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Bidirectional two-sample Mendelian randomization reveals causal link between genetic blood metabolites and tuberculosis

Zhenyu Shi[1†], Chenyi Zhao[1†], Xiaowen Yu[2†], Dingding Zhao[1] and Yongqiang Li[1*]

Abstract

Tuberculosis (TB), caused by infectious agent *Mycobacterium tuberculosis* (*Mtb*) seriously poses a great threat to health. An array of metabolites generated by metabolic pathways are essential for *Mtb* pathophysiology. However, a specific causal relationship between TB and human metabolites remains indistinct. This study aimed to investigate the relationship between 1400 metabolites and TB by Mendelian randomization (MR) analysis. In this study, a total of 1400 metabolites were utilized as exposure factors, while TB-related data served as the outcomes. And TwoSampleMR package and R software were adopted to perform this MR analysis. Various regression fitting methods were employed to conduct MR analysis, including inverse variance weighted (IVW), MR-Egger, weighted median, simple mode, and weighted mode. In addition, potential biases arising from linkage disequilibrium and weak instrumental variables were considered. Metabolites that failed to meet the criteria in both the heterogeneity and pleiotropy tests were considered to have no substantial causal influence on the results, ensuring the robustness and reliability of our analysis. IVW analysis showed that six human metabolites exhibited a significant causal influence ($P < 0.05$) on TB. Among them, dodecanedioate, myristoleate (14:1n5), and 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE(*p*-16:0/20:4) demonstrated a strong causally positive effect on TB, indicating that with the increase of these metabolites, TB progressed robustly. Glycerol 3-phosphate, sphingomyelin (d18:1/20:2, d18:2/20:1, and d16:1/22:2), and 2-methylserine were significantly negatively associated with TB, an increase in these metabolites inhibited TB progression. This is the first time to reveal the causal effects of human metabolites on TB through MR, and the metabolites may be potential biomarkers candidate for TB diagnosis, and monitoring these metabolites might have great clinic significance for TB diagnosis and treatment in the future.

Keywords Mendelian randomization, TB, Metabolites, Casual effect

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Introduction

Tuberculosis (TB) is a leading infectious disease that causes death worldwide, which mainly affects the respiratory system (Li et al. 2023; Schito et al. 2015). The World Health Organization's Global TB Report 2023 states that there will be 10.6 million new cases of TB worldwide with an incidence of 133/100,000 people, 748,000 new cases of TB in China in 2022 (compared with 780,000 in 2021), and an estimated 52/100,000 TB incidence rate. One billion deaths have been estimated to have occurred due to this disease in the last 200 years. Additionally, the incidence of the disease is slowly decreasing despite substantial global efforts to enhance prevention, diagnosis, and treatment (Cardona et al. 2020). TB substantially jeopardizes public health worldwide and strains medical resources (Dheda et al. 2016). The intricate pathophysiological pathways that propel the progression of TB remain incompletely elucidated, necessitating further study to achieve a comprehensive understanding of TB.

Metabolomics, which involves the identification and quantitation of small-molecule metabolites, is an omics approach that provides a powerful tool for phenotypic characterization. This methodology enables the identification of metabolites that can modify the phenotypic characteristics of cells and organisms (Guijas et al. 2018; Yu et al. 2023). The regular utilization of metabolome measurements facilitates the comprehension of pathophysiological processes underlying disease progression (Bujak et al. 2015).Recently, it has been successfully employed in the study of the pathogenesis of certain diseases, such as ovarian cancer (OC) and lung cancer (LC) (Huang et al. 2024; Li et al. 2025). In addition, early detection and involvement are crucial for the effective control of TB and metabolomics contributes to the early diagnosis and staging of TB (Jiang et al. 2021; Koen et al. 2018). However, the specific relationship between metabolites and TB remains unclear and the metabolic analysis of TB is constrained by a limited sample size and inadequate diversity among patient cohorts, significantly compromising the universal applicability of the derived research findings. In addition, given potential confounding factors such as substance abuse, complications, genetic differences and so on, definitively linking metabolic disturbances to the initiation or progression of TB is a formidable challenge. Therefore, the interaction between the two still needs to be clarified by further studies. Notably, advances in metabolomics methodologies enabling high-throughput profiling of blood metabolites have begun to address these limitations. Shin et al. conducted an extensive investigation into the genetic loci that influence human metabolism. This comprehensive exploration involved the recruitment of 7824 individuals from two demographic cohorts in Europe. They identified 486 metabolites with genetic effects on human serum metabolites. Following rigorous quality control measures, a detailed analysis of 486 metabolites was performed. Notably, 309 of these metabolites have been previously identified, whereas the remaining 177 remain unidentified, offering insights into the complex characteristic of human metabolism (Shin et al. 2014). Chen et al. conducted a genome-wide association study focusing on 1091 blood metabolites and 309 metabolites (Chen et al. 2023). The GWAS database assigned catalog numbers, GCST90199621 to GCS90201020, and 1,400 blood metabolites. These prior studies have provided critical resources for mechanistic investigations.

Mendelian randomization (MR) is a powerful tool in epidemiological research, which leverages genetic variation as instrumental variable to evaluate the casual relationship between risk factors and particular diseases(Shen et al. 2025; Zhang et al. 2023). It is expected that the chosen genetic variant is related to risk factors, but not to any confounding factors related to risk factors or the result in any way. The term, instrumental variables, is defined based on these three assumptions (Burgess et al. 2015). MR aims to reduce confounding and potential deviations of reverse causality (Skrivankova et al. 2021). Genetic variants are precisely measured and reported in most cases, ensuring the absence of bias and errors. This accuracy is particularly advantageous for evaluating risk factors associated with long-term effects (Hu et al. 2019).

Consequently, this study intends to use bidirectional, two sample MR analysis to analyze relevant data to investigate the relationship between 1400 metabolites and TB, identifying metabolites that may have a causal relationship with TB occurrence.

Materials and methods

Data source and software

Sources of information regarding human metabolites were obtained from the GWAS database (https://gwas.mrcieu.ac.uk/), which includes 1400 metabolites with catalog numbers GCST90199621 to GCST90201020. Datasets pertaining to TB were designated using the ukb-b-15,622. The TwoSampleMR package of R software (version 4.3.1) was essential. In addition, for the graphical display of MR analysis and results, we used a series of R software packages including "devtools, ieugwasr, gwasglue, VariantAnnotation, remotes, ComplexHeatmap, circlize, reshape2, BiocManager, TwoSampleMR, grid, readr, and forestploter."

Selection of instrument variable

The selection of single nucleotide polymorphisms (SNPs) related to 1400 metabolites adhered to the criterion $P < 5 \times 10^{-6}$. We imposed $r^2 < 0.001$ and $kb > 1000$ to ensure the independence of the SNPs. The SNPs selected

through the above steps were regarded as instrumental variables for subsequent analyses.

Remove weak instrumental variables (IVs)

There is no strong correlation between weak instrumental variables and exposure factors, or IVs only explain a small fraction of phenotypic variation; this is classified as weak IV. To mitigate the occurrence of bias caused by potential weak IVs, the F-statistic was utilized to evaluate the strength of IV (Feng et al. 2022). The F-statistic can be calculated using the formula $F = \beta^2/SE^2$. Standard $F < 10$ was used to filter all weak IVs.

MR analysis

The TwoSampleMR package in R software (version 4.3.1) was used for analysis. The inverse-variance weighted (IVW) method was used for bidirectional MR analysis, followed by instrument coordination and selection. When heterogeneity is present, the IVW technique employs a random effects model; otherwise, it uses a fixed-effects model. We used the IVW analysis results to ascertain whether there was a substantial causal association between exposure and outcome. Notably, if each variable satisfied all three requirements for valid IVs, the IVW technique provided a cogent assessment of causality (Fig. 1). The IVW estimate was obtained by calculating the gradient of weighted linear regression. The evaluation created using this method would be highly accurate because there would be no directional pleiotropy or heterogeneity between exposure and outcome (Kintu et al. 2023). To reduce the potential bias introduced by a single model, the MR-Egger, simple-mode, weighted-median, and weighted-mode methods were used as complementary and reference methods for the IVW model. The possibility of a reasonably consistent causal association between exposure and results exists when the causal orientations of these five models demonstrate alignment.

Heterogeneity, horizontal pleiotropy, reverse MR analysis

Cochran Q and MR-Egger intercept tests were used to assess heterogeneity and horizontal pleiotropy(Luan et al. 2025). In the event of significant heterogeneity, the causal relationship may be unstable. According to the horizontal pleiotropy theory, SNPs may directly affect outcomes through mechanisms unrelated to exposure. If horizontal pleiotropy is significant, the causal effects are ineffective. Heterogeneity in IVs arising from diverse analysis platforms, experiments, and populations may influence the outcomes of MR analysis. Heterogeneity was assessed using the IVW and MR-Egger tests; if the *P*-value was < 0.05, heterogeneity existed in this study. An IV is said to have pleiotropy if factors, rather than exposure factors, affect the result. Pleiotropy leads to deterioration of independence and exclusivity. The MR-Egger intercept test was used to detect data pleiotropy and evaluate the stability of the results. $P < 0.05$ indicated pleiotropy. Regarding TB as an exposure and metabolites as outcomes, we conducted reverse MR. In the results, $P < 0.05$ suggested a connection between the exposure and the outcome; conversely, there is no apparent causal relationship. $\beta > 0$ demonstrated that the exposure is a risk factor; conversely, it is a protective factor.

Results

Data and detailed information

In this study, metabolite-related data were obtained from the GWAS database, which contains 1400 metabolites (Suppl 1. Detailed Information of 1400 Human

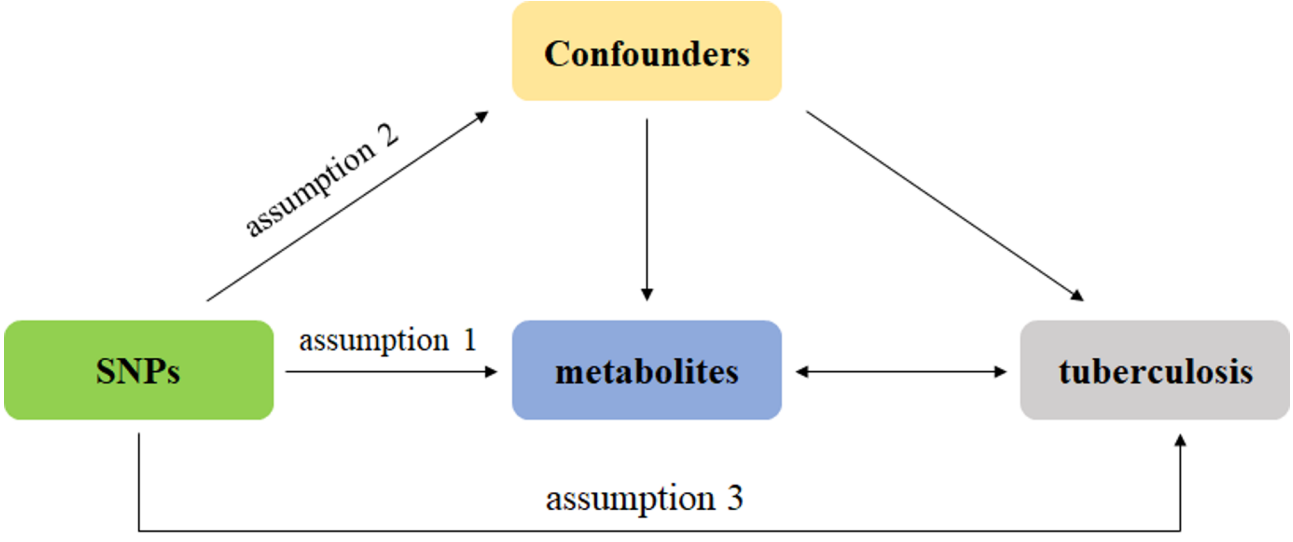

Fig. 1 MR study design diagram: Assumption 1, SNPs are closely related with exposure; Assumption 2, SNPs do not depend on confounding variables; Assumption 3, SNPs only affect outcome through exposure. SNP, single nucleotide polymorphism; MR, Mendelian randomization

Metabolite Data). The GWAS dataset for TB was coded ukb-b-15,622 based on the sequencing of 462,933 samples by Ben Elsworth in 2018. Sequencing data pertaining to TB were derived from 2,277 individuals of European ancestry, comprising all related datasets.

Selection of IVs and exclusion of weak IVs

The standard $P < 5 \times 10^{-6}$ was used to filter all the metabolites from the GWAS dataset for SNPs using. We made the following assumptions about the linkage disequilibrium parameters: $r^2 < 0.001$ and kb > 10,000. If the number of metabolites was insufficient for the MR analysis, they were excluded. F > 10 was used to filter SNPs following and F value range for all SNPs was obtained ranging from 19.503 to 5308.55 (Suppl 2. List of filtered SNPs, F > 10).

MR analysis and test results

MR analysis showed a plausible causal relationship between TB and 96 metabolites ($P < 0.05$). Figure 2 shows a flowchart of the MR analysis. Furthermore, as the statistical significance of numerous metabolites falls short of optimality, there is a considerable likelihood of Type II errors. For the purposes of this study, we prioritized reducing the probability of Type I errors, which inevitably leads to a certain level of decreased statistical power, by balancing the appearance of Type I and Type II errors. Figure 3 shows a heatmap of the *p*-values of the IVW, MR-Egger, simple mode, weighted median, and weighted mode methods for the 96 metabolites mentioned above. Ultimately, six metabolites successfully passed the heterogeneity and horizontal pleiotropy tests. Reverse MR analysis of these six metabolites and TB implied that TB had no obvious causal relationship with these six metabolites, suggesting that TB cannot affect these six metabolites. The 6 metabolites passing the test ultimately which are connected to TB are showed in Fig. 4.

Figures 5 and 6 show the regression of the SNPs of the six metabolites using the IVW, MR-Egger, simple mode, weighted median, and weighted mode methods. Figure 5 indicates that dodecanedioate, myristoleate (14:1n5), and 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (*p*-16:0/20:4) have a positive causal effect on TB, and the increase in these three metabolites promotes TB. Figure 6 shows that glycerol 3-phosphate, sphingomyelin (d18:1/20:2, d18:2/20:1, and d16:1/22:2),2-methylserine have a negative causal effect on TB, and an increase in these three metabolites suppresses TB. Figures 7 and 8 are the forest plots. The ordinate represents IVs (SNPs) and the abscissa represents effect values. In the plots, each SNP effect value and combined effect of all SNPs are shown, with effect values > 0 indicating risk factors and those < 0 indicating protective factors. Consequently, dodecanedioate, myristoleate (14:1n5) and

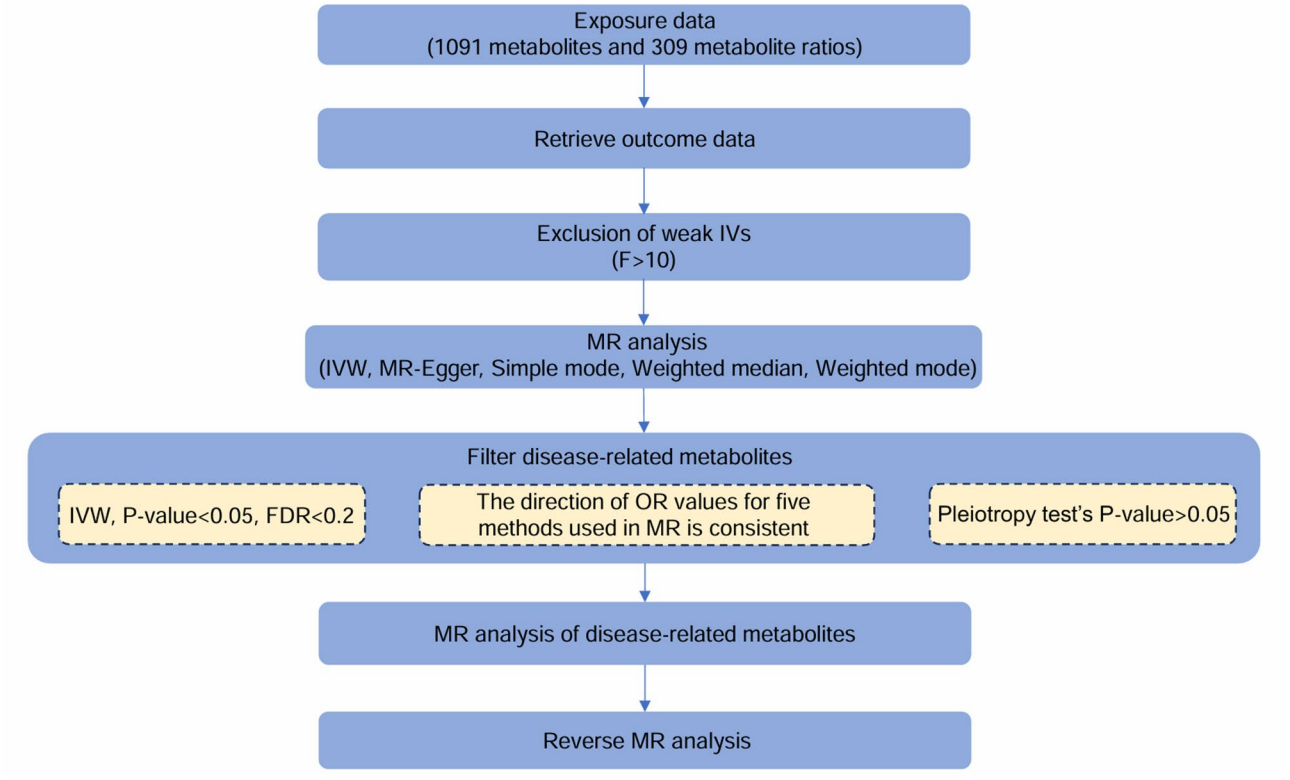

Fig. 2 Flow chart for MR analysis. IVW, inverse weighted variance; MR, Mendelian randomization

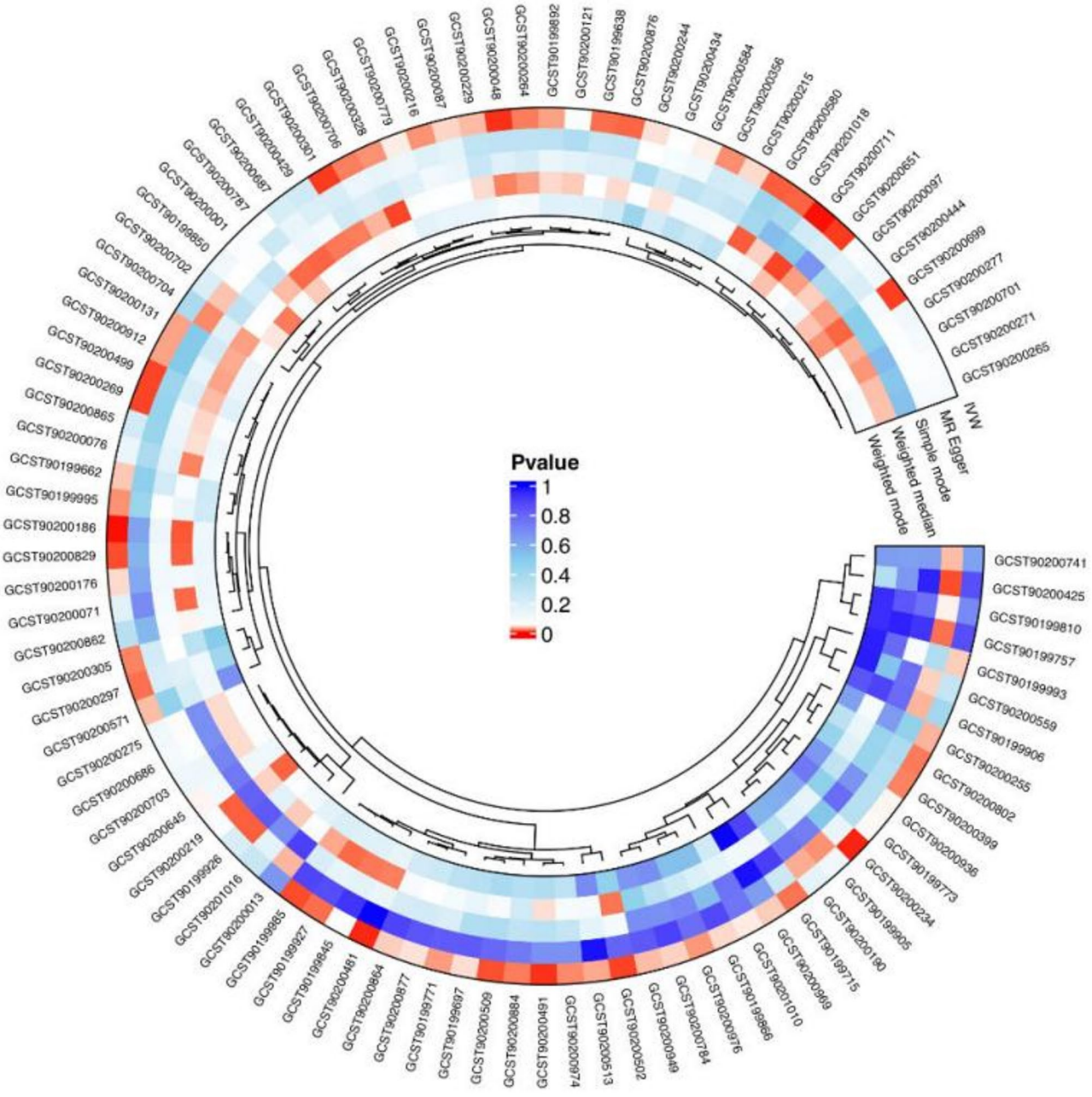

Fig. 3 The heatmap displays the *P*-values for each of the 96 metabolites using various techniques

1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (*p*-16:0/20:4) demonstrate a causal protective effect on TB (Fig. 7). Glycerol 3-phosphate, sphingomyelin (d18:1/20:2, d18:2/20:1, and d16:1/22:2) and 2-methylserine demonstrate a causal risk effect on TB (Fig. 8).

The results of the leave-one-out sensitivity analysis is presented in Supplementary Material 3.

Discussion

Globally, TB persists as the primary cause of death among adults with infectious diseases and annually accounts for over 10 million new cases. Ongoing advancements in diagnostic techniques, such as the use of rapid molecular testing and whole-genome sequencing of both sputum and non-sputum samples are anticipated to substantially contribute to reversing this trend (Furin et al. 2019). In our study, we determined the association between 1400 metabolites and TB using MR. Dodecanedioate, myristoleate (14:1n5), and

id.exposure	nsnp	method	pval	OR(95% CI)
GCST90199638	8	Weighted median	0.060	0.998 (0.997 to 1.000)
Glycerol 3-phosphate	8	Inverse variance weighted	**0.014**	0.998 (0.997 to 1.000)
	8	Weighted mode	0.183	0.998 (0.996 to 1.001)
GCST90199697	10	Weighted median	0.124	1.001 (1.000 to 1.003)
Dodecanedioate	10	Inverse variance weighted	**0.041**	1.001 (1.000 to 1.003)
	10	Weighted mode	0.337	1.001 (0.999 to 1.004)
GCST90199715	9	Weighted median	0.098	1.002 (1.000 to 1.004)
Myristoleate (14:1n5)	9	Inverse variance weighted	**0.015**	1.002 (1.000 to 1.003)
	9	Weighted mode	0.883	1.000 (0.997 to 1.003)
GCST90199995	10	Weighted median	0.130	0.999 (0.998 to 1.000)
Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)	10	Inverse variance weighted	**0.022**	0.999 (0.998 to 1.000)
	10	Weighted mode	0.236	0.999 (0.998 to 1.001)
GCST90200048	7	Weighted median	**0.016**	1.002 (1.000 to 1.003)
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (p-16:0/20:4)	7	Inverse variance weighted	**0.003**	1.002 (1.001 to 1.003)
	7	Weighted mode	0.090	1.002 (1.000 to 1.004)
GCST90200087	7	Weighted median	0.073	0.998 (0.996 to 1.000)
2-methylserine	7	Inverse variance weighted	**0.039**	0.998 (0.997 to 1.000)
	7	Weighted mode	0.146	0.997 (0.994 to 1.001)

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Fig. 4 Six successfully evaluated metabolites. nsnp: Number of SNPs analyzed; pval (*P*-value): Statistical significance threshold set at $P < 0.05$; OR, Odds ratio; OR > 1 indicates increased risk, OR < 1 indicates reduced risk; CI, Confidence interval

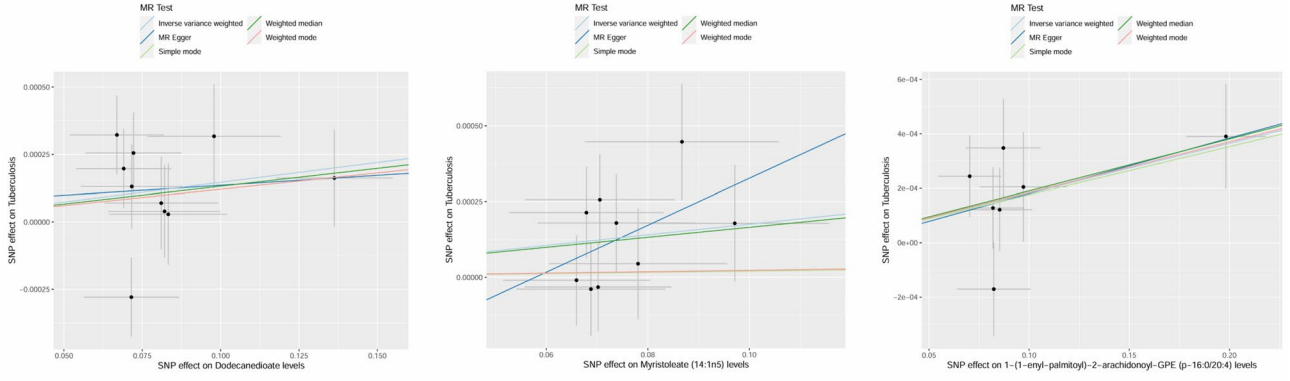

Fig. 5 Metabolite scatter plots with a forward direction. Vertical axis: The effect value of SNP on TB; Horizontal axis: The effect value of SNP on dodecanedioate, myristoleate (14:1n5), and 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (*p*-16:0/20:4) levels; Colored lines represent the results of MR analysis based on five methods

1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE(*p*-16:0/20:4) promote TB, while glycerol 3-phosphate, sphingomyelin (d18:1/20:2,d18:2/20:1,d16:1/22:2), and 2-methylserine suppress TB, which contributes to a better understanding of TB prevention.

Dodecanedioate, a human metabolite and conjugate base of dodecanedioic acid, is derived through the deprotonation of both its carboxy groups. Dodecanedioate is in reverse order and oxidizes peroxisomes and mitochondria, which is a potential substrate for treating reperfusion injuries and congenital dysbolism, predominantly affecting the restoration of the citric acid cycle (Jin et al. 2015).

Myristoleate, also known as tetradecenoate, is a plant metabolite and the conjugate base of myristoleic acid. Although existing research indicates that myristoleate play a role in the pathogenesis of certain diseases, such as Nonalcoholic fatty liver disease (NAFLD) (Guo et al. 2023), its potential effects on TB remain unexplored. In this study, we performed MR analysis of myristoleate and TB and found that high levels of myristoleate can inhibit its occurrence. However, research on the relationship between TB and myristoleate is insufficient, and the mechanism by which myristoleate inhibits the occurrence of TB remains unclear; therefore, the further study of protective mechanism of myristoleate are needed.

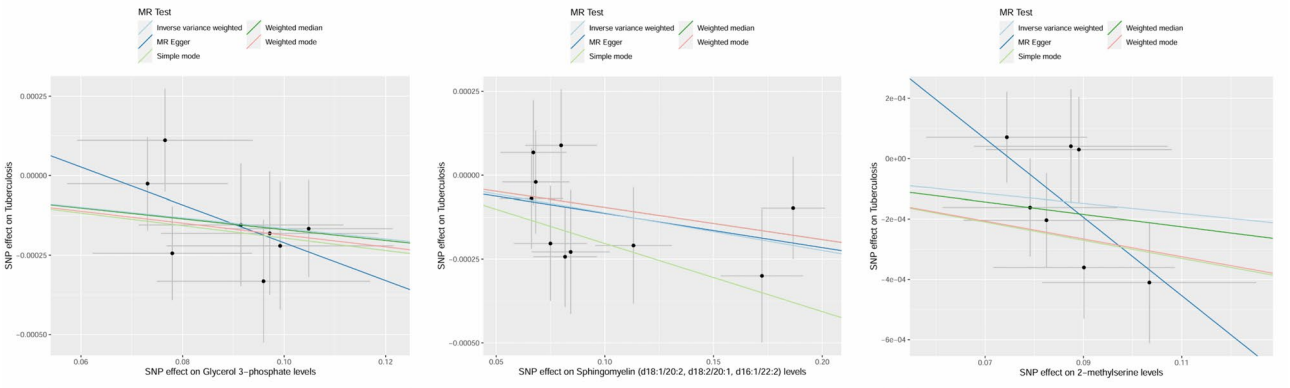

Fig. 6 Metabolite scatter plots with a negative direction. Vertical axis: The effect value of SNP on TB; Horizontal axis: The effect value of SNP on glycerol 3-phosphate, sphingomyelin (d18:1/20:2, d18:2/20:1, and d16:1/22:2) and 2-methylserine levels; Colored lines represent the results of MR analysis based on five methods

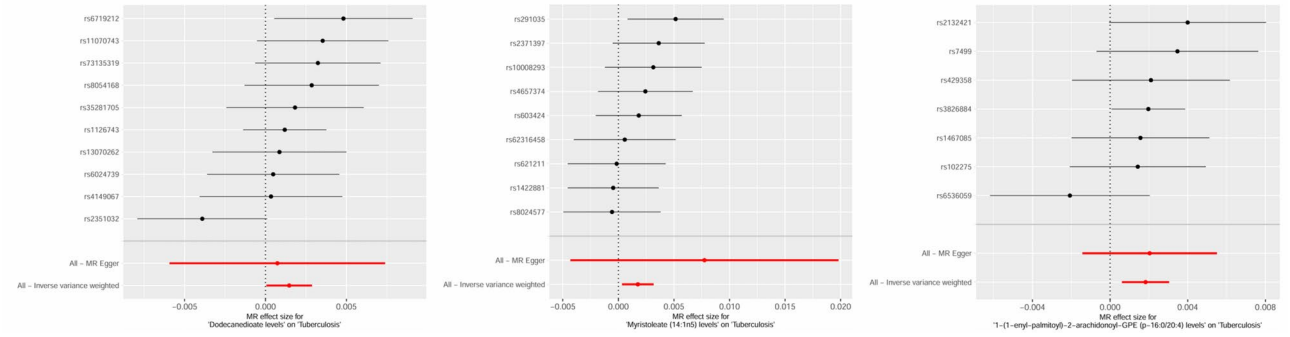

Fig. 7 Forest plots of metabolites with a forward direction. Vertical axis: SNP identifier; Horizontal axis: In MR analysis, the effect sizes of dodecanedioate, myristoleate (14:1n5), and 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (*p*-16:0/20:4) levels on TB; Black lines: Confidence intervals of the effect sizes for each SNP; Black dots: Point estimate for each SNP; Red lines: The causal estimate using all IVs

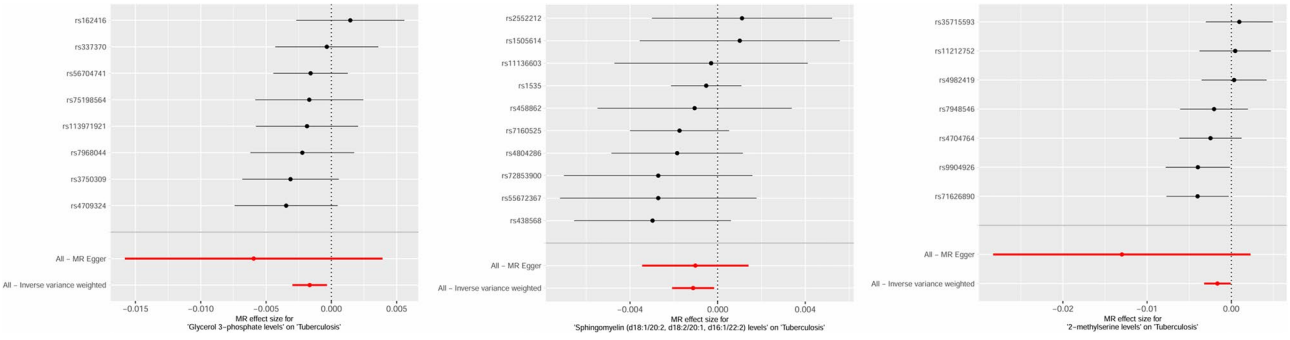

Fig. 8 Forest plots of metabolites with a negative direction. Vertical axis: SNP identifier; Horizontal axis: In MR analysis, the effect sizes of glycerol 3-phosphate, sphingomyelin (d18:1/20:2, d18:2/20:1, and d16:1/22:2),2-methylserine levels on TB; Black lines: Confidence intervals of the effect sizes for each SNP; Black dots: Point estimate for each SNP; Red lines: The causal estimate using all IVs

There is a relationship between 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE(*p*-16:0/20:4) and TB. We demonstrated that TB is progressed with an increase in 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE(*p*-16:0/20:4). The influencing mechanisms need to be studied further.

Glycerol 3-phosphate is a conserved three-carbon sugar that is an essential constituent of energy-producing reactions, such as glycolysis and glyceride biosynthesis. G3P can be obtained by glycerol kinase-mediated glycerol phosphorylation or G3P dehydrogenase (G3Pdh)-mediated dihydroxyacetone phosphate phosphorylation (Mandal et al. 2011). During microbial aerobic metabolism, glycerol kinase (GlpK), an ATP-dependent enzyme, phosphorylates glycerol to G3P (Larrouy-Maumus et al. 2013). Mukherjee et al. found that G3P promotes metastatic metabolism of tumors as a precursor to membrane

and signaling components; Willson et al. demonstrated the stability of HIF-1α in neutrophils is strengthened by mitochondrial ROS produced by G3P shuttling (Willson et al. 2022). Larrouy-Maumus et al. showed that Rv1692 is a D, L-glycerol 3-phosphatase, a unique haloacid dehalogenase superfamily phosphatase that can decompose phosphoglycerol into glycerol and inorganic phosphate through omics analysis and kinetic characterization. Metabolomic profile and functional research has demonstrated that *Rv1692* functions as the catalytic enzyme responsible for glycerophospholipid recycling and catabolism. The absence of *Rv1692* results in accumulation of G3P and lipid polar heads containing G3P (Larrouy-Maumus et al. 2013). We demonstrated that glycerol 3-phosphate can inhibit the occurrence of TB. Therefore, we speculated that a reduction in *Rv1692* expression may reduce the incidence of TB. In *Mtb*, the polyphosphatylase, PPK1, is responsible for the synthesis of poly(P), whereas the extracellular polyphosphatases, PPX1 and PPX2, and the GTP synthetase PPK2 account for the hydrolysis of poly(P). Liquid chromatography-tandem mass spectrometry reveals that the intracellular G3P levels in *Mtb* mutant strains with accumulated poly(P) and PPX1 or PPK2 deficiency are significantly reduced. Therefore, the accumulation of polyphosphate in *Mtb* is associated with the expression of G3P-related genes. The accumulation of poly(P) related to PPX1 or PPK2 deficiency leads to alterations in the expression of genes involved in G3P homeostasis, resulting in decreased G3P levels in cells (Chuang et al. 2016). In our study, we found that G3P inhibits the occurrence of TB and acts as a protective factor. Therefore, a decrease in PPX1 or PPK2 leads to a decrease in G3P levels, thereby increasing the risk of TB.

Sphingomyelin is a sphingolipid composed of phosphocholine (or ethanolamine phosphate) attached to the C-1 hydroxyl group of ceramides. By using palmitic acid and serine, sphingomyelin can synthesize sphingosine, followed by the synthesis of fatty acids CoA and phosphocholine. Its metabolites, such as ceramide (Cer), sphingosine (Sph), and sphingosine 1-phosphate (S1P), are signaling molecules that can act as first and/or second messengers to regulate cell life activities, such as cell growth, differentiation, aging, apoptosis (Zhang et al. 2022). Management of cell proliferation and apoptosis involves sphingomyelin metabolites, such as Cer and Sph. These compounds negatively regulated cell growth, suppressed cell proliferation, and promoted apoptosis. Conversely, their downstream metabolite S1P exerts stimulatory effects on cell growth and inhibits apoptosis (Momchilova et al. 2022). Cer/Sph and S1P constitute significant metabolic homeostasis, which is important for regulating the total amount of each lipid as well as the overall balance at the cellular level; therefore, the intricate balance between these metabolites is linked to cellular survival, demise, and functionality (Kihara et al. 2007). We hypothesized that the sphingomyelin metabolites, Cer and Sph, could inhibit the growth of *Mtb* and promote apoptosis, thereby inhibiting the occurrence of TB. Another sphingomyelin metabolite, S1P, stimulates cell growth, inhibits apoptosis, and promotes the growth of *Mtb*, thereby promoting its progression. We propose that Cer and Sph are dominant in the competition between Cer, Sph, and S1P, and that sphingomyelin inhibits cell growth, promotes apoptosis, and inhibits the growth and development of *Mtb*, thereby inhibiting the development of TB. However, further experimental evidence is required.

The 2-methylserine is a hydroxyl amino acid in which serine is replaced by a methyl group at position 2. The most common metabolic pathways of amino acids require the presence of a α-hydrogen atom, which is not open to amino acids with one tertiary carbon atom, thereby the metabolism of amino acids is of great significance. α-methylserine metabolism occurs naturally in the amisocetin (Wilson and Snell 1962). However, the metabolic process and mechanism of action of 2-methylserine in TB remain unclear and further research is needed.

The metabolism of 2-methylserine falls within the realm of amino acid metabolism, which is a crucial biochemical pathway for the survival of *Mtb*. These amino acids serve as essential nutrients for pathogens, providing both carbon and nitrogen sources, and substantially influence the life activities of *Mtb* (Yelamanchi and Surolia 2021). Existing information indicates that amino acid metabolism plays a pivotal role in governing the immune response following infection. Infection prompts the upregulation of metabolic pathways that restrict the availability of nutrients, and subsequently suppress effective immune responses. In addition, compared to patients with latent TB with normal body mass index, the immune cell profile in patients with latent TB with low body mass index was altered, including a decrease in T and B cells, emphasizing the importance of nutrients in the immune system, which is consistent with studies that nutritional supplementation can enhance the immune response after infection (Crowther and Qualls 2020). This concurs with the present study. Our study indicates that 2-methylserine is a protective factor against TB and can inhibit its progression. Therefore, we speculate that a certain amount of 2-methylserine could be supplemented to enhance the immune response after infection, thereby alleviating the clinical response.

Sphingomyelin and myristoleate are lipids. Lipid metabolism is an indispensable biochemical process that allows *Mtb* to harness host-derived lipids as an alternative nutritional source during infection. Beyond merely degrading host lipids, *Mtb* demonstrates proficiency in

utilizing lipids, particularly cholesterol, to facilitate its entry into macrophages (Moopanar and Mvubu 2020). A complicated pathogen-host interaction arises from the complex and dynamic interactions of host lipid homeostasis, immunological response, and lipid metabolism in *Mtb*. Imaging and metabolic analysis techniques have shown that *Mtb* binds to foam cells first and utilizes a variety of physiological systems to use exogenous fatty acids and cholesterol (Lovewell et al. 2016). Amaral et al. found that lung necrosis and bacterial burden were substantially increased in Gpx4-deficient mice after infection with *Mtb*, whereas lung necrosis and bacterial burden of transgenic mice overexpressing the Gpx4 enzyme were substantially reduced. Furthermore, macrophages deficient in Gpx4 displayed exacerbated necrosis upon in vitro infection with *Mtb*. However, this necrosis was attenuated by administration of the lipid peroxidation inhibitor, ferrostatin-1. In our study, sphingomyelin was a protective factor; therefore, we speculate that sphingomyelin may be involved in this process. In addition, metabolic changes under drug stress mainly occur in lipid metabolism and redox homeostasis of *Mtb*, and a decrease in the activity of the tricarboxylic acid cycle is beneficial to lipid anabolism (Goossens et al. 2020). The elevation of lipid anabolism contributes to the thickening of cell walls, thereby decreasing sensitivity to the majority of TB drugs (Goossens et al. 2020). Therefore, we speculated that the metabolism of myristoleate may contribute to the thickening of the cell wall in *Mtb*, thereby reducing its sensitivity to most drugs and promoting the development of TB.

Although we determined that six metabolites have a causal relationship with TB, there are some limitations to this study. Firstly, a MR analysis was performed using GWAS data specific to the European population as both the exposure and outcome groups to reduce the potential confounding effect of ethnicity on outcomes. However, there are issues with the generalization of the findings because ethnic variables might introduce bias into conclusions that are applied to the entire human population. Secondly, the current study lacked conclusive evidence regarding the fundamental mechanisms and molecular processes underlying the influence of metabolites on the pathogenesis. Notably, the identified six metabolites in our study have not been documented in the Human Metabolome Database (HMDB) and the blood metabolites concentration data from TB patients was unavailable. This absence of external validation weakens the causal claims. Additionally, this study was not integrated with clinical trials, which limits its applicability and validity. Therefore, the next step in our work plan is to collect clinical samples and conduct metabolomic validation. Thirdly, the statistical power related to the metabolite analysis was generally low, indicating a heightened risk of committing Type II errors or falsely accepting a null hypothesis. Finally, the selection of TB as an exposure factor is rather broad, and its appropriateness for studying potential infections and complications remains uncertain.

Conclusions

Our MR study revealed a potential causal association between six metabolites and TB, offering initial evidence for the role of metabolite dysregulation in its pathogenesis. These findings underscore the importance of metabolites in the development of this infectious disease. We found that glycerol 3-phosphate, sphingomyelin (d18:1/20:2,d18:2/20:1, d16:1/22:2), and 2-methylserine are promising markers for filtering and preventing TB in clinical practice. These metabolites, as promising biomarkers, warrant further clinical validation to elucidate their roles in the disease process.

Abbreviations

TB	Tuberculosis
Mtb	*Mycobacterium tuberculosis*
MR	Mendelian randomization
OC	Ovarian cancer
LC	Lung cancer
SNPs	Single nucleotide polymorphisms
IVs	Instrumental variables
IVW	Inverse-variance weighted
NAFLD	Nonalcoholic fatty liver disease
G3Pdh	G3P dehydrogenase
GlpK	Glycerol kinase
Cer	Ceramid
Sph	Sphingosine
S1P	Sphingosine 1-phosphate
HMDB	Human metabolome database

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-025-01901-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Not applicable.

Author contributions

CZ and XY contributed to the conceptualization; CZ, XY and DZ collected the clinical samples; LX, GZ, MW, XC, CZ, XY, DZ, YL and ZS wrote, edited, and reviewed this paper. All authors reviewed the manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate
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Competing interests
The authors declare no competing interests.

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