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Article

Metabolic Aging as an Increased Risk for Chronic Obstructive Pulmonary Disease

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Abstract: Both aging and chronic obstructive pulmonary disease (COPD) are strongly associated with changes in the metabolome; however, it is unknown whether there are common aging/COPD metabolomic signatures and if accelerated aging is associated with COPD. Plasma from 5,704 subjects from COPDGene and 2,449 subjects from SPIROMICS were profiled using the Metabolon global metabolomics platform (1,013 annotated metabolites). Post-bronchodilator spirometry measures of airflow obstruction (forced expiratory volume at one second (FEV₁)/forced vital capacity (FVC) < 0.7) were used to define COPD. Elastic net regression was trained on never and former smokers with normal spirometry and no emphysema to create a metabolomic age score which was validated in SPIROMICS subjects. Our metabolic age score was strongly associated with chronic age in the validation cohort (correlation coefficient 0.8). COPD subjects with accelerated aging (> 7 years difference between metabolic and actual age) had more severe disease compared with those who had decelerated aging (< -7 years difference between metabolic and actual age). COPD and aging metabolites were shared more than expected ($P < 0.001$) with overrepresented included amino acid and glutathione metabolism pathways. These findings suggesting a common mechanism between aging and COPD and that COPD is associated with accelerated metabolic aging.

Keywords: aging; COPD; metabolic age

1. Introduction

Persistent respiratory symptoms and airflow limitation due to chronic obstructive pulmonary disease (COPD) makes the disease a strong negative impactor of quality of life, as well as a leading cause of death and hospitalizations both worldwide and in the United States [1,2]. These symptoms are usually caused by repeated exposure to noxious particles or gases, including cigarette smoke, that damage the airways and lungs, ultimately resulting in airway and/or alveolar abnormalities [3]. COPD is also characterized by many comorbidities including muscle wasting, cardiovascular disease, osteoporosis, and depression [4]. This suggests that COPD is a generalized systemic disease not just limited to the lungs, and that there are subsequent downstream changes to molecular pathways, including disturbances to the metabolome. Many of these comorbidities are also associated with age; however, COPD patients often present with these comorbidities earlier than those at a similar age without COPD, indicating that COPD might be a disease of early aging [5]. In this study we examine the metabolomic signatures of COPD and aging to identify the shared and distinct pathologic signatures of each disease.

The aging metabolome is now well established and includes changes in energy metabolism (e.g., decreasing nicotinamide adenine dinucleotide (NAD⁺)), lipids (e.g., those carried by low-density lipoprotein (LDL) particles) such as ceramides and sphingomyelins), and amino acids [6]. For instance, in a large NMR study of 135 metabolites from 26,065 individuals of Northern European ancestry (Finns and Estonians), age-specific and sex-specific metabolic fingerprints were identified from the serum (Finns) or plasma (Estonians) with significant differences observed with age in males and females for lipoproteins, cholesterol, and triglyceride levels, whereas atherogenic metabolites and certain amino acids were only found to be increased in females during the time of menopausal transition [7]. In a different small untargeted LC-MS study of 126 plasma metabolites from 15 young and 15 elderly individuals there were 14 compounds associated with age [8]. The metabolites that declined with age included antioxidants and compounds associated with high physical activity (e.g., carnosine, UDP-acetyl-glucosamine, NAD⁺, and leucine), whereas metabolites that increased with age included those that were associated with a decline in renal and liver functions. In another more comprehensive LC-MS/MS study of 2,578 plasma metabolites from 150 healthy humans from age 30 to 100 years, there were age-related metabolites that were gender and non-gender specific including multiple lipid species that decreased with aging [9]. These studies illustrate some of the systemic age-related metabolic changes that are common across multiple different metabolomics platforms.

The relationship between individual metabolites and COPD has also been explored, particularly for spirometry tests of lung function. Specifically, three metrics obtained from these tests are typically used: forced expiratory volume in 1 second (FEV₁), FEV₁ percentage of predicted (FEV₁%), and the FEV₁/Forced Vital Capacity (FVC) ratio (FEV₁/FVC) (see review [10]). Multivariate models using more than one metabolite have also been used to predict COPD. Pinto-Plata et al. used machine learning methods to identify distinct plasma metabolomic profiles between survivors and non-survivors of COPD two years prior to their death [11]. These profiles were also characterized by differences in energy metabolism pathways. In Godbole et al 2022 [12], metabolomic scores with 132 and 129 metabolites were used to predict FEV₁ and emphysema respectively. These models performed better than models with clinical covariates alone. COPD metabolites were enriched in arginine biosynthesis; aminoacyl-tRNA biosynthesis; and glycine, serine, and threonine metabolism. Others have created metabolomic scores to predict COPD acute exacerbations [13], as well as a number of other diseases including coronary heart disease [14,15], type 2 diabetes [16], incident heart failure [17], and pulmonary arterial hypertension [18]. Many of these scores were derived with regularized regression models, a family of statistical methods which avoid overfitting from datasets with many features by penalizing and constraining the coefficient estimates. Others used machine learning methods, such as Random Forest, which may be powerful but have the disadvantage of less interpretability. When creating scores to predict pulmonary arterial hypertension, Alotaibi et al found relatively similar metabolites identified across 6 methods including elastic net and several machine learning methods. Furthermore, they found that elastic net selected more metabolites that were not as highly inter-correlated compared to those that conventional linear regression or random forest identified, suggesting that elastic net may perform better in capturing metabolites from a variety of different pathways, while also being more efficient and interpretable [18].

Elastic net has also been used by others to create -omic age scores. This began with Horvath [19] and Hannum et al. [20] who both trained Elastic Net models on transcriptomic data to predict age, arriving at transcriptomic age scores. Horvath found accelerated age, or when the transcriptomic age score was greater than the chronological age, in cancer tissues. Hannum et al found that the rate of transcriptomic age was impacted by gender and genetic variants. Since then, many have created age scores using a variety of -omic markers and the concept has been named “biological” age. Many have also found that accelerated biological age is associated with a number of health outcomes. These studies have been reviewed in Rutledge et al. [21] and Li et al. [22]. Although elastic net has been used to create age and COPD metabolite scores, there have been no investigations into the overlap between the two.

This study investigates the overlap between the aging and COPD metabolome and address some of the limitations of previous studies by using two independent, well-phenotyped case-control

populations with 8,153 subjects who have comprehensive plasma metabolomics profiling > 1,000 annotated metabolites. The study is the largest COPD metabolomics study to date. It also creates a normal lung function aging score as well as a COPD metabolome score and then contrasts the metabolites that make up aging scores with COPD scores to identify common and distinct aging and COPD metabolic features as well as identify COPD subjects who have accelerated aging.

2. Materials and Methods

Cohorts

The Genetic Epidemiology of COPD (COPDGene) (ClinicalTrials.gov Identifier: NCT00608764), is a NIH-sponsored multicenter cohort of never, former, and current smokers 45-80 years old. Written Informed consent was obtained from all subjects involved in the study. Details of the COPDGene study are provided elsewhere [23]. In brief, this study enrolled 10,198 non-Hispanic white and African American participants with at least 10 pack-years of smoking, and 465 individuals with no smoking history. Metabolomic profiling was performed on 5,704 participants from the second visit of the study.

The Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) (ClinicalTrials.gov Identifier: NCT01969344) is a NIH-sponsored multicenter cohort of never, former, and current smokers 40-80 years old. Written Informed consent was obtained from all subjects involved in the study. Details of the cohort are provided elsewhere [24]. In brief, this study recruited 2,771 participants with at least 20 pack-years of smoking and 202 participants who were never smokers. 73% of participants self-identified as non-Hispanic white. 2,449 had plasma available for metabolomic profiling of baseline fasting blood samples. Metabolomic profiling as performed on the baseline blood draw of the study.

Clinical Data and Definitions

COPD was defined using post-bronchodilator FEV₁/FVC with a case defined as FEV₁/FVC < 0.7. Emphysema was quantitated by CT scan at total lung capacity using low attenuation area (LAA) voxels < -950 Hounsfield units. GOLD 0 grade (FEV₁ ≥ 80% & FEV₁/FVC ≥ 0.7), no emphysema by LAA < 5%, and never smoking or former smokers who stopped smoking at least 5 years ago were used to define control subjects. A never smoker was defined as someone who smoked < 100 lifetime cigarettes. A former smoker was defined as someone who had quit smoking and not smoked a cigarette in the past 30 days.

Metabolomic Profiling and Processing

Plasma samples from both cohorts were profiled using Metabolon (Durham, NC, USA) Global Metabolomics Platform, as described previously, although profiling for each cohort occurred approximately 6 months apart [12]. Metabolite values were batch normalized, within each study, by dividing by the median metabolite value for each metabolite within a batch. After batch normalization, metabolite principal components showed a significant reduction in association with batch, so no further normalization was needed[25]. For this analysis, metabolites were excluded if missing in >20% of subjects and we only examined metabolites that had the same missing pattern in both studies (e.g., medications and nicotine metabolites). This resulted in 831 metabolites of the 1,314 metabolites identified in both studies. The missing values of these 831 metabolites were then imputed with k- nearest neighbor imputation (kNN; k = 10) using the R package 'impute', and then log₂-transformed for all analyses. The other metabolites that were excluded from analysis had discrepant missing patterns: 122 metabolites had <20% missing samples in one study and 20–80% missing in the other, and 361 metabolites had at least one cohort with ≥80% missing samples.

Software, Statistical and Bioinformatics Analysis

The statistical software R Version 4.0.2 was used for all analyses. The R package glmnet was used for ElasticNet, stats for over-representation analysis and univariate linear models, and table1 for creating characteristic tables. The coefficients from ElasticNet are used as weights to develop a final score and are interpretable in terms of the linear relationship between the metabolite and the outcome. For the aging score, the elastic net model was trained on COPDGene never and former smokers (subjects who quit at least 5 years ago, had less than 5% emphysema, and normal spirometry). We then tested the score on SPIROMICS subjects meeting the same criteria. The final score was then applied to other independent former and current smokers in each of the cohorts, looking separately at those without obstruction (GOLD 0) and with less than 5% emphysema compared (described as current/former smokers without COPD and emphysema) to those who do not meet those criteria (described as current/former smokers with COPD or emphysema). For the lung obstruction score, all COPDGene metabolite data were used to train an elastic net model predicting continuous FEV₁/FVC. It was then tested on all SPIROMICS data. For both scores, 10-fold cross validation was performed in the training data set to obtain the penalty parameter (λ), which corresponded to the largest lambda that minimizes MSE plus one standard error. This was iterated across alpha values of 0 to 1 at a 0.05 interval to identify the alpha that produced the best performing model. The metabolite score models did not include clinical covariates. Predictions were created with the metabolite coefficients and the set of metabolites selected by the ElasticNet procedure.

To assess the difference between metabolomic age and actual age, a spline was fitted between age_{actual} and age_{metabolomic} using the smooth.spline function from the stats R package. The age difference is then calculated by age_{metabolomic} – age_{spline}. To compare the subjects on the tail ends of age difference while maintaining sufficient sample size, we used a cutoff of 7 years in former and current smokers with COPD or emphysema to select about 10% of the subjects who were most accelerated or decelerated metabolic age. Specifically, subjects with accelerated age were defined as age_{metabolomic} – age_{spline} > 7 years and those having decelerated age as age_{metabolomic} – age_{spline} < -7 years. We examined differences in the demographic and clinical characteristics between these two groups, using a t-test for continuous variables and a Chi-squared test for categorical/binary variables. This was repeated separately in current smokers without COPD and emphysema. A chi-squared test was used to test the probability that the overlap in metabolites happened by chance.

Pathway Analysis

Pathway analysis was conducted by a Fisher's test to determine over-representation of sub- and super-pathways based on metabolites of interest. The sub- and super-pathways used were annotated and provided by Metabolon (Durham, NC, USA). 129 of the 831 metabolites used in our analysis have not been annotated yet. The Fisher's test p-values were also adjusted using the Benjamini-Hochberg procedure to account for multiple-testing [26].

Univariate Associations

To identify metabolites associated with age and FEV₁/FVC, simple univariate linear models without any other covariates were conducted with each phenotype. For association with FEV₁/FVC, all subjects were used for analysis. The analysis with age was repeated separately in never/former smokers without emphysema or COPD and in current/former smokers with COPD or emphysema.

3. Results

Demographic Characteristics

Demographics and clinical characteristics for the COPDGene never/former smoker without COPD or emphysema training, SPIROMICS never/former smoker without COPD or emphysema testing, and COPDGene and SPIROMICS other former and current smoker application groups are presented in **Table 1**. In general, the current smokers without COPD or emphysema were slightly

younger, more likely to be women and African American, and less likely to have coronary artery disease ($p < 0.001$).

Table 1. Characteristics of COPDGe and SPIROMICS subjects.

	never/former smokers without COPD or emphysema		current smokers without COPD or emphysema		former/current smokers with COPD or emphysema		
	COPDGe ne (N=1,346)	SPIROMI CS (N=413)	COPDGe ne (N=818)	SPIROMI CS (N=323)	COPDGe ne (N=3,540)	SPIROMI CS (N=1,681)	P- value
Age (years), mean (SD)	65.4 (9.25)	62.9 (9.51)	59.1 (6.33)	55.2 (8.82)	66.2 (8.56)	65.0 (8.05)	< 0.001
Race White/Black/Other %	87.4/12.6 /0	81.8/12.3/ 5.9	42.7/57.3 /0	55.7/38.7/ 5.6	71.3/28.7 /0	80.5/15.2/ 4.3	< 0.001
Gender, Male, n (%)	582 (43.2%)	181 (43.8%)	387 (47.3%)	154 (47.7%)	1888 (53.3%)	945 (56.2%)	< 0.001
Smoking Status Never/Former/Current %	29.3/70.7 /0	40/60/0	0/0/100	0/0/100	0/65.2/34 .8	0/67.3/32. 7	NA
Num. recent exacerbations	0 (0, 1.00)	0 (0, 1.00)	0 (0, 1.00)	0 (0, 2.00)	0 (0, 2.00)	0 (0, 2.00)	< 0.001
GOLD stage, n (%)							NA
GOLD 0	951 (70.7%)	248 (60.0%)	818 (100%)	323 (100%)	514 (14.5%)	130 (7.7%)	
GOLD 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	507 (14.3%)	334 (19.9%)	
GOLD 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1063 (30.0%)	667 (39.7%)	
GOLD 3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	565 (16.0%)	347 (20.6%)	
GOLD 4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	235 (6.6%)	142 (8.4%)	

	never/former smokers without COPD or emphysema		current smokers without COPD or emphysema		former/current smokers with COPD or emphysema		P- value
	COPDGe ne (N=1,346)	SPIROMI CS (N=413)	COPDGe ne (N=818)	SPIROMI CS (N=323)	COPDGe ne (N=3,540)	SPIROMI CS (N=1,681)	
PRISm	12 (0.9%)	0 (0%)	0 (0%)	0 (0%)	656 (18.5%)	61 (3.6%)	
Never smoker	383 (28.5%)	165 (40.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
FEV₁ (Liters)	2.71 (0.700)	2.85 (0.697)	2.69 (0.671)	2.89 (0.704)	1.86 (0.792)	1.86 (0.823)	NA
Emphysema%	1.59 (1.85)	1.64 (1.39)	0.883 (1.06)	0.997 (0.903)	8.01 (10.6)	10.4 (11.2)	NA
FVC (Liters)	3.45 (0.876)	3.62 (0.882)	3.45 (0.884)	3.71 (0.908)	2.98 (0.967)	3.38 (1.06)	NA
FEV₁/FVC	0.787 (0.0493)	0.788 (0.0505)	0.784 (0.0487)	0.782 (0.0487)	0.616 (0.151)	0.542 (0.147)	NA
History of diabetes, n (%)	169 (12.6%)	54 (13.1%)	125 (15.3%)	25 (7.7%)	670 (18.9%)	231 (13.7%)	< 0.001
History of stroke, n (%)	20 (1.5%)	14 (3.4%)	28 (3.4%)	10 (3.1%)	130 (3.7%)	66 (3.9%)	0.003 23
History of heart attack, n (%)	56 (4.2%)	17 (4.1%)	32 (3.9%)	5 (1.5%)	244 (6.9%)	123 (7.3%)	< 0.001
History of coronary artery disease, n (%)	86 (6.4%)	24 (5.8%)	32 (3.9%)	7 (2.2%)	346 (9.8%)	172 (10.2%)	< 0.001
Chronic Bronchitis, n (%)	46 (3.4%)	30 (7.3%)	116 (14.2%)	74 (22.9%)	643 (18.2%)	367 (21.8%)	< 0.001

Exacerbations included those treated with antibiotics and/or corticosteroids in the 12 months prior to the visit; shown are n (percentage), mean (standard deviation), or median (5th, 95th percentiles); spirometry volumes are in post-bronchodilator therapy and in liters; GOLD 0 (FEV₁ ≥ 80% & FEV₁/FVC ≥ 0.7) | GOLD 1 (FEV₁ ≥ 80% & FEV₁/FVC < 0.7) | GOLD 2 (50% ≤ FEV₁ < 80% & FEV₁/FVC < 0.7) | GOLD 3 (30% ≤

never/former smokers without COPD or emphysema		current smokers without COPD or emphysema		former/current smokers with COPD or emphysema		P- value
COPDGe	SPIROMI	COPDGe	SPIROMI	COPDGe	SPIROMI	
ne	CS	ne	CS	ne	CS	
(N=1,346)	(N=413)	(N=818)	(N=323)	(N=3,540)	(N=1,681)	

FEV₁ < 50% & FEV₁/FVC < 0.7) | GOLD 4 (FEV₁ < 30% & FEV₁ /FVC < 0.7) | PRISm (Preserved Ratio, Impaired Spirometry) (FEV₁/FVC ≥ 0.7 but FEV₁ < 80%); history of diabetes, stroke, heart attack, and coronary artery disease based on subject self-report; chronic Bronchitis defined by answers to questions about both cough and phlegm.

Metabolomic Age Score

Elastic net (alpha 0.05) selected 378 metabolites for the age model. When testing this model using independent never and former smoker control subjects without COPD or emphysema, the predicted-actual age correlations were 0.875 in COPDGene and 0.806 in SPIROMICS (Figure 1). Leucine, Isoleucine and Valine Metabolism was the most overrepresented sub-pathway and Amino acids were the most overrepresented super-pathway (Supplemental table 1B&C).

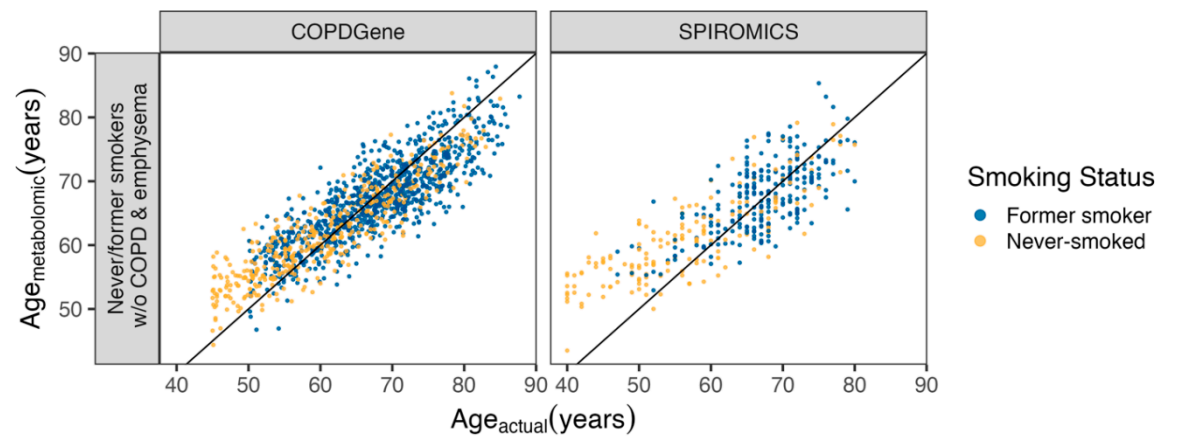


Figure 1. Metabolite age score. The age model was trained on COPDGene never (yellow) and former (blue) smokers without COPD and emphysema (left panel) and tested on SPIROMICS never and former smokers without COPD and emphysema (right panel). The subjects’ actual ages are on the x-axis and predicted metabolomic age on the y-axis.

Differences between COPD Subjects with Accelerated and Decelerated Metabolomic Age

To evaluate whether metabolic age difference was associated with COPD, a spline was fit between actual and metabolomic age (Figure 2). Current and former smokers with COPD or emphysema with accelerated age (age_{metabolomic} – age_{spline} > 7) were compared to those with decelerated age (age_{metabolomic} – age_{spline} < -7) (Table 2). Using a spline allowed us to compare subjects on each tail end of the age difference within the same age group more equivalently, particularly in younger subjects where the sample size was smaller. Age_{spline} did not differ greatly from Age_{actual}, with a mean difference of 0.5462 years and 25th and 75th percentiles of -1.6768 and 2.6501 years respectively. Those with accelerated metabolic age were more likely to be white (p < 0.001), female (p < 0.001), former smokers (p < 0.001), have more severe COPD by GOLD grade (p < 0.001), worse spirometry and more

emphysema ($p < 0.001$), and to have higher rates of diabetes and cardiological comorbidities. The same comparison in current smokers without COPD or emphysema (**Supplement table 2** and **Supplement figure 1**) showed that subjects without COPD or emphysema with accelerated metabolic age were also more likely to be white and female. They also were more likely to have had a stroke and chronic bronchitis, as well as greater rates of other comorbidities that did not reach statistical significance.

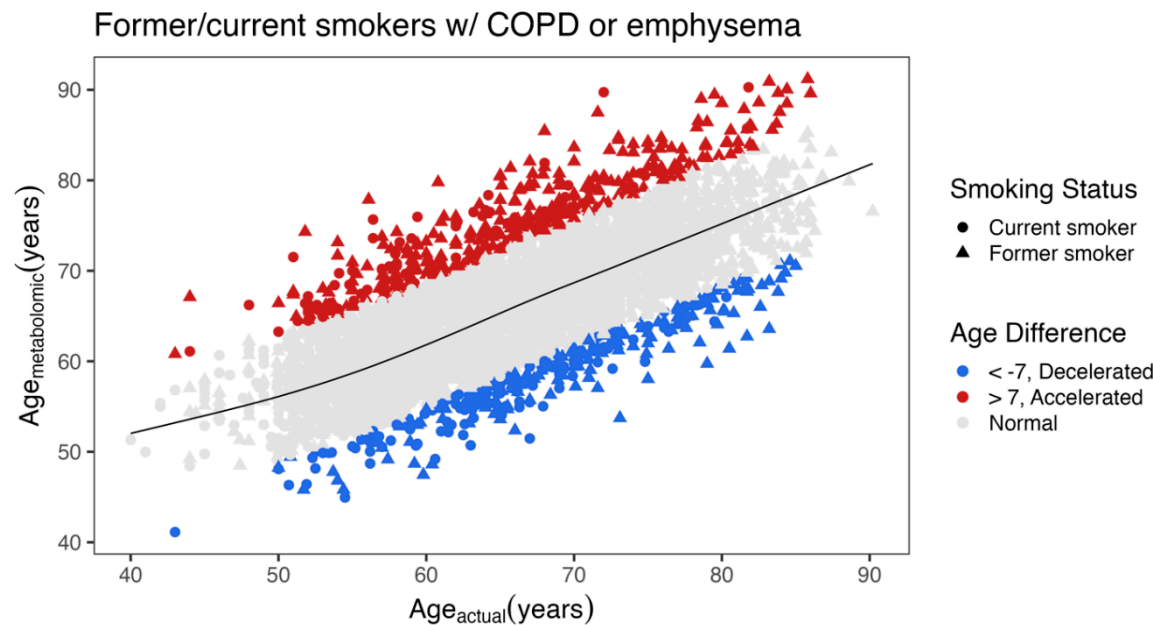


Figure 2. Age acceleration in former (triangle) and current (circle) smokers with COPD or emphysema. A spline was fitted between age_{actual} and $age_{metabolomic}$ (black line). Age difference is calculated by $age_{metabolomic} - age_{spline}$, with subjects having accelerated metabolic age (red) having a difference greater than 7 years and those having decelerated age (blue) having a difference less than -7 years.

Table 2. Accelerated age ($age_{metabolomic} - age_{spline} > 7$) vs. decelerated age ($age_{metabolomic} - age_{spline} < -7$) in current and former smokers with COPD or emphysema.

	Decelerated (N=277)	Accelerated (N=400)	P-value
Age (years), mean (SD)	67.9 (8.32)	65.3 (8.56)	< 0.001
Metabolomic age (years)	58.3 (5.79)	75.2 (6.18)	NA
Race White/Black/Other %	45.1/53.4/1.5	88.3/10.5/1.4	< 0.001
Gender, Male, n (%)	197 (71.1%)	172 (43.0%)	< 0.001
Smoking Status Former/Current %	57.8/42.2	75.0/25.0	< 0.001
Exacerbations	0 (0, 2.00)	0 (0, 2.00)	0.0178
GOLD stage, n (%)			

	Decelerated (N=277)	Accelerated (N=400)	P-value
GOLD 0	38 (13.7%)	27 (6.8%)	< 0.001
GOLD 1	65 (23.5%)	45 (11.3%)	
GOLD 2	90 (32.5%)	131 (32.8%)	
GOLD 3	41 (14.8%)	93 (23.3%)	
GOLD 4	10 (3.6%)	50 (12.5%)	
PRISm	33 (11.9%)	54 (13.5%)	
FEV ₁ (liters)	2.00 (0.801)	1.60 (0.724)	< 0.001
Emphysema%	7.16 (9.45)	10.8 (12.4)	< 0.001
FVC	3.21 (0.967)	2.87 (0.938)	< 0.001
FEV ₁ /FVC	0.613 (0.136)	0.554 (0.160)	< 0.001
History of diabetes, n (%)	43 (15.5%)	96 (24.0%)	0.00879
History of stroke, n (%)	12 (4.3%)	29 (7.3%)	0.157
History of heart attack, n (%)	7 (2.5%)	59 (14.8%)	< 0.001
History of coronary artery disease, n (%)	11 (4.0%)	79 (19.8%)	< 0.001
Chronic Bronchitis, n (%)	52 (18.8%)	87 (21.8%)	0.396

Exacerbations included those treated with antibiotics and/or corticosteroids in the 12 months prior to the visit; shown are n (percentage), mean (standard deviation), or median (5th, 95th percentiles); spirometry volumes are in post-bronchodilator therapy and in liters; GOLD 0 (FEV₁ ≥ 80% & FEV₁/FVC ≥ 0.7) | GOLD 1 (FEV₁ ≥ 80% & FEV₁/FVC < 0.7) | GOLD 2 (50% ≤ FEV₁ < 80% & FEV₁/FVC < 0.7) | GOLD 3 (30% ≤ FEV₁ < 50% & FEV₁/FVC < 0.7) | GOLD 4 (FEV₁ < 30% & FEV₁/FVC < 0.7) | PRISm (Preserved Ratio, Impaired Spirometry) (FEV₁/FVC ≥ 0.7 but FEV₁ < 80%); history of diabetes, stroke, heart attack, and coronary artery disease based on subject self-report; chronic Bronchitis defined by answers to questions about both cough and phlegm.

A metabolomic Lung Obstruction Score

We next evaluated metabolites associated with obstruction on spirometry. 461 metabolites were selected by elastic net with an alpha of 0.1 for the lung obstruction metabolomic score model. Correlations between actual and metabolome predicted FEV₁/FVC were 0.705 in COPDGene training and 0.651 in SPIROMICS testing (Figure 3).

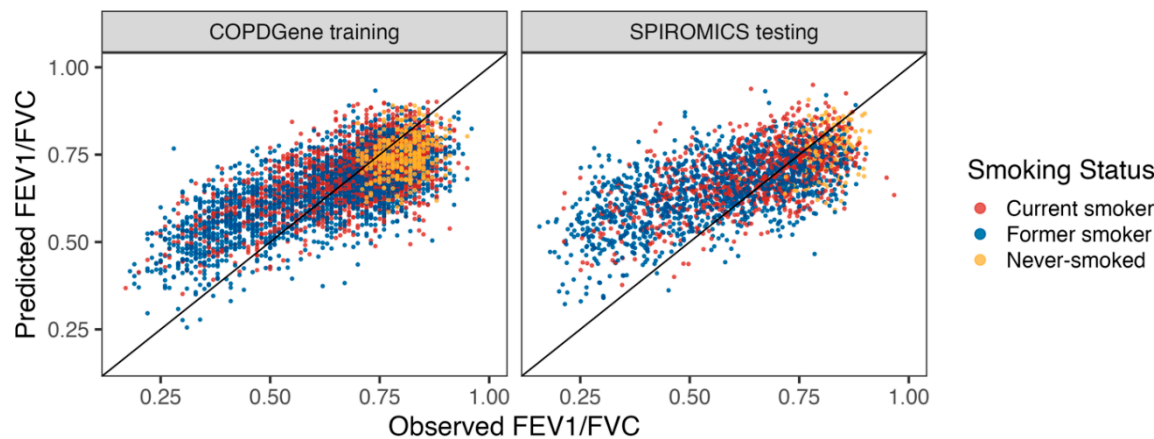


Figure 3. Metabolite based lung obstruction score. Shown on the x- and y-axis is observed and predicted FEV₁/FVC respectively. COPDGene (left) was used as the training dataset (cor = 0.705) and SPIROMICS (right) as the testing dataset (cor = 0.651), with an alpha of 0.1.

Overlap between the Age and COPD Metabolome Scores

Between the elastic net models of age and lung obstruction, there was a higher than expected by chance overlap in the metabolites selected (**Figure 4**; $P < 0.001$). Amino acids were overrepresented in both the lung obstruction and age models although from different sets of amino acids (**Supplement table 1C&E**). For example, metabolites in the FEV₁/FVC model, but not in an aging model included dimethylarginine, and specific sphingolipids and ceramides. Metabolites in both models included creatinine, urea, cortisol.

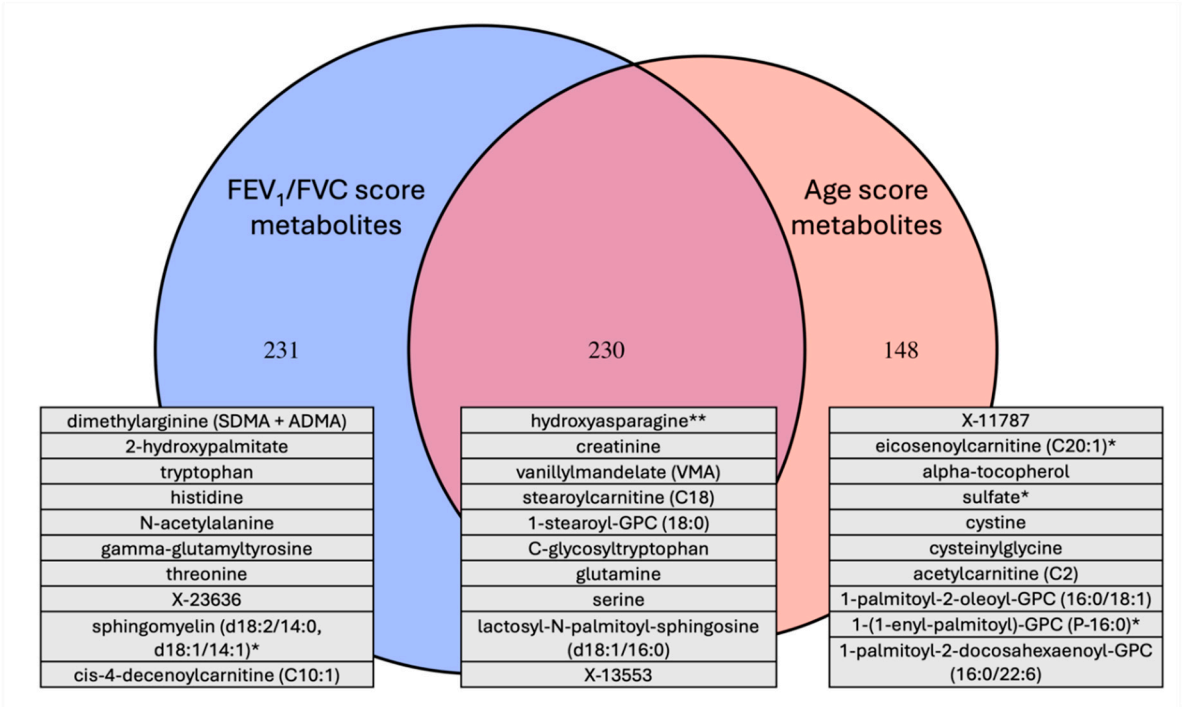


Figure 4. Overlap between metabolites in the COPD and age metabolite score models. There were 230/831 common to both models, 231/831 included only in the COPD model and 148/831 only in the age model. The 10 largest beta coefficient metabolites for age (overlap and age only) or obstruction (FEV₁/FVC score).

Overlap between Metabolite Univariate Associations with Age and COPD

In general, most metabolites univariately associated with age were also associated with lung obstruction with similar trend and p-value (**Figure 5, Supplemental Table 1F**). Examples of the metabolites that were higher with age and obstruction are vanillylmandelate, hydroxyasparagine, and arabonate/xylonate. Others such as androstenodiol and homoarginine were lower with age and amount of obstruction. These metabolites that were significant and concordant in both age and COPD were highly enriched in the Androgenic Steroids sub-pathway and in the nucleotide super-pathway (**Supplemental Table 1G&H**). Only a few significant metabolites were significant for only age or COPD or were discordant with age and obstruction severity. Those significant for only age include 3-methylglutaconate, N,N-dimethyl-pro-pro, dimethylarginine, and urea. Those only significantly associated with FEV₁/FVC include valine and methyl-4-hydroxybenzoate sulfate. The metabolites 2-O-methylascorbic acid, myo-inositol, choline, tetrahydrocortisol glucuronide were among those that were significantly associated with both age and FEV₁/FVC but with trends in discordant directions. These discordant metabolites were enriched in several sub-pathways including the Urea cycle; Arginine and Proline Metabolism (p-value = 0.002) and corticosteroids (p-value = 0.014), although these were no longer significant following multiple-testing corrections (**Supplemental Table 1I**).

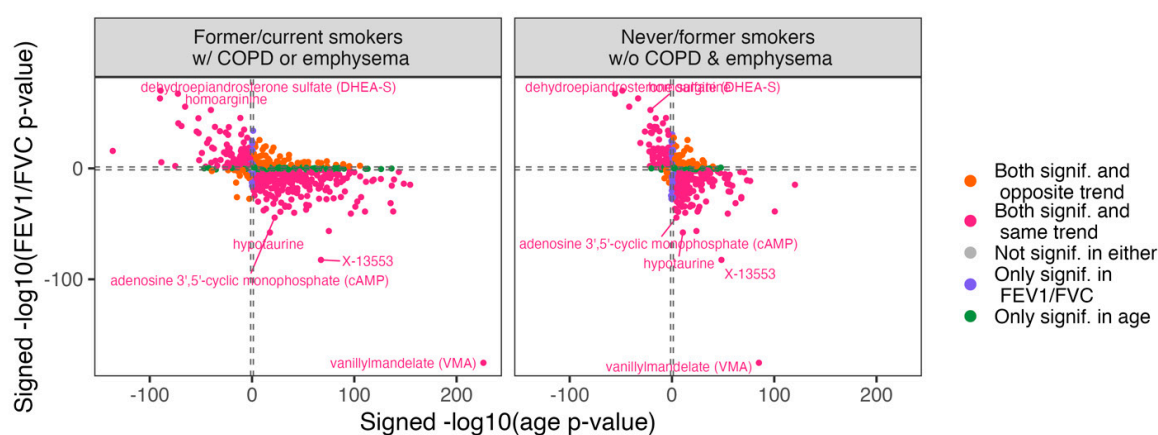


Figure 5. Overlap of metabolite associations with age and lung obstruction in never and former smokers without COPD or emphysema (left panel) and in former and current smokers with COPD or emphysema (right panel). The sign of the coefficient estimate is multiplied by the $-\log_{10}(\text{p-value})$, with the x-axis showing results from univariate metabolite association with age and the y-axis showing results from univariate metabolite association with FEV₁/FVC. Dashed lines at $\log_{10}(0.05)$ and $-\log_{10}(0.05)$ on both axes distinguish proteins that were statistically significant.

4. Discussion

COPD is a disease that occurs primarily in older persons. While COPD has been postulated to be a disease of accelerate lung aging for some time (PMID: 19136405), this is the first study to show that many of the metabolic signatures of COPD and age overlap and that accelerated aging is associated with worse COPD. Although aging is a complex multisystem process, López-Otín et al. described aging in reference to multiple hallmarks of aging. These hallmarks include: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [27,28]. Some of these features have been described in COPD. For instance, other biomarkers of aging such as telomere shortening [29] and oxidative damage to DNA [30,31], and mTOR pathway [32,33] are associated with both aging and COPD or emphysema. In this study we also find evidence for shared pathology with deregulated nutrient signaling with many amino acids and carnitines associated with both age and COPD. We also find evidence for altered intercellular signaling as with androstenodiol and sphingolipids. The strongest positive

association between age and COPD was VMA, which is a metabolite of catecholamines and may reflect elevated physiologic or psychologic stress [34].

Dimethylarginine (DMA) was one of 231 metabolites selected in the lung obstruction score but not in the age score. DMA is a product of methylated arginine residue protein degradation and may play a role in inflammation through inhibition of nitric oxide synthases [35]. We have previously reported an association with DMA and COPD in Godbole et al. 2022, as well as with the arginine pathway. As we noted in Godbole et al. 2022, several small studies and mouse models have also identified this relationship [35–39]. Other strong markers for both age and COPD were anabolic steroids such as androstenediol, albeit in an opposite direction where lower levels were associated with more severe COPD and older age. Lower levels of these hormones may explain with both older age and COPD are associated with lower muscle mass.

We identified several metabolites that had significant univariate associations only with COPD, or metabolites that were significantly associated with both age and COPD but in discordant directions. These findings point to some biological processes unique to COPD separate from healthy aging. Those only significantly associated with FEV₁/FVC include valine and methyl-4-hydroxybenzoate sulfate. Valine, involved in BCAA degradation, has previously been found to distinguish between patients with advanced COPD (GOLD grade 4) from healthy controls and is associated with increased systemic inflammation [40]. The discordant metabolites included 2-O-methylascorbic acid, myo-inositol, choline, tetrahydrocortisol glucuronide and represented the urea cycle; Arginine and Proline Metabolism and corticosteroids sub-pathways.

A major limitation of this study is that it is observational, and thus is not clear whether any of these pathways can be modified to prevent COPD progression. For instance, there have been mixed results with androgenic steroids to treat COPD and mTOR targeted interventions have not been successful for COPD. While diminished energy metabolism as evidence by amino acids and carnitine pathways, this may be secondary to the general inactivity of COPD patients, possibly from ventilatory limitation which prevents normal energy use. It would be useful to see if these biomarkers improve after COPD patients undergo a combine physical and nutritional rehabilitation. We did not use any clinical or demographic covariates in our analyses, which could potentially change the performance of the age and obstruction scores and change the results of the univariate associations. However, our univariate associations can provide a sense of metabolomic correlations with lung obstruction and age without the effect of covariates. We also did not adjust for lifestyle factors including diet, exercise, or medications, which also could have strong effects on the metabolome. Both our age and lung obstruction scores had poorer performance at the lower range of each outcome. This is likely due to smaller sample sizes of younger subjects and subjects with strong lung obstruction and would likely improve with greater sample sizes.

5. Conclusions

This study provides strong molecular evidence for shared pathophysiology of aging in COPD including diminished nutritional functioning (amino acids, carnitine), impaired androgenic signaling, and increased stress and inflammation (VMA, sphingolipids, DMA). People who have accelerated metabolic aging also have more severe COPD, thus any interventions that reduce aging, might also diminish COPD progression.

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