Mass spectrometry-based proteomics to evaluate the circulating proteome in idiopathic pulmonary fibrosis (IPF)

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INTRODUCTION

- Quantifying the circulating proteome may provide insights into the pathobiology of IPF and uncover candidate diagnostic and prognostic biomarkers.
- Studies of the peripheral blood proteome in IPF have employed approaches that are limited to target primers (e.g. aptamer-based technologies,[1] proximity extension assays[2]).

AIM

- We present the first multicenter study to use mass spectrometry-based methods to identify proteins related to the presence or severity of IPF.

METHODS

- The IPF cohort comprised 300 patients from the multi-center IPF-PRO Registry.[3] Controls (n=100) without known lung disease[4] were of similar age, sex and smoking status distribution to the IPF cohort.
- Plasma taken at enrollment was processed with an automated liquid handling platform and measured using Evosep One (liquid chromatography) coupled to Orbitrap Exploris (mass spectrometry). Two patients were excluded as their samples did not meet quality control criteria.
- Raw data processing was performed using Spectronaut 14 with a deep experimental spectral library to quantify peptide sequences and assign these precursors to protein groups. Protein data were $\log_2$ transformed and missing values were imputed. Batch correction was performed with pyCombat.
- Linear regression was used to compare protein abundances in the IPF versus control cohorts and, among the IPF cohort, to determine proteins associated with disease severity (FVC % predicted, DLco % predicted).
- The Benjamini–Hochberg method was used to control the false discovery rate (FDR) at 5%.
- For case-control results, pathway enrichment analyses on gene level were performed with clusterProfiler using canonical pathways from Reactome.

CONCLUSIONS

- Mass spectrometry-based proteomic analysis confirmed proteins previously associated with IPF and revealed new candidates for investigation as biomarkers.
- Our data suggest that the circulating proteome is not highly influenced by antifibrotic therapies.
- Future analyses will examine protein-outcomes associations and changes in the circulating proteome as IPF progresses.

RESULTS

Cohort characteristics at enrollment

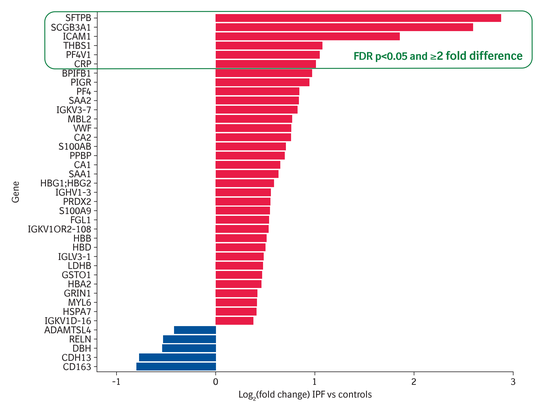
	IPF (n=299)	Control (n=99)
Age (years)	70 (65, 75)	66 (63, 71)
Male	223 (75%)	73 (74%)
White	284 (95%)	99 (100%)
Past smoker	201 (68%)	67 (68%)
FVC % predicted	69.7 (60.9, 80.2)	–
DLco % predicted	40.5 (31.6, 49.4)	–
Antifibrotic drug use		
Nintedanib	45 (15%)	–
Pirfenidone	97 (32%)	–

Values are median (Q1, Q3) or n (%).

Protein abundance in IPF versus control cohorts

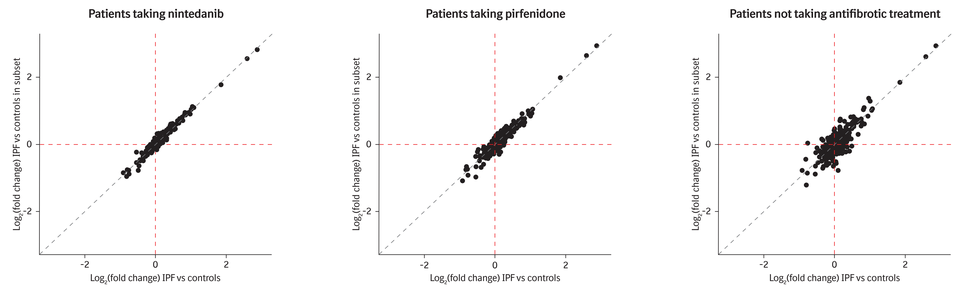
- 761 protein groups corresponding to 736 unique genes were detected.
- 168 protein groups were significantly different in abundance in the IPF versus control cohorts (FDR p<0.05).
- 21 protein groups were ≥1.5-fold different and 39 were ≥1.3-fold different.

Protein groups (n=39) with different abundances between IPF and control cohorts with FDR-adjusted p<0.05 and ≥1.3-fold difference($|\log_2$(fold change)$|\geq$0.38)

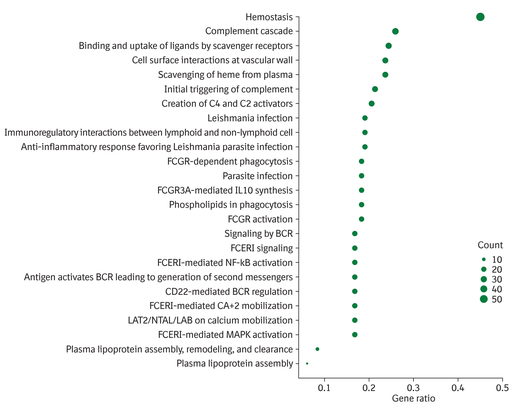


Protein groups are labeled by corresponding gene name.

Protein abundance in IPF versus control cohorts by antifibrotic treatment status



Enriched pathways in IPF versus control cohorts

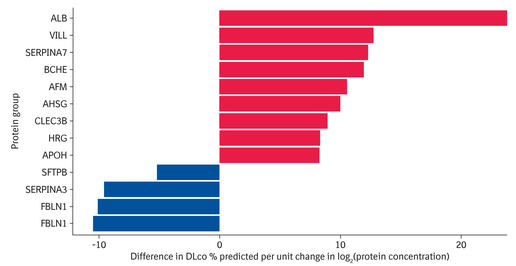


Pathways shown with FDR-adjusted p<0.01, ranked by gene ratio.
BCR, B-cell receptor. FCERI, Fc epsilon receptor I. FCGR, Fc-gamma receptor.

Proteins associated with disease severity

- One protein (ApoA1) was statistically (FDR-adjusted p<0.05) and clinically (difference in disease severity measure ≥5%) associated with FVC % predicted.
- 13 proteins were associated with DLco % predicted (see figure below).
- Protein–disease severity associations were similar in effect size after adjustment for antifibrotic drug use.

Proteins associated with DLco % predicted (FDR-adjusted p<0.05 and difference in DLco % predicted ≥5%)



Gene names are shown. Two protein groups related to the gene FBLN1 are shown representing unique protein groupings based on the detected peptides (Uniprot ID P23142, Uniprot ID B1AHL2.)

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IPF-PRO Registry enrolling centers: Albany Medical Center, Albany, NY; Baylor College of Medicine, Houston, TX; Baylor University Medical Center at Dallas, Dallas, TX; Cleveland Clinic, Cleveland, OH; Columbia University Medical Center/New York Presbyterian Hospital, New York, NY; Duke University Medical Center, Durham, NC; Froedtert & The Medical College of Wisconsin Community Physicians, Milwaukee, WI; Houston Methodist Lung Center, Houston, TX; Lahey Clinic, Burlington, MA; Loyola University Health System, Maywood, IL; Lynchburg Pulmonary Associates, Lynchburg, VA; Medical University of South Carolina, Charleston, SC; National Jewish Health, Denver, CO; NYU Medical Center, New York, NY; Piedmont Healthcare, Austell, GA; Pulmonary Associates of Stamford, Stamford, CT; PulmonIx LLC, Greensboro, NC; Renovatio Clinical, The Woodlands, TX; Salem Chest and Southeastern Clinical Research Center, Winston Salem, NC; South Miami Hospital, South Miami, FL; St. Joseph's Hospital, Phoenix, AZ; Stanford University, Stanford, CA; Temple University, Philadelphia, PA; The Oregon Clinic, Portland, OR; Tulane University, New Orleans, LA; UNC Chapel Hill, Chapel Hill, NC; University of Alabama at Birmingham, Birmingham, AL; University of California, Davis, Sacramento, CA; University of California Los Angeles, Los Angeles, CA; University of Chicago, Chicago, IL; University of Cincinnati Medical Center, Cincinnati, OH; University of Louisville, Louisville, KY; University of Miami, Miami, FL; University of Michigan, Ann Arbor, MI; University of Minnesota, Minneapolis, MN; University of Pennsylvania, Philadelphia, PA; University of Pittsburgh, Pittsburgh, PA; University of Virginia, Charlottesville, VA; UT Southwestern Medical Center, Dallas, TX; Vanderbilt University Medical Center, Nashville, TN; Vermont Lung Center, Colchester, VT; Wake Forest University, Winston Salem, NC; Washington University, St. Louis, MO; Weill Cornell Medical College, New York, NY; Wilmington Health and PMG Research, Wilmington, NC; Yale School of Medicine, New Haven, CT.

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