Novel Lipidomes Profile and Clinical Phenotype Identified in Pneumoconiosis Patients

Xiaoyang Chen (✉ 1073356693@qq.com)

Second Affiliated Hospital of Fujian Medical University <https://orcid.org/0000-0002-2533-1928>

Liyong Shi

Second Affiliated Hospital of Fujian Medical University

Xiaofang Dai

Second Affiliated Hospital of Fujian Medical University

Furong Yan

Second Affiliated Hospital of Fujian Medical University

Yujun Lin

Second Affiliated Hospital of Fujian Medical University

Lianshun Lin

Second Affiliated Hospital of Fujian Medical University

Yongquan Zhang

Second Affiliated Hospital of Fujian Medical University

Yiming Zeng

Second Affiliated Hospital of Fujian Medical University

Research article

Keywords: pneumoconiosis, lipidomics, phenomes, trans-omics

Posted Date: January 18th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-814567/v2>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Pneumoconiosis is a group of occupational lung diseases caused by inhalation of particles of coal, asbestos, silica, or similar substances, leading to fibrosis and loss of lung function. It is the most serious and common occupational disease in the world with an increased incidence but poor prognosis. To further investigate the regulatory mechanisms of lipidomic profiles in pneumoconiosis, we measured plasma lipidomic profiles between pneumoconiosis patients and health. Compared with healthy people, the circulating levels of phosphatidylethanolamine (PE) significantly increased while phosphatidylcholines (PC) and lysophosphatidylcholines (lyso PC) decreased in patients with pneumoconiosis. Clinical trans-omics analyses demonstrated that phenomes in pneumoconiosis was closely correlated with multiple lipids. Our data suggested that trans-omic analysis between clinical phenomes and lipidomes might have the potential to uncover the heterogeneity of lipid metabolism of pneumoconiosis patients and to screen out clinically significant phenome-based lipid panels with potential diagnostic application.

1 Introduction

Pneumoconiosis refers to a series of pulmonary diseases caused by inhalation of mineral dust, usually found in certain occupations. Being widespread globally, it poses life-threatening risk to public health worldwide. China has the largest working population in the world and in parallel with this, there were more than 975000 cases of occupational illness occurred in 2018, and 90% of that were pneumoconiosis^[1]. Currently, pneumoconiosis diagnosis mainly depends on reliable dust exposure history and chest X-rays^[2], however, both methods have low sensitivity and specificity. The high incidence and mortality are related to improper occupational protection, and lack of early diagnostic methods and effective treatments. The main pathological features of pneumoconiosis include chronic pulmonary inflammation and progressive pulmonary fibrosis, which can lead to respiratory and/or heart failure^[3]. The pathogenesis of pneumoconiosis remains unknown. We observed the majority of pneumoconiosis patients have dyslipidemia characterized by decreased levels of serum cholesterol (CHO), triglyceride (TAG), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol.

Lipids are the fundamental components of biological membranes as well as the metabolites of organisms, which play critical roles in cellular energy storage, structure, and signaling^[4, 5], mainly including subclasses of phosphatidic acid (PA), phosphatidylcholines (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidylserine (PS). Lipidomics has been accepted as a large scale lipid-related research methodology in numerous human lifestyle-related diseases, such as atherosclerosis^[6], diabetes^[7], Alzheimer's disease^[8], lung transplantation^[9] and cancer^[10].

Recent progress in lipidomic technology uncovered detailed lipid profiles and revealed that lipids were involved in the pathophysiology of human respiratory diseases. Studies have shown that sterols, including cholesterol, steroid hormones and bile acids, play important roles on different lung diseases,

such as acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), asthma and so on. Karagiorga et al. ^[11, 12] found that cholesterol in pulmonary surfactant of ARDS patients was significantly increased (up to 65%) which inhibits the reversible conversion between monolayer and bilayer of alveolar surfactant, resulting in the dysfunction of alveolar surfactant. Many studies of asthma have identified common lipid biomarkers of this disease mainly including phingomyelin, glycerophospholipids, ceramide, long-chain unsaturated fatty acids, phosphatidylcholine^[13–15]. In terms of chronic obstructive disease, studies have shown that decreased high-density lipoprotein Apo A β and Apo A α as well as increased serum amyloid protein (SA) levels are important indicators for abnormal lipid metabolism in acute exacerbation of COPD ^[16].

Therefore, lipids play important roles in lung diseases. As an important branch of metabolomics, lipidomics is a delicate study of all lipids aiming at comprehensive analysis of lipids in the biological system. The application of lipidomics in lung diseases provides the opportunity for gaining novel insights into biochemical mechanism. Clinical trans-omics is defined as a new subject to integrate clinical phenomes with multi-omics in multiple dimensions^[17] and becomes a novel approach for the discovery of disease-specific biomarkers and therapeutic targets^[18, 19].

In this study, we showed the unique research on pneumoconiosis regarding lipidomics, which preliminarily revealed the differences of lipidomic profiles between patients of pneumoconiosis and healthy people. In addition, using clinical trans-omics, we initially reveal a close correlation between clinical phenome and specific groups of lipid elements. Our research may shed light on a potential diagnostic approach of pneumoconiosis.

2 Methods And Materials

2.1 Chemical agents

The internal standard mix were subscribed from Internal Standards Kit for Lipidyzer™ Platform (SCIEX, MA, USA). The acetonitrile, water, isopropanol, ammonium acetate and ammonium hydroxide were HPLC grade and obtained from Sigma-Aldrich (St. Louis, MO, USA)^[20].

2.2 Patient population

The study, designed as a case-control approach, was approved by the Ethical Evaluation Committee of 2nd Affiliated Hospital of Fujian Medical University (ethical code B2020-293). The subjects were provided with informed consent for clinical data collection and lipids analysis before all the other procedures. The study included 48 pneumoconiosis patients diagnosed by mineral dust exposure history and chest imaging or pathology, and 48 other healthy people. 48 patients were recruited during September 2019 to September 2020. Participants of healthy controls were blood donors from the 2nd Affiliated Hospital of Fujian Medical University. Subjects with other Lipid metabolism disorders such as hyperthyroidism, diabetes mellitus, liver or kidney diseases and communicable disease such as active tuberculosis were

excluded. Fasting blood was drawn from healthy controls and pneumoconiosis patients on the day of entering hospital to harvest plasma. All the clinical data, including symptoms, signs, laboratory tests, images, pathologic information were collected and followed up.

2.3 Clinical phenotype

A total of 15 clinical phenomes were collected an pneumoconiosis group, including BMI, history of dust exposure, smoking and pneumothorax, mediastinal lymph node calcification, lung function, FEV₁, FEV1%, pH, PCO₂, PO₂, respiratory failure, restrictive ventilation dysfunction, complications and modified British medical research council (mMRC) questionnaire.

2.4 Lipid extraction for mass spectrometry analysis

The plasma samples (20 µL each) were precipitated by addition of isopropanol (400 µL each) precooled to 4°C and then mixed with lipid internal standard mix. Samples were vortex mixed for 1 min. After 10 min of incubation at room temperature, samples were stored overnight at -20°C to improve protein precipitation. Next day, the sample was centrifuged at 14 000g for 15 min. The supernatant was collected (200 µL) in a sample injection bottle and stored at -80°C awaiting MS analysis^[20].

2.5 Identification of lipidomic profiles

In the study, lipid profiling was detected by AB SCIEX QTRAP 4500 LC-MS/MS system. The extracted samples were injected onto a Waters Acquity UPLC BEH HILIC column (100 mm × 2.1 mm, 1.7 µm), which was fitted with a Waters Acquity UPLC BEH HILIC VanGuard Pre column(2.1mm×5mm, 1.7um,) at 35°C and then eluted. A phase was 95% acetonitrile containing with 10 mmol/L ammonium acetate, and B phase was 50% acetonitrile solution containing with 10 mmol/L ammonium acetate. The ammonium hydroxide was added to adjust the B phase pH until it is equal to the A phase pH. Flow rate was 0.5mL/min. During the gradient elution, B phase started with 0.1% and increased to 20% during 10 min, then linearly increased to 98% during 10min to 11 min, and 98% of B phase was held for 2 mins, and returning to the initial conditions 0.1% in 13.1 min. The analyze finally stop in 16 min. The positive electrospray ionization (ESI+) mode injection volume was 1 µL, and the negative electrospray ionization (ESI-) mode injection volume was 10 µL. The electrospray ionization (ESI) was operated in product ion scan mode in which N2 was used as dissolvent, cone and collision gas (curtain gas: 35psi, atomizer gas: 50psi, auxiliary gas: 60psi, ion spray voltage is: 5500V, declustering potential: 80V, the entrance energy: 10V, collision energy:15V). Up to 1000 lipids of plasma samples were carried out in our study.

2.6 Comprehensive analyses of lipidomic profiles

MultiQuant software (AB SCIEX) was used to process data, after lipids were identified by mass spectrometry. Further, MetaboAnalyst software 5.0 (www.metaboanalyst.ca) was utilized for conducting multivariate statistical analysis, cluster analysis, dimensionality reduction, and making heat map.

2.7 Trans-nodule analyses cross phenome and lipid network layers

The type-specific lipids were identified as more than one and half times elevated or declined significantly compared with pneumoconiosis subtypes (fold change > 1.5 and P value < .05). The expression quantitative trait locus (eQTL) model was utilized to evaluate trans-nodules between lipidomic profiles and clinical phenomes.

2.8 Statistics analysis

Data were presented as mean $\pm$ SD. The means of each group were used for calculation and comparison. Statistical significance of differences between two or three groups was determined by Student's t-test or one-way ANOVA test, respectively. Statistical significance was affirmed when P-value < 0.05. We ranked 15 clinical phenomes of pneumoconiosis patients. Volcano maps showed the significantly elevated or declined lipids in pneumoconiosis patients. A VIP plot was further exploited to sort the lipids according to their importance to differentiate groups. To explore the correlation between lipid elements and clinical phenomes, we applied the lipid-quantitative trait loci model modified from eQTL model.

Continuous variables are presented as median and interquartile range for non-parametric data, and as mean and standard error for parametric data. Differences between groups were analyzed by Student's t-test for parametric values. Measurements were analyzed using SPSS software version 24.0 (SAS Institute, Cary, NC, USA). All tests were two-sided, and P values <0.05 were considered statistically significant.

3 Results

3.1 Subject Characteristics

Forty-eight male pneumoconiosis patients were enrolled in the present study, age from 33 to 77 (57.31 $\pm$ 10.04) years old. The clinical phenomes of pneumoconiosis are BMI, history of dust exposure, smoking and pneumothorax, mediastinal lymph node calcification, lung function, FEV₁, FEV1%, pH, PCO₂, PO₂, respiratory failure, restrictive ventilation dysfunction, complications and modified British medical research council (mMRC) questionnaire.

The characteristics and blood data of the study subjects are shown in Table 1. Pneumoconiosis patients had significantly lower levels of serum cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and lower body mass index (BMI) than healthy groups (P all $\leq$ 0.05). In pneumoconiosis patients, there were 50% with complication(s), 56.25% with mediastinal lymph node calcification on chest CT, 60.42% was severe smoker, 68.75% dust exposure year was more than 15, 79.17% with abnormal lung function, 37.50% with respiratory failure, 56.25% with BMI $\leq$ 18.4 in malnutrition.

Table 1
Characteristics and blood data of patients with pneumoconiosis as compared with healthy control (*P*-values)

Characteristics	Pneumoconiosis (n=48)	Healthy (n=48)	<i>t</i> -value	<i>P</i> -value
Age, year	57.31±10.04	53.67±9.98	1.784	0.078
BMI, kg/m ²	19.20±2.45	22.91±2.87	-6.799	0.000
T-Chol, mg/dl	4.63±0.90	5.50±0.80	-5.050	0.000
TG, mg/dl	1.12±0.40	1.67±1.08	-3.276	0.002
HDL-Chol, mg/dl	1.21±0.33	1.40±0.37	-2.621	0.010
LDL-Chol, mg/dl	3.27±0.90	3.66±0.60	-2.518	0.014

3.2 Plasma lipidomic profiles of patients with pneumoconiosis

Total 426 Lipid elements of plasma were identified qualitatively and quantitatively. All the identified lipid species were grouped into 11 lipid classes, mainly including 107 PEs, 65 PCs, 12 sphingomyelins (SM), 3 cholesterols (CE), 3 ceramides (CER), 2 diacylceramides (DCER), 5 hexosylceramides (HCER), 195 triacylglycerols (TAG), 16 lysophosphatidylcholines (LPC), 1 lysophosphatidylethanolamines (LPE), 17 free fatty acids (FFA) (Fig. 1). Compared with the healthy control (Fig. 1a), the difference in constitution of those 11 lipid classes between healthy controls and pneumoconiosis patients were shown in Fig. 1b. The volcanic map demonstrated the clear patterns of lipid elements significantly increased and declined between healthy controls and pneumoconiosis (Fig. 1c) with 26 significantly increased and 30 decreased lipid elements in patients with pneumoconiosis (detail list as Table 2). We noticed that majority of those elevated lipid elements were PE (69%) and FFA (15%), while PC (23%), TAG (23%) and LPC (17%) declined in pneumoconiosis.

Table 2
Lipid elements significantly elevated and declined alone in patients with
pneumoconiosis as compared with healthy control (*P*-values)

Elevated ≥1.5fold			Declined ≤2/3 fold		
Lipids	Fold	P-value	Lipids	Fold	P-value
PE(16:0/16:1)	2.68	0.00	PC(18:1/20:5)	0.67	0.03
PE(18:1/18:3)	2.65	0.00	LPC(20:0)	0.66	0.00
FFA(20:4)	2.46	0.03	TAG(58:7/FA18:0)	0.65	0.02
FFA(22:4)	2.43	0.00	PC(16:0/20:5)	0.64	0.03
TAG(55:4/FA18:2)	2.33	0.02	LPC(18:0)	0.63	0.00
PE(18:1/18:2)	2.19	0.00	TAG(58:9/FA18:1)	0.63	0.01
PE(18:1/18:1)	2.07	0.00	PC(14:0/22:6)	0.61	0.00
PE(16:0/18:3)	2.03	0.00	LPC(22:6)	0.61	0.00
PE(18:2/18:2)	2.02	0.00	PE(14:0/20:4)	0.61	0.00
FFA(20:5)	1.99	0.00	TAG(56:6/FA22:6)	0.59	0.05
PE(O-18:0/22:4)	1.97	0.03	PC(18:2/20:5)	0.57	0.00
PE(16:0/18:2)	1.90	0.00	TAG(56:7/FA16:0)	0.57	0.04
FFA(20:3)	1.88	0.01	CER(24:1)	0.56	0.00
PE(16:0/22:4)	1.83	0.01	TAG(58:7/FA16:0)	0.55	0.04
PE(16:0/18:1)	1.82	0.00	PC(20:0/22:6)	0.54	0.00
PE(18:2/20:2)	1.79	0.01	LPC(14:0)	0.54	0.00
PE(18:0/22:4)	1.73	0.04	TAG(55:1/FA16:0)	0.52	0.01
PE(18:0/16:1)	1.68	0.02	HCER(24:1)	0.49	0.00
PE(P-18:0/22:4)	1.63	0.00	PC(14:0/20:5)	0.48	0.02
TAG(55:4/FA18:1)	1.63	0.02	TAG(56:8/FA16:0)	0.47	0.02
PC(16:0/16:1)	1.61	0.01	LPC(20:5)	0.46	0.00
PE(18:0/16:0)	1.59	0.00	HCER(20:1)	0.45	0.00
PE(18:0/18:3)	1.56	0.02	PE(P-18:0/20:5)	0.44	0.01
PE(18:1/20:4)	1.55	0.00	PE(P-18:1/20:5)	0.40	0.02
PE(18:1/20:3)	1.51	0.01	PE(O-16:0/20:5)	0.39	0.01

Elevated ≥1.5fold			Declined ≤2/3 fold		
PC(18:0/16:1)	1.50	0.02	PE(P-16:0/20:5)	0.36	0.03
			DCER(22:0)	0.36	0.00
			PC(20:0/20:5)	0.34	0.00
			CER(22:1)	0.33	0.00
			HCER(22:1)	0.32	0.00

By orthogonal partial least squares discrimination analysis (OPLS-DA), variable important in project (VIP) scores were used to select important lipids. Top 50 lipid elements were defined on the basis of VIP scores: PE (14:0/20:4, 16:0/16:1, 16:0/18:1, 16:0/18:2, 16:0/18:3, 16:0/20:4, 18:0/16:0, 18:0/18:0, 18:1/18:1, 18:1/18:2, 18:1/18:3, 18:1/20:3, 18:1/20:4, 18:2/18:2, 18:2/20:2, P-16:0/22:6, P-18:0/20:5, P-18:0/22:6, P-18:0/22:4, P-18:1/22:6, O-16:0/20:5, O-16:0/22:6), PC (20:0/22:6, 20:0/20:5, 20:0/20:4, 18:2/20:5, 16:0/22:6, 16:0/16:1, 16:0/16:0, 14:0/22:6, 14:0/20:4), LPC (22:6, 22:5, 20:5, 20:4, 20:2, 20:1, 20:0, 18:0, 16:0, 14:0), CER (22:1, 24:1), HCER (24:1, 22:1, 20:1), DCER (22:0), FFA (14:0, 22:4) (Listed in Table 3). Among these, 15 lipid elements were upregulated, while 18 lipids showed a significant downregulation in pneumoconiosis patients when compared with healthy group (Fig. 2). There was a clear distribution of top 50 lipid elements in pneumoconiosis group, compared with the healthy control (Fig. 3).

Table 3
Lipid elements significantly elevated and declined in pneumoconiosis patients compared with healthy control among the top 50 lipid elements defined on VIP scores (*P*-values)

Elevated ≥1.5fold			Declined ≤2/3 fold		
Lipids	Fold	P-value	Lipids	Fold	P-value
PE(16:0/16:1)	2.68	0.00	LPC(20:0)	0.66	0.00
PE(18:1/18:3)	2.65	0.00	LPC(18:0)	0.63	0.00
FFA(22:4)	2.43	0.00	PC(14:0/22:6)	0.61	0.00
PE(18:1/18:2)	2.19	0.00	LPC(22:6)	0.61	0.00
PE(18:1/18:1)	2.07	0.00	PE(14:0/20:4)	0.61	0.00
PE(16:0/18:3)	2.03	0.00	PC(18:2/20:5)	0.57	0.00
PE(18:2/18:2)	2.02	0.00	CER(24:1)	0.56	0.00
PE(16:0/18:2)	1.90	0.00	PC(20:0/22:6)	0.54	0.00
PE(16:0/18:1)	1.82	0.01	LPC(14:0)	0.54	0.00
PE(P-18:0/22:4)	1.63	0.00	HCER(24:1)	0.49	0.00
PE(16:0/16:1)	1.61	0.01	LPC(20:5)	0.46	0.00
PE(18:0/16:0)	1.60	0.00	HCER(20:1)	0.45	0.00
PE(18:1/20:4)	1.55	0.00	PE(P-18:0/20:5)	0.44	0.00
PE(18:1/20:3)	1.51	0.01	PE(O-16:0/20:5)	0.39	0.00
			DCER(22:0)	0.36	0.00
			PC(20:0/20:5)	0.34	0.00
			CER(22:1)	0.33	0.00
			HCER(22:1)	0.32	0.00

3.3 Trans-omic profiles between clinical phenomes and lipidomes

We next analyzed the correlation of lipid profiles with clinical phenomes from pneumoconiosis patients. Circulating levels of lipid elements were integrated with 15 clinical phenomes from patients with pneumoconiosis. Combined lipidomes (VIP#1) with clinical phenomes measured by simulating the expression quantitative trait locus (eQTL) model. With the aim to identify disease phenotype-specific lipid elements, circulating levels of lipid elements were integrated with clinical phenomes in patients with

pneumoconiosis patients. The correlation of varied lipid elements and clinical phenotype were clearly displayed in Fig. 4a and 4b, which showed that pH, lung function, mediastinal lymph node calcification and complication were highly correlated with lipid elements (VIP=1). Furthermore, up-regulated PE was corresponded to pH, smoking history and mediastinal lymph node calcification. PC was corresponded to dust exposure history, BMI, pneumothorax and mediastinal lymph node calcification (Fig. 4c).

4 Discussion

Lipid plays an important role in lung pathology and physiology. Although the composition and involvement of the lipidome in various disease is still poorly understood, abnormal lipid metabolism has been reported in pulmonary diseases including asthma, COPD and lung cancer^[22–24]. Lipids comprise diverse classes of components which are critically involved in cellular structure, signaling and energy storage^[25]. However, the potential role of lipid in pneumoconiosis pathogenesis remains unclear.

We observed that most of pneumoconiosis patients have poor nutritional status and bodies. We assume that it is related to hypoxia and poor lung function. We analyzed the possible reasons as follows: (1) Long-term hypoxia and chronic inflammatory infiltration may lead to gastrointestinal congestion. Recurrent pneumonia and dyspnea with antibiotics and theophylline and other drugs may reduce the absorption, transformation and synthesis of exogenous cholesterol and triglyceride in small intestinal mucosa cells^[26]; (2) liver cell hypoxia leads to energy dysmetabolism and decreasing the source of cholesterol and triglyceride synthesis raw materials, such as fatty acid, Acetyl coenzyme A, ATP, NADPH⁺, H⁺, and the synthesis rate of endogenous cholesterol and triglyceride decreased^[27]; (3) The lipid decomposition of pneumoconiosis increases, due to lung fibrosis. Thus, it causes a large amount of ATP consumption and increased body catabolism, and lipolysis^[28].

Lipidomic profiles between health and pneumoconiosis had never been defined before, although a majority of pneumoconiosis patients had decreased levels of serum CHO, TAG, HDL and LDL. With the rapid development of biotechnology on analytical chemistry, lipidomics has been used as a tool to define the lipid profiling of lung tissue and plasma, and the relationship between lipid characterization and lung function^[24, 29]. We investigated the plasma lipidomic profiles of patients with pneumoconiosis. Our study showed that certain types of PEs and FFAs were significantly higher in pneumoconiosis than healthy control, whereas some of PCs, lysoPCs were significantly lower.

PCs and PEs are the most abundant phospholipids in cell membranes and play essential structural and signaling roles. PCs play significant roles in lung surfactant phospholipid metabolism and is the major phospholipid comprising around 80% of surfactant lipid. Alterations in their composition can cause reduced elasticity, leading to an overall decrease in lung compliance^[30, 31]. Changes in the component phospholipids, including PCs have been described increased in plasma of patients with pulmonary fibrosis^[28]. Lv et al demonstrated that plasma levels of PC and lysoPC were increased while PE and lysoPE were decreased in patient with lung cancer^[21]. Gao et al also found that there was an increase of

PC and lysoPC in acute exacerbation of chronic pulmonary (AECOPD), acute pulmonary embolism (APE) and severe acute pneumonia (SAP) [29]. However, the trend of PE, PC and lysoPC in patient with pneumoconiosis were different from the results of the above researches in other pulmonary diseases. The up-regulated PE and down-regulated PC in this study might be a potential criterion to distinguish the pneumoconiosis from healthy and even other lung diseases. Although the reason and mechanism of changed lipidomics in pneumoconiosis are not clear, it may be of great significance to find the pathogenesis of pneumoconiosis.

In our study, we also found that FFA (22:4) was significantly higher in pneumoconiosis than healthy control. Guenther et al. reported that elevated plasma FFA levels resulted in increased activation of the classic proinflammatory IKK/I κ B/NF- κ B pathway and increased expression of inflammatory cytokines (tumor necrosis factor- α , interleukin-1 β and interleukin-6) [32]. Rudi et al. reported that activation of FFA4, a member of the G protein-coupled receptor family, that responded to FFA, resulted in a decrease in lung resistance [33]. At present, researches on FFA and FFA receptors are mostly focused on metabolic disorders such as diabetes and obesity, whereas few attentions were paid to respiratory diseases. Recent study found that FFA4 was highly expressed in lung epithelial cells [34], and that FFA acting via FFA4 receptors might have some beneficial effects on airway epithelial repair after naphthalene-induced airway injury [35]. These researches suggested that it may also be a potential target for understanding pathogenesis and treating respiratory diseases including pneumoconiosis.

There was lack of research in linking the lipidome data with clinical phenomes. Like other omics analysis, most genomic data were not tied with clinical information, which showed limited value for clinical precision medicine [36]. In order to integrate lipid profiles with clinical phenomes, clinical lipidomics is a novel extension of lipidomics to study lipid profiles, pathways and networks by characterizing and quantifying the total lipidomes in patients, and to link the lipidomics components to clinical phenomics [37]. Our study made the descriptive clinical phenomes scored by DESS system [38], which had been performed in patients with pulmonary embolism, acute pneumonia, and AECOPD, based on clinical transcriptomics principle [29], to converting descriptive information of clinical phenomes into digital information [39].

In this study, through eQTL model and integrated lipidomic and phenomic profiles, we discovered clinical phenotypes can be associated with a variety of altered lipid elements. Lipidomic profiles with about 426 lipids in 11 classes in pneumoconiosis, were correlated with clinical phenotypes, and it turned out that all altered lipids (VIP $\geq$ 1.0) had strong correlations with pH, FEV₁, mediastinal lymph node calcification and complications. Furthermore, we also found that PE was corresponded to pH, smoking history and mediastinal lymph node calcification while PC was closely correlated with BMI, dust exposure history, pneumothorax and mediastinal lymph node calcification separately.

One of the pathophysiological characteristics of pneumoconiosis was macrophage accumulation. Recent studies had showed that macrophages were fundamental for the repair of organs that were injured due to

ischemia. In response to hypoxia, acute-phase macrophages activate hypoxia-inducible transcription factors that adjust cellular metabolism via glycolysis, the tricarboxylic acid cycle, oxidative phosphorylation, lipids, amino acids pathways leading to metabolic acidosis and decreased pH^[40]. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), is a lipid marker of alveolar type 2 epithelial cells. Recently, a study revealed SiO₂ exposure could result in decreased DPPC secretion, affecting the normal function of alveoli, and lung function potentially [41]. There were studies confirmed that altered lipid metabolism (PCs and lysoPCs) is associated with decreased lean body mass [42]. These studies were consistent with our experimental results.

There was one research indicating that PEs were expressed at a higher level in smokers with COPD, compared with smokers without COPD. Altered lipids with a high fold change between smokers with and without COPD showed high correlations with lower lung function and inflammation in sputum^[43]. In our study, PEs were highly expressed in smoking patients. The mechanism and the diagnostic and therapeutic value of elevated PE were not clear now. Wu et al. found that phospholipid remodeling was critical for stem cell pluripotency by facilitating mesenchymal-to-epithelial transition. They also showed that PE binding to Pebp1 enhanced the interaction of Pebp1 with IKK α / β and reduced the phosphorylation of IKK α / β and consequently, the activation of NF- κ B signaling. Collectively, studies reveal an unforeseen connection between phospholipids and cell pluripotency and also highlights the importance of phospholipids in diseases^[44].

As to clinical phenotypes, pneumothorax and calcification of mediastinal and hilar lymph nodes were commonly found in pneumoconiosis. At present, there is no study of lipidomic profiles on pneumothorax, and the cause of pneumoconiosis with pneumothorax at high risk is still unclear. In our study we found that decrease of PCs was closely correlated with it, may be a new point to find out the mechanism of pneumothorax in pneumoconiosis. Although the mechanism of calcification of mediastinal lymph nodes was not clear, it might because macrophage accumulation and altered lipid metabolism in pneumoconiosis present with similar dystrophic calcification^[45].

There were also limitations in this study. First, the sample size was small. Second, therapeutic factors were not explored, and most corresponding groups of patients should be studied in future to explore medical influence on lipidomes. Third, it is not clear whether the correlation is positive or negative by the eQTL model for trans-omics.

In conclusion, we found that altered lipid panels between pneumoconiosis patients and healthy people by qualitatively and quantitatively measured plasma lipidomic profiles. Levels of PE and FFA elements meaningfully improved while PC and lysoPC reduced in patients with pneumoconiosis. Clinical trans-omics analyses demonstrated that some phenomes have strong correlation with lipids, although those needed to be further confirmed by bigger studies including large population of patients in multicenters. Consequently, our data suggested that trans-omic profiles between clinical phenomes and lipidomes might have the potential value to reveal the heterogeneity of lipid metabolism among pneumoconiosis patients and to identify meaningful phenome-based lipid panels as biomarkers.

Declarations

Acknowledgements

The work was supported by National Key Research and Development Program of China (2019YFC0121705), Quanzhou City Science & Technology Program of China (2018N008S, 2019C025R, 2020N034S) and Bethune Medical Science Research Foundation (SCZ134DS).

Conflict of interest

The authors declare that no conflicts of interest.

Data availability

The authors declare that the data and material have free access.

Author contribution

Xiaoyang Chen and Liyong Shi contributed to research design, methodology, data analysis and writing original draft. Xiaofang Dai, Furong Yan and Yongquan Zhang contributed to clinical material collection, data curation and visualization. Yujun Lin and Lianshun Lin contributed to writing, review and editing. Yiming Zeng and Xiaoyang Chen contributed to project administration and funding acquisition. All authors discussed the results and revised the manuscript.

Consent to participate and consent to publish

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given consent for their blood samples and clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

References

1. The Lancet, Improving occupational health in China. Lancet, 2019;394: 443.
2. Krefft Silpa, Wolff Jenna, Rose Cecile, Silicosis: An Update and Guide for Clinicians. Clin Chest Med, 2020; 41: 709-722.
3. Qi Xian-Mei, Luo Ya, Song Mei-Yue et al. Pneumoconiosis: current status and future prospects. Chin Med J (Engl), 2021; 134(8): 898-907.

4. Grunt Thomas W, Interacting Cancer Machineries: Cell Signaling, Lipid Metabolism, and Epigenetics. *Trends Endocrinol Metab*, 2018, 29 (2): 86-98.
5. Chen Y, Ma Z, Shen X, et al. Serum Lipidomics Profiling to Identify Biomarkers for Non-Small Cell Lung Cancer. *BioMed Research International*, 2018, (2018):1-16.
6. Didangelos A, Stegemann C, Mayr M. The-omics era: proteomics and lipidomics in vascular research. *Atherosclerosis*, 2012, 221(1):12-17.
7. Shetty SS, Kumari S. Fatty acids and their role in typediabetes (Review). *Experimental and Therapeutic Medicine*, 2021, 22(1): 706.
8. Huynh K, Lim W, Giles C, et al. Concordant peripheral lipidome signatures in two large clinical studies of Alzheimer's disease. *Nat Commun*. 2020. 11(1): 5698
9. Widder S , Gawish R , Watzenboeck M , et al. Multi-Omics Correlations Reveal Lipid Species Involved in Lung Allograft Adaptation. *The Journal of Heart and Lung Transplantation*, 2021, 40(4):S333-S334.
10. Park JK, Coffey NJ, Limoges A, Le A. The Heterogeneity of Lipid Metabolism in Cancer. *Adv Exp Med Biol*. 2021. 1311: 39-56.
11. Karagiorga G, Nakos G, Galiatsou E, Lekka ME. Biochemical parameters of bronchoalveolar lavage fluid in fat embolism. *Intensive Care Med*. 2006. 32(1): 116-23.
12. Leonenko Z, Gill S, Baoukina S, et al. An elevated level of cholesterol impairs self-assembly of pulmonary surfactant into a functional film. *Biophys J*. 2007. 93(2): 674-83.
13. Guo Chenglin,Sun Lina,Zhang Linlin et al. Serum sphingolipid profile in asthma.*J Leukoc Biol*, 2021, 110: 53-59.
14. Hough KP, Wilson LS, Trevor JL, et al. Unique Lipid Signatures of Extracellular Vesicles from the Airways of Asthmatics. *Sci Rep*. 2018. 8(1): 10340.
15. Wang S, Tang K, Lu Y, et al. Revealing the role of glycerophospholipid metabolism in asthma through plasma lipidomics. *Clin Chim Acta*. 2021. 513: 34-42.
16. Lin Tian-Lai,Chen Wei-Wen,Ding Zhi-Rong et al. Correlations between serum amyloid A, C-reactive protein and clinical indices of patients with acutely exacerbated chronic obstructive pulmonary disease.*J Clin Lab Anal*, 2019, 33 (4): e22831.
17. Wang X. Clinical trans-omics: an integration of clinical phenomes with molecular multiomics. *Cell Biol Toxicol*. 2018. 34(3): 163-166.
18. Song D, Yang D, Powell CA, Wang X. Cell-cell communication: old mystery and new opportunity. *Cell Biol Toxicol*. 2019. 35(2): 89-93.
19. Qiao T, Wang X. A new light of proteomics in cell biology and toxicology. *Cell Biol Toxicol*. 2019. 35(4): 289-291.
20. Sarafian MH, Gaudin M, Lewis MR, et al. Objective set of criteria for optimization of sample preparation procedures for ultra-high throughput untargeted blood plasma lipid profiling by ultra performance liquid chromatography-mass spectrometry. *Anal Chem*. 2014. 86(12): 5766-74.

21. Lv J, Gao D, Zhang Y, et al. Heterogeneity of lipidomic profiles among lung cancer subtypes of patients. *J Cell Mol Med*. 2018. 22(10): 5155-5159.
22. Li WJ, Zhao Y, Gao Y, et al. Lipid metabolism in asthma: Immune regulation and potential therapeutic target. *Cell Immunol*. 2021. 364: 104341.
23. Liu D, Meister M, Zhang S, et al. Identification of lipid biomarker from serum in patients with chronic obstructive pulmonary disease. *Respir Res*. 2020. 21(1): 242.
24. Zhu Z, Zhang L, Lv J, Liu X, Wang X. Trans-omic profiling between clinical phenoms and lipidomes among patients with different subtypes of lung cancer. *Clin Transl Med*. 2020. 10(4): e151.
25. Zehethofer N, Bermbach S, Hagner S, et al. Lipid Analysis of Airway Epithelial Cells for Studying Respiratory Diseases. *Chromatographia*. 2015. 78(5-6): 403-413.
26. McGarry Trudy, Biniecka Monika, Veale Douglas J et al. Hypoxia, oxidative stress and inflammation. *Free Radic Biol Med*, 2018, 125: 15-24.
27. Xiao W, Wang RS, Handy DE, Loscalzo J. NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. *Antioxid Redox Signal*. 2018. 28(3): 251-272.
28. Nambiar S, Clynick B, How BS, et al. There is detectable variation in the lipidomic profile between stable and progressive patients with idiopathic pulmonary fibrosis (IPF). *Respir Res*. 2021. 22(1): 105.
29. Gao D, Zhang L, Song D, et al. Values of integration between lipidomics and clinical phenomes in patients with acute lung infection, pulmonary embolism, or acute exacerbation of chronic pulmonary diseases: a preliminary study. *J Transl Med*. 2019. 17(1): 162.
30. Agudelo CW, Samaha G, Garcia-Arcos I. Alveolar lipids in pulmonary disease. A review. *Lipids Health Dis*. 2020. 19(1): 122.
31. Agassandian M, Mallampalli RK. Surfactant phospholipid metabolism. *Biochim Biophys Acta*. 2013. 1831(3): 612-25.
32. Boden G, She P, Mozzoli M, et al. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. *Diabetes*. 2005. 54(12): 3458-65.
33. Prihandoko R, Kaur D, Wiegman CH, et al. Pathophysiological regulation of lung function by the free fatty acid receptor FFA4. *Sci Transl Med*. 2020. 12(557).
34. Miyauchi S, Hirasawa A, Iga T, et al. Distribution and regulation of protein expression of the free fatty acid receptor GPR120. *Naunyn Schmiedebergs Arch Pharmacol*. 2009. 379(4): 427-34.
35. Lee KP, Park SJ, Kang S, et al. ω -3 Polyunsaturated fatty acids accelerate airway repair by activating FFA4 in club cells. *Am J Physiol Lung Cell Mol Physiol*. 2017. 312(6): L835-L844.
36. Rubin MA. Health: Make precision medicine work for cancer care. *Nature*. 2015. 520(7547): 290-1.
37. Lv J, Zhang L, Yan F, et al. Clinical lipidomics: a new way to diagnose human diseases. *Clin Transl Med*, 2018, 7(1):12.
38. Shi L, Zhu B, Xu M, Wang X. Selection of AECOPD-specific immunomodulatory biomarkers by integrating genomics and proteomics with clinical informatics. *Cell Biol Toxicol*. 2018. 34(2): 109-

123.

39. Chen H, Song Z, Qian M, Bai C, Wang X. Selection of disease-specific biomarkers by integrating inflammatory mediators with clinical informatics in AECOPD patients: a preliminary study. *J Cell Mol Med*. 2012. 16(6): 1286-97.
40. Thorp EB. Macrophage Metabolic Signaling during Ischemic Injury and Cardiac Repair. *Immunometabolism*. 2021. 3(2).
41. Liu S, Yang R, Chen Y, et al. Development of Human Lung Induction Models for Air Pollutants' Toxicity Assessment. *Environ Sci Technol*. 2021. 55(4): 2440-2451.
42. Papandreou C, García-Gavilán J, Camacho-Barcia L, et al. Circulating Metabolites Associated with Body Fat and Lean Mass in Adults with Overweight/Obesity. *Metabolites*. 2021. 11(5).
43. Telenga ED, Hoffmann RF, t'Kindt R, et al. Untargeted lipidomic analysis in chronic obstructive pulmonary disease. Uncovering sphingolipids. *Am J Respir Crit Care Med*. 2014. 190(2): 155-64.
44. Wu Y, Chen K, Xing G, et al. Phospholipid remodeling is critical for stem cell pluripotency by facilitating mesenchymal-to-epithelial transition. *Sci Adv*. 2019. 5(11): eaax7525.
45. Bargagli E, Rosi E, Pistolesi M, Lavorini F, Voltolini L, Rottoli P. Increased Risk of Atherosclerosis in Patients with Sarcoidosis. *Pathobiology*. 2017. 84(5): 258-263.

Figures

Figure 1

The proportion (%) of 11 main lipid elements of healthy controls (a) and pneumoconiosis (b). Volcanic map (c) between healthy controls with pneumoconiosis. The lipid elements were identified on the basis of statistical significance. The abscissa represents log values of fold changes, where the left side of the first dotted line perpendicular to the abscissa represents $<2/3$ -fold changes and the right side of the second dotted line represents >1.5 -fold changes. The vertical coordinate represents $-\log_{10}$ (P-value). The upper side of the dotted line perpendicular to the ordinate stands for P-value less than .05, as compared with healthy controls.

Figure 3

The heatmap describes the top 50 lipid elements at the high concentration and the degree of lipid elements increased from blue (low) and red (high).

Figure 4

The specific correlation between clinical phenotypes and altered lipid elements of pneumoconiosis patients illustrated by a, b and c. (a) Heatmap describes the correlation increased from blue (low) to red (high). (b) Venn's diagrams, the more overlaps, the greater the correlation, which showed that pH, lung function, mediastinal lymph node calcification and complication were highly correlated with lipid elements. (c) Stacked bar plot showing mean relative abundance of lipids in clinical phenomes. The longer column indicating the stronger correlation.