

Research Article

Causal Effects of Sarcopenia-related Traits on Ischemic Stroke: A Two-sample Mendelian Randomization Study

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Abstract

Objective: The stroke itself can cause sarcopenia, loss of muscle, and weaker strength is commonly experienced in some patients after ischemic stroke. However, the causal effect of sarcopenia on stroke is unclear, and previous studies focusing on ischemic stroke, particularly in its types were rare.

Methods: We propose that sarcopenia is a risk factor for stroke, suggesting that maintaining skeletal muscle mass may help prevent stroke. Using a two-sample Mendelian randomization (MR) approach, we analyzed sarcopenia-related traits and their impact on ischemic stroke. This analysis utilized summary-level data from publicly available genome-wide association studies (GWAS) focusing on European populations.

Results: In the results, inverse variance weighted analysis showed that sarcopenia-related traits were significant with ischemic stroke: whole body fat-free mass may increase risk for cardioembolic stroke (OR: 1.28 per SD increase in genetically determined left handgrip strength, 95% CI: 1.11-1.49, $P=1.14\times10^{-3}$) and reduce risk for large-artery atherosclerosis and small vessel stroke (OR: 0.76, 95% CI: 0.61-0.95, $P=0.014$, OR: 0.79, 95% CI: 0.65-0.97, $P=0.026$, respectively), and handgrip strength may reduce risk for small vessel stroke (OR: 0.60, 95% CI: 0.41-0.87, $P=7.29\times10^{-3}$; OR: 0.57, 95% CI: 0.40-0.82, $P=2.30\times10^{-3}$, left handgrip strength and right handgrip strength respectively).

Conclusion: We concluded that there was an overall inverse association of sarcopenia-related traits with large-artery atherosclerosis and small-vessel stroke and a positive association with cardiogenic embolism.

Keywords: Sarcopenia, whole body fat-free mass, grip strength, ischemic strokes, Mendelian randomization

Received: May 6, 2024

Revised: June 26, 2024

Accepted: July 26, 2024

Published: August 9, 2024

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Citation: Chen Y, Wu J, Yang Y, Xiong M, Yu X, Lei S. Causal Effects of Sarcopenia-related Traits on Ischemic Stroke: A Two-sample Mendelian Randomization Study. *Clin Mol Epidemiol*, 2024; 1: 9.

1 INTRODUCTION

Sarcopenia is a progressive skeletal muscle disease primarily characterized by the loss of muscle mass and function due to aging^[1]. This condition is linked to adverse outcomes such as falls, functional decline, frailty, and mortality^[2]. According to the European Working Group on

Sarcopenia in Older People (EWGSOP), the incidence of sarcopenia was 1.6% in the European Male Ageing Study^[3]. In a study of 4,000 Chinese community-dwelling older adults aged 65 and above, the average annual incidence over four years was 3.1% using EWGSOP criteria^[4]. stroke is characterized by the sudden onset of a focal neurological deficit due to a disruption in the blood supply to the brain.

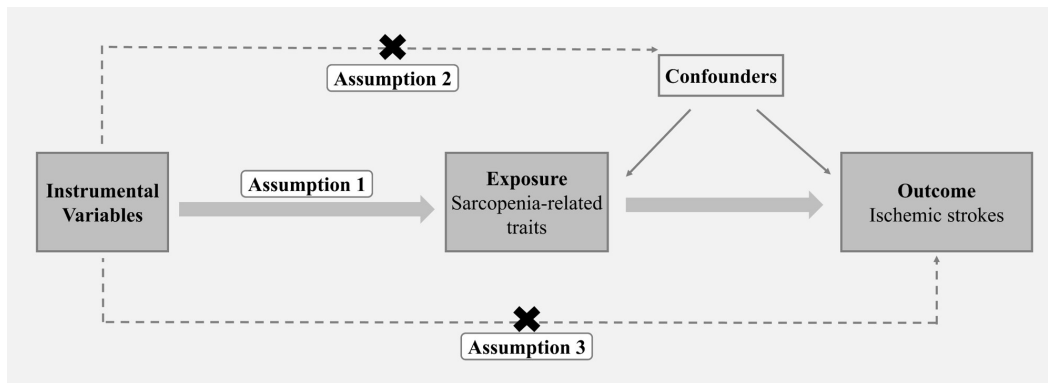


Figure 1. Overview of studies in MR analysis. Assumption 1: The genetic instrument should be reliably associated with the exposure; Assumption 2: The genetic instrument should be associated with the outcome only through the exposure; Assumption 3: The genetic instrument should not be associated with other factors that affect the outcome.

Globally, stroke is a primary disease that causes death and disability, and stroke patients are rapidly aging in recent years. In Japan, which has the world's largest aging population, the average age of onset of stroke has increased over the past 20 years^[5]. Strokes can either be ischemic (an occlusion of a blood vessel) or hemorrhagic (a rupture of a blood vessel)^[6]. Ischemic strokes (IS) have been further categorized into subtypes according to the mechanism of injury. These subtypes include cardioembolic stroke (CES), large-artery atherosclerosis stroke (LAS), small vessel occlusive disease (small vessel stroke, SVS), stroke of other determined cause, and stroke of undetermined cause^[7].

The stroke itself can cause sarcopenia. Increasing evidence suggests that patients with stroke are susceptible to promoting sarcopenia^[8]. However, it's not entirely clear what the effects of muscle mass and muscle strength are in having a stroke. The study shows that individuals with sarcopenia are more likely to develop new CVDs than their peers without sarcopenia, according to a survey of a Chinese population^[9]. In a similar Mendelian randomized (MR) analysis of body composition and cardiovascular disease, fat-free mass (FFM) index was briefly described as negatively correlated with the incidence of ischemic stroke, but no further correlation analysis was made for the subtypes of ischemic stroke^[10]. Faced with the heavy social burden and such high mortality of ischemic stroke^[6], reducing the risk of stroke by improving the muscle status of susceptible populations may be an effective and economical approach. We have aimed at the idea that sarcopenia is the risk factor for stroke to amount of skeletal muscle may be a preventive factor for stroke.

The associations between sarcopenia and stroke detected from observational studies have not been inferred in the causal effects. An alternative strategy to investigate potential causal relationships between an exposure and an outcome, especially when randomized control trials are not feasible, practical, or ethical, is Mendelian randomization is Mendelian randomization analysis^[11]. Previous studies have evaluated that fat free mass and handgrip strength

was associated with several diseases (e.g. coronary artery disease and type 2 diabetes)^[12,13], but have not yet been evaluated for ischemic disease. We therefore implemented the single-variable as well as multivariable two-sample MR design to assess the association of genetic predisposition to sarcopenia-related traits including FFM and handgrip strength (left handgrip strength (GSL); right handgrip strength (GSR)) with ischemic stroke and its main subtypes.

MR analysis as an instrumental variable (IV) analysis with genetic instruments, has been widely used to explore causal effects between disease and environmental factors, and is immune to confounders mixed in observational studies. All MR studies rely on 3 basic assumptions (Figure 1): the genetic instrument (i) should be reliably associated with the exposure, (ii) should be associated with the outcome only through the exposure, and (iii) should not be associated with other factors that affect the outcome^[14]. To our knowledge, we are the first study to use MR analysis to explore the causal link between the disease status of sarcopenia and IS.

2 MATERIALS AND METHODS

2.1 Sarcopenia-related Traits

Whole body FFM is the weight of other body components other than fat, including muscle mass body water, protein, glycerol, and soft tissue mineral mass^[15]. Muscles are a major part of FFM, hence it is also known as lean body mass. Bioelectrical impedance analysis is used to measure FFM and has been widely recognized as a reliable way to measure muscle mass in the body^[16].

There is no single method to evaluate sarcopenia and weakness, but because grip strength is relatively simple and straightforward to measure, it has been widely used to measure the overall muscle health of patients with sarcopenia^[17]. Grip strength is measured by calibrating the hand size of the hydraulic hand dynamometer^[18]. Because absolute grip strength may be more correlated with muscle strength than relative grip strength, absolute grip strength is

Table 1. Associations Between Sarcopenia-related Traits and Stroke

Exposure	Outcome	All IVs-IVW			Removed IVs-IVW		
		SNPs	OR (95% CI)	P Value	SNPs	OR (95% CI)	P Value
FFM	AIS	401	0.976 (0.883, 1.078)	0.628	378	0.996 (0.902, 1.099)	0.930
	CES	401	1.283 (1.104, 1.491)	0.001	380	1.293 (1.102, 1.516)	0.002
	SVS	404	0.792 (0.645, 0.973)	0.026	382	0.765 (0.618, 0.946)	0.014
	LAS	402	0.761 (0.611, 0.947)	0.014	379	0.759 (0.605, 0.952)	0.017
GSL	AIS	153	0.924 (0.785, 1.088)	0.343	148	0.940 (0.796, 1.109)	0.461
	CES	153	1.091 (0.798, 1.492)	0.584	148	1.059 (0.771, 1.454)	0.723
	SVS	152	0.598 (0.411, 0.871)	0.007	147	0.617 (0.412, 0.924)	0.019
	LAS	153	0.669 (0.447, 1.000)	0.050	148	0.693 (0.453, 1.060)	0.091
GSR	AIS	165	0.997 (0.852, 1.166)	0.967	163	0.999 (0.842, 1.185)	0.990
	CES	165	1.167 (0.863, 1.577)	0.316	163	1.160 (0.853, 1.577)	0.345
	SVS	164	0.570 (0.398, 0.818)	0.002	162	0.574 (0.394, 0.837)	0.004
	LAS	165	0.792 (0.537, 1.167)	0.238	163	0.806 (0.533, 1.218)	0.306

used instead of relative grip strength (absolute grip/weight) to reflect muscle strength^[19].

2.2 GWAS Data Sources

GWAS summary statistics of grip strength and whole body FFM were obtained from the UKB study, in which the sample size of whole body FFM was 331,291, the left grip strength was 461,089 and the right grip strength was 461,026^[18]. IS are based on publicly available aggregated statistical data on the genetic association of IS and their subtypes from the MEGASTROKE Consortium, and the sample consisted of 446,696 people of European descent, including any ischemic stroke (AIS) patients regardless of subtype ($n=34,217$), and ischemic stroke subtypes (CES, $n=7,193$; SVS, $n=5,386$; LAS, $n=4,373$)^[20]. The MEGASTROKE project received funding from sources specified at <http://www.megastroke.org/acknowledgments.html>. The information on genome-wide association study (GWAS) summary data sets used in our research was summarized in Table S1.

2.3 Selection of Genetic Predictors

First, we selected independent SNPs (linkage disequilibrium $r^2 < 0.001$ with a clumping window of 1Mb) that were strongly associated with the exposure $P < 5 \times 10^{-8}$) to satisfy the key assumptions of MR^[21]. We then obtained the corresponding effect estimates of these SNPs from outcome GWAS summary statistics. For SNPs unavailable in the outcome data, we used proxy SNPs highly correlated ($r^2 > 0.8$) with the original SNPs. Palindromic SNPs (allele pairs coded as A/T or C/G) were aligned based on allele and effect allele frequency. The F statistic for SNPs on exposures was computed to validate the strength of the selected instrumental variables (IVs). Additionally, we applied MR-Egger regression to examine the horizontal pleiotropy effects of selected IVs^[22], and the intercept that deviates from the origin may provide evidence for potential pleiotropic effects across the IVs. We also performed MR-PRESSO to remove

pleiotropic IVs^[23] and detect pleiotropy by assessing outliers among the selected IVs.

2.4 MR Analysis

We performed MR analysis to examine the causal effect of sarcopenia-related traits on ischemic stroke outcome. The principal analysis was conducted using the inverse-variance weighted (IVW) method, which was calculated as the SNP-outcome association effect size divided by the SNP-exposure association effect size. This regression is weighted by the inverse-variance of SNP-stroke association, and the regression line is fixed to zero^[24]. And the IVW fixed model was considered if there was insignificance in the heterogeneity test among the selected IVs.

In addition, to exclude the potential pleiotropic effects, the IVW analysis was repeated after removing some SNPs included in the analysis according to the following criteria: a) remove the proxy SNPs used in these types of strokes that failed to match; b) remove the SNPs associated with these types of strokes ($P < 5 \times 10^{-8}$); c) remove the SNPs associated with body fat-related traits.

2.5 Sensitivity Analyses

Multiple sensitivity analyses were conducted to test the reliability of the analysis results. We used MR-Egger, weighted median, and maximum likelihood methods to calculate estimates for comparison with the IVW estimates. MR-Egger allows some SNPs to affect the outcome through mechanisms other than modification of the exposure and provides a formal test for directional pleiotropy through its intercept. The weighted median estimate tends to give valid estimates when at least 50% of the information comes from valid IVs^[25]. The maximum likelihood method, which uses the maximum likelihood function to estimate probability distribution parameters, is a traditional method known for its low standard error^[26]. Additionally, we used the MR-PRESSO outlier test to identify outlier SNPs that could have

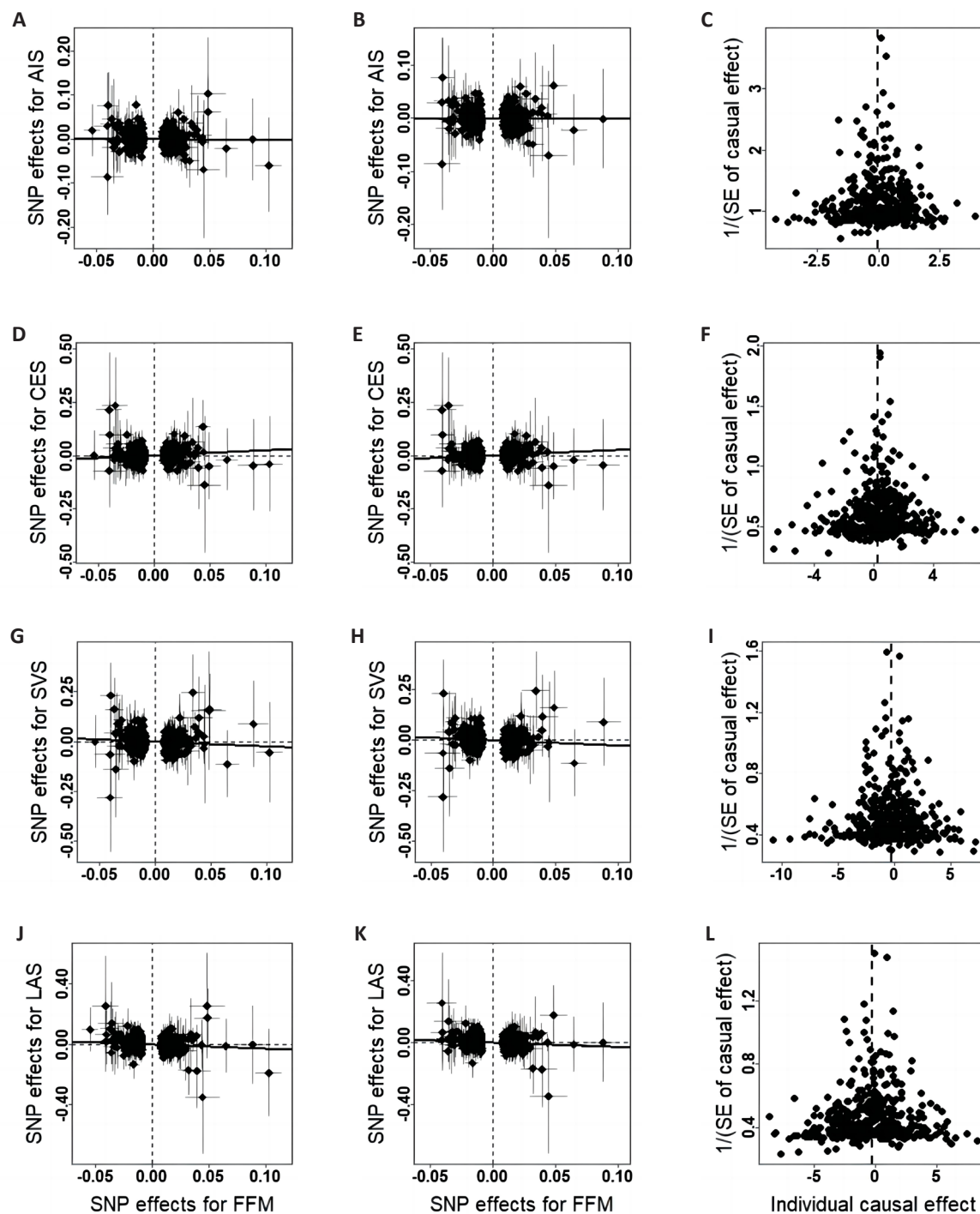


Figure 2. Scatter plots and funnel plots of the relationship between whole body FFM and IS. A/D/G/J, scatter plots of effects estimated by the crude IVW method; B/E/H/K, scatter plots of effects estimated by the repeated IVW method; C/F/I/L, funnel plots of effects estimated by the repeated IVW method.

pleiotropic effects. This method regresses SNP-outcome on SNP-exposure and uses the square of the residuals to identify outliers^[23].

The overview of our analysis was presented in Figure 1. The analyses were performed using R statistical software (Version 4.2.0) with the R packages “TwoSampleMR” version 0.5.6 and “MRPRESSO” version 0.6.0.

3 RESULT

In total, we obtained 418, 157, and 176 linkage disequilibrium-independent SNPs that achieved genome-

wide significance level ($P < 5 \times 10^{-8}$) from body FFM, left handgrip strength, and right handgrip strength, respectively. The F statistics for individual selected SNPs were all larger than 10, and those SNPs explained 8.8%, 1.6% and 1.7% of the phenotypic variation in FFM, left handgrip strength and right handgrip strength respectively. The summary information of selected SNPs was shown in Tables S2-S7.

The associations between each genetically predicted risk factor and ischemic stroke using IVW method are shown in the Table 1 and Figures 2-4. IVW analysis showed that whole body FFM may increase the risk of CES and reduce the risk

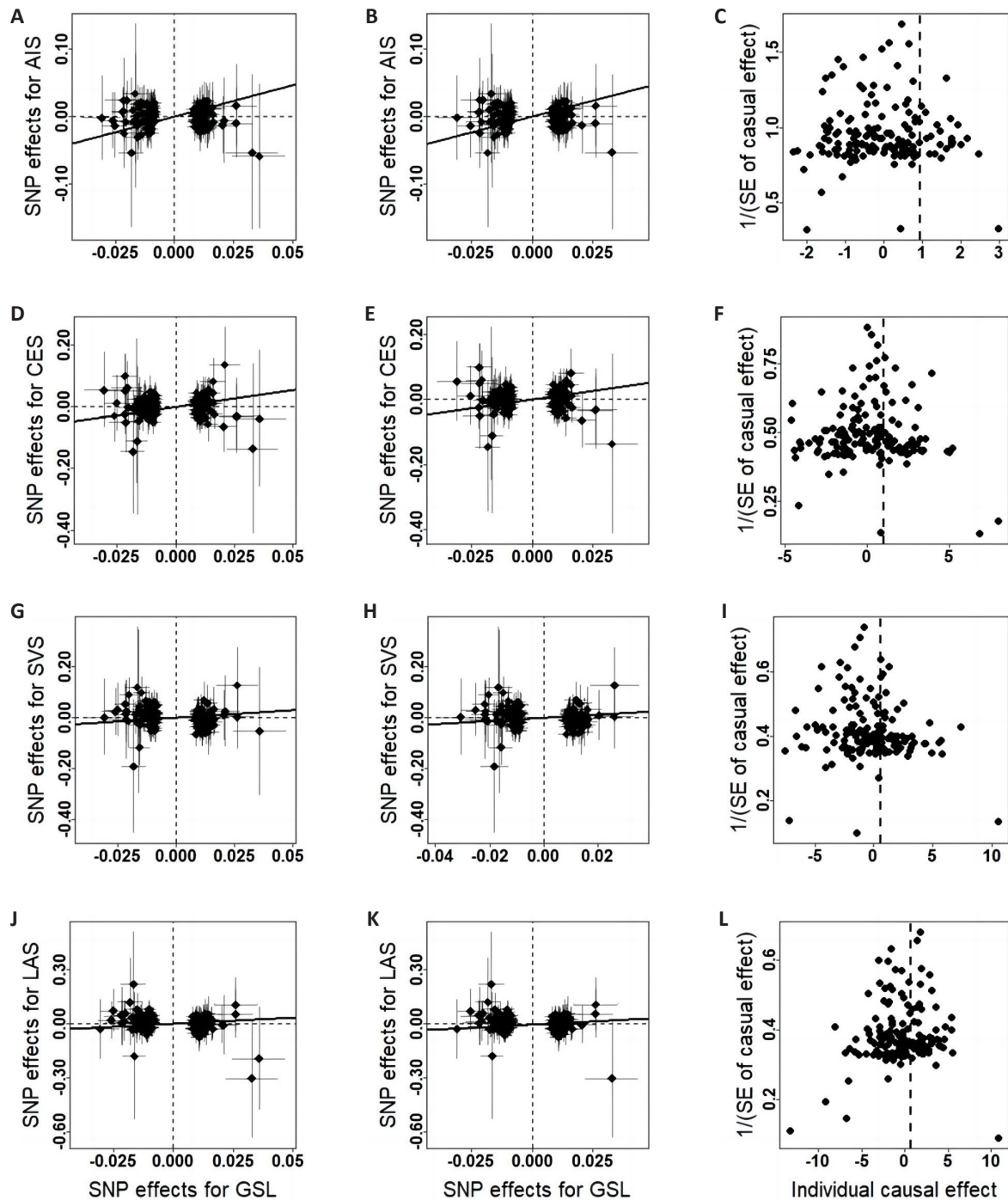


Figure 3. Scatter plots and funnel plots of the relationship between hand GSL and IS. A/D/G/J, scatter plots of effects estimated by the crude IVW method; B/E/H/K, scatter plots of effects estimated by the repeated IVW method; C/F/I/L, funnel plots of effects estimated by the repeated IVW method.

of large-artery atherosclerosis and small vessel occlusive disease, but handgrip strength may reduce risk of small vessel occlusive disease. The odds ratio of CES, SVS and LAS per 1 SD increase in genetically predicted whole body FFM were 1.28 (95% CI=1.10-1.49, $P=1.14 \times 10^{-3}$), 0.79 (95% CI=0.65-0.97, $P=0.03$) and 0.76 (95% CI=0.61-0.95, $P=0.01$) respectively. And left handgrip strength and right handgrip strength increase per unit in log odds, the OR of SVS was 0.60 (95% CI=0.41-0.87, $P=7.29 \times 10^{-3}$) and 0.57 (95% CI=0.40-0.82, $P=2.30 \times 10^{-3}$) respectively. In repeated IVW analysis, for the significant causal pairs, the direction of association was consistent, i.e., the FFM was significantly and

positively with CES but was inversely associated with large artery stroke and small vessel stroke.

Results were similar in a sensitivity analysis based on MR-Egger, weighted median, and maximum likelihood methods as shown in the Table 2. Some outlier SNPs were identified after performed MR-PRESSO, and the exclusion of the outliers did not essentially change the results for sarcopenia-related traits and ischemic stroke (Table 3). There was no evidence of directional pleiotropy (all P values for MR-Egger intercept > 0.1) among the selected IVs.

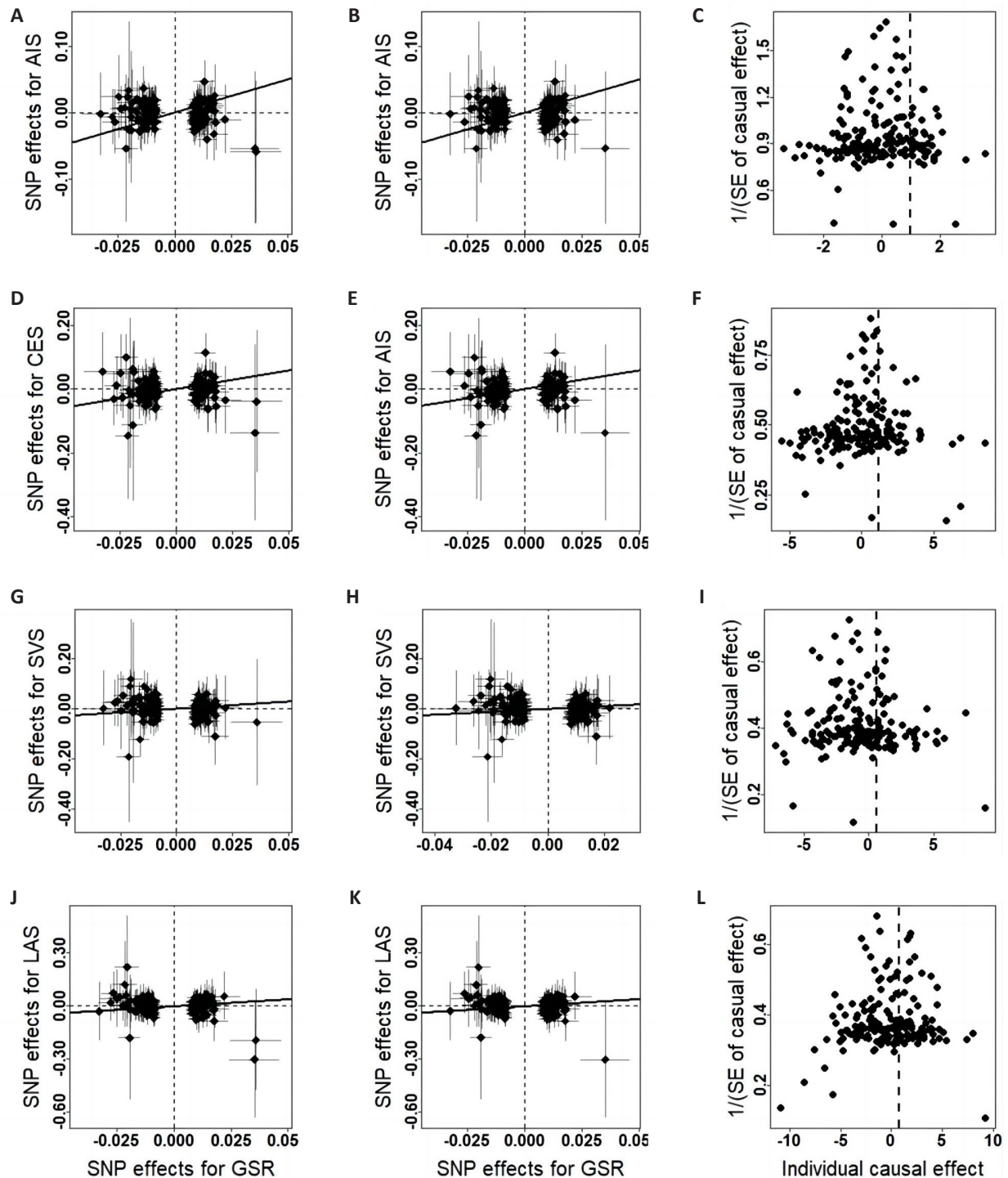


Figure 4. Scatter plots and funnel plots of the relationship between hand GSR and IS. A/D/G/J, scatter plots of effects estimated by the crude IVW method; B/E/H/K, scatter plots of effects estimated by the repeated IVW method; C/F/I/L, funnel plots of effects estimated by the repeated IVW method.

4 DISCUSSION

Few studies have evaluated the effect of sarcopenia-related symptoms as an independent risk factor on the occurrence and development of stroke. This study showed an overall inverse association of genetically predicted sarcopenia-related traits with LAS and SVS and a positive association of genetically with CES, despite the *P* value is not significant in the causal association between grip strength and LAS. We found no causal association between IS and these related traits before being categorized into subtypes. Less muscle mass

and weaker muscle strength may increase stroke risk through affected inflammatory signaling pathways^[27] and hypertension^[28].

Skeletal muscle contraction releases myokines, which can block inflammatory signaling pathways triggered by chronically elevated levels of pro-inflammatory adipokines^[27]. Muscle mass can be increased through exercise training, and long-term exercise can reduce cardiovascular risk by secreting various anti-inflammatory myokines in skeletal muscle^[29]. Muscle-secreted irisin can protect neurons by inhibiting the ROS-NLRP3

Table 2. Sensitivity Analysis for Genetic Associations Between Sarcopenia-related Traits and Strokes

Method		AIS		CES		SVS		LAS	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
MR-Egger	FFM	0.977 (0.754, 1.267)	0.862	1.217 (0.81, 1.827)	0.345	0.842 (0.492, 1.441)	0.531	0.912 (0.514, 1.620)	0.755
Weighted median		1.095 (0.963, 1.244)	0.164	1.403 (1.103, 1.786)	0.006	0.873 (0.653, 1.166)	0.358	0.869 (0.639, 1.183)	0.373
Maximum likelihood		0.976 (0.902, 1.056)	0.541	1.288 (1.107, 1.498)	0.001	0.793 (0.662, 0.949)	0.012	0.761 (0.626, 0.925)	0.006
MR-Egger	GSL	1.190 (0.620, 2.287)	0.602	1.204 (0.338, 4.290)	0.775	0.301 (0.059, 1.521)	0.148	0.891 (0.167, 4.765)	0.893
Weighted median		0.949 (0.737, 1.222)	0.683	1.165 (0.736, 1.841)	0.515	0.530 (0.302, 0.930)	0.027	0.708 (0.380, 1.316)	0.275
Maximum likelihood		0.924 (0.784, 1.090)	0.349	1.094 (0.797, 1.501)	0.578	0.598 (0.409, 0.875)	0.008	0.671 (0.446, 1.009)	0.055
MR-Egger	GSR	1.086 (0.552, 2.137)	0.811	1.109 (0.325, 3.787)	0.869	0.425 (0.095, 1.903)	0.265	0.417 (0.081, 2.160)	0.299
Weighted median		1.058 (0.837, 1.338)	0.635	1.212 (0.774, 1.897)	0.401	0.511 (0.3, 0.87)	0.013	0.825 (0.462, 1.475)	0.516
Maximum likelihood		0.997 (0.85, 1.168)	0.968	1.171 (0.863, 1.588)	0.311	0.568 (0.394, 0.819)	0.002	0.795 (0.537, 1.177)	0.251

Table 3. MR-PRESSO Analysis for Genetic Associations Between Sarcopenia-related Traits and Strokes

Outcome	Outcome	OR (95% CI)	Estimate	SD	P value	Global Test-P Value	Outliers
FFM	AIS	1.026 (0.938, 1.123)	0.026	0.046	0.571	<0.01	rs11065979, rs3843751, rs42044, rs4341996, rs57635800
	CES					0.198	*
	SVS	0.844 (0.694, 1.026)	-0.170	0.100	0.089	<0.01	rs10958683, rs11065979, rs4980067
	LAS	0.784 (0.632, 0.973)	-0.243	0.110	0.028	<0.01	rs58857770
GSL	AIS					0.662	*
	CES					0.506	*
	SVS					0.093	*
	LAS					0.241	*
GSR	AIS					0.036	*
	CES					0.360	*
	SVS					0.180	*
	LAS					0.107	*

Nores: * Means no significant outliers.

inflammatory signaling pathway^[30]. Minn YK's findings suggest that increased skeletal muscle mass may have a protective effect against brain white matter changes and silent infarctions in community-dwelling men without stroke or dementia^[31]. However, it is unfortunate that their study did not measure inflammatory cytokines such as IL-6 and IL-10.

Hypertension was a significant risk factor for ischemic stroke^[32]. FFM could be the be a determinant of blood pressure levels^[33], and a previous studies consisting of 2,671 children showed that for every 1% increase in FFM, the risk of developing high blood pressure was reduced by 9%^[34]. Four population-based cohort studies from Germany found significant positive associations between changes in FFM and incident hypertension, as well as

significant inverse associations with the normalization of blood pressure^[35]. We suspect that gaining FFM reduces the risk of ischemic stroke, in part by preventing hypertension.

The training exercise is often accompanied by an increase in muscle strength. Grip strength is widely used to measure overall muscle strength in patients with weakness or sarcopenia. Low grip strength is a component of weakness that predicts negative health outcomes, and is associated with an increased risk of mortality in many studies^[36-38]. Grip strength is a strong predictor of cardiovascular mortality and a moderately strong predictor of incident cardiovascular disease^[39]. A linear relationship was found between grip strength and all-cause mortality, cardiovascular disease, coronary heart disease

and stroke in a certain range^[40]. Results from a study of three prospective cohorts support grip strength as an independent predictor of stroke in middle-aged and elderly European, American, and Chinese populations^[41].

Notably, FFM appears to be a risk factor for CES in our study. Cardiogenic embolism is recognized to account for about 15% to 20% of all IS in clinical search. The most common underlying cause of CES is atrial fibrillation (AF), which increases the risk of ischemic stroke by 3 to 5 times. It is estimated that AF accounts for 15% of all strokes worldwide^[42]. A few epidemiologic studies have assessed body composition and suggested that FFM, and not just fat mass, may be responsible for the increased AF risk^[43,44]. Therefore, we hypothesized why FFM could positively affect AF and thus increase the risk of cardiogenic embolism, this also may explain that FFM-associated SNPs were not statistically significantly associated with AIS before being categorized into subtypes.

This study has some potential limitations to be noted. Firstly, we performed the sensitivity analysis combined with complementary MR methods aimed to minimize the risk of confounding and pleiotropy, but it is still the present possibility to bias the results. For example, the included SNPs in the whole body FFM instrument may impact ischemic stroke risk through other pathways. Secondly, we used body FFM rather than appendicular lean mass to measure muscle mass, which may not be entirely appropriate as this measurement could be biased by the inclusion of other non-fat soft tissues, such as the lungs, liver, and other organs. Additionally, relative muscle mass (appendicular lean mass/height²) may be a more appropriate measure of muscle mass compared to absolute muscle mass^[2]. Thirdly, our research was primarily based on participants of European ancestry and may not be generalizable to other populations. However, we believe the causal effects of grip strength and lean mass are likely similar across different populations, though they may have greater relevance in certain groups. Fourthly, because the MR analyses used summary statistics, we could not obtain the original individual indicators, meaning differences in quality control and selection criteria of GWAS at the individual level may affect our results. Lastly, we cannot determine the clinical significance of the results since MR should be interpreted as testing for causation rather than indicating the size of any causal effect. Even if the effect size is small and not clinically significant, it may still be important for population health.

Acknowledgements

The study was supported by Natural Science Foundation of China (No.82173529, No.82173598, No.82103922 and No.82373587). We would like to thank MR-base dataset for providing relevant publicly available summary statistics. We thank all the Neale Lab and MRC-IEU

consortium studies for making the summary association statistics data publicly available and we are grateful of all the investigators and participants in UK Biobank for the public individual-level data sets and their contribution to those studies.

Conflicts of Interest

The authors declared no conflict of interest.

Author Contribution

The authors made contributions to the conception and interpretation of the study and were involved in revising the manuscript. Lei S and Chen Y designed the study and interpreted the data, and Chen Y and Wu J drafted the manuscript. Chen Y, Yang Y, Xiong M, Yu X and Chen Y made a substantial contribution to the acquisition and analysis of the data. Lei S revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript.

Abbreviation List

AIS, Any ischemic stroke
CES, Cardioembolic stroke
EWGSOP, European Working Group on Sarcopenia in Older People
FFM, Fat-free mass
GWAS, Genome-wide association studies
GSL, Hand grip strength (left)
GSR, Hand grip strength (right)
IS, Ischemic strokes
IV, Instrumental variable
IVW, Inverse-variance weighted
LAS, large-artery atherosclerosis stroke
MR, Mendelian randomization
SVS, Small vessel stroke

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