The Plasma Metabolite-Mediated Relationship between Inflammatory Cytokines and Acute Respiratory Distress Syndrome

Keywords
inflammatory cytokines, plasma metabolites, mediation, Acute Respiratory Distress Syndrome, Mendelian Analysis

Abstract

Introduction
Biomarkers of acute respiratory distress syndrome (ARDS) can provide precise treatment options. It is a clinical syndrome of diffuse lung inflammation and edema, usually leading to acute respiratory failure. We use Mendelian randomization (MR) and mediation analysis to infer the potential impact of inflammatory factors and metabolites on ARDS.

Material and methods
The summary statistics of 1400 plasma metabolite traits and 41 inflammatory cytokine traits were obtained from publicly available GWAS. Inverse Variance Weighted were adopted for bidirectional MR analysis to infer causal relationships. Several sensitivity analyses were also used to ensure reliable MR results. Mediation analysis was used to determine the pathway from inflammatory factors to ARDS mediated by plasma metabolism and the proportion of mediation effects explained by plasma metabolites were estimated.

Results
MR analysis revealed the causal effects of 5 inflammatory cytokines and 18 plasma metabolites on ARDS. Reverse MR analysis shows that ARDS has no effect on these 5 inflammatory cytokines. In addition, we screened 18 pathogenic metabolites associated with ARDS. Based on known pathogenic metabolites above, it was observed that through mediation analysis that Ceramide levels and Alpha tocopherol to sulfate ratio may mediate the causal pathway from inflammatory factors to ARDS, with mediation ratios of 14.1% and 17.7%, respectively (p□0.05).

Conclusions
The increase of Ceramide levels and Alpha-tocopherol to sulfate ratio respectively will reduce the risk of ARDS. Our research provide further insights into the complex interactions between inflammatory cytokines and metabolites in the development of ARDS, promoting the development of innovative strategies for ARDS prevention and treatment.

The Plasma Metabolite-Mediated Relationship between Inflammatory Cytokines and Acute Respiratory Distress Syndrome

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ABSTRACT

Background

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Conclusions

The increase of Ceramide levels and Alpha-tocopherol to sulfate ratio respectively will reduce the risk of ARDS. Our research provide further insights into the complex interactions between inflammatory cytokines and metabolites in the development of ARDS, promoting the development of innovative strategies for ARDS prevention and treatment.

Keywords: Mendelian Analysis, Acute Respiratory Distress Syndrome, inflammatory cytokines, plasma metabolites, mediation

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a common clinical syndrome of acute respiratory failure as a result of diffuse lung inflammation and oedema. ARDS can be precipitated by a variety of causes[1]. The injury of pulmonary vascular endothelial cells is an important pathological change in the early stage of ARDS [2]. Endotoxin, inflammatory mediators and physical trauma can all cause endothelial cell damage. It is a clinical syndrome of diffuse lung inflammation and edema, usually leading to acute respiratory failure [3, 4]. ARDS is an acute lung injury induced by risk factors such as pneumonia, non-pulmonary infection, trauma, blood transfusion, burn, aspiration or shock [5]. At present, there is no single examination to identify or exclude diagnosis, largely due to the heterogeneity of ARDS, manifested in its causes, manifestations, and response to treatment [6, 7]. ARDS is a syndrome, not a specific pathological entity, currently identified only by clinical criteria, and diagnosis requires the presence of new or worsening respiratory distress and bilateral chest imaging abnormalities for 7 days or less [8].

Although some ARDS triggers can be self-limiting, while others do not have specific treatments, timely identification and treatment of reversible damage (such as infection, type I hypersensitivity) is essential [3]. Inflammatory factors play an important role in the occurrence and development of ARDS, but there is currently no unified research on the specific magnitude of their impact and whether they have the potential to serve as biomarkers. However, research has found that IL-1 β can increase endothelial permeability by inhibiting the transcription of VE cadherin [9]. The IL-18 levels in ARDS patients with sepsis are significantly higher than those in patients with sepsis alone, indicating that IL-18 in sepsis is a key cytokine leading to lung injury [10, 11]. In a randomized controlled trial, it was also found that plasma IL-18 levels were associated with mortality in sepsis induced ARDS [12]. The research results indicate

that inflammatory cytokines play a crucial role in the development of sepsis induced lung injury, and they synergistically interact to promote the progression of inflammation [13].

Metabolomics can reveal the correlation between metabolites or metabolic pathways and physiological and pathological changes, providing new information for the study of disease mechanisms [14]. Multiple studies have shown that metabolites and metabolic pathways are closely related to ARDS, and metabolic disorders often occur in ARDS patients [15, 16]. In the early stages of ARDS, neutrophils migrate from the pulmonary vascular system to the air chamber and can release various harmful metabolic mediators, including reactive oxygen species, proteases, and pro-inflammatory lipid derived mediators such as prostaglandins and leukotrienes [17]. Plasma metabolites, as small molecules in human blood components, play an important role in human health. They are direct or indirect products of various metabolic activities in the body, including all chemical changes that support life, including energy production, construction of essential substances, and waste disposal [18]. The abnormal levels of these metabolites can reflect oxidative stress and inflammatory status[19],

Mendelian randomization (MR) analysis is an effective method for evaluating potential causal relationships between exposure and outcomes using genetic variation as an instrumental variable (IV); Compared with traditional observational studies, MR analysis is relatively independent of unmeasured confounding factors and can significantly reduce confounding effects; Mediation analysis is used to evaluate the impact of exposure on results through mediation [20]. This study aims to conducted MR analysis based on publicly available genome-wide association studies (GWAS) to evaluate the causal relationship between inflammatory cytokines, plasma metabolites, and ARDS risk, and determined that the pathway from inflammatory cytokines to ARDS is mediated by plasma metabolites.

MATERIALS and METHODS

Hypothesis Diagram of Causal Relationship between Inflammatory Factors and ARDS

This study has two main components, as shown in Figure 1. (1) MR analysis of 41 inflammatory cytokines on ARDS and reverse MR analysis of ARDS on 41 inflammatory cytokines (Step 1 of Figure 1); (2) Analyze the mediating role of plasma metabolites in cytokine mediated ARDS in 1900 (Figure 1, Step 2). Single nucleotide polymorphisms (SNPs) were defined as IVs. SNP refers to the variation of a single nucleotide in the genome, which is the most common form of genetic variation in the genome. SNP exists widely in genome and can be used to study the relationship between genes and traits and diseases. MR analysis is based on three core assumptions: IV is closely related to exposure factors; IV is not related to confounding factors; IV will not directly affect the results, it can only affect the results through exposure.

Data Source

The genetic data of cytokines comes from the latest summary data of GWAS database, in which Zhao et al. sorted and analyzed the genome-wide genotypes of 14824 participants with European ancestry, including 91 circulating inflammatory cytokines [21]. The ARDS data was obtained from the FinnGen J10 Acute Respiratory Distress Syndrome summary dataset. This dataset can be accessed on https://www.finngen.fi/en, including 387 cases of ARDS and 447467 participants. The data information of 1400 metabolites were obtained from the research of Chen et al. in the GWAS database[22], which includes 1091 blood metabolites and 309 metabolite ratios.

Tool Variable Selection

SNPs associated with certain risk factors in GWAS can be used as IVs to test their causal effects on different outcomes. The selection of IV is based on three key assumptions: IVs must be significantly correlated with exposure; IVs cannot be associated with any known confounding factors that may alter the association between exposure and outcomes; IVs must be independent of the outcome and can only affect the outcome through their impact on exposure. The relationships study between exposure to outcome used in this study include the following four types: bidirectional MR analysis of inflammatory cytokines in ARDS, MR analysis of inflammatory cytokines to plasma metabolites, and MR analysis of plasma metabolites to ARDS. To

replace exposure factors, we first selected SNPs ($P<5 \times 10^{-8}$) significantly correlated with exposure factors as the threshold; In order to maximize the accuracy of IVs for each exposure factor, we removed some weak instrumental variables using F-statistic>10 as the standard [23, 24], and selected SNPs that could accurately simulate exposure (inflammatory factors, plasma metabolites, and ARDS); Linkage disequilibrium (LD) means that the allele distribution of two or more SNP loci in a population does not meet the expected independent distribution, that is, some alleles tend to be inherited together rather than independently distributed. Finally, we excluded SNPs with LD in MR analysis, and the LD of SNPs closely related to exposure factors should meet $R^2<0.001$ and distance>10000kb. To ensure that each IV is aligned with the same effector allele, exposure and outcome data were cross checked and palindromic SNPs were removed prior to analysis. The palindromic SNPs are SNPs with A/T or G/C alleles.

MR Analysis

(1) Phase One

To estimate the causal effect of inflammatory cytokines on ARDS, we conducted bidirectional MR analyses separately. The inverse variance weighting (IVW) method is a fundamental analysis method[25]. When the P-value of IVW results is less than 0.05 and the direction of IVW is consistent with that of MR Egger, weighted median, simple mode, and weighted mode, then the results have statistical significance. MR results are represented by odds ratios (ORs) and corresponding 95% confidence intervals (CI). To evaluate the reverse causal relationship between ARDS and inflammatory cytokines, we used ARDS as the "exposure" and inflammatory cytokines associated with ARDS as the "outcome" to conduct MR analysis. SNPs significantly associated with ARDS ($P < 5 \times 10^{-8}$) were selected as IVs for MR analysis. Based on the P-value of IVW greater than 0.05, the inflammatory factors identified in the forward MR analysis were further identified as exposure factors for ARDS.

(2) Phase two

Through bidirectional MR analysis, inflammatory cytokines with significant causal effects on ARDS were included in further mediation analysis. Furthermore, we

investigated whether plasma metabolites have a causal effect on ARDS. Immediately, we conducted multiple two-step MR analyses to explore whether plasma metabolites are mediating factors in the pathway from inflammatory cytokines to ARDS. coefficient product method was used to evaluate indirect effects and the standard error of indirect effects was determined using delta method[26].

Sensitivity Analysis

Cochran Q test was conducted to evaluate the heterogeneity of each SNP[27] and scatter plots of SNPs-exposure association and SNPs-outcome association were generated to visualize the test results. “leave one out” analysis were performed to evaluate whether each SNP will affect the results (by sequentially excluding each SNP and performing IVW on the remaining SNPs to assess the potential impact of specific variants on the estimated values) [28]. Furthermore, we used MR Egger regression to test and correct for potential horizontal pleiotropy.

All analyses were conducted using R (v4.3.3) statistical software. MR analysis was performed using the R software package 'TwoSampleMR'.

RESULT

The Association between All Inflammatory Cytokines and the Risk of ARDS

Table S1 showed the SNPs considered in the regional target analysis of inflammatory cytokines. According to the above screening threshold, the F-statistic of all SNPs obtained is greater than 10, reducing potential weak tool bias. After removing palindromic SNPs, a total of 1597 SNPs were used as IVs. Next, we will use these SNPs as IVs replacement inflammatory cytokines for "exposure", ARDS as the "outcome" for MR analysis, IVW as our main method, and MR Egger, weighted median, simple mode, and weighted mode served as auxiliary judgment methods. The IVW results showed that Fibroblast growth factor 19 levels (OR=0.497, p=0.002), Interleukin-8 levels (OR=0.555, p=0.043), and Neurturin levels (OR=0.506, p=0.008) were significantly negatively correlated with ARDS; TNF beta levels (OR=1.459, p=0.003) and Urokinase type plasminogen activator levels (OR=1.544, p=0.027) were significantly positively correlated with ARDS (**Figure 2 and Figure 3**). In the MR

analysis of ARDS, the Cochran Q test results for these 5 inflammatory cytokines showed no heterogeneity in IVs in the MR analysis , No significant intercept was observed in the MR Egger test results, confirming the absence of pleiotropy bias in IVs in MR analysis (**Figure 4，Table S2、S3**). In addition, the "leave one out" method demonstrated the robustness and reliability of our research results, as no single SNP had a significant impact on the results **(Figure 5**). ARDS was assumed as an "exposure", and 1154 SNPs related to ARDS were obtained as IVs (**Table S4**) and 91 inflammatory cytokines were assumed as "outcomes" according to the above SNP screening criteria. Reverse MR analysis was performed, and the results showed that there was no reverse causal relationship between the genetic prediction of ARDS and the inflammatory cytokines obtained from forward MR analysis. However, there was a significant positive correlation between ARDS and tumor necrosis factor ligand superfamily member 12 levels, monocyte chemoattractant protein-4 levels, neurotrophin-3 levels, C-X-C motif chemokine 9 levels, beta norve growth factor levels, and C-C motif chemokine 19 levels. Relationship (**Table S5**).

Two Step Correlation Analysis of Inflammatory Cytokines Mediating ARDS through Plasma Metabolite Pathway

Firstly, 31638 SNPs were obtained according to the above SNP screening criteria to replace plasma metabolites as "exposure" (**Table S6**), and ARDS was used as the "outcome" for MR analysis of 1400 metabolites to ARDS. The IVW analysis results showed that a total of 73 metabolites were associated with ARDS (**Figure 6**). By using sensitivity analysis, our results showed no horizontal pleiotropy or heterogeneity, and the IVs of each metabolite were relatively robust (**Tables S7 and S8**).

Secondly, five inflammatory cytokines, screened by MR analysis of inflammatory cytokines to ARDS, were used as "exposures", and 73 metabolites screened by MR analysis of metabolites to ARDS, were used as "outcomes". These two positive results were subjected to MR correlation analysis. IVW test results showed that there were 18 potential causal associations (5 inflammatory cytokines and 18 metabolites, see **Tables S9**). Through sensitivity analysis, we further confirm that our results do not have horizontal pleiotropy or heterogeneity.

Mediation analyses of potential plasma metabolites

To elucidate the potential mechanisms underlying the occurrence and progression of ARDS, we employed mediation analysis to identify the potential pathways involved in metabolic mediated inflammatory cytokines leading to ARDS. We analyzed the 18 metabolites mentioned above to determine their mediating roles in the 5-inflammatory-cytokine-ARDS pathways mentioned above. Finally, we identified 2 metabolites involved in 2 mediating relationships between 2 inflammatory cytokines and ARDS. According to **Table 1**, the total effect of TNF beta levels on ARDS is 0.377 (95% CI: 0.131-0.623), indicating that the causal effect of TNF beta levels on ARDS is positive, that is, an increase in TNF beta levels increases the risk of ARDS, and this effect is statistically significant. The total effect of FGF19 levels on ARDS is -0.699 (95% CI: -1.143--0.255). The causal effect of FGF19 levels on ARDS is negative, indicating that elevated FGF19 levels may reduce the risk of ARDS, and this effect is also statistically significant. The direct effect on ARDS is -0.581 (95% CI: -0.918--0.243), and the level of Ceramide (d18:1/17:0, d17:1/18:0) has a negative effect on ARDS, indicating that an increase in Ceramide (d18:1/17:0, d17:1/18:0) levels reduces the risk of ARDS. The direct effect of Alpha tocopherol to sulfate ratio on ARDS is -0.675 (95% CI: -1.17--0.175). An increase in Alpha tocopherol to sulfate ratio is associated with a reduced risk of ARDS, indicating its protective effect on ARDS. Specifically, the mediating role of Ceramide (d18:1/17:0, d17:1/18:0) levels in TNF beta levels to ARDS is (β=-0.053, SE=0.032), with a mediation ratio of 14.1%; The effect of Alpha tocopherol to sulfate ratio on Fibroblast growth factor 19 levels and ARDS mediation is (β=0.124, SE=0.029) with a mediation ratio of 17.7%.

DISCUSSION

ARDS is closely related to the immune system and inflammatory response, like whether local or systemic inflammation has a direct promoting effect on lung injury[4]. The inflammatory cytokines in bronchoalveolar lavage fluid of ARDS disease model are closely related to the severity of inflammation [29]. There are increasing reports on the relationship between inflammatory cytokines and ARDS. Previous studies have shown

that the recruitment of circulating immune cells into the alveolus triggered by chemokines can lead to endothelial and epithelial damage. For example, during the migration of monocytes to the lungs, IFN β-dependent release of TNF-related apoptosis inducing ligand induces epithelial cell apoptosis[30].

Metabolomics can reveal the correlation between metabolites or metabolic pathways and physiological and pathological changes, providing new information for the study of disease mechanisms[14]. Metabolites are downstream products of various intracellular biomolecules, including genes and protein transporters, which enable metabolomics to provide us with information on the metabolic status of the body's health and disease, as well as to identify biomarkers of drug response [16, 31]. Multiple studies have shown that metabolites and metabolic pathways are closely related to ARDS. The metabolic changes such as oxidative stress metabolism and energy level disorders described in the pathology of ARDS patients are consistent with the metabolic information changes generated by ARDS experiments and clinical studies[16, 32].

The study also showed that cytokines-induced lung inflammation leads to changes in metabolic patterns such as decreased energy status, ATP depletion, and significantly increased glycolytic activity, which are closely associated with disease phenotypes[33-35]. Therefore, we investigated the causal effects of inflammatory cytokines on ARDS. Assuming plasma metabolites as potential mediators for fractionation. The results support the mediating role of metabolites in the pathogenesis of ARDS driven by inflammatory cytokines.

With the wide availability of GWAS data, MR mediation analysis can use a large number of public data resources for analysis. These rich data resources enable researchers to analyze a wide range of exposure, mediation and outcome variables. MR mediation analysis provides a powerful and reliable method for causal inference and mediation effect analysis by combining the advantages of genetics and statistics. MR analysis uses genetic variations as IVs to assess potential causal relationships between exposure and outcomes, which can minimize the influence of confounding factors on causal estimation[36]. The combination of mediation analysis and MR analysis is used to evaluate the impact of exposure on outcomes through mediation, which can prevent

bias in estimated values due to unmeasured confounding between exposure, mediation, or outcomes[37]. Therefore, we conducted mediation analysis through two-step MR to evaluate the causal relationship between inflammatory cytokines, plasma metabolites, and ARDS, and determined that the pathway from inflammatory cytokines to ARDS is mediated by plasma metabolites. In a two-step MR study supported by large-scale GWAS and FinnGen data resources, we identified 24 important associations indicating the potential causal effects of 24 unique inflammatory cytokines and plasma metabolites on the risk of ARDS. Among the 5 inflammatory cytokines, three may have a direct effect on the risk of ARDS, while two may have a mediating effect through the plasma metabolite pathway.

In bidirectional MR analysis studies, Fibroblast growth factor 19 levels, Interleukin-8 levels, Neurturin levels, TNF beta levels, and Urokinase type plasminogen activator levels are influencing factors for ARDS risk; And tumor necrosis factor alignment and superfamily member 12 levels, Monocyte chemoattractant protein-4 levels, Neurotrophin-3 levels, C-X-C motif chemokine 9 levels, beta serve growth factor levels, and C-C motif chemokine 19 levels, in turn, may be the result of ARDS progression. In addition, there were 19 associations between plasma metabolites and ARDS risk in two-step MR analysis, of which 2 were shown to have mediating effects in the cytokine to ARDS pathway through mediation analysis (TNF beta levels → Ceramide (d18:1/17:0, d17:1/18:0) levels → ARDS; Fibroblast growth factor 19 levels → Alpha tocopherol to sulfate ratio → ARDS. These results may suggest that changes in the levels of the first 5 inflammatory cytokines may play a central role in inducing ARDS mechanisms, among which TNF beta levels and Fibroblast growth factor 19 levels may indirectly affect ARDS through metabolite mediated pathways. Huijuan Ouyang et al suggest that Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) are characterized by pulmonary microvascular endothelial cells (PMVECs) barrier dysfunction and proinflammatory cytokine influx into lung tissue, resulting in pulmonary oedema. Ceramide overproduction is an important mediator of pulmonary hyperinflammation and pulmonary oedema in ALI [38]. Mu Hu et al indicated that α -Tocopherol (α -TOH)

reduces the inflammation and oxidative stress of lung tissue by inhibiting the NF-κB signaling pathway, thereby alleviating the lipopolysaccharide (LPS) -induced ALI [39]. These findings provide further insights into the complex interactions between inflammatory cytokines and metabolites in the development of ARDS, promoting the development of innovative strategies for ARDS prevention and treatment. If future case-control studies nested in large population cohorts are validated, our results may make new contributions to the development of risk stratification of ARDS based on metabolites and the determination of new therapeutic targets, thus significantly improving the management and treatment strategies of ARDS.

However, the study has some limitations. The potential for confounding or bias in MR analyses, especially due to unmeasured pleiotropy. Due to the predominantly European dataset used, demographic stratification bias may have been introduced, limiting the generalizability of the conclusions to other racial groups. Therefore, further research involving diverse racial groups is warranted. Additionally, while several metabolites with a causal relationship with ARDS were identified, there remain unproven metabolites whose role in the disease is not fully understood, hampering a comprehensive analysis and interpretation of the findings. Although we have explored the mediating role of plasma metabolites between different cytokines and the risk of ARDS, considering the direct effects of various cytokines and plasma metabolites, as well as the differences in the effects of certain metabolites not acting as mediators, the mechanisms by which different cytokines affect ARDS still require further refinement.

Abbreviations

ARDS	Acute respiratory distress syndrome
MR	Mendelian randomization
ALI	Acute lung injury
SNPs	Single nucleotide polymorphisms
IV	Instrumental variable
GWAS	Genome-wide association studies
LD	Linkage disequilibrium

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Not applicable.

Authors' Contributions

Conception and design of the work: ZYL and FF. Acquisition, analysis and interpretation of data: ZYL. Drafting of the manuscript: ZYL and FF. Critical revision for important intellectual content: ZYL. All authors approved the final version of this manuscript.

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Availability of data and materials

The datasets generated for this study can be found at https://risteys.finregistry.fi/, and https://gwas.mrcieu.ac.uk/.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

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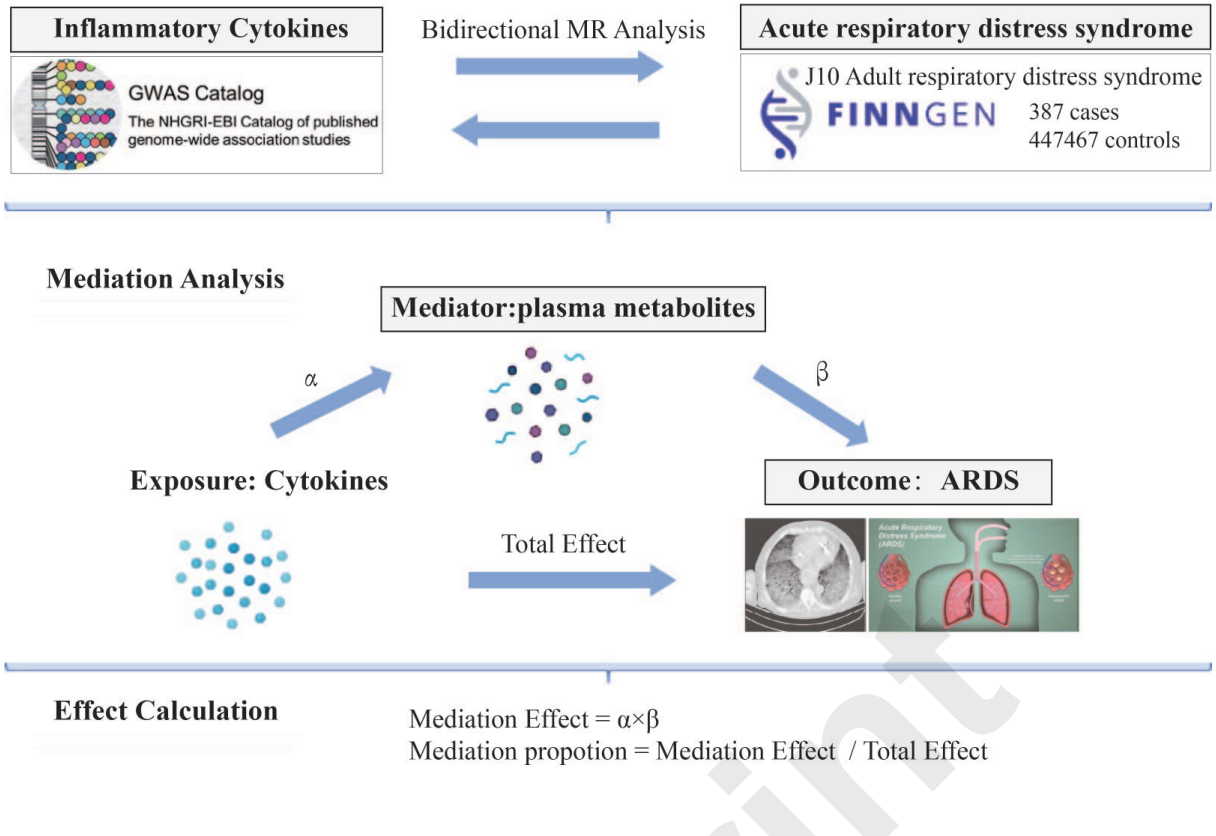
Inflammatory Cytokines
GWAS Catalog
The NHGRI-EBI Catalog of published genome-wide association studies
Bidirectional MR Analysis
Acute respiratory distress syndrome
J10 Adult respiratory distress syndrome
FINNGEN
387 cases
447467 controls
Mediation Analysis
Mediator:plasma metabolites
α
β
Exposure: Cytokines
Outcome: ARDS
Total Effect
Effect Calculation
Mediation Effect = α×β
Mediation propotion = Mediation Effect / Total Effect

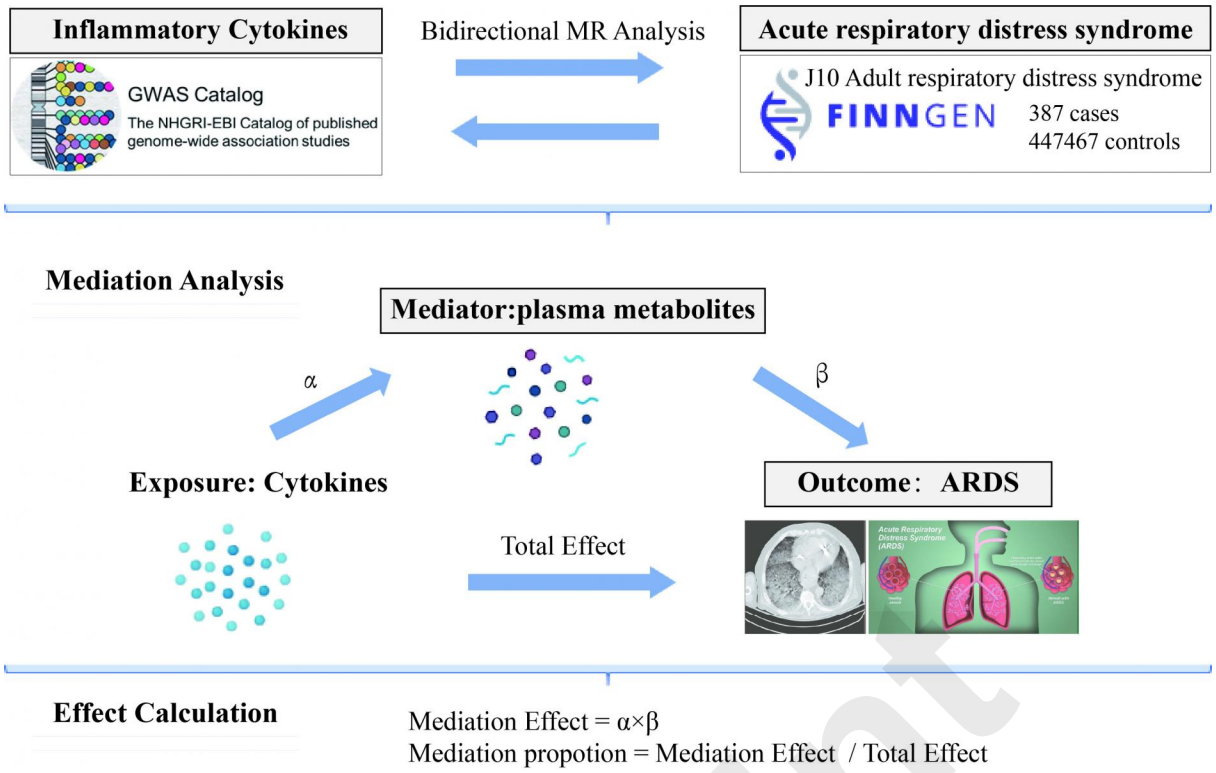

Figure 1. Experimental Flowchart.

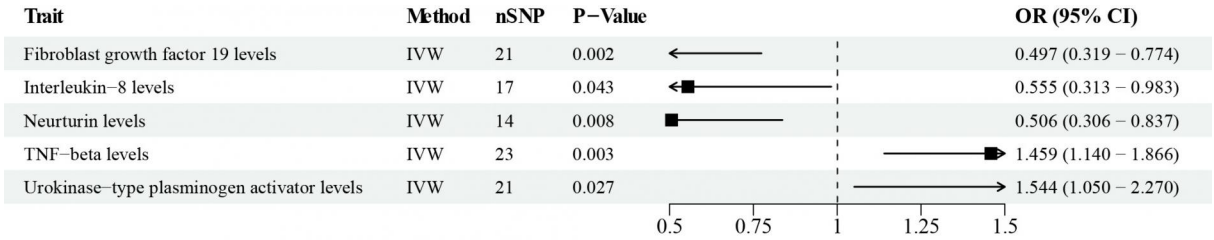

Figure 2. MR analysis of inflammatory cytokines and ARDS.

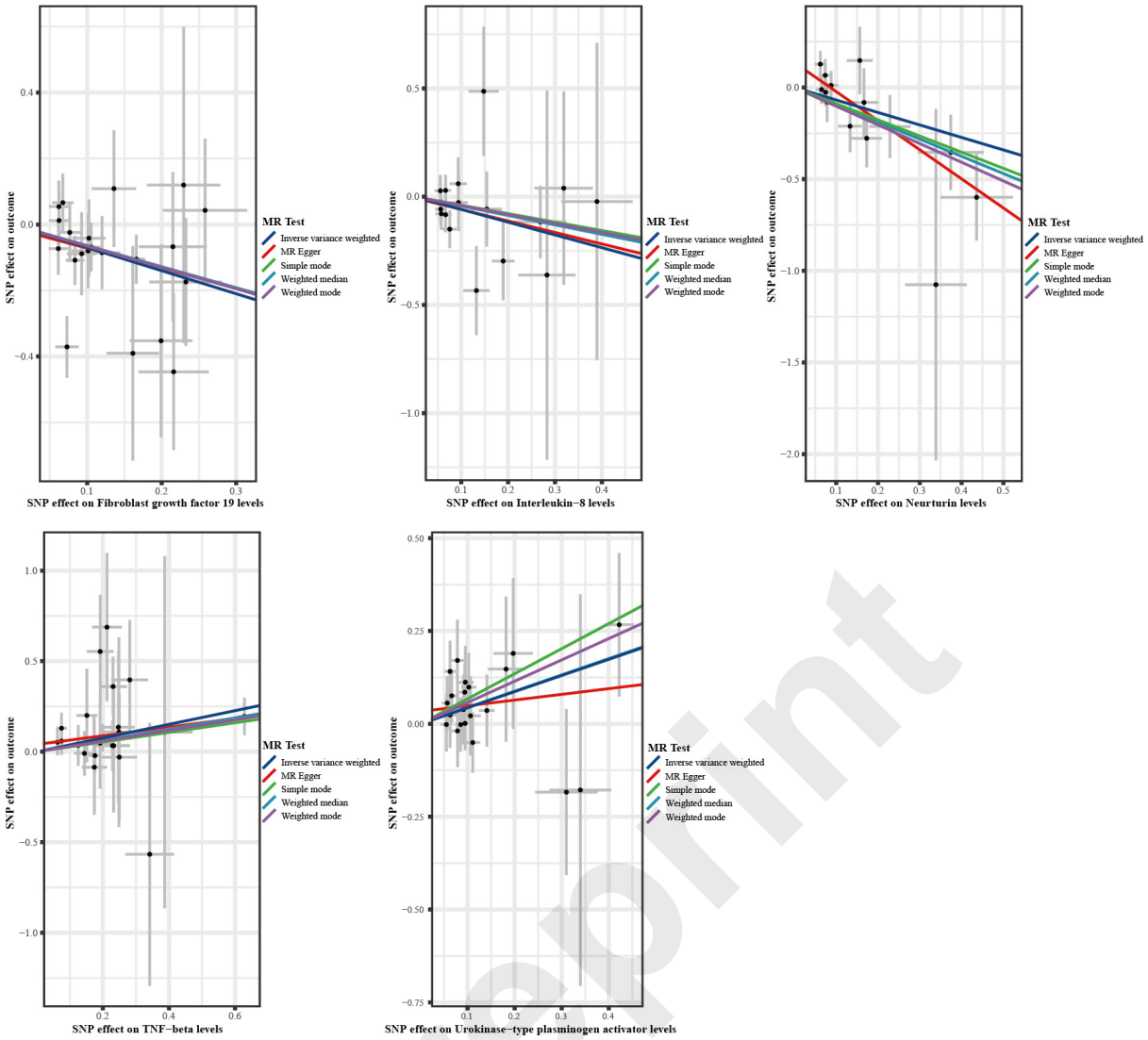

Figure 3 Scatter plot of MR analysis of inflammatory cytokines and ARDS.

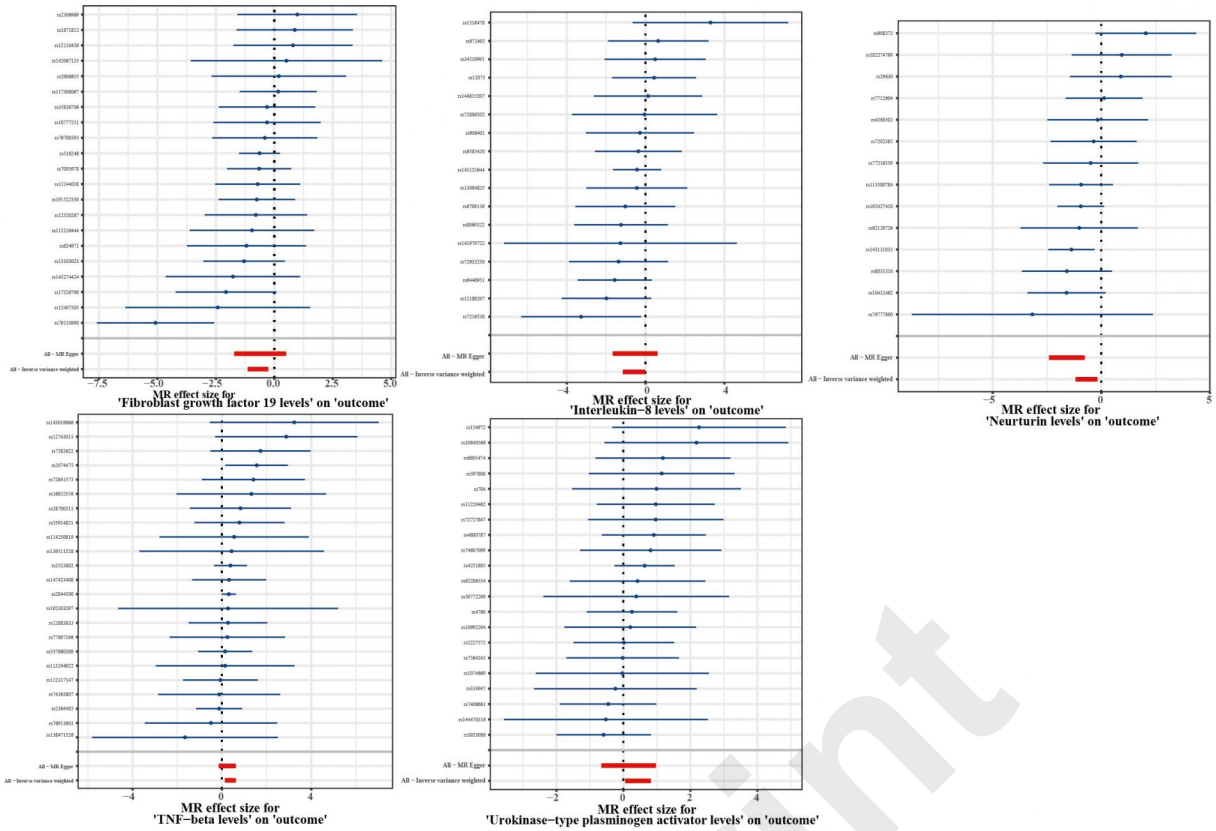

Figure 4 Forest plot: Visualization of Cochran's Q test results and MR Egger intercept method results.

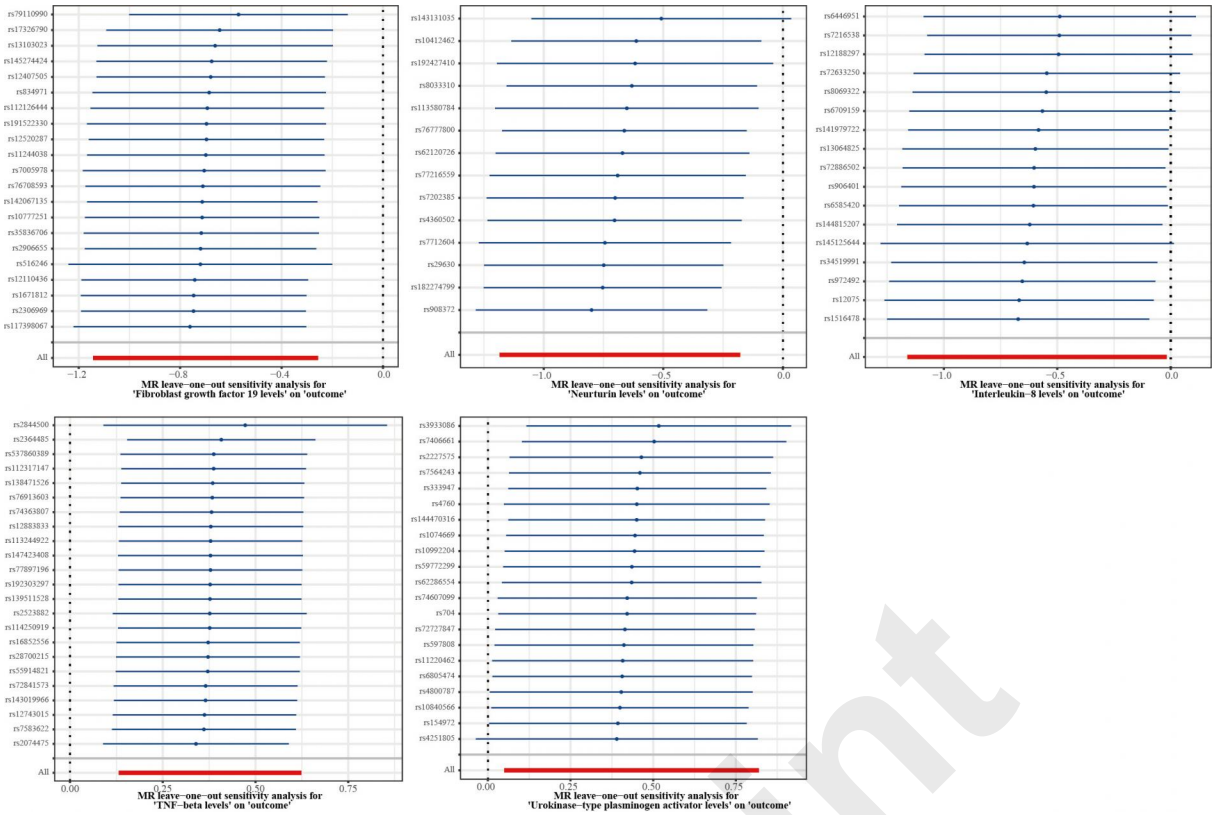

Figure 5 Forest chart: Stability test results using the "leave one method" Two Step Correlation Analysis of Inflammatory Cytokines Mediating ARDS through Plasma Metabolite Pathway.

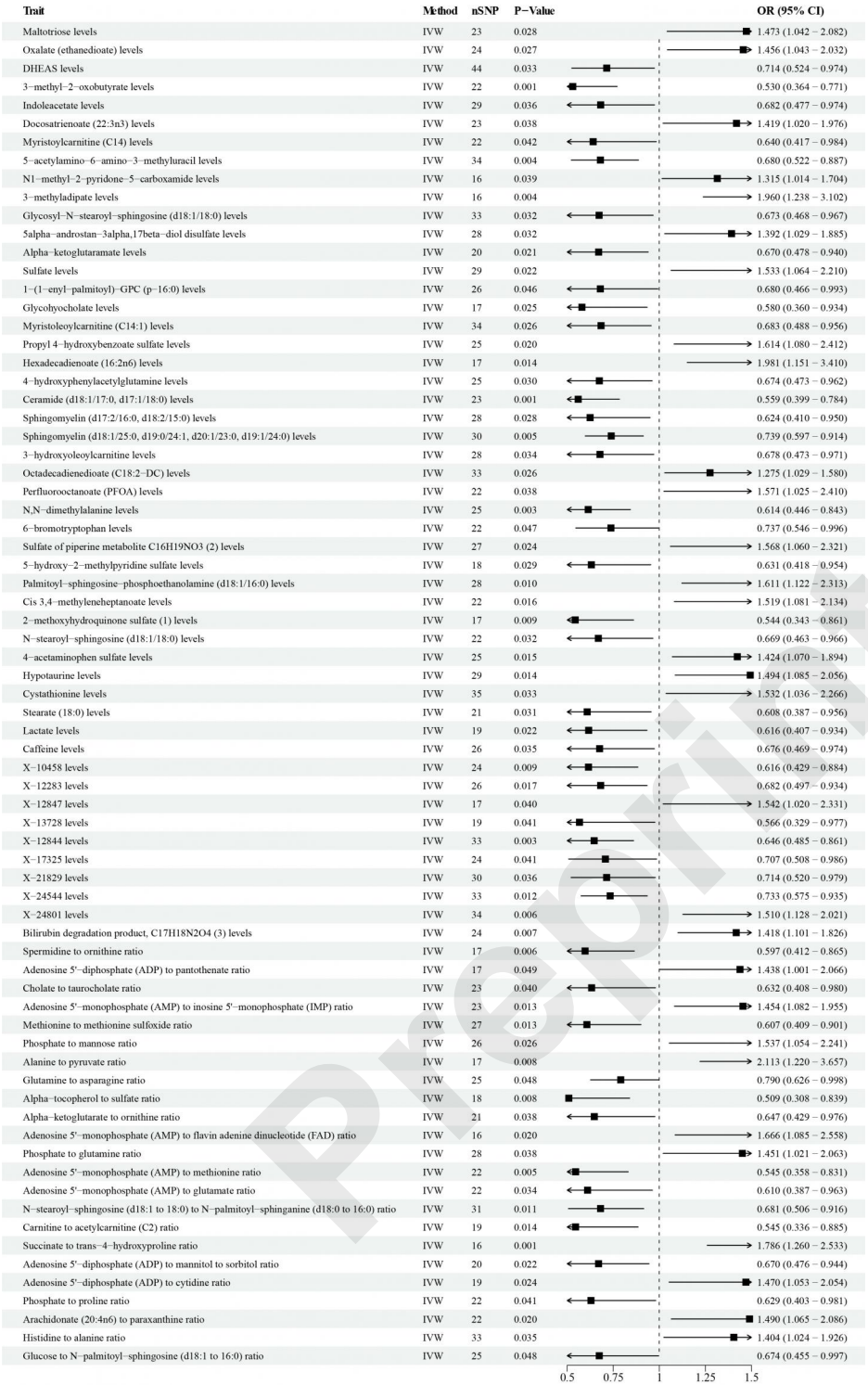

Figure 6 Causal relationship between plasma metabolites and ARDS.