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RESEARCH

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The causal relationship between anxiety and tinnitus severity: a Mendelian randomization study

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Abstract

Background The link between anxiety and tinnitus severity has garnered significant scholarly interest, with numerous studies identifying a positive correlation. Despite this, the genetic basis of this relationship remains underexplored. Leveraging publicly accessible GWAS data, this study employs Mendelian randomization to elucidate the genetic causality between anxiety and tinnitus severity.

Methods This research analyzed single nucleotide polymorphisms (SNPs) related to anxiety and tinnitus severity from the UK Biobank, utilizing aggregated data from genome-wide association studies (GWAS). Instrumental variables linked to anxiety were meticulously selected. The study implemented several Mendelian randomization techniques, including "mr_ivw," "mr_egger_regression," "mr_weighted_median," "mr_simple_mode," and "mr_weighted_mode," to assess the causal impact of anxiety on tinnitus risk through odds ratios (ORs). Sensitivity analyses, including MR-Egger and the leave-one-out method, were conducted to ensure result stability. The F-value was also calculated to ascertain the strength of the instrumental variables.

Results Analysis identified five SNPs as instrumental variables. The calculated ORs and 95% confidence intervals from MR-Egger regression, weighted median, and inverse variance weighting showed no statistically significant causal relationship between anxiety and severe tinnitus, with all *P*-values exceeding 0.05. This lack of statistical significance, consistent across various methods, indicates no genetic causality between anxiety and tinnitus severity. Furthermore, no evidence of heterogeneity ($P = 0.80$) or horizontal pleiotropy ($P = 0.31$) was found, reinforcing the robustness of the instrumental variables ($F > 10$).

Conclusion Our Mendelian randomization analysis reveals no genetic causality between anxiety and tinnitus severity, suggesting the need for further research into the multifaceted etiology of tinnitus.

Highlights

1. Investigating Genetic Causality: This study delves into the genetic basis of the relationship between anxiety and tinnitus severity, aiming to establish a theoretical foundation for clinical practices.
2. Findings from Mendelian Randomization: Our Mendelian randomization analysis reveals the absence of a genetic causal link between anxiety and tinnitus severity, challenging previous assumptions within the field.

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3. Call for Further Research: Despite the high clinical prevalence of tinnitus, its etiology remains elusive. This underscores the need for more comprehensive research to unravel its complex causes and improve patient outcomes.

Keywords Anxiety, Mendelian randomization, Tinnitus

Introduction

Tinnitus is a common symptom in otolaryngology, where patients often perceive sounds of varying loudness in their ears even without external acoustic or electrical stimuli. Numerous factors influence the severity of tinnitus [1, 2]. High-pitched tinnitus can cause distress and anxiety, severely affecting patients' sleep and work. A systematic review and meta-analysis showed that the prevalence of tinnitus in adults is related to age (9.7% in adults aged 18–44 [95% CI, 7.412.5%]; 13.7% in adults aged 45–64 [95% CI, 11.0–17.0%]; and 23.6% in the population aged ≥ 65 [95% CI, 19.4–28.5%]; $P < 0.001$) and is not related to gender (14.1% in males [95% CI, 11.6–17.0%]; 13.1% in females [95% CI, 10.5–16.2%]), with an adult prevalence rate of about 10 to 25% [3]. However, a retrospective study showed that tinnitus severity has different risk factors across genders [4]. Anxiety refers to anxiety disorders, characterized by anxious emotions as the main manifestation, including tension, fear, etc. Research has found that adolescents with autism or ADHD are at increased risk of anxiety, and parental abuse can also promote the occurrence of anxiety symptoms in youth [5, 6]. Clinically, anxiety and depression often coexist, with depression characterized by significant and persistent low mood, common in the elderly, and associated with increased risk of morbidity and mortality [7]. Anxiety and tinnitus both show low treatment compliance, significantly affecting individuals' overall quality of life, and bringing substantial economic burden and disease to patients. The causal relationship between anxiety and the severity of tinnitus has been of great concern for a long time. A study cohort of 8539 people showed that the prevalence of tinnitus and anxiety was significantly higher than in the normal population [8]. Patients with acute tinnitus have a higher risk of anxiety, while those with chronic tinnitus often exhibit symptoms of depression [9]. Although most studies indicate that anxiety and tinnitus severity are related, there are few related large samples and genetic-level causal inference studies [1, 10, 11].

Mendelian Randomization (MR) studies employ genetic variations following the principle of random allocation of alleles to offspring. This study, based on whole-genome sequencing data, effectively reduces bias and is akin to randomized controlled trials (RCTs), serving as a statistical method to reveal causal relationships [12, 13]. Numerous factors contribute to the severity of tinnitus, and to date, no studies have utilized Mendelian randomization (MR) to investigate the causal relationship between anxiety and the severity of tinnitus. Therefore, this study employs the MR method to explore whether there is a causal relationship between anxiety and the severity of tinnitus。

Materials and methods

Data sources and study design

Through a search of genome-wide association studies (GWAS), anxiety was identified as the exposure variable, and the severity of tinnitus as the outcome variable. The summary data for anxiety and tinnitus severity were sourced from the UK Biobank within GWAS (ht tps://pa n.ukbb.b roadinstitute.org.2020). The study includes data on 1173 anxiety cases and 9,810,481 SNPs; and 1293 cases of severe tinnitus with 9,812,495 SNPs, as shown in (Table 1). This research utilizes MR analysis to verify causal relationships, employing standardized summary statistics from GWAS. The necessary information for the exposure and outcome factors in the GWAS includes the SNP or the chromosome (CHR) and the specific location on the chromosome (POS), effect allele frequency (eaf), beta (the effect size, representing the standard error of the impact of each allele on the outcome in this study, expressed as the odds ratio (OR), analyzed in log(OR) format), the standard error (SE), and the significance indicator of the SNP in the exposure factor (*P*-value). The initial GWAS received approval from the relevant institutional

Table 1 GWAS Data Summary

variable	ID	Sample size	SNP	Population	Sex	Year
Anxiety	ukb-e-20506_CSA	1173	9,810,481	South Asian	Males and females	2020
Tinnitus severity/ nuisance	ukb-e-4814_CSA	1293	9,812,495	South Asian	Males and females	2020

review board. This study was approved by the Ethics Committee of XX University (approval number: XXX). Patients gave their consent through an informed consent procedure, which was reviewed by the Ethics Committee of XX University, confirming that this study complies with the ethical standards laid out in the 1964 Declaration of Helsinki. To ensure effective instrumental variables (IVs), Mendelian randomization studies must adhere to three core assumptions: a. The chosen instrumental variables are significantly related to the exposure. b. The instrumental variables are not related to the outcome. c. The instrumental variables are not related to confounding factors. The designed MR model is used to assess the risk of severe tinnitus due to anxiety (Fig. 1).

Instrumental variable selection

Initially, we used the R software (version 4.3.1) and the "TwoSampleMR" package to import the exposure and outcome variables. We filtered out SNPs with $P < 5 \times 10\text{^} - 8$ [14], a genetic distance of 10,000 kb, and a linkage disequilibrium parameter (r2) < 0.001, which yielded only two SNPs, deemed too small a sample size. Consequently, we relaxed the selection threshold to $P < 5 \times 10\text{^} - 7$ and set the linkage disequilibrium parameter (r2) at a threshold of 0.01, using a genetic distance of 1000 kb [15]. The selected SNPs were then searched in the PhenoScanner database (www.phenoscanner.medschl.cam.ac.uk/) to exclude SNPs associated with confounding factors. Finally, the extracted 5 SNPs were merged with the SNPs from the severe tinnitus database, eliminating palindromic sequence SNPs. The obtained SNPs served as instrumental variables representing the exposure factor (Table 2).

MR analysis

Mendelian randomization analyses were conducted utilizing five methods: inverse variance weighting (IVW), Egger regression (mr_egger_regression), weighted median (mr_weighted_median), simple mode (mr_simple_mode), and weighted mode (mr_weighted_mode).

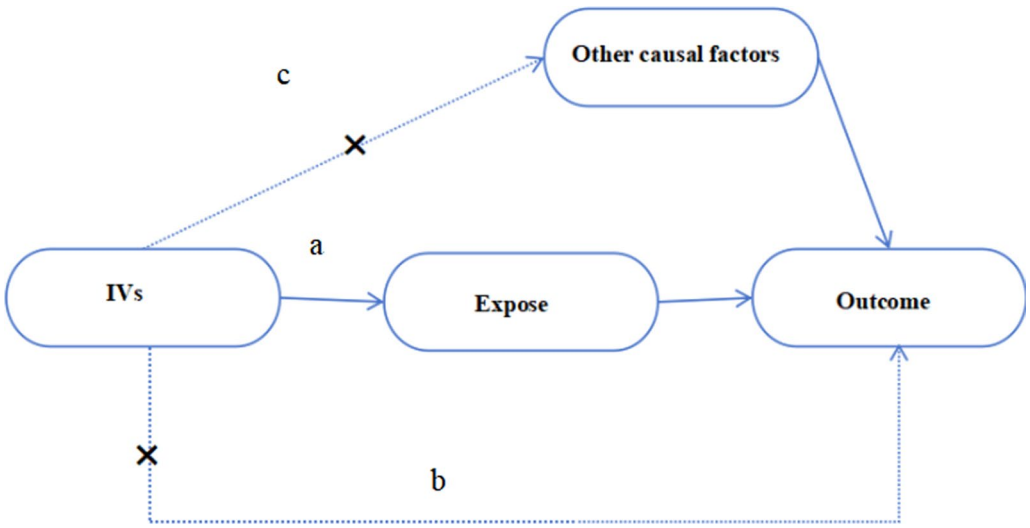

Fig. 1 Directed acyclic graphs for the classical Mendelian randomization designs. The arrows denote causal relations between two variables, pointing from the cause to the effect. The causal pathway is blocked if "X" is placed in the arrowed line. MR, Mendelian randomization

Table 2 The information for final tool variables

SNP	CHR	POS	EA	OA	EAF	*β*-value	SE-value	*P*-value	R^2	*F*-value
rs12330593	3	127,205,545	A	G	0.98764	0.7166	0.137	0.1771	0.012537206	27.35977196
rs144385275	12	38,482,542	G	C	0.9462	0.3704	0.07218	0.4943	0.013968095	26.33347667
rs62053319	16	85,786,810	C	T	0.98551	0.7418	0.1236	0.653301	0.015715676	36.01942009
rs72673891	4	106,814,302	G	A	0.97278	0.5508	0.0912	0.704001	0.016066475	36.47524238
rs75155228	3	184,347,463	C	G	0.98144	0.6748	0.1308	0.8576	0.016589064	26.61550188

chr: chromosome, EA: effect allele, OA: other allele, EAF: effect allele frequency, β: allele effect size, SE: standard error

Sensitivity analysis

The Racial Mendelian Randomization R package was utilized to perform Cochran's Q test on SNPs that met all three hypotheses, assessing heterogeneity among individual genetic variants. A *P*-value of Cochran's Q test less than 0.05 indicates the presence of heterogeneity, suggesting that the relationship between exposure and outcome is influenced by factors such as varying ages and genders. In such cases, the IVW random effects model is considered the gold standard for the final results of Mendelian randomization. Otherwise, the IVW fixed effects model is adopted as the gold standard, with forest plots employed to visualize the results of the heterogeneity test. Additionally, the Egger-intercept method for Mendelian randomization pleiotropy and the Mendelian Randomization-PRESSO test were applied to examine potential violations of Mendelian randomization assumptions due to horizontal pleiotropy. For the Egger-intercept, a *P*-value less than 0.05 indicates the presence of horizontal pleiotropy, signifying that the selected instrumental variables significantly affect the outcome through pathways other than the exposure, thereby violating hypotheses b and c. Conversely, a *P*-value greater than 0.05 suggests that the exposure does not significantly affect the outcome through other pathways. The leave-one-out approach was employed as a sensitivity analysis to determine whether any of the final SNPs were outliers, with the stability of results further verified by examining asymmetry in funnel plots.

Weak instrumental variables test

The F-statistic was calculated to evaluate the potential bias from weak instrumental variables in the selected SNPs, further validating the association hypothesis. This was determined using the formula $F = R^2 \times (N-K)/(K \times (1-R^2))$, where N is the sample size of the exposure, *K* is the number of instrumental variables, and R^2 is the proportion of exposure variance explained by the instrumental variables. R^2 is calculated as $2 \times (1\text{-MAF}) \times MAF \times \beta^2$, where MAF is the minor allele frequency, β represents the effect of the allele, and SD is the standard deviation. The standard error (SE) is used for individual SNPs. An *F*-value greater than 10 indicates strong instrumental variables, whereas a value less than 10 suggests weak instrumental variables [16].

Results

Two-sample mendelian randomization outcomes

The results of this study reveal that the odds ratios (ORs) and 95% confidence intervals (CIs) obtained from MR-Egger regression and weighted median methods, alongside the inverse variance weighted (IVW) method, were 0.643 (95% CI 0.330–1.251) and 0.965 (95% CI 0.766–1.216),Our study results demonstrate no causal relationship between anxiety and the severity of tinnitus. As illustrated in (Fig. 2). Additionally, the Cochran-Q test produced a *P*-value greater than 0.05, indicating a lack of heterogeneity among the instrumental variables and leading to the predominant use of the fixed-effects model IVW for analysis. The IVW method's findings suggest that the effect of anxiety on tinnitus severity was not statistically significant, indicating that within the studied population, anxiety does not have a discernible impact on the severity of tinnitus. Moreover, the effect sizes and confidence intervals derived from the MR-Egger and weighted median (WME) methods were consistent with those obtained from the fixed-effects model IVW method. The scatter plot results show that the instrumental variables closely related to anxiety and the severity of tinnitus disease are stable, but the slope directions of the five methods are inconsistent, which cannot indicate a correlation between the two, as shown in (Fig. 3).

Heterogeneity and horizontal pleiotropy

The analysis of heterogeneity in the Mendelian randomization outcomes, concerning the impact of anxiety on tinnitus severity through the IVW method, yielded a *P*-value of 0.80, which is greater than 0.05, indicating an absence of significant heterogeneity. Furthermore, the horizontal pleiotropy test, conducted using the Egger-intercept method, resulted in a *P*-value of 0.31, also exceeding 0.05 (Table 3).This suggests that the instrumental variables did not significantly affect the outcome through pathways other than the exposure of interest. The stability of these findings was confirmed by the leave-one-out sensitivity analysis. Forest plots depicting the instrumental variables, along with the results of the leave-one-out test, are presented in (Fig. 4), providing a visual representation and further validation of these results.

Assessment of instrumental variables

Using the R^2 and *F*-statistic formulas, the total R^2 value was determined to be 0.014975303, and the *F*-statistic was calculated to be 30.56. These results confirm that the five instrumental variables selected for this study are considered strong. The R^2 value, although relatively low, suggests a modest but non-negligible proportion of the exposure variance is explained by the instrumental variables. The *F*-statistic, significantly exceeding the threshold of 10, indicates that the instrumental variables are sufficiently strong to provide reliable estimates, minimizing the potential bias from weak instrument bias in the Mendelian randomization analysis.

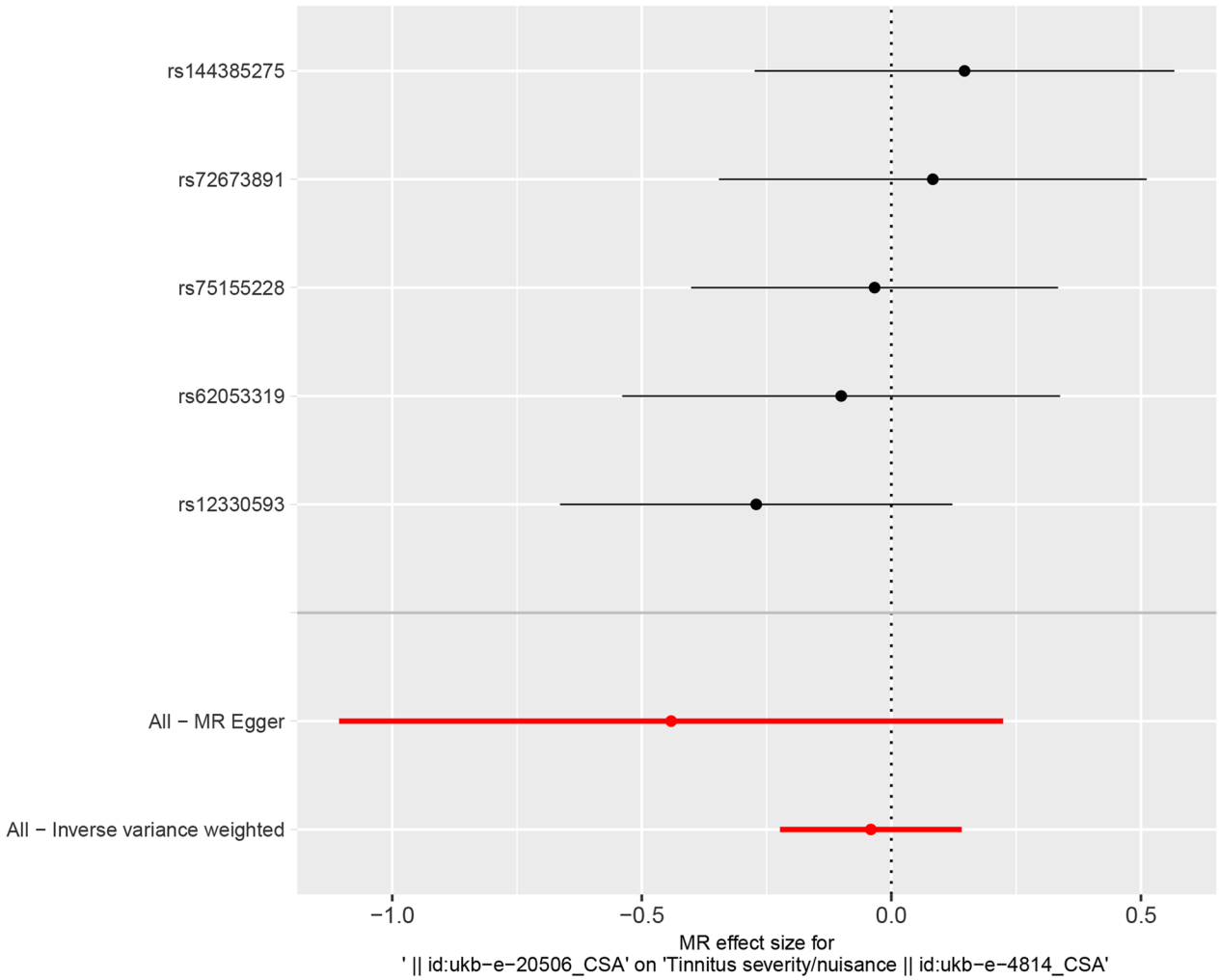

Fig. 2 Showing assessment of the effects of SNP-related anxiety on tinnitus severity using IVW, Mr-egg regression

Discussion

The prevalence of tinnitus is estimated at 20%, with severe cases affecting 3.8% of the population. Numerous studies have suggested a link between anxiety states and tinnitus, proposing an anxiety-insomnia-tinnitus pathway, with significant combined effects on tinnitus severity [17–32]. Understanding the etiology of tinnitus is crucial for its prevention, diagnosis, and treatment. Mechanistically, anxiety triggers the hypothalamic–pituitary–adrenal axis in the inner ear, leading to excessive cortisol release. This affects the concentration of potassium ions in the vascular stria, which in turn triggers tinnitus [33]. Additionally, the prevalence of tinnitus is associated with several factors, including hearing loss, advanced age, male gender, high BMI, deprivation, hypertension, smoking history, and comorbidities. Genetic susceptibility to neuroticism and schizophrenia also predicts tinnitus severity [34]. Causal relationships have been established between tinnitus susceptibility and brain volume changes, with specific correlations to the cortical region of the left parahippocampal gyrus and gray matter volume [35]. A randomized controlled trial demonstrated the efficacy of dietary and physical activity interventions in improving tinnitus severity and quality of life for patients with obesity-associated tinnitus [36]. Extensive research, including a retrospective analysis of data from 1452 chronic tinnitus patients, revealed that factors such as anxiety, auditory hypersensitivity, sleep disorders, depression, and ear fullness contribute to chronic tinnitus severity. This comprehensive overview underscores the complex interplay of physiological, genetic, and lifestyle factors in tinnitus, highlighting the need for a multifaceted approach to its management [37].

Our study results demonstrate no causal relationship between anxiety and the severity of tinnitus. A Mendelian randomization study on factors contributing to

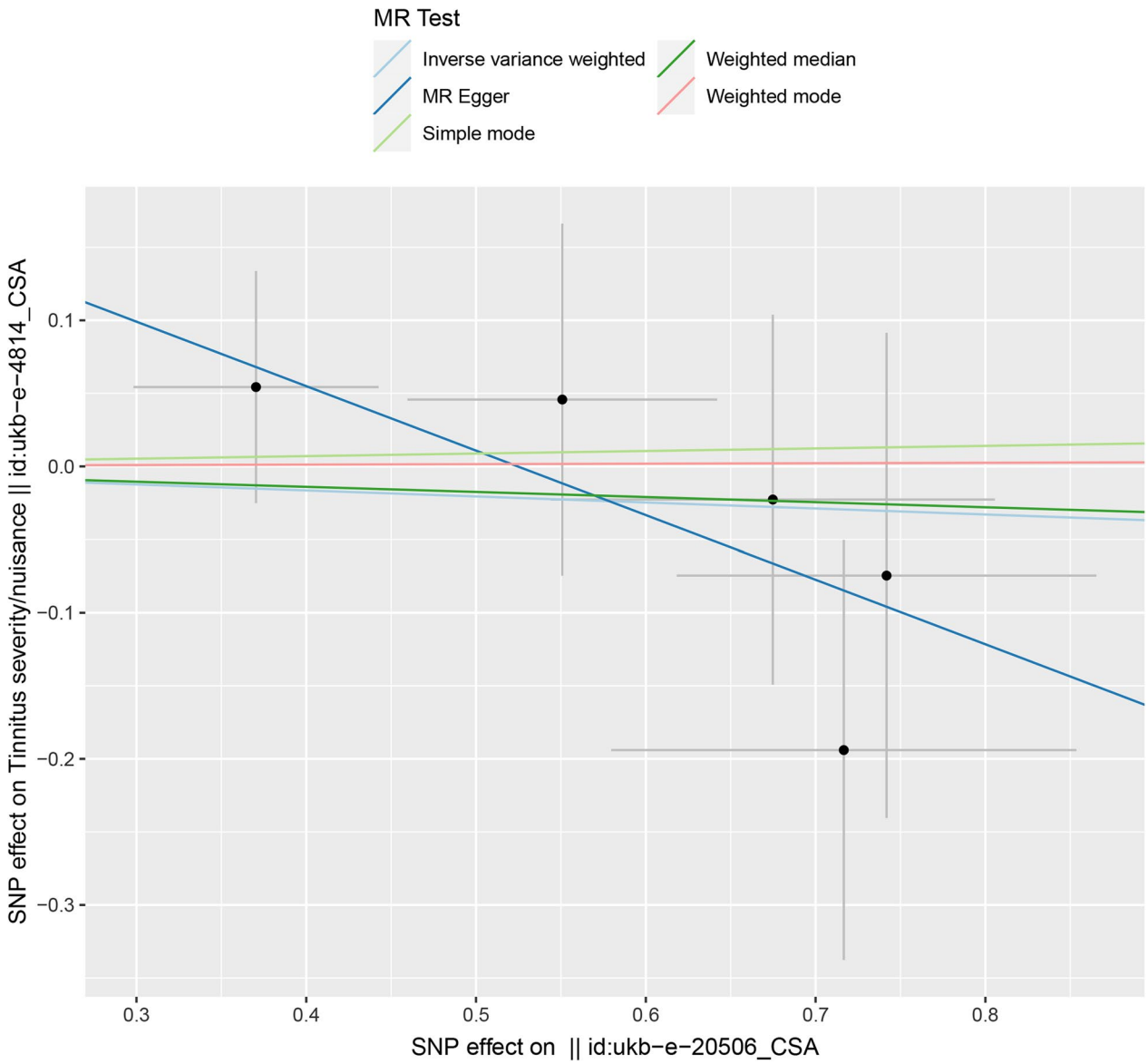

Fig. 3 Scatter plot, depicting the two-sample MR analysis

Table 3 Sensitivity test results

Exposure	Outcome	Cochran's Q	Q_df	Pval	Egger-intercept	Se	*P* val
Anxiety	Tinnitus severity	MR-Egger:0.969	3	0.809	0.231	0.188	0.308
		IVW:2.472	4	0.649			

tinnitus suggests that neuroticism and schizophrenia could predict the causal relationship of tinnitus severity [34]. Previous studies have shown that neither gender nor age has a significant correlation with the severity of tinnitus [38]. However, other research has found that the severity of tinnitus is not affected by age but is influenced by gender, with more pronounced effects in women [39]. Additionally, studies on the relationship between age, gender, and anxiety indicate that both age and gender contribute to the onset of anxiety [40]. Scholars have proposed that the severity of tinnitus has a genetic component, being associated with specific genes [41]. Using MR

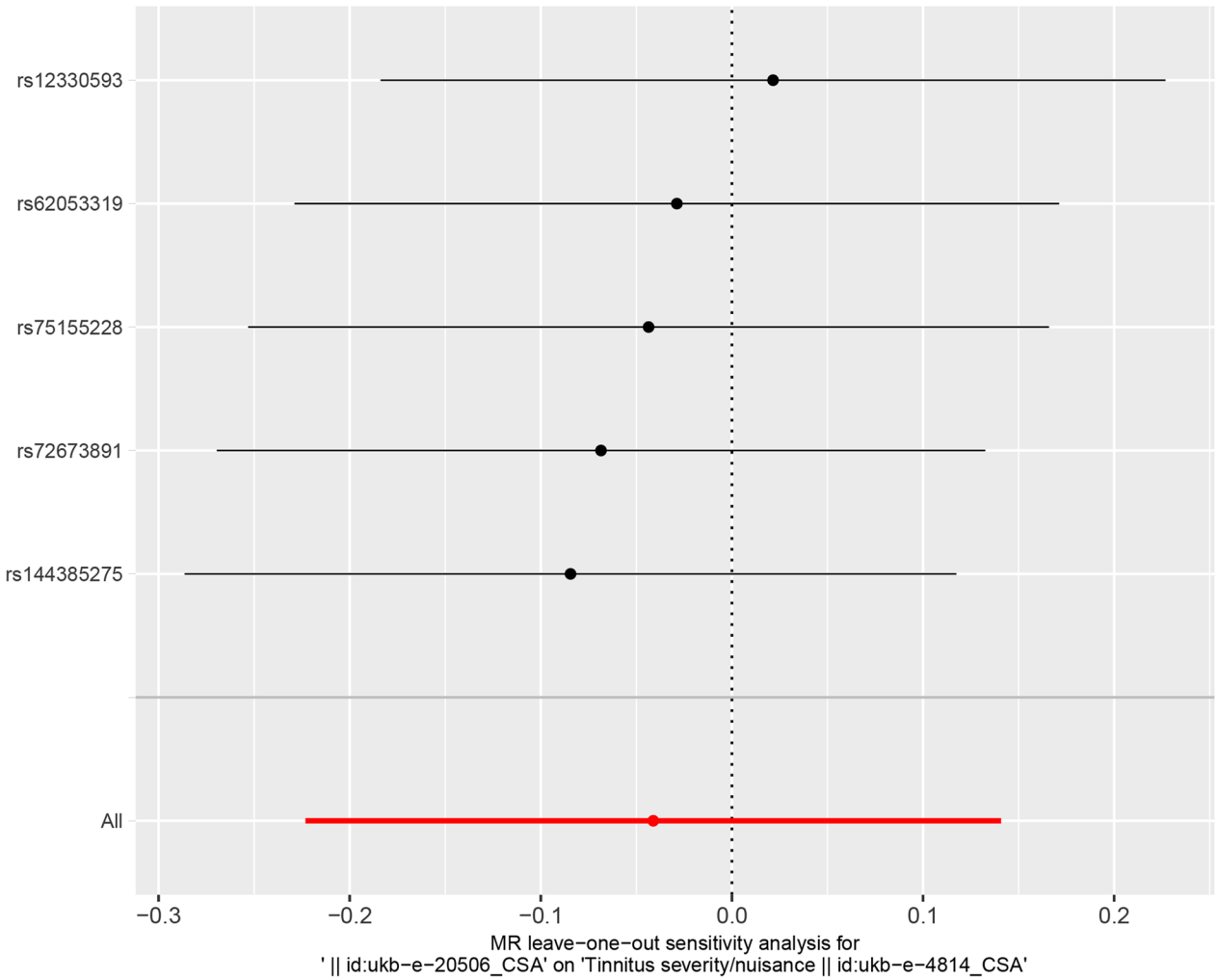

Fig. 4 The forest map based on the analysis result of" leave-one-out method"

analysis and searching for relevant instrumental variables on the PhenoScanner website, we found no SNPs related to confounding factors, conducted sensitivity analyses, and excluded heterogeneity and horizontal pleiotropy. Thus, our study conclusions are reliable, indicating that anxiety is not a risk factor for increasing the severity of tinnitus. Our MR study results do not align with clinical observational studies for several reasons: 1. Clinical observational studies inevitably collect data with confounding factors that affect outcomes; 2. The samples in clinical observational studies might differ from our MR randomized samples, with many studies expanding sample sizes by including multiple ethnicities, while our study only included South Asian population samples; 3. The result efficacy of clinical observational studies is low, and they can only suggest potential associations between exposure and outcome, not definitive causal relationships, and there is a possibility of reverse causation.

This study has several advantages: 1. MR theoretically eliminates confounding bias and controls for reverse causation, making the results more reliable; 2. Our data come from the UK Biobank database, which has a large sample size, and the data are publicly available and accessible; 3. Our study results show no heterogeneity or horizontal pleiotropy, with consistent results under the IVW and additional MR methods, indicating stability in the leave-one-out analysis.

However, our study has certain limitations. Firstly, the dataset selected was from the South Asian population, limiting the applicability of our results to other populations. Secondly, the database did not categorize by gender or age, preventing us from obtaining related data for stratified analysis, which should be explored further in the future.

In summary, we are the first to use the MR analysis method to assess the causal relationship between anxiety

and the severity of tinnitus, obtaining negative results, which are inconsistent with previous studies. Most past research was cross-sectional, lacking randomized controlled trials, while MR analysis resembles a natural RCT, making our evidence more persuasive and suggesting that previous studies may have been influenced by confounding factors or reverse causation. Therefore, we recommend that clinicians consider anxiety less in the treatment and etiology of severe tinnitus and that researchers conduct stratified analyses on age, gender, and other factors with updated and supplementary GWAS data, as well as expand research into other mechanisms in this field.

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Author contributions
Conceptualization, L.Y.; methodology, L.Y.; software, L.Y., L.Y.; validation, L.Y., Y.D., and L.Y..; formal analysis, L.Y., Y.D..; investigation,L.Y. resources, L.Y.; writing—original draft preparation, L.Y..; writing—review and editing, L.Y., Y.D.; visualization,L.Y.; supervision, L.Y.; project administration, Y.D..; funding acquisition, L.Y.. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The GWAS ID number of the datasets available in this study can be obtained directly to the corresponding authors.

Declarations

Ethics approval and consent to participate
This study Was approved by the Ethics Committee of the First Affiliated Hospital of Dali University (Approval NO:DFY20230310001),Patients were consented by an informed consent process that was reviewed by the Ethics Committee of First Affiliated Hospital of Dali university and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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