

# Causal Relationship between Cholelithiasis and Scoliosis: A Two-Sample Mendelian Randomization Study

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**Abstract:** To assess the potential causal association between cholelithiasis and scoliosis, we conducted a two-sample Mendelian randomization (MR) analysis employing three complementary approaches: inverse variance weighting (IVW), weighted median, and MR-Egger regression. We additionally performed heterogeneity and horizontal pleiotropy assessments, along with a leave-one-out sensitivity analysis to evaluate the stability of the findings. The IVW analysis indicated a significant causal effect of cholelithiasis on scoliosis, with an odds ratio (OR) of 1.143 (95% CI: 1.011–1.293,  $p = 0.033$ ). Tests revealed no evidence of heterogeneity or horizontal pleiotropy, and the sensitivity analysis confirmed the robustness of the results. Our findings suggest that gallstone disease may act as a causal risk factor for scoliosis, highlighting the need for increased clinical vigilance in patients diagnosed with cholelithiasis.

**Keywords:** Cholelithiasis; Scoliosis; Mendelian Randomization.

## 1. Introduction

Scoliosis is a multifactorial condition characterized by lateral curvature of the spine accompanied by vertebral rotation, shoulder asymmetry, pelvic tilt, and abnormal body alignment, all of which significantly impair patients' quality of life. Despite ongoing research, its underlying pathogenesis remains poorly understood [1–2]. Cholelithiasis, one of the most common gastrointestinal diseases in Western populations, arises from a complex interaction between genetic predisposition, lifestyle factors, and dietary habits, contributing to specific pathogenic pathways [3–4]. Raasck et al. [5] documented a case involving a spontaneous extensive spinal epidural hematoma secondary to choledocholithiasis, leading to tetraplegia and respiratory compromise. Although anecdotal, such findings may suggest a potential link between gallstone disease and spinal pathology.

Mendelian randomization (MR) uses genetic variants, typically single nucleotide polymorphisms (SNPs), as instrumental variables to assess causality based on genome-wide association study (GWAS) data. Since these variants are determined at conception and remain stable, MR helps reduce confounding and reverse causation [6–7]. In this study, we conducted a two-sample MR analysis to explore the potential causal association between cholelithiasis and scoliosis.

## 2. Data and Methods

### 2.1. Data Sources

We obtained GWAS data for cholelithiasis (ebi-a-GCST90018819) and scoliosis (finn-b-M13\_SCOLIOSIS) from the IEU OpenGWAS project (<https://gwas.mrcieu.ac.uk/>). We would like to thank the authors of Sakae S et al. [8] for providing the cholelithiasis dataset, which includes 26,122 cases and 461,431 controls, comprising a total of

24,173,391 SNPs. The scoliosis dataset includes 1,168 cases and 164,682 controls, with a total of 16,380,270 SNPs. This study was a re-analysis of publicly available GWAS data and therefore required no special ethical approval.

### 2.2. Selection Conditions for SNPs

SNPs were screened with a genome-wide significance threshold ( $p < 5 \times 10^{-8}$ ) to select SNPs significantly associated with exposure, while those directly associated with outcome were excluded ( $p < 5 \times 10^{-8}$ ). F statistics were calculated for each SNP ( $F = [(n-k-1)/k] \times [R^2/1-R^2]$ ) value, and SNPs with weak instrumental variables (F value less than 10) were excluded, where  $n$  represents sample size,  $k$  represents number of SNPs (F value of a single SNP was calculated,  $k=1$ ), and  $R^2$  represents variance explained by the SNP. The F statistics of the instrumental variables used in this study were all greater than 10, indicating that the instrumental variables had a significant effect on exposure and could provide reliable MR results [9].

### 2.3. Screening and Processing of SNPs

Genome-wide significant SNPs associated with cholelithiasis ( $p < 5 \times 10^{-8}$ ) were initially selected from the pooled GWAS dataset. To ensure the independence of instruments, linkage disequilibrium pruning was performed using European ancestry reference data from the 1000 Genomes Project, applying a threshold of  $r^2 < 0.001$  within a 10,000-kb window [10]. The resulting independent SNPs were then extracted from the scoliosis GWAS dataset, and effect alleles were harmonized across the two datasets. For palindromic SNPs, allele frequencies were examined to mitigate potential strand ambiguity; those with a minor allele frequency greater than 0.42 were excluded due to strand inference uncertainty.

## 2.4. Causality Verification Methods

The primary causal inference between cholelithiasis and scoliosis was assessed using the inverse variance weighted (IVW) method, with a significance threshold of  $p < 0.05$  considered indicative of a potential causal relationship [11]. Given the IVW method's vulnerability to horizontal pleiotropy, we supplemented the analysis with two additional approaches: the weighted median and MR-Egger methods. The weighted median estimator yields consistent results if at least 50% of the genetic instruments are valid, while MR-Egger regression can provide unbiased estimates even when all instruments violate MR assumptions, assuming the pleiotropic effects are independent of the instrument strength [12–13].

## 2.5. Sensitivity Analysis

To test result stability, heterogeneity was assessed via Cochran's Q in the IVW model, while horizontal pleiotropy was evaluated using the MR-Egger intercept and MR-PRESSO global tests [14–15]. If significant pleiotropy was

detected, the MR-PRESSO outlier test was applied to identify and remove outlier SNPs, after which the causal estimates were re-evaluated using the remaining instruments. In addition, a leave-one-out sensitivity analysis was conducted to determine whether individual SNPs disproportionately influenced the MR results. All analyses were carried out using the "TwoSampleMR" R package (version 0.5.8).

## 3. Results

### 3.1. Instrumental Variables

We screened 49 SNPs from the GWAS data for cholelithiasis that were closely related ( $p < 5 \times 10^{-8}$ ) and not chained unbalanced ( $r^2 < 0.001$  in a 10,000-kb window), and the F value for each SNP was calculated to be greater than a critical value of 10, indicating that none of these SNPs was a weak instrumental variable (for details, see Table 1). In the process of reconciling exposure data and outcome data, Removing the following SNPs for being palindromic with intermediate allele frequencies: rs6544721, this SNP will not be included in subsequent studies.

**Table 1.** Information on SNPs ultimately screened from GWAS data of Cholelithiasis (n=49).

ID	SNP	Effect_Allele	Other_Allele	B	SE	P	F
1	rs9427110	A	G	0.0512	0.0082	4.01E-10	38.98631767
2	rs1497406	G	A	0.0487	0.0088	3.70E-08	30.62616219
3	rs75331444	A	G	0.8073	0.0192	1.00E-200	1767.939697
4	rs6544721	C	G	-0.0637	0.0101	3.47E-10	39.77737477
5	rs28473566	A	G	-0.2047	0.0127	4.99E-58	259.7934776
6	rs1105880	G	A	0.0726	0.0087	4.95E-17	69.63614744
7	rs1260326	C	T	0.0819	0.0084	1.29E-22	95.0625
8	rs55726838	A	G	-0.3894	0.0509	1.90E-14	58.52700893
9	rs13427681	C	G	0.0495	0.0084	3.20E-09	34.72576531
10	rs55932961	A	G	-0.0847	0.0103	2.39E-16	67.62267886
11	rs4681515	G	A	-0.1251	0.0082	8.53E-53	232.7485128
12	rs6794817	T	C	0.0564	0.0103	4.90E-08	29.98359883
13	rs10025454	A	T	-0.0479	0.0085	1.70E-08	31.75653979
14	rs12500824	G	A	0.0525	0.0084	4.70E-10	39.0625
15	rs2290846	A	G	0.116	0.0092	1.41E-36	158.979206
16	rs10462337	T	C	-0.0543	0.0098	3.40E-08	30.70064556
17	rs351864	G	C	-0.0657	0.0104	3.14E-10	39.90837648
18	rs12528678	T	G	0.0824	0.0113	3.63E-13	53.17378025
19	rs4148805	A	G	-0.1386	0.011	1.62E-36	158.76
20	rs4724803	G	A	0.0551	0.0098	1.79E-08	31.61193253
21	rs714583	A	T	-0.0864	0.0091	1.43E-21	90.14563459
22	rs7786376	G	A	0.058	0.0101	9.79E-09	32.9771591
23	rs13280055	A	G	0.113	0.0148	2.36E-14	58.29528853
24	rs2954021	G	A	0.0453	0.0082	2.82E-08	30.51888757
25	rs2081687	C	T	-0.1177	0.009	5.88E-39	171.0282716
26	rs12547281	A	G	0.0716	0.0115	3.97E-10	38.76415879
27	rs686030	A	C	0.1158	0.0135	1.22E-17	73.5782716
28	rs600038	C	T	0.0618	0.0097	1.78E-10	40.59134871
29	rs11239549	G	A	0.1054	0.0102	7.08E-25	106.7777778
30	rs12245367	T	C	-0.0713	0.0083	8.37E-18	73.79430977
31	rs1535	G	A	0.0511	0.0084	1.23E-09	37.00694444
32	rs56363382	T	C	0.1141	0.0143	1.57E-15	63.66477578
33	rs7979473	G	A	0.0762	0.0083	5.53E-20	84.28567281
34	rs16961277	G	A	-0.0757	0.0125	1.46E-09	36.675136
35	rs28929474	T	C	0.3236	0.0346	9.34E-21	87.47114838
36	rs11644920	T	A	0.0682	0.0086	2.71E-15	62.88858843
37	rs1558902	A	T	0.0528	0.0087	1.25E-09	36.83234245
38	rs11866219	C	A	-0.0478	0.0085	1.97E-08	31.62408304
39	rs17138478	A	C	0.076	0.0112	1.27E-11	46.04591837
40	rs369298568	C	T	-0.0827	0.0146	1.57E-08	32.08524113
41	rs62129966	A	C	-0.1553	0.0132	5.05E-32	138.4187902
42	rs7599	G	A	-0.06	0.0088	8.39E-12	46.48760331
43	rs708686	T	C	0.0839	0.0089	5.44E-21	88.86769347
44	rs601338	A	G	0.077	0.0097	2.16E-15	63.0141354
45	rs1800961	T	C	0.3186	0.0246	2.09E-38	167.7340869
46	rs1321940	G	A	-0.0483	0.0084	8.87E-09	33.0625
47	rs5757135	T	C	0.0464	0.0084	3.77E-08	30.51247166
48	rs4821943	G	A	0.048	0.0084	1.10E-08	32.65306122
49	rs2076211	T	C	-0.0616	0.0099	4.27E-10	38.71604938

### 3.2. Causal Relationship Between Cholelithiasis and Scoliosis

The results obtained by MR analysis showed a causal

relationship between cholelithiasis and scoliosis (IVW OR=1.143, 95% CI=1.011-1.293, p= 0.033), as shown in Table 2. In addition, the results of the scatterplot (Figure 1) and the forest plot (Figure 2) also indicated that cholelithiasis increases the risk of scoliosis.

Table 2. MR regression results of the 3 methods.

Method	$\beta$	SE	OR (95% CI)	P
Inverse variance weighted	0.134	0.063	1.143(1.011-1.293)	0.033
Weighted median	0.108	0.089	1.113(0.935-1.326)	0.228
MR Egger	0.068	0.091	1.070(0.896-1.279)	0.458

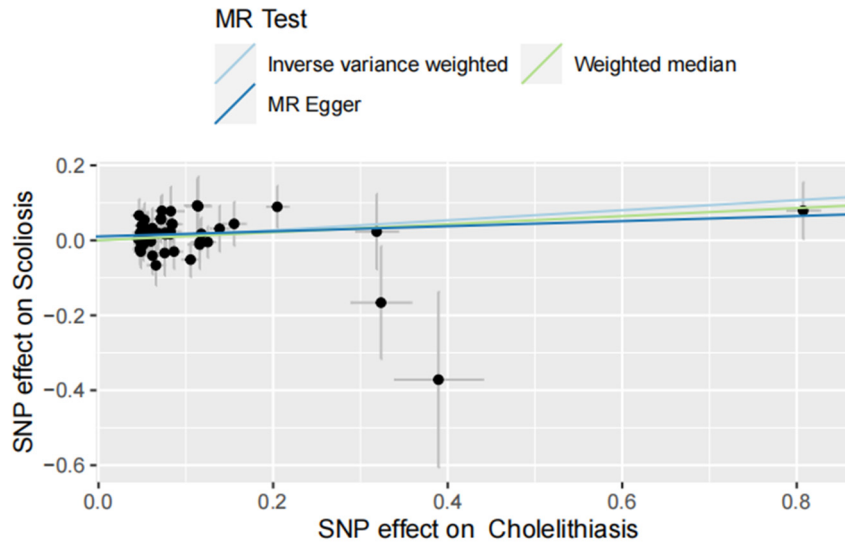


Figure 1. Scatter plot of cholelithiasis and scoliosis.

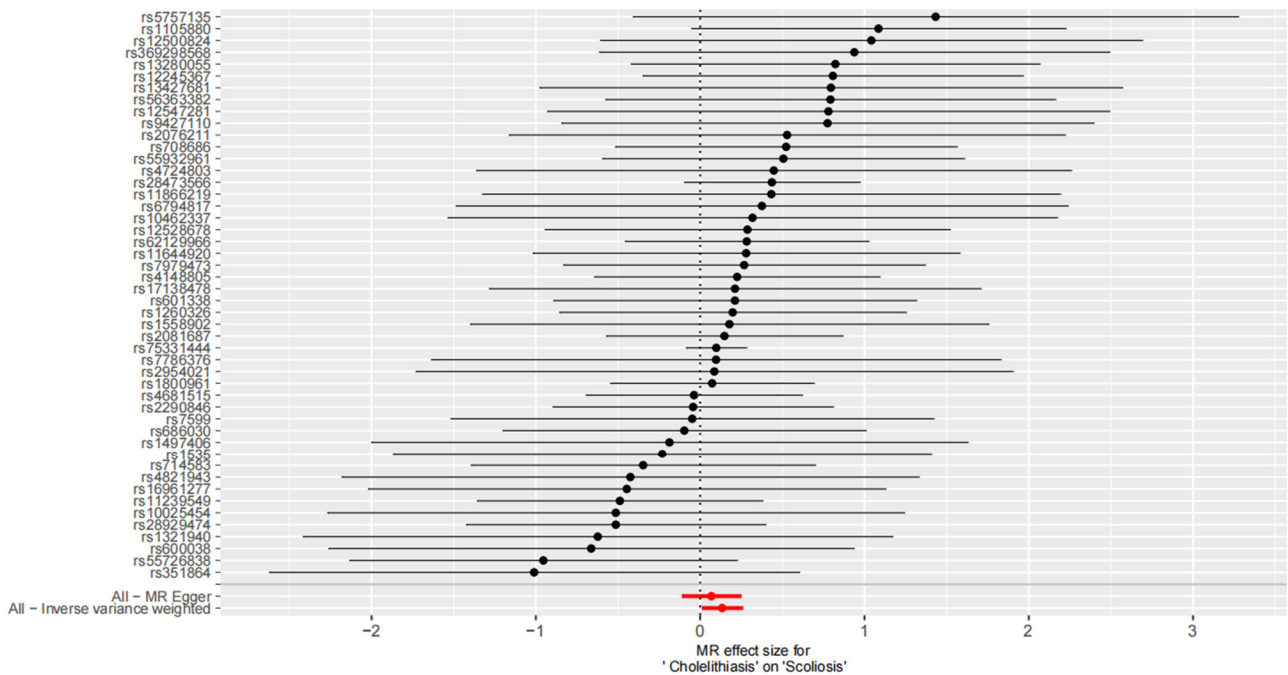


Figure 2. Forest plot of cholelithiasis and scoliosis.

### 3.3. Sensitivity Analysis

Cochran's Q test under the IVW model indicated no significant heterogeneity (p = 0.981), and the funnel plot (Figure 3) showed a symmetrical distribution of estimates. The MR-Egger intercept (p = 0.323) and MR-PRESSO global

test (p = 0.985) suggested no horizontal pleiotropy, with no outlier SNPs detected. Leave-one-out analysis confirmed that removing individual SNPs did not substantially impact the causal estimate, supporting the findings' robustness (Figure 4).

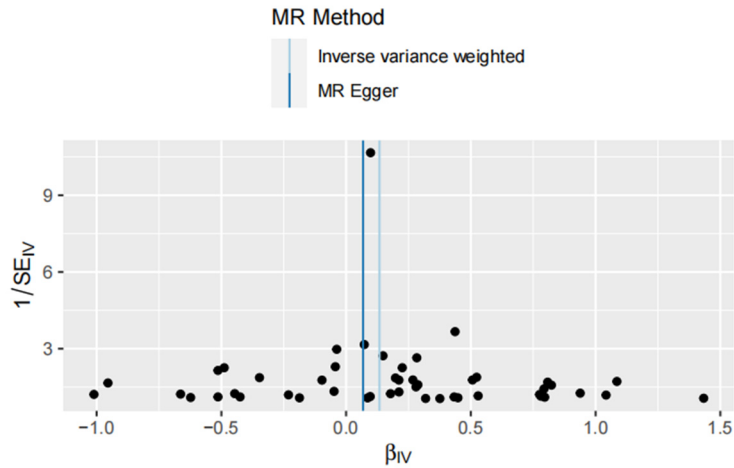


Figure 3. Funnel plot of cholelithiasis and scoliosis

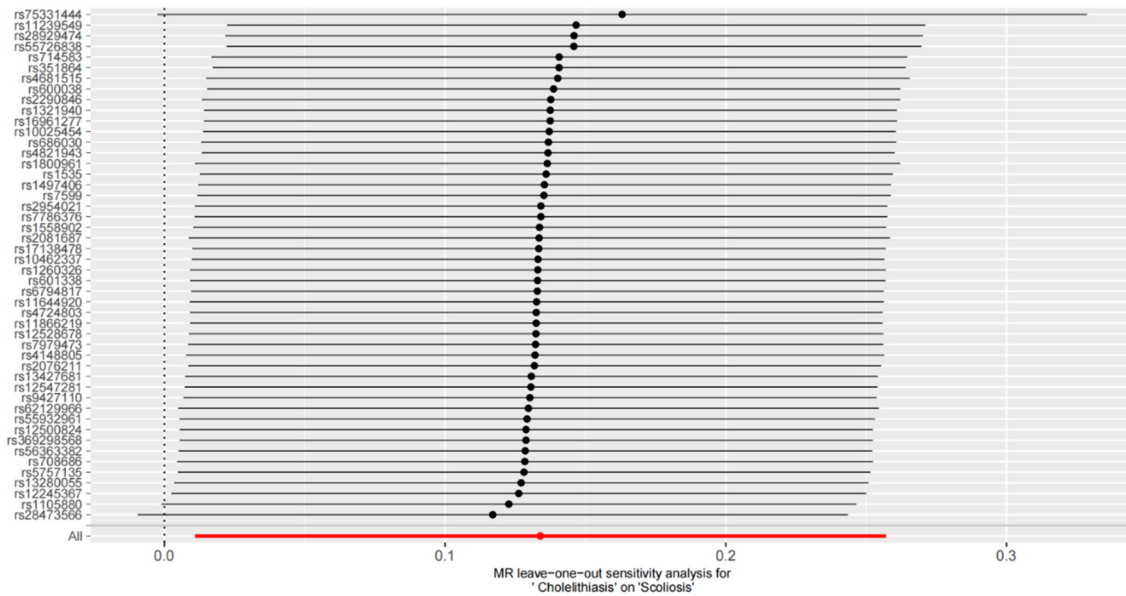


Figure 4. Analysis of cholelithiasis and scoliosis by the leave-one-out method

## 4. Discussion

Scoliosis is a three-dimensional spinal deformity characterized by lateral curvature and vertebral rotation, and is typically classified into idiopathic, congenital, or secondary forms [16]. Although factors such as angiogenesis, inflammation, extracellular matrix remodeling, and biomechanical stress have been implicated in its development, many aspects of its pathogenesis and associated risk factors remain unclear [17]. This study aimed to explore the potential causal association between cholelithiasis and scoliosis using MR. Our two-sample MR analysis yielded an odds ratio of 1.143 (95% CI: 1.011–1.293,  $p = 0.033$ ), indicating a statistically significant causal link, whereby individuals with cholelithiasis may have a 14.3% higher risk of developing scoliosis compared to the general population. Clinically, scoliosis can lead to asymmetries in shoulder height, thoracic and lumbar posture, and overall body alignment, often accompanied by chest and back pain. In more severe cases, structural changes to the spine may affect thoracic organs such as the heart and lungs, resulting in compromised cardiopulmonary function [18]. The condition may also

impair patients' ability to perform daily activities, reduce social adaptability, and contribute to psychological distress. While conditions such as Marfan syndrome, cerebral palsy, and neurofibromatosis have been suggested as possible contributors, definitive evidence identifying specific comorbidities that elevate scoliosis risk is still lacking [19]. Cholelithiasis, characterized by the presence of gallstones in the biliary system, is a prevalent gastrointestinal disorder manifesting with symptoms such as abdominal pain, nausea, vomiting, and jaundice, and poses a substantial healthcare burden globally [20–21]. Some recent reports have indicated a possible link between cholelithiasis and spinal pathology. Building on this observation, our study applied genetic epidemiological methods to evaluate causality, and the findings support a positive relationship between cholelithiasis and the development of scoliosis. Sensitivity analyses further confirmed the robustness of this association. These results suggest that individuals with cholelithiasis may benefit from early screening and clinical attention for scoliosis, which could enable timely intervention and potentially mitigate disease progression.

Given the observed association between cholelithiasis and an increased risk of scoliosis, several clinical and public

health recommendations can be proposed. Patients diagnosed with cholelithiasis should be encouraged to seek timely medical attention, adhere to regular monitoring and treatment plans, and maintain a positive and proactive approach to disease management. Considering that cholelithiasis is closely linked to dietary and lifestyle factors, public education efforts should emphasize the importance of healthy eating habits and raise awareness about the potential musculoskeletal implications of gallstone disease, including scoliosis. Since many individuals with cholelithiasis experience mild or asymptomatic presentations that do not necessitate immediate intervention, preventive strategies for scoliosis should be incorporated into the routine care of these patients. This could include scheduled follow-up visits, periodic clinical evaluations, health education programs, and, when appropriate, guidance on the use of preventive measures such as spinal orthoses. Furthermore, the underlying biological mechanisms connecting cholelithiasis to scoliosis remain unclear and warrant further investigation. A deeper understanding of this causal pathway may contribute to more effective prevention and targeted treatment strategies in the future.

This study has some limitations. First, the GWAS data used were exclusively from individuals of European ancestry, which may restrict the applicability of the results to other populations. Second, although sensitivity analyses did not indicate significant horizontal pleiotropy, it remains challenging to entirely eliminate the possibility of residual pleiotropic effects influencing the results. Lastly, the sample sizes of the available GWAS datasets were relatively modest, and future studies incorporating larger and more diverse cohorts will be essential to validate and extend our findings.

## 5. Conclusion

In summary, this study analyzed GWAS data using a two-sample MR analysis method to reveal cholelithiasis as a risk factor for the development of scoliosis from a genetic perspective.

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