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ORIGINAL RESEARCH

Metabolomics Reveals Dysregulated Sphingolipid and Amino Acid Metabolism Associated with Chronic Obstructive Pulmonary Disease

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Purpose: Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease presenting as multiple phenotypes, such as declining lung function, emphysema, or persistent airflow limitation caused by several risk factors, including cigarette smoking and air pollution. The inherent complexity of COPD phenotypes propounds difficulties for accurate diagnosis and prognosis. Although metabolomic profiles on COPD have been reported, the role of metabolism in COPD-related phenotypes is yet to be determined. In this study, we investigated the association between plasma sphingolipids and amino acids, and between COPD and COPD-related phenotypes in a Korean cohort.

Patients and Methods: Blood samples were collected from 120 patients with COPD and 80 control participants who underwent spirometry and quantitative computed tomography. The plasma metabolic profiling was carried out using LC-MS/MS analysis.

Results: Among the evaluated plasma sphingolipids, an increase in the metabolism of two specific sphingomyelins, SM (d18:1/24:0) and SM (d18:1/24:1) were significantly associated with COPD. There was no significant correlation between any of the SMs and the emphysema index, FVC and FEV_1 in the COPD cohort. Meanwhile, Cer (d18:1/18:0) and Cer (d18:1/24:1) were significantly associated with reduced FEV_1. Furthermore, the levels of several amino acids were altered in the COPD group compared to that in the non-COPD group; glutamate and alpha AAA were substantial associated with emphysema in COPD. Kynurenine was the only amino acid significantly associated with reduced FEV_1 in COPD. In contrast, there was no correlation between FVC and the elevated metabolites.

Conclusion: Our results provide dysregulated plasma metabolites impacting COPD phenotypes, although more studies are needed to explore the underlying mechanism related to COPD pathogenesis.

Keywords: targeted sphingolipids, amino acids, sub-phenotypes, lung function

Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease with multiple pathological features, such as inflammation, emphysema, chronic bronchitis, and altered lung function.[1,2] The underlying mechanisms are complex, and there are currently no effective biomarkers of COPD progression, severity, and mortality. A better understanding of the pathogenesis of each COPD-related phenotype will enable improved diagnosis and treatment.

Metabolomics is an emerging science that involves analyzing endogenous low-molecular-weight metabolites (≤1500 Da) in a biological specimen. The metabolome interacts with and reflects the activity of the genome, epigenome, and proteome, but is also influenced by factors such as diet, lifestyle, and medications. Providing a snapshot of gene function,

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enzyme activity, and physiological changes, metabolomics may help inform the heterogeneity of COPD-related phenotypes. While metabolomics analysis of biofluids (serum, plasma, or urine) and spirometry testing of pulmonary function can discriminate between healthy controls and patients with COPD, the associations with clinical COPD-related phenotypes have not yet been fully characterized.

Several metabolomics studies have demonstrated metabolic dysregulation in COPD.[3,4] A common observation in patients with COPD is the dysregulation of sphingolipid metabolism. A growing body of evidence indicates that sphingolipids play a key role in the pathogenesis of several lung diseases, such as asthma, acute lung injury, emphysema, COPD, and cystic fibrosis.[5–8] Ceramides are generated through hydrolysis by sphingomyelinases and produce sphingomyelins through sphingomyelin synthase.[9] Dysregulation of ceramide and sphingomyelin metabolites is strongly associated with COPD.[10] Bowler et al reported that levels of some specific sphingomyelins, such as SM (d18:1/16:0), were inversely associated with emphysema severity, in contrast to other sphingomyelins, such as SM (d18:1/18:0).[10] Low baseline plasma sphingomyelin levels have been associated with worse COPD, while high levels have been linked with the rapid progression of emphysema.[11,12] Similarly, levels of specific ceramides were inversely correlated with emphysema severity, especially Cer (d18:1/16:0).[10]

Furthermore, amino acids, which are the building blocks of proteins, play a vital role in the intermediary metabolism. Active metabolism of amino acids occurs during exercise, with altered levels of several amino acids reported in skeletal muscle and plasma. However, evidence supports dysregulated amino acid metabolism in patients with COPD even at rest,[13,14] suggesting that an abnormal amino acid profile may be a significant risk factor for COPD.

Several studies have investigated the associations between clinical features of COPD and targeted metabolism in various populations.[15–18] However, studies examining associations between sphingolipid metabolite dysregulation, impaired amino acid profiles, and COPD in Asian populations are relatively scarce. Therefore, the aim of the present study was to utilize a targeted, quantitative mass spectrometry-based approach for determining plasma levels of sphingolipids and amino acid metabolites associated with poor clinical outcomes, including rate of lung function decline and increased emphysema index determined using chest CT scan, in a cohort of Korean patients with COPD.

Materials and Methods

Study Design and Population

Participants were divided into COPD (n=120) and non-COPD (n=80) groups according to the standards of the Global Initiative for Chronic Obstructive Lung Disease, which defines COPD as a ratio of post-bronchodilator forced expiratory volume to forced vital capacity (FEV1/FVC) of < 0.70. Two hundred participants (132 men and 68 women), aged 50–89 years, were enrolled in the Chronic Obstructive Pulmonary Disease in Dusty Areas (CODA) study,[19,20] which was designed to observe clinical outcomes of Koreans with COPD near cement plants in the following regions: Gangneung (GN), Danyang (DY), Donghae (DH), Samcheok (SC), Yeongwol (YW), and Jecheon (JC). Data and biological specimens collected during baseline examinations of the CODA study between 2012 and 2016 were used in this study. Participants with known or suspected cancer or recent (within 3 months) hospitalization were excluded from the study; however, patients with comorbid conditions such as atherosclerosis or other lung diseases were included.

A medical interview was conducted as part of the baseline examination and participants completed a survey questionnaire. The questionnaire included demographic factors, medical and smoking history, lifestyle factors, current medications, exacerbation history, and respiratory symptoms during the past year. A physical examination, blood/urine sampling, spirometry, and computed tomography (CT) scan were performed for all participants. All participants in this study provided written informed consent. This study was approved by the Kangwon National University Hospital IRB (KNUH 2020–06-007) and all study protocols were conducted according to the Institutional Review Board of Kangwon National University Hospital.

Measurements

Spirometry was performed using standardized equipment according to the recommendations of the American Thoracic Society and European Respiratory Society guidelines.[21] Spirometry was performed before and 15 min after inhalation of

two puffs of salbutamol to assess the bronchodilator response. The protocols of data collection in the CODA cohort were previously described in detail. The spirometry results were expressed as percentages of predicted values based on the Korean population.[22]

Volumetric CT scan measurements were obtained using a 16-multidetector CT scanner (Somatom Sensation 16; Siemens Medical Systems, Bonn, Germany), based on the protocol used in the Korean Obstructive Lung Disease (KOLD) study.[23] The emphysema index, defined as the percentage of low attenuation area in the lung (≤ 950 Hounsfield units, %LAA-950HU), and airway wall thickness, defined as the percentage of mean wall area measured in two segmental bronchi, were derived using in-house software from the KOLD study.

Targeted Metabolomics

Plasma samples (100 μL) were mixed with 300 μL chloroform/methanol (1/2, v/v) and an internal standard solution. The internal standard contained 10 μM[13] C_5-glutamine, 0.4 μM serotonine-d_4, 0.6 μM dopamine-d_4, 2 nM tryptophan-d_5, 6 nM serine-d_3, 50 nM lysine-d_8, and 50 nM Cer (d18:1/17:0), and was normalized for the LC-MS/MS method. Liquid-liquid extraction was performed by incubating the mixture for 15 min at 4 °C, followed by centrifugation at 14,000 rpm for 15 min. The lipid phase was collected for sphingolipid measurement and dried under vacuum. The aqueous phase was used for chemical derivatization of amino acids using phenyl isothiocyanate. The derivatized amino acids were further extracted with 5 mM ammonium acetate in methanol and dried under vacuum. The dried matter was reconstituted with either methanol or H_2O/acetonitrile (50/50, v/v) prior to LC-MS/MS analysis.

Amino acids and sphingolipids were analyzed using a 1290 Infinity UHPLC system (Agilent Technologies, Palo Alto, CA, USA) with a Qtrap 5500 LC-MS/MS system (AB Sciex, Framingham, MA, USA). The injection volume was 3 μL and samples were ionized using a turbo ion spray interface. For amino acids analysis, a reverse-phase column (Zorbax Eclipse XDB-C18100 ×2 mm; Agilent Technologies) was used with mobile phases A and B consisting of 0.2% formic acid in H_2O and acetonitrile, respectively. The flow rate was 500 μL/min and the column temperature was 50 °C. The separation gradient was as follows: 0% B for 0.5 min, 0 to 95% B for 5 min, 95% B for 1 min, 95 to 0% B for 0.5 min, and 0% B for 2.5 min. For sphingolipids, a reverse-phase column (Pursuit 5 C18, 150×2.1 mm; Agilent Technologies) was used with mobile phases A (5 mM ammonium formate/MeOH/tetrahydrofuran [500/200/300, v/v/v]) and B (5 mM ammonium formate/MeOH/tetrahydrofuran [100/200/700, v/v/v]). The LC flow rate was 200 μL/min and the column temperature was 35 °C. The separation gradient was as follows: 50% B for 5 min, 50 to 70% B for 3 min, 70% B for 7 min, 70 to 90% B for 7 min, 90% B for 3 min, 90 to 50% B for 0.1 min, and 50% B for 4.9 min. Multiple reaction monitoring was performed in positive ion mode, and the extracted ion chromatogram corresponding to the specific transition for each analyte was used for quantification. Data analysis was performed using Analyst 1.5.1 software.

Statistical Analysis

Comparison of baseline characteristics between COPD and non-COPD groups was performed using Student's *t*-test and chi-square test. Categorical variables were described as N (%). Continuous variables were reported as the mean ± standard deviation (SD). Since sphingolipid metabolism and amino acid profiles were not normally distributed, they were analyzed by log-transformation. A linear regression model was used to determine the effects of COPD, emphysema, lung function (FEV_1, FVC), sphingolipid metabolism and amino acid profiles, adjusting for age, sex, smoking status, pack years, GOLD grade, Charlson Comorbidity Index (CCI), and drug history. All analyses were performed using SAS version 9.4 (SAS Institute, Inc. Cary, NC, USA), and statistical significance was set at $P < 0.05$.

Results

Korean COPD Cohort Demographics and Clinical Characteristics

The baseline clinical characteristics of the study participants (n=200) are summarized in Table 1. Participants were recruited from rural regions of South Korea near cement plants, with 80 (40%) and 120 (60%) participants in the non-COPD and COPD groups, respectively. The average age of the participants was 72.14 ± 6.66 years (non-COPD group = 70.71 ± 7.41 years, COPD group = 73.09 ± 5.96 years). Overall, 66% of the participants were male and 34% were

Table 1 Demographics Data for Participants Included in the Current Study

	All (n=200)	Non-COPD (n=80)	COPD (n=120)	*p-value
		*Mean ± SD or N (%)		
Age, mean (SD)	72.14±6.66	70.71±7.41	73.09±5.96	**0.0176**
50–59	11(5.5)	7(8.8)	4(3.3)	
60–69	45(22.5)	26(32.5)	19(15.8)	
70–79	127(63.5)	40(50.0)	87(72.5)	
80–89	17(8.5)	7(8.8)	10(8.3)	
Gender				0.4360
Male	132(66.0)	48(60.0)	84(70.0)	
Female	68(34.0)	32(40.0)	36(30.0)	
Smoking				**0.0471**
Current	57(28.5)	17(21.3)	40(33.3)	
Former	63(31.5)	23(28.7)	40(33.3)	
Never	80(40.0)	40(50.0)	40(33.3)	
Pack-years	19.06±22.94	16.03±21.50	21.11±23.74	0.1283
BMI	23.22±3.01	24.02±3.05	22.69±2.86	**0.0020**
Region				0.4050
GN	15(7.5)	7(8.8)	8(6.7)	
DY	19(9.5)	10(12.5)	9(7.5)	
DH	5(2.5)	2(2.5)	3(2.5)	
SC	33(16.5)	8(10.0)	25(20.8)	
YW	81(40.5)	34(42.5)	47(39.2)	
JC	47(23.5)	19(23.8)	28(23.3)	
Baseline examination, year				**0.0307**
2012	133(66.5)	53(66.3)	80(66.7)	
2013	36(18.0)	11(13.7)	25(20.8)	
2014	22(11.0)	8(10.0)	14(11.7)	
2015	3(1.5)	3(3.8)	0(0.0)	
2016	6(3.0)	5(6.2)	1(0.8)	
Gold grade				**<0.0001**
0	80(40.0)	80(100.0)	0(0.0)	
1	52(26.0)	0(0.0)	52(43.3)	
2	58(29.0)	0(0.0)	58(48.3)	
3 or 4	10(5.0)	0(0.0)	10(8.4)	
Drug History				
ICS/LABA	12(6.0)	2(2.5)	10(8.3)	0.0888
Lung Function				
FVC (L)	2.92±0.80	2.94±0.79	2.90±0.82	0.7726
FVC (%)	96.69±19.23	97.44±19.48	96.18±19.13	0.6526
FEV_1 (L)	1.92±0.61	2.22±0.59	1.72±0.54	**<0.0001**
FEV_1 (%)	87.19±23.04	100.21±21.85	78.50±19.57	**<0.0001**
FEV_1/FVC	65.77±10.33	75.66±4.75	59.17±7.32	**<0.0001**
Emphysema index	6.13±6.68	3.22±3.73	7.86±7.42	**<0.0001**
Comorbidity				
CCI	0.63±1.01	0.84±1.23	0.46±0.79	**0.0047**
Inflammatory				
CRP	0.31±0.72	0.29±0.68	0.33±0.74	0.7739
IL-6	2.70±3.64	2.44±3.03	2.84±3.94	0.5025
IL-8	17.28±21.29	18.99±29.23	16.35±15.37	0.4337

Notes: *Data are presented as n or mean ± SD. *P*-values were determined using Student's *t*-test or the chi-square test to compare categorical variables. The bold values denote statistical significance at the $p < 0.05$ level.

Abbreviations: BMI, body mass index; Regions: GN, Gangneung; DY, Danyang; DH, Donghae; SC, Samcheok; YW, Yeongwol; JC, Jecheon; Drug History: ICS/LABA, inhaled corticosteroids/ long-acting beta agonists; Comorbidity: CCI, Charlson Comorbidity Index.

female. Among them, 28.5% of participants were current smokers, 31.5% were former smokers, and 40% were nonsmokers. The COPD group had a significantly higher number of current and former smokers than the non-COPD group (p-value = 0.0471). The body mass index (BMI) was significantly lower in the COPD group than in the non-COPD group (p-value =0.002). Furthermore, GOLD severity was assigned as stage 1 in 52 (26.0%), stage 2 in 58 (29.0%), and stage 3 or 4 in 10 (5.0%) participants. The COPD group had a significantly decreased value of FEV_1/FVC than that in the non-COPD group (p-value = 0.0001). The emphysema index was significantly higher in the COPD group than that in the non-COPD group (p-value =0.0001). In cases with comorbidity, CCI was estimated and it was statistically significant in COPD (p-value = 0.0047). Participants with a drug history of inhaled corticosteroids (ICS) and long-acting beta-agonists (LABA) were evaluated; it did not have a statistically significant (p-value = 0.0888) influence. In addition, the peripheral levels of C-reactive protein (CRP), interleukin (IL)-6, and IL-8 were altered in the COPD group compared to that in the non-COPD group; these are markers of systemic inflammation status and the differences were not significant.

Geometric Analysis of Specific Plasma Sphingolipid Classes and Amino Acid Profiles of COPD Patients

Sphingolipids are highly associated with COPD prevalence.[24] Therefore, the associations between specific sphingolipid classes and COPD-related phenotypes were investigated. The list of sphingolipids included Cer (d18:0/14:0), Cer (d18:0/16:0), Cer (d18:0/18:0), Cer (d18:0/18:1), Cer (d18:0/20:0), Cer (d18:0/24:0), Cer (d18:0/24:1), SM (d18:0/18:0), SM (d18:0/18:1), SM (d18:0/16:0), SM (d18:0/24:0), and SM (d18:0/24:1). Additionally, several reports have indicated that patients with COPD exhibit disturbed amino acid metabolism.[3,25,26] Thus, the amino acid profiles of the COPD and non-COPD cohorts were compared. The results of the geometric analysis indicated that sphingolipids SM (d18:0/16:0), SM (d18:0/24:0), and SM (d18:0/24:1) were significantly associated with COPD (p-value: 0.0200, 0.0392, and 0.0027, respectively) (Table 2).

Associations Between Specific Plasma Sphingolipid Classes and Amino Acids with COPD-Related Phenotypes

Relationships were investigated between specific sphingolipids and amino acids with COPD-related phenotypes, represented by different ranges of emphysema index, FEV_1, and FVC values (Table 3). First, the plasma sphingolipids, Cer (d18:1/18:0) and Cer (d18:1/24:1) were significantly associated with reduced FEV_1 (p-value: 0.0203 and 0.0366, respectively) however, no Cer was significantly associated with the emphysema index and FVC. Overall, no significant

Table 2 Statistical Correlations Between Plasma Metabolites and COPD

	Non-COPD (n=80)		COPD (n=120)		*p-value of t-test	Linear Regression	
	LSmean	SD	LSmean	SD		beta	*p-value
Ceramides (nM)							
C14	2.70	0.05	2.78	0.05	0.3411	0.084	0.3411
C16	5.61	0.04	5.61	0.03	0.8987	0.008	0.8987
C18	4.80	0.07	4.76	0.05	0.7525	0.033	0.7525
C18_1	3.03	0.06	3.00	0.05	0.7956	0.023	0.7956
C20	4.91	0.07	4.94	0.05	0.7681	0.028	0.7681
C24	7.92	0.06	7.96	0.05	0.6808	0.037	0.6808
C24_1	7.09	0.06	7.05	0.05	0.6547	0.040	0.6547
SM18_0	9.46	0.05	9.53	0.04	0.3057	0.076	0.3057
SM18_1	8.70	0.05	8.76	0.04	0.4468	0.054	0.4468
SM16_0	11.10	0.04	11.18	0.03	0.1743	0.079	0.1743
SM24_0	9.72	0.06	9.90	0.04	**0.0357**	**0.177**	**0.0357**
SM24_1	10.51	0.05	10.60	0.04	**0.0265**	**0.086**	**0.0265**

(*Continued*)

Table 2 (Continued).

	Non-COPD (n=80)		COPD (n=120)		*p-value of t-test	Linear Regression	
	LSmean	SD	LSmean	SD		beta	*p-value
Amino acids (μM)							
Glycine	4.68	0.10	4.73	0.07	0.7166	−0.050	0.7166
Alanine	6.05	0.09	5.97	0.07	0.5640	−0.078	0.5640
Serine	4.12	0.09	4.12	0.07	0.9783	−0.003	0.9783
Proline	3.68	0.09	3.62	0.07	0.6830	−0.055	0.6830
Valine	7.43	0.06	7.29	0.04	0.0894	−0.139	0.0894
Threonine	4.32	0.09	4.26	0.07	0.6767	−0.055	0.6767
Taurine	2.99	0.10	3.01	0.07	0.8997	0.017	0.8997
t4_OH_Pro	1.27	0.14	1.00	0.10	0.1722	−0.268	0.1722
Leucine	6.26	0.06	6.12	0.05	0.1158	−0.146	0.1158
Isoleucine	5.27	0.07	5.09	0.05	0.0701	−0.172	0.0701
Asparagine	3.66	0.09	3.62	0.07	0.7211	−0.045	0.7211
Aspartate	2.74	0.11	2.77	0.08	0.8780	−0.023	0.8780
Glutamine	5.92	0.08	5.92	0.06	0.9854	0.002	0.9854
Glutamate	4.69	0.11	4.70	0.08	0.9693	0.006	0.9693
Methionine	7.11	0.13	6.87	0.10	0.2158	−0.238	0.2158
Histidine	2.72	0.09	2.85	0.07	0.2937	0.134	0.2937
Alpha_AAA	0.84	0.11	0.83	0.08	0.9415	0.012	0.9415
Phenylalanine	4.05	0.05	3.97	0.04	0.3114	−0.074	0.3114
Arginine	2.93	0.10	3.00	0.07	0.6114	0.072	0.6114
Ac_Orn	−0.23	0.12	0.11	0.09	0.0552	0.338	0.0552
Citrulline	4.00	0.10	4.09	0.08	0.5656	0.085	0.5656
Tyrosine	3.35	0.09	3.33	0.06	0.8644	−0.021	0.8644
ADMA	−1.70	0.09	−1.54	0.06	0.1981	0.160	0.1981
SDMA	−1.33	0.10	−1.18	0.07	0.2771	0.149	0.2771
Tryptophan	4.54	0.06	4.48	0.04	0.4283	−0.069	0.4283
Kynurenine	1.01	0.08	1.05	0.06	0.7455	0.039	0.7455
Ornithine	5.39	0.07	5.17	0.05	0.0251	−0.229	0.0251
Met_SO	6.80	0.06	6.67	0.04	0.1056	−0.129	0.1056

Notes: *This *p*-value was used for the Student's *t*-test and chi-square test for the analyses of the non-COPD and COPD groups followed by linear regression analysis, respectively. Here, bold values indicates a statistically significant correlation with a *p*-value less than 0.05. The data was adjusted for age, smoking, pack years, BMI, Gold grade, drug history, and CCI and then analyzed using log transformation.

Table 3 Statistical Correlations Between Plasma Metabolites and COPD Phenotypes, Represented by the Emphysema Index, FEV_1, and FVC

	Emphysema		FEV_1		FVC	
	Beta	*p-value	Beta	*p-value	Beta	*p-value
Ceramides (nM)						
C14	0.002	0.6198	0.010	0.8677	0.024	0.5297
C16	0.001	0.7500	−0.053	0.1914	−0.031	0.2367
C18	−0.002	0.7321	**−0.109**	**0.0203**	−0.064	0.1524
C18_1	−0.001	0.7956	−0.027	0.6452	−0.005	0.8955
C20	−0.003	0.5472	−0.082	0.1990	−0.047	0.2495
C24	−0.001	0.7397	−0.081	0.1718	−0.053	0.1582
C24_1	0.001	0.8425	**−0.123**	**0.0366**	−0.035	0.3635
SM18_0	−0.004	0.2664	−0.002	0.9702	−0.006	0.8432
SM18_1	−0.002	0.5824	−0.029	0.5328	−0.013	0.6654

(*Continued*)

Table 3 (Continued).

	Emphysema		FEV_1		FVC	
	Beta	***p-value**	**Beta**	***p-value**	**Beta**	***p-value**
SM16_0	0.002	0.5882	−0.0003	0.9947	−0.007	0.7756
SM24_0	0.003	0.4815	−0.018	0.7449	−0.009	0.8063
SM24_1	0.002	0.6800	0.018	0.7221	0.016	0.6246
Amino acids (μM)						
Glycine	−0.006	0.3301	0.030	0.7387	0.003	0.9533
Alanine	−0.011	0.0844	0.012	0.8900	−0.040	0.4878
Serine	−0.009	0.1411	0.006	0.9419	−0.026	0.6314
Proline	−0.012	0.0575	0.001	0.9902	−0.049	0.3957
Valine	−0.0003	0.9489	−0.020	0.7198	−0.032	0.3625
Threonine	−0.0006	0.9213	−0.048	0.5849	−0.042	0.4572
Taurine	−0.006	0.3361	−0.002	0.9784	−0.010	0.8626
t4_OH_Pro	−0.007	0.4609	−0.197	0.1306	−0.096	0.2503
Leucine	0.0001	0.9894	0.027	0.6619	−0.036	0.3639
Isoleucine	−0.004	0.4310	−0.056	0.3779	−0.064	0.1142
Asparagine	−0.009	0.1187	0.027	0.7499	−0.055	0.3062
Aspartate	−0.014	0.1304	−0.007	0.9414	0.013	0.8458
Glutamine	−0.006	0.3138	−0.027	0.7270	−0.049	0.3243
Glutamate	**−0.020**	**0.0055**	−0.094	0.3596	−0.053	0.4176
Methionine	−0.004	0.6853	0.007	0.9580	0.034	0.6779
Histidine	−0.009	0.1345	0.086	0.3097	0.017	0.7524
Alpha_AAA	**−0.015**	**0.0451**	−0.009	0.9287	−0.013	0.8516
Phenylalanine	0.005	0.1904	−0.050	0.3055	−0.024	0.4429
Arginine	−0.014	0.1398	0.095	0.3111	−0.001	0.9803
Ac_Orn	−0.003	0.7510	−0.047	0.6910	0.003	0.7163
Citrulline	−0.008	0.2555	−0.013	0.8925	−0.027	0.6699
Tyrosine	−0.008	0.1666	0.015	0.8542	−0.042	0.4336
ADMA	−0.007	0.2140	−0.088	0.2870	−0.072	0.1774
SDMA	−0.005	0.4598	−0.072	0.4255	−0.080	0.1677
Tryptophan	−0.002	0.5637	0.029	0.6168	0.013	0.7170
Kynurenine	0.001	0.8120	**−0.036**	**0.0213**	−0.035	0.4899
Ornithine	−0.003	0.5142	−0.001	0.9835	−0.003	0.9485
Met_SO	0.004	0.2383	−0.038	0.4802	−0.021	0.5487

Notes: *This *p*-value was used for the Student's *t*-test and chi-square test for the analyses of the metabolites and COPD phenotypes followed by linear regression analysis, respectively. Here, bold values indicate a statistically significant correlation with a *p*-value less than 0.05. The data was adjusted for age, smoking, pack years, BMI, Gold grade, drug history, and CCI and then analyzed using log transformation.

correlation was identified between any SM and the emphysema index, FVC and FEV_1 in the COPD cohort. Next, associations were investigated between amino acid metabolism and COPD-related phenotypes, represented by different ranges of emphysema index, FEV_1, and FVC values. Furthermore, glutamate and alpha AAA were significantly associated with emphysema in COPD (*p*-value: 0.0055 and 0.0451, respectively). Of all amino acids, only kynurenine was significantly associated with reduced FEV_1 in COPD (*p*-value: 0.0437). On the other hand, none of the amino acids correlated with FVC.

Discussion

This study investigated dysregulation of targeted sphingolipid and amino acid metabolism in a Korean COPD cohort living near cement plants. Interestingly, specific ceramide and sphingomyelin metabolite dysregulation was observed, some of which was significantly associated with worse lung function in patients with COPD. Additionally, significantly

elevated levels of some amino acids were detected in patients with COPD. Thus, the results demonstrated a relationship between specific sphingolipid and amino acid metabolism patterns and COPD-related phenotypes in a Korean cohort.

Sphingolipids are important structural components of cellular membranes. They interact with several proteins to regulate a wide-range of cellular processes, including cell death, proliferation, differentiation, autophagy, and migration.[27,28] Sphingolipids are found in all types of eukaryotic cells and are a unique category of lipids that contain a backbone of sphingoid bases. Based on the O-linked R group, sphingolipids are sub-classified into sphingosines, sphingomyelins, ceramides, or glycosphingolipids.[29] Chronic airway inflammation is a known key pathophysiology in COPD.[30] In addition, sphingolipids have been recognized to be involved in the inflammatory process.[9] In this study, most of the ceramides and sphingomyelins were increased in COPD patients, and some specific sphingomyelins were significantly associated with COPD, such as SM (d18:1/24:0), and SM (d18:1/24:1). Ceramides can also be metabolized to ceramide 1-phosphate and sphingosine 1-phosphate, and both were known as anti-inflammatory sphingolipids.[9] Although the levels of ceramide 1-phosphate and sphingosine 1-phosphate were not measured in this study, more increased expressions of sphingomyelins comparing to those of ceramides in COPD may indicate that ceramides are mainly metabolized to sphingomyelin, not to ceramide 1-phosphate and sphingosine 1-phosphate. Therefore, beyond the identification of the sphingolipid metabolome as a biomarker for worse prognosis for patients with COPD, this metabolomic analysis provides important preliminary data that can be used to study some of the mechanisms that participate in COPD pathogenesis. As the determinants of the variation between the individuals for most metabolites are unknown, the mechanisms underlying most of these associations remain unclear. Nevertheless, more studies are needed, including human and animal studies that examine the manipulation of diet, physical activity, and other lifestyle factors, to gain a full understanding of the normal inter-individual variation. A molecular understanding of the differences that affect the metabolome may lead to a better understanding of COPD.

Circulating amino acids are the main components involved in several physiological functions, such as gluconeogenesis, protein synthesis, cell signaling, and immunity.[31] Growing evidence supports the view that an abnormal amino acid profile may be a significant risk factor for COPD.[32,33] Previous studies have reported decreased serum concentrations of many amino acids in COPD patients compared to healthy individuals.[13,15] Additionally, lower amino acid concentrations were associated with worse clinical outcomes in the active smoking group. Furthermore, some amino acid concentrations were inversely associated with exacerbation frequency in COPD patients.[34] Under certain conditions, skeletal muscle is the major source of amino acids for other tissues. However, plasma free amino acid concentrations are balanced between exogenous uptake and metabolites produced during protein synthesis and breakdown.[35] Several studies have suggested that amino acid profiles are disturbed in the plasma and skeletal muscle of patients with COPD.[13,14,25,36]

Glutamate and alpha AAA were significantly associated with emphysema in COPD. Glutamate and alpha-AAA concentrations are significantly altered in association with emphysema and COPD severity.[15,37] According to Ubhi et al, in emphysematous patients of the ECLIPSE cohort, the levels of glutamine, serine, histidine, arginine, proline, asparagine, aspartic acid, glycine–proline, and lysine were increased, compared to that in the non-emphysematous patients.[15] However, the concentration of aminoadipic acid decreased in patients with severe COPD (GOLD IV) and emphysema.[15] Analysis of the serum from 30 COPD patients and 30 former and never-smokers identified 41 metabolites as markers of COPD, using PLS-DA.[38] These included lower glutamate levels, and elevated arginine and phenylalanine levels.[38] Furthermore, our study findings indicated that kynurenine was significantly associated with reduced FEV_1 in COPD (Table 3). Our results coincide with a report showing elevated kynurenine levels in COPD patients with a decline in lung function.[39] Importantly, whether kynurenine or other amino acids mechanistically contribute to lung function decline or to other COPD phenotypes remains unknown. Kynurenine, known to be associated with systemic inflammation, is accompanied with induced indoleamine 2,3-dioxygenase activity and tryptophan catabolism. In addition, the elevated levels of circulating kynurenine reported in non-COPD subjects might be associated with increased Kyn/Trp ratios, as observed in tuberculosis, pulmonary arterial hypertension, and lung cancer cases.[40–42] Therefore, the ability of this marker to effectively discriminate COPD should be confirmed in additional studies that include other lung pathologies.

This study had several limitations that could influence the context of the results. Prolonged storage of samples in biobanks can lead to artificial alterations in metabolite levels resulting in data bias in metabolomics experiments.[43] However,

we used samples that satisfied the collection and storage criteria defined in the earlier metabolomics study.[44] The amino acids and lipids in human plasma are rarely altered within the first seven years of storage.[45] Second, although several metabolites were significantly associated with emphysema and lung function indicators in patients with COPD, the relatively small sample size limited the statistical power of the analysis and selection of statistical methods. Thus, causality needs to be confirmed with further studies. Third, although correlations between metabolite changes and lung function were adjusted for age, smoking status, and BMI, exogenous factors such as diet, alcohol, or medication may also lead to metabolic changes. Additional studies should assess the possible effects of these factors. Forth, the results of this study did not reveal the biological mechanisms underlying the detected metabolite changes or explore the power of dynamic metabolites to predict disease progression and survival, which are clinically significant. Nonetheless, the study identified metabolites associated with lung function that might influence the pathogenesis of COPD and provides a potential basis for drug development.

Conclusion

In conclusion, our study provides important information regarding associations between plasma sphingolipid and amino acid metabolism and lung function indicators of patients with COPD. The presence of COPD-specific metabolic changes supports the use of relevant metabolites as biomarkers in COPD. Future studies should investigate the potential relationship between these metabolites and COPD pathogenesis, thus laying the groundwork for application of molecular biomarkers for disease diagnosis, prediction of disease progression and severity, and selection of appropriate treatments.

Data Sharing Statement

Data from this study will be made available by the corresponding author (Woo Jin Kim) upon reasonable request.

Ethics Approval and Informed Consent

This study was approved by the Kangwon National University Hospital IRB (KNUH 2020-06-007) and all study protocols were conducted according to the Institutional Review Board of Kangwon National University Hospital. All participants in this study provided written informed consent. Also, this study complies with the Declaration of Helsinki.

Consent for Publication

All authors agreed to this publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in relation to this work.

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