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ORIGINAL RESEARCH

Exploring the Causal Role of miR-941 in Chronic Rhinosinusitis: Insights from Transcriptomics and Genomics

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Purpose: Despite significant advancements in the epigenetics of chronic rhinosinusitis (CRS), particularly in the domain of microRNA (miRNA), little is known about miRNAs that play a causal role in CRS. This study aims to identify miRNAs with a causal relationship to CRS and explore their potential clinical value and mechanisms in CRS.

Methods: We conducted small RNA sequencing on blood and nasal samples to find miRNAs with consistent expression differences in CRS. These miRNAs were confirmed via qRT-PCR and assessed for clinical relevance through Spearman correlation and statistical analysis used to evaluate diagnostic accuracy. Bidirectional Mendelian randomization (MR, a genetic causal inference method) analyzed their causal links to CRS. Target genes of causally significant miRNAs were identified using miRWalk, and their mechanisms were explored through pathway enrichment and validation studies.

Results: We identified differentially expressed miRNAs in blood and nasal tissues using a $|\log2(\text{Fold Change})| > 0.58$ and P-value < 0.05 threshold. Following False Discovery Rate correction, hsa-miR-941 was identified as an upregulated miRNA in both CRS patient samples. The experimental validation of miR-941 expression closely matched sequencing results. Spearman and statistical analysis associated miR-941 expression changes with CRS severity and diagnostic accuracy. Bidirectional MR demonstrated a significant association of miR-941 with CRS risk, without evidence of reverse causality. Target gene and Western blot assays suggested miR-941's potential influence on CRS through the PI3K/AKT pathway.

Conclusion: There is a positive causal relationship between hsa-miR-941 and CRS, making hsa-miR-941 a valuable target for the diagnosis and treatment of CRS. These findings position miR-941 as a valuable biomarker and therapeutic target, providing new opportunities for precision medicine in CRS treatment. miR-941 may exert its effects by modulating the PI3K/AKT signaling pathway.

Keywords: chronic rhinosinusitis, transcriptomics, genomics, Mendelian randomization, miR-941

Introduction

Chronic rhinosinusitis (CRS) is a prevalent and highly heterogeneous inflammatory disease of the upper respiratory tract, affecting over 10% of adults worldwide. It has two distinct forms: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). It is concerning that CRS imposes a significant health burden on patients and has a notable socioeconomic impact.[1–3] The treatment of CRS encompasses various accepted approaches, such as intranasal corticosteroids, allergen immunotherapy, antihistamines, and endoscopic sinus surgery.[4] Nevertheless, the underlying causes of CRS are intricate, and a significant proportion of patients experience persistent or recurrent conditions, especially those with CRSwNP.[5,6] Over the past few decades, there has been notable advancement in comprehending the pathophysiology of CRS. However, the precise molecular mechanisms remain elusive, impeding the development of new therapeutic

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strategies. Therefore, it becomes imperative to further elucidate the key molecules in the pathogenesis of CRS and identify potential biomarkers to develop new treatment strategies.

In recent years, increasing evidence supports the close association between the dysregulation of microRNAs (miRNAs) and the pathogenesis of CRS.[7] miRNAs are small nucleotide sequences that play a key role in regulating gene expression, various molecular pathways, and about 60% of protein-coding genes.[8,9] They are involved in almost every biological event known today and play a pivotal role in the pathogenesis of diseases,[10,11] such as psoriasis,[12] atopic dermatitis,[13] and inflammatory bowel disease.[14] Notably, certain miRNAs exhibit multifunctional roles across various inflammatory disorders. For example, hsa-miR-941 has been identified as a biomarker for predicting treatment response to oral corticosteroids (OCS) in severe asthma and as a therapeutic target in psoriatic arthritis by regulating osteoclast differentiation.[15,16] This functional versatility highlights the potential of miRNAs as therapeutic agents across diseases. Mature miRNAs are not only found in cells but also whole blood, serum, plasma, breast milk, saliva, and even tears in the form of circulating miRNAs.[17,18] Due to their unique expression profiles, key regulatory roles, high stability, and specificity to targets in biological samples,[19] miRNAs in CRS have attracted increasing attention. Many studies have been conducted on miRNAs as potential diagnostic and therapeutic biomarkers for CRS.[20,21] Some miRNAs have been developed as targets for the treatment of some intractable diseases like stroke, and some miRNA-targeted drugs have entered the clinical stages.[22,23] Significant breakthroughs have been made in the last five years and multiple studies have demonstrated the involvement of miRNAs at the molecular and phenotypic levels in mediating the occurrence and development of CRS. However, miRNA research in the field of CRS is still in its infancy. Abnormal expression of miRNAs can influence disease progression by activating pro-inflammatory signaling axes or mediating the function of immune cells (eg eosinophils) or tissue inflammation and remodeling.[24–27] There are still considerable gaps in understanding the causal effect of miRNAs on CRS. Overall research is still at an early stage, with relatively few papers on the role of miRNA in CRS. Most of these papers only discuss the correlation between nasal tissue-level miRNAs and CRS, but fail to explore the impact of circulating miRNAs on the pathogenesis of CRS, nor prove their causal effects. Therefore, there is a considerably long way to the development and application of clinical trials.[28]

Mendelian randomization (MR) is an epidemiological approach that uses genetic variants as instrumental variables to investigate causal relationships between exposures (eg, circulating miRNA levels) and outcomes. This method offers two major advantages over traditional observational studies. First, it substantially reduces confounding bias, as genetic variants are randomly distributed during meiosis, making them independent of common confounders such as environmental factors and lifestyle choices. Second, MR inherently avoids reverse causality, as germline genetic variants remain unaffected by the onset or progression of disease.[29,30] Three key assumptions are required for MR analysis: (I) the genetic variants used as IVs for MR related to exposure must be strongly associated with the exposure; (II) these genetic variants should not be associated with any confounders that could influence the outcome; (III) the genetic variants must affect the outcome only through the exposure, not through other pathways. The MR analysis has been widely utilized to explore the causal relationship between molecular traits (such as miRNA or protein exposures) and phenotypes.[31–34] Previous MR studies have obtained a reliable causal relationship between miRNAs and COVID-19[35] as well as between leukocytes and CRS.[36] However, there is still a lack of MR studies that specifically investigate the causal relationship between miRNAs and CRS.

In this study, the causal relationship between miR-941 and CRS was elucidated for the first time through high-throughput small RNA sequencing and MR analysis of blood and nasal tissue, revealing its key role in the pathogenesis of CRS. Furthermore, its potential mechanisms and clinical significance were explored through bioinformatics and experimental methods (Figure 1). These findings not only provide new perspectives for understanding the molecular mechanisms of CRS but also suggest the clinical potential of miR-941 as a diagnostic biomarker and therapeutic target. Specifically, miR-941's sensitivity and specificity in CRS diagnosis and disease progression monitoring make it an important candidate biomarker with clinical application prospects. Additionally, miR-941 may regulate the PI3K/AKT signaling pathway, making it a potential therapeutic target for CRS. This study opens up new directions for the application of miRNA-based precision medicine strategies in CRS treatment and provides valuable insights for future clinical interventions.

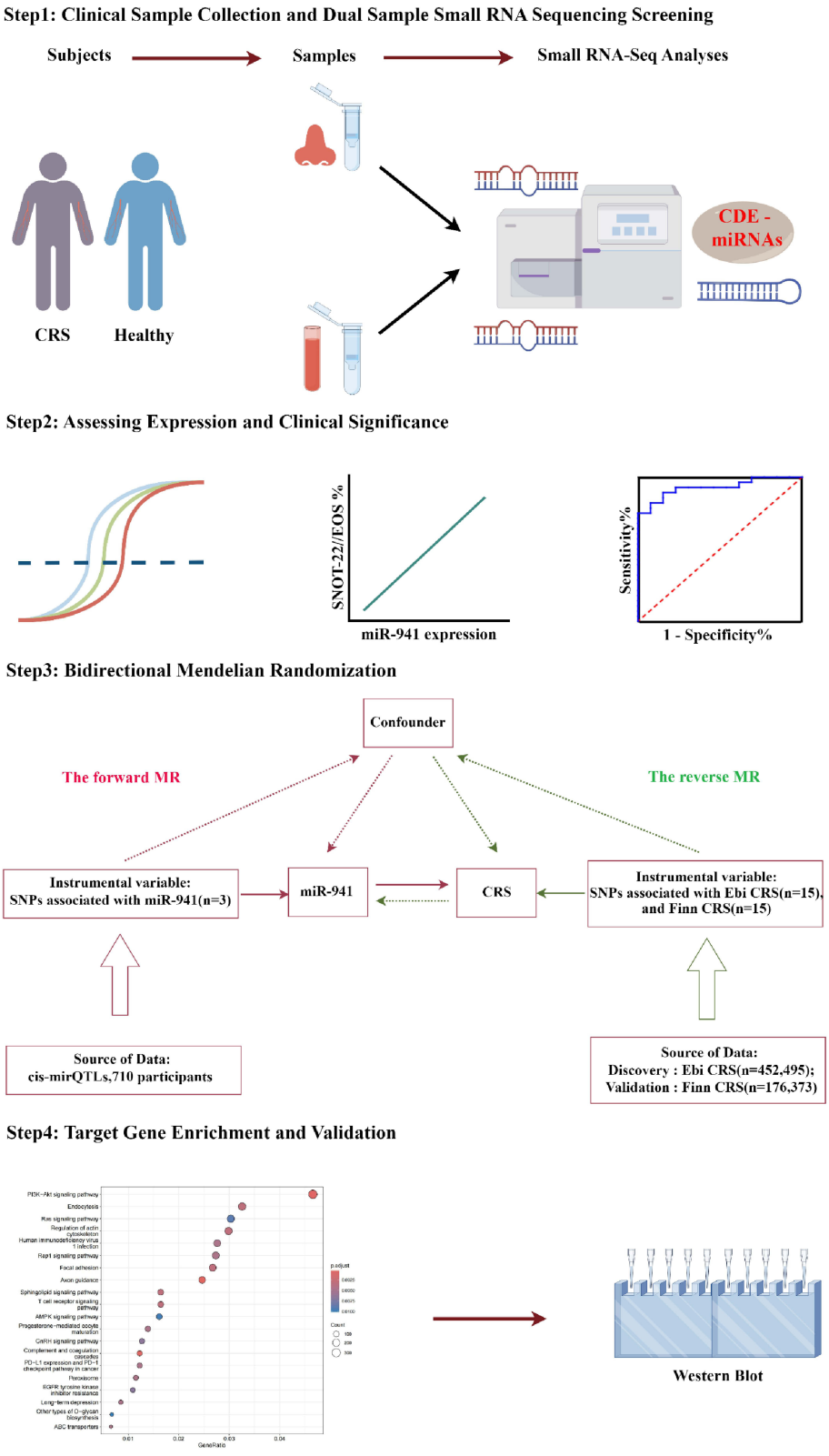

Figure 1 Schematic representation of the study design (By Figdraw).
Abbreviations: CDE-miRNAs, miRNAs with consistent differential expression; SNOT-22, Sino-Nasal Outcome Test-22; MR, Mendelian randomization; CRS, chronic rhinosinusitis; SNPs, single nucleotide polymorphisms; QTL, quantitative trait locus.

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Materials and Methods

Clinical Tissue Sample Collection

13 patients with deviated nasal septum and 28 patients with CRSwNP who were treated at the Yongchuan Chinese Medicine Hospital Affiliated to Chongqing Medical University, were recruited from April 2023 to October 2023.Nasal polyp specimens from CRSwNP patients diagnosed according to EPOS 2020, and middle turbinate or uncinate process tissues from patients with deviated nasal septum, were collected during surgery and stored at −80°C.[2] To avoid potential confounding factors, patients with fungal sinusitis, recurrent acute rhinosinusitis, cystic fibrosis, granulomatosis with polyangiitis, autoimmune diseases, or myelodysplastic syndrome, as well as those who had recently (within the last month) used antibiotics, immunomodulators, or corticosteroids, were excluded. Additionally, all participants in the control group had no history of chronic rhinosinusitis or allergic rhinitis. All participants underwent computed tomography (CT) and/or magnetic resonance imaging (MRI) scans prior to surgery to assess the nasal condition.

Clinical Blood Sample Collection

At enrollment or on the second day of enrollment, 2.5 mL of fasting blood samples were collected in the morning from the 41 participants using a blood collection needle into BD PAXgene Blood RNA Tubes (Becton, Dickinson and Company, USA). After that, the tubes were inverted 8–10 times and then placed at room temperature for 12 hours before being frozen at −20°C for 24 hours, and subsequently stored at −80°C.

Small RNA Sequencing

Total RNA was extracted from nasal cavity tissues of 6 patients with deviated nasal septum and 7 CRSwNP patients, as well as their corresponding blood samples, using Trizol reagent (Accurate Biology, China). Blood miRNAs were purified using the PAXgene Blood miRNA Kit (QIAGEN, Germany). RNA concentration and integrity were assessed using NanoDrop 2000 (Thermo Fisher Scientific Inc, USA) and Agilent 2100, LabChipGX (PerkinElmer, USA) to ensure that only qualified samples were used for small RNA sequencing. The following quality control parameters were applied: RNA concentration >50 ng/μL, RNA Integrity Number (RIN) >6.3, OD260/280 >1.8, and total RNA >1 μg. The clustering of index-coded samples was carried out on a cBot Cluster Generation System using the TruSeq PE Cluster Kit v4-cBot-HS (Illumina) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina NovaSeq 6000 platform, specifically: (1) removal of adapter sequences; (2) removal of sequences shorter than 18 or longer than 30 nucleotides; (3) removal of low-quality sequences based on quality score thresholds for each sample; (4) removal of reads with ≥10% unknown bases (N). The remaining reads were utilized to detect known miRNAs and predict novel miRNAs by comparison with the Genome and known miRNAs from miRBase.[37] Randfold tools were employed for the secondary structure prediction of novel miRNAs. miRNA expression levels were quantified as transcripts per million. Multiple comparisons were corrected using the Benjamini-Hochberg procedure, with the False Discovery Rate (FDR) serving as the key criterion for the final CDE-miR.

Quantitative Real-Time PCR (qRT-PCR) Assay

Total RNA was extracted from tissue samples using Trizol reagent (Accurate Biology, China), and blood miRNAs were purified using the PAXgene Blood miRNA Kit (QIAGEN, Germany). Total RNA was then reversely transcribed using the First Strand cDNA Synthesis Kit (Sangon Biotech, China), followed by qRT-PCR according to the parameters of the SYBR Green Fluorescent Quantitative Premix Kit (TIANGEN, China). Specific qRT-PCR primers are detailed in Supplementary Table S1. With U6 as an internal reference, the relative gene levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Clinical Significance Analysis of CDE-miRs

Receiver operating characteristic (ROC) curve analysis was performed using GraphPad Prism 9 (GraphPad Software, USA) to estimate the accuracy of CDE-miRs in distinguishing CRS patients from healthy subjects by calculating the area

under the curve (AUC). Furthermore, using data from CRS patients,[11] Spearman correlation analysis was employed to assess the relationship between CDE-miRs and clinical parameters.

MR Analysis

The "TwoSampleMR" R package for bidirectional MR analyses was utilized to clarify the causal relationship between CDE-miRs and CRS.[38] Initially, CDE-miRs were regarded as the exposure and CRS as the outcome, with single nucleotide polymorphisms (SNPs) as IVs to assess the causal effect of CDE-miRs on CRS. According to previous studies,[32,35] to integrate the datasets, SNPs independently associated with miRNAs (r^2<0.4 and kb<10) were extracted from publicly available data sources, with a significance threshold of P<5e-6. These SNPs were then matched based on genetic coordinates with the outcome dataset (genome-wide association study [GWAS] data for CRS). Cis variants within a 1 Mb distance of the start and end of the miRNAs were also identified. This integration process was carried out using the TwoSampleMR R package, which aligns SNPs from the exposure (cis-mirQTLs) and outcome (GWAS data for CRS) datasets based on proximity and significance. These aligned SNPs were then used as IVs for MR analysis. In summary, cis-mirQTLs data extracted from 710 healthy blood donors were utilized as exposure data for CDE-miRs.[32] The outcome GWAS data for CRS were derived from the Integrative Epidemiology Unit Open GWAS project (ID: ebi-a-GCST90018823) and the FinnGen consortium with 8,524 CRS cases, obtained from hospital records (ICD-8, ICD-9, or ICD-10 codes), and 167,849 non-cases.[39] Furthermore, we regarded CRS as the exposure and miRNAs as the outcome to explore potential reverse causal effects. SNPs associated with CRS (p-value<5e-06, r^2>0.001, KB<10000) were used as IVs for the outcome MR.[39]

To achieve stable and reliable causal relationships, on one hand, the MR pleiotropy residual sum and outlier (MR-PRESSO) test was employed to remove SNPs with high heterogeneity before conducting the MR analysis;[40] on the other hand, six different MR methods were utilized, including inverse-variance weighted (IVW), Weighted median, MR Egger, Weighted mode, Simple mode, and MR-PRESSO. Considering the effectiveness of IVW in overcoming the impact of heterogeneity on causal effects, we also adopted IVW as the primary method to evaluate the causal effects, in line with previous studies.[39,41] Moreover, heterogeneity was tested using Cochran's Q, horizontal pleiotropy was assessed with MR-Egger intercepts, sensitivity analyses were conducted using the leave-one-out approach,[41] and the F value of each IV was examined to identify the presence of weak instruments.[42] A robust causal relationship was assumed when the MR results between exposure and outcome simultaneously met the following criteria: (I) P-value < 0.05 according to the IVW method; (II) MR estimations, including IVW, Weighted median, MR Egger, Weighted mode, Simple mode, and MR-PRESSO were directionally consistent; (III) MR-Egger intercept test indicated an absence of horizontal pleiotropy. Finally, the causal effects of exposures on outcomes were described as odds ratios (OR) and 95% confidence intervals (95% CI).

Target Gene Prediction, Functional Annotation, and Validation

Consistent with previous research,[24] miRwalk v3.0 was employed to predict potential target genes of CDE-miRs that have a causal relationship with CRS. The predicted target genes were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using the "clusterProfiler" R package to identify significantly enriched pathways. The expression levels of proteins in the most significantly enriched pathways were then examined through Western blotting (WB).

WB

Total protein was extracted from nasal cavity tissues using RIPA Lysis Buffer containing protease inhibitors (Beyotime, China). Protein concentration was determined using the BCA Protein Assay Kit and normalized. The proteins were denatured by heating at 95°C for 5 minutes and then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). Afterward, the membrane was blocked with 5% non-fat milk on a shaker at room temperature for 1.5 hours, and then incubated with primary antibodies overnight at 4°C, followed by washings with TBST and incubation with horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 hours. Protein bands were visualized using an electrochemical

luminescence (ECL) kit and quantified using Image J software. With GAPDH as the internal control, the relative protein expression was represented as the ratio of the target protein to GAPDH band intensity. Specific details regarding antibody use are provided in Supplementary Table S2.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 9. Quantitative data conforming to a normal distribution were presented as mean ± standard deviation, while those not conforming to a normal distribution were presented as median (interquartile range). For data that followed a normal distribution, comparisons between two groups were performed using independent sample t-tests, otherwise, the Mann–Whitney *U*-test was used. Categorical data were described as frequencies and percentages, and differences between groups were assessed using the chi-square test or Fisher's exact test. The relationships between various indicators were evaluated using Spearman correlation analysis. P-value < 0.05 was considered statistically significant.

Results

Patient Characteristics

The demographic and clinical characteristics between the CRS patients and the control group were compared. Except for a significantly higher percentage of eosinophils in peripheral blood (EOS%) in CRS patients, there were no statistically significant differences in gender, age, and other aspects between the two groups, demonstrating comparability (Supplementary Table S3).

Differential Expression of miRNAs in Whole Blood and Nasal Tissue Between CRS and Control Patients

Small RNA sequencing of whole blood indicated that based on the threshold of |log2(Fold Change)| >0.58 and P-value < 0.05, we identified 150 differentially expressed miRNAs (DE-miRs) between CRS patients and controls (Figure 2A, C, and Supplementary Table S4). This threshold was selected based on previous miRNA expression studies.[43] After FDR correction, hsa-miR-941 was identified as the only upregulated miRNA showing a significant difference (Table 1). Similarly, small RNA sequencing of nasal tissue showed 177 DE-miRs between CRS patients and controls (Figure 2B, D, and Supplementary Table S5). After FDR correction, 6 downregulated and 2 upregulated DE-miRs were identified, making a total of 8 DE-miRs (Table 2). After molecular intersection and expression trend determination of DE-miRs in whole blood and nasal tissue, it was found that hsa-miR-941 was the only miRNA that showed significant differences and consistent upregulation. Figure 2E shows the expression levels of hsa-miR-941 between controls and CRS patients in the sequencing data.

Validation of miR-941 Expression and its Clinical Significance

hsa-miR-941 expression in the whole blood and nasal tissue of CRSwNP patients and the control group was validated through qRT-PCR, which showed consistent high expression with the small RNA sequencing result. miR-941 expression in both whole blood and nasal tissue was higher in the CRSwNP group than in the control group (Figure 3A). The correlation analysis between miR-941 and EOS% revealed that in CRSwNP patients, the miR-941 expression in whole blood was positively correlated with blood EOS% (r=0.381, p = 0.046), while there was no correlation between miR-941 expression in nasal tissue and blood EOS% (r = 0.190, p = 0.334) (Figure 3B). The correlation analysis between miR-941 and Sino-Nasal Outcome Test-22 (SNOT-22) scores showed that in CRSwNP patients, the miR-941 expression in whole blood was positively correlated with SNOT-22 scores (r = 0.470, p = 0.012), and a similar positive correlation was observed in nasal tissue (r = 0.476, p = 0.010) (Figure 3C). ROC analysis for miR-941 in whole blood revealed an AUC of 0.929, and in nasal tissue, an AUC of 0.915, both indicating that miR-941 expression levels can effectively distinguish CRS patients from the normal controls (Figure 3D).

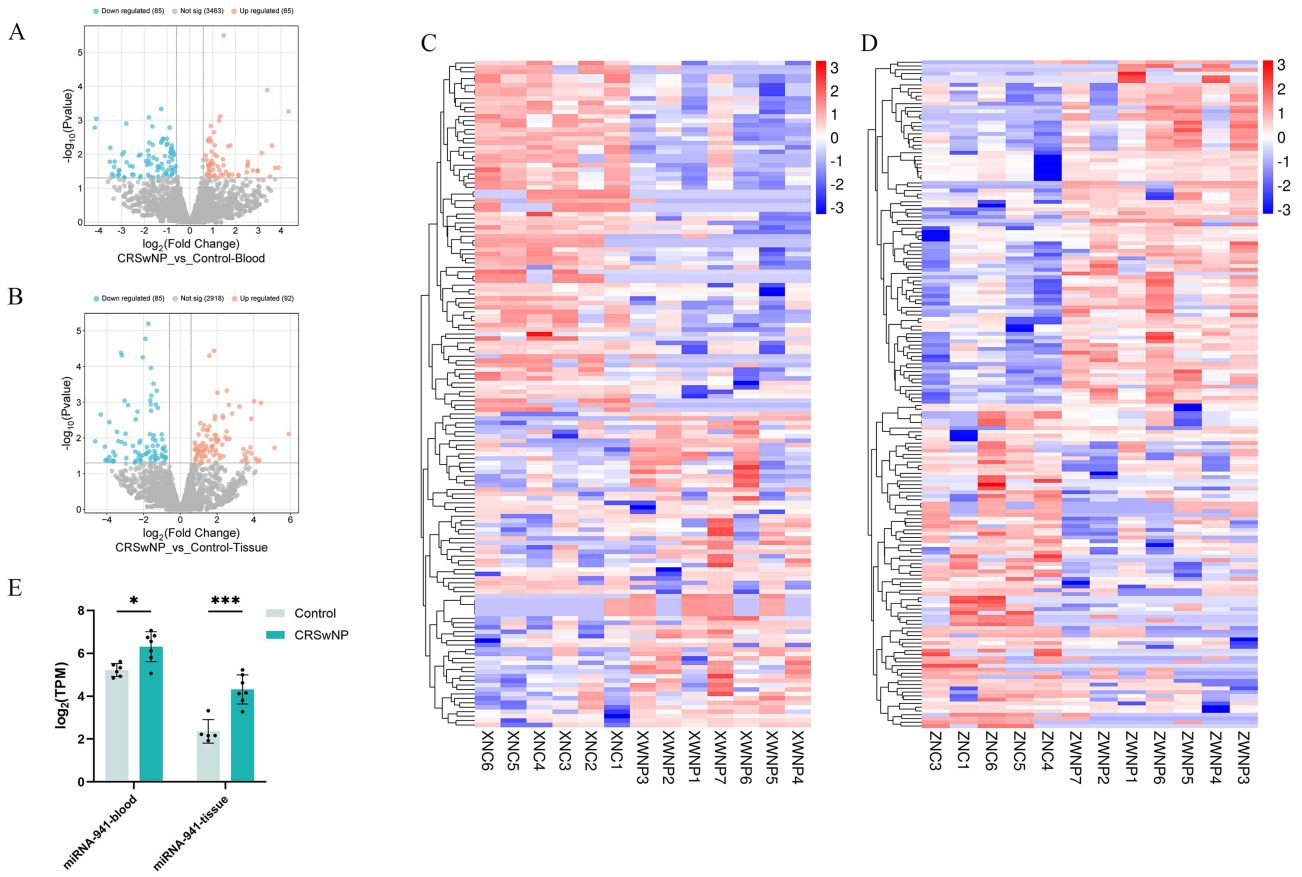

Figure 2 Differentially expressed miRNAs between CRSwNP and Control patients in blood and nasal tissue samples. A, (**C**) Volcano plot (**A**) and heatmap (**C**) of miRNA expression in human blood showing differences between CRSwNP patients and normal controls. B, (**D**) Volcano plot (**B**) and heatmap (**D**) of miRNA expression in human nasal tissue showing differences between CRSwNP patients and normal controls. E. The expression of hsa-miR-941 in the sequencing data. *$p < 0.05$, ***$p < 0.001$.
Abbreviations: ZWNP, From tissue samples of Chronic Rhinosinusitis with Nasal Polyps; XWNP, From blood samples of Chronic Rhinosinusitis with Nasal Polyps; ZNC, From tissue samples of normal controls; XNC, From blood samples of normal controls.

Characteristics of Genetic Variants Used in MR

Based on the aforementioned selecting criteria for IVs, a total of 3, 15, and 15 SNPs were identified to be strongly associated with CDE-miRNAs (hsa-miR-941), Ebi CRS, and Finn CRS, respectively. All SNPs had an F-statistic ≥ 10, indicating no weak instruments in the MR analysis. The detailed information of the included IVs is presented in Supplementary Tables S6 and S7.

Causal Effect of miR-941 on CRS

The univariable MR analysis unveiled the causal effect of hsa-miR-941 on CRS (Figure 4A and B). The IVW results obtained using the Ebi CRS dataset from the IEU consortium as the outcome indicated that higher levels of miR-941 were associated with an increased risk of CRS [OR, 1.301; 95% CI, 1.134 to 1.493; P < 0.001]. The IVW analysis using the Finn validation dataset also revealed the causal effect [OR, 1.203; 95% CI, 1.045 to 1.386; P = 0.010]. The OR values from various MR methods were ≥ 1 for both the Ebi CRS and Finn datasets, suggesting a consistent causal effect, indicating that elevated miR-941 was a potential cause of CRS. Moreover, the MR-Egger intercept test and Cochran's Q results for both datasets showed no significant horizontal pleiotropy or heterogeneity (P > 0.05). Additionally, the leave-one-out analyses (Figure 4C) for both datasets indicated that the estimates from various MR methods were not affected by any single SNP, robustly supporting the stability of the IVW and overall MR results.

Table 1 Comparative Values of Each Whole Blood miRNA Transcript Between CRSwNP (n=7) and Control (n=6) (Show FDR Top Ten)

#ID	P-value	FDR P-value	Log2fc	Regulated
hsa-miR-941	3.19E-06	0.012	1.485	up
hsa-miR-200a-3p	1.28E-04	0.232	3.396	up
hsa-miR-6747-3p	4.61E-04	0.457	−1.257	down
hsa-miR-30c-2-3p	5.54E-04	0.457	4.323	up
hsa-miR-877-5p	7.64E-04	0.457	1.331	up
hsa-miR-25-5p	8.24E-04	0.457	−1.792	down
novel_miR_383	9.08E-04	0.457	−4.101	down
novel_miR_1426	1.01E-03	0.457	1.283	up
novel_miR_209	1.25E-03	0.457	−2.785	down
hsa-miR-151b	1.47E-03	0.457	0.923	up

Notes: log2FC represents the log2 fold change (CRSwNP vs Control); positive values indicate upregulation, and negative values indicate downregulation. FDR P-values are adjusted for multiple comparisons; values < 0.05 indicate significant differences. The "Regulated" column shows whether a miRNA is upregulated ("up") or downregulated ("down") in the CRSwNP group. Significance is denoted by an asterisk (*), where FDR < 0.05.

Table 2 Comparative Values of Each Tissue miRNA Transcript Between CRSwNP (n=7) and Control (n=5) (Show FDR Top Ten)

#ID	P-value	FDR P-value	Log2fc	Regulated
hsa-miR-582-3p	6.33E-06	0.020	−1.736	down
hsa-miR-338-3p	1.68E-05	0.024	−1.903	down
hsa-miR-21-3p	3.65E-05	0.024	1.843	up
novel_miR_2310	4.14E-05	0.024	−3.226	down
novel_miR_1319	4.87E-05	0.024	−3.177	down
hsa-miR-941	4.98E-05	0.024	1.581	up
hsa-miR-1249-3p	5.53E-05	0.024	−2.043	down
hsa-miR-582-5p	1.09E-04	0.042	−1.593	down
hsa-miR-338-5p	3.04E-04	0.104	−1.458	down
hsa-miR-223-3p	4.73E-04	0.134	2.536	up

Notes: log2FC represents the log2 fold change (CRSwNP vs Control); positive values indicate upregulation, and negative values indicate downregulation. FDR P-values are adjusted for multiple comparisons; values < 0.05 indicate significant differences. The "Regulated" column shows whether a miRNA is upregulated ("up") or downregulated ("down") in the CRSwNP group. Significance is denoted by an asterisk (*), where FDR < 0.05.

Causal Effect of CRS on miR-941

The reverse MR analysis investigated the potential causal impact of CRS on hsa-miR-941 expression (detailed in Figure 5A and B). The IVW analysis utilizing the Ebi CRS dataset as the exposure indicated no causal effect of CRS on miR-941 expression [OR, 1.069; 95% CI, 0.925 to 1.236; P = 0.364]. Furthermore, IVW results derived from the Finn validation dataset also demonstrated no causal relationship [OR, 1.113; 95% CI, 0.982 to 1.260; P = 0.093]. Consistency was observed across the remaining five MR analytical approaches for both Ebi CRS and Finn datasets, indicating no causal link between CRS and miR-941. Similarly, the MR-Egger intercept test and Cochran's Q test results from both datasets demonstrated no significant horizontal pleiotropy or heterogeneity (P > 0.05). Additionally, the leave-one-out analyses (Figure 5C) for both datasets affirmed that the overall effect estimations of the MR methods were not influenced by any specific genetic variant. These findings collectively reinforce the stability of the reverse MR results.

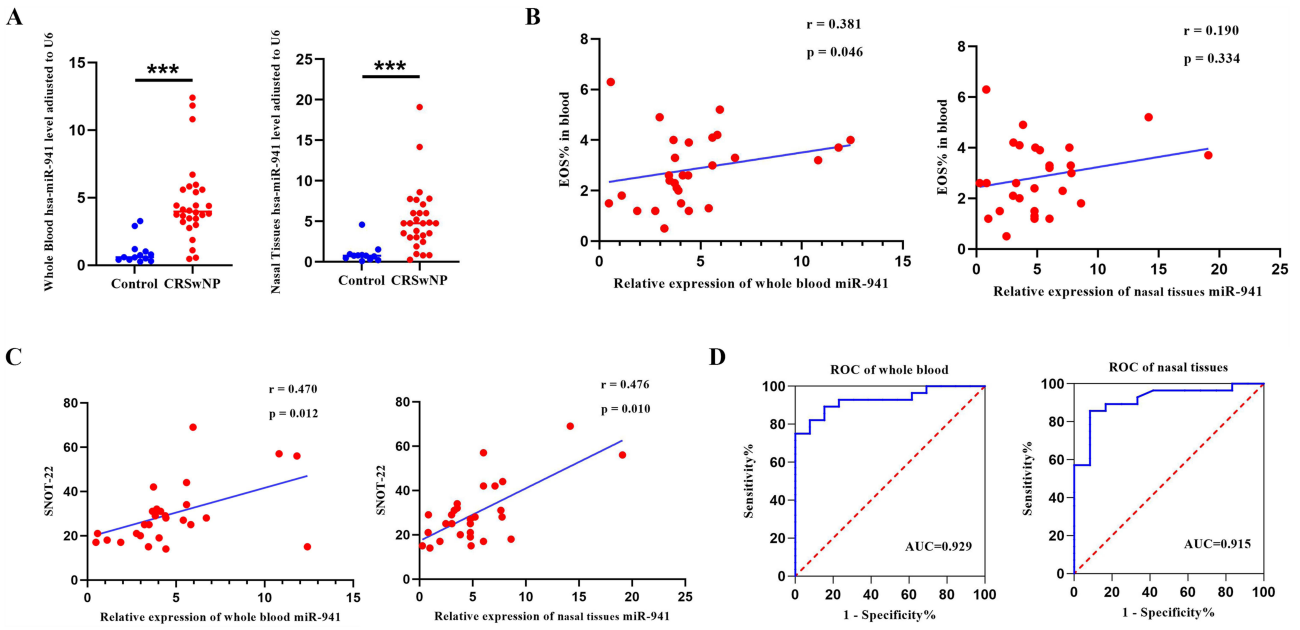

Figure 3 Upregulation of miR-941 in CRSwNP Patients in both whole blood and nasal tissue. To validate the expression of miR-941 and investigate its association with CRSwNP, we employed qRT-PCR, Spearman correlation analysis, and ROC curve assessment. (**A**) The expression of hsa-miR-941 was measured in whole blood (n=28) and nasal tissue (n=28) from CRSwNP patients and compared to control subjects' whole blood (n=13) and nasal tissue (n=12), with normalization to U6. (**B**) Spearman correlation analysis between hsa-miR-941 expression and EOS% in the whole blood and nasal tissue of CRSwNP patients. (**C**) Spearman correlation analysis between hsa-miR-941 expression and SNOT-22 scores in the whole blood and nasal tissue of CRSwNP patients. (**D**) ROC curves for hsa-miR-941 expression were plotted for both whole blood and nasal tissue in CRSwNP patients (n=28) versus control subjects (n=13 for blood, n=12 for tissue). The curves include the area under the curve (AUC) along with sensitivity and specificity values. ***$P < 0.001$.

Abbreviations: miR, microRNA; CRSwNP, Chronic Rhinosinusitis with Nasal Polyps;qRT-PCR, quantitative real-time PCR.

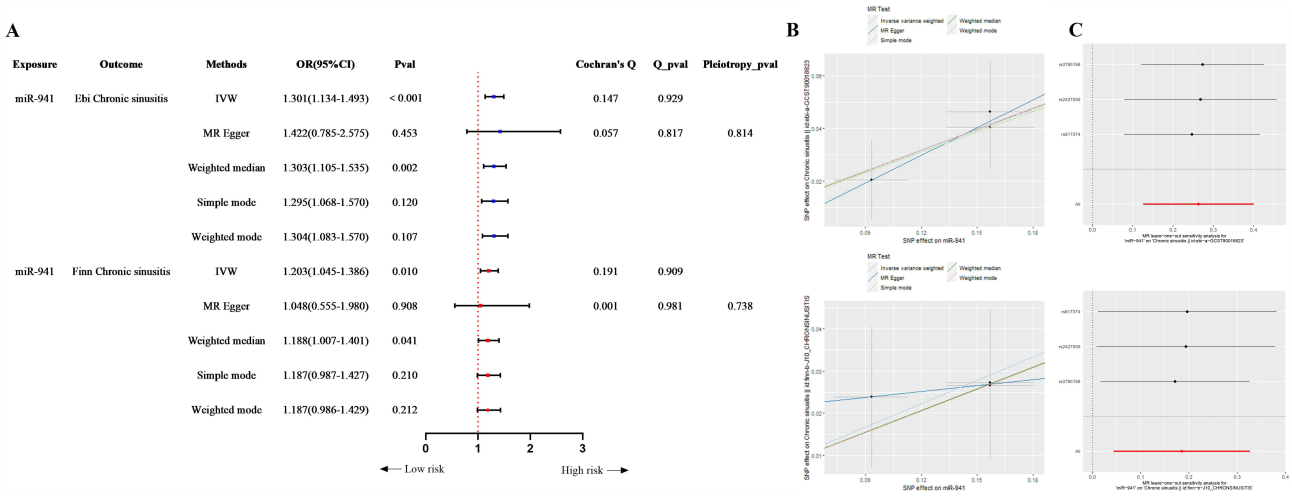

Figure 4 Univariate MR estimates of miR-941 on CRS. (**A**) The MR forest plot for Ebi chronic sinusitis and FinnGen chronic sinusitis as outcomes and miR-941 as the exposure. (**B**) Scatter plots showing the relationship between SNP effects on miR-941 and Ebi chronic sinusitis (top) and FinnGen chronic sinusitis (bottom), with each line representing the estimated MR effect according to different methods. (**C**) The leave-one-out estimate of miR-941 on Ebi chronic sinusitis(top), and miR-941 on FinnGen chronic sinusitis(bottom). Data are presented as β with the 95% confidence interval.

Abbreviations: MR, Mendelian randomization; CRS, chronic rhinosinusitis; OR, odds ratios; CI, confidence intervals; IVW, inverse-variance weighted.

The PI3K-Akt Signaling Pathway is the Most Significantly Enriched Pathway for Target Genes of Hsa-miR-941

The results of the KEGG enrichment analysis indicated that the PI3K-Akt signaling pathway was the most significantly enriched pathway for miR-941's target genes (Figure 6A). The expression of key proteins in the PI3K-Akt signaling

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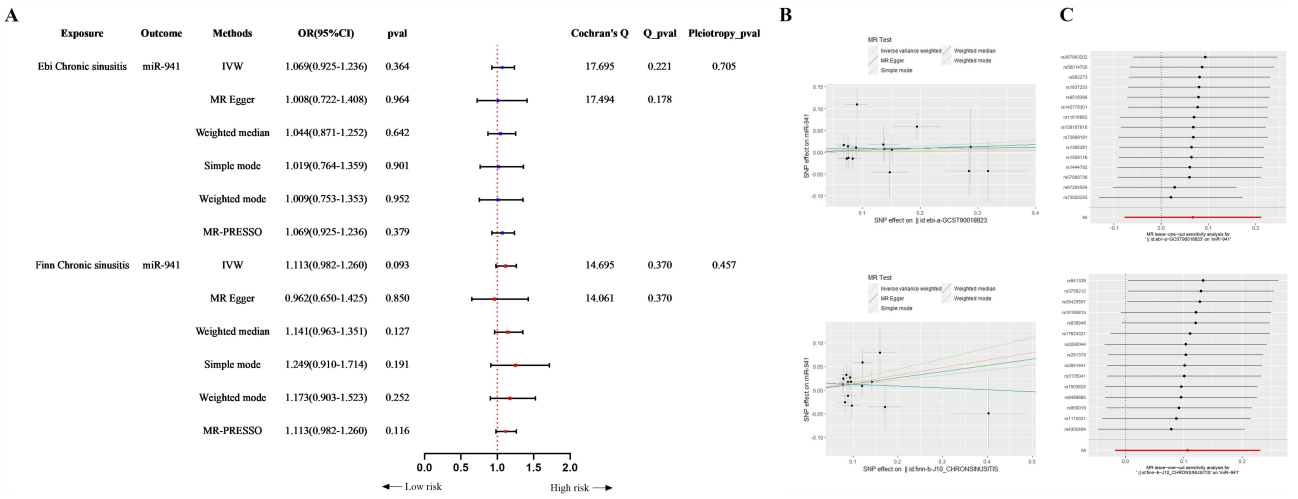

Figure 5 Univariate MR estimates of CRS on miR-941. (**A**) The MR forest plot for miR-941 as the outcome in Ebi chronic sinusitis and FinnGen chronic sinusitis as exposures. (**B**) Scatter plots of SNP effects on Ebi chronic sinusitis versus miR-941 (top), and FinnGen chronic sinusitis versus miR-941 (bottom), with the slope of each line corresponding to the estimated MR effect per method. (**C**) The leave-one-out estimate of Ebi chronic sinusitis versus miR-941 (top), and FinnGen chronic sinusitis versus miR-941 (bottom). Data are presented as β with the 95% confidence interval.
Abbreviations: CRS, chronic rhinosinusitis; OR, odds ratios; CI, confidence intervals; IVW, inverse-variance weighted; MR-PRESSO, MR pleiotropy residual sum and outlier.

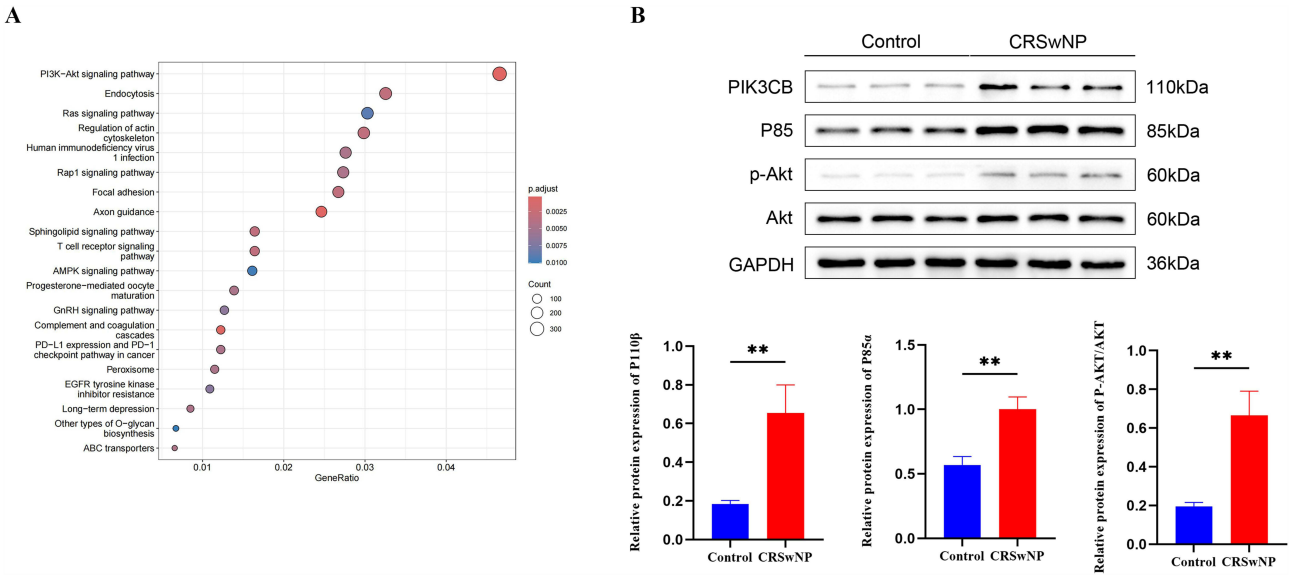

Figure 6 Pathway enrichment and expression validation of miR-941 downstream target genes. (**A**) KEGG analysis revealing the top 20 most critical pathways enriched by miR-941 downstream targets. (**B**) Western blot (n=3) validation of expression levels for key markers within the most significantly enriched pathway: the PI3K-AKT signaling pathway. **P <0.01.
Abbreviation: CRSwNP, Chronic Rhinosinusitis with Nasal Polyps.

pathway in CRSwNP patients and the control group was detected using WB, which revealed that the PI3K-Akt signaling pathway was significantly activated in CRS patients (Figure 6B). This suggests that miR-941 likely plays a crucial role in the pathogenesis of CRS by activating the PI3K-Akt signaling pathway.

Discussion

CRS is an intricate and multifactorial disease and its pathogenesis is not fully understood. Research on miRNAs that may play a significant role in the pathogenesis of CRS is still in its infancy, and the specific causal miRNAs in CRS remain largely unknown.[28,44–46] Through small RNA sequencing of blood and nasal tissues, bidirectional MR analysis, and experimental validation, this study is the first to reveal the upregulation of miR-941 in the blood and nasal tissues of CRS

patients and its positive correlation with CRS severity. Our findings also confirm a direct causal relationship between miR-941 and CRS and illustrate that miR-941 may exert its effects on CRS through the activation of the PI3K-Akt signaling pathway. To our knowledge, this is the first study that combines small RNA sequencing of blood and nasal tissues with MR analysis to investigate the role of miRNAs in CRS. This not only helps further research to better understand the miRNA expression profiles in the blood and nasal tissues of CRS patients compared to control groups but also clarifies the pathogenesis of CRS, providing a theoretical basis for developing targeted CRS treatment strategies in the future.

To date, several studies have focused on sequencing the miRNA expression profiles in CRS. Xuan et al through small RNA sequencing of nasal tissues revealed that the miRNA expression profile of CRSwNP patients was completely different from that of controls.[46] Zhang et al also demonstrated, through miRNA microarray analysis of nasal tissues and experimental validation, that the upregulation of miR-125b could lead to an increase in mucosal eosinophils.[47] Through transcriptome sequencing of clinical nasal tissues, Bu et al[48] and Li et al[11] revealed the dysregulation of miRNA-mRNA regulatory networks between CRSwNP patients and control groups. The dysregulated miRNAs and their regulatory networks may become potential targets for novel CRSwNP treatment strategies in the future. Additionally, the role of individual miRNAs in CRS has also been studied. Wu et al found that miR-200a-3p could exert anti-inflammatory and anti-EMT effects in CRS by targeting ZEB1, offering a new target for protecting nasal epithelial cells from tissue remodeling.[25] Luan et al demonstrated that miR-21-5p could exacerbate the type 2 inflammation process in CRSwNP by targeting the GLP1R/IL-33 signaling axis, also highlighting its potential of miR-21-5p as a disease treatment target.[24]

These studies have to some extent demonstrated that changes in miRNA expression in nasal tissue may play a key role in the pathogenesis and progression of CRS. However, they almost exclusively focused on the changes in miRNA expression in nasal tissue, with insufficient attention paid to the changes in blood miRNA expression profiles and their potential roles in CRS. So far, only a study by He et al analyzed the miRNA expression profiles in blood samples of CRSwNP patients and control individuals.[43] Nonetheless, He et al solely used blood samples but failed to integrate them with nasal tissue expression. Circulating miRNAs in the blood can reflect organ or tissue damage to a certain extent. The potential regulation of circulating miRNAs in Th2-type inflammation makes them promising diagnostic biomarkers and new therapeutic targets for upper respiratory tract inflammation. Considering the availability of clinical samples, it is imperative to give due consideration to circulating miRNAs in blood for clinical diagnosis and treatment.[43,49–51] In this study, whole blood samples were used to detect circulating miRNAs. On the one hand, observing miRNA expression changes in whole blood favors the development of disease biomarkers. On the other hand, analysis of specific blood components is challenging in clinical practice. In contrast, whole blood is more easily obtained and collected, with 200–1000 times more RNA content than plasma.[52,53] Related studies have shown consistent miRNA expression in whole blood, plasma, and serum, so all three biological fluids can be used to assess miRNA expression.[52,54] Furthermore, dysregulated specific miRNAs in tissues may also be secreted into the blood.[49] Currently, there is confusion as to whether changes in miRNAs are drivers of CRS or merely byproducts of CRS. In such a context, this study aims to provide new insights into the molecular mechanisms of CRS by combining miRNA expression profiles in blood and nasal tissue with advanced MR methods.

This study was mainly to explore the role of miRNAs in the pathogenesis of CRS, rather than starting from any specific target miRNA. By sequencing small RNA in the blood and nasal tissue of CRS patients and control individuals, we identified 150 and 177 DE-miRs in blood and tissue, respectively, based on the threshold of $|\log2(FC)|>0.58$ and P value <0.05. The result was in line with the results of Xuan's sequencing study in nasal tissue, further demonstrating significantly different miRNA expression profiles in the nasal tissues of CRS patients and controls.[46] However, to reduce false positivity due to multiple comparisons, this study ultimately used $|\log2(FC)|>0.58$; FDR <0.05 as the criteria for key DE-miRs. Eventually, a consistently upregulated CDE-miR was found in the blood and nasal tissue of CRS patients: hsa-miR-941. Consistently, in sequencing nasal tissues of CRSwNP patients, Bu et al showed an elevated expression of miR-941 compared to the control group.[48] The consistent upward trend of miR-941 in blood and nasal tissue prompted us to further explore the potential relationship between miR-941 and CRS, thereby better assessing the potential of miR-941 as a key driver in the pathogenesis of CRS. miR-941 expression was detected using qRT-PCR on an expanded sample size, consolidating the reliability of the upward trend of miR-941 expression. Spearman correlation analysis and ROC curve

further confirmed that the expression change of miR-941 was closely related to CRS severity and had high clinical diagnostic accuracy. This aligned with previous findings that the expression of certain specific miRNAs, such as miR-200a-3p, was associated with clinical parameters of CRS and may serve as a diagnostic and prognostic biomarker.[25]

Additionally, based on the results above and to further broaden our understanding of the biological significance of miR-941, we conducted a comparative analysis of its role in other inflammatory and respiratory diseases. Studies have reported that in ulcerative colitis (UC), genome-wide circulating miRNA expression profiling revealed that miR-941 is significantly upregulated in UC patients and is associated with UC susceptibility loci, suggesting its involvement in intestinal inflammation and immune regulation.[55] In psoriatic arthritis (PsA), miR-941 is upregulated in circulating $CD14^+$ monocytes, where it promotes osteoclast differentiation and function by inhibiting WNT16, with its expression level positively correlating with disease activity, indicating that it not only serves as a biomarker but also has the potential to be a therapeutic target.[16] In severe asthma, comparison of the serum and lung tissue miRNA expression profiles between patients treated with and without oral corticosteroids (OCS) revealed significant differences in the expression of several miRNAs, including miR-941, suggesting that miR-941 may be involved in regulating airway inflammation or related signaling pathways associated with drug response.[15] These cross-disease comparisons further confirm the important role of miR-941 in various inflammatory and respiratory conditions and provide broader biological evidence and potential clinical applications for the sustained upregulation of miR-941 and its association with disease severity observed in CRS.

Previous studies in the CRS field related to miRNAs mainly focused on the expression patterns of miRNAs in CRS, their potential biological functions in inflammation, EMT, and tissue remodeling, and their feasibility as diagnostic biomarkers. Few studies have explored the causal relationship between miRNAs and CRS.[11,25,46–48] To fill this gap and provide high-level genetic evidence for a direct causal relationship between miRNAs and CRS, we conducted a bidirectional MR analysis to further explore the association between miR-941 and CRS. With miR-941 as the exposure and the IEU consortium and Finn's CRS data as the two outcomes, the MR results robustly supported that higher levels of miR-941 were associated with an increased risk of CRS, indicating a direct causal relationship between miR-941 and CRS. The reverse MR analysis also indicated no causal relationship between CRS and miR-941. By applying the MR approach to explore the causal relationship between miR-941 and CRS, our study further reveals the pathogenesis of CRS and provides more reliable support for the therapeutic potential of miR-941 in clinical applications. miRNAs can regulate the expression of target genes by binding to the 3'UTR of these target genes, thus affecting several biological processes including signal pathway transmission, and participating in the pathophysiology of diseases.[56,57] Hence, the downstream target genes of hsa-miR-941 were predicted using miRwalk. The key signaling pathways potentially regulated by miR-941 were analyzed by KEGG enrichment analysis of target genes. The levels of proteins in the most significantly enriched PI3K/AKT pathway were examined using WB, which implied that the PI3K/AKT pathway was activated in CRSwNP. Consistent with these findings, previous studies have also reported upregulated PI3K/mTOR protein expression in nasal polyps.[58] In one study, protein expression levels of the PI3K/Akt pathway in the nasal mucosa were found to be significantly higher in CRSwNP patients compared to normal controls.[59] Several in-depth basic studies have demonstrated that IL-21 induces pyroptosis in nasal mucosal cells by activating the PI3K/Akt/mTOR signaling pathway, while inhibition of PI3K/Akt can effectively prevent pyroptosis.[60] Inhibition of the PI3K signaling pathway can significantly reduce the production of growth factors and effectively alleviate nasal inflammation.[61] Furthermore, activation of PI3K/AKT signaling is closely associated with the EMT process, inflammatory cell infiltration, and epithelial cell apoptosis in nasal polyps.[62] These finding offers valuable insights for elucidating the mechanism of miR-941 in CRS and lays a theoretical foundation for the development of new therapeutic strategies.

Compared with existing studies, the novelty of this study is as follows. First, the small RNA sequencing analysis of blood and nasal tissue was integrated, providing a more comprehensive perspective for studying the miRNA expression profiles in CRS. Second, this study is the first in the CRS field to explore the role of a specific miRNA in disease pathogenesis from a causal perspective, demonstrating the causal effect of miR-941 on CRS and offering a new potential target for CRS treatment. Furthermore, this study combined advanced molecular biology techniques with genetic epidemiological research methods, providing a new perspective and methodology for subsequent research on the mechanisms of CRS and other diseases. Of course, despite these advantages, some limitations still exist. First, the size

of the population used for sequencing and validation in our study is relatively small, which limits the statistical power and generalizability of the results to some extent. To address this limitation, we performed FDR correction, validated the results through qRT-PCR, and incorporated genomic evidence. However, future studies should validate our findings in larger and more diverse populations to further confirm their generalizability. Second, although the MR analysis is a powerful tool for exploring causal relationships, further experimental methods, such as the construction of lentiviral vectors, are needed to verify the specific functions and mechanisms of hsa-miR-941. For example, by using over-expression and knockdown experiments, employing CRISPR/Cas9 technology to establish gene-editing models to further confirm the causal role of miR-941, and by combining immunofluorescence and flow cytometry to comprehensively analyze the function of miR-941 in specific cell types, its effects on inflammatory responses and the activation of other signaling pathways can be evaluated, thereby revealing its role in the pathogenesis of CRS in a more direct manner. Finally, the study identified a relatively small number of CDE-miRs after correction. Future research should continue to explore the role of other miRNAs, which will help to reveal more aspects of the complex pathogenesis of CRS.

Conclusion

In summary, transcriptomic and genomic evidence supports a positive causal relationship between hsa-miR-941 and CRS, making it a valuable target for the diagnosis and treatment of CRS. Our study finds that miR-941 may exert its effects by modulating the PI3K/AKT pathway, which not only deepens our understanding of the pathogenesis of CRS but also provides a theoretical basis for developing novel diagnostic and therapeutic strategies. Moreover, this study is the first to integrate small RNA sequencing data from both blood and nasal tissues and to explore the role of a specific miRNA in CRS from a causal perspective, demonstrating the broad applicability of multi-omics approaches in elucidating the molecular mechanisms underlying inflammatory diseases and offering new avenues for exploring the mechanisms of other inflammatory conditions and developing personalized treatments.

Abbreviations

CRS, Chronic rhinosinusitis; CRSwNP, Chronic rhinosinusitis with nasal polyps; CRSsNP, Chronic rhinosinusitis without nasal polyps; miRNA, microRNA; MR, Mendelian randomization. IVs, instrumental variables; FDR, False Discovery Rate; qRT-PCR, Quantitative Real-Time PCR; ROC, Receiver operating characteristic; AUC, area under the curve; SNPs, single nucleotide polymorphisms; GWAS, genome-wide association study; MR-PRESSO, MR pleiotropy residual sum and outlier; IVW, inverse-variance weighted; KEGG, Kyoto Encyclopedia of Genes and Genomes; WB, Western blotting; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; ECL, electrochemical luminescence; DE-miRs, differentially expressed miRNAs; SNOT-22, Sino-Nasal Outcome Test-22; OR, odds ratios; CI, confidence intervals.

Data Sharing Statement

mirQTLs data can be directly downloaded from the supplementary materials of the published article;[30] GWAS data on chronic sinusitis sourced from Ebi is publicly accessible, and can be retrieved through the IEU website by searching for the GWAS ID: ebi-a-GCST90018823 (https://gwas.mrcieu.ac.uk/). The summary-level data from FinnGen are publicly available (https://finngen.gitbook.io/documentation/). Other data from this study can be obtained by making a reasonable request to the corresponding author.

Statement of Ethics

Since the original GWAS studies included in this research have been approved by the respective ethics review committees and all participants have signed informed consent forms, an ethical approval is not required for the two-sample MR analysis based on GWAS summary-level data. Moreover, the experimental portion of our study was approved by the Ethics Committee of Yongchuan Chinese Medicine Hospital Affiliated to Chongqing Medical University (Ethics Opinion Number: 2023-004-01) and conducted following the Declaration of Helsinki. All patients provided informed consent at enrollment.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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