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Title

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Journal

Seminars in Arthritis and Rheumatism, 51(5)

ISSN

0049-0172

Authors

Juge, Pierre-Antoine
Solomon, Joshua J
van Moorsel, Coline HM
et al.

Publication Date

2021-10-01

DOI

10.1016/j.semarthrit.2021.07.002

Peer reviewed

MUC5B PROMOTER VARIANT rs35705950 AND RHEUMATOID ARTHRITIS ASSOCIATED INTERSTITIAL LUNG DISEASE SURVIVAL AND PROGRESSION

Pierre-Antoine Juge[1], Joshua J. Solomon[2], Coline H.M. van Moorsel[3], Romain Garofoli[1], Joyce S. Lee[4], Fabienne Louis-Sydney[1], Jorge Rojas-Serrano[5], Montserrat I. González-Pérez[5], Mayra Mejia[5], Ivette Buendia-Roldán[5], Ramcés Falfán-Valencia[6], Enrique Ambrocio-Ortiz[6], Effrosyni Manali[7], Spyros A. Papiris[7], Theofanis Karageorgas[8], Dimitrios Boumpas[8], Katarina M. Antoniou[9], Prodromos Sidiropoulos[10], Athina Trachalaki[11], Joanne J. van der Vis[3], Anna Jamnitski[3], Jan C. Grutters[3], Caroline Kannengiesser[12], Raphaël Borie[13], Leticia Kawano-Dourado[13], Lidwine Wemeau-Stervinou[14], René-Marc Flipo[15], Hilario Nunes[16], Yurdagul Uzunhan[16], Dominique Valeyre[16], Nathalie Saidenberg-Kermanac'h[17], Marie-Christophe Boissier[17], Christophe Richez[18,19], Thierry Schaeverbeke[18,19], Tracy Doyle[20], Paul J. Wolters[21], Marie-Pierre Debray[22], Catherine Boileau[12], Raphaël Porcher[23,24], David A. Schwartz[4], Bruno Crestani[13], Philippe Dieudé[1]

1. Université de Paris, AP-HP, Hôpital Bichat Claude-Bernard, Service de Rhumatologie, DMU Locomotion, INSERM UMR1152, Paris, France

2. Department of Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, National Jewish Health, Denver, CO, USA

3. St Antonius ILD center of excellence, St Antonius ziekenhuis, Nieuwegein, The Netherlands

4. Department of Medicine, University of Colorado School of Medicine, Aurora, CO, USA

5. Interstitial Lung Disease & Rheumatology Unit, Instituto Nacional de Enfermedades Respiratorias, Ismael Cosío Villegas, México City, México

6. HLA Laboratory, Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas. Mexico City, Mexico

7. 2nd Pulmonary Medicine Department, University Hospital of Athens "Attikon", National and Kapodistrian University of Athens, Greece

8. Rheumatology and Clinical Immunology Unit, 4th Department of Internal Medicine, University Hospital of Athens "Attikon", National and Kapodistrian University of Athens, Greece

9. PS Department of Respiratory Medicine & Laboratory of Molecular & Cellular Pneumonology, Faculty of Medicine, University of Crete, Crete, Greece

10. Department of Rheumatology, Faculty of Medicine, University of Crete, Crete, Greece

11. Internal Medecine, University College of London, London, United Kingdom

12. Université de Paris, AP-HP, Hôpital Bichat Claude-Bernard, Service de Génétique, INSERM UMR1152, Paris, France

13. Université de Paris, AP-HP, Hôpital Bichat Claude-Bernard, Service de Pneumologie, DMU Victoire, INSERM UMR1152, Paris, France

14. CHRU de Lille, Service de Pneumologie et Immuno-Allergologie, Centre de compétence des maladies pulmonaires rares, FHU IMMINENT, Lille, France

15. CHU de Lille, Service de Rhumatologie, Lille, France

16. Université de Paris, AP-HP, Hôpital Avicenne, Service de Pneumologie, Bobigny, France

17. Université de Paris, AP-HP, Hôpital Avicenne, Service de Rhumatologie, Bobigny, France

18. CHU de Bordeaux, service de rhumatologie, Bordeaux, France

19. Immuno ConcEpT, CNRS UMR_5164, Bordeaux, France

20. Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA

21. Department of Medicine, University of California, San Francisco, CA, USA

22. Université de Paris, AP-HP, Hôpital Bichat Claude-Bernard, Service de Radiologie, Paris, France

23. Université de Paris, CRESS, INSERM, INRA, F-75004 Paris, France

24. Centre d'Épidémiologie Clinique, AP-HP, Hôpital Hôtel-Dieu, F-75004 Paris, France

Corresponding author: Philippe Dieudé

Rheumatology Department, Bichat Hospital, APHP, Paris Diderot University

46, rue Henri Huchard 75018, Paris, France

+33 (0) 1.40.25.74.08

philippe.dieude@aphp.fr

ABSTRACT

Background

The major risk factor for idiopathic pulmonary fibrosis (IPF), *MUC5B* rs35705950, was found to be associated with rheumatoid arthritis-associated interstitial lung disease (RA-ILD). Whilst the *MUC5B* rs35705950 T risk allele has been associated with better survival in IPF, its impact on RA-ILD prognosis remains to be determined. Our objective was to explore the influence of *MUC5B* rs35705950 on survival and progression in RA-ILD.

Methods

Through an international retrospective observational study, patients with RA-ILD were genotyped for the *MUC5B* rs35705950 variant and consecutive pulmonary function tests (PFTs) findings were collected. Longitudinal data up to a 10-year follow-up were considered and analyzed using mixed regression models. Proportional hazards and joint proportional hazards models were used to analyze the association of baseline and longitudinal variables with lung transplant-free survival. Significant progression of RA-ILD was defined as at least an absolute or relative 10% decline of forced vital capacity at 2 years from baseline.

Results

Out of 321 registered patients, 261 were included in the study :139 women (53.3%), median age at RA-ILD diagnosis 65 years (interquartile range [IQR] 57 to 71), 151 ever smokers (59.2%). Median follow-up was 3.5 years (IQR 1.3 to 6.6). Mortality rate was 32% (95%CI 19 to 42) at 10 years. The *MUC5B* rs35705950 variant did not impact lung transplant-free survival (HR for the T risk allele carriers=1.26; 95%CI 0.61 to 2.62; P=0.53). Decline in pulmonary function at 2 years was not influenced by *MUC5B* rs35705950 (OR=0.95; 95%CI 0.44 to 2.05; P=0.89), irrespective of the HRCT pattern.

Conclusion

In this study, the *MUC5B* rs35705950 promoter variant did not influence transplant- free survival or decline in pulmonary function in patients with RA-ILD.

KEY WORDS

Rheumatoid Arthritis
Interstitial Lung Disease
MUC5B
Genetics

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic auto-immune disease that can be associated with extra-articular manifestations in up to 50% of patients (1). Among them, interstitial lung disease (ILD) can be observed in 10 to 50% of patients with RA depending on ILD definition and screening tool (2, 3). RA-ILD is a severe condition associated with a 2 to 10 times increased mortality compared to patients with RA without ILD (3-5). However, RA-ILD is a heterogeneous disease with variable evolution. Progressive disease is observed in 40 to 50% of patients with RA-ILD, in limited case series, based on high-resolution computed tomography (HRCT) of the chest or pulmonary function tests (PFTs) (6-8). To date, few studies have identified factors associated with progressive disease and/or survival with contradictory results. Clinical features (e.g. age, sex, tobacco smoking), HRCT pattern (usual interstitial pneumonia (UIP) pattern) or extent, forced vital capacity (FVC), diffusing lung capacity for carbon monoxide (DLCO) have been associated with disease progression or worse survival (7, 9-13).

Recently, we found that there is a shared genetic background between RA-ILD and idiopathic pulmonary fibrosis (IPF) including rs35705950, a *MUC5B* promoter variant, representing the major genetic risk factor in both diseases (14, 15). In previous studies, the *MUC5B* rs35705950 T risk allele has been controversially associated with better survival among patients with IPF (16, 17). Our aim was to investigate whether the *MUC5B* rs35705950 variant influenced ILD progression or survival in patients with RA-ILD.

METHODS

Patients

This international retrospective observational study included patients with RA-ILD and available *MUC5B* rs35705950 genotypic data from France, United States, Mexico, Netherlands and Greece. All included patients fulfilled the 2010 European League Against Rheumatism-American College of Rheumatology (EULAR-ACR) and/or 1987 ACR revised criteria for RA (18, 19). In each participating center, ILD was established by HRCT of the chest and classified as UIP, probable UIP (combined under the heading "RA-UIP") or inconsistent with UIP (RA-nonUIP) according to international criteria by expert radiologists at ILD centers (20). All available PFT data from RA-ILD onset to the last follow-up were retrospectively collected through a systematic chart review. In addition, clinical data and information about death and/or lung transplant were collected. The institutional review boards at each institution approved the protocol and all patients provided written informed consent.

Genotyping

Genotyping of the *MUC5B* rs35705950 single nucleotide polymorphism involved the use of TaqMan Genotyping Assays (Applied Biosystems) as previously reported (14).

Statistical analysis

Follow-up was counted from the date of diagnosis of RA-ILD that was considered as the baseline date. Longitudinal data up to a 10-year follow-up were considered in the analysis. Longitudinal data were analyzed using mixed regression models, with the time effect flexibly modelled using splines to allow possibly nonlinear subject-specific evolutions. Confidence

intervals for these models were obtained by bootstrapping. To account for informative censoring due to death or lung transplantation, only measurements prior to lung transplantation were analyzed, and joint models for the longitudinal data and survival data processes were used (21). This approach jointly modelled the longitudinal data with a mixed model and the survival data (time to transplantation or death) assuming proportional hazards for the effect of covariates. The baseline hazard was modelled with B-splines. In a sensitivity analysis, random country effects were added to the flexible mixed model. Average slopes of decline in FVC and DLCO during the 10-year period after RA-ILD diagnosis were obtained from the joint models with a linear time effect and expressed as % per year with their 95% confidence interval (95% CI). The association of time-fixed variables (e.g. age or sex) with death or lung transplantation was assessed with Cox proportional hazards models, whereas for time-varying FVC and DLCO, or their current slope, estimates were obtained from the joint model described above.

We defined a significant deterioration of pulmonary function as an absolute decline of at least 10% in FVC (expressed in percent of the predicted value) or a relative decline in FVC of at least 10%, at two years from baseline (7, 20, 22-24). A sensitivity analysis using a definition of an absolute or a relative decline of 5% in FVC was also performed.

Given the observational nature of the study and irregular measurements of the longitudinal data, we used individual predictions obtained from the flexible mixed model (best linear unbiased predictions, BLUP) to impute the baseline and 2-year predicted FVC, as well as significant deterioration of pulmonary function for each patient of the study. Briefly, the mixed regression models with smooth time effects were used to derive individual-level predictions of FVC at relevant timepoints. The same procedure was used for DCO at baseline. For individuals with available data at baseline (114 individuals for FVC and 101 for DLCO), we

then assessed how well the predictions matched with observed values, visually over the entire follow-up, and by computing correlation coefficients for baseline values (we obtained 0.982 for FVC and 0.977 for DLCO). We therefore considered that the BLUP imputation were reliable for further analysis. Individuals who underwent lung transplantation or who died before 2 years from baseline were considered as experiencing a significant decline of pulmonary function. Those with shorter follow-up were censored at the date of last contact, and inverse probability of censoring weighting (IPCW) was used to correct for missing outcome at two years (25, 26). Briefly, IPCW consists in analyzing patients with outcome known at two years after weighting by the inverse of the estimated probability of not being censored at 2 years. This allows for reconstructing a population at 2 years which has the same structure as the population that we would have observed if there was no censoring. In the absence of any evidence that baseline variables affected the risk of censoring, IPCW weights were constructed using Kaplan-Meier estimator. Given the limited number of events (n = 30 in the whole cohort), we did not use multivariable models and only marginal associations are reported. We did not impute missing data, except for FVC and DLCO (by using BLUPs, as explained above). Our choice was based on the only very few data missing for chest HRCT patterns, among the variables and the fact that we did not use multivariable analyses.

Analyses were performed using the R statistical software version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Joint models were fitted with the JM package (version 1.4-8) (27).

Patient and Public Involvement Statement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. We have invited patients to help us develop our dissemination strategy.

RESULTS

Out of 321 patients with RA-ILD and available genetic data, 60 patients could not be analyzed (22 patients had unknown age at the time of RA-ILD diagnosis and 38 patients had no PFTs measured during the study period, see figure S1). Baseline characteristics of included and excluded patients are reported in table S1. In the end, 261 patients were included in this study.

Characteristics of the patients with RA-ILD included

Baseline characteristics of included patients are presented in table 1. Briefly, 139 (53.3%) were women with a mean age at RA-ILD diagnosis of 65 years (interquartile range [IQR] 57 to 71). UIP or probable UIP pattern was identified in 128 (51.4%) patients. FVC and DLCO at RA-ILD diagnosis were 78.2% of the predicted (IQR 64.2 to 100.7) and 56.0% of the predicted (IQR 46.5 to 72.0), respectively. The median follow-up was 3.5 years (IQR 1.3 to 6.6). Three or more FVC measurements were collected in 181 (69.3%) patients. During the follow-up, 56 (23.2%) patients died and 4 (3.4%) underwent lung transplant. Mortality rate was 12% (95% CI 7 to 17) at 5 years and 32% (95% CI 19 to 42) at 10 years.

MUC5B rs35705950 genotyping

The minor allele frequency of the T risk allele in the overall RA-ILD population was found to be increased (27.2%) compared to that observed in the general population and in good agreement with previous reported frequencies in the RA-ILD population (15, 28).

Transplant-free survival according to *MUC5B* rs35705950 genotypes

Kaplan-Meier curves of all patients with RA-ILD and of the subgroup of patients with RA-UIP are presented in figures 1 and 2. No difference in transplant-free survival was detected according to *MUC5B* rs35705950 genotype among patients with RA-ILD (hazard ratio (HR) for *MUC5B* rs35705950 T risk allele carriers 1.26, 95% CI 0.61 to 2.63, *P* = 0.53) (Figure 1). When the analysis was restricted to patients with RA-UIP, no difference was identified according to *MUC5B* rs35705950 genotypes (HR for *MUC5B* rs35705950 T risk allele carriers 0.86, 95% CI 0.29 to 2.51, *P* = 0.78) (Figure 2).

Other factors associated with death or lung transplantation at 10 years of RA-ILD duration

A lower FVC and/or DLCO at the time of RA-ILD diagnosis significantly increased the risk of death or lung transplantation at 10 years of RA-ILD duration; for each one percent reduction in FVC and DLCO at baseline (expressed in % of the predicted value), the risk of death or transplantation increased by 4% and 10%, respectively (HR = 1.04, 95% CI 1.02 to 1.06, *P* = 0.0004 and HR = 1.10 95% CI 1.06 to 1.15, *P* = <0.0001, respectively) (Table 2). A higher risk of death or lung transplantation at 10 years of RA-ILD duration was observed for male sex but it did not reach statistical significance (HR = 2.00, 95% CI 0.96 to 4.16, *P* = 0.064) (Table 2). In sub-analyses stratified by chest HRCT patterns, there was no difference in transplant-free survival according to age (Table 2). A higher risk for death or lung transplantation at 10 years of RA-ILD duration was observed for male sex in patients with non UIP patterns but it did not reach statistical significance (HR = 3.08, 95% CI 0.99 to 4.76, *P* = 0.053) (Table 2). Analyses modelling age as a continuous variable are available in Table S2. Lung transplant-free survival analyze including the 38 patients that were excluded because of no PFTs measured during the study period (n = 299) showed similar results (Table S3).

Changes in Pulmonary Function Over Time

Observed *vs* predicted (BLUP) FVC and DLCO data are presented in figure S2. Over ten years following diagnosis, an average of 5 longitudinal measurements per patient were available (1292 data points in total, 708 (54.8%) after two years, 279 (21.6%) after five years and 76 (5.9%) after eight years). Overall, there was slow decline in FVC over the ten-year follow-up (Figure 3A), with an average decline of 0.8% per year (95% CI 0.3 to 1.4). In addition, an important inter-patient FVC variability was present at baseline (standard deviation of random subject effect 21.9%), as well as during the study time. Variation due to the country of origin was smaller (standard deviation of random effect 8.4%). DLCO showed a significant decline of 1.8% per year on average (95% CI 1.2 to 2.3) (Figure 3B). Analyses stratified by *MUC5B* genotype are available in Figure 3, Panel C (FVC) and Panel D (DLCO).

PFTs deterioration at 2 years of RA-ILD duration (decline in FVC) according to *MUC5B* rs35705950 genotypes

Among the 256 patients with available FVC data, a 10% or more FVC decline at 2 years was observed for 33 patients (12.9%), corresponding to 49 (19.1%) after IPCW (86 patients being censored before 2 years).

No association was detected between the *MUC5B* rs35705950 variant and FVC decline at 2 years (OR for the T risk allele 0.95, 95% CI 0.44 to 2.05, *P* = 0.89) (Table 3). No association was detected between HRCT patterns (notably UIP and probable UIP), age or male sex and FVC decline (Table 3).

When the analysis was performed according to the HRCT pattern (*i.e.* RA-UIP and RA-nonUIP patterns), the *MUC5B* rs35705950 variant did not influence FVC decline at 2 years in patients with RA-UIP nor in patients with RA-nonUIP (OR for the T risk allele 0.70, 95% CI 0.22 to 2.21, *P* = 0.54 and 1.48, 95% CI 0.45 to 4.89, *P* = 0.52, respectively) (Table 3).

Analyses modelling age as a continuous variable are available in Table S4. Sensitivity analysis using a definition of an absolute or a relative 5% decline in FVC demonstrated similar findings (Table S5).

Slope of decline in FVC according to *MUC5B* rs35705950 genotypes

We observed no significant difference on the slope of decline in FVC at 2 years between the *MUC5B* rs35705950 T risk allele carriers and non-carriers (difference in average decline 0.6% per year, 95% CI -0.4 to 1.6, *P* = 0.25) (Table S6). No difference was identified according to age at RA-ILD > 65 years, sex or HRCT pattern (Table S6).

Sub-analyses in patients with the RA-UIP pattern found that age at RA-ILD diagnosis and male sex were significantly associated with the slope of decline in predicted FVC: *P* = 0.016 and *P* = 0.014, respectively whereas no association was detected in patients with RA-nonUIP (*P* = 0.97 and *P* = 0.30, respectively) (Table S6). No difference in the slope of decline in predicted FVC was detected according to *MUC5B* rs35705950 in patients with RA-UIP (difference in average decline 0.9% per year, 95% CI -0.6 to 2.3, *P* = 0.24) and in patients with RA-nonUIP (difference in average decline 0.3% per year, 95% CI -1.5 to 2.1, *P* = 0.78).

Slope of decline in DLCO according to *MUC5B* rs35705950 genotypes

A trend was observed for a greater decline in DLCO in *MUC5B* rs35705950 T risk allele carriers (difference in average decline 0.9% per year, 95% CI -0.05 to 1.8, *P* = 0.062) (Table S7). Age >

65 years old was associated with a greater decline in DLCO (difference in average decline 1.0% per year, 95% CI 0.02 to 1.9, *P* = 0.044) (Table S7). No difference was detected according to sex or HRCT pattern.

In the sub-analyses performed according to HRCT pattern, a similar trend was detected for a greater decline in *MUC5B* rs35705950 T risk allele carriers in RA-UIP (*P* = 0.082) but not in RA-nonUIP (*P* = 0.41) (Table S7). No significant difference was detected according to age and sex in patients with RA-UIP and RA-nonUIP (Table S7).

DISCUSSION

This is the first study to evaluate whether the presence of *MUC5B* rs35705950 T risk allele affects progression of ILD and transplant-free survival in patients with RA. We did not observe any impact of the *MUC5B* rs35705950 promoter variant on lung transplant-free survival nor pulmonary function deterioration, expressed using the decline of FVC, at 2 years in patients with RA-ILD. A greater decline in DLCO was observed for the T risk allele carriers was observed but did not reach statistical significance.

Although RA-ILD is associated with increased morbidity and mortality, little is known about its natural history. Evolution and prognosis of RA-ILD varies among patients (9). Identification of markers that predict RA-ILD progression would be relevant in the context of the recent benefit of antifibrotic therapy in systemic sclerosis-related ILD and pooled autoimmune disease-related ILDs having a progressive fibrosis phenotype (29-31).

To date, several baseline phenotypic characteristics at baseline have been reported to be associated with poor prognosis in RA-ILD: older age, male sex and tobacco smoking, UIP and probable UIP HRCT patterns, subpleural distribution, higher baseline ILD extent and PFTs findings (low FVC at baseline, low DLCO at baseline, FVC rate of decline ≥ 10% within the 6 first months and DLCO rate of decline ≥ 15% within the 6 first months) (7, 9-13). However, given the heterogeneity of the above cited studies, notably regarding the definition of progressive RA-ILD, stratification of patients at high risk of RA-ILD progression remains challenging for clinicians, illustrating the need for identification of new risk factors.

The impact of the *MUC5B* rs35705950 variant on ILD progression is controversial. In IPF, the *MUC5B* rs35705950 T risk allele has been associated with a slower progression and an improved survival (16, 17, 32) . One explanation for this protective effect would be that non-carriers of the risk allele have an increased frequency of *TERT* mutations (32). Indeed, shorter

telomeres were associated with declined survival in patients with IPF (32, 33). Conversely, in a recent study, Dudbrige *et al.* found that the paradoxical association of *MUC5B* rs35705950 T risk allele with increased survival could indeed be due to index event bias, and that the risk allele is in fact associated with worse survival of patients with IPF (34).

Outside IPF, in a study that included 248 patients with CTD-ILDs which included 62 patients with RA-ILD, no association was observed between *MUC5B* rs35705950 and FVC decline whereas this allele was associated with an increase in transplant-free survival (28). Lastly, two studies failed to identify a contribution of the *MUC5B* rs35705950 T allele and severity and progression of ILD in systemic sclerosis (35, 36). Given the strong relationship between the *MUC5B* rs35705950 T risk allele and the UIP and probable UIP HRCT patterns, the relatively low prevalence of this fibrotic HRCT phenotype in CTD-ILDs could explain this lack of association. Moreover, in. patients with idiopathic NSIP, significantly reduced survival was observed in patients carrying the *MUC5B* rs35705950 T risk allele (17).

RA-ILD differs from other CTD-ILDs by sharing several similarities with IPF: *i)* clinical characteristics (older age, male sex), *ii)* high prevalence of UIP and probable UIP HRCT pattern, *iii)* poor prognosis and *iv)* common environmental and genetic risk factors (15, 37-40). These similarities would lead to the hypothesis that IPF and RA-ILD may share common prognostic factors. Unlike IPF, we did not find any impact of the *MUC5B* rs35705950 variant on survival in RA-ILD. While the association between the *MUC5B* rs35705950 variant and RAILD is restricted to the UIP HRCT pattern (15), we failed to identify any contribution of the risk allele even when analysis was restricted to the HRCT RA-UIP pattern. The differences between the impact of this risk allele on IPF and RA-ILD could be explained by factors related to the chronic inflammatory rheumatic underlying condition which could impact the putative influence of *MUC5B* rs35705950 on RA-ILD progression and could contribute to the strong

heterogeneity in the RA-ILD course. Indeed, the RA disease activity and disease modifying anti-rheumatic drugs (DMARDs) could influence the course of ILD (39, 41-43). Of interest, even though statistical significance was not reached, the OR point estimate of 0.70 for FVC deterioration of the *MUC5B* rs35705950 T risk allele among patients with RA-UIP, is concordant with a possible protective effect of the risk allele as reported in IPF. Sensitivity analyses using a definition of a decline of 5% instead of 10% in FVC found similar results with a OR point estimate of 0.45 for FVC deterioration of the *MUC5B* rs35705950 T risk allele among patients with RA-UIP. In aggregate, these findings indicate that, in order to increase statistical precision, very large samples may be required to definitely exclude *MUC5B* rs35705950 as a prognosis factor of RA-ILD (notably in patients with a HRCT UIP or probable UIP HRCT pattern).

RA-ILD is an insidious disease with a probable extended preclinical stage. To date, there are no recommendation about the screening of such preclinical ILD in patients with RA. As RA-ILD is mostly diagnosed when pulmonary symptoms occur, our study could not assess *MUC5B* rs35705950 impact on preclinical RA-ILD progression. However, baseline PFT values were relatively balances according to *MUC5B* rs35705950 genotype suggesting that it did not impact preclinical RA-ILD progression.

The study has some limitations. First, the low occurrence of significant progression may have decreased the study's statistical power. The numbers get even smaller when we analyzed the UIP versus nonUIP subgroups. Sub-analyses regarding the decline of DLCO showed trends without reaching statistical significance meaning that a weak effect of the *MUC5B* rs35705950 variant could be completely ruled out. Second, other factors of interest such as ILD extent and HRCT fibrotic features were not considered. Third, the retrospective design of the study did not allow us to assess the variations of RA disease activity during the follow-up

period and individual therapeutic sequences of DMARDs that could both impact the course of ILD. Similarly, smoking status data was not available across the follow-up and therefore could not be included in the analysis. Fourth, significant differences between included and excluded patients (age at RA diagnosis and *MUC5B* genotype distribution) may have introduced a selection bias. Another potential selection bias may have been induced by the inclusion of patients with less aggressive phenotypes as only individuals living long enough to be included in this study were analyzed. Patients with severe and acute disease may have been underrepresented. The lower mortality rate observed in this study mays be induced by this immortal time bias. As death status was collected through medical records review, some non-hospitalized death may also have not been captured. Fifth, to date, *MUC5B* rs35705950 is the only common variant that has been associated with RA-ILD and our aim was to investigate its role as a prognosis factor. Other potential genetic risk factors such as *HLA-DRB1* shared epitope *(HLA-DRB1*SE)* were not investigated in this study. However, the exact contribution of *HLA-DRB1*SE* in the risk of ILD occurrence in patients with RA is controversial (44-46). Nonetheless, according to the association of *HLA-DRB1*SE* with a severe RA prognosis, a dedicated study with a relevant power of detection examining the role of this candidate locus in the RA-ILD prognosis would be of interest. Last, our single genetic variant analysis did not allow us to perform a regression-based adjustment using GWAS data that was recently proposed to adjust for index event bias (34).

In conclusion, in this large international multiethnic observational study, the *MUC5B* rs35705950 promoter variant, the major risk factor for RA-ILD, was not found to be associated with transplant-free survival or with progression of RA-ILD. Precision medicine in RA-ILD, with the goal of an early therapeutic intervention, requires additional studies to identify predictors of ILD progression in patients with RA.

Acknowledgements

We would like to thank all the patients included in this study for accepting to participate.

Author contributions

PAJ, BC and PD conceived the study idea and developed it. PAJ, JS, CvM, RG, JSL, FLS, JRS, MIGP, MM, IBR, RFV, EAO, EM, SAP, TK, DB, KMA, PS, AT, JJvdV, AJ, JG, CK, RB, LWS, RMF, HN, YU, DV, NSK, MCB, CR, TS, TD, PJW and DAS collected the data. RP directed the statistical analysis. PAJ, BC and PD wrote the initial draft. All the authors critically revised the manuscript and approved the final version before submission.

Conflict of interest

PAJ reports personal fees from BMS, outside the submitted work. JSL reports grants from NIH, personal fees from Genentech, personal fees from Celgene outside the submitted work. RB reports grants and personal fees from Roche, grants and personal fees from Boerhinger Ingelheim, outside the submitted work. LWS reports personal fees and non-financial support from Roche, personal fees and non-financial support from Boehringer-Ingelheim, personal fees from Janssen-Cilag, personal fees from Bristol-Myers-Squibb, outside the submitted work. RMF reports grants and personal fees from Roche Chugai, grants and personal fees from Abbvie, personal fees from Bristol-Meyers Squibb, grants and personal fees from Pfizer, outside the submitted work. YU reports personal fees from Roche, personal fees from Bohringer Ingelheim, non-financial support from Oxyvie, outside the submitted work. DV reports personal fees from Roche, personal fees from Bohringer Ingelheim, personal fees from Astra Zenecca, outside the submitted work. PJW reports grants from Genentech, grants from Medimmune, outside the submitted work. DAS reports grants from NIH-NHLBI, grants

REFERENCES

1.   Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years. Ann Rheum Dis. 2003;62(8):722-7.

2.   Doyle TJ, Lee JS, Dellaripa PF, Lederer JA, Matteson EL, Fischer A, et al. A roadmap to promote clinical and translational research in rheumatoid arthritis-associated interstitial lung disease. Chest. 2014;145(3):454-63.

3.   Hyldgaard C, Hilberg O, Pedersen AB, Ulrichsen SP, Lokke A, Bendstrup E, et al. A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality. Ann Rheum Dis. 2017;76(10):1700-6.

4.   Olson AL, Swigris JJ, Sprunger DB, Fischer A, Fernandez-Perez ER, Solomon J, et al. Rheumatoid arthritis-interstitial lung disease-associated mortality. Am J Respir Crit Care Med. 2011;183(3):372-8.

5.   Bongartz T, Nannini C, Medina-Velasquez YF, Achenbach SJ, Crowson CS, Ryu JH, et al. Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. Arthritis Rheum. 2010;62(6):1583-91.

6.   Habib HM, Eisa AA, Arafat WR, Marie MA. Pulmonary involvement in early rheumatoid arthritis patients. Clinical rheumatology. 2011;30(2):217-21.

7.   Solomon JJ, Chung JH, Cosgrove GP, Demoruelle MK, Fernandez-Perez ER, Fischer A, et al. Predictors of mortality in rheumatoid arthritis-associated interstitial lung disease. Eur Respir J. 2016;47(2):588-96.

8.   Zamora-Legoff JA, Krause ML, Crowson CS, Ryu JH, Matteson EL. Patterns of interstitial lung disease and mortality in rheumatoid arthritis. Rheumatology (Oxford). 2017;56(3):344-50.

9.   Zamora-Legoff JA, Krause ML, Crowson CS, Ryu JH, Matteson EL. Progressive Decline of Lung Function in Rheumatoid Arthritis-Associated Interstitial Lung Disease. Arthritis Rheumatol. 2017;69(3):542-9.

10.   Jacob J, Hirani N, van Moorsel CHM, Rajagopalan S, Murchison JT, van Es HW, et al. Predicting outcomes in rheumatoid arthritis related interstitial lung disease. Eur Respir J. 2019;53(1).

11.   Hyldgaard C, Ellingsen T, Hilberg O, Bendstrup E. Rheumatoid Arthritis-Associated Interstitial Lung Disease: Clinical Characteristics and Predictors of Mortality. Respiration. 2019;98(5):455-60.

12.   Assayag D, Lubin M, Lee JS, King TE, Collard HR, Ryerson CJ. Predictors of mortality in rheumatoid arthritis-related interstitial lung disease. Respirology. 2014;19(4):493-500.

13.   Kawano-Dourado L, Doyle TJ, Bonfiglioli K, Sawamura MVY, Nakagawa RH, Arimura FE, et al. Baseline Characteristics and Progression of a Spectrum of Interstitial Lung Abnormalities and Disease in Rheumatoid Arthritis. Chest. 2020.

14.   Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;364(16):1503-12.

15.   Juge PA, Lee JS, Ebstein E, Furukawa H, Dobrinskikh E, Gazal S, et al. MUC5B Promoter Variant and Rheumatoid Arthritis with Interstitial Lung Disease. N Engl J Med. 2018;379(23):2209-19.

16.   Peljto AL, Zhang Y, Fingerlin TE, Ma SF, Garcia JG, Richards TJ, et al. Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. JAMA. 2013;309(21):2232-9.

17.   van der Vis JJ, Snetselaar R, Kazemier KM, ten Klooster L, Grutters JC, van Moorsel CH. Effect of Muc5b promoter polymorphism on disease predisposition and survival in idiopathic interstitial pneumonias. Respirology. 2016;21(4):712-7.

18.   Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

19.   Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis and rheumatism. 1988;31(3):315-24.

20.   Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;198(5):e44-e68.

21.   Tsiatis AA, Davidian M. Joint modeling of longitudinal and time-to-event data: an overview. Statistica Sinica. 2004:809-34.

22.   Wijsenbeek M, Cottin V. Spectrum of Fibrotic Lung Diseases. N Engl J Med. 2020;383(10):958-68.

23.   Richeldi L, Ryerson CJ, Lee JS, Wolters PJ, Koth LL, Ley B, et al. Relative versus absolute change in forced vital capacity in idiopathic pulmonary fibrosis. Thorax. 2012;67(5):407-11.

24.   Khanna D, Mittoo S, Aggarwal R, Proudman SM, Dalbeth N, Matteson EL, et al. Connective Tissue Disease-associated Interstitial Lung Diseases (CTD-ILD) - Report from OMERACT CTD-ILD Working Group. J Rheumatol. 2015;42(11):2168-71.

25.   Vock DM, Wolfson J, Bandyopadhyay S, Adomavicius G, Johnson PE, Vazquez-Benitez G, et al. Adapting machine learning techniques to censored time-to-event health record data: A general-purpose approach using inverse probability of censoring weighting. Journal of biomedical informatics. 2016;61:119-31.

26.   Tsiatis AA. Semiparametric theory and missing data. New York: Springer; 2006. xvi, 383 p. p.

27.   Rizopoulos D. JM: An R package for the joint modelling of longitudinal and time-to-event data. Journal of Statistical Software (Online). 2010;35(9):1-33.

28.   Newton CA, Oldham JM, Ley B, Anand V, Adegunsoye A, Liu G, et al. Telomere length and genetic variant associations with interstitial lung disease progression and survival. Eur Respir J. 2019;53(4).

29.   Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, et al. Nintedanib in Progressive Fibrosing Interstitial Lung Diseases. N Engl J Med. 2019;381(18):1718-27.

30.   Wells AU, Flaherty KR, Brown KK, Inoue Y, Devaraj A, Richeldi L, et al. Nintedanib in patients with progressive fibrosing interstitial lung diseases-subgroup analyses by interstitial lung disease diagnosis in the INBUILD trial: a randomised, double-blind, placebo-controlled, parallel-group trial. Lancet Respir Med. 2020.

31.   Bruni T, Varone F. The adoption of nintedanib in systemic sclerosis: the SENSCIS study. Breathe (Sheff). 2020;16(2):200005.

32.   Dressen A, Abbas AR, Cabanski C, Reeder J, Ramalingam TR, Neighbors M, et al. Analysis of protein-altering variants in telomerase genes and their association with MUC5B common variant status in patients with idiopathic pulmonary fibrosis: a candidate gene sequencing study. Lancet Respir Med. 2018;6(8):603-14.

33.   Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A. 2008;105(35):13051-6.

34. Dudbridge F, Allen RJ, Sheehan NA, Schmidt AF, Lee JC, Jenkins RG, et al. Adjustment for index event bias in genome-wide association studies of subsequent events. Nat Commun. 2019;10(1):1561.

35. Volkmann ER, Tashkin DP, Roth MD, Li N, Charles J, Mayes M, et al. The MUC5B promoter variant does not predict progression of interstitial lung disease in systemic sclerosis. Semin Arthritis Rheum. 2020;50(5):963-7.

36. Stock CJ, Conti C, Montero-Fernandez A, Caramori G, Molyneaux PL, George PM, et al. Interaction between the promoter MUC5B polymorphism and mucin expression: is there a difference according to ILD subtype? Thorax. 2020;75(10):901-3.

37. Juge PA, Borie R, Kannengiesser C, Gazal S, Revy P, Wemeau-Stervinou L, et al. Shared genetic predisposition in rheumatoid arthritis-interstitial lung disease and familial pulmonary fibrosis. The European respiratory journal. 2017;49(5).

38. Juge PA, Crestani B, Dieude P. Recent advances in rheumatoid arthritis-associated interstitial lung disease. Curr Opin Pulm Med. 2020;26(5):477-86.

39. Kelly CA, Saravanan V, Nisar M, Arthanari S, Woodhead FA, Price-Forbes AN, et al. Rheumatoid arthritis-related interstitial lung disease: associations, prognostic factors and physiological and radiological characteristics--a large multicentre UK study. Rheumatology (Oxford). 2014;53(9):1676-82.

40. Saag KG, Kolluri S, Koehnke RK, Georgou TA, Rachow JW, Hunninghake GW, et al. Rheumatoid arthritis lung disease. Determinants of radiographic and physiologic abnormalities. Arthritis Rheum. 1996;39(10):1711-9.

41. Sparks JA, He X, Huang J, Fletcher EA, Zaccardelli A, Friedlander HM, et al. Rheumatoid Arthritis Disease Activity Predicting Incident Clinically Apparent Rheumatoid Arthritis-Associated Interstitial Lung Disease: A Prospective Cohort Study. Arthritis Rheumatol. 2019;71(9):1472-82.

42. Juge PA, Lee JS, Lau J, Kawano-Dourado L, Rojas Serrano J, Sebastiani M, et al. Methotrexate and rheumatoid arthritis associated interstitial lung disease. Eur Respir J. 2020.

43. Fernandez-Diaz C, Castaneda S, Melero-Gonzalez RB, Ortiz-Sanjuan F, Juan-Mas A, Carrasco-Cubero C, et al. Abatacept in interstitial lung disease associated with rheumatoid arthritis: national multicenter study of 263 patients. Rheumatology (Oxford). 2020.

44. Migita K, Nakamura T, Koga T, Eguchi K. HLA-DRB1 alleles and rheumatoid arthritis-related pulmonary fibrosis. J Rheumatol. 2010;37(1):205-7.

45. Furukawa H, Oka S, Shimada K, Sugii S, Ohashi J, Matsui T, et al. Association of human leukocyte antigen with interstitial lung disease in rheumatoid arthritis: a protective role for shared epitope. PLoS One. 2012;7(5):e33133.

46. Shirai Y, Honda S, Ikari K, Kanai M, Takeda Y, Kamatani Y, et al. Association of the RPA3-UMAD1 locus with interstitial lung diseases complicated with rheumatoid arthritis in Japanese. Ann Rheum Dis. 2020.
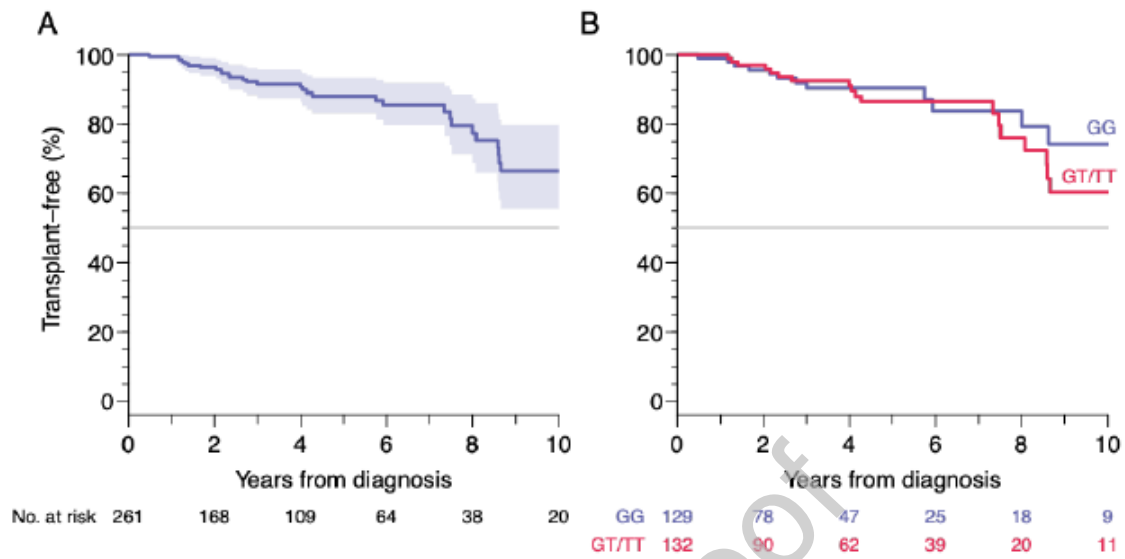
FIGURES LEGEND

Figure 1. Transplant-free survival of all patients with RA-ILD (A) and according to *MUC5B* rs35705950 genotypes (B). No difference was found between both genotypes (hazard ratio for GT/TT *vs*. GG 1.26, 95% CI 0.61 to 2.63, *P* = 0.53). RA-ILD: rheumatoid arthritis-associated interstitial lung disease.

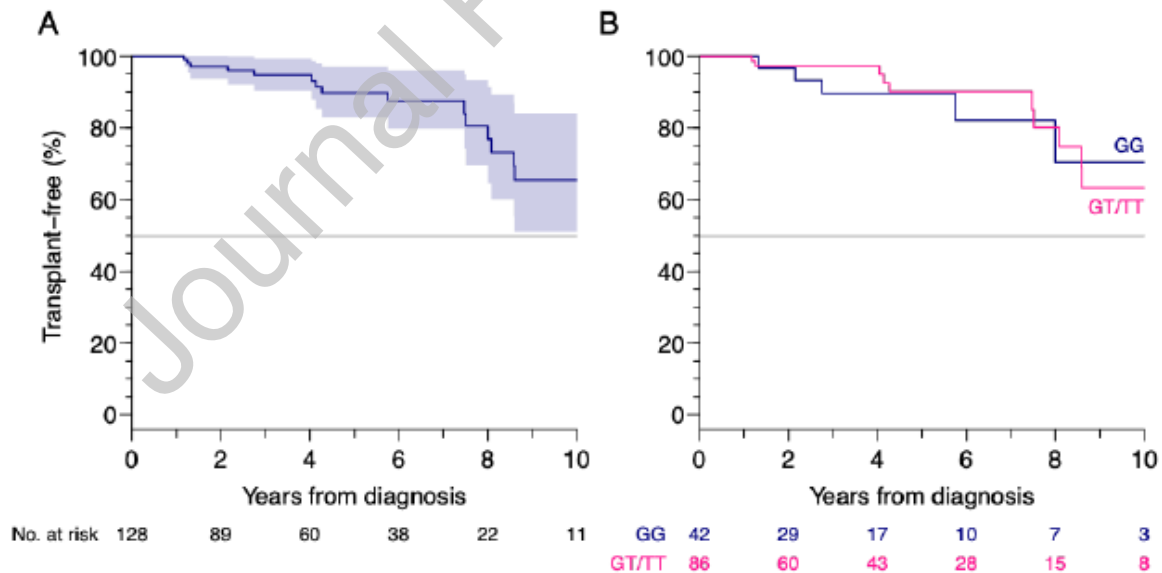**Figure 2. Transplant-free survival of patients with RA-ILD having a UIP or probable UIP chest HRCT pattern: all patients (A) and stratified by *MUC5B* rs35705950 genotypes (B).** No difference was found between both genotypes (hazard ratio for GT/TT *vs*. GG 0.86, 95% CI 0.29 to 2.51, *P* = 0.78).RA-ILD: rheumatoid arthritis-associated interstitial lung disease; UIP: usual interstitial pneumonia.
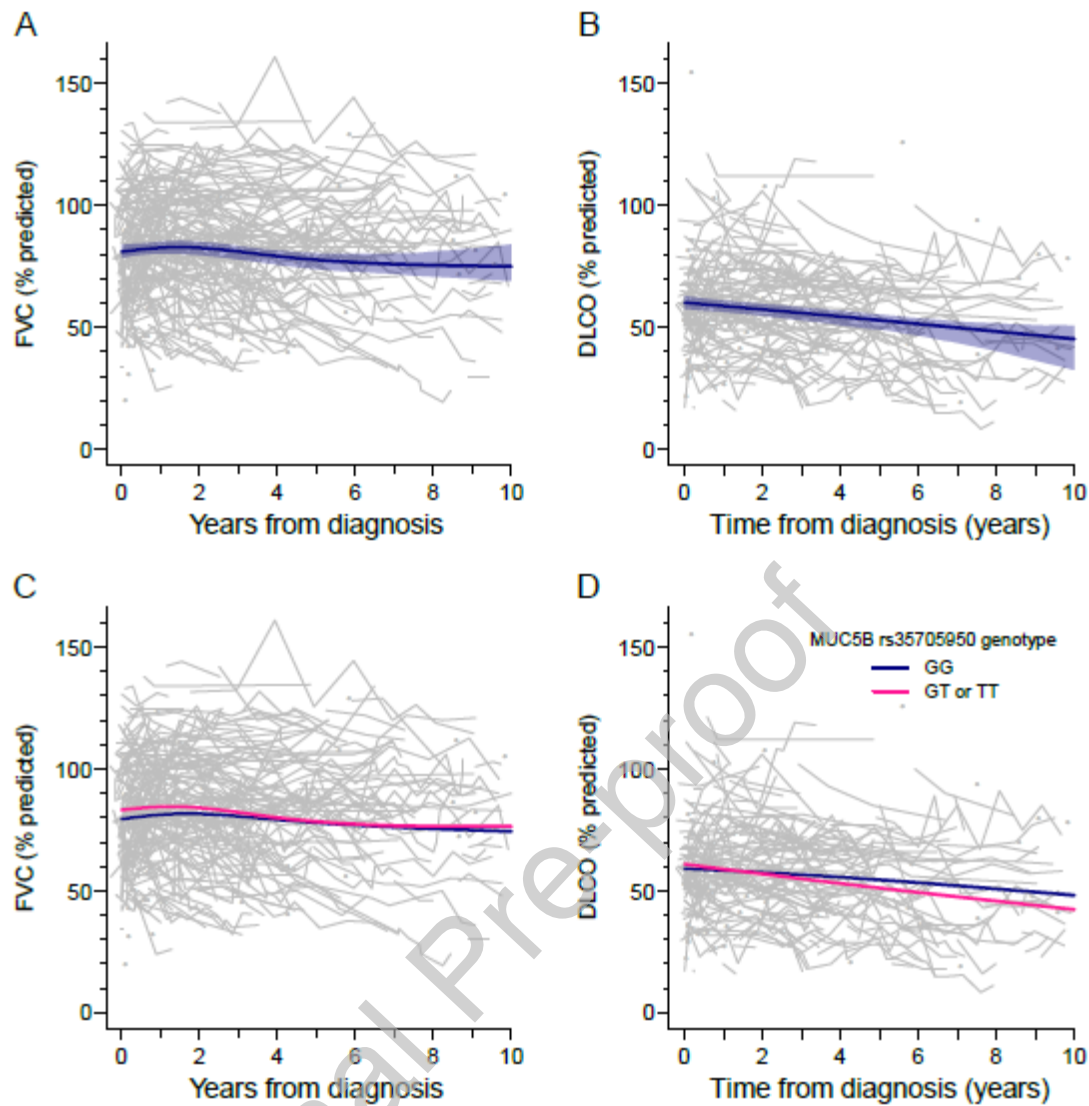
Figure 3. Evolution of FVC (A and C) and DLCO (B and D) during the 10 years following diagnosis. The blue lines show the mean trajectory, and blue shaded areas their bootstrap 95% confidence intervals. On panel A, although the curves seem linear, there was some evidence of non-linear effect of time ($P < 0.0001$), and the best-fitting model was a natural spline with 3 degrees of freedoms. On panel B, there was also evidence for non-linearity ($P = 0.0002$), with the best-fitting natural spline having 2 degrees of freedom. Analyses stratified by *MUC5B* rs35705950 genotype are shown on Panel C for FVC and D for DLCO. FVC: forced vital capacity; DLCO: diffusing capacity of lung for carbon monoxide. Shaded area around the line represents the 95%CI.

Table 1. Characteristics of patients with RA-ILD at diagnosis.

Characteristics	Whole sample (n=261)	MUC5B rs35705950 genotypes		
		GG (n=129)	GT or TT (n=132)	P
MUC5B rs35705950 genotypes — no. (%)				—
GG	129 (49.4)	129 (100.0)	0 (0)	
GT	122 (46.7)	0 (0)	122 (92.4)	
TT	10 (3.8)	0 (0)	10 (7.6)	
Age at diagnosis (years) — Median [IQR]	65 [57 - 71]	62 [54–69]	66 [61–74]	0.0004
Women — no. (%)	139 (53.3)	77 (59.7)	62 (47.0)	0.047
RF-positive — no. (%)	181 (75.4)	89 (74.2)	92 (76.7)	0.76
No. missing	21	9	12	
ACPA-positive — no. (%)	172 (73.8)	88 (73.9)	84 (73.7)	>0.99
No. missing	28	10	18	
Erosive status — no. (%)	101 (41.2)	51 (42.9)	50 (39.7)	0.70
No. missing	16	10	6	
Ever smoker — no. (%)	151 (59.2)	74 (59.2)	77 (59.2)	>0.99
No. missing	6	4	2	
Chest HRCT patterns — no. (%)				<0.0001
UIP*	128 (51.4)	42 (33.6)	86 (69.4)	
NonUIP	121 (48.6)	83 (66.4)	38 (30.6)	
No. missing	12	4	8	
FVC (% predicted)				
Observed — Median [IQR]	78.2 [64.2–100.7]	78.8 [63.8–103.3]	77.8 [66.0–98.9]	0.89
No. missing	147	69	78	
BLUP* — Median [IQR]	82.2 [65.9–97.2]	83.0 [65.2–96.9]	82.1 [69.2–98.2]	0.63
No. missing	5	2	3	
DLCO (% predicted)				
Observed — Median [IQR]	56.0 [46.5–72.0]	57.3 [44.8–71.2]	55.0 [48.9–70.1]	>0.99
No. missing	160	75	85	
BLUP* — Median [IQR]	57.5 [48.2–70.7]	58.3 [48.4–69.3]	57.2 [48.0–71.7]	0.85
No. missing	24	13	11	

IQR: interquartile range; RF: rheumatoid factor; ACPA: anticitrullinated peptides antibodies; HRCT: high-resolution CT scan; UIP: usual interstitial pneumonia; FVC: forced vital capacity; BLUP: best linear unbiased predictions; DLCO: diffusing capacity of lung for carbon monoxide.
* includes UIP and probable UIP HRCT patterns.
** Best linear unbiased predictions obtained from joint models were used to infer a baseline value for all patient who had at least one measurement during the study period (5 patients had no FVC measurements, and 24 no DLCO measurement).

Table 2. Factors associated with death or lung transplantation at 10 years of RA-ILD duration.

Characteristics	All patients (N = 261)		UIP***(N = 128)		NonUIP (N = 121)		Interaction††
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	P
Age at diagnosis							
≤ 65 years*	1		1		1		
> 65 years	1.29 (0.62 to 2.66)	0.49	1.78 (0.63 to 5.02)	0.28	0.63 (0.17 to 2.34)	0.49	0.29
Sex							
Female	1		1		1		
Male	2.00 (0.96 to 4.16)	0.064	1.41 (0.50 to 3.97)	0.51	3.08 (0.99 to 9.66)	0.053	0.31
Chest HRCT patterns**							
UIP***	0.82 (0.39 to 1.72)	0.60	—	—	—	—	—
NonUIP	1		—	—	—	—	
MUC5B rs35705950 genotypes							
GG	1		1		1		
GT or TT	1.26 (0.61 to 2.62)	0.53	0.86 (0.29 to 2.51)	0.78	1.59 (0.53 to 4.76)	0.41	0.48
Baseline FVC† (per % predicted decline)	1.04 (1.02 to 1.06)	0.0004	1.05 (1.01 to 1.09)	0.017	1.05 (1.02 to 1.09)	0.005	0.60
Baseline DLCO‡ (per % predicted decline)	1.10 (1.06 to 1.15)	<0.0001	1.09 (1.03 to 1.16)	0.003	1.12 (1.05 to 1.19)	0.0006	0.50
Longitudinal measurements (joint models)							
Current FVC (per % predicted decline)	1.04 (1.02 to 1.06)	<0.0001	1.05 (1.01 to 1.09)	0.0008	1.05 (1.02 to 1.09)	0.001	0.98
Slope of FVC (per % predicted/year)††	1.08 (0.89 to 1.30)	0.44	1.04 (0.84 to 1.28)	0.71	1.07 (0.90 to 1.27)	0.44	0.84
Current DLCO (per % predicted decline)	1.10 (1.06 to 1.15)	<0.0001	1.09 (1.04 to 1.15)	0.001	1.10 (1.04 to 1.17)	0.002	0.82
Slope of DLCO (per % predicted/year)††	1.30 (0.77 to 2.20)	0.33	1.09 (0.63 to 1.89)	0.76	1.28 (0.49 to 3.35)	0.61	0.77

The table presents hazard ratios and 95% confidence intervals. For longitudinal measurements, joint models were used.

HR: Hazard ratio; 95%CI: 95% confidence interval; UIP: Usual interstitial pneumonia; HRCT: high-resolution CT scan; FVC: forced vital capacity; DLCO: diffusing capacity of lung for carbon monoxide.

* 65 years was the median age

** 12 missing chest HRCT patterns

*** includes UIP and probable UIP HRCT patterns

† 5 missing data;

‡ 24 missing data.

††Interaction *P*-values assess whether the association with death or lung transplant varies between UIP and NonUIP.

Table 3. Association of baseline characteristic with the probability of significant deterioration of pulmonary function at 2 years, as defined by a decline of 10% in FVC.

Characteristics	All patients (*N* = 256)			UIP** (*N* = 126)			NonUIP (*N* = 118)		
	Events (%)	OR (95% CI)	*P*	Events (%)	OR (95% CI)	*P*	Events (%)	OR (95% CI)	*P*
Age at diagnosis									
≤ 65 years*	19.3	1		13.1	1		27.6	1	
> 65 years	18.7	0.96 (0.44 to 2.08)	0.92	21.9	1.85 (0.60 to 5.74)	0.28	13.9	0.42 (0.12 to 1.53)	0.19
Sex									
Female	21.8	1		23.5	1		21.4	1	
Male	15.8	0.67 (0.31 to 1.48)	0.33	12.3	0.46 (0.15 to 1.42)	0.18	23.2	1.11 (0.35 to 3.57)	0.86
Chest HRCT patterns[†]									
UIP**	17.4	0.74 (0.34 to 1.63)	0.45	—	—	—	—	—	—
NonUIP	22.2	1		—	—	—	—	—	—
MUC5B rs35705950 genotypes									
GG	19.4	1		22.5	1		19.9	1	
GT or TT	18.6	0.95 (0.44 to 2.05)	0.89	15.8	0.70 (0.22 to 2.21)	0.54	26.9	1.48 (0.45 to 4.89)	0.52

The table presents percent of events (**significant deterioration**), and odds ratios and 95% confidence intervals in the inverse probability of censoring (IPC)-weighted samples. Five patients could not be evaluated for FVC since they had no measurements (only DLCO data was available for them).-Interaction test (differential effect according to UIP status): *P* = 0.19 for age > 65 years, *P* = 0.27 for sex and *P* = 0.38 for *MUC5B* rs35705950 genotypes. OR: Odds ratio; 95%CI: 95% confidence interval; UIP: usual interstitial pneumonia; HRCT: high-resolution CT scan.

* 65 years was the median age

** includes UIP and probable UIP HRCT patterns

† 12 missing chest HRCT patterns