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ARTICLE

The Causal Relationship Between Serum Metabolites and Sjogren's Syndrome: A Mendelian Randomization Study

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Abstract

Aims/Background Sjogren's syndrome (SS) is a highly prevalent autoimmune disease with potentially serious consequences if left untreated, but methods for early detection and prevention of SS remain limited. This study aims to investigate the causal relationships between serum metabolites and SS using Mendelian randomization (MR), focusing on identifying key metabolic pathways and biomarkers that contribute to SS pathogenesis.

Methods We used a two-sample MR approach to investigate the causal relationships between serum metabolites and SS. The primary method for estimating these causal effects was inverse variance weighting (IVW), with results presented alongside their corresponding 95% confidence intervals (CIs). Sensitivity analyses included the Cochran's Q statistical analysis and MR-Egger method. Furthermore, an enrichment analysis of metabolic pathways was applied to the identified metabolites.

Results Thirty-seven serum metabolites that have causal links with SS, encompassing 7 metabolite ratios and 30 single metabolites (4 unknown and 26 known), were identified. Metabolite ratios, reflecting the balance between specific metabolites, were analyzed to identify metabolic shifts that may contribute to SS pathogenesis. Among the 26 known metabolites, 12 are protective factors and 14 are risk factors. The levels of cis-4-decenoate (cDA) (10:1n6) (odds ratio [OR] = 1.125; 95% CI = 1.026–1.233; p = 0.012) is positively correlated with the incidence of SS, whereas the levels of butyrate/isobutyrate (4:0) (OR = 0.822; 95% CI = 0.701–0.963; p = 0.016) are negatively correlated with the SS incidence. Most of these metabolites are associated with lipid and amino acid metabolism. Among lipids, the strongest risk-increasing factor was 2,3-dihydroxy-2-methylbutyrate (OR = 1.307; 95% CI = 1.054–1.621; p = 0.015), while the strongest risk-decreasing factor was hexadecadienoate (16:2n6) (OR = 0.774; 95% CI = 0.635–0.944; p = 0.011). Among amino acids, the strongest risk-increasing factor was N-acetylproline (OR = 1.178; 95% CI = 1.024–1.355; p = 0.022), and the strongest risk-decreasing factor was N-acetylserine (OR = 0.802; 95% CI = 0.694–0.926; p = 0.003). Furthermore, these metabolites are predominantly enriched in the arginine and proline metabolism pathway.

Conclusion This study helped enhance our comprehension of the causal relationship between serum metabolites and SS, showing that some metabolites may influence the risk and development of this disease. These insights offer novel perspectives for the development of SS prediction and diagnosis.

Key words: Mendelian randomization analysis; metabolomics; Sjogren's syndrome; causality

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Introduction

Sjogren's syndrome (SS) is a chronic inflammatory autoimmune disease characterized by lymphocyte infiltrating exocrine glands (Carvajal Alegria et al, 2015).

Based on epidemiological research from 2013, the global incidence rate of SS is approximately 0.007%, while the figure stands at 0.043% in the European population (Qin et al, 2015). The main clinical manifestations of SS are xerostomia, xerophthalmia, fatigue, joint pain and parotid glands swelling (Mariette and Criswell, 2018), whereas some patients may also experience systemic complications involving the lungs, kidneys, nervous and other systems (Sato-Fukuba et al, 2023), which impose significant financial strain on both patients and society at large. Such adverse repercussions underscore the urgencydrive an exigency for early detection and prevention of SS.

Metabolites are defined as intermediates and products of metabolism, playing a particularly significant role in various diseases. The latest advancements in metabolomics research have explored some connections between human metabolites and SS. By identifying different metabolites and developing diagnostic models, researchers have discovered combinations of numerous metabolic biomarkers that have the potential to effectively identify, diagnose and assess SS (Vaz et al, 2024; Zhang et al, 2023). For example, high performance liquid chromatography-electrospray ionization-quadrupole time of flight-mass spectrometry methodology was used to discover 69 new metabolite alterations in SS patients, related to phospholipids, fatty acids, and amino acids (Xu et al, 2021). A non-targeted gas chromatography-mass spectrometry (GC-MS) serum metabolomics method was also conducted to examine the alteration, determining 21 distinct serum metabolites (Fernández-Ochoa et al, 2020). Despite the identification of numerous metabolites in previous studies, the causal effect of serum metabolites on SS has not yet been comprehensively and directly assessed. Among the numerous metabolites studied in the context of autoimmune diseases, cis-4-decenoate (cDA) and butyrate/isobutyrate have emerged as particularly noteworthy. cDA has been implicated in mitochondrial dysfunction, oxidative stress, and immune dysregulation (Amaral et al, 2016; Barrera et al, 2021; Schuck et al, 2010), all of which play a role in the pathogenesis of SS. Butyrate, a short-chain fatty acid, is known for its anti-inflammatory properties and its ability to regulate immune responses and maintain intestinal barrier integrity (Hodgkinson et al, 2023). These unique properties make them prime candidates for further investigation in SS pathogenesis. While numerous metabolites were analyzed in this study, we focused on cDA and butyrate/isobutyrate due to their significant associations with SS and their distinct biological roles in immune regulation and metabolic dysfunction. Their mechanistic links to SS pathogenesis would provide a unique direction for uncovering potential biomarkers and therapeutic targets.

Mendelian randomization (MR) study is an analytical approach that utilizes genetic variations as instrumental variables (IVs) for causal inference (Pierce et al, 2011). Genetic variants connected with phenotypes are randomly distributed during meiosis, and appropriate ones which meet three hypotheses can be used as IVs. By excluding the effect of confounders on outcomes, MR can avoid the bias and restriction of the traditional research methods. Therefore, MR methods were used in our study to explore the potential causality between human serum metabolites

and SS using the genome-wide association studies (GWAS) data obtained from FinnGen GWAS and Canadian Longitudinal Study on Aging (CLSA).

Methods

Study Design

Three key assumptions must be satisfied to achieve valid outcomes in MR analysis. Specifically, genetic variants as IVs must satisfy: (1) correlation hypothesis: the IVs have reliable association with the risk factor studied; (2) independence hypothesis: the IVs are not connected with confounders, either known or unknown; and (3) exclusive restriction hypothesis: the IVs affect outcomes only by risk factors with no other direct causal pathways intervened. Their causal effects were primarily gauged through the inverse variance weighting (IVW) approach alongside a 95% confidence interval (CI). Sensitivity analyses were combined the Cochran's Q statistical analysis and MR-Egger method. Furthermore, an enrichment analysis of metabolic pathways was applied for the identified metabolites (Fig. 1).

Data Sources

The summary data for serum metabolites was obtained from the GWAS Catalog database (http://www.ebi.ac.uk/gwas), which involved 1091 serum metabolites and 309 metabolite ratios from 8299 European participants in the CLSA (Chen et al, 2023). Metabolite ratios reflecting the balance between specific metabolites could indicate the metabolic shifts that may contribute to diseases, and the metabolites can be categorized into eight super-pathways, involving lipids, amino acids, carbohydrates, peptides, vitamins, nucleotides, energy metabolism products, and xenobiotics.

The data of SS were obtained from FinnGen GWAS (https://r11.finngen.fi). The phenotype for SS is "Sicca syndrome [Sjögren]" (Kurki et al, 2023), and the data set obtained includes data of 402,090 individuals, consisting of 2735 cases and 399,355 controls from the European population.

Instrumental Variables

The meticulous selection and validation of single nucleotide polymorphisms (SNPs) as IVs significantly enhances the accuracy and reliability of MR analyses. Firstly, to satisfy the correlation assumption, strongly associated SNPs with the exposure were selected rooted in a genome-wide significance threshold of $p < 1 \times 10^{-5}$. This ensures that the chosen SNPs were robustly linked to the exposure factor. Secondly, to ensure compliance with the independence assumption, selected SNPs were screened against known confounders, such as age, sex, and body mass index (BMI). Additionally, SNPs exhibiting horizontal pleiotropy based on Egger intercept tests were excluded from analysis. The linkage disequilibrium threshold was set at $r^2 < 0.001$, and SNPs separated by less than 10,000 kb were excluded to mitigate correlated effects (Emdin et al, 2017). CLSA GWAS data provides the metabolite names each SNP belong to, and metabolite-specific SNPs were ensured again using METAL. For instance, SNP rs2185152 was mapped to the metabolite called cis-4-decenoate (10:1n6). This ensured that SNPs were directly relevant to

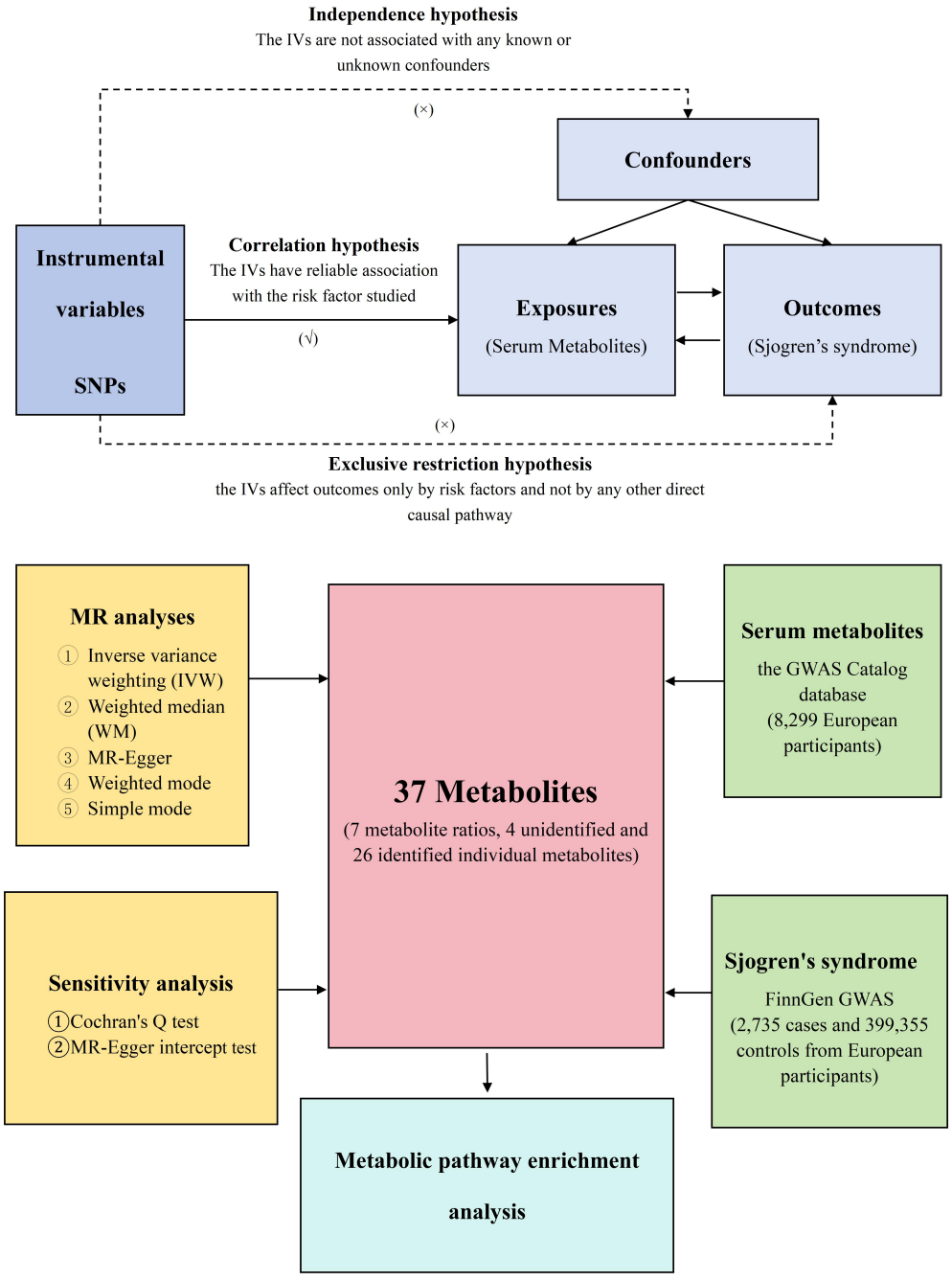

Fig. 1. Three key assumptions and the flowchart of the MR study design. The flowchart outlines the process of selecting genetic variants as IVs, ensuring that they meet the correlation, independence, and exclusive restriction assumptions. Each step of the analysis, from data sourcing to sensitivity analyses, is depicted to provide a comprehensive overview of the study framework. GWAS, genome-wide association studies; IVs, instrumental variables; MR, Mendelian randomization; SNPs, single nucleotide polymorphisms. The diagram was created with Microsoft Office Word manufactured by Microsoft (Redmond, WA, USA).

the metabolite under investigation. Lastly, the screened SNPs were extracted from the outcome variable data, and an F-test was performed to evaluate weak IVs. To confirm the strength of the IVs, we calculated F-statistics for all SNPs. The SNPs of F-values <10 were excluded to lessen the potential effect of weak instruments

(Burgess et al, 2011). The formula is as follows: $F = R^2 \times (n-2)/(1-R^2)$, $R^2 = 2 \times EAF \times (1-EAF) \times \beta^2$ (n, sample size; R, the fraction of specific variance; β, estimate of the genetic influence of exposure SNP; EAF, effect allele frequency) (Pierce et al, 2011). Only SNPs with F-statistics >10 were retained to ensure robust causal inference. The calculated F-statistics for each SNP ranged from 19.5 to 671.9, with all selected SNPs exceeding the threshold of F >10. For example, SNP rs9933684 had an F-statistic of 22.1, while SNP rs28415528 showed an F-statistic of 671.9. This procedure adheres to the exclusive restriction assumption of MR, ensuring that the selected SNPs influence the outcome variable solely through the exposure variable.

MR Analysis

We employed multiple MR methods, including IVW, MR-Egger, weighted median, simple mode and the weighted mode methods to ensure the robustness of our causal effect estimates. Each of these methods was selected for its specific strengths and its ability to address potential violations of MR assumptions. The IVW method serves as the primary method due to its statistical efficiency under the assumption that all IVs are valid and there is no horizontal pleiotropy. This approach provides the most reliable estimates when MR assumptions are met. In the MR-Egger method, MR-Egger regression accounts for potential directional horizontal pleiotropy by estimating a non-zero intercept, which reflects pleiotropic effects. Although it has reduced power compared to IVW, MR-Egger method provides a safeguard against pleiotropy-driven biases. The weighted median method generates robust causal estimates even when up to 50% of the IVs are invalid. By weighting each SNP based on the inverse of its variance, this method reduces the influence of outlier SNPs (Bowden et al, 2016). The simple mode leverages the estimation of each single SNP's causal effect to form a cluster and estimate the causal effect value of the largest SNP cluster to judge the existence of causality. In the weighted mode, the same process in the simple mode is used, except that a weight is assigned to each SNP, and by calculating the weighted average of each SNP, the influence of different SNPs on the results is comprehensively evaluated.

The consistency of causal effect estimates across these methods was evaluated to ensure robustness. Causal effect estimates from IVW, MR-Egger, weighted median, simple mode and weighted mode methods showed alignment in directionality and magnitude, reinforcing the reliability of our findings. The causal effect was interpreted using odds ratios (ORs) with 95% CIs. R Studio (version 4.3.3, manufactured by Posit located in Boston, MA, USA) was utilized throughout the data analysis. A p-value < 0.05 was considered statistically significant.

Sensitivity Analysis

Sensitivity analyses were applied to guarantee the stability. Cochran's Q statistical analysis is a method used to test whether there is a significant difference between the frequencies or ratios of three or more groups of matches. This method was applied in this research to detect the degree of heterogeneity among the IVs, with a p-value > 0.05 showing no significant heterogeneity (Bowden et al, 2018).

Egger's intercept test was used to examine horizontal pleiotropy, and the intercept represented the average multivariate estimate of all IVs. If the intercept is close to zero, the Egger regression model is very close to that of IVW. However, if the intercept is far from zero, there may be horizontal pleiotropy among these IVs. A p-value < 0.05 indicates the existence of horizontal pleiotropy, suggesting that the IVs may influence the outcome through pathways except the exposure, thus going against the independence assumption of the MR assumptions (Bowden et al, 2015). Sensitivity analyses were performed using the leave-one-out method to observe outliers and the funnel plots to assess symmetry to evaluate the stability of the results (Gronau and Wagenmakers, 2019). The leave-one-out sensitivity analysis was conducted for each SNP to determine its influence on the overall causal effect estimate.

Metabolic Pathway Enrichment Analysis

The enrichment analysis on the connected metabolic pathways was conducted using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg) was chosen as the pathway library. The hypergeometric test was adopted, with a significance level set as 0.01.

Results

MR Analysis Results

We ultimately found 37 serum metabolites potentially causally linked to SS, encompassing 7 metabolite ratios and 30 individual metabolites (4 unidentified and 26 identified) (Figs. 2,3). Among the identified metabolites, 12 were associated with a lower risk of SS, while 14 were connected with a higher risk (Figs. 4,5). The results for each metabolite causal analysis and calculated F-statistics are provided in **Supplementary Table 1**. The effect size of each SNP for 26 identified metabolites on SS is shown in **Supplementary Fig. 1**.

These metabolites primarily belonged to lipids and amino acids (Fig. 6). Among the 12 identified lipids, the 2,3-dihydroxy-2-methylbutyrate (OR = 1.307; 95% CI = 1.054–1.621; $p = 0.015$) was found as the strongest risk-increasing factor, while the hexadecadienoate (16:2n6) (OR = 0.774; 95% CI = 0.635–0.944; $p = 0.011$) was identified as the strongest risk-decreasing factor. Additionally, among the 9 identified amino acids, N-acetylproline (OR = 1.178; 95% CI = 1.024–1.355; $p = 0.022$) was the strongest risk-increasing factor, and N-acetylserine (OR = 0.802; 95% CI = 0.694–0.926; $p = 0.003$) was the strongest risk-decreasing factor. Additionally, the level of cis-4-decenoate (cDA) (10:1n6) (OR = 1.125; 95% CI = 1.026–1.233; $p = 0.012$) was found to be positively connected with the risk for SS, while the level of butyrate/isobutyrate (4:0) (OR = 0.822; 95% CI = 0.701–0.963; $p = 0.016$) presented inverse correlation with the risk for SS.

Sensitivity Analysis Results

Multiple sensitivity analyses were applied to ensure the stability of the results. The p-values for Cochran's Q heterogeneity statistical analyses all exceeded 0.05,

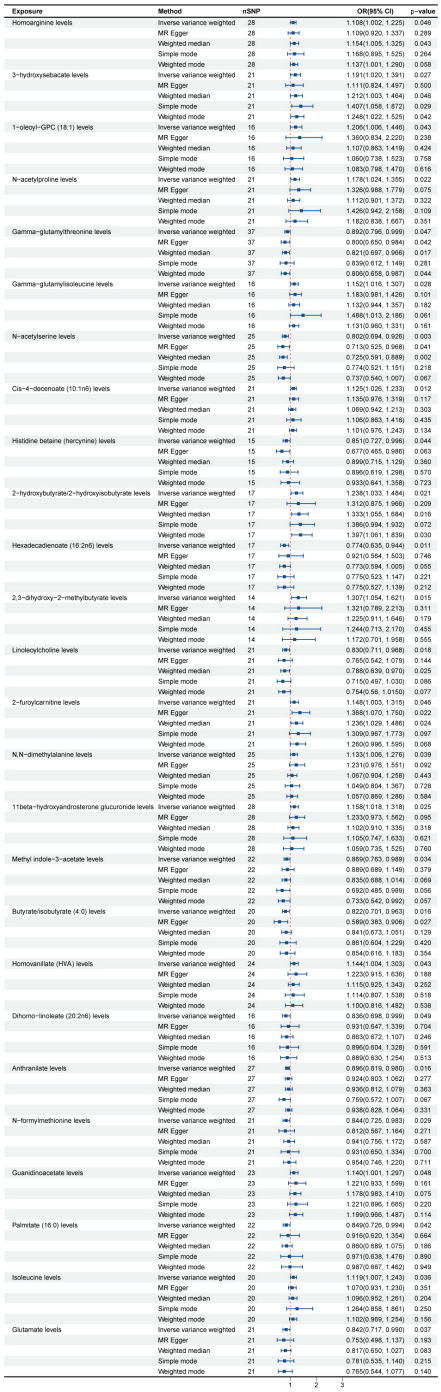

Fig. 2. Forest plot of the results of 26 identified metabolites. Forest plot summarizing the causal effects of 26 identified serum metabolites on SS using five MR methods: IVW, MR-Egger, weighted median, weighted mode, and simple mode. Each line represents the effect estimate with corresponding 95% confidence intervals for each MR method. The plot highlights the consistency of causal effect estimates across methods, with IVW being the primary approach for inference. Statistical significance is indicated where confidence intervals do not include zero. IVW, inverse variance weighting; MR, Mendelian randomization; nSNP, Number of single nucleotide polymorphism used in MR; OR, odds ratio; 95% CI, 95% confidence interval; SS, Sjogren's syndrome; GPC, glycerophosphocholine. The diagram was created with R Studio 4.3.3 manufactured by Posit (Boston, MA, USA).

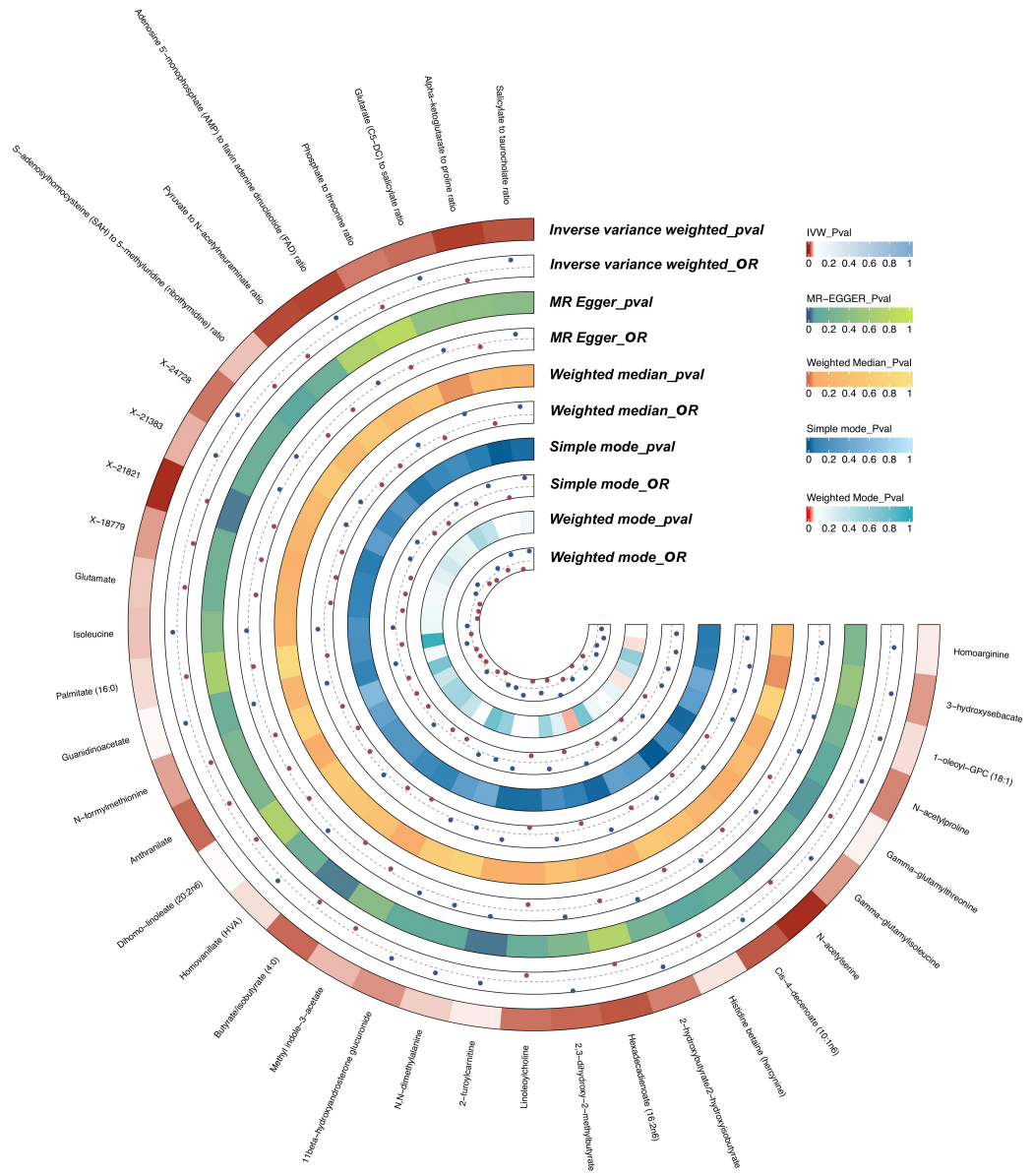

Fig. 3. Heat map of the results of 37 identified metabolites. Heat map summarizing the results of the MR analysis for 37 identified metabolites using five MR methods. Each cell represents the statistical significance and effect direction for a specific metabolite-method combination. MR, Mendelian randomization; OR, odds ratio. The diagram was created with R Studio 4.3.3 manufactured by Posit (Boston, MA, USA).

indicating no underlying heterogeneity in the results. The *p*-values for the MR Egger intercept also all exceeded 0.05, suggesting that the study results were not driven by pleiotropy at the genetic level. The results for Cochran's Q heterogeneity and MR-Egger pleiotropy statistical analysis are shown in **Supplementary Table 2**. Additionally, the leave-one-out analysis results showed no individual SNP having a disproportionate impact on the outcome. Funnel plots were used to assess potential bias due to asymmetry, and no significant deviations were observed. These analyses confirm the robustness of the results (**Supplementary Fig. 1**).

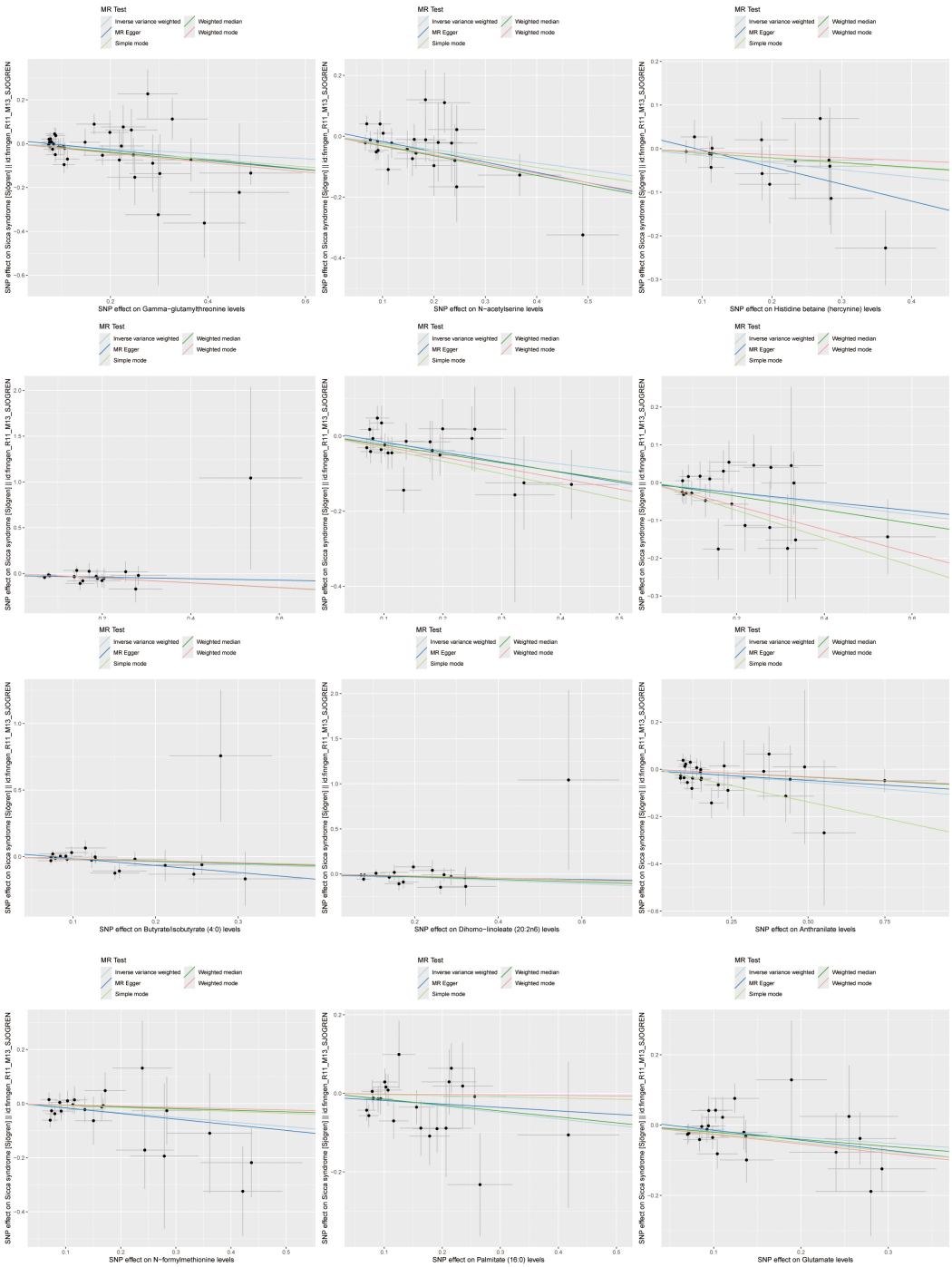

Fig. 4. Scatter plot of 12 risk-decreasing metabolites on SS. Scatter plot illustrating the relationship between SNP-exposure and SNP-outcome associations for 12 significant risk-decreasing metabolites. Each point represents an SNP, with lines showing the causal effect estimates derived from different MR methods (IVW, MR-Egger, weighted median, weighted mode, and simple mode). The consistency of these estimates across methods supports the robustness of the causal inference. The slope of the IVW line indicates the primary causal effect estimate, while the spread of points reflects heterogeneity among the SNPs. IVW, inverse variance weighting; MR, Mendelian randomization; SNP, single nucleotide polymorphism; SS, Sjogren's syndrome. The diagram was created with R Studio 4.3.3 manufactured by Posit (Boston, MA, USA).

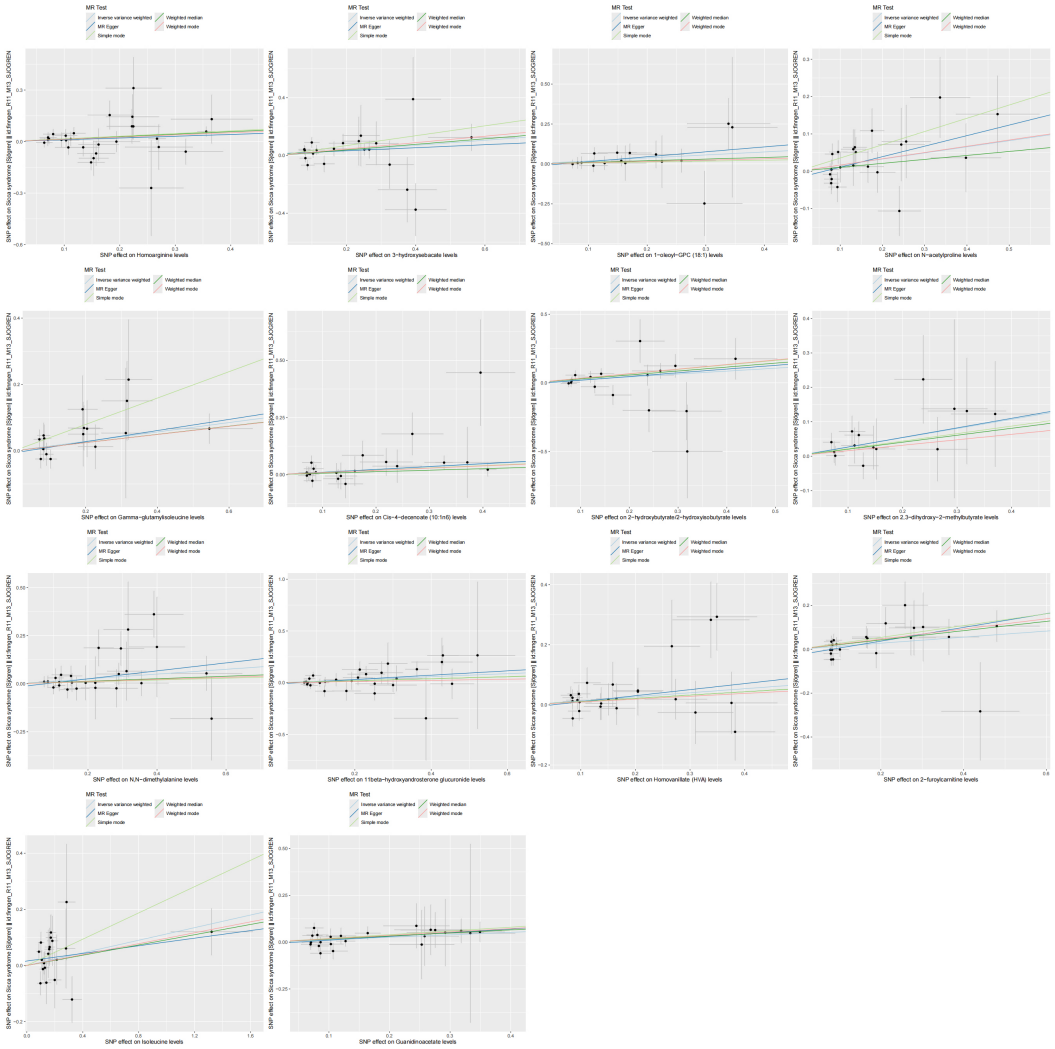

Fig. 5. Scatter plot of 14 risk-increasing metabolites on SS. Scatter plot illustrating the relationship between SNP-exposure and SNP-outcome associations for 14 key risk-increasing metabolites. Each point represents an SNP, with lines showing the causal effect estimates derived from different MR methods (IVW, MR-Egger, weighted median, weighted mode, and simple mode). The consistency of these estimates across methods supports the robustness of the causal inference. The slope of the IVW line indicates the primary causal effect estimate, while the spread of points reflects heterogeneity among the SNPs. IVW, inverse variance weighting; MR, Mendelian randomization; SNP, single nucleotide polymorphism; SS, Sjogren's syndrome. The diagram was created with R Studio 4.3.3 manufactured by Posit (Boston, MA, USA).

Metabolic Pathway Enrichment Analysis Results

We applied a metabolic pathway enrichment analysis on 26 identified metabolites. The results indicated that the most primary metabolic pathway was arginine and proline metabolism, while other involved pathways included glycine and serine metabolism; valine, leucine and isoleucine degradation; and tryptophan metabolism (Fig. 7).

Fig. 6. Categorization of all 37 metabolites based on their involvement in specific metabolic pathways. The majority of metabolites are associated with lipid and amino acid pathways, emphasizing their potential roles in SS pathogenesis. Metabolites are grouped into broader categories for clarity, reflecting their biological significance. SS, Sjogren's syndrome. The diagram was created with R Studio 4.3.3 manufactured by Posit (Boston, MA, USA).

Discussion

This study provides a preliminary MR analysis to reveal the causal relationship between serum metabolites and SS. Our team is also the first group to investigate the relationship between serum metabolites and SS risk through pathway enrichment analysis. A total of 37 metabolites, potentially causal to SS, were discovered, including 7 metabolite ratios and 30 individual metabolites (4 unidentified and 26 identified). The known metabolites are primarily involved in lipid and amino acid metabolic pathway. The arginine and proline metabolism was found as the most prominent metabolite pathway following the enrichment analysis.

cDA is a risk factor for SS due to its role in mitochondrial dysfunction and immune dysregulation (Barrera et al, 2021), which is consistent with our findings. Studies have shown that cDA disrupts mitochondrial energy metabolism, redox balance, and calcium homeostasis, leading to activation of inflammatory pathways (Amaral et al, 2016; Schuck et al, 2010). This mitochondrial dysfunction exacerbates inflammatory responses by releasing damage-associated molecular patterns and promoting oxidative stress (Barrera et al, 2021), both of which are implicated in SS pathogenesis. In the salivary glands, cDA-associated mitochondrial dysfunction contributes to reduced fluid secretion and glandular hypofunction (Huang et al, 2024), which are hallmark features of SS.

Butyrate is generated by intestinal microbiota as a result of fermenting dietary fiber (Coccia et al, 2024). A previous research showed that individuals with SS had a decreasing population of anti-inflammatory butyrate-producing microorganisms

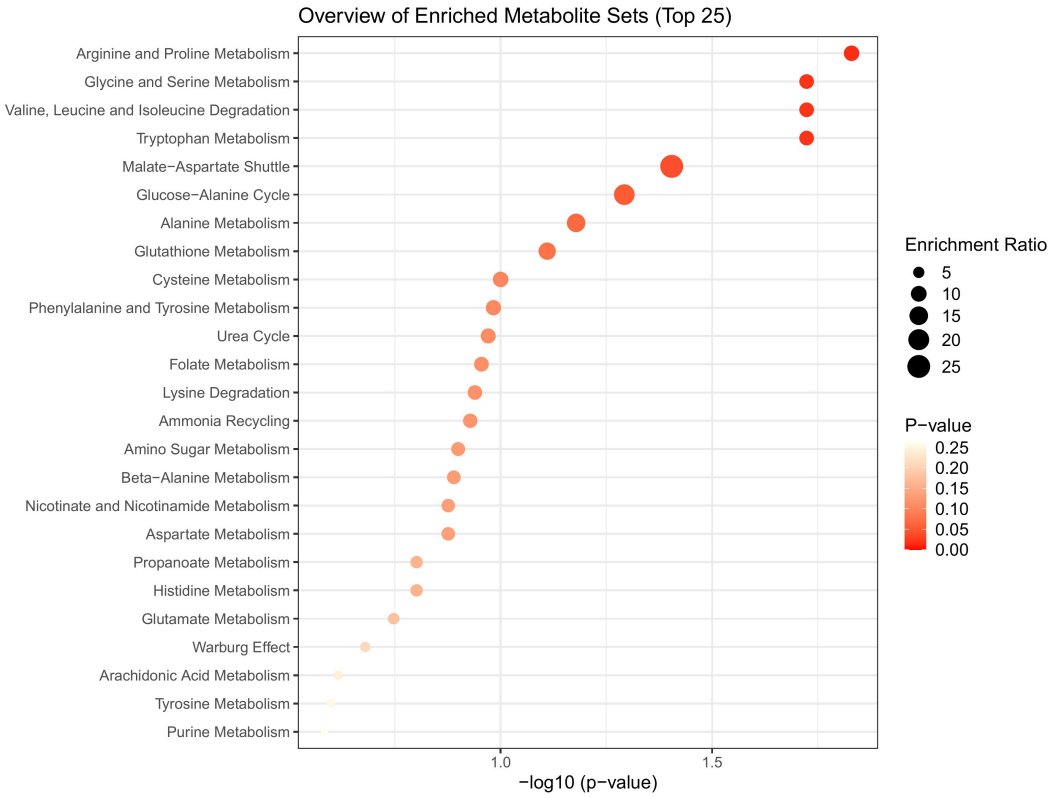

Fig. 7. Bubble chart of the enrichment pathways of metabolites identified in KEGG database. The size of each bubble corresponds to the significance level of pathway enrichment (*p*-value), while the color gradient reflects the pathway impact score. The arginine and proline metabolism pathway was identified as the most significant, alongside other pathways such as glycine and serine metabolism; valine, leucine, and isoleucine degradation; and tryptophan metabolism. KEGG, Kyoto Encyclopedia of Genes and Genomes. The diagram was created with MetaboAnalyst 6.0 manufactured by Xia Lab (Montreal, Canada).

(Wang et al, 2022). Maintaining optimal butyrate levels can reduce inflammation, maintain intestinal barrier integrity, and promote a healthy microbiota (Hodgkinson et al, 2023). Butyrate may exert its influence on the occurrence and development of SS through these pathways. Crucially, the research conducted by Kim et al (2021) confirmed that butyrate plays important roles in SS mouse models. Following intraperitoneal injection of butyrate, a reduction in inflammation of the salivary glands and a rise in the flow rate of saliva were observed. More crucially, butyrate was found to ameliorate SS by enhancing interleukin-10-generating B cells and reducing interleukin-17-generating B cells. It was discovered that the use of butyrate had an SS-relieving effect by diminishing the interleukin-17-producing B cells while increasing the generation of B cells that produce interleukin-10 (Kim et al, 2021).

The enrichment analysis highlighted the arginine and proline metabolism pathway is the most significantly associated with SS. The identified metabolites within the arginine and proline metabolism pathway show potential links to autoimmune and inflammatory pathways relevant to SS. Arginine plays a pivotal role in the regulation of endothelial cell survival under oxidative stress conditions, contributing to

the modulation of vascular inflammatory environment observed in SS pathogenesis. Specifically, arginine metabolism influences nitric oxide production, a critical mediator of vascular tone and immune responses, and its dysregulation has been linked to autoimmune diseases, including SS (Suschek et al, 2003). Moreover, alterations in arginine metabolism could affect T-cell activation and differentiation, thereby contributing to the inflammatory and autoimmune environment of SS (Canè et al, 2024). In another way, arginine metabolism can modulate macrophage polarization, shifting between pro-inflammatory (M1) and anti-inflammatory (M2) states, which are critical in the context of autoimmune diseases (Rath et al, 2014). Recent study has also highlighted the cross-talk between arginine and gut microbiota, which could influence systemic immune responses (Nüse et al, 2023). Dysbiosis in SS patients has been linked to altered microbial metabolism, potentially affecting systemic arginine availability and downstream immune regulation.

Proline also exhibits a dual role in maintaining redox homeostasis. As both a pro-oxidant and a reactive oxygen species eliminator, proline plays a key role in regulating intracellular redox conditions, thereby protecting cells from oxidative stress damage (Krishnan et al, 2008). Oxidative stress has been shown to exacerbate autoimmune conditions, and proline's protective effects could help mitigate this process. Additionally, proline metabolism is closely linked to collagen synthesis, which is significant given the glandular damage and fibrosis observed in SS (Phang, 2021). Proline, on the other hand, has been implicated in regulating fibroblast activation and extracellular matrix remodeling (Kay et al, 2022), processes that are often dysregulated in SS and contribute to glandular dysfunction. These interactions underscore the multifaceted role of the arginine and proline metabolic pathway in SS pathogenesis, particularly in immune regulation and oxidative stress control.

Among the lipid metabolites, hexadecadienoate (16:2n6) was the strongest risk-decreasing factor for SS. This metabolite is a long-chain polyunsaturated fatty acid, playing important roles in developing immune system (Miles et al, 2021). It was also identified as one of the factors distinguishing insulin-sensitive individuals from those who are insulin-resistant, regardless of whether they are lean or overweight (Diboun et al, 2021). Instead, 2,3-dihydroxy-2-methylbutyrate was the strongest risk-increasing factor, originating from branched-chain amino acid metabolism, indicative of worsening clinical status and mitochondrial dysfunction, highlighting the potential role of disrupted energy metabolism in SS (Niehaus et al, 2023). On the other hand, amino acid derivatives such as N-acetylproline and N-acetylserine were significantly associated with SS. N-acetylproline, an acetylated derivative of proline, was the strongest risk-increasing factor. Protein N-acetylation represents a well-preserved post-translational modification that serves as a protective shield for intracellular proteins against proteolysis. This modification can arise through specific N-acetyltransferases. There has been recognition that acetyltransferases may play a crucial role in the onset of early vascular and endothelial dysfunctions, potentially instigating inflammation and oxidative stress (Di Pietrantonio et al, 2023), resulting in tissue damage in SS. N-acetylserine, the strongest risk-decreasing factor, is derived from serine metabolism, and could influence immune tolerance mechanisms, as serine metabolism is integral to T-cell function and one-

carbon metabolism (Ma et al, 2017). These findings suggest that the interplay between energy metabolism, immune regulation, and oxidative stress is central to SS pathogenesis and warrants further investigation.

Four unidentified metabolites were also associated with SS in our analysis. Although their exact biological roles remain unclear, these metabolites could represent novel biomarkers or unknown metabolic pathways implicated in SS pathogenesis. Further experimental studies are necessary to characterize these metabolites and their potential contributions to disease development.

Several limitations of this research should be highlighted: firstly, this study primarily used data from individuals of European ancestry, limiting the generalizability of findings obtained to other ethnic groups, since differences in genetic architecture and environmental factors across different ethnic and racial populations could influence metabolite profiles and their associations with SS. Future studies involving diverse cohorts are needed to validate our findings and assess their applicability to broader populations. Secondly, some metabolites discovered potentially involved in the development of SS remain unidentified, hindering our ability to provide a clear explanation of their roles. Thirdly, pathway databases such as KEGG and MetaboAnalyst rely on pre-defined metabolic networks, which may not comprehensively capture all biological interactions or pathway redundancies. The robustness of these findings depends on the accuracy and completeness of these databases, and alternative pathway enrichment tools might yield slightly different results. Additionally, the identified associations between metabolites and pathways are correlative, and caution should be exercised in interpreting them as causal. Non-causal relationships, such as reverse causation or confounding factors, cannot be completely excluded. Another limitation lies in the reliance on global metabolomic data, which may not fully reflect tissue-specific metabolic changes. For example, the metabolic activity in salivary glands, a primary target in SS, may differ from that observed in systemic circulation. Future studies incorporating tissue-specific metabolomic analyses or experimental validation are needed to confirm these findings.

Conclusion

To summarize, this MR study demonstrated the causal relationship between 37 metabolites (7 metabolite ratios, 4 unidentified and 26 identified individual metabolites) and the risk of SS. Most of these metabolites belonged to lipids and amino acids metabolic pathways. The most primary metabolic pathway was the arginine and proline metabolism pathway. We hope that the results of this study will offer fresh perspectives on the pathogenesis of SS, enabling the use of these metabolites as potential diagnostic biomarkers to identify high-risk populations for early prevention and SS incidence reduction.

Key Points

- This study investigates the causal relationships between serum metabolites and Sjogren's syndrome (SS) using Mendelian randomization (MR) analysis.
- Lipids- and amino acids-based metabolism pathways were identified as key contributors to SS pathogenesis.
- Among the metabolites, hexadecadienoate (16:2n6) and N-acetylproline were highlighted for their significant associations with SS risk and protection, respectively.
- The study reveals the important role of metabolites and metabolic pathways in SS.
- These findings provide potential biomarkers and therapeutic targets for SS, advancing our understanding of SS pathogenesis.

Abbreviations

SS, Sjogren's syndrome; MR, Mendelian randomization; IVW, inverse variance weighting; GC-MS, gas chromatography-mass spectrometry; cDA, cis-4-decenoate; IVs, instrumental variables; GWAS, genome-wide association studies; CLSA, Canadian Longitudinal Study on Aging; SNP, single nucleotide polymorphism; ORs, odds ratios; CIs, confidence intervals; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Availability of Data and Materials

All data included in this study are available upon request by contacting the corresponding author.

Author Contributions

YQL: research planning, data extraction, result analysis, original draft writing and manuscript revision. LC and XHH: data extraction, result analysis and figure creation. YW: research design, research planning and supervision. All authors contributed to revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted based on publicly available datasets, including GWAS and the FinnGen database. All data utilized in this research were derived from studies that had obtained appropriate approvals from ethical committees and informed consent from participants during their original data collection. As this analysis involved secondary use of anonymized, publicly accessible data, no additional ethical

approval was required. The study complies with all relevant ethical guidelines for research involving human genetic data.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.magonlinelibrary.com/doi/suppl/10.12968/hmed.2024.0968.

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