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The causal relationship between ever smoked and frozen shoulder
A two-sample Mendelian randomization

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Abstract
To investigate the causal relationship between ever smoked and frozen shoulder using a Mendelian randomization (MR) approach. Pooled data from a large-scale genome-wide association study were used. Genetic loci that were independent of each other and associated with ever smoked and frozen shoulder in populations of European ancestry were selected as instrumental variables. Inverse variance weighting was used as the primary analysis method. Weighted median and MR-Egger were used as complementary analysis methods to assess causal effects. To explore the causal relationship between ever smoked and frozen shoulder. Sensitivity test analysis was performed using heterogeneity test, multiple validity test, and leave-one-out analysis to explore the robustness of the results. Inverse variance weighting results of ever smoked showed an OR = 2.49, 95% CI = 1.05–5.91, P = .038, indicating that ever smoked is a risk factor for a frozen shoulder. And the test revealed no heterogeneity and pleiotropy, and the sensitivity analysis also showed robust results. This study used two-sample MR analysis to analyze and explore the genetic data, and the results showed a higher prevalence of frozen shoulder in patients with ever smoked, suggesting that active control of ever smoked may reduce the occurrence of frozen shoulder.

Abbreviations: GWAS = genome-wide association study, IVW = inverse variance weighting, MR = Mendelian randomization, SNP = single nucleotide polymorphism.

Keywords: ever smoked, frozen shoulder, Mendelian randomization

1. Introduction

Frozen shoulder, also known as adhesive capsulitis,[1] is a pathological condition characterized by pain and limited joint motion in the shoulder.[2] There are usually no significant findings in the patient's history, clinical examination, or imaging evaluation to explain the loss of motion or pain.[3,4] Ever smoked is a common risk behavior[5,6] and has been found to contribute to the development and progression of many diseases.[7,8] In recent years, several studies have investigated the relationship between ever smoked and frozen shoulder, but the findings have been mixed.[9,10] Therefore, the causal relationship between ever smoked and periarthritis still needs further investigation.

In traditional epidemiological research methods, the causal inferences obtained are considered to be of limited value because of the influence of confounding factors and reverse causality.[11] In contrast, Mendelian randomization (MR), a genetic epidemiological method, is a useful tool to assess the causal role of ever smoked and frozen shoulder.[12] By using genetic variants such as single nucleotide polymorphism (SNP) as instrumental variables that can modify disease risk factors or exposures, MR studies can strengthen causal inferences about exposure-outcome associations.[13] According to Mendel's law of inheritance, genetic variants are not susceptible

to confounding factors because they are randomly assigned during gamete formation.[14] In addition, confounding factors and reverse causality correlations can be minimized because genotypes cannot change with disease progression.[15]

To this end, we conducted a two-sample MR study to examine the association of genetic susceptibility to ever smoked with frozen shoulder risk factors. We aimed to provide important evidence for the causal role of ever smoked in causing a frozen shoulder.

2. Data and methods

2.1. Data sources

The largest sample size of genome-wide association study (GWAS) data for ever smoked and frozen shoulder was obtained through the IEU OpenGWAS project (mr cieu. ac. uk). The website was accessed on 2023-06-06. The final population source for all data was Europe, of either sex. Including ever smoked (ieu-b-4858) containing 7,933,821 SNPs with a sample size of 99,996; frozen shoulder (ebi-a-GCST90000512) containing 15,184,371 SNPs with a sample size of 4,51,099. This study was a re-analysis of previously collected and published data and therefore did not require additional ethical approval.

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2.2. Conditioning of SNP as an instrumental variable

First, the instrumental variables were highly correlated with exposure, with F > 10 as a strong correlation criterion.[16] Secondly, the instrumental variable was not directly correlated with the outcome, but only influenced the outcome through exposure, meaning that there was no genetic pleiotropy. In this study, the MR-Egger regression model with a non-zero intercept

term ($P < .05$) indicated the absence of genetic pleiotropy.[17] Third, instrumental variables were not related to unmeasured confounding.[18] Finally, the human genotype-phenotype association database Phenoscanner V2 was searched for phenotypes associated with instrumental variables at genome-wide significance levels to determine whether these SNPs were associated with potential risk factors.[19]

Table 1

Information on the final screening of ever smoked SNPs from GWAS data (n = 6).

ID	SNP	Effect_Allele	Other_Allele	β	SE	P	F
1	rs12731986	T	C	−0.0121	0.0022	2.17E-08	30
2	rs12874797	T	C	−0.0194	0.0034	1.06E-08	32
3	rs2011487	A	C	−0.0142	0.0021	3.96E-11	45
4	rs7829715	C	T	−0.0125	0.0021	4.12E-09	35
5	rs846184	T	C	0.0179	0.0032	1.54E-08	31
6	rs9287372	G	A	−0.0148	0.0021	4.05E-12	49

GWAS = genome-wide association study, SNP = single nucleotide polymorphism.

Table 2

MR regression results of the 3 methods.

Method	β	SE	OR (95% CI)	P
IVW	0.912	0.441	2.49 (1.05–5.91)	.039
WME	1.011	0.558	2.75 (0.92–8.20)	.070
MR-Egger	3.708	2.581	440.77 (0.15–10902.67)	.263

IVW = inverse variance weighting, MR = Mendelian randomization, WME = Weighted median.
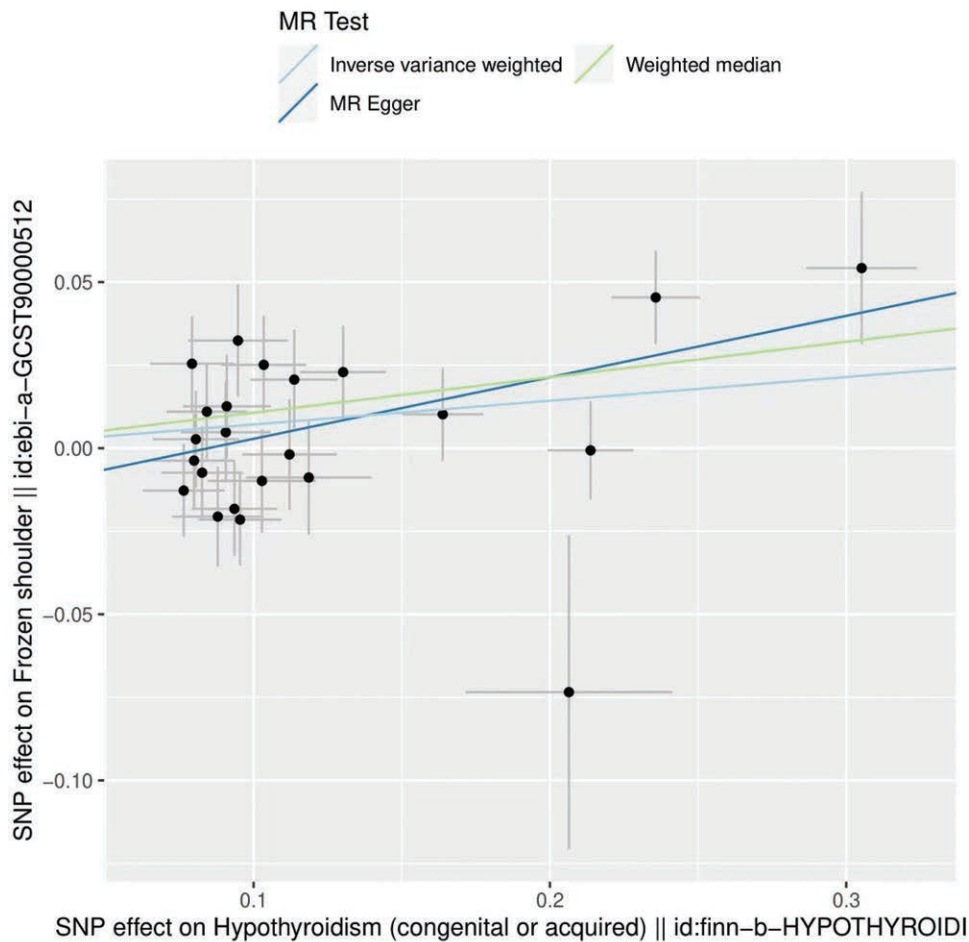

Figure 1. Scatter plot of ever smoked and frozen shoulder.

2.3. SNP screening

Significant SNPs were screened from the GWAS summary data of ever smoked ($P < 5 \times 10^{-8}$ was used as the screening condition)[20]; the linkage disequilibrium coefficient r^2 was set to 0.001 and the width of the linkage disequilibrium region was 10,000 kb to ensure that each SNP was independent of each other.[21] The above-screened ever smoked related SNPs were extracted from the GWAS summary data of frozen shoulder, while SNPs directly related to the outcome index were excluded ($P < .05$). The F value of each SNP was calculated, and SNPs with weak instrumental variables (F value less than 10) were excluded.[22] And the human genotype-phenotype association database was queried to screen for potentially associated risk factor SNPs and to exclude them.[23]

2.4. Causality validation methods

The causal relationship between exposure (ever smoked) and outcome (frozen shoulder) was verified mainly using inverse variance weighting (IVW), supplemented by weighted median and MR-Egger MR analysis methods, using SNPs as instrumental variables.

2.5. Sensitivity analysis

Various methods were used for sensitivity analysis. First, the Cochran Q test was used to assess the heterogeneity among the individual SNP estimates, and a statistically significant Cochran Q test proved that the analysis was significantly heterogeneous. Second, the MR pleiotropy residual sum and outlier (MRPRESSO) was used to validate the results in the IVW model, correct for the effects of outliers, and if outliers existed, they were excluded and the analysis was repeated. Third, the MR-Egger intercept test was used to test the horizontal multiplicity of SNPs. If the intercept term in the MR-Egger intercept test analysis was statistically significant, it indicated that the MR analysis had significant horizontal multiplicity. Fourth, leave-one-out analyses were performed by removing a single SNP at a time to assess whether the variation drove the association between the exposure and outcome variables. Fifth, funnel plots and forest plots were constructed to visualize the results of sensitivity analyses. $P < .05$ suggests a potential causal relationship for MR analysis and is statistically significant. All statistical analyses were performed using the "TwoSampleMR" package in R software version 4.3.0.

3. Results

3.1. Instrumental variables

The current study screened 6 SNPs that were strongly associated with ever smoked ($P < 5 \times 10^{-8}$) without chain imbalance ($r^2 < 0.001$, kb = 10,000). 6 SNPs remained by taking intersection with SNPs in the GWAS pooled data from frozen shoulder and also excluding SNPs directly associated with outcome
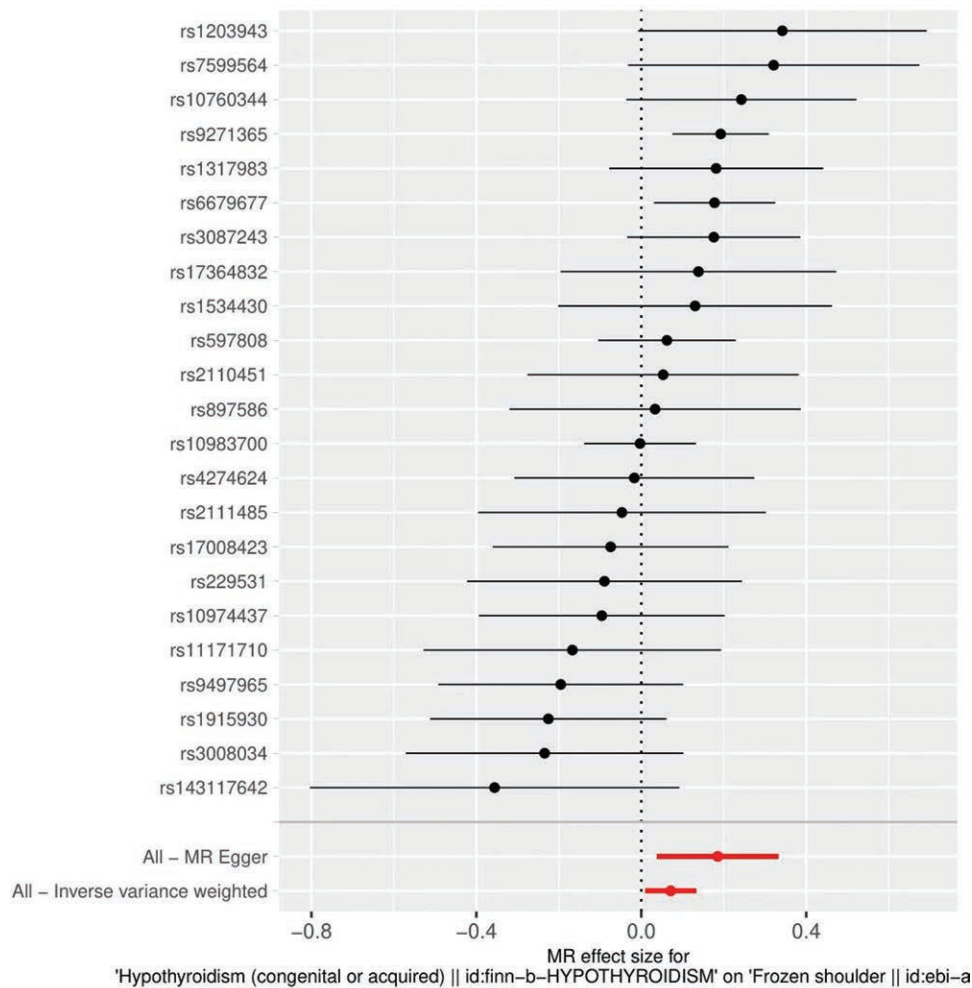

Figure 2. Forest plot of ever smoked and frozen shoulder.

indicators. In our study, each SNP had an F value greater than 10, indicating no weak instrumental variables (Table 1). We searched the human genotype-phenotype association database and no potentially relevant risk factor SNPs were found.

3.2. Causal relationship between ever smoked and frozen shoulder

The results of IVW showed a causal relationship between ever smoked and frozen shoulder. IVW: OR = 2.49, 95% CI = 1.05–5.91, $P = .038$. MR-Egger and weighted median show results in the same direction as IVW results (Table 2). We can see from both the scatter plot (Fig. 1) and the forest plot (Fig. 2) that ever smoked increases the risk of developing frozen shoulder.

3.3. Sensitivity analysis

The heterogeneity test (Cochran Q test, $P = .697$) was performed using the IVW method and the results suggested that there was no heterogeneity. A funnel plot was drawn to show the heterogeneity results, as shown in Figure 3. MR-PRESSO was used to screen for SNPs that might cause heterogeneity, and no SNPs were found to cause heterogeneity in the results. The results of the global test by MR-PRESSO suggested that there was no pleiotropy ($P = .377$). The IVW method was used by default for the leave-one-out method, and as seen in Figure 4, the results of the remaining SNPs after eliminating any of them were on the right side of the valid line, indicating that the results were robust.

4. Discussion

Some studies suggest that ever smoked may be an observable risk factor for frozen shoulder, but the causality of this association is unclear. Our MR study aimed to reveal a causal relationship between ever smoked and frozen shoulder. The results of the two-sample MR showed that ever smoked is a risk factor for frozen shoulder. Previous history of ever smoked IVW results showed an OR (95% CI) of 2.49 (1.05–5.91) and a higher probability of having frozen shoulder in the ever smoked population.

Cogan et al[9] found that tobacco use increased the incidence of frozen shoulder with an OR (95% CI) of 1.33 (1.30–1.36), $P < .001$, after retrospectively analyzing a cohort of 165,937 patients.

However, Lee et al[10] used univariate and multifactorial logistic regression to analyze risk factors in 262 patients with advanced frozen shoulder, and the results of the study suggested that ever smoked was not associated with the incidence of frozen shoulder. Cohen et al[24] also found that ever smoked was not a risk factor for the development of frozen shoulder by comparing 166 patients with frozen shoulder, with 129 patients diagnosed with rotator cuff tears and 251 control subjects. Zhang et al[25] used logistic regression for multivariate analysis of clinical data from 592 patients who underwent arthroscopic rotator cuff repair, and multivariate analysis was performed and found that ever smoked was also not a risk factor for the development of frozen shoulder.

The present study confirmed a causal relationship between ever smoked and the occurrence of frozen shoulder from a genetic perspective. The results of the present study are consistent with Cogan's conclusion that ever smoked is a risk factor for the development of frozen shoulder. ever smoked
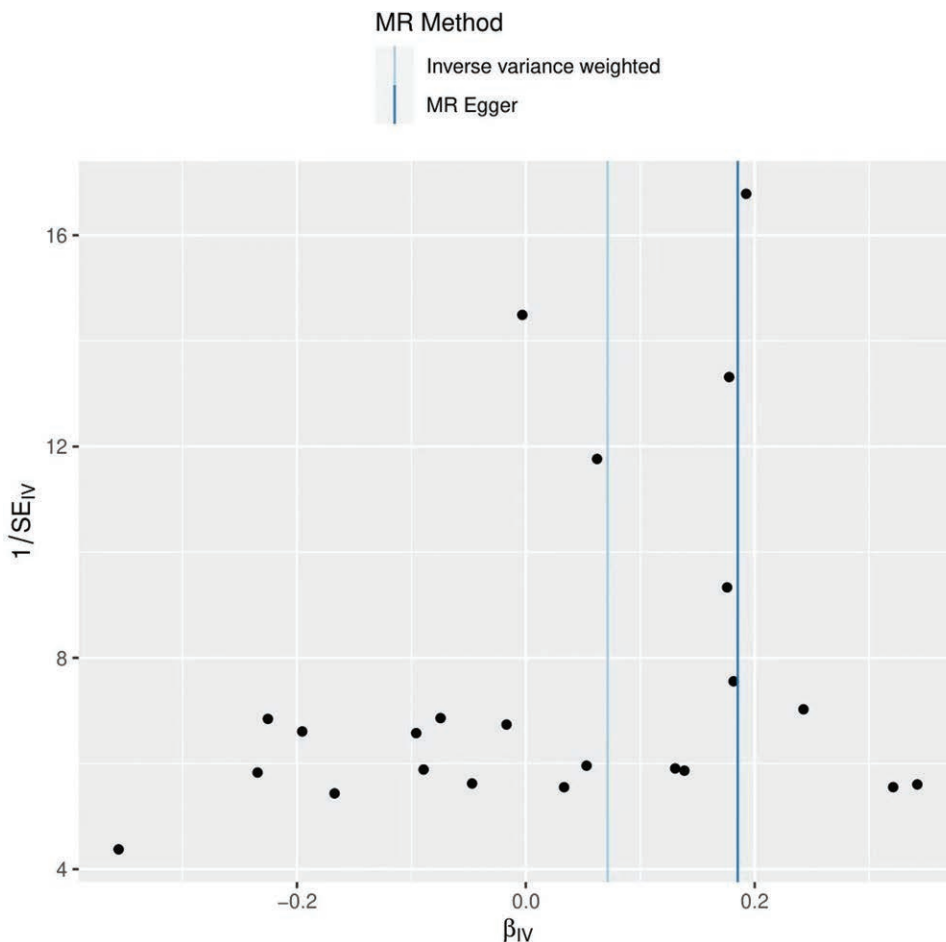

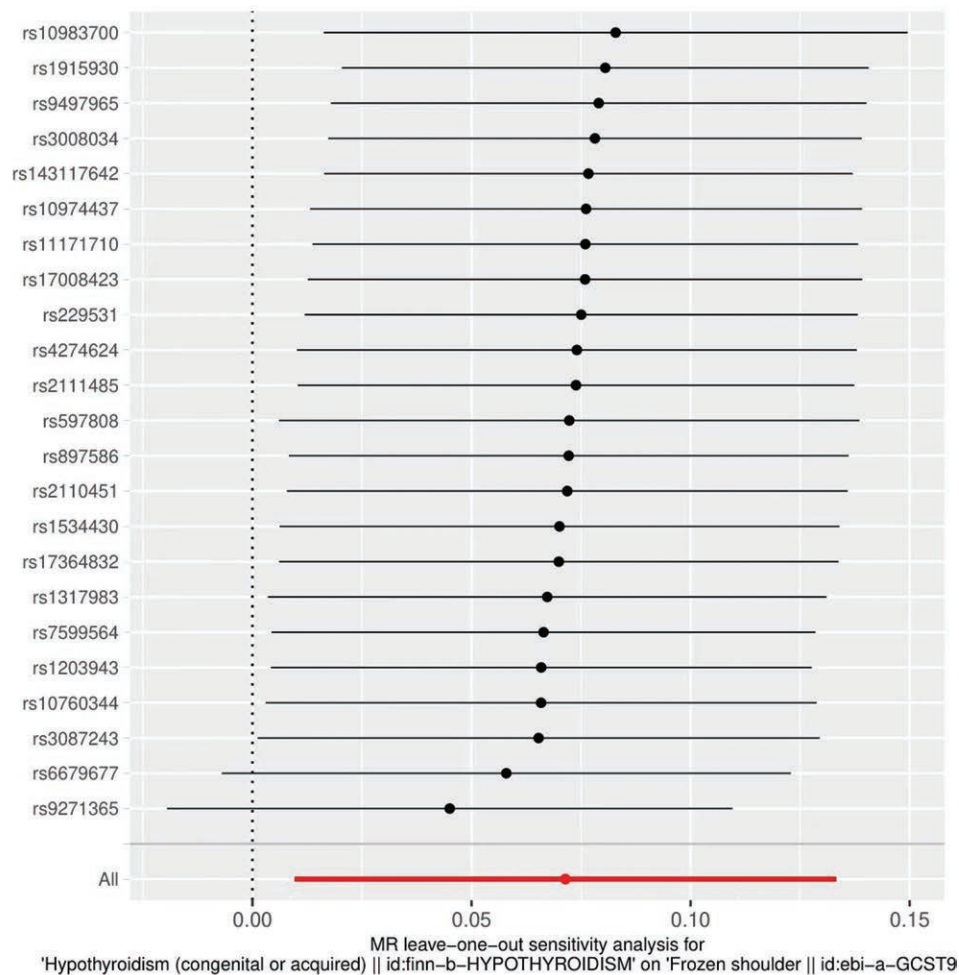Figure 3. Funnel plot of ever smoked and frozen shoulder.

Figure 4. Analysis of ever smoked and frozen shoulder by the leave-one-out method.

population is more likely to develop frozen shoulder than non-ever smoked population. Therefore, there should be increased publicity about the harmful effects of ever smoked to reduce the number of smokers and thus reduce the prevalence of frozen shoulder. Patients with frozen shoulder should be asked to quit ever smoked to speed up the healing process. In addition, screening for frozen shoulder should be increased for the ever smoked population, early detection of patients with frozen shoulder and timely treatment, which is beneficial to the prognosis of patients.

Lee et al retrospectively found that ever smoked does not affect the incidence of frozen shoulder, probably because retrospective studies are susceptible to confounding factors and reverse causality, and therefore the causal inferences obtained are considered of limited value. In contrast, Mendelian randomization (MR) analysis is a new epidemiological approach that uses genetic variation as an instrumental variable for exposure to enhance causal inferences. This approach reduces the effects caused by confounding factors.[26]

At the same time, this study has some limitations. First, since all data were obtained from a population of European ancestry, the results do not represent a truly random population sample and are not applicable to other so races. Second, although various sensitivity analyses have been performed in this study to test the hypotheses of the MR study, it is also difficult to completely rule out horizontal pleiotropy of instrumental variables. Finally, the current sample size of GWAS data is still not large enough, and more in-depth studies using more GWAS data are needed in the future.

5. Conclusion

In summary, this study used a two-sample MR analysis to analyze and explore the genetic data, and the results showed that the prevalence of frozen shoulder was higher in the ever smoked population, suggesting that reducing the ever smoked population may reduce the occurrence of frozen shoulder.

Author contributions

Conceptualization: Yong-kang Wei.
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Validation: Guang-hua Deng.
Visualization: Guang-hua Deng.
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Writing – review & editing: Yong-kang Wei.

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