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The MUC5B promoter polymorphism and telomere length in patients with chronic hypersensitivity pneumonitis

Brett Ley, MD[1,*], **Chad A. Newton, MD**[2,3], **Isabel Arnould**[1], **Brett M. Elicker, MD**[4], **Travis S. Henry, MD**[4], **Professor Eric Vittinghoff, PhD**[5], **Professor Jeffrey A. Golden, MD**[1], **Professor Kirk D. Jones, MD**[6], **Kiran Batra, MD**[7], **Jose Torrealba, MD**[8], **Professor Christine Kim Garcia, MD**[2,3], and **Professor Paul J. Wolters, MD**[1]

[1]Department of Medicine, University of California San Francisco, California

[2]Eugene McDermott Centre for Human Growth and Development, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

[3]Dept of Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

[4]Department of Radiology and Biomedical Imaging, University of California San Francisco, California

[5]Department of Pathology, University of California San Francisco, California

[6]Department of Epidemiology and Biostatistics, University of California San Francisco, California

[7]Department of Radiology, University of Texas Southwestern Medical Center, Dallas, TX, USA

[8]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA

SUMMARY

Background—Patients with hypersensitivity pneumonitis (HP) may develop lung fibrosis, which is associated with reduced survival. Families with pulmonary fibrosis can present with members diagnosed with idiopathic pulmonary fibrosis (IPF) or chronic HP (cHP), suggesting that fibrotic HP may share risk factors with IPF.

[*]Corresponding Author: 505 Parnassus Ave, Box 0111, San Francisco, CA 94143; brett.ley@ucsf.edu; Phone: 415-502-2361, Fax: 415-502-1321.

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Methods—In an observational study of two independent cohorts of patients with cHP (UCSF n=145, UTSW n=72), we measured two common single nucleotide polymorphisms associated with IPF (MUC5B rs35705950 & TOLLIP rs5743890) and peripheral blood leukocyte telomere length and evaluated their associations with cHP disease, survival, and clinical-radiograph-pathologic features.

Findings—The frequency of the MUC5B minor allele, but not the TOLLIP minor allele, was significantly increased in cHP patients in both cohorts (UCSF MAF 24.4% & UTSW MAF 32.3%) compared to healthy controls (MAF 10.7%; p-values for comparison = <0.0001 for both cohorts) and similar to IPF (UCSF MAF 33.3% & UTSW MAF 32.0%, p-values for comparison=0.10 & 0.95, respectively). The MUC5B minor allele (adjusted OR 1.91, p=0.045) and shorter telomere length (adjusted OR 0.23, p=0.002) were associated with extent of radiographic fibrosis and other measures of lung remodeling and fibrosis in the combined cHP cohorts. Shorter telomere length had a significant association (adjusted HR 0.18, p=0.001) with reduced survival in the combined cHP cohorts.

Interpretation—The MUC5B promoter polymorphism rs35705950 and shorter telomere length are associated with extent of fibrosis in cHP. Shorter telomere length is associated with histopathology findings typical of usual interstitial pneumonia and reduced survival in cHP.

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INTRODUCTION

Hypersensitivity pneumonitis (HP) is an inflammatory lung disease caused by inhalational exposure to a variety of organic antigens that has a heterogeneous clinical presentation and natural history.[1] Heterogeneity of HP is represented most commonly by a clinical classification system that uses the temporal designations of acute, sub-acute, and chronic HP (cHP).[1] Lung fibrosis is the feature that most clearly identifies patients with HP that will have a chronic, progressive course and a poor prognosis.[2] In fact, fibrotic HP often has a clinical presentation similar to idiopathic pulmonary fibrosis (IPF), the most deadly form of idiopathic interstitial pneumonia, including progressive lung fibrosis and reduced survival.[2,3]

The development of lung fibrosis in HP is thought to be due to persistent lung injury resulting from ongoing antigen exposure and inflammation. Epidemiologic studies confirm that only a subset of individuals exposed to an inciting antigen develop HP, suggesting a possible genetic contribution to the disease.[4–7] However, the genomic factors that predispose individuals with HP to developing lung fibrosis are not known.[1] Several single nucleotide polymorphisms (SNPs) have been associated with predisposition to IPF, [8–12] and two of these, the mucin 5B (MUC5B) rs35705950 and toll interacting protein (TOLLIP) rs5743890 SNPs, have also been associated with survival in established IPF.[8,9] In addition, variants in genes associated with telomere maintenance have been linked to IPF,[11,13–19] and short telomere lengths measured in peripheral blood leukocytes (PBLs) have been associated with poorer survival in patients with IPF.[20] Families with pulmonary fibrosis often include members diagnosed clinically with cHP, suggesting that telomere dysfunction may be a risk factor for the development of chronic fibrotic HP. [13–19,21]

We propose here that genomic risk factors associated with the development and progression of IPF may also be associated with the development of fibrosis and reduced survival in cHP. To test this, we measured two SNPs that have been associated with a predisposition to IPF and survival in IPF (MUC5B rs35705950 and TOLLIP rs5743890) as well as PBL telomere length in two well-characterized cohorts of patients with cHP, and specifically examined their association with (1) cHP (compared to IPF and controls), (2) radiographic and histopathologic features of lung remodeling (e.g., honeycombing and traction bronchiectasis) and fibrosis (e.g., reticulation, fibroblast foci) in cHP, and (3) survival in cHP.

METHODS

Study Design and Populations

Hypersensitivity pneumonitis cohorts—This is an observational cohort study of patients with cHP drawn from two academic interstitial lung disease (ILD) centers: the University of California San Francisco (UCSF) and the University of Texas Southwestern (UTSW). Both cohorts are clinical registries of consecutive patients seen in ILD clinic enrolled from October 2001–April 2016 (UCSF) and from August 2005–August 2016 (UTSW). Baseline clinical information and blood samples were collected at the time of enrollment, and longitudinal data was recorded corresponding to clinical follow-up. The diagnosis in both cohorts were made by in-person multidisciplinary team discussion (MDD) according to available guidelines.[22,23] All patients with a MDD diagnosis of cHP who had a DNA sample collected from peripheral blood were included in the study. A diagnosis of cHP required a compatible clinical presentation and either (1) HRCT features of sub-acute/chronic HP[24,25] AND identification of a plausible exposure or (2) surgical lung biopsy histopathology most consistent with cHP.[26–28] In all cases, an alternative connective tissue disease was excluded.

Clinical information collected from both cohorts included demographics, pulmonary function tests, and radiologic and histopathologic studies. Ethnicity was self-reported. The date of death was recorded for both cohorts and confirmed using the United States Social Security Death Index for the UCSF cohort. Lung transplants and date of lung transplants were recorded.

Control cohorts—SNP frequencies in cHP patients were compared to two control populations: (1) patients with IPF identified from UCSF and UTSW and (2) the European population available from the 1000 Genomes Project Phase3, version 1[29]. We compared telomere length in cHP patients to IPF patients from UCSF and UTSW.[20]

Measurements

DNA—PBL genomic DNA was isolated in the UCSF cohort using the Gentra Puregene cell kit and in the UTSW cohort using Autopure LS (both from Qiagen, Valencia, CA, USA). MUC5B rs35705950 and TOLLIP rs5743890 SNPs were measured using the Taqman SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) independently at both institutions. PBL telomere length was measured for both cohorts at UTSW using quantitative

PCR as previously described; samples were excluded for pre-specified DNA quality and concentration or sample volume criteria. [15,20,30] Telomere length was expressed as the natural logarithm-transformed ratio of the telomere to single gene copy (log T/S), the difference between the observed and expected for age, and the age-adjusted percentile.[15,20,30]

Radiology—All available high-resolution computed tomography images of the chest (HRCTs) were reviewed by expert chest radiologists (B.M.E. or T.S.H. for UCSF and K.B.for UTSW). Radiologists scored HRCTs for pattern (definite, possible, or inconsistent with usual interstitial pneumonia)[22]; semi-quantitative extent of fibrosis as the percentage of total lung volume involved (none, mild [< 10%], moderate [10–50%], and severe [>50%])[31]; and presence or absence of honeycombing, traction bronchiectasis, upper/mid-lung predominance of abnormalities, peribronchovascular distribution of abnormalities, ground glass opacities outside of areas of fibrosis, consolidation, profuse micronodules, cysts away from areas of honeycombing, and significant (defined as three or more lobes) mosaic perfusion and/or air-trapping.[22,32]

Histopathology—In the UCSF cohort, surgical lung biopsies are reviewed prospectively in MDD at the time of diagnosis and scored by an expert lung pathologist (K.J.D.) using a structured pathology data collection form (see supplemental Figure 1). In the UTSW cohort, surgical lung biopsies were reviewed and scored by a separate expert lung pathologist (J.T.) specifically for this study using the same form.

Statistical Analysis

Bivariate associations of genotype (coded as a binary response variable for any minor allele vs. homozygous wild-type) with binary clinical, radiologic, and histopathologic variables were evaluated using the Chi-squared or Fisher's exact test where appropriate; and with continuous clinical variables and telomere length (log T/S) using Student's t test. Telomere length (log T/S) association with binary clinical, radiologic, and histopathologic variables was evaluated using Student's t test; telomere length association with continuous clinical variables was evaluated with Pearson's correlation. Statistical significance (i.e., alpha level) was determined using the Benjamini-Hochberg procedure for multiple testing within categories of comparison (e.g., MUC5B and radiographic features) using a false discovery rate of 0.05.[33]

For the SNP genotype association analyses, minor allele frequency (MAF) was calculated in each population as the total number of minor alleles divided by the total number of alleles in the population. MAF in the cHP populations was compared to IPF and control populations using the Chi-squared test. For the primary analysis, comparisons were made in the population restricted to non-Hispanic white individuals to control for confounding by ethnicity (i.e. population stratification). All associations were considered significant at alpha < 0.05 without correction for multiple comparisons. MAFs were reported along with exact binomial 95% confidence intervals (CIs).

Primary survival analyses were evaluated as time-to-death from any cause with right censoring for alive at end of study or lung transplantation using Cox proportional hazards

models. Transplant-free survival (where lung transplantation is considered equivalent to death in Cox models) and Fine-Gray competing risks regression models (where lung transplant is considered a competing risk) were performed to evaluate sensitivity of results to the method of handling of lung transplantation. In the individual cohorts, survival associations were evaluated both unadjusted and adjusted for age, sex, baseline forced vital capacity (FVC) % predicted, and diffusing capacity of the lung for carbon monoxide (DLCO) % predicted. For the combined cohort, a stratified Cox proportional hazards model was used to evaluate survival and transplant-free survival; for the competing risks analyses, cohort was included as a covariate. Kaplan Meier plots were constructed comparing survival and transplant-free survival by telomere length using an age-adjusted percentile cut-off of 10%, and groups were compared using the log-rank test. The 10% cutoff was chosen because the vast majority (>80%) of patients with telomere-maintenance machinery mutations have telomere length <10th percentile, and this cutoff represents a biologically relevant group of patients in which manifestations of telomeropathies are more prevalent.[19]

To evaluate the independent association of telomere length and MUC5B SNP with radiographic extent of fibrosis in the combined cohort, a multivariate ordinal logistic regression model was constructed with the outcome variable extent of radiographic fibrosis (0-none, 1-mild = 0–10%, or 2-moderate to severe = >10%). Potential confounders were selected based on prior knowledge and published literature to include potential causes of both lung fibrosis and shorter telomere length (common causes of MUC5B genotype and fibrosis were not suspected). These common causes included age, sex, and smoking history. We then examined univariate models for each potential confounder and included those with p-value < 0.05 for inclusion in the final multivariate model. This resulted in the final model including age (in years), sex (male vs. female), MUC5B SNP (any minor allele vs. homozygous wild-type), telomere length (in log T/S), and cohort (UCSF vs. UTSW). Interactions were assessed by testing the statistical significance of product terms between cohort and MUC5B or telomere length.[34]

RESULTS

Cohort characteristics

In the UCSF cohort, 145 patients with cHP were genotyped for the MUC5B rs35705950 and TOLLIP rs5743890 SNPs; telomere length was measured in 129 (89%) after exclusions for pre-specified quality criteria. In the UTSW cohort, 72 patients with cHP were genotyped for MUC5B rs35705950 and TOLLIP rs5743890 SNPs; all had PBL telomere length measured. The mean age was nearly 60 years in both cohorts, 61% were female in the UCSF and 47% in the UTSW cohort, and nearly half were ever-smokers in both cohorts (Table 1). The majority of patients identified as non-Hispanic white in both cohorts (85% in UCSF and 90% in UTSW). A family history of ILD was more common in the UTSW cohort (23.6%) than in the UCSF cohort (10.3%). Diagnosis was informed by either transbronchial lung biopsy or surgical lung biopsy in 70% or more of the cases, more than two-thirds had a potential antigen exposure identified, and nearly all patients had a HRCT pattern inconsistent with usual interstitial pneumonia (UIP). Of those with available HRCT scans

for review, 79% and 94%, had radiographic signs of fibrosis in the UCSF and UTSW cohorts, respectively.

MUC5B, TOLLIP, and Telomere Length Associations with Clinical Features

The presence of any MUC5B rs35705950 minor allele (genotype TT or GT compared to GG) was associated with older age at diagnosis in both cohorts (UCSF: 65.6 vs. 61.7 years, p=0.037; UTSW: 63.6 vs. 57.2 years, p=0.004), but not sex, smoking history, or an identifiable exposure. The presence of any TOLLIP rs5743890 minor allele (genotype GG or AG compared to AA) was not associated with age, sex, smoking history, or identifiable exposure. Shorter telomere length was significantly associated with older age in the UCSF cohort (r = −0.31, p=0.0003) but not significantly in the UTSW cohort (r = −0.19, p=0.10). Telomere length was not associated with sex, smoking history, exposure identification, or MUC5B rs35705950 genotype in either cohort.

MUC5B and TOLLIP SNP Association Analysis—In non-Hispanic white patients with cHP, the MUC5B rs35705950 minor allele frequency was increased in both the UCSF (MAF = 24.4% [95%CI 19.2–30.3]) and UTSW (MAF = 32.3% [95%CI 24.4–41.1]) cohorts compared to a publically available European healthy control population (MAF = 10.7%, p = < 0.0001 for comparison in both cohorts) and was comparable to patients with IPF (Table 2). The TOLLIP rs5743890 MAF was not significantly different in cHP patients compared to IPF or healthy controls (Table 2). Results for SNP MAF associations were similar for the entire cohort (i.e. not restricted to the non-Hispanic white population, see supplemental Table 1).

Telomere Length—The mean telomere length was longer in the UCSF cHP cohort compared to the UTSW cHP cohort (mean log T/S 1.53+/−0.37 vs. 1.30+/−0.33, p = < 0.0001; mean observed-expected for age difference 0.026+/−0.358 vs. −0.203+/−0.0328, p = <0.0001, **see** Table 1). Compared to IPF, telomere length was longer in the combined cohort of cHP patients (mean log T/S in HP 1.45+/−0.37 vs. in IPF 1.32+/−0.35, p = 0.0005; mean observed-expected for age difference in cHP −0.058+/−0.364 vs. in IPF −0.166+/−0.360, p = 0.0034).

MUC5B, TOLLIP, and Telomere Length Associations with Radiographic Features—For the two cohorts, 189 HRCTs were available for scoring by a radiologist (119/145 [82%] in the UCSF cohort and 70/72 [97%] in the UTSW cohort). In the combined cohort, the MUC5B rs35705950 minor allele was associated with moderate-severe radiographic fibrosis (p=0.009) and the presence of traction bronchiectasis (p=<0.001), but not a pattern consistent with definite or possible UIP or presence of radiographic honeycombing (Table 3). Among features inconsistent with UIP, the MUC5B minor allele was associated with the absence of diffuse ground glass opacities but not upper-mid lung distribution, peribronchovascular distribution, micronodules, or mosaic perfusion/air-trapping.

In the combined cohort with HRCT scores and telomere length measurements (n=175), shorter telomere length was associated with moderate to severe radiographic fibrosis

(<0.0001), presence of honeycombing (p=0.002), and presence of traction bronchiectasis (p=0.0007). Among the inconsistent with UIP features, shorter telomere length was associated with the absence of diffuse ground glass opacities but not upper-mid lung distribution, peribronchovascular distribution, micronodules, or mosaic perfusion/air-trapping. The TOLLIP rs5743890 minor allele was not associated with any HRCT feature (data not shown). Results were consistent across individual cohorts (supplement Table 2). In the combined UCSF and UTSW HP cohorts, the MUC5B minor allele (adjusted OR 1.91, p=0.045) and shorter telomere length (adjusted OR 0.23, p=0.002) were independently associated with extent of radiographic fibrosis, after adjustment for age and sex (Table 4). There was no evidence for a significant interaction between MUC5B and telomere length, MUC5B and cohort, or telomere length and cohort on extent of radiographic fibrosis.

MUC5B, TOLLIP, and Telomere Length Associations with Histopathologic Features—A total of 75 surgical lung biopsies were available for histopathologic scoring (54/145 [37%] in the UCSF cohort and 16/72 [22%] in the UTSW cohort). In the combined cohort, the MUC5B rs35705950 minor allele was not significantly associated with features of lung remodeling or fibrosis (Table 3), nor was it associated with any typical features of HP histopathology such as lymphocytic interstitial infiltrate, interstitial granulomas, or small airway disease.

In the combined cohort with histopathology scores and telomere length measurements (n=70), a shorter telomere length was associated with the presence of any histopathologic fibrosis (p=<0.0001), microscopic honeycombing (p=0.002), a heterogeneous distribution of fibrosis (p=0.0006), and a moderate to marked profusion of fibroblastic foci (p=0.003). Telomere length was not associated with any typical features of HP histopathology such as lymphocytic interstitial infiltrate, interstitial granulomas, or small airway disease. The TOLLIP rs5743890 minor allele was not associated with any of these histopathologic features (data not shown). Results were consistent across individual cohorts (supplement Table 2).

MUC5B, TOLLIP, Telomere Length and Survival—The presence of any minor allele for MUC5B rs35705950 SNP was of borderline statistical significance with worse survival (censoring for lung transplant) in the combined UCSF & UTSW cHP cohorts (adjusted HR 2.01, 95% CI 0.97–4.20, p=0.061) (Table 5). The TOLLIP rs5743890 SNP was not associated with survival in either cohort or the combined cohort. Shorter PBL telomere length was associated with worse survival (censoring for lung transplant) in both cohorts of cHP patients and the combined cHP cohort (adjusted HR 0.18, 95%CI 0.06–0.51, p=0.001) (Figure 2). Results were similar for transplant-free survival and when treating lung transplant as a competing risk (supplemental Tables 3 & 4 and Figure 2). There were no significant interactions for MUC5B and telomere length, MUC5B and cohort, or telomere length and cohort on survival.

DISCUSSION

This is the first study reporting on MUC5B rs35705950 and TOLLIP rs5743890 single nucleotide polymorphisms and peripheral blood leukocyte telomere length associations with

clinical features and outcomes in patients with cHP. Shorter PBL telomere length and the MUC5B promoter variant rs35705950, but not the TOLLIP variant rs5743890, were found to be associated with fibrosis in cHP patients. Shorter PBL telomere length is also strongly associated with histopathology features typical of usual interstitial pneumonia and reduced survival in cHP patients. Prior to this study, the MUC5B SNP appeared to confer specific risk for idiopathic interstitial pneumonia and familial pulmonary fibrosis.[35] We speculate that the MUC5B SNP and telomere dysfunction may predispose HP patients to development of lung remodeling and fibrosis, which in turn leads to worse outcomes.

The MUC5B promoter polymorphism rs35705950 minor allele has been repeatedly shown to occur more frequently in both sporadic and familial forms of IPF and thus far is the strongest identified genetic risk factor for IPF.[8,10–12,35] How this polymorphism contributes to IPF pathogenesis remains unclear. The minor allele causes increased MUC5B production by distal bronchiolar epithelial cells, and MUC5B protein accumulates in the honeycomb cysts of IPF lungs.[10,36,37] The prevailing theories are that over-production of MUC5B impairs mucociliary clearance, contributes to lung injury or epithelial cell stress and/or disrupts reparative mechanisms in the distal lung, leading to lung fibrosis.[38]

The MUC5B rs35705950 SNP was not associated with radiographic or histopathologic features common to HP such as air trapping on HRCT, or histopathologic quantification of airway centered inflammation or fibrosis, granulomas, or lymphocytic interstitial inflammation. In contrast, the SNP was associated with HRCT evidence of moderate-severe fibrosis and traction bronchiectasis. In contrast to IPF, where the MUC5B minor allele is associated with better survival,[9] a statistical trend toward poorer survival was found among patients with cHP. We hypothesize that the mechanisms by which MUC5B promotes pulmonary fibrosis in its idiopathic forms are similarly active in patients who develop cHP, and that the combination of injury from inflammation in HP in the context of excess MUC5B production increases the risk of developing lung remodeling and fibrosis in HP.

Telomere dysfunction has been implicated in the pathogenesis of both sporadic and familial forms of IPF. Mutations in several telomere-associated genes have been identified in sporadic and familial IPF,[13–19,21,39] and telomeres are short in the alveolar epithelial cells (AEC) of these patients[40,41]. Telomere dysfunction isolated to AECs causes lung remodeling and fibrosis in mouse models.[42,43] In addition to typical IPF presentations, many families contain members with a clinical diagnosis of cHP, suggesting shared pathobiology of telomere dysfunction in IPF and cHP.[21,44] Consistent with this possibility, shorter PBL telomeres were associated with fibrosis and poor survival in cHP. Short PBL telomere length was also associated with radiographic and histopathologic changes characteristic of IPF including radiographic severity of fibrosis, traction bronchiectasis and honeycombing, and histopathologic evidence of microscopic honeycombing, heterogenous distribution, and fibroblast foci. Similar to IPF, shorter telomere length was a strong predictor of poor survival in cHP.[20] Given these findings, we speculate that persistent inflammation in HP could be a driver of accelerated AEC turnover, contributing to telomere shortening in these cells, and, in susceptible individuals (i.e. those born with shorter telomeres or with an impaired ability to maintain telomeres), eventually leads to elements of lung remodeling and fibrosis similar to those seen in IPF. This hypothesis requires experimental examination.

No association was found between the TOLLIP rs5743890 SNP minor allele and predisposition, fibrosis, or survival in patients with cHP. In contrast to prior studies, there was no association between TOLLIP rs5743890 in IPF compared to controls.[8,11] However, the smaller sample size of this study may have limited the statistical power for this comparison. [8,11]

Our findings are associations, and do not prove a causative role for MUC5B or telomere dysfunction in cHP. There may be alternative explanations for the findings. Recent studies have highlighted the problem of misclassification of cHP and IPF diagnoses.[45,46] Therefore, it is possible that some patients diagnosed with cHP actually had IPF, leading to an enrichment in the MUC5B minor allele and shorter telomere lengths in the cHP study cohorts. However, this is unlikely considering diagnoses were established by multidisciplinary consensus, most were supported by histopathology, had identified exposures, had HRCT features inconsistent with a UIP pattern, and were consistent across two ILD centers. Even if true, this explanation would call into question the validity of clinical classification of ILD's and would argue for the importance of developing molecular classification systems, at least in regards to risk stratification. It should also be noted that we observed numeric differences in MUC5B and TOLLIP MAFs between our two cHP cohorts. While we believe these differences are related to random variation and differences in distribution of fibrotic severity between the two cohorts, we cannot exclude the possibility of a contribution from different MAFs in the local non-Hispanic white populations.

In regards to the telomere length findings, shorter telomeres in PBLs in cHP could alternatively be a marker of telomere attrition in PBLs resulting from persistent inflammation rather than a surrogate marker of AEC telomere length.[47] The link between inflammation and telomere attrition is complex. However, since telomere length was measured in PBLs originating from bone marrow, and it is unlikely that the bone marrow was affected by localized pulmonary inflammation, we believe it is more likely that PBL telomere length represents a surrogate marker for the "starting point" of AEC telomere length. Future studies should examine this possibility as well as the telomere lengths in various cell types of HP lungs to establish a causative role. Finally, because our genotype frequency analysis was restricted to the non-Hispanic white population, our results may not be generalizable to other racial/ethnic groups.

In conclusion, the MUC5B promoter polymorphism and shorter peripheral blood leukocyte telomere length are associated with fibrosis and reduced survival in cHP. These findings may have a role in risk stratification of patients with cHP, and provide insights into its pathogenesis. Future studies are needed to elucidate the role of MUC5B and telomere biology in the pathogenesis of cHP, and whether protein biomarkers that have been associated with IPF also have a role in defining an IPF-like endotype in non-IPF fibrotic lung diseases including cHP.[48]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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RESEARCH IN CONTEXT

Evidence before this study

We searched Pubmed for studies published before January 14, 2017 with the search terms “MUC5B”, “TOLLIP”, “telomere length”, “hypersensitivity pneumonitis”, and “alveolitis, extrinsic allergic”. No studies were identified reporting associations with the mucin 5B (MUC5B) rs35705950 or toll interacting protein (TOLLIP) rs5743890 single nucleotide polymorphisms (SNPs) and hypersensitivity pneumonitis (HP). Two studies reported peripheral blood leukocyte (PBL) telomere length in HP, but did not examine associations with clinical features or outcomes.

Added value of this study

This is the first study to demonstrate that the MUC5B SNP rs35705950 minor allele, but not the TOLLIP SNP rs5743890, is associated with risk of chronic HP and extent of fibrosis. It also demonstrates that shorter PBL telomere length is associated with extent of fibrosis, microscopic honeycombing, and reduced survival in chronic HP.

Implications of all the available evidence

The MUC5B SNP rs35705950 minor allele and shorter PBL telomere length are associated with greater extent of fibrosis, and shorter PBL telomere length is associated with histopathologic findings typical of usual interstitial pneumonia and reduced survival in patients with chronic HP. These findings suggest their potential role in risk stratification of patients with chronic HP, and potentially shared pathobiology between IPF and chronic HP.

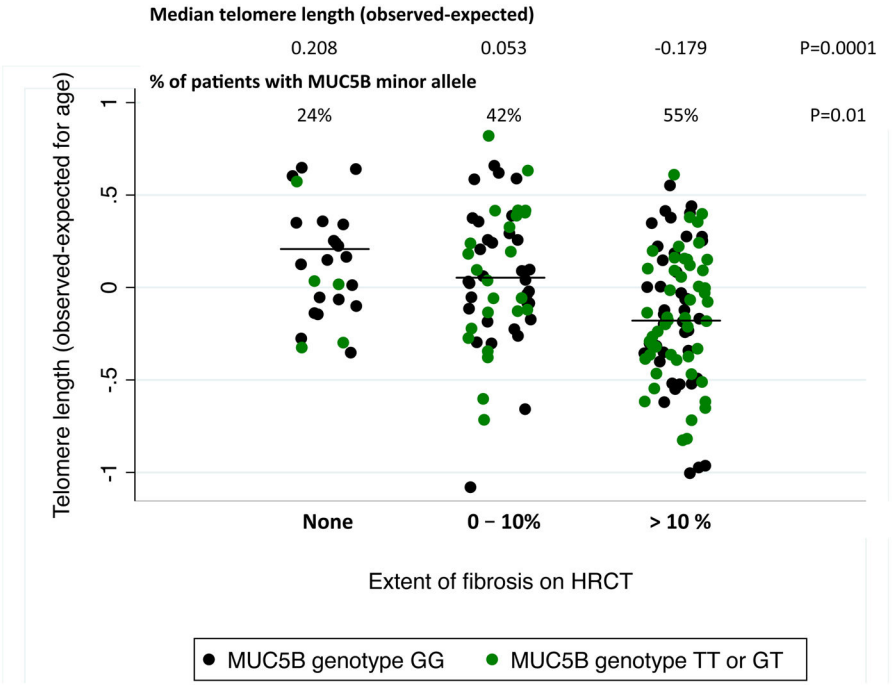

Figure 1.

Telomere length distribution (observed – expected for age) and mucin 5B (MUC5B) single nucleotide polymorphism rs35705950 grouped by extent of fibrosis on HRCT in the combined cohort of patients with chronic hypersensitivity pneumonitis

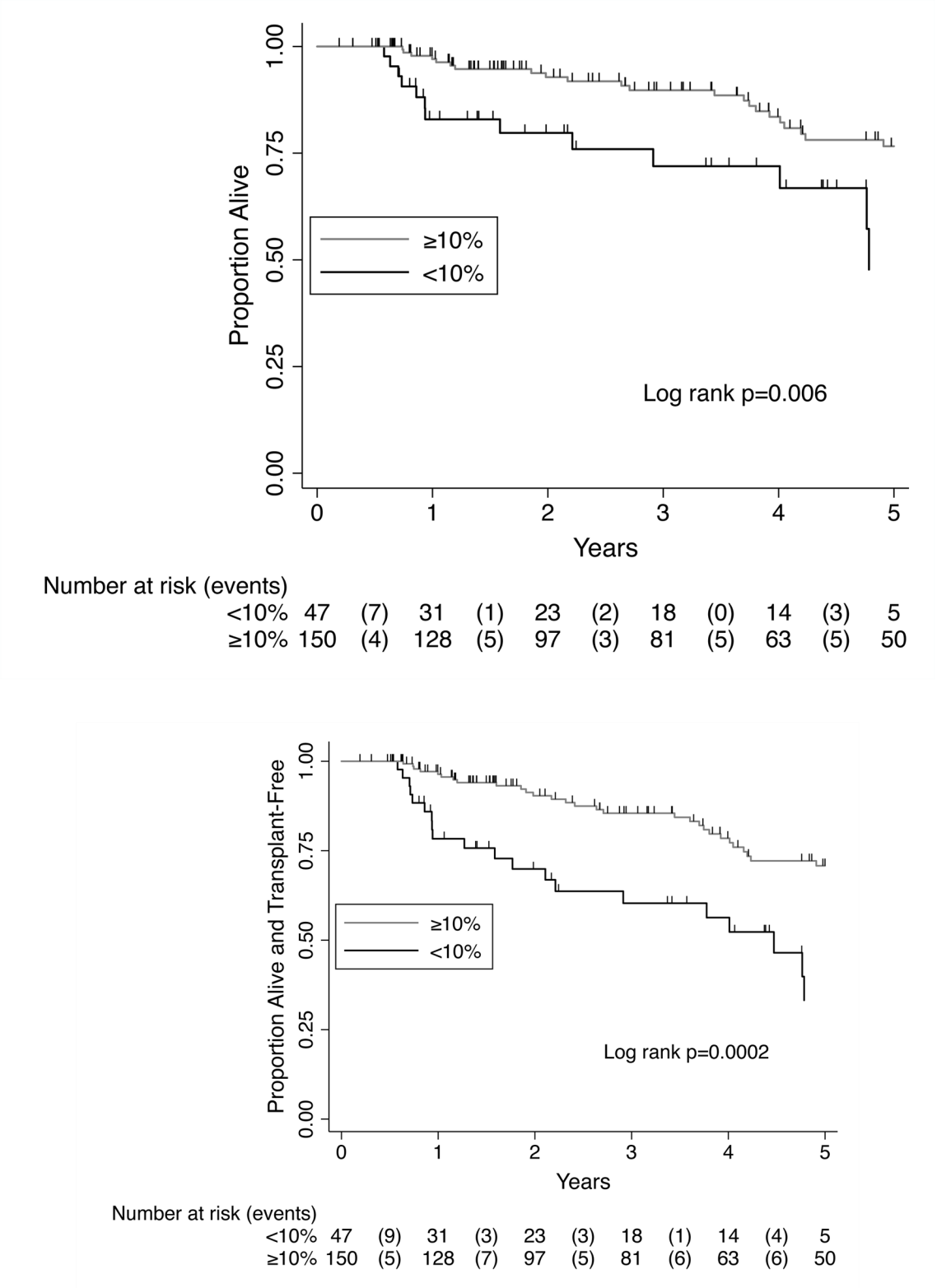

Figure 2.

Survival (A) and transplant–free survival (B) in the combined cohort of patients with chronic hypersensitivity pneumonitis with peripheral blood leukocyte telomere less than or greater than the 10^{th} percentile for age

Table 1

Characteristics of patients with chronic hypersensitivity pneumonitis

Characteristic	UCSF (n=145)	UTSW (n=72)	p-value
Age, mean (SD)	63.3 (11.2)	60.6 (9.8)	0.081
Female Sex, n (%)	89 (61.4)	34 (47.2)	0.048
Race, n (%)			
White, non-Hispanic/Latino	123 (84.8)	65 (90.3)	0.27
Hispanic or Latino	13 (9.0)	4 (5.6)	
Black	2 (1.4)	2 (2.8)	
Asian	4 (2.8)	1 (1.4)	
Other/unknown	3 (2.1)	0	
Ever-smoker, n (%)	75 (51.7)	36 (50.0)	0.81
Family history, n (%)	15 (10.3)	17 (23.6)	0.009
Lung biopsy, n (%)	114 (78.6)	51 (70.8)	0.21
Surgical lung biopsy	94 (64.8)	36 (50.0)	0.036
Transbronchial lung biopsy	30 (20.7)	15 (20.8)	0.98
Pulmonary Function Tests, mean (SD)			
FVC, % predicted	67 (17)	66 (19)	0.82
DLCO, % predicted	48 (17)	50 (18)	0.57
Antigen identified, n (%)	100 (69.0)	54 (75.0)	0.36
Avian	73 (50.3)	31 (43.1)	0.31
Bird	41 (28.3)	21 (29.2)	
Down	21 (14.5)	10 (13.9)	
Mold	21 (14.5)	11 (15.3)	0.88
Other	2 (1.4)	7 (9.7)	
Unknown	45 (31.0)	18 (25)	
Multiple	15 (10.3)	5 (6.9)	0.42
Telomere length measured, n (%)	129 (89.0)	72 (100)	
mean log T/S (SD)	1.53 (0.37)	1.30 (0.33)	<0.001
MUC5B genotype, n (%)			
GG	85 (58.6)	34 (47.2)	0.14
GT	55 (37.9)	32 (44.4)	
TT	5 (3.4)	6 (8.3)	
TOLLIP genotype, n (%)			
AA	109 (75.2)	48 (66.7)	0.34
AG	33 (22.8)	23 (31.9)	
GG	3 (2.1)	1 (1.4)	

Characteristic	UCSF (n=145)	UTSW (n=72)	p-value
HRCT available for scoring, n (%)	119 (82)	70 (97)	
Fibrosis extent, semi-quantitative, n (%)			
None	25 (21)	4 (6)	<0.001
Mild (< 10%)	39 (33)	21 (30)	
Moderate (10–50%)	49 (41)	27 (39)	
Severe (> 50%)	6 (5)	18 (26)	
Honeycombing, n (%)	18 (15)	24 (34)	0.002
UIP Pattern, n (%)			
Definite	4 (3.4)	5 (7)	0.13
Possible	10 (8.4)	54 (16)	
Inconsistent	105 (88.2)	11 (77)	
Inconsistent Features, n (%)			
Upper-mid lung predominance	44 (37)	26 (37)	0.98
Peribronchovascular distribution	63 (53)	36 (51)	0.84
Diffuse ground glass	52 (44)	34 (49)	0.52
Micronodules	12 (10)	7 (10)	0.99
Mosaic perfusion/air-trapping	69 (58)	40 (57)	0.91

Abbreviations: UCSF = University of California San Francisco; UTSW = University of Texas Southwestern; FVC = forced vital capacity; DLCO = diffusing capacity of the lung for carbon monoxide; HRCT = high-resolution computed tomography; UIP = usual interstitial pneumonia; log T/S = Telomere length expressed as the natural logarithm of the telomere to single gene copy ratio

Table 2

MUC5B rs35705950 and TOLLIP rs5743890 single nucleotide polymorphism minor allele frequency comparisons in non-Hispanic white populations of chronic hypersensitivity pneumonitis, idiopathic pulmonary fibrosis, and non-diseased controls

SNP	Chronic Hypersensitivity Pneumonitis				Idiopathic Pulmonary Fibrosis			Control*	
	n	MAF (%)(95% CI)	P-value, HP vs. IPF	OR (P-value), HP vs. Control	n	MAF (%)(95% CI)	OR (P-value), IPF vs. Control	n	MAF (%)(95% CI)
MUC5B (rs35705950)									
UCSF	123	24.4 (19.2–30.3)	0.09	2.27 (<0.0001)	147	33.3 (28.0–39.0)	3.10 (<0.0001)	503	10.7 (8.9–12.8)
UTSW	65	32.3 (24.4–41.1)	0.95	3.01 (<0.0001)	126	32.0 (26.3–38.2)	2.99 (<0.0001)	503	10.7 (8.9–12.8)
TOLLIP (rs5743890)									
UCSF	123	14.6 (10.5–19.7)	0.96	1.03 (0.88)	128	14.5 (10.4–19.4)	1.02 (0.93)	503	14.2 (12.1–16.6)
UTSW	65	19.2 (12.8–27.1)	0.27	1.35 (0.20)	126	14.8 (10.6–19.8)	1.03 (0.87)	503	14.2 (12.1–16.6)

Footnote:

*1000 Genomes Project, Phase 3, version 1, European population[29]

Abbreviations: SNP = single nucleotide polymorphism; HP = hypersensitivity pneumonitis; IPF = idiopathic pulmonary fibrosis; MAF = minor allele frequency; CI = confidence interval; OR = odds ratio; MUC5B = mucin 5B; TOLLIP = Toll interacting protein; UCSF = University of California San Francisco; UTSW = University of Texas Southwestern

Table 3

MUC5B single nucleotide polymorphism rs35705950 and peripheral blood leukocyte telomere length associations with radiographic and histopathologic features in the combined cohort of patients with chronic hypersensitivity pneumonitis

	MUC5B (rs35705950)			**Telomere Length**		
Radiology	TT/GT	GG	Total		Total	
(n)	87	102	189		175	
	% with feature			mean log T/S		
Feature	TT/GT	GG	p-value[1]	with	without	p-value[2]
Fibrosis (moderate-severe)	63	44	**0.009** *	1.34	1.58	**<0.0001** *
Definite or possible UIP pattern	17	15	0.63	1.31	1.48	0.03
Honeycombing	24	21	0.58	1.29	1.49	**0.002** *
Traction bronchiectasis	89	63	**<0.001** *	1.39	1.61	**0.0007** *
Diffuse ground glass opacities	36	54	**0.012** *	1.53	1.37	**0.004** *
Upper – mid lung distribution	32	41	0.202	1.45	1.45	0.97
Peribronchovascular distribution	45	59	0.055	1.48	1.41	0.20
Micronodules	7	13	0.183	1.48	1.45	0.76
Air-trapping/mosaic perfusion	59	57	0.807	1.49	1.39	0.10
Histopathology	TT/GT	GG	Total		Total	
(n)	29	46	75		70	
	% with feature			mean log T/S		
Feature	TT/GT	GG	p-value[1]	with	without	p-value[2]
Fibrosis (any)	90	74	0.097	1.32	1.83	**<0.0001** *
Microscopic honeycombing	45	17	0.010	1.18	1.52	**0.002** *

	MUC5B (rs35705950)			Telomere Length		
Heterogenous distribution of fibrosis	80	67	0.23	1.32	1.67	**0.0006** *
Subpleural distribution of fibrosis	38	17	0.046	1.27	1.47	0.10
Fibrobast Foci (moderate-marked)	34	15	0.052	1.15	1.50	**0.003** *
Lymphocytic interstitial inflammation	90	80	0.29	1.42	1.43	0.95
Interstitial granulomas/giant cells	52	72	0.079	1.49	1.32	0.089
Small airway disease	72	76	0.72	1.42	1.43	0.97

Footnote:

1 Chi2 test

2 Student's t test

* Indicates statistical significance using the Benjamini-Hochberg procedure for multiple testing by quadrant of the table.

Abbreviations: MUC5B = mucin 5B; UCSF = University of California San Francisco; UTSW = University of Texas Southwestern; log T/S = Telomere length expressed as the natural logarithm of the telomere to single gene copy ratio

Table 4

Multivariate ordinal logistic regression model for extent of radiographic fibrosis in the combined cohort of patients with chronic hypersensitivity pneumonitis (n = 175)

Predictor*	OR	95% CI	p-value
MUC5B, rs35705950, TT/GT vs. GG	1.91	1.02–3.59	0.045
Telomere length, per 1 unit change in log T/S	0.23	0.09–0.59	0.002
Age, per one year	1.02	0.99–1.05	0.22
Sex, male	2.51	1.34–3.32	0.004

Footnote: Extent of radiographic fibrosis is based on radiologist visual score as follows: none, mild (< 10% of the lung), moderate to severe (≥10% of the lung). * Multivariate model included MUC5B any minor allele, telomere length in log T/S, age per year, male sex, and cohort (cohort p = 0.124); interactions by cohort for MUC5B p=0.24 and for telomere length p=0.34.

Abbreviations: MUC5B = mucin 5B; log T/S = Telomere length expressed as the natural logarithm of the telomere to single gene copy ratio; OR = odds ratio; CI = confidence interval

Table 5

MUC5B single nucleotide polymorphism rs35705950, TOLLIP single nucleotide polymorphism rs5743890, and peripheral blood telomere length associations with survival in patients with chronic hypersensitivity pneumonitis

	MUC5B (rs35705950) (TT/GT vs. GG)			TOLLIP (rs5743890) (GG/AG vs. AA)			Telomere Length[2] (log T/S)		
	n	HR (95% CI)	p-value	n	HR (95% CI)	p-value	n	HR (95% CI)	p-value
UCSF									
Unadjusted	142	1.14 (0.55–2.38)	0.72	142	0.91 (0.39–2.14)	0.83	126	0.22 (0.09–0.56)	0.001
Adjusted[1]	119	1.59 (0.67–3.74)	0.29	119	0.97 (0.39–2.38)	0.94	109	0.22 (0.06–0.72)	0.012
UTSW									
Unadjusted	72	4.20 (1.35–13.1)	0.013	72	0.78 (0.27–2.26)	0.65	72	0.07 (0.01–0.42)	0.003
Adjusted[1]	60	4.23 (0.71–25.1)	0.11	60	2.04 (0.39–10.6)	0.40	60	0.06 (0.01–0.59)	0.015
Combined[3]									
Unadjusted	214	1.75 (0.97–3.16)	0.063	214	0.86 (0.44–1.67)	0.65	198	0.17 (0.08–0.39)	<0.001
Adjusted[1]	179	2.01 (0.97–4.20)	0.061	179	0.87 (0.41–1.83)	0.70	169	0.18 (0.06–0.51)	0.001

Footnote:

[1] Adjusted for age, sex, forced vital capacity % predicted, and diffusing capacity of the lung for carbon monoxide % predicted

[2] Telomere length expressed as the natural logarithm of the telomere to single gene copy ratio

[3] Cox proportional hazards model stratified by cohort

Abbreviations: MUC5B = mucin 5B; TOLLIP = Toll interacting protein; HR = hazard ratio; CI = confidence interval; UCSF = University of California San Francisco; UTSW = University of Texas Southwestern