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Keywords
Plasma Fatty acid, Pneumonia, Mendelian randomization, Biomarkers, Machine learning algorithms

Abstract

Introduction

Fatty acids mediate pulmonary inflammation through cytokine regulation, interactions with the tryptophan-kynurenine pathway, and mediators like 18-HEPE that boost interferon-λ. To systematically dissect these mechanisms and their translational implications, we pioneered a novel framework integrating bidirectional two-sample mendelian randomization (MR) with multi-algorithm machine learning.

Material and methods

We obtained Single nucleotide polymorphisms (SNPs) significantly associated with eight plasma fatty acids from genome-wide association study (GWAS) summary statistics and used them as instrumental variables in bidirectional two sample MR across six pneumonia phenotypes (Pneumonia, Asthma related pneumonia, Bacterial pneumonia, Critical pneumonia, Viral pneumonia, and Lobar pnemonia). Causal estimates were calculated using inverse variance weighting (IVW), which combines SNP-specific Wald ratios weighted by the inverse of their variance, with MR-Egger and weighted median approaches for sensitivity analysis. Transcriptomic data were then analyzed by Lasso regression, support vector machine recursive feature elimination and random forest to identify fatty acid metabolism–related biomarker candidates.

Results

MR analysis suggests potential causal associations between omega-6 fatty acids and critical pneumonia (OR:1.28, CI:1.01-1.61, P=0.038), Linoleic acids (LA) and bacterial pneumonia (OR:0.85, CI:0.73-0.99, P=0.047), and Docosahexaenoic fatty acids (DHA) and pneumonia (OR:0.83, CI:0.74-0.93, P=0.002). Moreover, ACAA1 and OLAH, which are genes involved in fatty acid metabolism, were identified as potential candidate biomarkers for pneumonia.

Conclusions

Our study employed MR analysis to establish a causal link between omega-6, LA and DHA with pneumonia. Additionally, through transcriptomic analysis, we identified plasma fatty acid metabolism-associated biomarkers that may serve as diagnostic indicators for pneumonia.

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Causality analysis of plasma fatty acids with pneumonia: identifying diagnostic biomarkers through transcriptome-wide association study

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Abstract

Background: Fatty acids mediate pulmonary inflammation through cytokine regulation, interactions with the tryptophan-kynurenine pathway, and mediators like 18-HEPE that boost interferon-λ. To systematically dissect these mechanisms and their translational implications, we pioneered a novel framework integrating bidirectional two-sample mendelian randomization (MR) with multi-algorithm machine learning. This approach not only quantifies causal relationships between fatty acids and inflammatory outcomes but also identifies clinically actionable diagnostic biomarkers.

Methods: We obtained Single nucleotide polymorphisms (SNPs) significantly associated with eight plasma fatty acids from genome-wide association study (GWAS) summary statistics and used them as instrumental variables in bidirectional two sample MR across six pneumonia phenotypes (Pneumonia, Asthma related pneumonia, Bacterial pneumonia, Critical pneumonia, Viral pneumonia, and Lobar pnemonia). Causal estimates were calculated using inverse variance weighting (IVW), which combines SNP-specific Wald ratios weighted by the inverse of their variance, with MR-Egger and weighted median approaches for sensitivity analysis. Transcriptomic data were then analyzed by Lasso regression, support vector machine recursive feature elimination and random forest to identify fatty acid metabolism–related biomarker candidates.

Results: MR analysis suggests potential causal associations between omega-6 fatty acids and critical pneumonia (OR:1.28, CI:1.01-1.61, P=0.038), Linoleic acids (LA) and bacterial pneumonia (OR:0.85, CI:0.73-0.99, P=0.047), and Docosahexaenoic fatty acids (DHA) and pneumonia (OR:0.83, CI:0.74-0.93, P=0.002). Moreover, ACAA1 and OLAH, which are genes involved in fatty acid metabolism, were identified as potential candidate biomarkers for pneumonia.

Conclusions: Our study employed MR analysis to establish a causal link between omega-6, LA and DHA with pneumonia. Additionally, through transcriptomic analysis, we identified plasma fatty acid metabolism-associated biomarkers that may serve as

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diagnostic indicators for pneumonia.

Keywords: Plasma Fatty acid, Pneumonia, Mendelian randomization, Biomarkers, Machine learning algorithms

Highlights

1. **Significant Associations Found:** MR analysis reveals potential causal associations between specific fatty acids and pneumonia subtypes.
2. **Machine-Learning for Biomarker Discovery:** Applies machine-learning algorithms to identify diagnostic biomarkers among differentially expressed genes.
3. **Novel Biomarker Candidates:** Identifies ACAA1 and OLAH, genes in fatty acid metabolism, as potential pneumonia biomarkers.

Table (1): List of Abbreviations

Abbreviation	Definition
MR	Mendelian randomization
GWAS	Genome-wide association study
SNP	Single nucleotide polymorphism
IVW	Inverse variance weighting
OR	Odds ratio
LA	Linoleic acids
DHA	Docosahexaenoic acid
PUFA	Polyunsaturated fatty acid
FA	Fatty acid
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acids
T2D	Type 2 diabetes
COPD	Chronic obstructive pulmonary disease
BMI	Body mass index
IEU	Integrative epidemiology unit
IV	Instrumental variable
MR-PRESSO	Mendelian randomization pleiotropy residual sum and outlier
FDR	False discovery rate
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
LASSO	Least absolute shrinkage and selection operator
SVM-RFE	Support vector machine recursive feature elimination
CIBERSORT	Cell-type identification by estimating relative subsets of

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	RNA transcripts
GSEA	Gene set enrichment analysis
ROC	Receiver operating characteristic
PCA	Principal component analysis
Treg	Regulatory T cell
NK	Neutral killer

Introduction

Pneumonia, a global health challenge driven by infectious pathogens, is aggravated by antibiotic resistance and chronic respiratory comorbidities [1]. This study focuses on pathogen-mediated mechanisms, distinguishing pneumonia from non-infectious pneumonitis caused by autoimmune disorders or chemical exposures [2]. Severe cases often progress to systemic complications, including coagulation abnormalities such as disseminated intravascular coagulation, distinct from coagulation necrosis [3]. These abnormalities synergize with cytokine storms, elevating cardiovascular mortality risk through endothelial dysfunction and myocardial suppression [4, 5]. Despite advances in therapy, pneumonia remains a leading global cause of morbidity and mortality, underscoring the urgency to elucidate pathogenic mechanisms and advance novel therapies [6].

Fatty acids are essential membrane phospholipids and bioactive mediators that regulate immune cell function and inflammatory signaling [7, 8]. Dysregulated lipid profiles, particularly imbalances in circulating polyunsaturated fatty acids (PUFAs), influence lung pathology through membrane-mediated immune cell dysfunction and the production of lipid signaling molecules [9]. This mechanistic duality is exemplified in chronic obstructive pulmonary disease, where skewed omega-3 to omega-6 PUFA ratios correlate with sustained airway inflammatio [10]. The interplay extends to acute infectious contexts, as observed in severe pneumonia patients exhibiting activation of the tryptophan-kynurenine axis [11]. During this process, indoleamine 2,3-dioxygenase catalyzes tryptophan conversion into immunomodulatory kynurenines that suppress T-cell activity, establishing a regulatory network with PUFA-derived inflammatory mediators, a phenomenon inversely associated with protective PUFA ratios [11-13].

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Experimental evidence underscores therapeutic opportunities, with murine models demonstrating omega-3 fatty acids attenuate bacterial pneumonia severity through coordinated cytokine modulation, reducing pro-inflammatory IL-6 while elevating anti-inflammatory IL-10 [14-16]. Parallel studies reveal butyrate-induced omega-3 metabolites exert antiviral effects via interferon-λ induction in viral pneumonia models [17]. Despite these mechanistic insights, translational challenges persist in distinguishing observational associations from causal relationships, particularly regarding pneumonia-specific lipid signatures and targeted therapeutic strategies.

To address these gaps, this study employed bidirectional two-sample Mendelian randomization (MR) to causally link eight plasma fatty acids to six distinct pneumonia phenotypes. The workflow of this study was illustrated in Fig. (1). Building on MR-derived causal evidence, we further applied three complementary machine-learning algorithms to transcriptomic data, identifying diagnostic biomarkers of fatty acid metabolism. This integrated methodology synergistically combined genetic causality analysis with multi-algorithm biomarker identification, establishing unified framework in pneumonia research that bridged mechanistic discovery and clinical translation.

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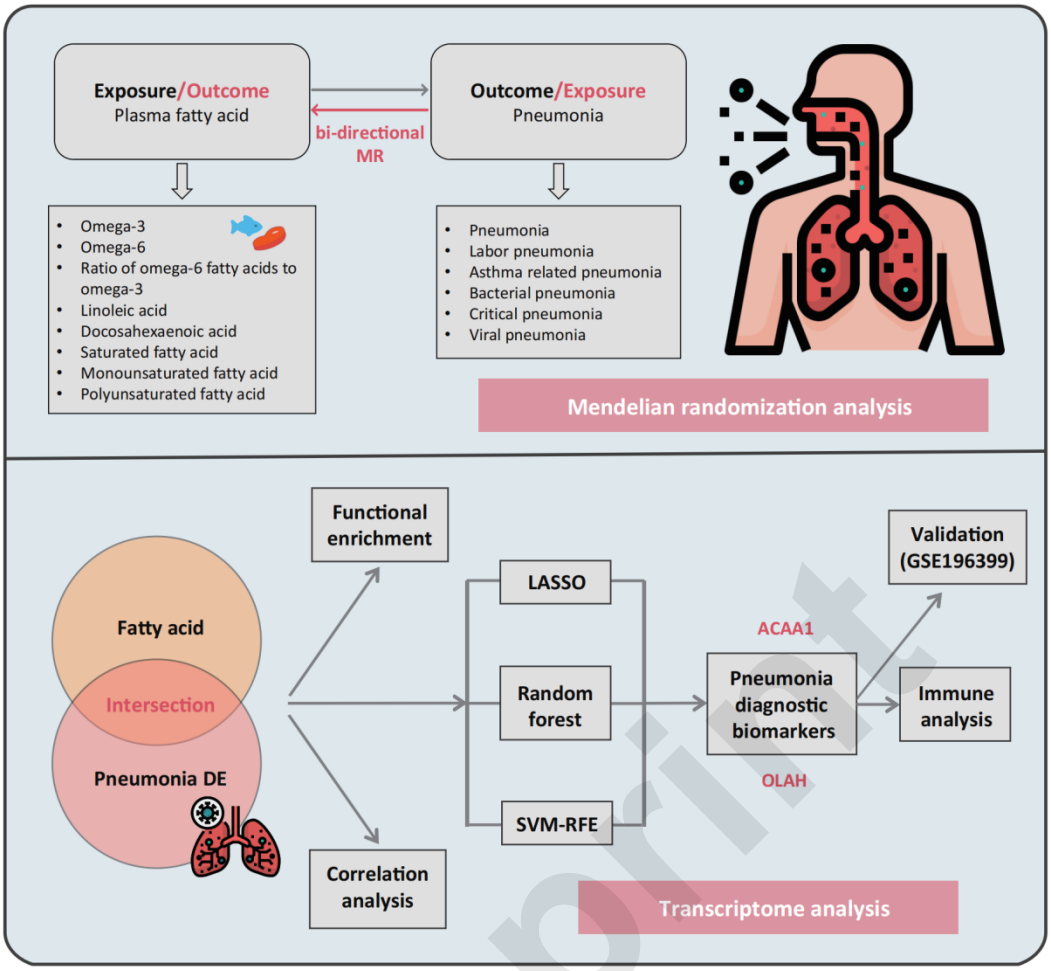

Fig. (1): Work flow of this study.

Material and methods

Study design

We first applied a two-sample MR framework to evaluate the causal effect of circulating plasma fatty acids on pneumonia risk. To address potential reverse causation, we then performed a reciprocal MR analysis [18], treating pneumonia liability as the exposure and plasma fatty acid levels as the outcome. In every MR analysis, we ensured that instrumental variables (IVs) satisfied the three core assumptions: strong association with the exposure, independence from confounders, and influence on the outcome exclusively through the exposure pathway [19]. To minimize bias from population stratification, all summary statistics were drawn from cohorts of European ancestry (Fig.

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2).

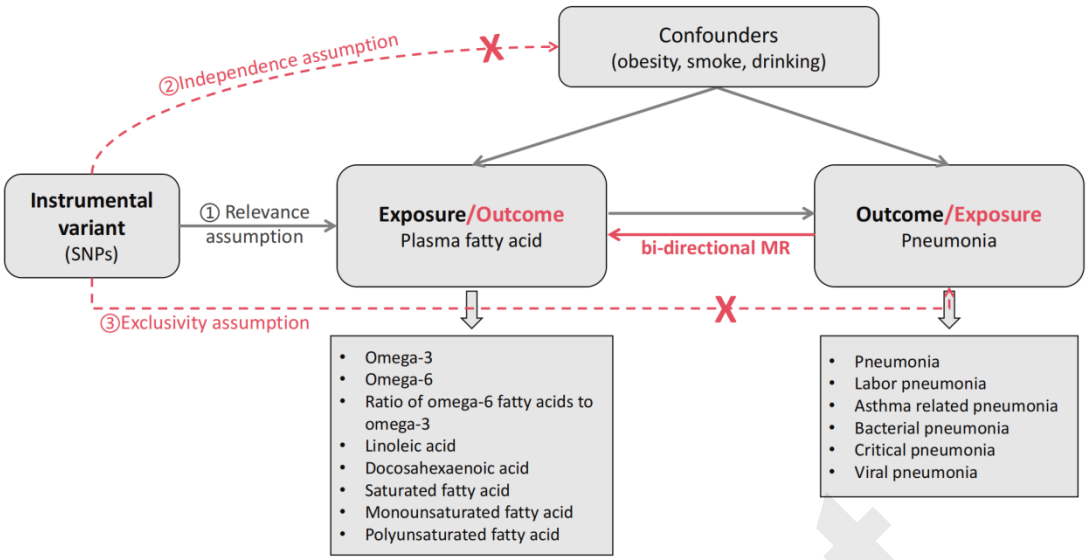

Fig. (2): Flow chart of the Mendelian randomization study design.

Data source

Genome-wide association study (GWAS) summary statistics for eight plasma fatty acid (FA) traits—omega-3, omega-6, omega-6/omega-3 ratio, linoleic acid (LA), docosahexaenoic acid (DHA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA—were obtained from the OpenGWAS platform. These data derive from 115,006 European ancestry participants in the UK Biobank. Pneumonia-related GWAS data were accessed via the IEU OpenGWAS repository and included overall pneumonia, severe pneumonia, lobar pneumonia, asthma-related pneumonia, viral pneumonia , and bacterial pneumonia. All summary statistics were drawn from cohorts of European ancestry, with detailed population descriptors provided in Table (2).

Table (2): Detailed information of the genome-wide association studies in our analysis.

Phenotype	PMID (ID)	Sample size (case/control)	Population

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Omega-3 fatty acid levels	35213538 (ebi-a-GCST90092931)	115006 /	European
Omega-6 fatty acid levels	35213538 (ebi-a-GCST90092933)	115006 /	European
Ratio of omega-6 fatty acids to omega-3 fatty acids	35213538 (ebi-a-GCST90092934)	115006 /	European
Linoleic acid levels	35213538 (ebi-a-GCST90092880)	115006 /	European
Docosahexaenoic acid levels	35213538 (ebi-a-GCST90092816)	115006 /	European
Saturated fatty acid levels	35213538 (ebi-a-GCST90092980)	115006 /	European
Monounsaturated fatty acid levels	35213538 (ebi-a-GCST90092928)	115006 /	European
Polyunsaturated fatty acid levels	35213538 (ebi-a-GCST90092939)	115006 /	European
Pneumonia	34594039 (ebi-a-GCST90018901)	480299 (16887/463412)	European
Asthma related pneumonia	/ (ukb-d-ASTHMA_PNEUMONIA)	361194 (5900/355294)	European
Bacterial pneumonia	/ (finn-b-J10_PNEUMOBACT)	196855 (7987/188868)	European
Critical pneumonia	/ (ieu-b-4978)	431365 (2758/428607)	European
Viral pneumonia	/ (finn-b-J10_VIRALPNEUMO)	189568 (700/188868)	European
Lobar pnemonia	/ (ukb-b-6576)	463010 (2359/460651)	European

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Instrumental variants selection

The criteria for selecting IVs were as follows: SNP Selection: SNPs were used as IVs, with a genome-wide significance threshold of $P < 5e-08$. However, for some pneumonitis-related traits, the number of SNPs meeting this threshold was limited. To increase the number of available SNPs, we relaxed the threshold to $P < 5e-06$, and for lobar pneumonia, a threshold of $P < 5e-05$ was applied. Clumping: SNPs were clumped to remove the effect of linkage disequilibrium ($r^2 < 0.001$, with a region length of 10,000 kb). F-Statistic: The F-statistic was used to assess the strength of IVs. SNPs with an F-statistic > 10 were retained, indicating strong instrument strength. Weak instruments were excluded. Allelic Consistency: SNPs associated with both exposure and outcome were aligned in terms of allelic direction. Palindromic SNPs and SNPs with inconsistent allelic directions were removed. MR-PRESSO Outliers: Outlier SNPs identified by the MR-PRESSO test were excluded from the analysis.

MR analysis

The primary causal estimates were obtained using the inverse variance weighted (IVW) method under a random effects model, supplemented by MR-Egger regression, weighted median, weighted mode, and simple mode analyses to assess robustness. Prior to MR, any SNPs associated with known confounders (BMI, waist measures, smoking, alcohol) were excluded. For reverse MR, the same procedure was followed with pneumonia as the exposure and individual fatty acid traits as outcomes.

Sensitivity Analysis

To assess the robustness of the identified associations, we conducted sensitivity analyses using three methods: heterogeneity test, horizontal pleiotropy test, and leave-one-out test. Cochran's Q test was used to assess SNP heterogeneity. If heterogeneity was detected ($P < 0.05$), a random-effects IVW model was applied. The presence of horizontal pleiotropy was evaluated using MR-Egger regression, with statistical significance of the intercept ($P < 0.05$) indicating pleiotropy. Additionally, MR-PRESSO was used to identify and remove outliers. A "leave-one-out" test was

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performed to evaluate the impact of individual SNPs on the causal associations by sequentially excluding each SNP.

Transcriptomic Data Acquisition and Differential Expression

We retrieved two gene expression datasets from the Gene Expression Omnibus (GEO): GSE40012 (training set: 16 pneumonia, 18 controls) and GSE196399 (validation set: 56 pneumonia, 21 controls). A curated list of 308 fatty acid metabolism-related genes was compiled from the MSigDB Hallmark, KEGG, and Reactome collections (version 2024.1). Differential expression between pneumonia and control samples was determined using the limma package in R, with thresholds of $|\log_2$ fold change$| > 1$ and adjusted $P < 0.05$ (Benjamini–Hochberg correction).

Functional enrichment Analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted in R with the clusterProfiler package. We controlled for multiple testing using the Benjamini-Hochberg method and considered pathways with adjusted $P < 0.05$ to be significantly enriched.

Feature Selection and Predictive Modeling

To identify key fatty acid-related genes predictive of pneumonia, we applied three machine learning approaches: least absolute shrinkage and selection operator (LASSO) regression for initial feature retention, support vector machine recursive feature elimination (SVM-RFE) with ten-fold cross-validation for iterative refinement, and random forest for ranking variable importance. The intersection of genes selected by all three methods constituted the final feature set used in model construction.

Immune infiltration analysis

Immune cell proportions were estimated using the CIBERSORT algorithm implemented in the IOBR R package, and single sample gene set enrichment analysis (ssGSEA) of immune-related functions was performed via the GSVA package.

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Differences between pneumonia and control groups were tested by Wilcoxon rank sum test with $P<0.05$ denoting significance.

Statistical Analysis

All analyses were executed in R version 4.4.1, utilizing the TwoSampleMR (0.6.8) [20], MRPRESSO (1.0) [21], limma [22], clusterProfiler [23] and IOBR [24] packages. Statistical significance was defined as two-sided $P < 0.05$ throughout.

Results

Causal relationship between fatty acids and pneumonia

Following rigorous selection and harmonization of genetic IVs, along with MR-PRESSO-mediated removal of pleiotropic outliers, we employed IVW MR to estimate causal effects of eight plasma fatty acids on pneumonia risk. We observed that higher omega-6 levels were associated with increased risk of severe pneumonia (OR=1.28, 95% CI: 1.01-1.61, P=0.038), whereas greater LA levels conferred protection against bacterial pneumonia (OR=0.85, 95% CI: 0.73-0.99, P=0.047). Similarly, elevated DHA was inversely related to overall pneumonia incidence (OR=0.83, 95% CI: 0.74-0.93, P=0.002). No statistically significant causal links were detected for the remaining fatty acids (Fig. 3). Consistent direction and magnitude across MR-Egger, weighted median, weighted and simple mode analyses reinforced these findings (Supplementary Table 1), and the complete lists of SNP instruments appear in Supplementary Tables (2-7) with corresponding scatter and forest plots in Supplementary Figures (1-2).

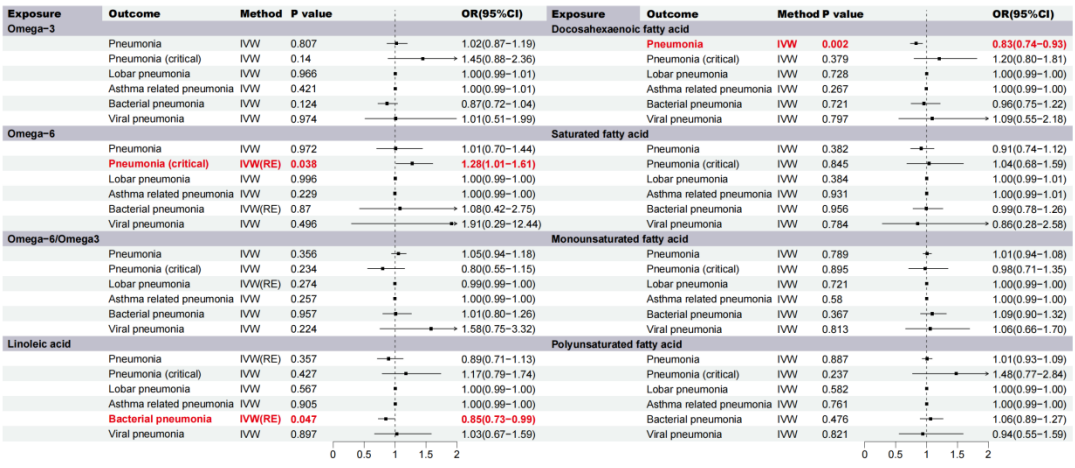

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Fig. (3): Univariable Mendelian randomization results of Plasma fatty acid on Pneumonia.

Sensitivity analysis

To verify robustness, we assessed heterogeneity and pleiotropy across instruments. For DHA, Cochran's Q test indicated no heterogeneity (P>0.05), whereas omega-6 and LA instruments displayed modest heterogeneity (P<0.05) but no evidence of horizontal pleiotropy by MR-Egger intercept (Table 3). All instruments demonstrated adequate strength (F>30; Supplementary Tables 8-10). Applying a random effects IVW model for heterogeneous traits did not materially alter effect estimates (P<0.05), and MR-PRESSO detected no additional outliers. Symmetrical funnel plots (Supplementary Figure 3) and stable leave-one-out analyses (Supplementary Figure 4) further support the reliability of these causal inferences (Supplementary Table 11).

Table (3): Sensitivity analysis of causal relationship between plasma fatty acid and pneumonia subtypes.

Exposure	Outcome	Heterogeneity				Pleiotropy
		MR-Egger Statistics Q	MR-Egger P-Value	IVW Statistics Q	IVW P-Value	P-Value
Docosahexaenoic acid levels	Pneumonia	11.74	0.698	14.78	0.541	0.102
Omega-6 fatty acid levels	Critical pneumonia	62.52	0.022	63.00	0.025	0.573
Linoleic acid levels	Bacterial pneumonia	48.62	0.030	49.33	0.034	0.499

Reverse causality between fatty acids and pneumonia

In reverse MR, we tested whether genetic liability to pneumonia subtypes influences fatty acid levels. Notably, higher genetic risk of viral pneumonia was causally linked to marginally lower omega-3 levels (OR=0.98, 95% CI: 0.97-0.99, P=0.006) and to an elevated omega-6/omega-3 ratio (OR=1.02, 95% CI: 1.01-1.04, P=0.005) (Fig. 4). A random effects IVW framework accounted for heterogeneity (P<0.05), and sensitivity plots (Supplementary Figures. 5-8) confirmed absence of bias or influential SNPs.

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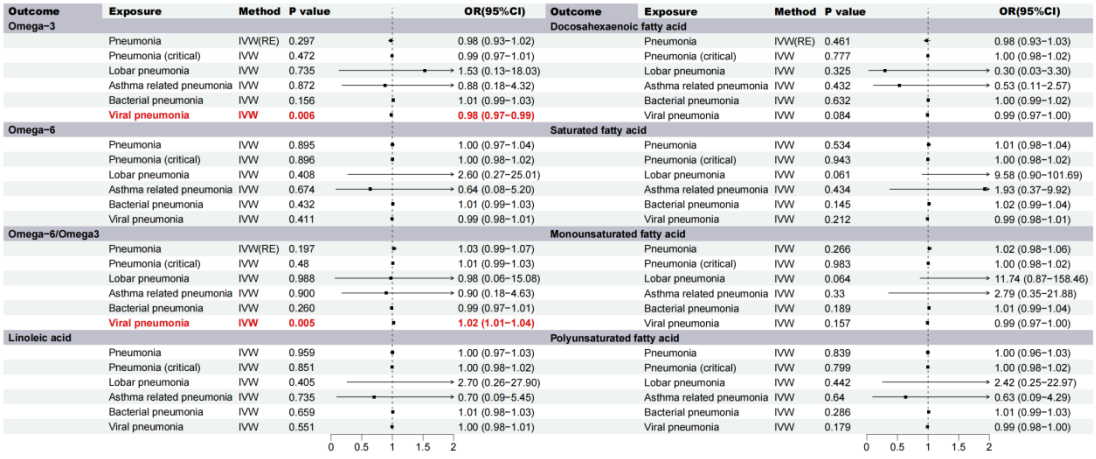

Fig. (4): Reverse Mendelian randomization estimates of Pneumonia on Plasma fatty acid.

Identification of Differential Genes Associated with Fatty Acid Metabolism

Analyzing GSE40012, we identified 549 genes differentially expressed between pneumonia patients and controls ($|\log_2 FC|>1$, $P<0.05$), including 251 up-regulated and 298 down-regulated (Fig. 5A). Intersection with our curated set of 308 fatty acid metabolism genes yielded 16 overlapping targets (Fig. 5B). Correlation analysis revealed strong positive co-expression among most of these 16 genes (Fig. 5C). GO enrichment highlighted their roles in fatty acid and unsaturated fatty acid metabolism, carboxylic acid biosynthesis, and localization to peroxisomes, outer mitochondrial membranes, and rough endoplasmic reticulum (Fig. 5D-F).

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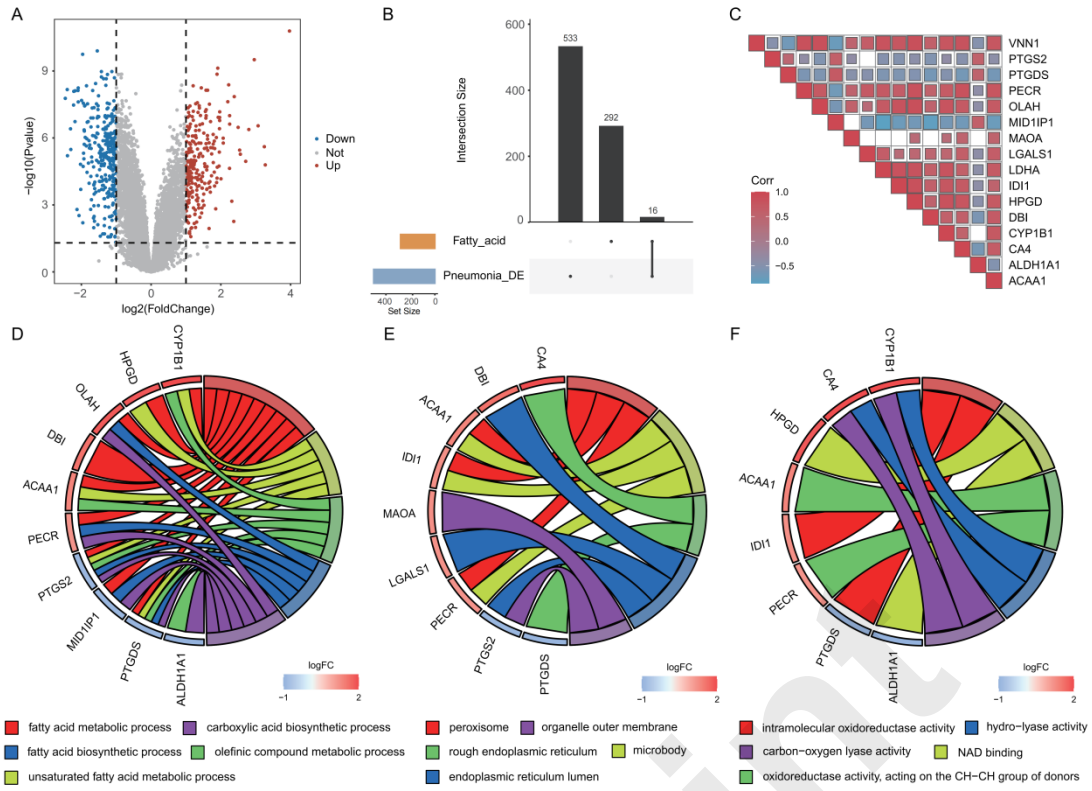

Fig. (5): Differential Gene Expression and Functional Enrichment Analysis of Fatty Acid Metabolism-Related Genes in Pneumonia. (A) Volcano plot depicting differential gene expression in pneumonia. (B) Intersection of fatty acid metabolism-related genes and pneumonia differential genes. (C) Correlation heatmap of differential genes associated with fatty acid metabolism. (D-F) GO enrichment map of 16 fatty acid metabolism differential genes enriched in BP, CC and MF.

BP: Biological Process, CC: Cellular Component, CC: Cellular Component

Identification of key fatty acid metabolism genes in pneumonia

In order to exclude unimportant genes and identify key genes associated with pneumonia, we employed three machine learning algorithms for gene selection. Starting with 16 fatty acid metabolism-related differential genes, we applied Lasso regression, which selected 9 genes (Fig. 6A-B), SVM-RFE selected 5 genes (Fig. 6C), and the random forest algorithm identified 7 genes (Fig. 6D-E). Only ACAA1 and OLAH were consistently selected by all three methods (Fig. 6F). In the training cohort (GSE40012), ROC AUCs were 0.969 for ACAA1 and 0.979 for OLAH (Fig. 6G); in

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validation (GSE196399), AUCs were 0.781 and 0.965, respectively (Fig. 6H). PCA based on these two genes demonstrated clear separation of cases versus controls (Fig. 6I), and expression differences were highly significant in both datasets (Fig. 6J-K).

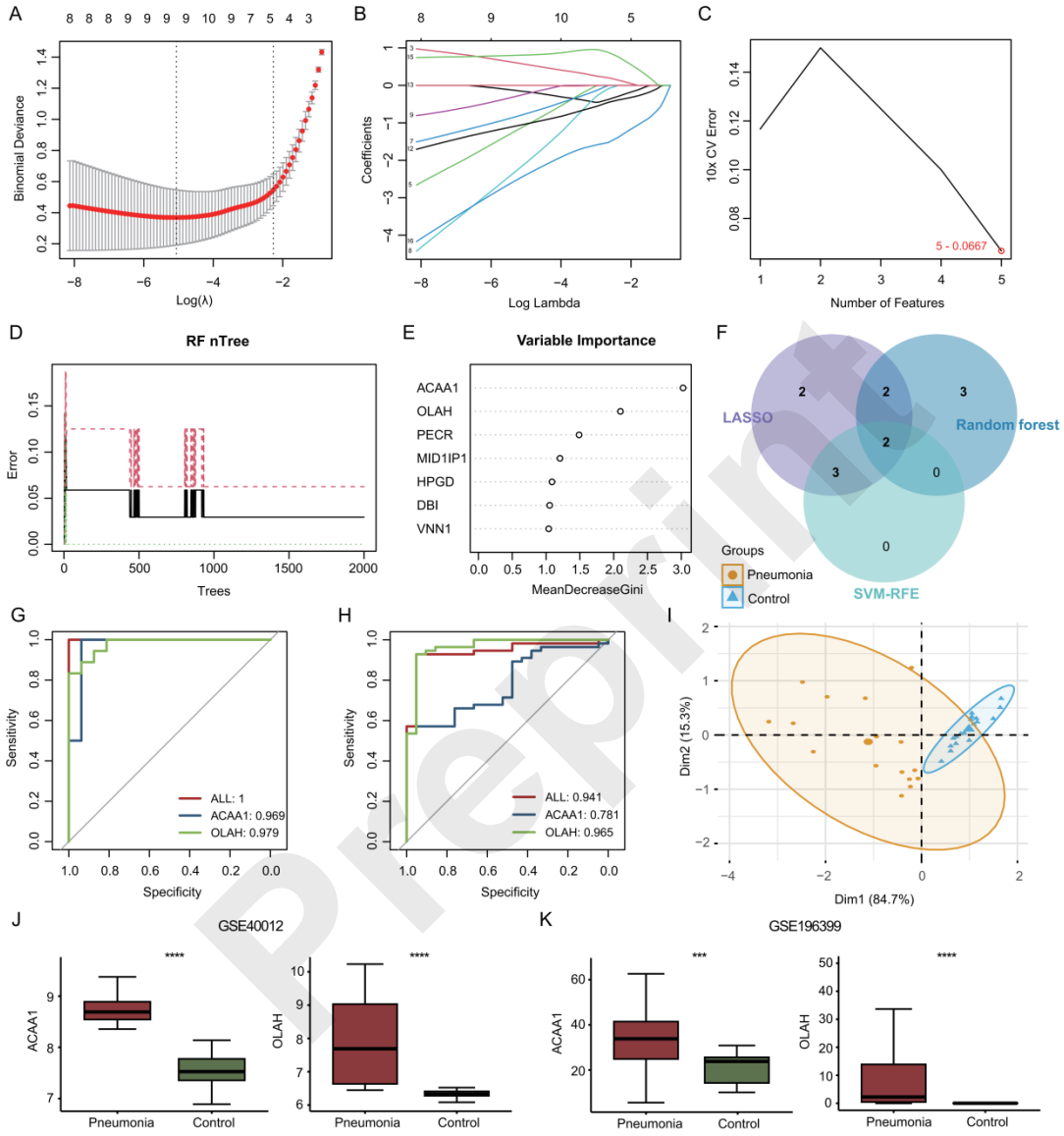

Fig. (6): Identification and validation of pneumonia diagnostic markers. (A) Lasso regression and (B) 10 fold cross validation diagram. (C) Error rate curve based on SVM-RFE algorithm with 10 fold cross validation. (D) The relationship between random forest error rate and the number of classification trees and (E) gene importance. (F) Genes shared by three machine learning models. (G) ROC curves of each signature gene in the training set (GSE40012) and (H) validation set (GSE196399) of the

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diagnostic markers. (I) PCA plot of training set (GSE40012). (J) Expression of signature genes in the training set (GSE40012) and (K) validation set (GSE196399) in pneumonia and normal samples.

Immune Cell Infiltration and Immune-Related Function in Pneumonia

To characterize immune microenvironment alterations in pneumonia, we first applied CIBERSORT to quantify immune cell proportions in pneumonia versus healthy lung tissues (Fig. 7A-B). Compared to controls, pneumonia tissues showed significant increases in neutrophils, monocytes, M0 macrophages, and gamma-delta T cells, accompanied by reduced regulatory T cells (Tregs), resting NK cells, and CD8+ T cells. Next, to explore broader functional implications, we performed single-sample gene set enrichment analysis (ssGSEA), which revealed distinct immune-related functional signatures and infiltration patterns between pneumonia patients and controls (Fig. 7C). Notably, ssGSEA further demonstrated that elevated expression of ACAA1 and OLAH correlated positively with macrophage infiltration but inversely with cytotoxic immune functions (Fig. 7D-E), suggesting these fatty acid metabolism-related genes may drive macrophage polarization while suppressing adaptive immunity during pneumonia pathogenesis.

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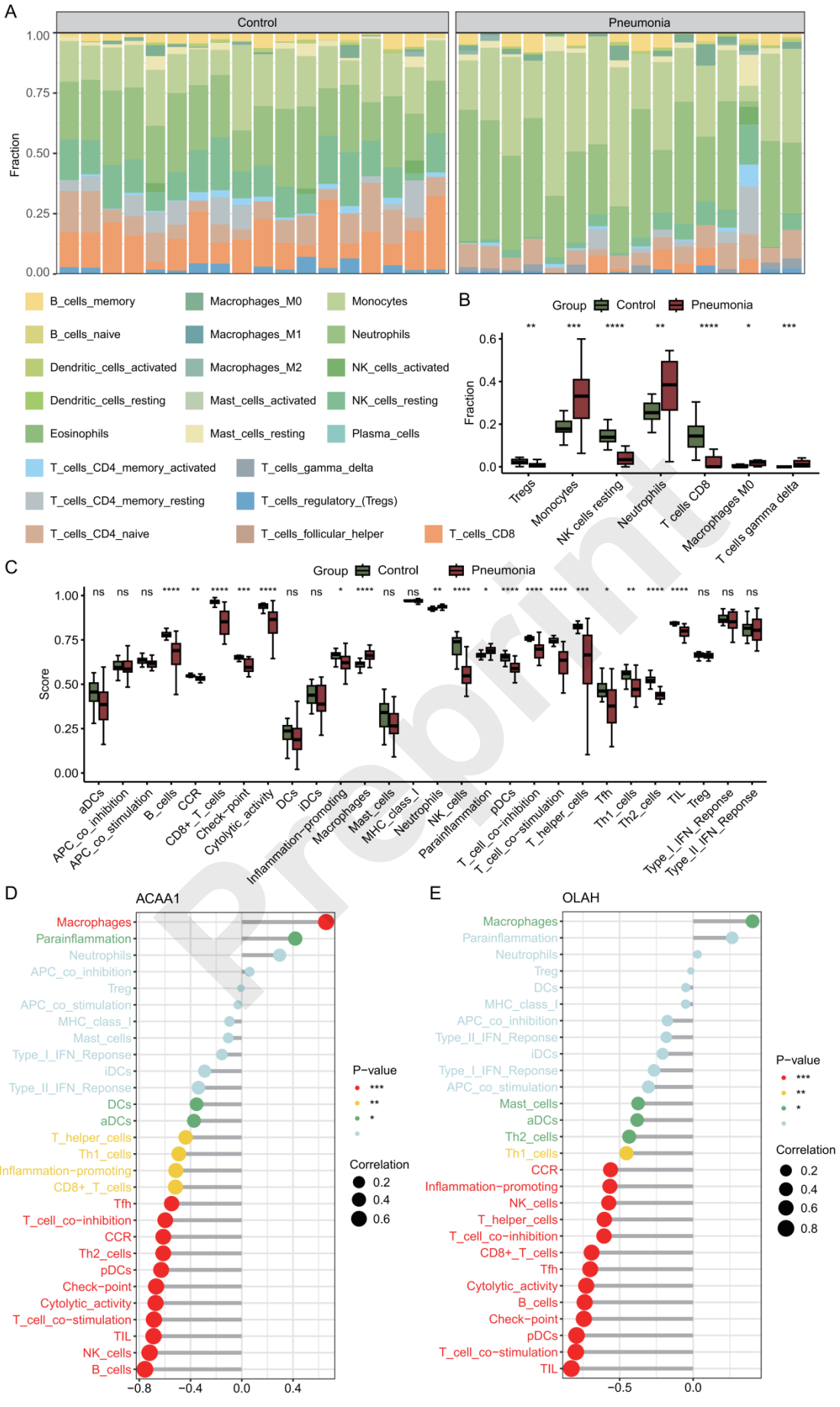
A
Control
Pneumonia
Fraction
1.00
0.75
0.50
0.25
0.00
B_cells_memory
B_cells_naive
Dendritic_cells_activated
Dendritic_cells_resting
Eosinophils
Macrophages_M0
Macrophages_M1
Macrophages_M2
Mast_cells_activated
Mast_cells_resting
Monocytes
Neutrophils
NK_cells_activated
NK_cells_resting
Plasma_cells
T_cells_CD4_memory_activated
T_cells_CD4_memory_resting
T_cells_CD4_naive
T_cells_gamma_delta
T_cells_regulatory_(Tregs)
T_cells_follicular_helper
T_cells_CD8
B
Group Control Pneumonia
Fraction
Tregs
Monocytes
NK cells resting
Neutrophils
T cells CD8
Macrophages M0
T cells gamma delta
C
Group Control Pneumonia
Score
aDCs
APC_co_inhibition
APC_co_stimulation
B_cells
CCR
CD8+_T_cells
Check-point
Cytolytic_activity
DCs
iDCs
Inflammation-promoting
Macrophages
Mast_cells
MHC_class_I
Neutrophils
NK_cells
Parainflammation
pDCs
T_cell_co-inhibition
T_cell_co-stimulation
T_helper_cells
Tfh
Th1_cells
Th2_cells
TIL
Treg
Type_I_IFN_Reponse
Type_II_IFN_Reponse
D
ACAA1
Macrophages
Parainflammation
Neutrophils
APC_co_inhibition
Treg
APC_co_stimulation
MHC_class_I
Mast_cells
Type_I_IFN_Reponse
iDCs
Type_II_IFN_Reponse
DCs
aDCs
T_helper_cells
Th1_cells
Inflammation-promoting
CD8+_T_cells
Tfh
T_cell_co-inhibition
CCR
Th2_cells
pDCs
Check-point
Cytolytic_activity
T_cell_co-stimulation
TIL
NK_cells
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OLAH
Macrophages
Parainflammation
Neutrophils
Treg
DCs
MHC_class_I
APC_co_inhibition
Type_II_IFN_Reponse
iDCs
Type_I_IFN_Reponse
APC_co_stimulation
Mast_cells
aDCs
Th2_cells
Th1_cells
CCR
Inflammation-promoting
NK_cells
T_helper_cells
T_cell_co-inhibition
CD8+_T_cells
Tfh
Cytolytic_activity
B_cells
Check-point
pDCs
T_cell_co-stimulation
TIL
P-value
Correlation

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Fig. (7): Analysis of immune cell infiltration in patients with pneumonia and normal controls. (A) Distribution and (B) differences in levels of immune cell infiltration between normal samples and pneumonia patients. (C) Differences in immune cell infiltration between normal and pneumonia patients using ssGSEA. (D) Association between the ACAA1, (E) OLAH and immune cell infiltration levels. The circle size indicates the correlation strength, with larger circles representing stronger correlations. The color reflects the p-value significance: red for $p < 0.001$, yellow for $p < 0.01$, and green for $p < 0.05$.

Discussion

In this study, we investigated the causal relationships between plasma fatty acids and pneumonia, with a specific emphasis on identifying key genes involved in fatty acid metabolism and their potential roles in disease pathogenesis. Our MR analysis revealed that LA and DHA are causally associated with a reduced risk of pneumonia, suggesting their protective effects. These findings support and extend earlier observational studies, which have reported anti-inflammatory and immunomodulatory effects of omega-3 and omega-6 fatty acids in various inflammatory conditions, including pneumonia [25, 26]. For instance, a large prospective cohort study demonstrated that every 1-gram increase in LA intake was associated with a 4% reduction in pneumonia risk [27], which is consistent with our MR-derived protective estimate for LA.

Our study further confirms the anti-inflammatory role of omega-3 fatty acids, particularly DHA, which are known to reduce the severity of inflammatory diseases by modulating immune responses [14]. While previous observational studies have suggested a beneficial association, our MR analysis strengthens the causal interpretation, overcoming potential confounding and reverse causation. On the other hand, omega-6 fatty acids are generally considered pro-inflammatory and have been associated with adverse outcomes in certain pneumonia subtypes [28, 29]. Our results support this view, as genetically predicted higher omega-6 levels were associated with

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increased risk of severe pneumonia, thereby aligning with earlier evidence and reinforcing the dualistic role of fatty acids in inflammation.

In animal models, omega-3 supplementation has been shown to mitigate pneumonia severity by suppressing microglial activation and inflammatory cytokine release through HMGB1 and TLR4/NF-κB signaling pathways [14, 30]. Furthermore, omega-3 and its derivatives have been shown to alleviate intestinal inflammation and modulate systemic immune responses in diseases such as ulcerative colitis [31, 32]. Short-chain fatty acids, another important class of fatty acids, exert anti-inflammatory effects by lowering colonic pH and limiting the growth of pathogenic bacteria [33]. These consistent findings across different disease models underscore the importance of fatty acid metabolism in modulating immune function and inflammatory responses.

We further explored potential molecular mediators linking fatty acids to pneumonia by applying machine learning algorithms. Through this approach, we identified ACAA1 and OLAH as key genes involved in fatty acid metabolism. ACAA1 plays a crucial role in mitochondrial β-oxidation, while OLAH is involved in fatty acid biosynthesis and homeostasis [34, 35]. Previous studies have linked ACAA1 variants with asthma and other inflammatory airway diseases, suggesting its involvement in immune regulation [36]. The identification of these genes complements and expands previous research by offering specific molecular targets through which fatty acids may exert their protective effects in pneumonia.

Infiltration analysis of immune cells revealed significant differences between pneumonia patients and healthy individuals. In line with previous reports, healthy controls exhibited higher infiltration of Tregs, resting NK cells, and $CD8^+$ T cells, whereas pneumonia patients showed increased infiltration of neutrophils, monocytes, M0 macrophages, and gamma-delta T cells. These findings are consistent with the established role of neutrophils and macrophages in pneumonia pathogenesis [37, 38]. In Streptococcus pneumoniae-induced pneumonia, for example, neutrophil accumulation is a hallmark of the early immune response, contributing to bacterial clearance but also to lung tissue damage and systemic spread [39, 40]. Macrophages act as first responders to pathogens and contribute to both host defense and

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inflammation by releasing cytokines and orchestrating immune responses [41-44]. The elevated levels of gamma-delta T cells in pneumonia samples also suggest a role in early immune activation, consistent with their known function in bridging innate and adaptive immunity [45].

In addition to lipid mediators, various acute-phase plasma proteins, including C-reactive protein, procalcitonin, surfactant proteins, and complement components, are key players in the pathophysiology of pneumonia [46-49]. These proteins are closely correlated with disease severity and outcomes [50]. Notably, their interplay with fatty-acid-driven cytokine modulation implies that integrating lipid and protein biomarkers into combined panels could significantly enhance the accuracy of pneumonia diagnosis and improve prognostication[51, 52].

While our MR framework reduces confounding and reverse causation, it is limited by reliance on peripheral blood-derived GWAS and transcriptomic data, which lack direct validation in lung tissue. Future studies should combine clinical phenotyping with analyses of lung biopsy or bronchoalveolar lavage specimens to validate these findings. Additionally, functional studies of ACAA1 and OLAH in animal models will be critical to translate these genetic insights into targeted therapies.

Conclusion

In summary, our bidirectional MR study establishes that genetically higher levels of LA and DHA reduce pneumonia risk, while elevated omega-6 increases susceptibility to severe disease. Integrating machine learning driven transcriptomic analysis, we pinpoint ACAA1 and OLAH as novel biomarkers linking fatty acid metabolism to pneumonia pathogenesis. Immune cell deconvolution further reveals a shift toward neutrophil and macrophage driven inflammation alongside reduced regulatory and cytotoxic T-cell subsets in patients. Together, these findings deepen our understanding of lipid-immune crosstalk in pneumonia and highlight promising targets for future diagnostic and therapeutic strategies.

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Supplementary Tables 1-3: Univariable MR estimates for plasma fatty acids across pneumonia phenotypes

Supplementary Tables 4-6: Genetic instruments used in MR analyses

Supplementary Table 7: Heterogeneity and pleiotropy test results (Cochran's Q, MR-Egger intercept)

Supplementary Tables 8-9: Instrument strength (F-statistics) and outlier removal details

Supplementary Table 10: Scatter plots and forest plots summary for key SNPs

Supplementary Table 11: Leave-one-out sensitivity analyses

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Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no potential conflicts of interest.

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None.

Authors' contributions

XBC, XYW, PAM and ZJG contributed to the study design. XBC conducted the literature search. PAM and ZJG acquired the data. XBC wrote the article and performed data analysis. XMW revised the article and gave the final approval of the version to be submitted. All authors read and approved the final manuscript.

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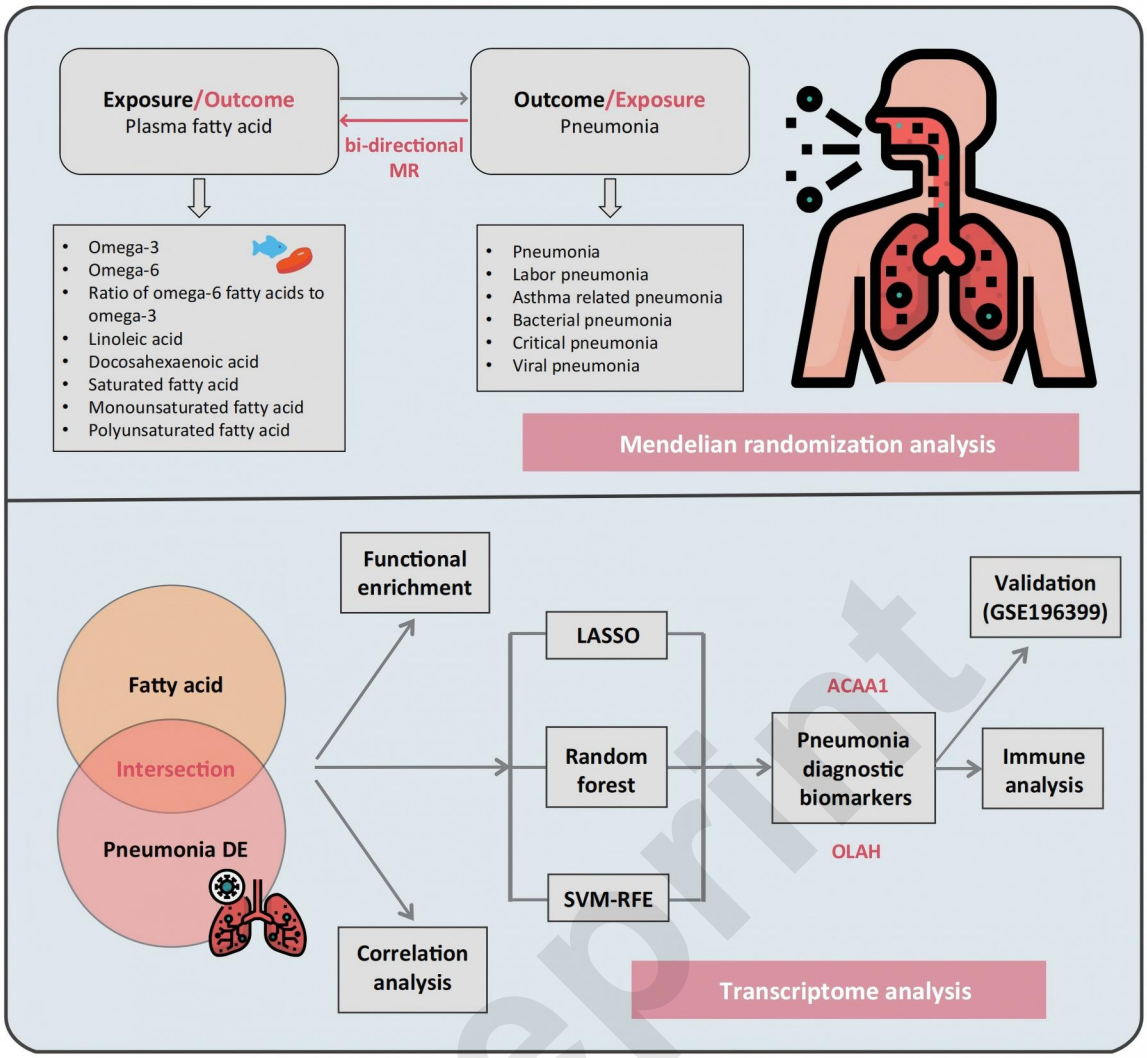
Exposure/Outcome
Plasma fatty acid
bi-directional MR
Outcome/Exposure
Pneumonia
Omega-3
Omega-6
Ratio of omega-6 fatty acids to omega-3
Linoleic acid
Docosahexaenoic acid
Saturated fatty acid
Monounsaturated fatty acid
Polyunsaturated fatty acid
Pneumonia
Labor pneumonia
Asthma related pneumonia
Bacterial pneumonia
Critical pneumonia
Viral pneumonia
Mendelian randomization analysis
Fatty acid
Intersection
Pneumonia DE
Functional enrichment
Correlation analysis
LASSO
Random forest
SVM-RFE
ACAA1
Pneumonia diagnostic biomarkers
OLAH
Validation (GSE196399)
Immune analysis
Transcriptome analysis

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