Original Research

Serum Proteins Associated with Emphysema Progression in Severe Alpha-1 Antitrypsin Deficiency

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for QUANTUM-1 Investigators *

Abstract

Computed tomography (CT) lung density is an accepted biomarker for emphysema in alpha-1 antitrypsin deficiency (AATD), although concerns for radiation exposure limit its longitudinal use. Serum proteins associated with emphysema, particularly in early disease, may provide additional pathogenic insights. We investigated whether distinct proteomic signatures characterize the presence and progression of emphysema in individuals with severe AATD and normal forced expiratory volume in 1 second (FEV_1). **QUANT**itative lung CT **UnM**asking emphysema progression in AATD (QUANTUM-1) is a multicenter, prospective 3-year study of 49 adults with severe AATD and FEV_1 post-bronchodilator values (Post-BD) $\geq$ 80% predicted. All participants received chest CT, serial spirometry, and contributed to the serum biobank. Volumetric imaging display and analysis (VIDA) software defined the baseline 15th percentile density (PD15) which was indexed to CT-derived total lung capacity (TLC). We measured 317 proteins using a multiplexed immunoassay (Myriad Discovery MAP® panel) in 31 individuals with a complete dataset. We analyzed associations between initial PD15/TLC, PD15/TLC annual decline, body mass index (BMI), and protein levels using Pearson's product moment correlation. C-reactive protein (CRP), adipocyte fatty acid-binding protein (AFBP), leptin, and tissue plasminogen activator (tPA) were found to be associated with baseline emphysema and all but leptin were associated with emphysema progression after adjustments were made for age and sex. All 4 proteins were associated with BMI after further adjustment for multiple comparisons was made. The relationship between these proteins and BMI, and further validation of these findings in replicative cohorts require additional studies.

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Introduction

Alpha-1 antitrypsin deficiency (AATD) predisposes individuals to early chronic obstructive pulmonary disease (COPD). COPD is one of the leading causes of medical hospitalizations and the third leading cause of death worldwide.[1] AATD individuals experience even more hospital visits and cost more to the health care system compared to the older and more comorbid cohort of patients with usual COPD.[2] The search for biomarkers that inform future clinical events is important. Unfortunately, only a few existing therapies (e.g., smoking cessation and oxygen therapy for hypoxemic individuals) reduce mortality or modify the natural history of disease. In addition, few novel drugs with systemic action are currently in clinical trials. Among the many problems with COPD drug development is the slow progression of disease in the majority of affected individuals. In addition, the many clinical phenotypes of disease complicate a comprehensive disease understanding.

AATD is the most common known genetic cause of COPD[3] that affects at least 100,000 Americans and over 3 million individuals worldwide. The blood level of alpha-1 antitrypsin is determined by each of the inherited alleles on SERPINA1, the gene that codes for alpha-1 antitrypsin. Various genotypes exist with the largest burden of disease ascribed to protease inhibitor ZZ (PiZZ) and protease inhibitor SZ (PiSZ).

Individuals with AATD present at an earlier age and have more rapid disease progression than individuals with usual COPD.[4] AATD individuals are at increased risk for developing COPD compared to the general population, especially when tobacco or environmental exposures exist, causing disease at younger age. Additionally, individuals with AATD have an emphysema-predominant phenotype that is more homogeneous than usual COPD. Intravenous alpha-1 antitrypsin, known as augmentation therapy, is currently approved by regulatory authorities for AATD individuals with forced expiratory volume in 1 second (FEV_1) in the range of 30%-65% predicted.[5] Observational studies have noted excess mortality in AATD and have shown a survival benefit in selected subgroups of individuals receiving augmentation therapy.[6,7] We hypothesized that biomarkers may characterize AATD individuals with rapid progression of emphysema, thereby creating the possibility of new, targeted therapy.

Most traditional biomarkers of COPD severity and progression have been related to lung function decline, measured as FEV_1.[8,9] Chest radiography is not sensitive enough to detect early emphysema. Computed tomography (CT) of the chest can reveal early emphysematous changes that are sometimes present in individuals with normal lung function who are minimally symptomatic. Emphysema is usually detected earlier in life and in a higher proportion of AATD affected individuals compared to individuals with usual COPD.

CT lung density has been utilized as a promising

biomarker in AATD.[10] In a mixed cohort of both usual COPD and AATD-associated COPD, assessment of emphysema progression was more sensitive by lung densitometry than FEV_1 and measurements of gas transfer.[11] For this reason, CT density has been allowed as a primary endpoint in trials of augmentation therapy efficacy by U.S. and European regulatory authorities.[5] Desmosine is the most promising biomarker in AATD-related COPD,[12] although several others have been evaluated, including fibrinogen,[13] matrix-metalloprotease-9,[14,15] and angiopoietin-like protein 4.[16]

In 2008, the National Institutes of Health Office of Rare Diseases and the National Heart, Lung, and Blood Institute funded the Rare Lung Disease Consortium to study radiographic emphysema decline in a cohort of PiZZ AATD individuals with normal lung function as defined by post-bronchodilator (post-BD) FEV_1% predicted ≥ 80% per the National Health and Nutrition Examination Survey III (NHANES III).[17] The primary hypothesis of the study, called **QUANT**itative lung CT **U**n**M**asking emphysema progression in AATD (QUANTUM-1), was to determine whether baseline CT density predicted a more rapid decline in FEV_1. Because the large biorepository of this carefully phenotyped cohort is available for additional projects, we analyzed this cohort to identify serum proteins that correlate with CT density decline in individuals with early stage AATD-associated COPD.

Methods

Study Design and Population

The 49 participants in QUANTUM-1 were enrolled between September 9, 2007 and December 11, 2008 following informed consent at 1 of 7 participating U.S. study centers and followed for 3 years. All participants had normal lung function at baseline as defined by post-bronchodilator FEV_1% predicted ≥80% per the NHANES III.[17] Baseline demographics are included in Table 1. No participant smoked during the time of biospecimen collection. At each visit, absence of significant smoke exposure was confirmed in all individuals by urinary cotinine. Participants had bimonthly telephone calls to assess exacerbations and serial site visits (at baseline, 6, 12, 18, 24, and 36 months) with chest CT, pulmonary function tests (spirometry, lung volumes, and diffusing capacity of

the lung for carbon monoxide (DLCO) tests, and serum collection to explore the natural history of disease progression over the course of 3 years. Although the protocol specified 50 individuals, funding ended once 49 participants were enrolled.

The annual changes in FEV_1 were calculated utilizing baseline, 6, 12, 18, 24, and 36-month visits. The PD15/TLC slope was calculated by endpoint analysis (36 months-baseline) based on acceptability of the CT scans for analysis. The exacerbation frequency for the year preceding the study was assessed at the baseline site visit using the AlphaNet exacerbation questionnaire.[18] This AATD-specific questionnaire was developed to address exacerbation type and frequency by a variety of exacerbation definitions and has been used in large cohorts of AATD individuals who receive augmentation therapy through AlphaNet.

Contiguous non-overlapping 0.6-1.0mm slice thickness multi-detector row CT scans were acquired at 80 mAs, 120 kVp. Images were reconstructed using a medium spatial frequency reconstruction algorithm (GE: Standard, Philips: B, Siemens: B30f, Toshiba: FC86). A Gammex phantom was used to assure the accuracy of each CT scanner in the study at 6-month intervals.

We included all participants with acceptable baseline and 36-month CT scans that could be analyzed using Apollo image analysis software (VIDA Diagnostics, Inc.). Briefly, the lungs were segmented in 3-dimensions from the chest wall and mediastinal components using Apollo. The CT-derived total lung capacity (TLC), mean lung density and the lowest percentile density at 15% (PD15) corrected for lung volume was derived from all air density voxels. Of the original cohort of 49 participants, 7 had missing data, and 11 had CT scans that did not follow the specific acquisition protocol. Thirty-one individuals remained eligible for analyses.

Blood Samples

A total of 50 ml of blood was drawn at the initial study visit for serum and plasma analysis. Serum samples were allowed to clot for 15 minutes per protocol, spun at 3500 rpm, and placed into a -80°C freezer until transported on dry ice to the central repository. Serum samples were stored at the University of Florida Alpha-1 Genetics Laboratory or at the Medical University of South Carolina Alpha-1 Registry at -80°C until analyzed.

Table 1. Demographic Features of the PiZZ Alpha-1 Antitrypsin Deficiency Cohort Enrolled and Excluded from Proteomic Analysis

Characteristic	Included Patients (N=31)	Range	Excluded Patients (N=18)	Range	P Value
Age, years[a]	52 ± 10	32 - 71	50 ± 9	35 - 69	0.45
Sex, number of females (%)	18 (58%)	N/A	14 (78%)	N/A	0.15
BMI, kg/m^{2a}	30 ± 7	21 - 48	28 ± 7	21 - 46	0.24
FEV$_1$ post-BD % predicted[a]	101 ± 12	76 - 123	97 ± 10	75 - 115	0.22
FEV$_1$/FVC post-BD	0.76 ± 0.1	0.5 - 0.9	0.76 ± 0.1	0.5 - 0.96	0.95
Baseline PD15, HU[a]	- 937 ± 27	- 980; - 846	- 937 ± 20	- 966; - 892	0.99
Mean loss of FEV$_1$, ml/year[a]	58 ± 85	- 300; + 80	32 ± 88	- 280; + 160	0.31
Baseline DLCO, adjusted for hemoglobin	23 ± 6	12 - 43	22 ± 7	11 - 40	0.53
DLCO slope	- 0.5 ± 0.8	- 2; + 1.5	- 0.7 ± 0.8	- 2.9; 0.66	0.49

[a]mean ± Standard Deviation.

BMI=body mass index; FEV$_1$=forced expiratory volume in 1 second; post-BD=post bronchodilator; FVC=forced vital capacity; PD15=percentile density at 15%; DLCO=diffusion capacity for carbon monoxide.

Primary Outcome Measures

We measured 317 proteins from serum obtained at the baseline visit using a multiplexed immunoassay (Myriad Discovery MAP® panel) in the 31 study individuals with a complete dataset. Values were expressed as mean ± standard deviation. We analyzed the associations between baseline PD15/TLC, PD15/TLC annual decline, and baseline protein levels using Pearson's product moment correlation. A *p*-value < 0.05 was considered statistically significant. Correlations were determined to be weak, moderate, or high when the r coefficient was in the range 0.2-0.39, 0.4-0.59, or 0.6-0.79, respectively. The two-tailed correlations were then adjusted for age, and sex. To reduce false positives due to multiple comparisons, we have further adjusted the raw *p*-values for a global false discovery rate (FDR) of less than 10%.[19] This method adjusts up the significant values for individual proteins to ensure that the expected number of false positives among positive results is no more than 10%, which is a common practice in high-throughput data analysis.

Pathway analysis of proteins correlated with PD15/TLC and/or its slope was performed using the UniProt number and Homo sapiens Reactome pathway analysis software.[20]

Results

The 31 eligible PiZZ participants had a mean age of 52 ± 10 years, a body mass index (BMI) of 30 ± 7 kg/m^2 (39% had BMI in the obese range), FEV$_1$/ forced vital capacity (FVC) post-BD of 0.76 ± 0.1, post-BD FEV$_1$ of 101 ± 12% predicted, baseline DLCO adjusted for hemoglobin 23 ± 6, and baseline PD15 of -937 ± 27 Hounsfield units (HU). A slight majority of participants were women (58%). No differences in baseline characteristics between the 31 included and the 18 excluded participants were found (Table 1). There were changes in radiographic and spirometric measurements over the 3-year study course, verifying progression of emphysema. Annual changes included a decline of FEV$_1$ of 58 ± 85 ml/year, decline of PD15 of 4 ± 13HU/year, increase of CT volume (CT TLC) of 80 ± 371 ml/year, decrease of PD15/ CT TLC of 0.75 ± 4.4 HU/ml/year, decline in DLCO of 0.5 ± 0.8, and rise in post-bronchodilator TLC measured by lung volumes of 212 ± 600 ml.

Exacerbation questionnaires at the baseline visit revealed that 47% of individuals had at least one exacerbation in the preceding year. However, over the 3 years of observation, only 51% had one or more events. A bivariate analysis of FEV$_1$ slope and PD15 slope adjusted for TLC in 31 individuals confirmed a significant association between spirometry and

CT measures. However, adjustment for age and sex, extinguished the significant correlation between FEV$_1$ decline and emphysema progression (p=0.21 after adjustment). Baseline emphysema showed wide variation among participants at baseline. All but 5 individuals had emphysema present, as defined by PD15 ≤ -910 HU, with a range of PD15 of -966 HU to -892 HU. Three of 5 participants with no emphysema by this definition were extremely obese (BMI>39), potentially affecting PD15 measurements.

From the 317 proteins evaluated, 31 were associated with baseline emphysema (Table 2) and 14 were associated with emphysema progression (Table 3) when unadjusted for age, sex, and multiple comparisons. Most of these proteins showed a moderate degree of correlation as measured by Pearson product moment

Table 2. Proteins Associated with Severity of Baseline Emphysema as Measured by Percentile Density at 15 Percent /Total Lung Capacity Slope

Protein	Unique and Stable Entry Identifier	Baseline PD15/TLC (r)	*p*-value	Degree of Correlation
6Ckine	O00585	-0.428	0.02	m
Adipocyte Fatty Acid-Binding Protein	P15090	-0.446	0.01	m
Aldose Reductase	P15121	0.368	0.04	w
Alpha-1 Antitrypsin	P01009	0.448	0.01	m
Alpha-1- Microglobulin	P02760	0.417	0.02	m
Angiogenin	P03950	0.404	0.02	m
Antileukoproteinase	P03973	0.400	0.03	m
Apolipoprotein D	P05090	0.501	0.004	m
Cadherin-1	P12830	0.393	0.03	w
Ceruloplasmin	P00450	-0.480	0.006	m
Chromogranin-A	P10645	0.369	0.04	w
Complement Factor H	P08603	-0.389	0.03	w
C-Reactive Protein	P02741	-0.445	0.01	m
Cystatin-A	P01040	0.395	0.04	w
Dickkopf-Related Protein 1	O94907	-0.371	0.04	w
Gelsolin	P06396	0.388	0.03	w
HE4	Q14508	0.472	0.007	m
IGFBP4	P22692	0.421	0.02	m
IGFBP6	P24592	0.551	0.001	m
Leptin	P41159	-0.654	0.00006	s
Macrophage-Derived Chemokine	O00626	-0.372	0.04	w
Macrophage Inflammatory Protein 3 Beta	Q99731	-0.358	0.047	w
Matrix Metalloproteinase-3	P35625	0.661	0.00005	s
Myoglobin	P02144	0.581	0.0006	m
Retinol-Binding Protein 4	P02753	0.415	0.02	m
Sex Hormone-Binding Globulin	P04278	-0.377	0.04	w
Stromal Cell-Derived Factor-1	P48061	-0.435	0.01	m
Thrombomodulin	P07204	0.408	0.02	m
Thyroxine-Binding Globulin	P05543	-0.490	0.005	m
Tissue Type Plasminogen Activator	P00750	-0.455	0.01	m
Transthyretin	P02766	0.381	0.03	w

IGFBP=insulin-like growth factor binding protein; m=moderate; s=strong; w=weak; PD15=percentile density at 15%; TLC=total lung capacity.

Table 3. Proteins Associated with Emphysema Progression as Measured by Percentile Density at 15 Percent/Total Lung Capacity Slope

Protein	Unique and Stable Entry Identifier	Baseline PD15/TLC (r)	*p*-value	Degree of Correlation
Adipocyte Fatty Acid-Binding Protein	P15090	-0.425	0.02	m
Angiopoietin-1	Q15389	0.433	0.01	m
Angiopoietin-Related Protein 4	Q9BY76	-0.477	0.007	m
Brain-Derived Neurotrophic Factor	P23560	0.374	0.04	w
C-Reactive Protein	P02741	-0.471	0.007	m
Growth-Regulated Alpha Protein	P09341	0.406	0.02	m
Omentin	Q8WWA0	-0.370	0.04	w
Osteoprotegerin	O00300	-0.379	0.04	w
Pancreatic Secretory Trypsin Inhibitor	P00995	-0.364	0.04	w
Plasminogen Activator Inhibitor 1	P05121	0.404	0.02	m
Sclerostin	Q9BQB4	-0.378	0.04	w
Sex Hormone-Binding Globulin	P04278	-0.559	0.001	m
Thrombospondin-4	P35443	-0.379	0.03	w
Trefoil Factor 3	Q07654	-0.513	0.003	m

m=moderate; s=strong; w=weak; PD15=percentile density at 15%; TLC=total lung capacity.

correlation. In unadjusted analyses, 3 proteins (C-reactive protein [CRP], adipocyte fatty acid binding protein [AFBP], and sex hormone-binding globulin) were associated with both baseline emphysema and emphysema progression as measured by the PD15/TLC and PD15/TLC slope, respectively (Table 4). Angiopoietin-related protein 4 showed a moderate correlation (r=-0.477, *p*=0.007) with emphysema progression. CRP and AFBP correlated strongly with BMI.

CRP, AFBP, leptin, and tissue plasminogen activator (tPA) were found to be significantly associated with BMI after controlling for multiple comparisons at global FDR < 10% and adjusting for age and sex (Table 5). If these proteins were not adjusted for multiple comparisons, all 4 were statistically significantly associated with baseline emphysema and all but leptin were associated with emphysema progression after adjustments were made for age and sex (Table 6 and Figure 1). In summary, the adjustment for multiple comparisons caused the strength of the associations of CT-measured emphysema with biomarkers to lessen such that none correlated with CT measures of emphysema (q value range 0.18-0.94).

Discussion

In individuals with rare diseases such as AATD, large, prospective, controlled, clinical trials are difficult to perform. Therefore, characterizing the correlation between biomarkers and important clinical features is of paramount importance, as the availability of reliable biomarkers might limit the size of required clinical trials and inform emphysema research about biologically relevant pathways.

Baseline PD15 values in our cohort (N=47) were -937 ± 25 HU, which is abnormal even in comparison to smoking participants with normal gas trapping.[21]

In individuals with AATD and preserved lung function, our unadjusted analysis showed that of 317 proteins assessed, 31 proteins were associated with baseline emphysema extent and 14 proteins were correlated with its progression. Only 3 of these 42 proteins correlated both with baseline emphysema extent and its progression: CRP, AFBP, and sex hormone-binding globulin. These 3 proteins are not related in pathway analysis; however, each has a previously described connection to emphysema.[22-26]

One of the important questions in the field of COPD and emphysema is whether the longitudinal change in FEV₁ correlates with the emphysema progression measured by PD15 utilizing CT. In our cohort of

Table 4. Proteins Correlated with Emphysema, Emphysema Progression, and Body Mass Index

Protein	PD15/TLC Baseline (r)	*p*-value	PD15/TLC/ year (r)	*p*-value	BMI (r)	*p*-value
C-Reactive Protein	-0.445	0.01	-0.471	0.007	0.634	0.0001
Adipocyte Fatty Acid-Binding Protein	-0.446	0.01	-0.425	0.02	0.683	0.00002
Sex Hormone-Binding Globulin	-0.377	0.04	-0.559	0.001	0.222	0.231

BMI=body mass index, PD15=percentile density at 15%; TLC=total lung capacity.

Table 5. Proteins Correlated with Emphysema, Emphysema Progression, and Body Mass Index Adjusted for Age, Sex, and False Discovery Rate

Protein	q-value PD15/TLC Baseline	q-value PD15/TLC/year	q-value BMI
C-Reactive Protein	0.18	0.28	0.039
Adipocyte Fatty Acid-Binding Protein	0.28	0.26	0.026
Leptin	0.26	0.94	0.006
Tissue Type Plasminogen Activator	0.26	0.37	0.098

BMI=body mass index, PD15=percentile density at 15%; TLC=total lung capacity.

Table 6. Proteins Correlated with Emphysema, Emphysema Progression, and Body Mass Index Adjusted for Age and Sex

| Protein	PD15/TLC Baseline (r)	*p*-value	PD15/TLC/ year (r)	*p*-value	BMI (r)	*p*-value
C-Reactive Protein	-0.532	0.002	-0.428	0.02	0.626	0.0002
Adipocyte Fatty Acid-Binding Protein	-0.430	0.02	-0.449	0.01	0.650	7.46E-05
Leptin	-0.446	0.01	-0.110	0.55	0.708	8.50E-06
Tissue Type Plasminogen Activator	-0.460	0.01	-0.404	0.02	0.584	0.0006

BMI=body mass index, PD15=percentile density at 15%; TLC=total lung capacity.

31 individuals followed for 3 years we found no association between decline in spirometry and CT emphysema measures in analyses adjusted for age and sex. More importantly, once corrected for age, sex, and multiple comparisons, striking associations between CT features and CRP, AFBP, leptin, and tPA with BMI were found. This finding is significant as we previously found that obesity (BMI>30) was associated with more exacerbations in this cohort of individuals.[23] Exacerbations are important patient-reported outcomes and are known to negatively affect COPD individuals and portend poor prognosis.[24] Our study lacked sufficient power for formal statistical pathway analysis. However, proteins correlated with PD15/TLC or its slope were often interrelated with

broad connections to known pathways of emphysema pathogenesis. These involved complement activation, retinoic acid transport, wingless-related integration site (WNT) signaling, pathways of hemostasis, and chemokine production. As discussed below, some of the pathways involved in metabolism of lipids and lipoproteins were also overexpressed.

CRP has been proposed as a biomarker for COPD because of clinical associations with FEV_1, exacerbations, comorbidities, hospitalization risk, and mortality.[22-24] Biochemically, CRP promotes complement fixation and may scavenge nuclear material from damaged circulating cells. In addition, CRP has several functions associated with host defense. The complement cascade is critical in connecting the

Figure 1. Proteins Correlated with Emphysema, Adjusted for Age, and Sex
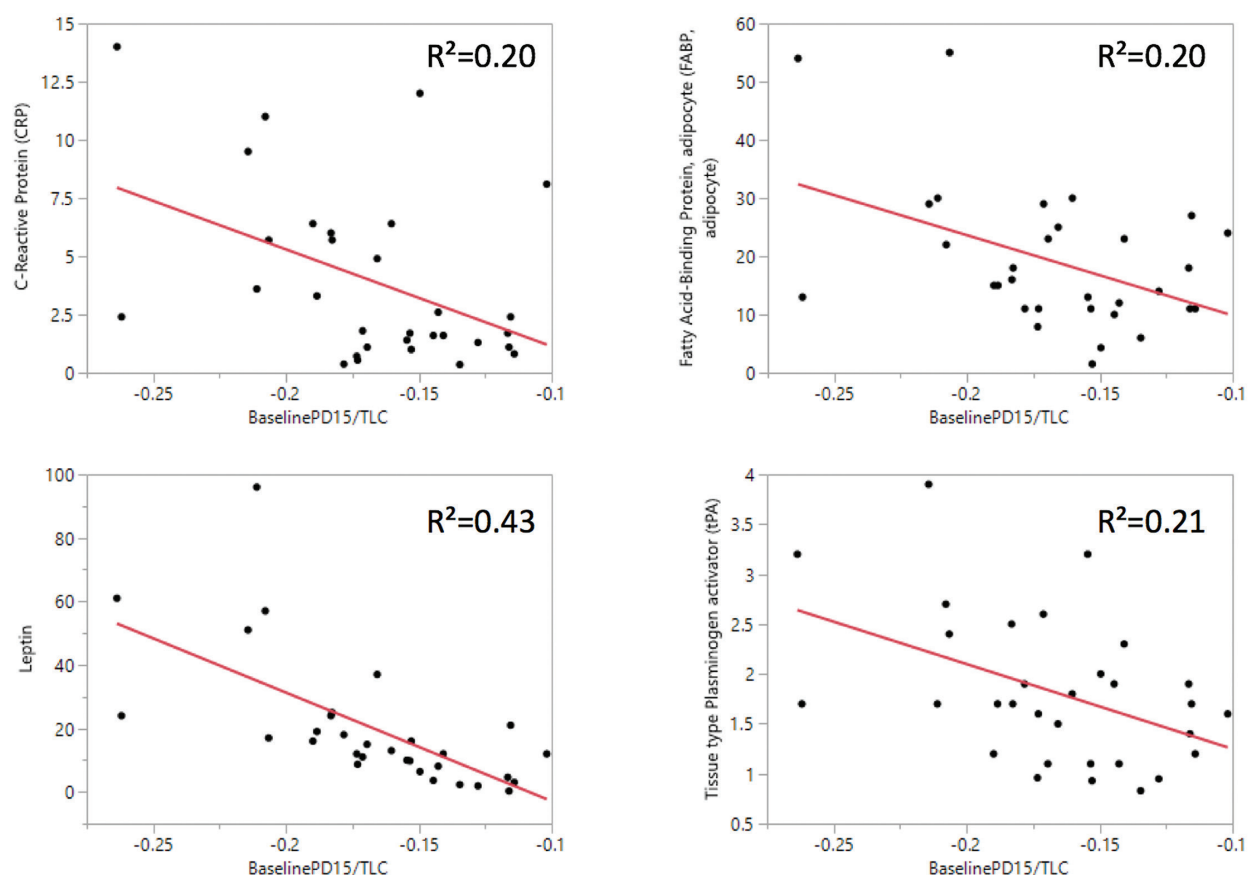
innate and adaptive immune response and has been studied in COPD.[25] Because CRP has been extensively studied in cardiac disease as a risk factor biomarker, the lack of specificity for emphysema will be an issue in further advancing this observation toward the clinic.

AFBP is a lipid transport protein in adipocytes which binds both long-chain fatty acids and retinoic acid. Given the long history of interest in retinoic acid therapy and alveolar growth, the association with rate of emphysema progression is interesting.

TPA plays an important role in tissue remodeling and degradation, by converting plasminogen to plasmin. Prior research confirms that the tPA levels are predictive of cardiovascular events[26] and recent publications reveal the potential of fibrinolytic agents as treatments for chronic respiratory diseases.[27]

Also, earlier analyses revealed that exacerbation frequency is increased in obese individuals with AATD.[23] In this exploratory analysis, we found that some proteins had very strong correlations with BMI

(CRP and AFBP). This association is not surprising as some view obesity as a chronic inflammatory state that further perpetuates many chronic diseases. In a clinical context, the prevalence of obesity is disproportionately high in individuals with COPD[28] and the cause and consequences of this association remains speculative. To date, studies investigating the impact of obesity on mortality in COPD individuals suggest that obesity is associated with a 30%-34% increase in relative risk of all-cause mortality in individuals with mild or moderate COPD compared to individuals with normal weight and comparable disease severity.[29] How much of the associated mortality from high BMI is from COPD comorbidities and how much may be from an impact on emphysema progression remains unknown. Recent analysis of the Behavioral Risk Factor Surveillance System data collected by the Centers for Disease Control and Prevention confirmed that comorbidities are commonly present in COPD individuals.[30]

Body composition abnormalities with higher

measurements of adiposity are related to greater functional limitations, particularly in women.[31] One of the reasons that obesity may be associated with worse outcomes in COPD may relate to systemic inflammation.[32] In the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort, patients exhibiting high circulating levels of 6 inflammatory biomarkers had worse health outcomes and were more obese compared to non-inflamed individuals.[33] In our study, metabolism of lipids and lipoproteins were implicated by finding different expression of adipocyte fatty acid-binding protein, aldose reductase, and angiopoietin-related protein 4. Angiopoietin-related protein 4 plays a role in several plausible AATD-associated COPD pathways[34] involving inflammation, lipid metabolism, and metabolic dysregulation. Therefore, linkage between lipid transport and systemic inflammation in COPD[35] is supported by these observations in the well-phenotyped QUANTUM-1 cohort of AATD individuals.

Sex hormone-binding globulin was also associated with emphysema and its progression, although the degree of correlation was smaller. This molecule has been associated with androgen binding and could participate in emphysema pathogenesis by altering the clearance rate of steroid hormones. We are unaware of other pathway associations for COPD.

The other 39 proteins that were associated with either baseline emphysema extent or emphysema progression play roles in many pathways that have been previously implicated in emphysema pathogenesis. Associations with programmed cell death showed activity for apoptotic signaling that has been well studied in emphysema.[36] We also found strong signals for retinoic acid transport. Although clinical trials of all-trans retinoic acid have proved negative[37] the retinoid cycle has been implicated in COPD pathogenesis. In this context, 2 proteins in our analysis regarded the retinoid cycle: retinol-binding protein 4 and transthyretin, both with significant correlations to baseline emphysema (of moderate and strong degrees).

Very little is known about the types of inflammatory events and their initiation, especially in early onset emphysema. In this regard, our finding that some chemokine receptors and chemokines were associated with emphysema extent raises the possibility that they contribute to the pathogenesis of emphysema.

The associated proteins in this study include 6Ckine, stromal cell-derived factor-1, macrophage inflammatory protein 3 beta, growth-regulated alpha protein, and macrophage-derived chemokine.

Linkages to autoimmunity have been studied extensively in clinical and experimental COPD.[38] Proteins involved in WNT signaling (sclerostin and dickkopf-related protein 1) were moderately expressed, as was metalloproteinase 3, a metalloproteinase previously identified to play a role in AATD-related emphysema.[39]

The hemostatic protein fibrinogen is the first serum biomarker validated in the COPD Biomarkers Qualification Consortium[40] and has known involvement in hemostatic pathways.[41] Fibrinogen reflects both COPD severity and progression by association with adverse clinical outcomes (e.g., exacerbation, hospitalization, and mortality) and is now a drug development tool in COPD. We saw significant upregulation (negative correlation with PD15/TLC) for tissue type plasminogen activator and downregulation of its inhibitor plasminogen activator inhibitor 1, signaling a pro-thrombotic state associated with emphysema.

This study has several limitations. First, the sample size was small and included only 31 individuals of the 49 participants originally enrolled in the study. The fact that included participants did not differ from excluded participants regarding demographic features lessens concerns about selection bias. Second, the associations reported were based on single measurements but not serial measures. Furthermore, the extent of inter-individual and intra-individual variation in the biomarker protein measurements in COPD patients is currently unclear. Few exacerbations were seen over the 3 years of the study, limiting correlations with this clinical outcome. Obesity with BMI >40 was present in 4 of 31 individuals and can impact CT density independent of chest restriction by radiation scatter. As such, though our findings are provocative and novel, their validity and generalizability will require replication in other cohorts.

Variation in CT measurement of lung volumes posed a challenge in this study, as it has characteristically in other studies of individuals with AATD.[5] Our study used state-of-the-art techniques to control CT acquisition, which have not materially advanced since 2008 when the images in this study were obtained. Although new methodologies that allow registration of

the same segments during inspiration and expiration to differentiate between small airways disease and emphysema are available, these were not used in this study as they have not been fully validated.[42]

Projects designed for discovery of novel biologic pathways that impact clinical disease are high risk with the hope of high reward. The challenge with rare disease cohorts such as AATD is that validation cohorts are limited and data are costly to acquire. Recognizing that validation of these findings is needed before routine use of these biomarkers in clinical trials can be endorsed, our findings are novel and, we believe, promising.

Conclusions

Prior publications confirm that PiZZ AATD individuals are at increased risk for developing COPD compared to the normal population and typically develop emphysema at a younger age, especially if exposed to tobacco smoke. We have shown that baseline CT density varies widely in the QUANTUM-1 cohort, which includes individuals with advanced emphysema despite normal lung function tests and some individuals with normal lung density as measured by densitometry. In addition, these preliminary data suggest that, at baseline, there is poor correlation between the rate of FEV_1 decline and the radiographic presence of emphysema.

In this cohort of AATD individuals, leptin, CRP, AFBP, and tPA were associated with baseline emphysema and all but leptin were associated with emphysema progression after controlling for age and sex. Strong correlations of these proteins with BMI were also found after adjustments for multiple comparisons were made.

While analysis of CRP and leptin in COPD is ongoing,[22,43,44] the observation regarding AFBP may prompt investigation regarding the regulation of systemic glucose and lipid metabolism in the pathogenesis of COPD. Similarly, more research investigating plasminogen activation in COPD is needed. Such investigations could suggest promising targets for COPD therapy.

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