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ORIGINAL RESEARCH

Proteomic Mediators of Chronic Obstructive Pulmonary Disease Phenotypes and Coronary Artery Calcification Burden in Ever Smokers

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BACKGROUND: Chronic obstructive pulmonary disease (COPD) increases cardiovascular disease risk. Coronary artery calcification (CAC) predicts cardiovascular events and mortality in COPD. We hypothesized that plasma proteins linked to pulmonary phenotypes mediate CAC burden.

METHODS: Pulmonary function, emphysema, airway wall thickening, Agatston CAC scores (inverse normal transformed), and relative abundance of 1305 plasma proteins (log-transformed) were assessed in 989 Phase 1 COPDGene (Genetic Epidemiology of COPD) participants. Proteins associated with both pulmonary phenotypes (FEV_1[forced expiratory volume in 1 second]%predicted, FVC [forced vital capacity], FEV_1/FVC, emphysema, airway wall thickness, wall area percentage) and CAC (false discovery rate $P \leq 0.20$) were evaluated using multivariable mediation. Model adjustments included sex, age, race, body mass index, smoking, comorbidities, and medications. Adjustment for pulmonary artery-to-aortic diameter ratio—a marker of pulmonary vascular pressure—was also explored. The95% bootstrap CIs that excluded zero were considered significant.

RESULTS: FEV_1%predicted ($P=0.026$) and FEV_1/FVC ($P=0.010$) were associated with CAC. After adjusting for FEV_1, visual emphysema, and visual airway wall thickening remained associated with CAC. Five proteins (TSP2 [thrombospondin-2], renin, MMP-7 [matrix metalloproteinase-7], ERBB1 [epidermal growth factor receptor], MIC-1 [macrophage inhibitory cytokine-1]) mediated the FEV_1%predicted and CAC association. All except MIC-1 mediated FEV_1/FVC and CAC. All except renin mediated quantitative airway wall thickness or wall area percentage and CAC. Additionally, α2-antiplasmin (alpha-2 antiplasmin) mediated airway wall thickness and CAC. ERBB1 mediated visual paraseptal emphysema and CAC. Pulmonary artery-to-aortic diameter ratio adjustment reduced or eliminated some mediation effects. ERBB1 remained an independent mediator across multiple phenotypes.

CONCLUSIONS: Six plasma proteins mediated associations between COPD phenotypes and CAC burden. These effects were partially influenced by pulmonary artery-to-aortic diameter ratio A, suggesting shared molecular pathways linking lung dysfunction to cardiovascular risk in COPD.

Key Words: chronic obstructive pulmonary disease ■ coronary artery calcification ■ coronary artery disease ■ plasma mediators ■ pulmonary artery-to-aorta ratio

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RESEARCH PERSPECTIVE

What Is New?

- We identified 6 circulating proteins that mediated associations between chronic obstructive pulmonary disease (identified by a range of measures of small airways disease or emphysema) and coronary artery calcification.
- These 6 proteins involved diverse biological processes including inflammation, angiogenesis, extracellular matrix remodeling, and thrombosis

What Question Should Be Addressed Next?

- Whether these 6 mediating proteins can be replicated in different cohorts or predict progression of coronary atherosclerosis should be assessed in future studies.

Nonstandard Abbreviations and Acronyms

CXCL	C-X-C motif chemokine ligand
ERBB1	epidermal growth factor receptor
MIC-1	macrophage inhibitory cytokine-1 (growth differentiation factor-15; GDF-15)
MMP-7	matrix metalloproteinase-7
mMRC	modified Medical Research Council Dyspnea Scale
PA/A	pulmonary artery-to-aorta ratio
PRM	parametric response mapping
TSP2	thrombospondin-2
UMAP	Uniform Manifold Approximation and Projection

Chronic obstructive pulmonary disease (COPD) is associated with pulmonary inflammation and alveolar destruction but also has adverse systemic health consequences. COPD is associated with increased risk of significant chronic comorbid diseases, including cardiovascular disease and coronary artery disease (CAD), even after adjusting for cigarette smoke exposure.[1,2] Patients with more severe COPD exhibit greater rates of cardiovascular morbidity and mortality compared with those with less severe disease.[3,4]

Coronary artery calcification (CAC), a marker of atherosclerosis, is prognostic of cardiovascular events and is independently associated with all-cause mortality in patients with COPD. Mortality risk is nearly 3-fold greater in patients with high CAC burden and severe COPD.[5] In addition, data from the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-Points) cohort study showed that CAC scores were greater in individuals with COPD compared with non-COPD ever-smokers or never-smokers and CAC was associated with an increased burden of dyspnea, exercise intolerance, and mortality.[6]

Although shared risk factors such as age, smoking, and lifestyle contribute to both COPD and cardiovascular disease, the relationship between the two is likely to extend beyond these shared risk factors. Chronic low-grade systemic inflammation, driven by persistent lung inflammation in COPD, may contribute to an atherogenic environment. Bhatt et al.[7] investigated this potential link in 150 individuals with and 150 individuals without COPD with normal spirometry from the SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study) cohort and found an association between forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) or visual centrilobular emphysema and CAC. They also measured 10 candidate plasma molecules, implicated in the pathogenesis of atherosclerosis, hypothesized to mediate this relationship. They showed that MMP3 (matrix metalloproteinase-3), VCAM1 (vascular cell adhesion molecule-1), and CXCL9 (C-X-C motif chemokine ligand 9) mediated ~7% to 17% of the association between COPD and CAC,[7] consistent with an inflammatory overspill hypothesis. However, their analysis did not find any plasma biomarkers mediating radiographic markers of airway inflammation (such as airway wall thickening) and CAC. In other populations, including rheumatologic diseases, inflammatory biomarkers such as CRP (C-reactive protein), fibrinogen, and IL-6 (interleukin-6) are associated with an increased risk of coronary heart disease.[8,9]

Based on these findings, we aimed to identify protein mediators between COPD phenotypes and CAC using an untargeted approach in a large cohort with and without COPD and with a history of smoking. We hypothesized that plasma proteins associated with COPD phenotypes, determined by spirometry or quantitative radiographic markers of airway inflammation or emphysema, would mediate the increased CAC burden observed in COPD.

METHODS

Data Sharing Statement

The COPDGene (Genetic Epidemiology of COPD) deidentified participant data sets and data dictionaries are available for monitored public access through Database of Genotypes and Phenotypes. More information about the study and how to access COPDGene data is available at www.copdgene.org.

Participants and Study Design

We conducted a retrospective analysis of data from the COPDGene study (NCT00608764) at Phase 1 (2008–2011). A detailed description of the study design, as well as the inclusion and exclusion criteria, is available at www.copdgene.org.[10] Briefly, COPDGene enrolled current and former smokers (≥10 pack-years smoking history, no exacerbations within 30 days of enrollment) with and without a diagnosis of COPD. Participants self-identified as either non-Hispanic White or non-Hispanic African American. For this analysis, we included all participants for whom both fresh frozen plasma for protein profiling and CAC measurements were available (n=1055).[11] This included data from participants at 4 COPDGene study sites: National Jewish Health; the Lundquist Institute at Harbor-UCLA Medical Center; University of Iowa; and Baylor College of Medicine. The COPDGene protocol was approved by the institutional review boards at each of the 21 participating sites (see online supplement) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before study enrollment.

Measurements

All participants underwent standardized assessments, including the 6-minute walk test, postbronchodilator spirometry, quantitative computed tomography (CT), and questionnaires evaluating symptoms and medical history. Covariates included age, sex, smoking history, exacerbation history, and the use of medications such as statins, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers (ARBs). Additional covariates included symptom scores from the modified Medical Research Council dyspnea scale and the COPD Assessment Test.

Spirometry and Chest CT

The exposure variables for mediation modeling were COPD phenotypes measured by spirometry or chest CT scan.

Spirometry was performed before and after administration of a short-acting β_2-agonist (albuterol) using the EasyOne spirometer (ndd, Zurich, Switzerland), following the American Thoracic Society/European Respiratory Society recommendations.[12] To ensure quality and consistency across multiple centers, spirometry data were centrally reviewed by the COPDGene study team.[10] Out of 3 acceptable efforts, the greatest FEV_1 and FVC values were reported. COPD severity was classified using the GOLD spirometric grade.[1,13]

CT scans were performed at full inspiration (200 mAs), and at the end of normal expiration (50 mAs).[10] Airway wall thickening was assessed by segmental airway wall thickness calculated as average values in 6 segmental bronchi, segmental wall area percentage (=[outer bronchus area-airway luminal area]/outer bronchus area×100) obtained at the same sites as airway wall thickness, and the square root of wall area of a 10-mm perimeter airway.[14–16] Emphysema was assessed using the Thirona program (Thirona, The Netherlands, http://www.thirona.eu).[10] Parametric response mapping (PRM) provided data in 3 categories: emphysema percentage (a percentage of lung voxels ≤ −950 Hounsfield units [HU] on an end-inspiratory CT scan) and gas trapping percentage (percentage of lung voxels ≤−856 HU on expiratory CT).[16–18] Paraseptal emphysema was visually scored as absent, mild or substantial. Centrilobular emphysema was visually scored as absent, trace, mild, moderate, confluent, or advanced destructive, as previously described.[19] Bronchial wall thickening was visually scored as none, mild, and substantial. For analyses, clinically substantial centrilobular emphysema was defined at moderate, confluent, or advance destructive versus absent, trace, or mild; for paraseptal emphysema mild or substantial versus absent; and wall thickening as definite versus none or possible.

Coronary Artery Calcium Score

The outcome variable for mediation modeling was CAC score. CAC was assessed using ungated electrocardiographically gated chest CT on multidetector computed tomography scans, which are reliable for determining the presence of CAC and measurement of Agatston score.[20] CAC scoring was performed at the CT reading center at the Lundquist Institute on dedicated workstations by experienced technologists blinded to the participant's status. CAC is defined considering hyperattenuating lesions of at least 3 contiguous pixels with a density more than 130 HU and reported as the Agatston score, which is calculated by multiplying the lesion area by a density factor (between 1 and 4).[21] The total CAC score was quantified by summing individual lesion scores from each of 4 anatomical sites (left main coronary, left anterior descending, left circumflex, and right coronary arteries). A CAC score of 0 implies that presence of atherosclerotic plaque is highly unlikely and is consistent with a low 5-year risk for an atherosclerotic cardiovascular disease event. A positive test (CAC>0) confirms the presence of atherosclerotic plaque, and a CAC score>100 portends a high risk (>2%) of atherosclerotic cardiovascular disease event within the next 5 years.[21]

Protein Profiling

Fresh frozen plasma samples were collected using 8.5 mL P100 tubes (Becton Dickinson, BD)[22] and stored at 80 °C until assayed. Relative abundance of plasma proteins was quantified using SomaScan 1.3K (1305 aptamers). SomaScan is a multiplex aptamer-based

proteomics platform.[23] Quality assurance and data processing were conducted by SomaLogic, including hybridization and median normalization, SOMAmer calibration to remove intra-assay variation, and plate scaling to adjust for interplate signal differences. Samples that either did not pass SomaLogic's quality control or that were determined to have the incorrect study identification, were removed from the analysis. Because technical variability due to preanalytical factors, such as sample collection and processing can affect protein levels, we conducted a Uniform Manifold Approximation and Projection (UMAP) analysis to determine if there was any clustering of samples by scanner model. Because scanner model has a strong relationship with clinical center it controls for technical variability of both CT and protein variance introduced by clinical center processing. For statistical analyses, proteomic data were naturally log-transformed.

Statistical Analysis

Participants included in the analysis had (1) complete CAC measurements for all 4 major coronary arteries (left main, left anterior descending, left circumflex, and right coronary arteries); (2) SomaScan proteomic data; and (3) all relevant covariates. Forty-two participants were excluded due to incomplete CAC measurements, and 16 due to missing ≥1covariates required for the core models. In a subanalysis that included the pulmonary artery-to-aorta (PA/A) ratio, an additional 3 participants were excluded due to missing values. In addition, there were 5 participants missing spirometry, variables which were used both as an exposure (to define the COPD phenotype) and included as a covariate in anatomic CT-derived phenotype models; these 5 participants were excluded. For analyses involving parametric response mapping measurements, 50 participants had missing data and were excluded from that subanalysis (Figure 1).

To enhance the robustness of our analysis planned for mediation modeling, which assumes normality of residuals, we evaluated various transformations of CAC in our multivariable regression models to determine the optimal approach for model fit and residual normality. Based on this evaluation, ranked-based inverse normal transformation was selected. Multivariable linear regression was used to evaluate associations between spirometric or anatomic COPD phenotypes and CAC.

Multivariable regressions adjusted for age, sex, race, body mass index, current smoking, pack-years, scanner model, as well as self-reported diabetes, hypertension, high cholesterol, statin, ACE and ARB use. FEV_1 (L) was included as a covariate in the CT-derived phenotype models, and height was additionally included as a covariate in FVC models. To facilitate comparison across phenotypes with different measurement scales, we standardized (*Z* score) all continuous phenotypes and CAC scores in the regression models (fully standardized models). For models evaluating visual emphysema and visual airway wall thickness, only CAC was standardized (partially standardized models).

Mediation Analysis

We used an agnostic approach in evaluating the potential mediation of all 1305 proteins measured.[24] Joint significance of proteins in both paths *A* and *B* was used to identify proteins to evaluate in a formal test for mediated effect, regardless of a significant total effect. The test for joint significance was used to lower the risk of falsely identifying a significant indirect effect.[25] The test for mediated effect model with percentile bootstrap (5000 bootstrap samples) estimated the indirect effect size and a 95% CI to determine statistical significance.[26] The percentile bootstrap CI accounts for the nonnormal sampling distribution of the indirect effect estimator. For mediation analyses, log-transformed proteins, the COPD phenotype and inverse normal transformed CAC were each standardized (expressed as a Z score). When the total effect approaches 0, the resulting mediation percentages can become unstable, including inflation of percentage mediation (>100%) or negative percentage meditation.[24] For this reason, we chose to not report percentage mediation. Instead, for continuous phenotypes we reported the completely standardized indirect effect size and reported partially standardized effect size for binary phenotypes.

Based on the test for joint significance to identify potential mediating proteins, multivariable regression analyses for path *A* (COPD phenotype) and path *B* (CAC) were conducted (Figure S1). Proteins that had a significant association with both path *A* and path *B* at a false discovery rate of ≤0.20 were evaluated in multivariable mediation analysis using the test for mediated effect.[22] The multivariable regression model controlled for the same covariates as before with the addition of the COPD phenotype being evaluated included as a covariate for path *B*. Regression results were corrected for multiple testing using the Benjamini–Hochberg method to control the false discovery rate.[27] In the test for mediated effect, percentile bootstrap CIs were used to establish significant mediation, due to the potential for nonnormal distribution of the indirect effect estimator.

Where mediation analysis of single proteins identified multiple significant mediators per phenotype, we used parallel multiple mediator analysis to simultaneously evaluate each protein's effect while controlling for the association between all proteins included. We evaluated collinearity between proteins and if absent, unlike single protein mediation we used parallel multiple mediator analysis to estimate the total indirect effect of the proteins when taken together.

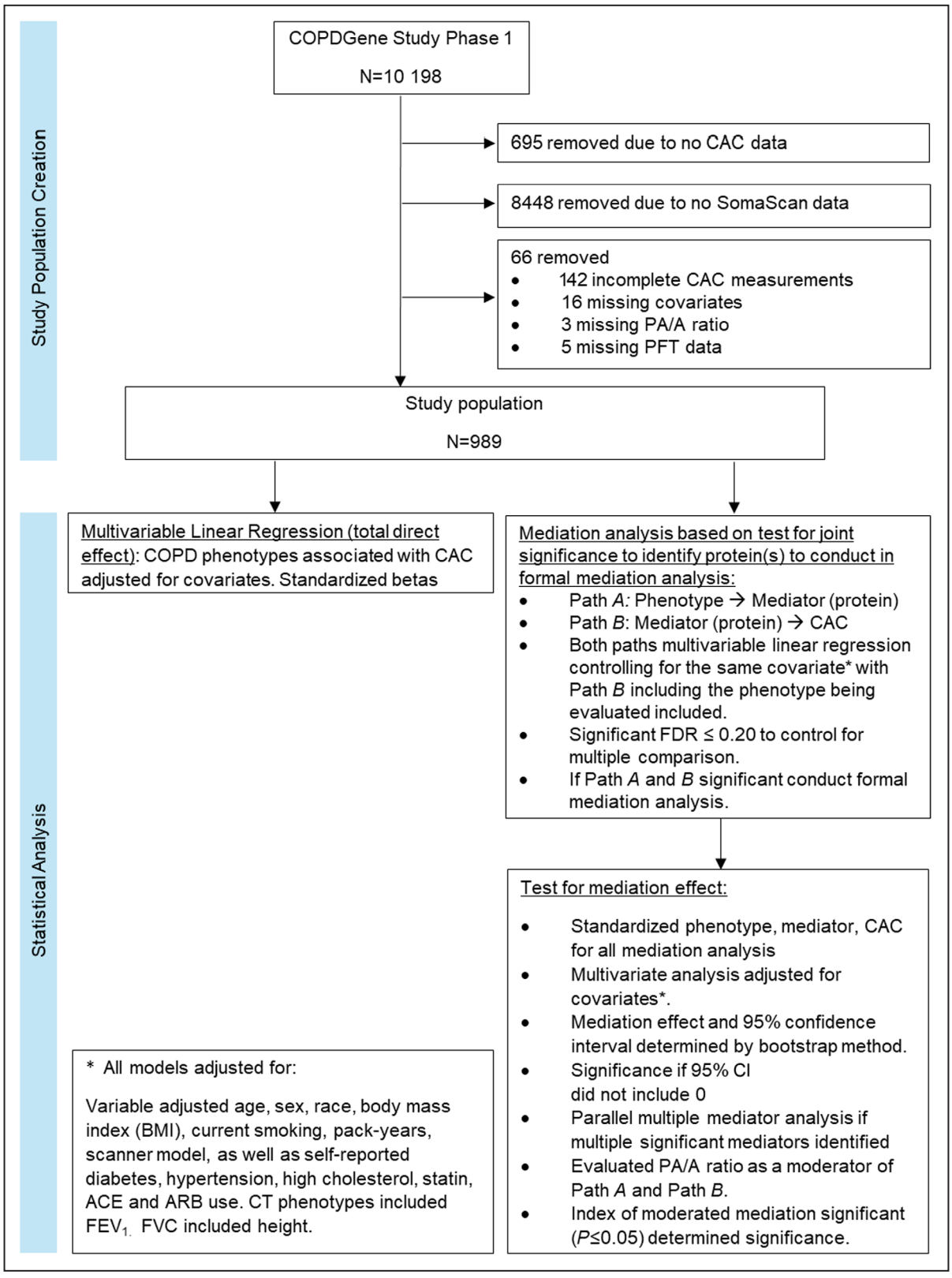

Figure 1. Consolidated Standards of Reporting Trials diagram and study design
ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CAC, coronary artery calcium; COPD, chronic obstructive pulmonary disease; CT, computed tomography; FDR, false discovery rate; FEV_1, forced expiratory volume in first s; FVC, forced vital capacity; PA/A, pulmonary artery-to-aorta ratio; and PFT, pulmonary function test.

Because some participants in our study population carried a risk for pulmonary hypertension, we conducted a sensitivity analysis including PA/A ratio as a covariate. In addition, we evaluated PA/A ratio as a moderator of path *A* or path *B*. If found to be a significant moderator, then a formal test of moderation mediation was performed (index of moderated mediation) to test whether PA/A changed the mediating effect of the protein. A significant index indicated that the strength of protein mediation varied depending on PA/A. In all models, PA/A ratio was included as a confounder for the paths that did not include moderation. Only nonstandardized estimates can be generated for moderation mediation analyses, as reported.[24]

All analyses were performed using SAS 9.4 (SAS Institute, Cary NC). For single mediation analysis the causalmed procedure was used and for multiple mediation and moderation mediation analysis the PROCESS macro (version 4.1) was used.[24] Figures were generated in R (version 4.3.3). Volcano plots were generated using ggplot2 (version4.0.2), patchwork (version 1.3.2), and figpatch (version 0.3.0). Forest plots were created with ggplot2 (version 4.0.2) and Spearman correlation heatmap using base R cor() function with ggcorrplot (version 0.1.4.1) with hierarchical clustering with complete linkage. UMAP version 0.2.10.0 was used to conduct UMAP analysis and ggplot2 (version 4.0.2) was used to create a UMAP figure.

We note that effect sizes are not directly comparable across models because the results from the moderated mediation analysis are presented as unstandardized estimates.

RESULTS

Demographics and Clinical Characteristics

Participants' physical and clinical characteristics are summarized in Table 1. Of 989 participants, 478 (48.3%) were male, and 101 (10.2%) identified as African American. A total of 388 participants (39.2%) reported current smoking, with a median smoking history of 40 pack-years. 426 (43.1%) participants had normal spirometry, 465 (47.0%) had COPD, and 98 (9.9%) had a preserved ratio impaired spirometry phenotype (Table 1). Additionally, 294 (29.7%) had visual centrilobular emphysema, 433 (43.8%) had visual paraseptal emphysema, and 377 (38.1%) had visual airway wall thickening. Pre-existing cardiovascular comorbidities included hypertension (n=428), hyperlipidemia (n=423), and diabetes (n=103). A total of 251 participants (25.4%) were prescribed ACE/ARBs, and 296 (29.9%) were taking statins.

Table 1. Participant Characteristics

Characteristic	Mean±SD, Median [IQR] or N (%)
Participants, n	989
Age, y	61.5±9.2
African American, n (%)	101 (10.2%)
Male sex, n (%)	478 (48.3%)
Body mass index, kg/m^2	28.7±6.1
Current smoker, n (%)	388 (39.2%)
American Thoracic Society pack-y, median [IQR]	40 [27.3]
PA/A ratio	0.8±0.1
PA/A ratio>1, n (%)	115 (11.6%)
CAC Agatston scores	
Total Agatston score (CAC), median ([IQR])	11±[182]
CAC positive, n (%)	556 (56.2%)
CAC grade	
0 very low risk	433 (43.8%)
1–10 minimal risk	61 (6.2%)
11–100 mild risk	170 (17.2)
101–400 moderate to high	196 (19.8%)
>400 high risk	129 (13.0%)
COPD phenotypes	
COPD Gold stage, n (%)	
Preserved ratio impaired spirometry	98 (9.9%)
Control	426 (43.1%)
1	91 (9.2%)
2	200 (20.2%)
3	114 (11.5%)
4	60 (6.1%)
FEV_1/FVC	0.7±0.2
FEV_1, L	2.3±0.9
FEV_1, %predicted	77±25.9
FVC, L	3.4±1.0
Visual centrilobular emphysema present, n (%)	294 (29.6%)
Visual paraseptal emphysema present, n (%)	433 (43.8%)
Visual airway wall thickening present, n (%)	377 (38.1%)
Square root of the wall area of a theoretical circular cross section of an airway with 10 mm luminal perimeter standardized airway wall thickness, mm	2.3±0.6
Airway wall thickness, mm	1±0.2
Wall area,(%)	49.9±8.2
PRM % gas trapping, median ([IQR])	9.3 ([18.8])
PRM % emphysema, median ([IQR])	0.5 ([4])
Comorbidities, n (%)	
High blood pressure	428 (43.3%)
High cholesterol	423 (42.8%)
Diabetes	103 (10.4%)

(*Continued*)

Table 1. Continued

Characteristic	Mean±SD, Median [IQR] or N (%)
Medication use, n (%)	
On angiotensin-converting enzyme/ angiotensin receptor blockers	251 (25.4%)
On statin	296 (29.8%)

Values are shown as mean±SD, median [IQR], and number (percentage). CAC indicates coronary artery calcification; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first s; FVC, forced vital capacity; IQR, interquartile range; PA/A, pulmonary artery to aorta ratio; and PRM, parametric response mapping.

Association Between COPD Phenotypes and CAC (Path *C*)

Multivariable linear regression analysis showed that both FEV_1%predicted (β(SE)=−0.063 (0.028); *P*=0.026) and FEV_1/FVC ratio (β(SE)=−0.078 (0.030); *P*=0.010) were significantly associated with CAC, whereas FVC was not (β(SE)=−0.031 (0.040); *P*=0.425; note that sex, age, and height, among others, were included in models with FVC) (Table 2). The β coefficients indicate that, for example, a 1-SD decrease in FEV_1%predicted was associated with a 0.063 SD increase in inverse normal transformed CAC, after adjusting for all covariates. In anatomic models, after further adjusting for FEV_1, only visual radiographic features remained significantly associated with CAC: visual paraseptal emphysema (β(SE)=0.223 (0.055); *P*<0.001), visual centrilobular emphysema (β(SE)=0.166 (0.072); *P*=0.022), and visual airway wall thickening (β(SE)=0.210 (0.067); *P*=0.002) (Table 2).

There were no significant associations between CAC and other CT-based variables including parametric response mapping % emphysema, parametric response mapping % gas trapping, 10-mm perimeter airway, airway wall thickness, or wall area % (*P*>0.05; Table 2).

To investigate the potential influence of pulmonary hypertension on multivariable association between COPD phenotype and CAC, we conducted a sensitivity analysis including PA/A ratio as a covariate. We found the association between FEV_1%predicted and CAC lost significance after adjusting for PA/A (β(SE)=−0.057 (0.031); *P*=0.067). However, FEV_1/FVC remained significantly associated with CAC (β(SE)=−0.073 (0.033); *P*=0.026), and all visual radiographic parameters retained their significance following PA/A adjustment (Table 2).

Protein Mediated Association Between COPD Phenotypes and CAC (Paths *A* and *B*)

The multivariable regression model was used to identify plasma proteins associated with both COPD phenotypes and CAC. Between 1 and 237 proteins

Table 2. Multivariable Linear Regression Analysis of the Association of COPD Phenotypes With CAC

Phenotype	Base multivariable model			With PA/A ratio	
	No.	Beta (SE)	*P* value	Beta (SE)	*P* value
FEV_1/FVC	989	−0.078 (0.030)	0.010*	−0.073 (0.033)	0.026*
FEV_1, %predicted	989	−0.063 (0.028)	0.026*	−0.057 (0.031)	0.067
FVC, L	989	−0.031 (0.040)	0.425	−0.019 (0.041)	0.634
Visual centrilobular emphysema, Y/N	989	0.166 (0.072)	0.022*	0.163 (0.073)	0.025*
Visual paraseptal emphysema, Y/N	989	0.223 (0.055)	<0.001*	0.225 (0.055)	<0.001*
Visual airway wall thickening, Y/N	989	0.210 (0.067)	0.002*	0.208 (0.068)	0.002*
Airway wall thickness, mm	989	0.030 (0.034)	0.374	0.029 (0.034)	0.400
Wall area, %	989	0.016 (0.034)	0.644	0.014 (0.034)	0.681
Square root of the wall area of a theoretical circular cross section of an airway with 10 mm luminal perimeter, mm	989	−0.013 (0.036)	0.724	−0.013 (0.036)	0.724
PRM % emphysema	939	0.028 (0.038)	0.474	0.026 (0.038)	0.505
PRM % gas trapping	939	0.053 (0.043)	0.219	0.049 (0.043)	0.252

Beta estimates are fully standardized beta estimate for continuous phenotype and semistandardized for binary. Covariates including in both models are adjusted for age, sex, race, body mass index, current smoking status, pack-year, scanner model, diabetes, history of hypertension, history of high cholesterol, and being on a statin or angiotensin-converting enzyme/angiotensin receptor blockers. In computed tomography phenotypes models, FEV_1 (L) was included. For FVC models, height was included. CAC indicates coronary artery calcification; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in first s; FEV_1/FVC, forced expiratory volume in first second/ forced vital capacity; FVC, forced vital capacity; PA/A, pulmonary artery to aorta ratio; and PRM, parametric response mapping.

*$P<0.05$.

Table 3. List of SomaScan Protein Found to Be Significant Mediators: Aptamer Meta Data

SeqID	Target	Target full name	Entrez gene symbol	Entrez gene ID	Uniprot number
2677-1-1	ERBB1	Epidermal growth factor receptor	*EGFR*	1956	P00533
4374-45-2	MIC-1	Growth/differentiation factor 15	*GDF15*	9518	Q99988
2789-26-2	MMP-7	Matrilysin	*MMP7*	4316	P09237
3396-54-2	Renin	Renin	*REN*	5972	P00797
3339-33-1	TSP2	Thrombospondin-2	*THBS2*	7058	P35442
3024-18-2	a2-antiplasmin	Alpha-2-antiplasmin	*SERPINF2*	5345	P08697

(median=45) were significantly associated (false discovery rate ≤0.20) with various COPD phenotypes (path *A*) (Table S1). Additionally, between 3 and 7 proteins (median=7) were significantly associated with CAC (path *B*) (Table S1). Proteins in common to both associations (paths *A* and *B*) were further evaluated using multivariable mediation analysis. In total, 6 common proteins significantly mediated associations between COPD phenotypes and CAC (Table 3 and Figure 2). Five proteins (TSP2 [thrombospondin-2], renin, MMP-7, ERBB1 [epidermal growth factor receptor], and MIC-1 [macrophage inhibitory cytokine-1]) mediated the relationship between FEV_1%predicted or FVC and CAC, and 4 of these (MMP-7, TSP2, renin, and ERBB1) mediated the association between FEV_1/FVC and CAC (Table 4 and Figure 3). ERBB1 was the only protein that significantly mediated the association between visual paraseptal emphysema and CAC (Table 4 and Figure 3). We also found 5 proteins (ERBB1, MIC-1, MMP-7, TSP2, and α2-antiplasmin [alpha-2 antiplasmin]) mediating a relationship between quantitative airway wall thickness and CAC; 4 of which (ERBB1, MIC-1, MMP-7, and TSP2) also mediated quantitative wall area % and CAC (Table 4 and Figure 3). Volcano plots for COPD phenotypes without significant mediating proteins are shown in Figure S2.

Because blood samples were collected over 4 years (2008–2011; with the >95% of samples collected in 2009 or 2010) and stored until analysis in 2017, we conducted a sensitivity analysis for our mediation models on sample storage duration (the interval between enrollment year and analysis). This adjustment resulted in only minor changes in effect estimates and did not alter the overall pattern of identified mediators. We additionally evaluated potential differences in sample handling by adjusting for scanner model (strongly relate to clinical center). UMAP analyses of the SomaScan data set demonstrated modest clustering by scanner model without complete separation of samples (Figure S3).

Table 5 shows the standardized mediation path estimates and direction of association of the mediation of paths *A* and *B* for protein and COPD phenotypes. The standardized path estimates of the mediated effects by each protein identified were generally similar across spirometric or imaging-based COPD phenotypes, in the range of ~0.07 to 0.24 for path *A* and ~ 0.09 to 0.12 for path *B*. The directionality was also generally similar for each protein or path. For example, we found that lower FEV_1%predicted was associated with a greater abundance of TSP2, renin, MMP-7, and MIC-1 (negative in path *A*) that mediated greater CAC (positive in path *B*); whereas lower FEV_1%predicted was associated with less ERBB1 abundance (positive in path *A*) mediating a lower CAC score (negative in path *B*). Similar trends were observed for proteins mediating FEV_1/FVC or FVC and CAC (Table 5).

The standardized path mediation estimates and directionality were similar for proteins mediating visual paraseptal emphysema or quantitative airway wall thickness or wall area % and CAC; less severe COPD phenotypes were associated with greater ERBB1 abundance and lower CAC, but more severe COPD phenotypes were associated with greater MIC-1, MMP-7, or TSP2 abundance mediating a greater CAC (Table 5). An exception to these patterns was mediation by α2-antiplasmin, where a greater airway wall thickness was associated with greater α2-antiplasmin abundance that mediated lower CAC, suggesting a potentially unique or compensatory biological role for this protein (Table 5).

Influence of PA/A Ratio on Mediated Proteins

Several mediating proteins that we identified are implicated in cardiovascular disease and pulmonary hypertension. To investigate this, we explored the role of PA/A as both a confounding variable and a potential moderator within the mediation models (Table S2 and Figure 3). When PA/A was included as a confounder, several significant path *A* and path *B* associations were no longer observed. This loss of significance was specific to models involving pulmonary function and emphysema phenotypes, whereas associations involving airway imaging phenotypes remained largely unaffected. Notably, the mediating effects of specific proteins were no longer

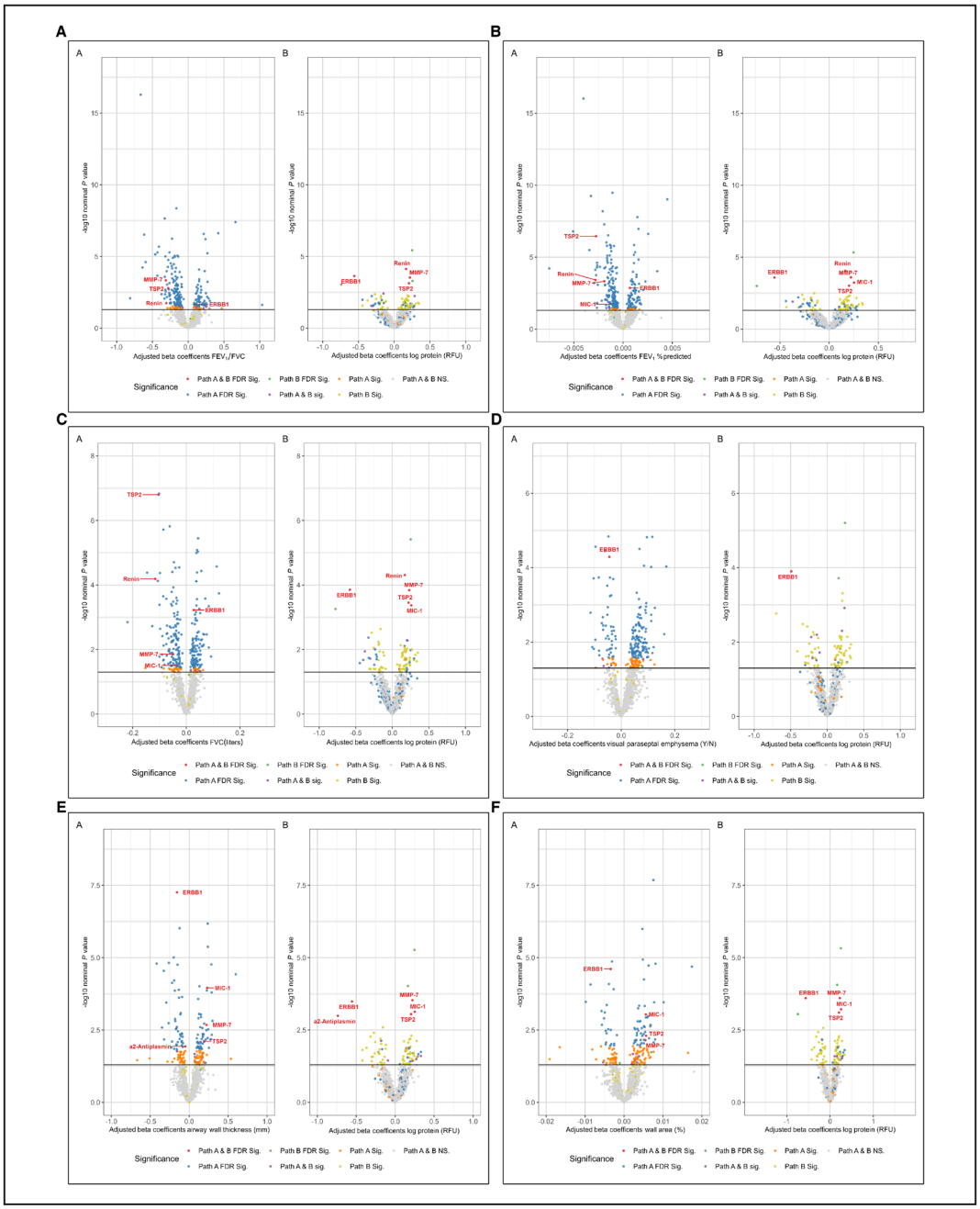

Figure 2. Volcano plots of protein associations used for joint-significance mediation screening across COPD phenotypes

Volcano plots show the associations of circulating proteins with COPD phenotypes (path *A*) and with CAC(path *B*) used to screen candidate mediators. Path *A* represents the regression of each protein on the COPD phenotype, whereas path *B* represents the regression of CAC on each protein. **A** through **F** correspond to the 6 COPD phenotypes that achieved joint significance. Within each panel, subpanel A displays the volcano plot for path *A*, and subpanel B displays the volcano plot for path *B*. The *x* axis represents the adjusted beta coefficients for the COPD phenotype (path *A*; subpanel A) or the adjusted beta coefficients for log-transformed protein abundance (path *B*; subpanel B). The *y* axis represents the –log10 (*P* value). The black horizontal line indicates the significance threshold, corresponding to a *P* value of 0.05. Proteins labeled in red indicate those that were significant in both path *A* and path *B* at an FDR≤0.20, meeting the joint-significance criteria for formal mediation testing. CAC indicates coronary artery calcium; COPD, chronic obstructive pulmonary disease; FDR, false discovery rate; FEV_1, forced expiratory volume in first s; FVC, forced vital capacity; and RFU, relative fluorescence unit.

Table 4. Standardized Mediation Analysis for Proteins Significantly Mediating the Association Between COPD Phenotypes and CAC

Phenotype	Mediator	Indirect effect beta	Bootstrap percentile 95% CI for indirect effect	Adjusted direct effect (c′) beta (SE)
FEV_1/FVC	MMP-7	−0.0125	−0.0230 to −0.0040	−0.0655 (0.0303)
	TSP2	−0.0105	−0.0222 to −0.0025	−0.0675 (0.0303)
	Renin	−0.0092	−0.0188 to −0.0017	−0.0687 (0.0302)
	ERBB1	−0.0083	−0.0179 to −0.0006	−0.0696 (0.0302)
FEV_1, %predicted	TSP2	−0.0156	−0.0290 to −0.0053	−0.0478 (0.0287)
	Renin	−0.0128	−0.0235 to −0.0044	−0.0505 (0.0285)
	MMP-7	−0.0118	−0.0219 to −0.0040	−0.0516 (0.0285)
	ERBB1	−0.0108	−0.0207 to −0.0030	−0.0526 (0.0284)
	MIC-1	−0.0074	−0.0163 to −0.0009	−0.0559 (0.0284)
FVC, L	TSP2	−0.0242	−0.0443 to −0.0088	−0.0071 (0.0399)
	Renin	−0.0208	−0.0366 to −0.0080	−0.0105 (0.0396)
	ERBB1	−0.0168	−0.0299 to −0.0055	−0.0145 (0.0395)
	MMP-7	−0.0120	−0.0251 to −0.0019	−0.0193 (0.0394)
	MIC-1	−0.0097	−0.0229 to −0.0007	−0.0217 (0.0394)
Visual paraseptal emphysema, Y/N	ERBB1	0.0235	0.0078 to 0.0449	0.1997 (0.0555)
Airway wall thickness, mm	ERBB1	0.0217	0.0089 to 0.0368	0.0086 (0.0345)
	MIC-1	0.0145	0.0044 to 0.0265	0.0159 (0.0342)
	MMP-7	0.0123	0.0038 to 0.0245	0.0181 (0.0341)
	TSP2	0.0098	0.0009 to 0.0217	0.0206 (0.0341)
	α2-antiplasmin	0.0092	0.0017 to 0.0192	0.0212 (0.0341)
Wall area, %	ERBB1	0.0171	0.0064 to 0.0308	−0.0014 (0.0340)
	MIC-1	0.0125	0.0035 to 0.0238	0.0032 (0.0340)
	MMP-7	0.0109	0.0027 to 0.0218	0.0048 (0.0339)
	TSP2	0.0104	0.0018 to 0.0225	0.0053 (0.0339)

Visual paraseptal emphysema are partially standardized estimates, with all other phenotype results fully standardized estimates. All models adjusted for the following covariates: age, sex, race, body mass index, current smoking, pack-years, scanner model, diabetes, hypertension, high cholesterol, and statin, angiotensin-converting enzyme/angiotensin receptor blocker use. Visual paraseptal emphysema, airway wall thickness and wall area percentage also included FEV_1; and FVC included height. To estimate the percentile CIs 5000 bootstraps were used. α2-antiplasmin indicates alpha 2 antiplasmin; CAC, coronary artery calcification; COPD, chronic obstructive pulmonary disease; ERBB1, epidermal growth factor receptor; FEV_1, forced expiratory volume in first s; FEV_1/FVC, forced expiratory volume in first s/forced vital capacity; FVC, forced vital capacity; MIC-1, macrophage inhibitory cytokine-1; MMP-7, matrix metalloproteinase-7; and TSP2, thrombospondin-2.

significant in certain models: MIC-1 and ERBB1 lost significance in FEV_1%predicted models, renin and ERBB1 in FEV_1/FVC models, and MIC-1 and MMP-7 in FVC models (Table S2 and Figure 3).

Although PA/A appeared to act as a confounder, its role as a moderator offered additional insights into the nature of these relationships. Investigation of PA/A as a moderator revealed it significantly influenced only path *A's* association between COPD phenotypes and with only the TSP2 protein (Table S3). The other proteins evaluated did not have any significant effect modification for path *A* or path *B* and are not reported. This significant effect modification was found with all phenotypes tested except FVC (*P*=0.5146) (Table S3). The index of moderated mediation, based on bootstrapped 95% CI, further confirmed that the indirect effect of TSP2 was significantly moderated by PA/A for both FEV_1%predicted (−0.1818 [95% CI, −0.4466 to −0.0144]) and FEV_1/FVC and (−0.2765 [95% CI, −0.6962 to −0.0021]) (Table S3).

Moderate PA/A values were 0.8305 (50th percentile) and high PA/A values were 0.9712 (84th percentile). For FEV_1/FVC, a significant moderating effect of PA/A on mediation by TSP was observed only at higher values of PA/A (−0.0658 [95% CI, −0.1441 to −0.0101]), whereas for FEV_1%predicted, a significant moderating effect was observed even at moderate PA/A (−0.0427 [95% CI, −0.0823 to −0.0123]). This difference may reflect that FEV_1%predicted is a stronger index of COPD severity than FEV_1/FVC.

Multiple Mediator Analysis

We performed a multiple mediator analysis to estimate both the total indirect mediation effect and the individual

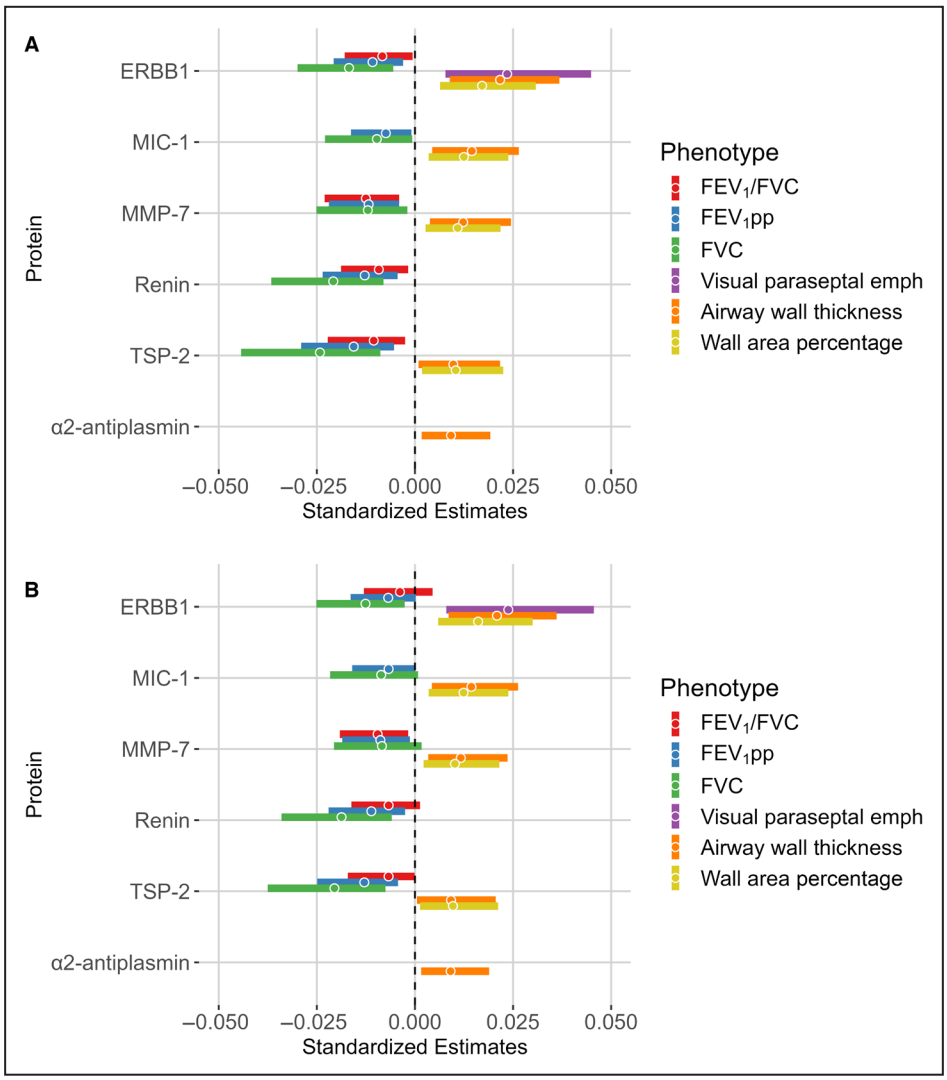

Figure 3. Forest plot of standardized indirect effects of proteins across COPD phenotypes
A, Displays mediation models without adjustment for the PA/A ratio, (**B**) shows models adjusted for PA/A ratio. Horizontal bars represent 95% bootstrap CIs, and white circles denote the standardized indirect effect estimates. Colors indicate the corresponding COPD phenotype. α2-antiplasmin indicates alpha 2 antiplasmin; COPD, chronic obstructive pulmonary disease; emph, emphysema; ERBB1, epidermal growth factor receptor; FEV_1, forced expiratory volume in first s; FEV_1pp, forced expiratory volume in 1 s percentage of predicted; FVC, forced vital capacity; MIC-1, macrophage inhibitory cytokine-1; MMP-7, matrix metalloproteinase-7; PA/A, pulmonary artery-to-aorta ratio; and TSP2, thrombospondin-2.

protein-specific indirect effect sizes, independent of other significant mediators. An evaluation of serial multiple mediation was conducted with the aid of the Search Tool for the Retrieval of Interacting Genes/Proteins database to guide the directionality of relationships; however, all serial mediation pathways were nonsignificant (P>0.05). Therefore, a multiple parallel mediation analysis was conducted. Many of the proteins identified as significant mediators showed moderate correlations with each other as well as with COPD phenotypes and CAC (Figure 4). We did not find any collinearity, but there were moderate correlations between ERBB1 and α2-antiplasmin (r=0.46), and among MIC-1, MMP-7, and TSP2 (r=0.18–0.45) (Figure 4). As a result, adjusting for all mediators simultaneously in the model weakened or eliminated some of the previously significant associations (Table S4).

After adjusting for all mediators in the FEV_1%predicted model, renin and ERBB1 remained significant mediators, with little to no change in their specific indirect effects (Table S4). In the FEV_1/FVC model, renin and ERBB1 retained their significance, whereas the indirect effects of TSP2 and MMP-7 were attenuated. Nevertheless, all 4 proteins remained significant mediators in this

Table 5. Standardized Mediation Path *A* and Path *B* Beta Estimates

Phenotype	Mediator	Path *A* estimate	Path *B* estimate
FEV_1/FVC	MMP-7	−0.1224	0.1029
	TSP2	−0.1129	0.0937
	Renin	−0.0742	0.1252
	ERBB1	0.0779	−0.1076
FEV_1, %predicted	TSP2	−0.1696	0.0923
	Renin	−0.1037	0.1242
	MMP-7	−0.1140	0.1040
	ERBB1	0.1011	−0.1070
	MIC-1	−0.0683	0.1094
FVC, L	TSP2	−0.2401	0.1003
	Renin	−0.1607	0.1293
	ERBB1	0.1495	−0.1119
	MMP-7	−0.1106	0.1080
	MIC-1	−0.0862	0.1124
Visual paraseptal emphysema, Y/N	ERBB1	−0.2473	−0.0947
Airway wall thickness, mm	ERBB1	−0.2028	−0.1069
	MIC-1	0.1332	0.1084
	MMP-7	0.1188	0.1035
	TSP2	0.1051	0.0930
	α2-antiplasmin	−0.0943	−0.0972
Wall area, %	ERBB1	−0.1578	−0.1083
	MIC-1	0.1143	0.1099
	MMP-7	0.1039	0.1047
	TSP2	0.1107	0.0940

Visual paraseptal emphysema are partially standardized estimates, with all other phenotype results fully standardized estimates. All models adjusted for the following covariates: age, sex, race, body mass index, current smoking, pack-y, scanner model, diabetes, hypertension, high cholesterol, and statin, angiotensin-converting enzyme/angiotensin receptor blocker use. Visual paraseptal emphysema, airway wall thickness and wall area percentage also included FEV_1; and FVC included height. α2-antiplasmin indicates alpha 2 antiplasmin; ERBB1, epidermal growth factor receptor; FEV_1, forced expiratory volume in first s; FEV_1/FVC, forced expiratory volume in first s/forced vital capacity; FVC, forced vital capacity; MIC-1, macrophage inhibitory cytokine-1; MMP-7, matrix metalloproteinase-7; and TSP2, thrombospondin-2.

model (Table S4). FVC, renin, TSP2, and ERBB1 retained significance. The total indirect effect beta of the 5 proteins associated with spirometric COPD phenotypes was greater for FVC (−0.0615) compared with FEV_1%predicted (−0.0416) or FEV_1/FVC (−0.0326) (Table S4). In the parallel multiple mediation analysis of airway imaging COPD phenotypes, the specific indirect effects of ERBB1 and MMP-7 were attenuated but remained significant, whereas the effects of MIC-1 and TSP2 lost significance (Table S4).

Because we found that MIC-1 showed the strongest correlations with other mediating proteins (Figure 4), we conducted an exploratory sensitivity analysis to assess the extent to which MIC-1 influenced specific indirect effects of other proteins in the multiple mediation models. To do so, MIC-1 was removed from the analysis; the results are presented in Table S5. Removing MIC-1 from the FEV_1%predicted and FVC models increased the strength of the indirect effects and restored statistical significance for all 4 protein mediators considered in either model (TSP2, renin, MMP-7, ERBB1). In contrast, for airway imaging COPD phenotypes, removing MIC-1 had no effect on the indirect effects of either TSP2 or α2-antiplasmin (cf. Table S4).

In the parallel multiple mediation analysis, including PA/A as a covariate, the mediation effects of the significant proteins identified in previous models were re-evaluated (Table S6). For FEV_1%predicted, the indirect effects of TSP2 and MMP-7 were attenuated but remained significant, and the mediation effect of renin was unchanged. In the FEV_1/FVC model, the effect sizes of all mediators were reduced, with TSP2 becoming nonsignificant, whereas MMP-7 remained a significant mediator. For FVC, the mediation effect of TSP2 remained unchanged, ERBB1 showed a slight reduction, and indirect effect of renin increased, although all 3 proteins remained significant mediators in this model.

In the parallel multiple mediation analysis of FEV_1%predicted and FEV_1/FVC, with PA/A included as a moderator of the indirect effect of TSP2, a similar pattern was observed; TSP2, renin, and MMP-7 remained significant mediators (Table S7). However, in the FEV_1/FVC model, the index of moderated mediation 95% CI includes 0, indicating the effect modification of FEV_1/FVC by PA/A on TSP2 mediation is not significant, whereas MMP-7 remained a significant mediator.

DISCUSSION

In 989 ever-smokers with and without COPD from the COPDGene study, we found a significant association between COPD phenotypes and CAC after adjusting for covariates including age, sex, race, smoking history, and comorbid conditions. Our untargeted analysis of 1305 proteins found that the association between COPD phenotypes and CAC was mediated by 6 circulatory proteins. Protein mediators were identified for both spirometric COPD phenotypes (FEV_1/FVC, FEV_1%predicted, and FVC adjusted for sex, age, and height) and chest CT-derived COPD phenotypes (visual parasepatal emphysema, quantitative airway wall thickness, and wall area %). In addition, we found that adjusting for PA/A, as indicator of pulmonary hypertension, attenuated some of the spirometry protein-mediated associations, but not protein-mediated associations with CT-derived phenotypes. However, using parallel multiple mediation analysis of FEV_1%predicted and FEV_1/FVC, including PA/A as a moderator of the indirect

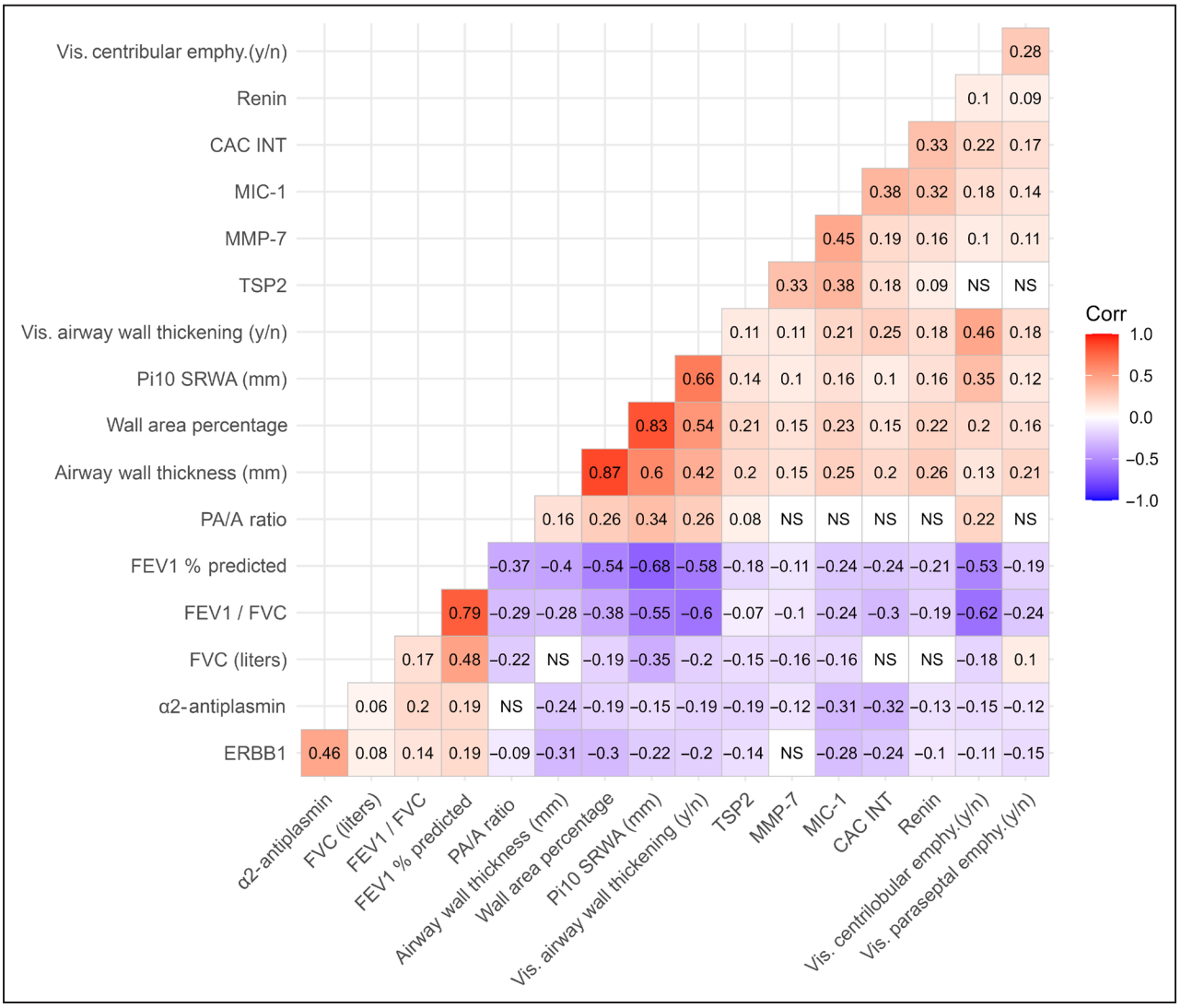

Figure 4. Heatmap of hierarchical clustering of COPD phenotypes, CAC, and candidate proteins included in mediation analyses

The heatmap displays the pairwise Spearman correlation matrix among COPD phenotypes, inverse normal transformed CAC, and proteins identified for mediation modeling. Color intensity represents the strength and direction of the correlation, with red indicating positive correlations and blue indicating negative correlations. Rows and columns were reordered using hierarchical clustering with the complete linkage method to group variables with similar correlation patterns. Cells labeled "NS" (white boxes) indicate correlations that were not statistically significant (P>0.05). CAC indicates coronary artery calcium; COPD, chronic obstructive pulmonary disease; emphy., emphysema; ERBB1, epidermal growth factor receptor; FEV_1, forced expiratory volume in first s; FVC, forced vital capacity; INT, inverse normal transformed; MIC-1, macrophage inhibitory cytokine-1; MMP-7, matrix metalloproteinase-7; PA/A, pulmonary artery-to-aorta ratio; Pi_{10}, square root of the wall area of a theoretical circular cross section of an airway with 10 mm luminal perimeter SRWA, standardized airway wall thickness; TSP2, thrombospondin-2; and Vis., visual.

effect of TSP2, 3 proteins (TSP2, renin, and MMP-7) were retained as significant mediators of spirometrically defined COPD and CAC. Overall, our data suggest a significant mediation between the cause of airway obstruction, airway inflammation, and pulmonary emphysema in ever-smokers by proteins that are associated with CAC.

Growing evidence indicates that COPD is associated with an increased risk of cardiovascular comorbidities, particularly CAD.[28] Individuals with a high CAC burden and advanced COPD have a nearly 3-fold higher risk of all-cause mortality than individuals without COPD and CAC.[5] Consistent with previous studies,[7] we found spirometric, airway inflammatory, and parenchymal disease COPD phenotypes were significantly associated with CAC, supporting the well-established epidemiological link between COPD and increased CAC[7,29–32]; these findings were generally retained after adjusting

for PA/A, suggesting that these variables may provide independent prognostic information for CAD beyond conventional chest CT measures.

Prior studies have demonstrated that systemic inflammatory markers are involved in both COPD and atherosclerotic cardiovascular disease.[8,9,33,34] Multiple plasma biomarkers have been explored in development and progression of CAC, including those related to calcium-phosphate and lipid metabolism, inflammation, renal function, and myocardial injury.[35] For example, phosphate, low-density lipoprotein, and total cholesterol are independently linked to CAC outcomes, even after adjusting for traditional cardiovascular risk factors including smoking history.[36] Additionally, lower adiponectin and greater osteoprotegerin are predictors of CAC progression.[37,38] Inflammatory markers such as sICAM-1 (soluble intercellular adhesion molecule-1), CRP, and fibrinogen also show modest associations with CAC, whereas findings on cardiac troponins (T and I) as indicators of myocardial injury remain inconsistent.[39,40] However, a comprehensive analysis of diverse biochemical markers and their links to CAC progression has yet to be fully explored. Although we did not find a mediating effect of these well-established atherogenic biomarkers, we did identify 6 proteins involved in chronic, low-grade systemic inflammation that could provide a link between lung injury in COPD and accelerated CAC development.

In an effort to identify circulating mediators, Bhatt et al[7] investigated 10 candidate biomarkers and found that 4 of them mediated 7% to 16% of the association between FEV_1/FVC and CAC, but none of the 10 targets mediated the association between emphysema phenotypes and CAC.[7] The 4 proteins reported by Bhatt et al.[7] to mediate the FEV_1/FVC-CAC association (MMP-3, VCAM1, CXCL5, and CXCL9) were also identified in our data set; however, they were associated with only COPD phenotypes (eg, FEV_1%predicted) and not with CAC.

In our study, we leveraged proteomics data from the large COPDGene cohort and identified 6 circulating proteins (TSP2, renin, MMP-7, MIC-1, ERBB1, and α2-antiplasmin) that mediated the association between COPD phenotypes and CAC. These proteins represent distinct yet interconnected biological processes, including inflammation, angiogenesis, tissue remodeling, and thrombosis. The fact that the 4 protein targets investigated by Bhatt et al.[7] were associated with COPD phenotypes in our data set but not with CAC is biologically informative.[41–44] It suggests that proteins linked to pulmonary obstruction, airway inflammation, or COPD-related immune activation do not necessarily participate directly in pathways driving coronary artery calcification. Whereas another study found that serum VCAM1 was associated with increased risk of cardiovascular events in COPD, this study did not investigate CAC.[44]

Several factors may explain the difference between studies in proteins mediating CAC. One difference is that we included ~3 times more participants and investigated ~1300 proteins using an untargeted proteomics approach, whereas Bhatt et al.[7] examined 10 candidate biomarkers. Study populations also differed: Bhatt et al.[7] included 25% never-smokers, whereas our participants all had a smoking history (median 40 pack-years). Bhatt et al.[7] also included 25% severe COPD with both significant spirometric impairment and emphysema, whereas our group tended to have less severe COPD and less % emphysema, on average. Another difference was that Bhatt et al.[7] used the Weston visual score for CAC, whereas we quantified CAC using the Agatston score method. Also, there were differences in proteomic platforms, including assay specificity, epitope or isoform targeting, or binding characteristics that may have contributed to these differences between studies.[22] Importantly, mediation analysis is more stringent than association testing alone, requiring a protein to be associated with both the COPD phenotype and CAC. Thus, although MMP-3, VCAM1, CXCL5, and CXCL9 were related to COPD phenotypes in our cohort, they did not emerge as significant mediators of CAC. Collectively, these findings suggest that only a subset of COPD-associated inflammatory proteins may also contribute to coronary calcification and that the biological pathways linking COPD to CAC are likely broader than systemic inflammation alone. Nevertheless, consistent with previous studies, neither study identified prominent inflammatory biomarkers such as CRP or fibrinogen as mediators of COPD and CAC associations. Next, we discuss the potential role and relevance of each identified mediator in the context of our findings and existing literature.

Inflammation and Angiogenesis Mediators

TSP2 emerged as a key mediator linking COPD phenotypes to CAC, retaining significance even after adjusting for PA/A, a risk factor for pulmonary hypertension. TSP2 is an extracellular glycoprotein expressed in endothelial and smooth muscle cells that regulates inflammation, angiogenesis and thrombosis.[45] Elevated circulating TSP2 is a predictor of CAD, with greater concentration linked to increased risk of heart failure-related death, all-cause mortality and recurrent hospitalization in patients with CAD.[46,47] In our study, the greater TSP2 abundance was associated with greater airway wall thickness, lower FEV_1%predicted, lower FVC, lower FEV_1/FVC and greater CAC. These findings support the growing evidence that TSP2 concentration may improve cardiovascular risk prediction when integrated with established clinical indicators.

We identified MIC-1 (also known as GDF15 [growth-differentiation factor 15]) as another significant mediator of COPD phenotypes and CAC. MIC-1 is a pleiotropic cytokine of the TGF-β (transforming growth factor beta) superfamily often involved in chronic inflammation and mucus secretion but also acts as a modulator of inflammation.[48] Elevated MIC-1 is associated with pulmonary inflammation, COPD exacerbations, increased CAC score, and poor cardiovascular outcomes.[48–52] We found that greater MIC-1 abundance was associated with lower FEV_1%predicted and FVC, increased airway wall thickness, and increased CAC. Our findings align with a previous publication from the COPDGene cohort, showing a strong positive association between GDF15 (measured by ELISA assay) and CAC in patients with Gold 2 to 4 COPD without self-reported cardiovascular disease.[51] Notably, the mediating effect of MIC-1 disappeared after adjusting for PA/A, suggesting that its role may be influenced by vascular strain or right heart strain in patients with COPD. Although we might anticipate PA/A would be greater in participants with more emphysema, we did not identify mediation of any emphysema variables by MIC-1. Also, MIC-1 was the protein most also strongly associated with the other identified mediators (Figure 4), suggesting that its effects on CAC could work through associations with other inflammatory variables. To that end, we found that removal of MIC-1 from the multiple mediation model increased the strength of the indirect effects and for TSP2 and MMP-7 (Table S5).

Tissue Remodeling and Matrix Degradation Mediators

MMP-7 was significantly associated with both CAC and COPD phenotypes. MMP-7, a member of a larger family of matrix metalloproteinases, contributes to extracellular matrix degradation, cytokine activation, and promotion of inflammation and atherogenesis.[53,54] Elevated MMP-7 concentration is found in smokers with COPD and in individuals with atherosclerotic disease, although its specific association with CAC has not been previously documented.[55–58] We found greater circulating MMP-7 abundance was associated with increased airway wall thickness, lower spirometric lung function, and greater CAC burden, supporting its potential role as a biomarker linking pulmonary and cardiovascular pathology. In multiple mediator analysis, MMP-7 maintained its specific indirect effect for FEV_1/FVC and airway inflammatory phenotypes, with consistent strength of association.

ERBB1/EGFR (epidermal growth factor receptor) was uniquely identified as a significant mediator across all COPD phenotypes, including visual radiographic features. ERBB1 is a transmembrane glycoprotein that can also be found as a cargo within extracellular vesicles and can undergo extracellular domain shedding to produce soluble forms.[59] ERBB1 signaling regulates multiple biological processes relevant to COPD and cardiovascular disease, including epithelial repair, cellular proliferation, inflammation, and tissue remodeling.[60,61] Experimental studies demonstrated that ERBB1 inhibition attenuates oxidative stress, macrophage infiltration, smooth muscle cell proliferation, and proinflammatory cytokine production within atherosclerotic lesions,[62] suggesting that ERBB1 contributes to the pathogenesis of vascular diseases. However, these experimental observations primarily reflect tissue-level EGFR signaling activity and may not directly correspond to circulating ERBB1 concentrations measured in blood. Interestingly, we found that greater circulating ERBB1 abundance was associated with better lung function (greater FEV_1%predicted, FEV_1/FVC and FVC adjusted for sex, age and height) and lower CAC score. Although this appears paradoxical relative to ERBB1 inhibition studies, circulating ERBB1 may reflect distinct biological processes compared with membrane-bound receptor activation. Soluble or extracellular vesicle-associated ERBB1 could represent a compensatory or protective response to ongoing tissue injury and epithelial remodeling and may potentially serve as marker of epithelial repair or altered ERBB1 signaling dynamics in chronic inflammatory conditions.[63–66] In COPD, increased circulating ERBB1 may therefore indicate preservation of epithelial integrity or activation of tissue-protective pathways rather than pathogenic receptor overactivation.

Additionally, in our study, ERBB1 was negatively correlated with most other identified mediators except α2-antiplasmin, further supporting the possibility that ERBB1 may reflect a biologically distinct and potentially protective pathway. These findings support the hypothesis that ERBB1 may exert pleiotropic and context-dependent effects, contributing to tissue repair and disease modulation under some conditions while promoting pathological remodeling when dysregulated or excessively activated at the tissue level.[67,68] Notably, in multiple mediator analysis, ERBB1 remained an independent mediator across all phenotypes, especially for FVC and airway inflammatory phenotypes.

Coagulation and Vascular Stress Mediators

Renin, a key component of the renin-angiotensin-aldosterone system, significantly mediated the association between COPD phenotypes and CAC. Renin contributes to systemic inflammation, lung fibrosis, and cardiovascular complications.[69,70] Inhibiting renin-angiotensin-aldosterone reduces risk of COPD exacerbations[70] and improves outcomes in terms of myocardial infarction and mortality.[71–73] In addition, polymorphisms in the renin-angiotensin system genes are predictive of

CAC progression in type 2 diabetes but only in the absence of treatment with ACE inhibitors or ARBs.[74] We found that greater renin abundance was associated with worse lung function and greater CAC score. Importantly, renin maintained its mediation effect even after adjusting for PA/A, and multiple mediator analysis confirmed that renin was an independent mediator specifically for pulmonary function phenotypes, with a particularly strong indirect effect on FVC.

α2-antiplasmin, a serine protease inhibitor that prevents plasmin-mediated fibrinolysis, was identified as a mediator linking airway wall thickness and CAC. Because fibrinogen is considered a key biomarker for emphysema and COPD exacerbations, the finding of increased 2-antiplasmin abundance in COPD could have clinical significance. Although little is known about its role in COPD pathophysiology, greater α2-antiplasmin abundance is associated with increased thrombotic risk in cardiovascular disease.[75,76] In our study, greater α2-antiplasmin abundance was positively associated with airway wall thickness but inversely associated with CAC score, suggesting a complex role in vascular remodeling and pulmonary pathology.

Influence of PA/A on Protein Mediators

Because several of our identified mediators are implicated in pulmonary hypertension,[77–83] and given the established association between pulmonary hypertension and both COPD and CAC,[84,85] we evaluated PA/A as a potential moderator. After adjusting for PA/A, several proteins mediating CAC with spirometric and emphysema COPD variables lost significance, whereas associations with airway inflammatory phenotypes remained largely unaffected. This finding suggests that vascular strain or right heart strain may affect specific protein-mediated pathways linking pulmonary obstruction to CAC in patients with COPD.

Moderated mediation analysis further demonstrated that PA/A only moderated the association of TSP2 with COPD phenotypes, particularly FEV_1%predicted. Parallel multiple mediation analysis showed that TSP2 remained a significant mediator for FEV_1%predicted even after accounting for other mediators, whereas its role in FEV_1/FVC was weakened when controlling for MMP-7. The clinical significance of this observation is unknown; nevertheless, it highlights TSP2 as a potentially independent and robust biomarker for CAD risk in patients with COPD.

This study has several limitations. First, it is cross-sectional in design, which limits causal inference. Longitudinal studies are necessary to validate our findings. Second, while we adjusted our models for comorbidities, they were self-reported. Third, protein measurements were obtained using a multiplex aptamer-based proteomics platform (SomaScan), which may produce data that differ from other protein profiling techniques or platforms. Replication of these findings in diverse cohorts and using different platforms is warranted. Finally, although mediation analysis provides valuable insight into potential biological pathways, it does not establish causality.

CONCLUSIONS

In conclusion, this study identified 6 circulating proteins that mediated the association between COPD phenotypes and CAC burden. These proteins are involved in diverse biological processes, including inflammation, angiogenesis, extracellular matrix remodeling, and thrombosis. The mediating effects of several proteins were attenuated after adjusting for the PA/A, suggesting that right heart strain associated with pulmonary hypertension may contribute to the development of CAC in patients with COPD. Notably, TSP2 demonstrated an independent mediating effect for FEV_1%predicted even after accounting for PA/A, whereas TSP2 and renin consistently mediated the association between impaired lung function and CAC. In contrast, ERBB1 emerged as an independent mediator across all COPD phenotypes, regardless of PA/A adjustment, mediating lower CAC with increasing COPD severity. These findings suggest that the relationship between COPD phenotypes and CAC may be mediated by single or multiple circulating proteins, depending on the specific COPD trait, and that these pathways may be modified by pulmonary vascular remodeling. Collectively, these results provide new insights into the complex biological mechanisms linking pulmonary to cardiovascular pathology in COPD and highlight potential circulating proteins for future risk stratification and therapeutic targeting.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S7
Figures S1–S3
CONSORT 2025 Checklist

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