not change the results in general, further studies are warranted to investigate whether different classifications of dyslipidaemia or diabetes are associated with the risk of IA formation. (6) Despite the causal role of tHcy on IA formation evaluated by MR, patients with ruptured IA might have acute changes in tHcy, confounding the estimation of ORs in the observational analyses. We are conducting a further study focusing on the association of tHcy with the development (growth/rupture), quantity and size of IA to elaborate on this topic.

CONCLUSION

In conclusion, we report genetic and epidemiological evidence that tHcy plays a causal role in IAs formation. We found that a genetic increase in tHcy was causally associated with a higher risk of IA. Real-world data revealed that tHcy increases the risk of IAs in a dose-dependent manner. Our results imply a tHcy-hypertension synergism in IA formation, but tHcy increases the risk of IAs independently, irrespective of hypertension status. Our findings may help uncover the mechanisms of IA pathophysiology and are expected to contribute to more aggressive screening for tHcy in future guidelines. Further studies including experimental research and longitudinal clinical trials are needed to better characterise the true effect size of tHcy on the risk of IA formation.

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