SUPPLEMENTARY MATERIALS

Methods

CT Scans:
HRCT scans were obtained utilizing a standardized protocol comprising of non-contiguous thin section high resolution CT images (2 cm intervals) on full inspiration in prone or prone and supine positions at centers across the United States. Digital images were obtained with study subject's consent from these centers and were uploaded to a secure, password-protected, IRB-approved study database for review by study radiologists. Results of study CT reviews were communicated to subjects. All subjects were offered the opportunity to speak with a trained genetic counselor.

Visual CT Review:
Technically inadequate imaging was denoted as such by reviewing radiologists, and these subjects were excluded from analyses (Figure 1, Figure S2). Pulmonary fibrosis was defined as reticular abnormality and/or subpleural irregularity or traction bronchiectasis with or without honeycombing—individual reviewers assigned each study with evidence of pulmonary fibrosis as "Probable" or "Definite" depending on extent of abnormality. "Definite" fibrotic ILD was identified by the presence of reticular abnormality with traction bronchiectasis, with or without honeycombing. "Probable" fibrotic ILD was defined as reticular abnormality, but with traction bronchiectasis that was mild or questionable. Predominant zonal distribution and axial distributions were noted. Pattern diagnoses were assigned based on standard criteria (e.g., usual interstitial pneumonia [UIP], nonspecific interstitial pneumonia [NSIP]), and the probability of each diagnosis was graded as possible, probable, or definite based on published criteria [1–4]. When a confident single diagnosis could not be made, multiple patterns were noted. A second radiologist performed over-reads on all HRCTs with fibrotic ILD as well as on 10% of other CT scans [5]; discrepancies were resolved by consensus interpretation.

Quantitative CT Review:
Lung segmentation was accomplished using a deep learning algorithm based on a fully convolutional DenseNet architecture [6]. The model was trained using full resolution CT on 1584 subjects with accompanying lung segmentations that had been verified visually. The training cohort did not overlap with study subjects and included both normal and diseased lungs.

An update of data-driven texture analysis (DTA), was used to detect and quantify lung fibrosis on CT in this work [7]. The updated method, called deepDTA, uses a convolutional neural network (CNN) trained as a binary classifier (Figure S3). The CNN architecture consists of three two dimensional convolutional layers, each of which is followed by average pooling operations. The pooled features are concatenated, then passed through two 1x1 convolutional layers before binary classification using a sigmoid activation function. The CNN was trained using 33x33 pixel image patches that had been labeled as either lung fibrosis or not. The training data was described previously and did not overlap with study data. The CNN operates in a fully convolutional fashion, so that input images may be of any size and classification is performed over sliding windows sized 33x33 pixels with a stride of 1 pixel.

deepDTA was validated by repeating previous experiments comparing forced vital capacity (FVC) and fibrosis scores from sequential CT (54 or 60 week follow-up) on pooled data (n=141) from two clinical trials of IPF (independent from the present study cohort) [8]. Spearman correlation coefficients between FVC percent predicted and deepDTA or DTA at baseline were similar (rho=0.46, $p<0.0001$ for both methods). Association between change in deepDTA and change in FVC at follow-up was somewhat stronger than for DTA (Spearman correlation rho=-0.53, $p<0.0001$ versus rho=-0.46, $p<0.0001$ for deepDTA and DTA, respectively). The minimal clinically important difference (MCID) of deepDTA using FVC as an anchor was estimated to be 3.8 (95% confidence interval [CI] 2.0-5.6). This is similar to the MCID we estimated for DTA, (3.4, 95% CI 0.5-6.3), but with slightly smaller confidence interval.

Quantitative CT Review with %HAA:
Percent high attenuation area (%HAA), the percentage of total lung volume with HRCT pixel intensity greater than -600 HU and less than -250 HU, has been used as a measure of interstitial lung disease on CT [9].

Blood Sample Processing:
A subset of these subjects recruited through the University of Colorado (n=332) consented to provide peripheral blood samples in addition to undergoing HRCT. DNA was extracted from Paxgene tubes of whole blood utilizing the Qiagen AllPrep kits per manufacturer's protocol (Qiagen #80204). Nucleic acid quantity and quality was assessed utilizing the Agilent 4200 TapeStation Instrument (Cat # G2991AA). Plasma was separated from tubes of whole blood by centrifugation (15 minutes at 1500 x g at room temperature). Plasma samples were stored at -80°C.

Genotyping:
The *MUC5B* promoter variant (rs35705950) and a common variant in *TERT* (rs2736100) were genotyped on the all study subjects for whom DNA samples were available utilizing 10 nanograms of genomic DNA and pre-designed Taqman genotyping assays (C___1582254_20 and C___1844009_10, respectively) on a ViiA™ 7 Real-Time PCR System and associated software (ThermoFisher Scientific) per manufacturers' instructions.

Autoantibody Testing:
Autoantibody testing was performed at the University of Colorado Excera BioLabs. The ANA testing was performed by indirect fluorescence assay and read by a trained technologist. Anti-CCP, Scl-70, Jo-1, SSA, and RNP were run by Inova ELISA. Rheumatoid factor (RF), C3, and C4 were measured by nephelometry. All assays have been validated to College of American Pathologists/Clinical Laboratory Improvements Amendments (CLIA) standards for clinical diagnostics.

Supplementary Figure Legends

Figure S1. Representative Pedigrees for Families in Cohort
A-C. Representative pedigrees from study subjects' families are displayed here. White shapes represent family members reported as unaffected with pulmonary fibrosis; black shapes represent family members reported or confirmed to be affected with pulmonary fibrosis. Circles represent females, while squares represent male family members. Shapes with lines through them represent deceased family members in the pedigree.

Figure S2. Enrollment and Screening Flowchart for Quantitative HRCT Analysis
Description of enrollment process and results for study subjects for whom quantitative HRCT analysis was performed.

Figure S3. deepDTA Convolutional Neural Network (CNN) Architecture
The model consists of three convolutional layers (with 256 6x6, 256 3x3, and 512 3x3 filters, respectively) each of which is followed by an average pooling operation. The resulting 1024 pooled features are passed through two 1x1 convolutional layers, sized 1024 and 256, respectively. The model output is a sigmoid activation to perform binary classification (fibrosis or not). Leaky rectified linear unit activations are used through and dropout was used in the last two layers to avoid overfitting during training.

Figure S4. Distribution of Raw Fibrosis Scores
Distribution of raw fibrosis scores among CT scans examined by quantitative HRCT analysis. The skewness of distribution of raw scores prompted logarithm transformation of data to facilitate statistical analyses.

Figure S5. Distribution of Logarithm of Raw Fibrosis Scores
Histogram of raw fibrosis scores (quantitative HRCT) after logarithm transformation, showing a more Gaussian distribution.

Figure S6. Distribution of Logarithm of High Attenuation Area (HAA) score
Histogram of logarithm transformation of HAA scores, showing a more Gaussian distribution.

Figure S7. Logarithm of HAA Score by Visual Diagnosis
Boxplots of logarithm of HAA scores for each visual diagnosis category.

Figure S8. Age Distributions of Subjects
Density plots of ages for FIP relatives cohort separated by visual diagnosis (No Fibrosis vs. PrePF), illustrating that PrePF subjects (blue) were older than those without fibrosis (red).

Supplementary Tables and Legends

Table S1. Summary of Characteristics of Study Subjects Used in Quantitative CT Analyses
* genotyping data not available for COPDGene subjects

	COPDGene Nonsmoking Controls (n=100)	**FIP Relatives in Dataset (n=402)**
Age, mean (95% CI)	57.2 [55.3, 59.0]	57.4 [56.5, 58.3]
Male, n (%)	47 (47%)	153 (38%)
rs35705950 minor allele frequency*	NA	0.22

Table S2. Patterns of Radiographic Abnormalities in Scans with any ILD (non-fibrotic or fibrotic)

***** Because a confident single diagnosis was relatively uncommon, most cases included consideration of several patterns. For this reason, the percentages add up to more than 100%.

Total with Any ILD	**93**
Cranio-caudal distribution	
Upper	18 (19%)
Middle	5 (5%)
Lower	55 (59%)
Diffuse	7 (8%)
Not noted	10 (11%)
Axial distribution	
Subpleural	69 (74%)
Diffuse	12 (13%)
Peribronchovasular	4 (4%)
Not noted	10 (11%)
Honeycombing?	**12 (13%)**
CT Pattern*	
UIP	**59 (63%)**
Possible	41 (70%)
Probable	9 (15%)
Definite	9 (15%)
NSIP	**46 (49%)**
Possible	41 (89%)
Probable	3 (7%)
Definite	2 (4%)
Sarcoid Pattern	**4 (4%)**
Hypersensitivity Pneumonitis Pattern	**19 Possible, 1 Probable (22%)**

Table S3. Patterns of CT Abnormalities in Scans with non-fibrotic ILD Findings

Non Fibrotic ILD	**16**
Cranio-caudal distribution	
Upper	7 (44%)
Middle	0 (0%)
Lower	1 (6%)
Diffuse	3 (19%)
Not noted	5 (31%)
Axial distribution	
Subpleural	2 (13%)
Diffuse	7 (44%)
Peribronchovasular	2 (13%)
Not noted	5 (38%)
Honeycombing	**0**
CT Pattern*	
UIP	**0**
Possible	*0*
Probable	*0*
Definite	*0*
NSIP	1 (6%)
Possible	*0*
Probable	*1 (100%)*
Definite	*0*
Sarcoid Pattern	**1 (6%)**
Hypersensitivity Pneumonitis Pattern	**5 possible, 1 probable (38%)**

Table S4. Autoantibody Testing in Screened Subjects

	No Fibrosis (n=238)	**PrePF (n=44)**	**p-value**
C3 [95% CI]	114.5 [111.2, 117.9]	120.7 [112.9, 128.5]	NS
C4 [95% CI]	34.7 [33.4, 36.1]	36.3 [32.8, 39.8]	NS
ANA positive (1:160 or greater)	82 (24.4%)	11 (25%)	NS
Rheumatoid Factor >20	19 (8%)	4 (9.3%)	NS
Jo-1 positive	1 (0.4%)	2 (4.5%)	NS
CCP3.1 positive	20 (8.5%)	2 (4.5%)	NS
Scl70 positive	6 (2.5%)	0 (0%)	NS
SSA positive	6 (2.5%)	1 (2.3%)	NS
RNP positive	2 (0.8%)	0 (0%)	NS
Any antibody positive	*109 (45.8%)*	*15 (34%)*	*NS*

Table S5. Analyses excluding non-fibrotic ILD cases.
* DNA available on 462 of these subjects (387 No Fibrosis, 75 PrePF subjects). ** Odds ratios reported in this table were calculated from a mixed effects logistic regression model including age (as a continuous variable), male sex, ever smoker (yes/no), and rs35705950 genotype. ***In the reported model, rs35705950 coded as a dominant allele.

	No ILD (n=401)	**PrePF (n=77)**	**p-value**	**OR [95% CI], controlling for family****	**Logistic regression p-value**
Age, mean (SD), years	55.7 (8.7)	65.9 (10.1)	$1.7x10^{-12}$	*1.15 [1.09, 1.21]*	*$8.8x10^{-7}$*
Male, %	36%	49%	0.05	*1.99 [0.96, 4.14]*	*0.06*
Ever smoker, %	26.1%	44%	0.003	*1.66 [0.76, 3.44]*	*0.18*
MUC5B Promoter Variant (rs35705950), MAF*	0.21	0.29	0.02	*2.15 [0.99, 4.69]*	*0.05***

Table S6. Analyses of all ILD versus no ILD.
* DNA available on 479 of these subjects (387 No ILD, 92 Any ILD subjects). ** Odds ratios reported in this table were calculated from a mixed effects logistic regression model including age (as a continuous variable), male sex, ever smoker (yes/no), and rs35705950 genotype. ***In the reported model, rs35705950 coded as a dominant allele.

	No ILD (n=401)	**Any ILD (n=93)**	**p-value**	**OR [95% CI], controlling for family****	**Logistic regression p-value**
Age, mean (SD), years	55.7 (8.7)	64.5 (10.2)	7.2×10^{-12}	*1.11 [1.07, 1.15]*	5.58×10^{-9}
Male, %	36%	49.5%	0.02	*1.87 [1.06, 3.31]*	*0.03*
Ever smoker, %	26.1%	45.6%	0.0003	*1.81 [1.01, 3.25]*	*0.05*
MUC5B Promoter Variant (rs35705950), MAF*	0.21	0.29	0.01	*1.87 [1.04, 3.36]*	*0.04***

Supplementary References

1 Chung JH, Chawla A, Peljto AL, *et al.* CT scan findings of probable usual interstitial pneumonitis have a high predictive value for histologic usual interstitial pneumonitis. *Chest* 2015;**147**:450–9. doi:10.1378/chest.14-0976

2 Silva CIS, Müller NL, Lynch DA, *et al.* Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. *Radiology* 2008;**246**:288–97. doi:10.1148/radiol.2453061881

3 Travis WD, Costabel U, Hansell DM, *et al.* An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013;**188**:733–48. doi:10.1164/rccm.201308-1483ST

4 Lynch DA, Sverzellati N, Travis WD, *et al.* Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med* 2018;**6**:138–53. doi:10.1016/S2213-2600(17)30433-2

5 Washko GR, Lynch DA, Matsuoka S, *et al.* Identification of Early Interstitial Lung Disease in Smokers from the COPDGene Study. *Acad Radiol* 2010;**17**:48–53. doi:10.1016/j.acra.2009.07.016

6 Iandola F, Moskewicz M, Karayev S, *et al.* DenseNet: Implementing Efficient ConvNet Descriptor Pyramids. 2014;:1–11. doi:10.1111/j.1360-0443.1993.tb03137.x

7 Humphries SM, Yagihashi K, Huckleberry J, *et al.* Idiopathic Pulmonary Fibrosis: Data-driven Textural Analysis of Extent of Fibrosis at Baseline and 15-Month Follow-up. *Radiology* 2017;**285**:270–8. doi:10.1148/radiol.2017161177

8 Humphries SM, Swigris JJ, Brown KK, *et al.* Quantitative high-resolution computed tomography fibrosis score: performance characteristics in idiopathic pulmonary fibrosis. *Eur Respir J* 2018;**52**. doi:10.1183/13993003.01384-2018

9 Ash SY, Harmouche R, Vallejo DLL, *et al.* Densitometric and local histogram based analysis of computed tomography images in patients with idiopathic pulmonary fibrosis. *Respir Res* 2017;**18**:45. doi:10.1186/s12931-017-0527-8