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ORIGINAL RESEARCH

Systemic Markers of Lung Function and Forced Expiratory Volume in 1 Second Decline across Diverse Cohorts

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Abstract

Rationale: Chronic obstructive pulmonary disease (COPD) is a complex disease characterized by airway obstruction and accelerated lung function decline. Our understanding of systemic protein biomarkers associated with COPD remains incomplete.

Objectives: To determine what proteins and pathways are associated with impaired pulmonary function in a diverse population.

Methods: We studied 6,722 participants across six cohort studies with both aptamer-based proteomic and spirometry data (4,566 predominantly White participants in a discovery analysis and 2,156 African American cohort participants in a validation). In linear regression models, we examined protein associations with baseline forced expiratory volume in 1 second (FEV_1) and FEV_1/forced vital capacity (FVC). In linear mixed effects models, we investigated the associations of baseline protein levels with rate of FEV_1 decline (ml/yr) in 2,777 participants with up to 7 years of follow-up spirometry.

Results: We identified 254 proteins associated with FEV_1 in our discovery analyses, with 80 proteins validated in the Jackson Heart Study. Novel validated protein associations include kallistatin serine protease inhibitor, growth differentiation factor 2, and tumor necrosis factor-like weak inducer of apoptosis (discovery $\beta = 0.0561$, $Q = 4.05 \times 10^{-10}$; $\beta = 0.0421$, $Q = 1.12 \times 10^{-3}$; and $\beta = 0.0358$, $Q = 1.67 \times 10^{-3}$, respectively). In longitudinal analyses within cohorts with follow-up spirometry, we identified 15 proteins associated with FEV_1 decline ($Q < 0.05$), including elafin leukocyte elastase inhibitor and mucin-associated TFF2 (trefoil factor 2; $\beta = -4.3$ ml/yr, $Q = 0.049$; $\beta = -6.1$ ml/yr, $Q = 0.032$, respectively). Pathways and processes highlighted by our study include aberrant extracellular matrix remodeling, enhanced innate immune response, dysregulation of angiogenesis, and coagulation.

Conclusions: In this study, we identify and validate novel biomarkers and pathways associated with lung function traits in a racially diverse population. In addition, we identify novel protein markers associated with FEV_1 decline. Several protein findings are supported by previously reported genetic signals, highlighting the plausibility of certain biologic pathways. These novel proteins might represent markers for risk stratification, as well as novel molecular targets for treatment of COPD.

Keywords: proteomics; biomarkers; airflow obstruction

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Chronic obstructive pulmonary disease (COPD) is a common respiratory disease with more than 328 million cases worldwide and is the third leading cause of death in the world (1, 2). COPD is defined by airflow obstruction that is not fully reversible and is characterized by accelerated lung function decline.

Although recent investigations have applied genome-wide association studies (GWASs) to identify novel disease pathways in lung function and COPD (3, 4), only a moderate amount of COPD susceptibility has been linked to genetic causes (5), suggesting that additional genetic and environmental determinants and gene–environment interactions remain to be identified. In developed countries, smoking is the primary exposure associated with COPD; however, a minority of smokers develop COPD (6). Defining molecular signatures through additional-omics studies reporting on processes downstream of genetics (e.g., transcriptomics and proteomics) may improve our understanding of COPD.

Although several studies have assessed focused protein biomarker panels in lung function and COPD (7–10), recently developed large-scale proteomic technologies may be applied to epidemiologic cohorts to simultaneously measure hundreds to thousands of proteins. Proteomic platforms facilitate analyses across broad swaths of the human proteome, which may better highlight disease pathways and processes. Specifically, aptamer-based proteomic technologies have been used to identify biomarkers associated with coronary artery disease (11, 12), Alzheimer's disease (13), and lung cancer (14).

Here, in discovery analyses, we evaluated protein associations with spirometry indices in six cohort studies and validate cross-sectional protein findings in an

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Data Sharing: Aptamer-based proteomic profiling results for all proteins measured in FHS, MESA, LSC, COPDGene, and SPIROMICS have been or are in process of being deposited in the database of Genotypes and Phenotypes (https://www.ncbi.nlm.nih.gov/gap). Primary data are available through the established application procedure. The data from the KORA study are subject to national data protection laws, and restrictions were imposed by the Ethics Committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. Data can be requested through an individual project agreement with KORA via the online portal KORA.PASST, and requests are subject to approval by the KORA Board. All other results and analytic methods are available within the manuscript or from the authors on request.

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This article has a related editorial.

This article has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

African American (AA) cohort. These data were leveraged to identify novel biomarkers and pathways associated with pulmonary function traits, including forced expiratory volume in 1 second (FEV_1) decline.

Methods

Study Populations

Our study included 6,722 participants from seven cohorts, with at least one spirometry and plasma SomaScan proteomic data from the same exam visit (study plan in Figure 1). Discovery cohorts included 4,566 participants from three population-based prospective cohort studies (FHS [Framingham Heart Study], KORA [Kooperative Gesundheitsforschung in der Region Augsburg], MESA [Multi-Ethnic Study of Atherosclerosis] lung study), a smokers' cohort (LSC [Lovelace Smokers Cohort]), and two COPD-enriched studies: the COPDGene Study and SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study). Given the limited diversity of the discovery study, we validated protein associations with cross-sectional lung function traits in 2,156 AA participants from the JHS (Jackson Heart Study). Longitudinal analyses of protein associations with FEV_1 decline included cohorts with follow-up spirometry measurements (FHS, KORA, LSC, and COPDGene). The supplementary methods in the data supplement detail cohort descriptions. The respective institutional review boards approved all study protocols; informed consent was obtained from all participants.

Spirometry Measurements

Baseline spirometry was performed in all cohorts, in accordance with American Thoracic Society and European Respiratory Society recommendations (15) (*see* data supplement).

Aptamer-based Proteomics Platform

The single-stranded DNA aptamer-based SomaScan proteomics platform was used to assay baseline plasma samples (16). Details on the SomaScan versions run in each cohort, including orthogonal support, can be found in the supplemental methods and Table E1 in the data supplement.

Statistical Analysis

Protein relative fluorescent unit values were inverse rank normalized because of nonnormal distribution. Although the SomaScan assay has no missing values and does not report the lower limit of detection (LLOD) for individual proteins, two recent publications have estimated the LLOD for each SOMAmer assay using buffer blank values and found that very few (<0.1%) assays are below the LLOD (17, 18). Proteins and pulmonary function traits (FEV_1, forced vital capacity [FVC], and FEV_1/FVC) were treated as continuous variables. We analyzed cross-sectional protein associations with lung function (FEV_1, FVC) in linear regression models adjusting for age, age^2, sex, height, height^2, race (when applicable), body mass index (BMI), pack-years, current smoking status, and proteomics plate and batch (when applicable). In the FEV_1/FVC linear regression models, we adjusted for age, sex, race, BMI, pack-years, smoking status, and proteomics plate and batch. We adjusted for COPD case/noncase status as a covariate in the models for COPDGene and SPIROMICS to account for the study design. Linear mixed-effects regression models (including pedigree-based kinship matrix to adjust for familial relatedness in FHS) were used to examine the association between protein levels at baseline exam and FEV_1 decline. The model included random intercept and fixed effects for time (continuous variable quantifying time period between each FEV_1 measurement and baseline), protein and its interaction with time, baseline age, sex, race (when applicable), height, current smoking status and pack-years, and proteomics plate and batch (when applicable). Results across

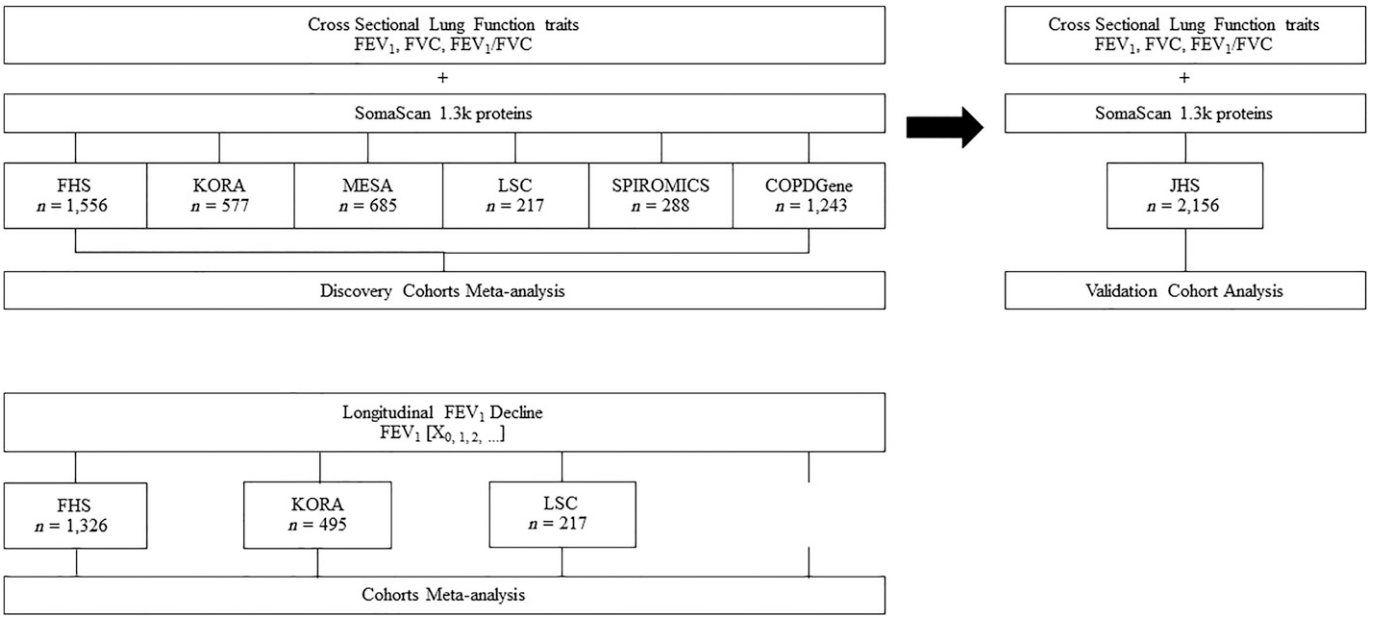

Figure 1. Flow chart of the proteomic analysis plan. COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 second; FHS = Framingham Heart Study; FVC = forced vital capacity; JHS = Jackson Heart Study; KORA = Kooperative Gesundheitsforschung in der Region Augsburg (Southern Germany); LSC = Lovelace Smokers Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; SPIROMICS = Subpopulations and Intermediate Outcome Measures in COPD Study.

discovery cohorts were combined in random-effects meta-analyses, and false discovery rate (19) Q-value adjusting for 1,305 proteins was used to denote significance ($Q < 0.05$). The Q-value was adjusted for the number of significant proteins per trait in discovery meta-analyses that were tested in the validation ($Q < 0.05$).

Pathway Analyses

Pathway analyses were run in ConsensusPath DB Release 35 (20, 21), accessed December 23, 2022. Details are in the data supplement.

Results

Study Sample: Cross-Sectional Lung Function Analyses

Characteristics of the discovery and validation study sample for cross-sectional analyses are presented in Table 1. We studied 4,562 participants from six cohorts in our discovery analyses. Average participant age in each study ranged from 54 to 68 years, with the COPDGene and SPIROMICS participants being slightly older. COPDGene, SPIROMICS, and LSC participants had greater smoking exposures and lower mean FEV_1. Discovery cohort participants were predominantly White, with a slight female predominance. We validated protein associations with cross-sectional lung function traits in 2,156 AA participants, having similar age distribution, female predominance, and less smoking exposure than other smoking- and COPD-enriched cohorts in discovery analyses. Four studies (FHS, COPDGene, KORA, and LSC) had two or more spirometry measurements in 2,777 participants, with average follow-up ranging from 5.4 to 6.9 years.

Protein Associations with Lung Function in Discovery Cohorts

In multivariable-adjusted models, we identified 254 proteins that associated with baseline FEV_1 in meta-analyses across six discovery cohorts ($Q < 0.05$; Figure 2, represented as colored circles, and Table E2). Of 254 proteins, 69 proteins met a Bonferroni-adjusted significance threshold ($P < 3.83 \times 10^{-5}$). Cohort level results for all lung function traits are reported in Table E3. We confirmed previously identified markers associated with COPD: C-reactive protein (22) ($\beta = -0.073$, $Q = 1.02 \times 10^{-16}$) and

Table 1. Characteristics of cohort participants with baseline spirometry

	Discovery						Validation
	Population-based			Smoking-enriched	COPD-enriched		Population-based
	FHS	KORA	MESA	LSC	COPDGene	SPIROMICS	JHS
N	1,556	577	685	217	1,243	288	2,156
Age, yr, mean (SD)	55 (10)	53.8 (4.3)	68.75 (9.3)	55.6 (8.7)	61.6 (9.3)	59.8 (9.3)	56 (13)
Male	716 (46)	263 (45.6)	303 (47.7)	46 (21.2)	609 (48.9)	148 (51.4)	837 (39)
Body mass index, kg/m^2, mean (SD)	27.4 (5.0)	27.5 (4.7)	29.42 (5.5)	27.3 (5.5)	28.7 (6)	28.1 (5.1)	32 (7)
Race, White	1,556 (100)	577 (100)	278 (43.7)	144 (66.4)	1,079 (86.8)	188 (65.3)	0 (0)
Race, Black-African	0 (0)	0 (0)	132 (20.8)	1 (0.5)	164 (13.2)	67 (23.3)	2,156 (100)
Race, Hispanic	0 (0)	0 (0)	225 (35.4)	64 (29.5)	0	27 (9.4)	0 (0)
Current Smoker	303 (19)	105 (18.2)	57 (9.0)	132 (60.8)	495 (39.8)	96 (33.3)	277 (13)
Never-smoker	252 (36)	221 (38.3)	250 (39.6)	0 (0)	37 (3.0)	49 (17)	1,433 (66)
Smoking pack-year history, mean (SD)	16.6 (21.4)	21.2 (20.0)	10.92 (18.8)	41.5 (19.6)	43.6 (25.5)	39 (29.8)	22.4 (21.5)
FEV_1, L, prebronchodilator, mean (SD)	2.9 (0.8)	3.3 (0.8)	2.47 (0.8)	2.5 (0.7)	2.2 (0.9)	2.3 (0.9)	2.40 (0.72)
FEV_1 % predicted, prebronchodilator, mean (SD)	92.7 (14.0)	102.8 (15.4)	92.76 (18.6)	88.1 (19)	74.3 (26.0)	79.4 (25.1)	92 (18)
FVC % predicted, prebronchodilator, mean (SD)	97.6 (13.2)	102.6 (12.9)	98.27 (16.7)	93.5 (14.9)	86.3 (18.1)	3.5 (1.0)	91 (17)
PRISm	101 (6)	20 (3.5)	49 (7.2)	17 (7.8)	119 (9.6)	10 (3.5)	341 (17)
GOLD 1	202 (13)	29 (5.0)	321 (78.3)	13 (6)	102 (8.2)	45 (15.6)	56 (2.6)
GOLD 2	164 (11)	18 (3.1)	79 (19.3)	31 (14.3)	245 (19.8)	60 (20.8)	116 (5.4)
GOLD 3	23 (1)	1 (0.2)	9 (2.2)	8 (3.7)	142 (11.5)	20 (6.9)	15 (0.7)
GOLD 4	1 (<1)	0 (0)	1 (0.2)	3 (1.4)	68 (5.5)	10 (3.5)	6 (0.28)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 second; FHS = Framingham Heart Study; FVC = forced vital capacity; GOLD = Global Initiative for Chronic Obstructive Lung Disease; JHS = Jackson Heart Study; KORA = Kooperative Gesundheitsforschung in der Region Augsburg (Southern Germany); LSC = Lovelace Smokers Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; PRISm = preserved ratio impaired spirometry; SD = standard deviation; SPIROMICS = Subpopulations and Intermediate Outcome Measures in COPD Study.
Entries represent *n* (%) unless otherwise noted. PRISm defined as $FEV_1/FVC \geq 0.7$ and FEV_1 % predicted according to Hankinson equations < 80%.

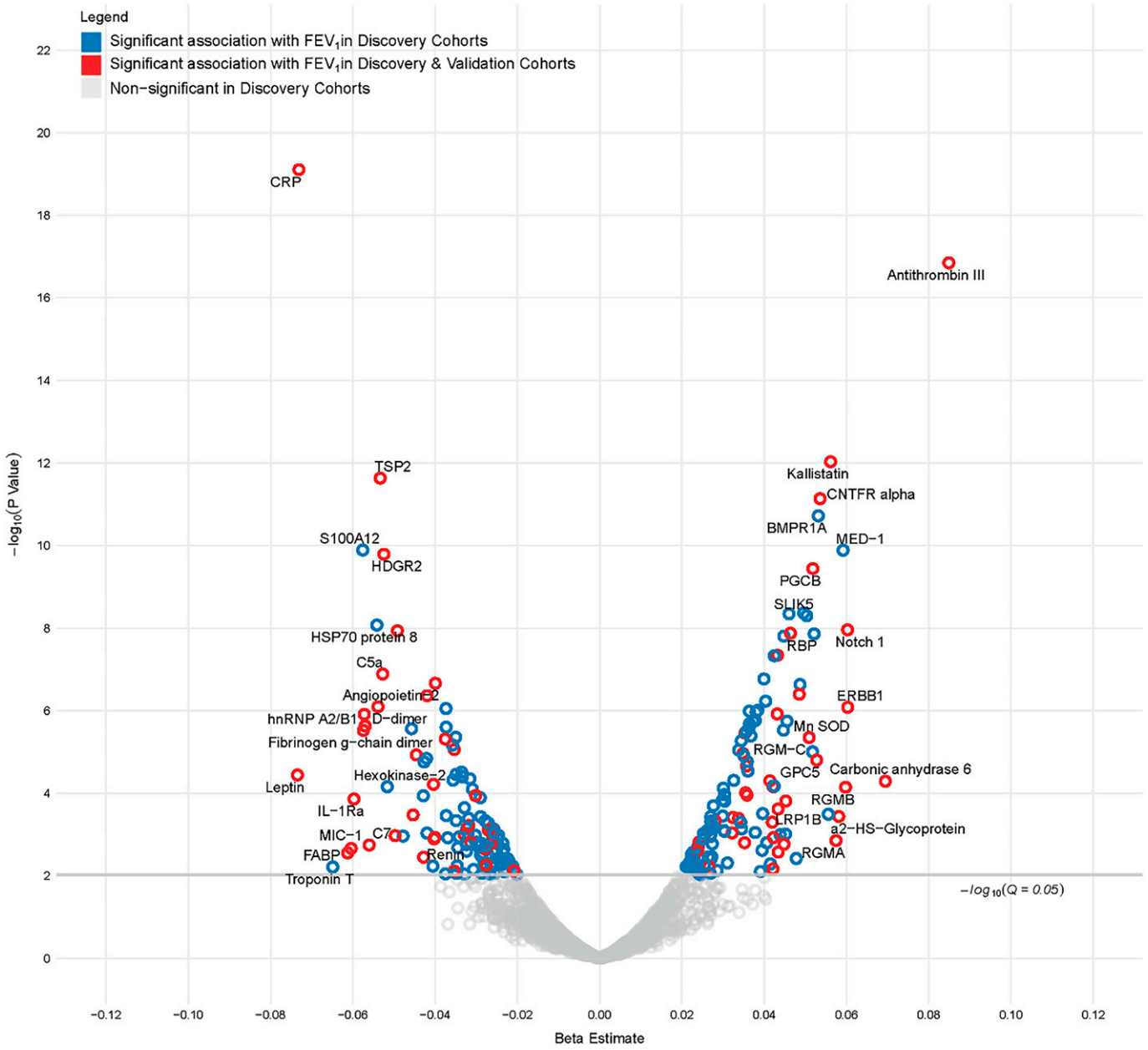

Figure 2. Protein associations with baseline forced expiratory volume in 1 second (FEV_1). Volcano plot showing multivariable-adjusted protein associations with FEV_1. All colored circles above the $-\log_{10} Q = 0.05$ line represent proteins with significant associations with FEV_1 in discovery cohort meta-analyses. Red circles highlight proteins associated with FEV_1 in both discovery and Jackson Heart Study validation analysis ($Q < 0.05$, accounting for testing associations in 254 significant proteins found in discovery). *See* Table E2 for details for all significant protein associations with FEV_1 in discovery and validation analyses. Cohort level results for all proteins and all traits in discovery and validation are shown in Table E3. Annotation of protein full name, UniProt, EntrezGene, and aptamer sequence IDs are included across all supplementary tables. BMPR1A = bone morphogenetic receptor type 1A; RBP = retinol binding protein; RGM = repulsive guidance molecule.

fibrinogen γ chain (23) ($\beta = -0.057$, $Q = 8.54 \times 10^{-5}$). Our findings confirmed a previously reported association of proteins in the TGF-β/BMP pathway with FEV_1 (5, 24, 25), including BMPR1A (bone morphogenetic receptor type 1A; $\beta = 0.053$, $Q = 4.14 \times 10^{-9}$), and multiple BMP coreceptors, such as RGM (repulsive guidance molecule) A, B, and C (RGMA: $\beta = 0.057$, $Q = 1.1 \times 10^{-2}$; RGMB: $\beta = 0.060$, $Q = 1.1 \times 10^{-3}$; and RGMC: $\beta = 0.052$, $Q = 2.27 \times 10^{-4}$, respectively).

Many protein associations with FEV_1 were novel, including kallistatin ($\beta = 0.056$, $Q = 4.05 \times 10^{-10}$), a serine protease inhibitor similar to alpha-1 antitrypsin in structure (26). Kallistatin binds prekallikrein ($\beta = 0.0326$, $Q = 8.63 \times 10^{-4}$) to produce

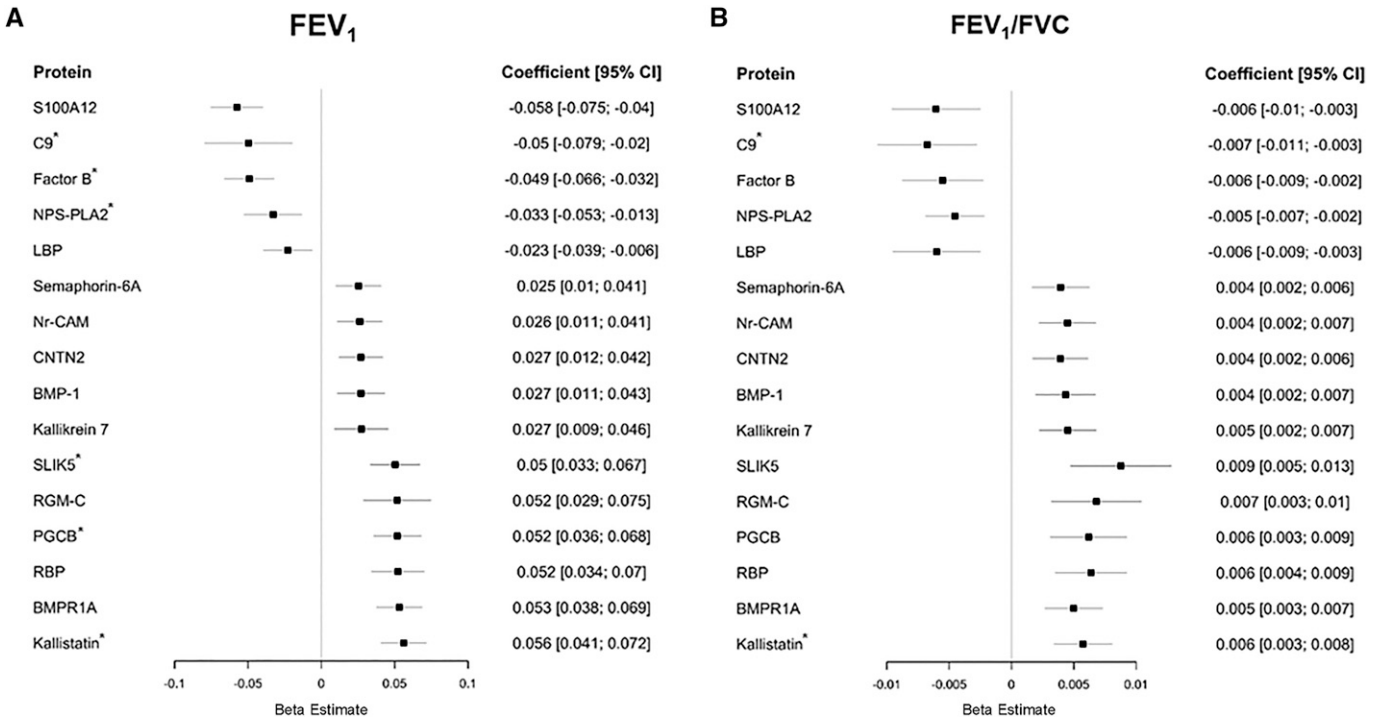

Figure 3. Proteins associated with both (*A*) forced expiratory volume in 1 second (FEV_1) and (*B*) FEV_1/forced vital capacity (FVC). Proteins associated with both FEV_1 and FEV_1/FVC in discovery cohort meta-analyses ($Q < 0.05$). Proteins are listed in order of ascending β estimate for FEV_1 association. *See* Tables E2 and E5 for details for all significant protein associations with FEV_1 and FEV_1/FVC in discovery and validation analyses, respectively. Cohort-level results are shown in Table E3. *Proteins associated with both FEV_1 and FEV_1/FVC in Jackson Heart Study validation analyses ($Q < 0.05$). BMPR-1A = bone morphogenetic receptor type 1A; CI = confidence interval; LBP = lipopolysaccharide binding protein; RBP = retinol binding protein; RGM = repulsive guidance molecule.

kallikreins, several of which (kallikreins 7, 11, and 14) associated with FEV_1 ($\beta = 0.027$, $Q = 2.31 \times 10^{-2}$; $\beta = 0.027$, $Q = 1.35 \times 10^{-2}$; $\beta = -0.034$, $Q = 6.01 \times 10^{-4}$, respectively). We identified other proteins in a coagulation cascade associated with FEV_1, including antithrombin III ($\beta = 0.084$, $Q = 9.26 \times 10^{-15}$). In addition, circulating levels of adipokines were related to FEV_1, including TIG2 (retinoic acid receptor responder protein 2 [27]; $\beta = -0.042$, $Q = 2.09 \times 10^{-5}$) and RBP4 (28) (retinol binding protein 4; $\beta = 0.052$, $Q = 9.98 \times 10^{-7}$). Although some proteins related to both FEV_1 and FVC, 56 proteins uniquely associated with FEV_1 only (FEV_1 $Q < 0.05$; FVC $Q > 0.05$). FVC results are shown in Table E4.

Sixteen proteins were associated with both FEV_1 ($Q < 0.05$) and FEV_1/FVC ($Q < 0.05$; Figure 3), many of which were highlighted above, including RBP, kallistatin, kallikrein 7, and RGM-C. Several innate immunity pathway participants also associated with both FEV_1 and FEV_1/FVC, including complement factors B and C9 (Factor B: FEV_1 $\beta = -0.049$, $Q = 9.44 \times 10^{-7}$ and FEV_1/FVC $\beta = -0.006$, $Q = 7.74 \times 10^{-4}$; C9: FEV_1 $\beta = -0.050$, $Q = 9.81 \times 10^{-3}$ and FEV_1/FVC $\beta = -0.0067$, $Q = 8.39 \times 10^{-4}$). Other immune-related proteins inversely associated with FEV_1/FVC and FEV_1 include S100A12 (FEV_1: $\beta = -0.058$, $Q = 2.12 \times 10^{-8}$; FEV_1/FVC: $\beta = -0.006$, $Q = 7.61 \times 10^{-4}$), a danger-associated molecular pattern molecule that binds to the receptor for advanced glycation end products to stimulate innate immune cells and inhibit metalloproteinase (29–31), and LBP (lipopolysaccharide binding protein: FEV_1 $\beta = -0.023$, $Q = 3.86 \times 10^{-2}$; FEV_1/FVC $\beta = -0.006$, $Q = 7.15 \times 10^{-4}$), an antibacterial factor with activity against gram-negative bacteria (32). Association of BMPR1A and RGM-C with both FEV_1 (Table E3) and FEV_1/FVC (Table E5) also highlights BMP-2 signaling (33).

Protein Associations with Lung Function in JHS as Validation

We evaluated the relationship of "discovered" proteins with lung function traits in JHS, an AA population-based cohort. We found that 80 of 254 proteins associated with FEV_1 in our discovery analyses were also significant in JHS ($Q < 0.05$, correcting for 254 tests; Figure 2, red circles). Twenty-seven additional proteins were nominally significant and directionally consistent in JHS. Top validated proteins include phospholipase A2, an arachidonic acid pathway metabolite (34); growth differentiation factor 2, a potent antiangiogenic factor (35); and tumor necrosis factor-like weak inducer of apoptosis, a cytokine previously associated with skeletal muscle atrophy (36). Of 22 protein associations with FEV_1/FVC reported in the discovery analyses, we confirmed that kallistatin and C9 were also associated with FEV_1/FVC in JHS ($Q < 0.05$, correcting for 22 tests, Figure 3 denoted by *). Tables E2, E4, and E5 detail results for FEV_1, FVC, and FEV_1/FVC in JHS together with discovery cohort results.

In exploratory analyses, we evaluated the association of all 1,305 proteins assayed by SomaScan with FEV_1, FVC, and

FEV_1/FVC across JHS participants. We observed 90 proteins associated with FEV_1 ($Q < 0.05$). Twenty-nine protein associations were unique to JHS and not significant in our discovery analyses ($Q > 0.05$). Furthermore, we found five proteins associated with FEV_1/FVC in JHS, not observed in discovery analyses. *See* Table E6 for protein associations across all traits in JHS ($Q < 0.05$, accounting for 1,305 protein tests).

Protein Associations with FEV_1 Decline

Our longitudinal analyses were limited to cohorts with follow-up spirometry measurements (FHS, KORA, LSC, and COPDGene only). Table 2 shows baseline characteristics of 2,777 participants. Overall, clinical characteristics of participants included in the longitudinal analyses are similar to those reported in Table 1. Average FEV_1 decline ranged from 30.3 ml/yr to 67.4 ml/yr across cohorts; expectedly, declines were faster in the smoking and COPD cohorts.

We examined relationships between baseline protein levels and rate of FEV_1 decline in ml/yr in multivariable-adjusted models. Fifteen proteins significantly associated with rate of change in FEV_1 ($Q < 0.05$; Figure 4 and Table E7). In multivariable analyses, including adjustments for sex, we observed that higher levels of female-associated sex hormones (follicular stimulating hormone [$\beta = 7.2$ ml/yr, $Q = 2.78 \times 10^{-4}$] and luteinizing hormone [$\beta = 6.7$ ml/yr, $Q = 2.73 \times 10^{-3}$]) were associated with slower rate of FEV_1 decline. Leptin, a hormone produced by adipose tissue, also associated with a slower rate of decline ($\beta = 8.0$ ml/yr, $Q = 1.14 \times 10^{-2}$). Model adjustments for BMI did not attenuate leptin association with FEV_1.

We identified 12 circulating proteins associated with faster lung function decline, including higher levels of neutrophil elastase inhibitors such as SLPI (secretory leukocyte protease inhibitor) and elafin (37) ($\beta = -4.3$ ml/yr, $Q = 0.040$; and $\beta = -4.3$ ml/yr, $Q = 0.049$, respectively). Angiogenic factors, such as VEGF (vascular endothelial growth factor) and angiogenin, also associated with accelerated lung function loss (VEGF; $\beta = -5.4$ ml/yr, $Q = 2.90 \times 10^{-2}$; angiogenin $\beta = -6.6$ ml/yr, $Q = 4.20 \times 10^{-2}$). Elevated levels of TF (tissue factor), a regulator of coagulation cascade (38) and inflammation (39), were also associated with more rapid FEV_1 decline ($\beta = -5.1$ ml/yr, $Q = 9.00 \times 10^{-3}$). Higher levels of proteins enriched within mucus-secreting cells, TFF2 (trefoil factor 2; $\beta = -6$ ml/yr, $Q = 3.21 \times 10^{-2}$) and REG4 (regenerating islet-derived protein 4; $\beta = -5.7$ ml/yr, $Q = 4.03 \times 10^{-2}$) (40) were associated with faster decline.

Look-Ups of Previously Reported Genetic Findings to Support Protein Associations

We identified 22 proteins in discovery analyses associated with lung function traits (FEV_1, FVC, FEV_1/FVC) that had previously reported genetic findings for lung function or COPD (24, 41–43). Table E8 summarizes protein–trait associations with corroborating gene–trait associations from prior genomewide association studies (GWAS) or whole genome sequencing (WGS) association studies, including recent novel findings such as tumor necrosis factor ligand superfamily member 12 (protein: TWEAK; gene: TNFSF12) and TYRO3 (Tyrosine-protein kinase receptor; protein: Dtk; gene: TYRO3) (3, 24, 42–44). Our results highlight IL1RL1 (interleukin-1 receptor-like 1 protein) association with more rapid FEV_1 decline, whereas rs12470864, an IL1RL1 coding gene variant, was recently associated with FEV_1/FVC in a large GWAS (3). Furthermore, a

Table 2. Baseline characteristics of cohort participants with follow-up spirometry

	Population-based		Smoking-enriched	COPD-enriched
	FHS	KORA	LSC	COPDGene
N	1,326	495	217 (100)	739
Interval between first and last spirometry, yr, mean (SD)	6.9 (1.1)	6.5 (0.5)	1.2 (0.3)	5.4 (0.7)
Age, yr, mean (SD)	54 (10)	53.7 (4.4)	55.6 (8.7)	61.6 (8.9)
Male	605 (45.6)	229 (46.3)	46 (21.2)	340 (46.0)
Body mass index, kg/m^2, mean (SD)	27.4 (5.0)	27.4 (4.6)	27.3 (5.5)	28.9 (5.9)
Race, White-European/Non-Hispanic	1,556 (100)	495 (100)	144 (66.4)	662 (89.6%)
Race, Black-African	0 (0)	0 (0)	1 (0.5)	77 (10.4%)
Race, Hispanic	0 (0)	0 (0)	64 (29.5)	0 (0)
Current smoker	241 (18.2)	80 (16.2)	132 (60.8)	255 (34.5)
Never-smoker	490 (37)	193 (39.0)	NA	27 (3.7)
Smoking pack-year history, mean (SD)	15.7 (20.1)	21.0 (19.9)	41.5 (19.6)	41.4 (25.2)
FEV_1, L, prebronchodilator, mean (SD)	2.90 (0.77)	3.3 (0.8)	2.54 (0.8)	2.3 (0.9)
FEV_1 % predicted, prebronchodilator, mean (SD)	93 (15)	103.1 (15.4)	88.1 (19)	78.1 (24.2)
FVC % predicted, prebronchodilator, mean (SD)	98 (13)	103.2 (12.9)	93.5 (14.9)	88.3 (17.5)
FEV_1 decline, ml/yr, mean (SD)	30.3 (25.2)	56.3 (40.8)	64.7 (37.1)	37.6 (55.3)
PRISm	82 (6)	18 (3.6)	17 (7.8)	69 (9.3)
GOLD 1	170 (13)	27 (5.5)	13 (6)	68 (9.2)
GOLD 2	136 (10)	16 (3.2)	31 (14.3)	135 (18.3)
GOLD 3	13 (1)	1 (0.2)	8 (3.7)	71 (9.6)
GOLD 4	0 (0)	0 (0)	3 (1.4)	20 (2.7)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 second; FHS = Framingham Heart Study; FVC = forced vital capacity; GOLD = Global Initiative for Chronic Obstructive Lung Disease; KORA = Kooperative Gesundheitsforschung in der Region Augsburg (Southern Germany); LSC = Lovelace Smokers Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; PRISm = preserved ratio impaired spirometry; SD = standard deviation; SPIROMICS = Subpopulations and Intermediate Outcome Measures in COPD Study.
Entries represent *n* (%) unless otherwise noted. PRISm defined as FEV_1/FVC ≥ 0.7 and FEV_1 % predicted according to Hankinson equations $< 80\%$.

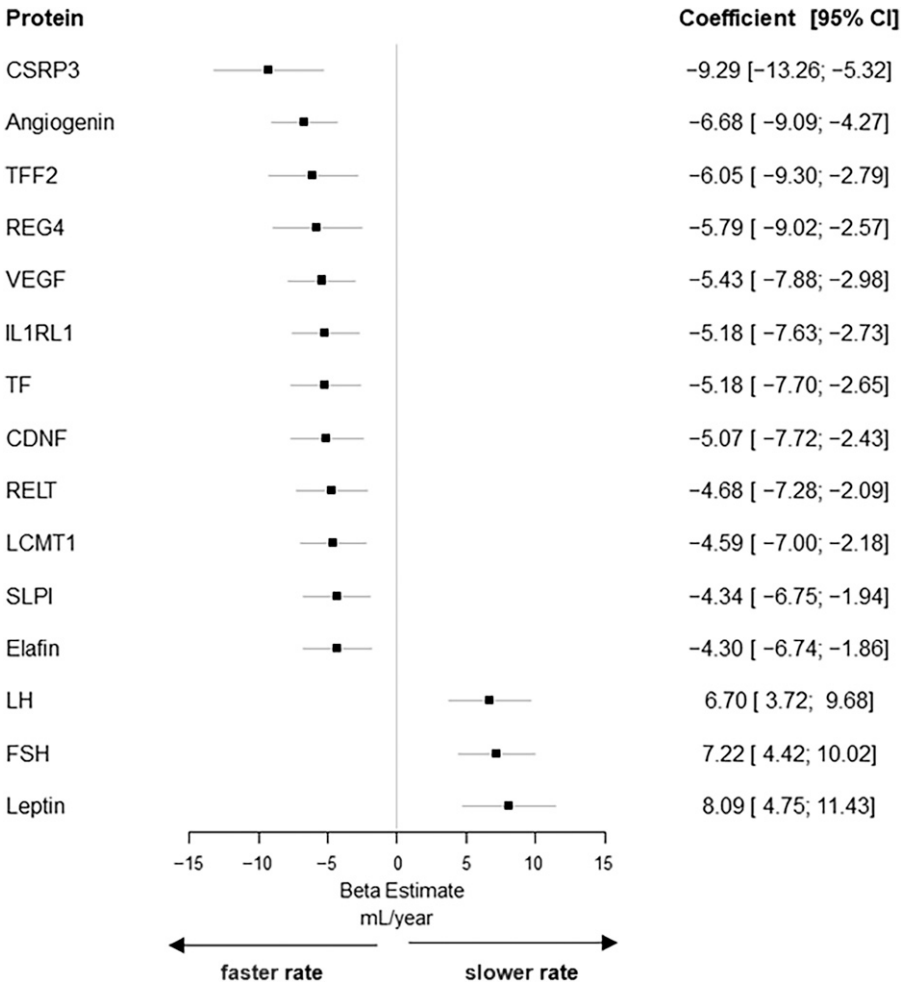

Figure 4. Proteins associated with rate of forced expiratory volume in 1 second (FEV_1) decline. Meta-analyses of linear mixed-effects models for FHS (Framingham Heart Study), KORA (Kooperative Gesundheitsforschung in der Region Augsburg [Southern Germany]), COPDGene, and LSC (Lovelace Smokers Cohort); $Q < 0.05$. Proteins listed in order of ascending β estimate for FEV_1 decline association. Models were adjusted for age, sex, height, smoking status and pack-years, and assay plate and batch (if applicable). Sample size varied because some proteins were not measured in SomaScan Version 1.1k for FHS batch 1 and KORA. β estimates >0 indicate higher levels of protein associated with slower rate of decline and <0 indicate higher levels of proteins associated with faster rate of decline. *See* Table E6 for details for all significant protein associations with FEV_1 decline, including annotation for protein full name, UniProt, EntrezGene, and aptamer sequence IDs. Cohort level results are shown in Table E3. FSH = follicular stimulating hormone; IL1RL1 = interleukin-1 receptor-like 1; LH = luteinizing hormone; REG4 = regenerating islet-derived protein 4; SLPI = secretory leukocyte protease inhibitor; TF = tissue factor; TFF2 = trefoil factor 2.

recent phenome-wide Mendelian randomization study in the MR-base database, which leveraged published protein quantitative trait loci as genetic instruments, identified several proteins with putative causal effects on FEV_1 and/or FVC, including fibulin 3 (protein: FBLN3; gene: EFEMP1, rs3791679), a protein found to be associated with FEV_1 across discovery and validation cohorts (Table E9) (45).

Pathway Analysis for Overrepresentation

Using ConsensusPathDB (20, 21), we tested proteins associated with lung function in our discovery analyses (FEV_1, FEV_1/FVC, and FEV_1 decline) for biological pathway overrepresentation (Table E10 and Figure E1). There was an overrepresentation of proteins associated with immune activation and tissue destruction, including: complement system and heat shock factor 1 dependent transactivation ($P < 0.001$); HSP90 chaperone cycle for steroid hormone receptors, IL1R signaling transduction, peptide hormone metabolism, renin-angiotensin-aldosterone system (RAAS), common pathway of fibrin clot formation, Ras activation upon $Ca2^+$ influx through NMDA receptor, hormone ligand-binding receptors, and potential therapeutics for severe acute respiratory syndrome (SARS) ($P < 0.01$); and selenium micronutrient network, regulation of complement cascade, complement and coagulation cascades, and regulation of IGF (Insulin-like Growth Factor) transport and uptake by IGFBPs (Insulin-like Growth Factor Binding Proteins), ($P < 0.05$).

Discussion

Leveraging large-scale proteomic profiling across multiple cohorts, our study identified

dozens of novel protein associations with lung function and lung function decline across a large, diverse study population. Although the vast majority of proteomic profiling efforts in cardiopulmonary disease have been performed in predominantly White populations (15, 16, 18), there are no studies evaluating pulmonary function in more racially diverse populations. We report cross-sectional protein associations with lung function in discovery analyses comprised of predominantly White participants, but we go on to validate protein findings in an AA cohort. Furthermore, in longitudinal analyses limited to cohorts with follow-up spirometry, baseline levels of more than a dozen proteins associated with the rate of FEV_1 decline. Our study highlights many known and novel proteins and associated pathways that may be relevant in COPD pathobiology in White and AA populations.

We found 254 protein associations with FEV_1 in discovery analyses across multiple cohorts and confirmed 80 protein findings in JHS with $Q < 0.05$. We replicated previously established COPD biomarker associations (46) with FEV_1, including C-reactive protein, IL-6, and fibrinogen, adding confidence to the validity of our study approach. Although previous publications highlight abnormal immune responses and extracellular matrix remodeling as critical mediators of COPD, our study further elucidates proteins and pathways that may be most relevant in processes and pathobiology across our diverse study population.

Several proteins showed a strong association with both FEV_1 and FEV_1/FVC, making them better candidates as COPD biomarkers. Kallistatin, a serine protease structurally similar to alpha-1 antitrypsin (26), was a marker associated with FEV_1 and FEV_1/FVC in discovery and validation cohorts. Although some studies report lower levels of kallistatin in several diseases (e.g., cardiovascular disease, cirrhosis, cancer, sepsis), our study is the first to associate lower levels with impaired lung function (47). Beyond its primary function to inhibit the tissue kallikrein–bradykinin pathway, animal models suggest it also inhibits inflammation, angiogenesis, and cancer cell migration (47). Specifically, it has been shown to inhibit VEGF signaling in animal models (48). VEGF is one of the proteins associated with faster decline, whereas several other pro- and antiangiogenic factors (angiogenin, angiopoietin-2, endoglin, GDF2) demonstrated significant relations with lung function. Our observations support the potential role of pathological vascular remodeling in airway disease.

Our findings represent an enhanced innate immune response. Cross-sectional results for FEV_1 and FVC support activation of the alternative complement pathway (e.g., C9, C7, C5a anaphylatoxin, and Factor B). Among proteins associated with both FEV_1 and/or FEV_1/FVC, we observe upregulation of antimicrobial proteins such as phospholipase A2 (49), LBP (29), S100A12 (30, 31), and S100A9 (50). Notably, phospholipase A2 is also involved in surfactant catabolism (51), as well as production of eicosanoid inflammatory mediators (34). The association of leukocyte elastase inhibitors (e.g., SLPI and elafin) (52, 53) with faster lung function decline may reflect a compensatory response to a greater burden of neutrophil elastases and proteases. The enrichment of innate immune mediators may elucidate mechanisms by which chronic bacterial colonization contributes to COPD.

Coagulation factors emerged as top associations across analyses, which may suggest dysregulation of coagulation favoring a procoagulant state in lung disease. Elevated TF levels were associated with more rapid lung function decline. TF is released in the setting of tissue injury and initiates the common extrinsic coagulation cascade to generate thrombin (54). Antithrombin was the most significantly associated protein with FEV_1 across our study cohorts, suggesting possible counterregulatory mechanisms to diminish coagulation. Protease nexin-1, a tissue thrombin inhibitor, has associated with FEV_1 and has been identified as a COPD susceptibility gene (55, 56).

Several proteins in extracellular matrix remodeling were highlighted across our cohorts, including MMP-12 (metalloproteinase-12), matrilysin, FBLN3 (fibulin-3), aggrecan, and brevican core protein. Genetic variants near or in the MMP-12 and FBLN3 genes have been associated with FEV_1 (3, 9) and moderate and severe COPD (43). Notably, animal model work suggests a role for MMP-12 in the pathogenesis of cigarette-induced emphysema (57, 58). Although previous studies implicated MMP-12 in lung function and COPD pathogenesis, none have reported circulating protein level associations with lung function. In aggregate, these data may support MMP-12 as a potential blood marker for COPD risk.

Although genetic studies have identified variants within the 1L1RL1 gene to be associated with FEV_1 and FEV_1/FVC, our study is the first to report 1L1RL1 (a.k.a. ST2) plasma protein association with lung function decline. The IL1RL1 protein transmembrane protein facilitates an inflammatory cascade by binding to IL-33; it can also be a soluble decoy receptor for IL-33 to downregulate inflammation. In transmembrane form, IL1RL1 may also represent a potential link between the airway epithelium and induction of Th2-type allergic responses leading to production of IgE (59). Consistent with this finding, our cross-sectional results demonstrate an inverse association of IgE with FEV_1 across the discovery and validation cohorts. Given that multiple GWASs have implicated IL1RL1 in asthma (60), IL1RL1 may represent a potential pathway in COPD–asthma overlap. A history of asthma was associated with higher IgE in the COPDGene study, further suggesting a role for COPD–asthma overlap as a risk factor for progression. However, other factors, such as blood eosinophils or bronchodilator responsiveness, were not associated with IgE or IL1RL1 (Figure E1). There are clinical trials underway targeting the IL1RL1/ST2 pathway as COPD and asthma treatments (61).

Our study highlights 22 proteins associated with lung function traits (FEV_1, FVC, and FEV_1 decline), supported by previous genetic findings for lung function and COPD (3, 24, 42, 44). Among these protein associations, several were validated in JHS, including CDH5 (cadherin 5), a vascular endothelial glycoprotein. Few studies have examined genetic risk factors in African ancestry; however, one multiethnic GWAS has identified variants near the CDH5 gene associated with FEV_1/FVC in a population of African ancestry, providing additional evidence to support the relevance of CDH5 in AA populations. A limitation of our look-ups is that the vast majority of genetic association studies have been conducted in predominantly European ancestry populations. Although complementary protein–gene trait associations in our discovery cohorts and the referenced genetic studies may highlight proteins and pathways most relevant to White populations, we believe protein findings validated in JHS may be generalizable to AA participants as well,

despite the lack of genetic data for African ancestry.

Limitations

Our study has several limitations. Although many cross-sectional findings were validated in the AA cohort, we lacked validation for our longitudinal results. Proteins unique to JHS also require validation in another AA cohort; however, proteomics data remain limited in AA populations. Although cohort study designs were heterogeneous, by combining results we detected significant protein associations with the rate of FEV_1 decline, despite the small effect size and small sample size. Such heterogeneity would bias results toward the null. In addition, cohorts had different lengths of follow-up and number of spirometries, and some cohorts only had two time points, which would likely bias toward a null result through more noise. Additional potential biases to the null result include heterogeneity of sample collection and storage among cohorts, some of which were stored for years in freezers before assays. Other publications have found that samples without repeated freeze–thaws and stored at a stable temperature ($-80^{\circ}C$ or $-30^{\circ}C$) are stable for long periods (62) and that the chronologic age of the subject has a much bigger effect on proteins than the storage time for human proteins (63). Another confounding factor that may bias toward null results is inhaled corticosteroid (ICS) use, which is strongly associated with COPD severity and has been shown to depress inflammatory proteins such as HSP70 and eotaxin (64). In a small 4-week trial of patients given inhaled budesonide, sputum nuclear levels of HSP70 and HSP90 were higher in subjects receiving ICS (65). Thus, some of the observed HSP90 pathway effects associated with severity of obstruction could be due to increased use of ICS in patients with more severe disease. We believe many findings highlight protein signals that are common across the entire study population—several of which were supported by previous GWAS findings. Our study was limited to prebronchodilator measurements because post-bronchodilator spirometry was unavailable across all cohorts. However, pre- and post-bronchodilator spirometry measures are highly correlated (66), and pre- and post-bronchodilator lung function are similarly associated with outcomes, such as general population mortality (67). Although this proteomics platform assays 1,305 proteins, it does not provide uniform pathway coverage; thus, the analytes assayed overall are a modest fraction of the human proteome.

Conclusions

We identified and validated dozens of novel circulating protein markers associated with cross-sectional lung function across a diverse study population and associated baseline levels of several proteins with the rate of FEV_1 decline. By leveraging this large-scale proteomic platform, we identified putative proteins and pathways that may be relevant in COPD development and provided additional biologic evidence to support previously reported genetic associations with lung function and COPD. Future efforts should be directed toward: *1*) replicating findings in more diverse populations; *2*) confirming technical specificity of more aptamers for target proteins/isoforms; *3*) examining the clinical utility of these biomarkers for prediction and risk stratification; *4*) elucidating the functional effects of these protein–phenotype associations, as well as other clinical features such as medication use, eosinophilia, bronchodilator responsiveness, etc.; and *5*) whether these proteins might represent novel disease markers for subtyping and risk stratification, together with novel molecular targets for treatment of COPD. ■

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