The Association between Statin Medication Use and Intracranial Aneurysm Risk: A Two- Way Mendelian Randomization Study

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Abstract. Recent observational studies have identifi ed a potential link between statin medication use and the risk of intracranial aneurysms (IAs). However, the causal relationship between these factors is not yet clear.Methods:We used a two-way Mendelian randomization approach to examine the relationship between genetically predicted statin medication use and the risk of IAs, as well as the reverse association. We incorporated data from genome-wide association studies of statin medication and IAs in a European population. Our analysis relied on random-effects inverse variance weighted estimation as the primary statistical method.Results:Neither statin medication use nor IA risk was signifi cantly associated with the other, according to ourfindings. The odds ratio (OR) for statin medication was 1.551 (95% confi dence interval [CI]: 0.895–2.685,P = 0.117), and the OR for IA risk was 1.020 (95% CI: 0.984–1.059, P = 0.281). Our results were consistent across different analytical methods, including MR-Egger regression and weighted median.Conclusions:These fi ndings suggest that there is no causal relationship between statin medication use and IA risk.

Keywords: Mendelian randomization, statin medication, intracranial aneurysms, Genome-Wide Association Study, data mining.

1. Introduction

Intracranial aneurysms (IAs) are a prevalent cerebrovascular disease that affects approximately 2.0–4.0% of the global adult population [1]. The rupture of IAs often leads to catastrophic subarachnoid hemorrhage (SAH), with a mortality rate of 15%-30% within one month and over one-third of survivors experiencing signifi cant neurological impairment [2]. Currently, it is widely accepted that arterial wall remodeling, driven by brain blood fl ow-induced arterial infl ammation, is the primary mechanism responsible for the development of brain aneurysms [3]. This process is initiated by the action of various infl ammatory mediators, including matrix metalloproteinases (MMPs), which degrade extracellular matrix and eliminate smooth muscle cells (SMCs), resulting in thinner walls and increased susceptibility to

aneurysm rupture [4]. In addition, macrophage infi ltration into the aneurysm wall is recognized as a crucial factor in the initiation and progression of IAs [5]. Given the importance of preventing and treating brain aneurysms, it is imperative to explore effective pharmacological interventions. Unfortunately, at present, no specifi c medication exists to mitigate the development and progression of brain aneurysms.

Statin medication belong to a class of drugs known as 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors. They have long been used as lipid-lowering agents. In addition to

their primary function, statin medication have been shown to exhibit multifaceted effects, including the reduction of

arterial plaques [6], alleviation of Alzheimer's disease [7], and treatment of coronary artery disease [8].Several recent studies suggest that statin medication may inhibit the progression and rupture of intracranial aneurysms (IAs) [9, 10]. However,observational studies have also reported no such effect of statin medication on IAs [11, 12]. Thus, the role of statin medication in the context of IAs remains elusive. Mendelian randomization (MR) uses single nucleotide polymorphisms (SNPs) as instrumental variables(IVs) to infer a causal relationship between two traits, with the advantage of minimizing confounding factors and bias due to reverse causation. In this study, we employed a two-sample MR design with a bidirectional approach to examine the potential bidirectional relationship between statin medication and IAs.

2. Methods

2.1 Ethical Statement

This study was based on publicly available aggregated statistical data, no new data were collected,therefore ethical approval was not required. A fl ow chart of the study is displayed in Figure 1.

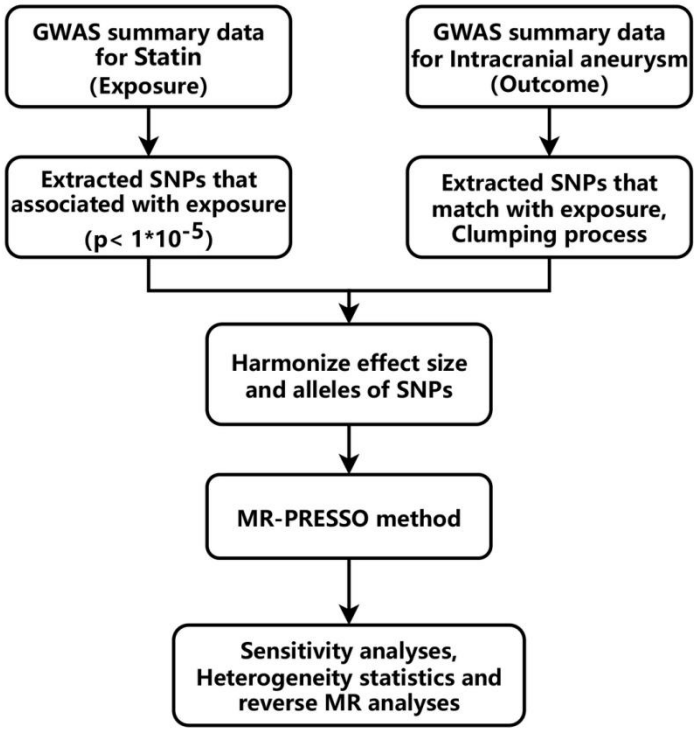

Fig .1 Flowchartofthestudydesign.MR,Mendelianrandomization

2.2 GWAS data source

In this study, we used publicly available GWAS summary statistics. The sample of statin users(RX_STATIN) was from the FinnGen consortium [13] (https://www.fi nngen.fi /en), which aims to collect and analyze genomic and health data from 500,000 participants in the Finnish biobank. The GWAS summary statistics for IAs were from a meta-analysis by Bakker et al [14]. that included both unruptureda

intracranial aneurysms (uIAs) and aneurysmal subarachnoid hemorrhage (aSAH) cases, with 7,495 diagnosed cases and 71,934 controls in the former and 1,838 diagnosed cases and 16,523

controls in the latter. To eliminate population stratifi cation bias, all GWAS summary statistics were retrieved from studies that only included individuals of European ancestry. In short, statin medication use was the

exposure, and IAs was the outcome. SNPs that were signifi cantly associated with statin medication use were selected as IVs based on strict inclusion and exclusion criteria. A series of sensitivity analyses were conducted to examine the signifi cance of the association between the two variables. Finally, a reverse MR analysis was performed to evaluate the potential impact of IAs on statin medication use.

2.3 Selection of IVs for MR analysis

We have implemented a series of quality control steps to select IVs as alternatives to statin. Firstly,we set the genome-wide signifi cance threshold $P=1.0 \times 10-5$ and the linkage disequilibrium threshold $P=5.0 \times 10-8$, with r2=0.1 and region length of 5000kb. This allowed us to obtain suffi cient IVs for simulating statin medication in the European population. We identifi ed the target genes of the active ingredients of statin medication using the DrugBank database (https://go.drugbank.com/). Next, we excluded SNPs that were inconsistent with alleles between exposure and outcome samples, as well as palindromic alleles. Third, we estimated the F statistic, which is used to assess the strength of association between IVs and outcomes relative to the risk of exposure. IVs with an F statistic less than 10 were considered weak IVs and were excluded [15]. Fourthly, we applied the MR-PRESSO global test to detect potential horizontal pleiotropy of SNPs and removed MR-PRESSO outliers to eliminate the infl uence of SNP polymorphism [16].

2.4 Statistical analysis

We analyzed the effects of statin medication on IAs through various statistical methods, including the fixed/random effect inverse variance weighted (IVW) test [17], weighted median (WM) method [18], and MR-Egger regression test [19]. Cochrane's Q test was used to assess heterogeneity among SNPs. If heterogeneity was present ($P< 0.05$), the random-effects IVW test provided a more conservative but more reliable estimate. The WM test can produce consistent estimates when more than 50% of the weights come from valid IVs [20]. The MR-Egger regression test allows for multiplicity in more than 50% of the IVs [19]. In addition, we conducted sensitivity analyses using MR-Egger and weighted median, which allowed us to accurately estimate causal relationships even if invalid SNPs were present. Finally, we conducted sensitivity analyses such as leave-one-out analysis and single SNP analysis, and tested for heterogeneity among IVW and MR-Egger methods using a funnel plot. This study applied odds ratios (OR) and 95% confi dence intervals (CI) to estimate the degree of causality, and presented p-values and standard errors of causal estimates. Signifi cant statistical signifi cance was defi ned as $p<0.05$. All statistical analyses were conducted using R (version 4.0.3). The IVW test, weighted median WM, and MR-Egger regression methods were performed using the "TwoSampleMR" package (version 0.5.7). The MR-PRESSO test was conducted using the "MRPRESSO" package (version 1.0).

2.5 Inverse Mendelian randomization analysis

To explore whether IAs had any causal effect on statin medication, we performed a reverse MR analysis(i.e., IAs as exposure and statin medication as outcome).

3. Results

3.1 Selection of instrumental variables

We selected fi ve SNPs signifi cantly associated with statin medication as genetic substitutes for statin therapy (Table 1). The F statistics of these IVs were all greater than 10. The MR-PRESSO global test detected evidence of multiplicity effects ($p= 0.938$), indicating the absence of weak

instrument bias. In the reverse MR analysis, we obtained nine SNPs signifi cantly associated with IAs, and the Fstatistics of these IVs were all greater than 10. In the MR-PRESSO global test, p<0.001. Therefore, we removed three outliers. After removing outliers, the IVs (Table 2) had a MR-PRESSO global test p value of 0.375. After these screening and quality control processes, we provided qualifi ed IVs forbidirectional two-sample MR.

Table 1. genetic substitutes for statin therapy

SNP	rs10942732	rs4704227	rs55810502	rs6861546	rs10043960
effect_allele.exposure	G	A	G	T	G
other_allele.exposure	A	G	A	C	A
effect_allele.outcome	G	A	G	T	G
other_allele.outcome	A	G	A	C	A
beta.exposure	0.0794	0.0945	0.0922	0.0451	0.0774
beta.outcome	0.0194	0.0288	0.0507	0.017	0.0719
eaf.exposure	0.3824	0.444	0.2533	0.2934	0.243
eaf.outcome	0.6386	0.6137	0.8071	0.7481	0.7756
remove	FALSE	FALSE	FALSE	FALSE	FALSE
palindromic	FALSE	FALSE	FALSE	FALSE	FALSE
ambiguous	FALSE	FALSE	FALSE	FALSE	FALSE
id.outcome	IAs	IAs	IAs	IAs	IAs
se.outcome	0.0354	0.0647	0.0529	0.0591	0.0553
pval.outcome	0.5827	0.4077	0.2373	0.6636	0.2123
outcome	IAs	IAs	IAs	IAs	IAs
se.exposure	0.0092	0.0109	0.0103	0.0199	0.0104
pval.exposure	7.44E-18	8.48E-26	3.07E-19	5.12E-06	1.27E-13
id.exposure	Statin	Statin	Statin	Statin	Statin
exposure	Statin	Statin	Statin	Statin	Statin
action	2	2	2	2	2
mr_keep	TRUE	TRUE	TRUE	TRUE	TRUE
samplesize.outcome	NA	NA	NA	NA	NA

Table 2. Tool variables for removing differential values

SNP	rs10893077	rs11646044	rs2417658	rs62349022	rs6798962	rs72705377
effect_allele.exposure	G	G	C	C	C	G
other_allele.exposure	A	T	T	T	T	A
effect_allele.outcome	G	G	C	C	C	G
other_allele.outcome	A	T	T	T	T	A
beta.exposure	-0.2538	-0.2051	-0.2178	-0.2468	-0.1876	-0.5121
beta.outcome	-0.015	-0.0179	0.0061	0.0045	-0.0117	0.001
eaf.exposure	0.709	0.5577	0.7103	0.2512	0.7757	0.9527
eaf.outcome	0.2179	0.426	0.2314	0.7872	0.1281	0.0896
remove	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
palindromic	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
ambiguous	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
id.outcome	Statin	Statin	Statin	Statin	Statin	Statin
chr	11	16	9	5	3	9

pos	123578396	75312548	108674392	7099868	157275513	7208683
se.outcome	0.0109	0.0091	0.0106	0.011	0.0134	0.0157
samplesize.outcome	NA	NA	NA	NA	NA	NA
pval.outcome	0.1671	0.0475499	0.564901	0.687	0.3825	0.9485
outcome	Statin	Statin	Statin	Statin	Statin	Statin
originalname.outcome	Statin medication	Statin medication	Statin medication	Statin medication	Statin medication	Statin medication
outcome.deprecated	Statin medication	Statin medication	Statin medication	Statin medication	Statin medication	Statin medication
mr_keep.outcome	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
data_source.outcome	igd	igd	igd	igd	igd	igd
se.exposure	0.054	0.0398	0.046	0.0517	0.0409	0.1094
pval.exposure	2.63E-06	2.53E-07	2.23E-06	1.84E-06	4.61E-06	2.86E-06
id.exposure	IAs	IAs	IAs	IAs	IAs	IAs
exposure	IAs	IAs	IAs	IAs	IAs	IAs
action	2	2	2	2	2	2
mr_keep	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE

3.2 Causal effect of statin medication on IAs

As shown in Table 3 and Figure 2A, the IVW analysis indicates that there is no signifi cant correlation between genetic prediction of statin medication and IAs (OR: 1.551, 95% CI: 0.895-2.685, P = 0.117).Similarly, the MR-Egger analysis did not reveal a signifi cant association (Table 3, P = 0.784), and neither

was there a signifi cant correlation observed in the weighted median approach (Table 3, p = 0.328).Therefore, there is no evidence to suggest a causal relationship between statin medication and IAs. In thereverse MR analysis, the IVW results indicate that there is no signifi cant correlation between genetic

prediction of IAs and statin medication (OR: 1.020, 95% CI: 0.984-1.059, P = 0.281), and other statisticalmethods did not reveal a signifi cant association (Table 3 and Figure 3A).

Table 3. Correlation between Statin measurement and internal aneurysms in MR analysis

Traits (outcome)	Exposure	MR method	No. SNP	SE	OR	95% CI	P
The forward MR analyses							
Intracranial aneurysms	Statin medication	IVW (fixed effects)	6	0.280	1.551	0.895-2.685	0.117
		MR Egger		1.493	1.564	0.084- 3.374	0.784
		Weighted median		0.324	1.373	0.727- 2.593	0.328
The reverse MR analyses							
Statin medication	Intracranial aneurysms	IVW (fixed effects)	6	0.011	0.992	0.970-1.014	0.46

		MR Egger		0.020	0.973	0.935-1.012	0.198
		Weighted median		0.016	0.996	0.966-1.028	0.825

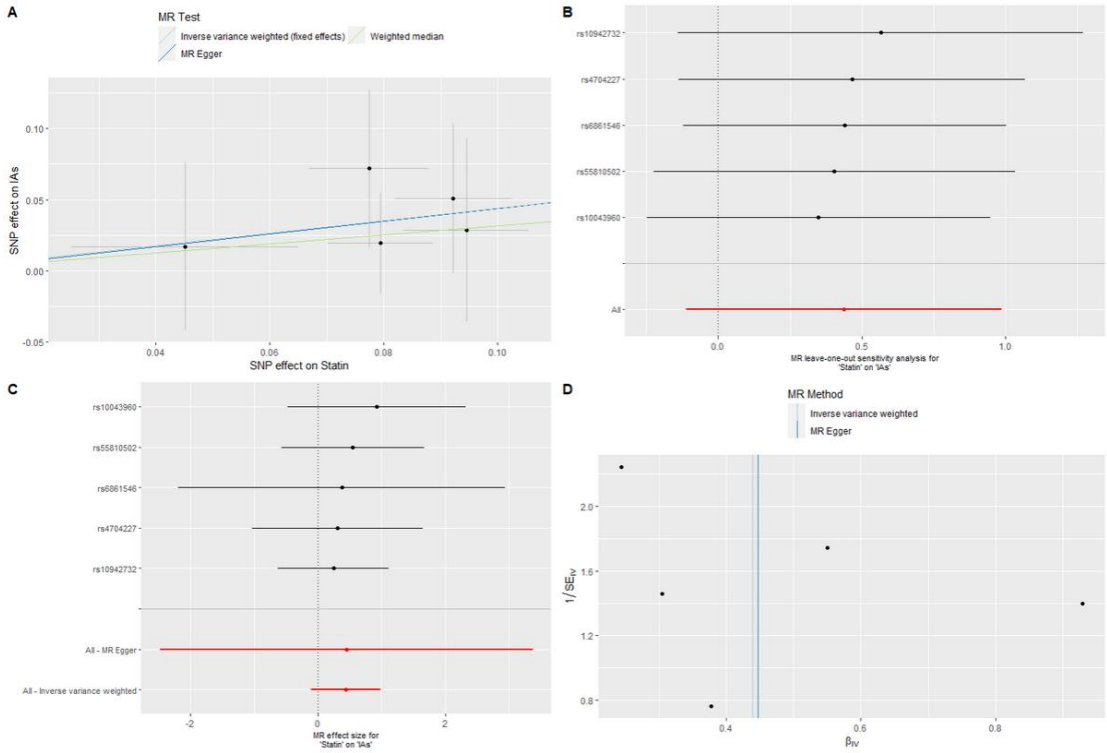

Fig .2 Forward MR analysis: the incidental effect of Statin medication on IAs

(A) Scatterplot showing the association between Statin medication and IAs. (B) Forest plot displaying the MR estimates and 95% confi dence intervals for each SNP. (C) Leave-one-out analysis to assess whether any single instrumental variable drives the causal effect. (D) Funnel plot used to detect whether the observed association shows signifi cant heterogeneity. (IVW, Inverse-variance weighting; IAs, Intracranial aneurysms; MR, Mendelian randomization; SNP, Single nucleotide polymorphism).

3.3 Sensitivity analysis

In both forward and reverse MR analysis, there were no signifi cant differences in the causal estimates of statin medication and IAs, suggesting that no individual IV drove a signifi cant causal association (Figures 2B and 3B). The Cochran's Q test in the IVW and MR-Egger methods (p>0.05, Table 4,Figures 2C and 3C) did not reveal any signifi cant heterogeneity, indicating that the results were not infl uenced by heterogeneity. The funnel plots (Figures 2D and 3D) showed a generally uniform distribution of SNPs, with no obvious outliers.

Traits (outcome)	exposure	Heterogeneity analyses			MR PRESSO Global test p	MR-Egger regression	
		method	Q	Q_pval		Intercept	p
The forward MR analyses							
Intracranial aneurysms	Statin medication	IVW	0.739	0.946	0.938	-0.001	0.996

		MR Egger	0.739	0.864			
The reverse MR analyses							
Statin medication	Intracranial aneurysms	IVW	19.055	0.163	0.511	-0.004	0.635
		MR Egger	18.715	0.132			

Table 4. Pleiotropy and heterogeneity analyses

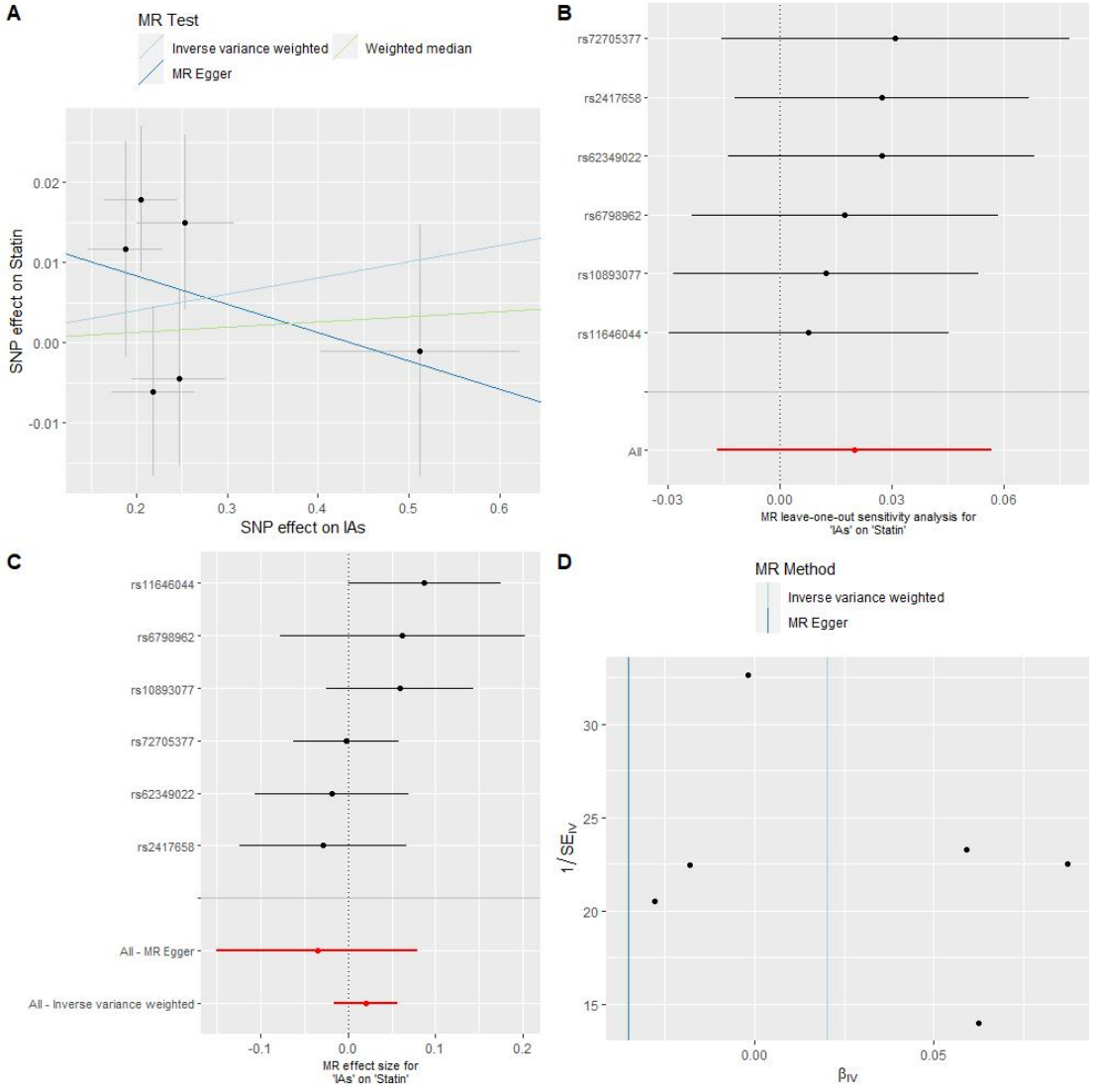

Fig .3 Reverse MR analysis: the incidental effect of IAs on Statin medication

Scatterplot showing theassociation between IAs and Statin medication. (B) Forest plot displaying the MR estimates and 95% confi dence intervals for each SNP. (C) Leave-one-out analysis to assess whether any single instrumental variable drives the causal effect. (D) Funnel plot used to detect whether the observed association shows signifi cant heterogeneity. (IVW, Inverse-variance weighting; IAs, Intracranial aneurysms; MR, Mendelian randomization; SNP, Single nucleotide polymorphism).

4. Discussion

In this study, we conducted a two-sample Mendelian randomization analysis to investigate the causal relationship between statin medication and IAs. Based on the evidence presented above, we found no causal relationship between statin medication and the risk of IAs. Previous randomized controlled trials and prospective cohort studies have suggested that statin medication may reduce the risk of IA progression and rupture [9,10]. However, some studies have reported

confl icting results. For example, statin medication did not improve the healing rate of aneurysms in a rabbit model of unruptured aneurysms [21]. Additionally, a single-center case-control study including 1200 patients showed no signifi cant benefi cial effect of statin medication on IA inhibition [22]. Our study

found no causal relationship between statin medication and the risk of IAs, indicating that they have no signifi cant inhibitory effect on IAs. Although statin medication may mediate cholesterol reduction and induce plaque regression and macrophage infi ltration in the infl ammatory process of IA formation [23],

we believe that the observed effect of statin medication on IAs in previous observational studies may have been biased by potential confounding factors and reverse causality. For example, there is a high overlap between smoking and statin use in European populations. Although our MR analysis had signifi cant advantages in avoiding confounding factors and reverse causality, our study had several limitations. Firstly, the GWAS summary data we used were limited to

participants of European descent, limiting the generalizability of our results. Secondly, if detailed information on statin use (including drug name, dose, and duration) was available, our results could be more specifi c and accurate. Thirdly, most IAs are more common in women than in men [24], but gender information was not complete in the original data. Our study did not analyze each gender separately, which may have affected our results. In summary, our study results do not support a causal relationship between statin use and the risk of IAs. Given the high mortality and disability rates associated with IAs, further investigations to explore the correlation between IAs and statin use are encouraged.

5. Abbreviations

IAs Intracranial aneurysms
MR Mendelian randomization
GWAS Genome-Wide Association Study
SAH Subarachnoid hemorrhage
SNPs single nucleotide polymorphisms
IVs instrumental variables
IVW Inverse Variance Weighted
WM weighted median

6. Declarations

Author Contributions

Conception and design: Peirun Wu, Shengjie Hao; Analysis and interpretation: Shengjie Hao,Zeyu Luo; Drafting the manuscript:Zhiyuan Xue, Jiaqi Cao; Statistics:Mengrui Wang,Bingjie Wang and Shiyu Wang. The author(s) read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Data Availability

The dataset provided in this study can be found in an online repository

[https://www.jianguoyun.com/p/DaYizUYQhKCkCRiIwpkFIAA]. The name and identifi cation number of the repository can be found in the supporting material

Confl ict of Interest

Not applicable

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