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Prospective Identification of Subclinical Interstitial Lung Disease in a Rheumatoid Arthritis Cohort Is Associated with the *MUC5B* Promoter Variant

To the Editor:

Interstitial lung disease (ILD) is common among patients with rheumatoid arthritis (RA), but ILD is often diagnosed late when there is already a heavy burden of lung disease (1). Among patients with RA without a known history of ILD, it is estimated that approximately one-third of patients have high-resolution computed tomography (HRCT) abnormalities suggestive of ILD (subclinical ILD) and more than half of these patients demonstrated radiologic progression (2). These estimates of disease are likely biased owing to small sample size and/or retrospective study design (2–5).

Recently, we discovered that the *MUC5B* promoter variant is a genetic risk factor for patients with established RA-ILD (6). Given this association, we hypothesized that the *MUC5B* promoter variant would also be associated with subclinical ILD among patients with RA. We performed a prospective study of subjects with RA without known ILD to determine the prevalence of and risk factors for subclinical ILD in RA.

Methods

Subjects with RA (2010 American College of Rheumatology criteria and/or a clinical diagnosis of RA by a board-certified rheumatologist) were identified from the outpatient rheumatology clinic at the University of Colorado and asked to participate if they had no clinical diagnosis of ILD. The institutional review board approved all protocols (COMIRB 16-1907), and all patients provided written informed consent.

All subjects filled out questionnaires, had blood samples collected, had measurements taken of lung function with spirometry (FVC) and $D_{L_{CO}}$, and had lung imaging performed with HRCT scans.

All HRCT scans were read independently by two chest radiologists using a scoring form. Qualitative findings of interstitial fibrosis were defined by the presence of irregular reticular opacities, traction bronchiectasis, and/or honeycombing. Discrepancies were resolved by consensus. Subclinical ILD was defined as subjects with RA without a diagnosis of ILD at the time of enrollment and who had interstitial fibrosis on a prospectively performed HRCT as determined by radiologist consensus.

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Table 1. Baseline Characteristics Associated with Radiologist-identified ILD and Quantitative Fibrosis Scores

Covariate	Total Cohort (*n* = 184)	Radiologist-identified Subclinical RA-ILD			Log Quantitative Fibrosis Score	
		Present (*n* = 38)	Absent (*n* = 146)	*P* Value*	Coefficient (95% CI)	*P* Value†
Age, yr	56.4 (44.2 to 66.6)	64.4 (57.0 to 69.8)	54.4 (40.0 to 64.4)	<0.001	0.04 (0.03 to 0.05)	<0.001
Sex, M	18	24	16	0.288	0.62 (0.21 to 1.03)	0.003
Non-Hispanic White	74	72	74	0.878	0.04 (-0.33 to 0.42)	0.822
Ever-smoker	51	61	49	0.207	0.67 (0.36 to 0.97)	<0.001
FVC, % predicted	100 (90 to 112)	94 (85 to 107)	101 (91 to 113)	0.122	−0.02 (−0.03 to −0.01)	<0.001
D_{LCO}, % predicted	87 (73 to 100)	79 (63 to 88)	90 (76 to 102)	<0.001	−0.02 (−0.03 to −0.02)	<0.001
UCSD Shortness of Breath Questionnaire‡	6 (2 to 17)	8 (3 to 18)	5 (2 to 17)	0.481	0.01 (0.00 to 0.02)	0.046
Rheumatoid factor	81	78	82	0.569	0.24 (−0.18 to 0.67)	0.262
Anti-CCP antibody	84	89	83	0.414	0.18 (−0.21 to 0.58)	0.365
RA duration, yr	8.5 (3 to 17)	10 (3 to 20)	8 (2 to 16)	0.325	0.01 (0.00 to 0.02)	0.135
Current methotrexate use	54	44	56	0.200	0.29 (−0.02 to 0.61)	0.068
Minor (T) allele frequency for MUC5B	0.10	0.17	0.08	0.012§	0.66 (0.28 to 1.04)	0.001
Quantitative fibrosis score	0.34 (0.17 to 0.73)	0.93 (0.55 to 1.95)	0.25 (0.13 to 0.55)	<0.001		

Definition of abbreviations: CCP = cyclic citrullinated peptide; CI = confidence interval; ILD = interstitial lung disease; IQR = interquartile range; RA = rheumatoid arthritis; UCSD = University of California San Diego.
Data expressed as median (IQR) or percentage.
**P* values from univariate logistic regression between covariate and outcome of radiologist-identified ILD.
†*P* values from univariate linear regression between covariate and outcome of log quantitative fibrosis score.
‡University of California San Diego Shortness of Breath Questionnaire (scale 0–120, with higher scores indicating more breathlessness) (8).
§Based on a dominant model (GG vs. GT/TT).

All HRCT scans were also analyzed using data-driven textural analysis (DTA) (7). This quantitative method provides an objective, continuous, subject-level fibrosis score, which is computed as the percentage of total lung volume classified as fibrotic by DTA.

Serum samples were tested by ELISA for rheumatoid factor (RF) (Inova Diagnostics, Inc.) and anti-CCP (cyclic citrullinated peptide) (Inova Diagnostics, Inc.). The *MUC5B* SNP rs35705950 was genotyped by TaqMan assay (Thermo Fisher Scientific) (6).

Statistical analysis. We analyzed the association between the *MUC5B* promoter variant and subclinical ILD using logistic regression models adjusted for age, male sex, and ever-smoking history based on biologic rationale. We performed an exploratory analysis to determine the relevance of potential RA-specific covariates and a sensitivity analysis using backward stepwise selection. Quantitative fibrosis score was log-transformed to achieve normality, and multivariable linear regression modeling of quantitative fibrosis score was performed using the same model-building strategy as described for radiologist-identified subclinical RA-ILD.

Results

A total of 194 subjects with RA consented for participation, 5 withdrew consent, and 5 did not complete the HRCT; 184 subjects with RA were included in the final analysis. The median age was 56.4 years, and subjects were predominantly women and ever-smokers (Table 1). Median lung function was normal with minimal symptoms of shortness of breath (8). The majority were RF and anti-CCP positive with a median RA duration of 8.5 years. Fifty-four percent were on methotrexate. The minor (T) allele frequency for the *MUC5B* promoter variant was 0.097.

Radiologist consensus for interstitial fibrosis was identified in 38 (21%) subjects and was associated with age, D_{LCO} % predicted, and the *MUC5B* minor allele (Table 1). Using quantitative imaging, the median DTA scores were higher in those with subclinical ILD than in those without subclinical ILD ($P < 0.001$). Higher DTA scores were also associated with older age, male sex, ever-smoking status, lower FVC % predicted, lower D_{LCO} % predicted, a higher breathlessness score, and the *MUC5B* minor allele (Table 1).

The primary analysis demonstrated that the *MUC5B* minor allele was an independent predictor of subclinical ILD after adjustment for age, male sex, and ever-smoking history (Table 2). The exploratory and reduced models demonstrated a similar association between the *MUC5B* minor allele and subclinical ILD. The association between the *MUC5B* minor allele and quantitative fibrosis score was also consistent across all three models. Other explanatory covariates included age and, depending on the model and outcome, RF positivity, male sex, ever smoking, and methotrexate use.

Table 2. Multiple Logistic Regression Models of Subclinical ILD and Multiple Linear Regression Models of Log-transformed Quantitative Fibrosis Score

Subclinical ILD	Age (*yr*)		Male Sex		Ever-Smoking		*MUC5B* Genotype (GT/TT)		RF Positive		CCP Antibody Positive		Current Methotrexate Use		RA Disease Duration (*yr*)	
	Odds Ratio	*P* Value	Odds Ratio	*P* Value	Odds Ratio	*P* Value	Odds Ratio	*P* Value	Odds Ratio	*P* Value	Odds Ratio	*P* Value	Odds Ratio	*P* Value	Odds Ratio	*P* Value
Primary model	1.07 (1.03 to 1.11)	<0.001	1.22 (0.45 to 3.28)	0.694	1.04 (0.46 to 2.39)	0.918	2.49 (1.01 to 6.12)	0.047	—	—	—	—	—	—	—	—
Expanded model	1.08 (1.04 to 1.13)	<0.001	1.40 (0.49 to 4.00)	0.533	1.50 (0.59 to 3.83)	0.400	2.71 (1.04 to 7.05)	0.041	0.31 (0.10 to 0.98)	0.046	1.94 (0.46 to 8.22)	0.367	0.45 (0.18 to 1.16)	0.098	0.99 (0.95 to 1.03)	0.597
Reduced model	1.07 (1.03 to 1.11)	<0.001	—	—	—	—	2.56 (1.04 to 6.28)	0.041	—	—	—	—	—	—	—	—

Quantitative Fibrosis	Age (*yr*)		Male Sex		Ever-Smoking		*MUC5B* Genotype (GT/TT)		RF Positive		CCP Antibody Positive		Current Methotrexate Use		RA Disease Duration (*yr*)	
	Coefficient	*P* Value	Coefficient	*P* Value	Coefficient	*P* Value	Coefficient	*P* Value	Coefficient	*P* Value	Coefficient	*P* Value	Coefficient	*P* Value	Coefficient	*P* Value
Primary model	0.03 (0.02 to 0.04)	<0.001	0.37 (0.03 to 0.71)	0.036	0.38 (0.12 to 0.65)	0.006	0.49 (0.16 to 0.82)	0.004	—	—	—	—	—	—	—	—
Expanded model	0.04 (0.03 to 0.05)	<0.001	0.38 (0.05 to 0.71)	0.026	0.32 (0.06 to 0.57)	0.016	0.60 (0.29 to 0.91)	<0.001	−0.12 (−0.45 to 0.21)	0.467	0.23 (−0.14 to 0.61)	0.224	0.27 (0.01 to 0.53)	0.043	−0.00 (−0.02 to 0.01)	0.553
Reduced model	0.03 (0.02 to 0.04)	<0.001	0.45 (0.11 to 0.79)	0.011	0.33 (0.06 to 0.60)	0.017	0.51 (0.19 to 0.84)	0.002	—	—	—	—	0.26 (−0.01 to 0.52)	0.058	—	—

Definition of abbreviations: CCP = cyclic citrullinated peptide; ILD = interstitial lung disease; RA = rheumatoid arthritis; RF = rheumatoid factor.
Primary model: Based on our hypothesis, we analyzed the association between the *MUC5B* promoter variant and subclinical ILD using logistic regression models adjusted for age, male sex, and smoking history (never, ever). Expanded model: Exploratory multivariable analysis adjusting for additional covariates of interest including RA disease duration, seropositivity to RF, seropositivity to CCP, and current methotrexate use. Reduced model: Sensitivity analysis performed by fitting a reduced model using backward stepwise selection (threshold $P < 0.1$; covariates considered included age, *MUC5B*, male sex, ever-smoking history, RA disease duration, seropositivity to RF, seropositivity to CCP, and current methotrexate use.

Conclusions

In this prospectively enrolled RA cohort without a prior diagnosis of ILD, the overall prevalence of subclinical ILD was 21%. Our findings support the association of the *MUC5B* promoter variant in subclinical RA-ILD, similar to our findings in established RA-ILD (6). Age was also associated with subclinical RA-ILD, but RA disease duration was not. The role of other RA-specific covariates (e.g., RF positivity and methotrexate use), male sex, and smoking history remains unclear in this cohort.

Subclinical radiologic abnormalities like these or those described in other cohorts (e.g., interstitial lung abnormalities and preclinical disease) have also been shown to be associated with the *MUC5B* promoter variant (9), are often progressive, and are associated with all-cause mortality (2, 5, 10). Our definition of subclinical ILD recognizes that these patients were not necessarily asymptomatic and therefore may have as-yet-unrecognized disease. However, this RA population was followed and recruited from a university rheumatology clinic and were not known to have ILD. Furthermore, the degree of symptoms and lung function impairment among those with subclinical ILD in this cohort was minimal and not to the extent observed in those with RA-ILD presenting for clinical care (11).

There was strong association between the radiologist-determined interstitial fibrosis and the quantitative fibrosis score ($P = 2 \times 10^{-10}$). Compared with the radiologist interpretation of subclinical ILD, quantitative fibrosis score was associated with lower lung function and more breathlessness. These data suggest that this quantitative technology may be more sensitive than radiologist interpretation for identifying early lung abnormalities.

To our knowledge, this is the largest prospectively screened cohort of diverse patients with RA with a standardized collection of questionnaires, pulmonary physiology, imaging, and biosamples. The primary limitation is the lack of a validation cohort. Despite this limitation, the clinical characteristics and *MUC5B* genotype of those with subclinical RA-ILD are consistent with what we have observed in established RA-ILD (6) and support the *MUC5B* promoter variant as a risk factor for subclinical RA-ILD. These findings should be tested in another cohort, and those with subclinical RA-ILD should be followed to understand the natural history of these findings. Early identification of ILD in RA may inform our understanding of disease pathogenesis and may provide a therapeutic window of opportunity to prevent progression to clinically significant RA-ILD. ■

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