CHEST

Original Research

DIFFUSE LUNG DISEASE

The Pulmonary Fibrosis-Associated *MUC5B* Promoter Polymorphism Does Not Influence the Development of Interstitial Pneumonia in Systemic Sclerosis

Anna L. Peljto, DrPH; Mark P. Steele, MD, FCCP; Tasha E. Fingerlin, PhD; Monique E. Hinchcliff, MD; Elissa Murphy, MS; Sofia Podlusky, BA; Mary Carns, MS; Marvin Schwarz, MD, FCCP; John Varga, MD; and David A. Schwartz, MD

Background: **More than 80% of patients with systemic sclerosis (SSc) develop lung involvement, most commonly interstitial pneumonia (IP). We recently identified a common variant in the promoter region of *MUC5B* (rs35705950) that has a significant effect on the risk of developing both familial and sporadic forms of IP. We hypothesized that this *MUC5B* promoter polymorphism is also associated with IP in subjects with SSc.**

Methods: **We examined the minor allele frequency of the *MUC5B* polymorphism among 231 subjects with SSc, 109 with IP, and 122 without IP. IP diagnosis was confirmed by HRCT imaging and defined as the presence of reticular infiltrates and/or honeycomb cysts. FVC and diffusing capacity of the lung for carbon monoxide (DLCO) were also assessed.**

Results: **We found no association between IP and the *MUC5B* polymorphism among subjects with SSc (OR = 1.1, *P* = .80). The frequencies of the *MUC5B* polymorphism among subjects with SSc with IP (10.6%) and without IP (9.4%) were similar to the frequency observed in a population of unaffected control subjects (9.0%). In secondary analyses, we found the *MUC5B* polymorphism was not significantly associated with either FVC (*P* = .42) or DLCO (*P* = .06). No association with SSc-associated IP was found even when we used a more conservative definition of IP (FVC ≤ 70% and evidence of reticulations or honeycombing vs SSc FVC > 70% and no evidence of reticulation or honeycombing).**

Conclusions: **Although SSc-associated IP is clinically, radiologically, and histologically similar to other forms of IP, it appears to have distinct genetic risk factors. This study highlights the genetic and phenotypic heterogeneity of IP in general.** ***CHEST 2012; 142(6):1584–1588***

Abbreviations: DLCO = diffusing capacity of the lung for carbon monoxide; HRCT = high-resolution CT; IIP = idiopathic interstitial pneumonia; IP = interstitial pneumonia; NSIP = nonspecific interstitial pneumonia; PFT = pulmonary function test; RNP = ribonucleoprotein; SSc = systemic sclerosis; SSc-IP = scleroderma-associated interstitial pneumonia; UIP = usual interstitial pneumonia

Scleroderma or systemic sclerosis (SSc) is a complex systemic autoimmune disease characterized by prominent immunologic, vascular, and fibrotic features. Although the pathogenesis of SSc remains incompletely understood, a combination of genetic risk factors and environmental exposures is implicated in triggering the development of tissue injury and damage.[1,2] Lung involvement is estimated to occur in > 80% of patients with SSc.[3] The most common forms of lung involvement are interstitial pneumonia (IP) and pulmonary vascular disease (pulmonary arterial hypertension). Scleroderma-associated interstitial pneumonia (SSc-IP) is primarily a fibrosing interstitial lung disease that is associated with the histologic patterns of nonspecific interstitial pneumonia (NSIP) or usual interstitial pneumonia (UIP) and has clinical and radiologic similarities to idiopathic interstitial pneumonia (IIP). However, in contrast to IIP, SSc-IP is more commonly NSIP, whereas a UIP histologic pattern is less frequent.[4] Furthermore, the prognosis of patients with SSc with IP is generally better than that of patients with idiopathic pulmonary fibrosis,

the most common form of IIP.[5] These observations raise questions regarding shared and distinct etiologic and pathologic events underlying the development of lung fibrosis in its idiopathic and SSc-associated forms.

High-resolution CT (HRCT) scan features of SSc-IP include ground-glass opacities, reticulations, subpleural crescent of increased densities, traction bronchiectasis, and occasionally fine honeycomb airspace disease. Risk factors for SSc-IP include higher creatine phosphokinase levels, hypothyroidism, and cardiac involvement.[6] Although the sensitivity is low and predictive value is uncertain, antitopoisomerase I (also known as anti-Scl-70), anti-U3 ribonucleoprotein (RNP), anti-U11/U12 RNP, anti-Th/To, and antihistone autoantibodies have also been associated with an increased risk of SSc-IP.[7-13] In contrast, SSc-IP is less common in patients with anticentromere antibodies.[14] Although some studies have identified genes that increase the risk of SSc-IP, it remains difficult to predict who will develop IP among patients with scleroderma.[15-18]

We have recently discovered a common variant in the promoter region of the *MUC5B* gene (rs35705950) that has a profound effect on the risk of developing familial and sporadic forms of IIP in two independent studies.[19,20] ORs for disease for subjects heterozygous and homozygous for the minor allele of this *MUC5B* single-nucleotide polymorphism (SNP) were 6.8 (95% CI, 3.9-12.0) and 20.8 (95% CI, 3.8-113.7) for familial ($P = 1.2 \times 10^{-15}$), and 9.0 (95% CI, 6.2-13.1) and 21.8 (95% CI, 5.1-93.5) for sporadic ($P = 2.5 \times 10^{-37}$) forms of IIP. Given the clinical, radiologic, and histologic similarities between SSc-IP and the forms of IIP associated with the common variant in the *MUC5B* promoter (rs35705950), we speculated that the *MUC5B* promoter polymorphism increases the risk of developing SSc-IP. To pursue this hypothesis, we studied the frequency of the *MUC5B* promoter polymorphism (rs35705950) among subjects with scleroderma with and without IP.

Manuscript received January 13, 2012; revision accepted April 13, 2012.

Affiliations: From the School of Public Health (Drs Peljto and Fingerlin), and the School of Medicine (Ms Murphy and Drs Schwarz and Schwartz), University of Colorado Denver, Aurora, CO; the Vanderbilt University School of Medicine (Dr Steele), Nashville, TN; and the Northwestern University Feinberg School of Medicine (Drs Hinchcliff and Varga and Mss Podlusky and Carns), Chicago, IL.

Funding/Support: This research was supported by the National Institutes of Health [Grants R01-HL095393, R01-HL097163, P01-HL092870, and RC2-HL101715].

Correspondence to: Anna L. Peljto, DrPH, University of Colorado, School of Public Health, 13001 E 17th Pl, MS B119, Aurora, CO 80045; e-mail: anna.peljto@ucdenver.edu

DOI: 10.1378/chest.12-0110

Materials and Methods

Study Population

This study population consisted of 333 subjects with SSc seen at the Northwestern Scleroderma Program and enrolled in the NUgene Project, each of whom was classified based on criteria proposed by LeRoy et al.[21] Subjects underwent a full evaluation, including pulmonary function tests (PFTs) and HRCT scan of the chest. HRCT imaging was available for 231 study subjects (69%), and standardized PFTs were available for 221 (66%). HRCT scans were reviewed by two of the study authors (J. V. and M. P. S.) and FVC was measured using standard methods.[22] For the purposes of the primary analysis, we defined IP based on HRCT imaging as presence of reticular infiltrates and/or honeycomb cysts, regardless of PFTs or other radiologic features. Exclusion criteria included significant exposure to known fibrogenic agents or an alternative cause for IP. Clinical and demographic characteristics of the subjects are summarized in Table 1. The samples in this study were obtained with the approval of the Northwestern University Institutional Review Board (IRB#STU00010003).

Genotyping Assay and Statistical Analysis

Taqman Genotyping Assays (Applied Biosystems) were used to evaluate the MUC5B promoter SNP, as has been described previously.[19] χ^2 Exact tests were computed to evaluate Hardy-Weinberg equilibrium among scleroderma cases. A goodness-of-fit χ^2 statistic was used to test for allelic association between the *MUC5B* minor allele and IP (honeycombing and/or reticulation). Student *t* test was used to test for an association between *MUC5B* carrier status and measures of pulmonary function.

Results

Subjects with and without IP had similar demographic characteristics in terms of sex, age, and smoking status (Table 1). However, subjects with IP were significantly less likely to have anticentromere antibodies ($P = .0004$), as has been previously reported by Kane et al.[14] The *MUC5B* SNP showed no evidence of a departure from Hardy-Weinberg equilibrium among those with IP ($P = .33$) or without IP ($P = .60$). We examined the minor allele frequency of the *MUC5B* minor allele among subjects with SSc with IP and without IP. We found no association between IP and the *MUC5B* polymorphism among subjects with scleroderma (OR = 1.1, $P = .80$). The frequencies of the *MUC5B* polymorphism among subjects with SSc with and without IP (10.6%, 9.4%) were similar to the frequency of the polymorphism that was previously reported in a population of unaffected control subjects (9.0%).[19] In secondary analyses, we examined the distribution of FVC and diffusing capacity of the lung for carbon monoxide (Dlco) values among subjects with one or more copies of the *MUC5B* polymorphism and subjects with no copies of the polymorphism (Figs 1A, 1B). The *MUC5B* polymorphism was not significantly associated with either FVC ($P = .42$) or Dlco ($P = .06$). Finally, we tested for association using a more stringent phenotype definition,

Table 1—*Characteristics of Subjects With SSc, With and Without IP on HRCT Scan*

Characteristic	IP	No IP	*P* Value
No.	109	122	…
Sex			.62
Female	88 (81)	104 (84)	
Male	21 (19)	18 (16)	
Age at diagnosis, mean (SD), y	47.3 (26.9)	44.2 (21.2)	.34
Smoking			.29
Never	69 (62)	65 (54)	
Former	36 (33)	50 (41)	
Current	4 (4)	7 (6)	
Clinical subtype			.61
Limited cutaneous	60 (55)	73 (60)	
Diffuse cutaneous	46 (42)	47 (39)	
Anticentromere antibodies			< .01
Yes	9 (8)	31 (25)	
No	81 (74)	65 (53)	
Anti-Scl-70 antibodies			.07
Yes	36 (33)	25 (20)	
No	67 (61)	84 (69)	

Data are presented as No. (%) unless otherwise noted. HRCT = high-resolution CT; IP = interstitial pneumonia; SSc = systemic sclerosis.

comparing study subjects with scleroderma with FVC ≤ 70% and evidence of reticulation or honeycombing on HRCT scan to subjects with SSc with FVC > 70% and no evidence of reticulation or honeycombing. The polymorphism was not associated with this more conservative definition of IP (OR = 1.1, $P = .95$). Subjects with one or more copies of the *MUC5B* polymorphism were similar to subjects with no copies of the polymorphism for evidence of IP on all PFTs and HRCT scan abnormalities (Table 2).

Discussion

Our findings indicate that scleroderma-associated IP is etiologically distinct from familial and sporadic forms of IIP. Although a variant in the promoter of *MUC5B* (rs35705950) is strongly associated with the development of familial and sporadic forms of IIP,[19,20] our results indicate that this promoter variant is not more frequently observed in individuals with SSc-IP. This finding supports a model of etiologic heterogeneity between IIP and SSc-IP. This may reflect etiologic heterogeneity between UIP and NSIP in general, since UIP was the primary form of IIP among cases in the study of IIP and *MUC5B*,[19] and previous studies have shown that the large majority of patients with SSc-IP have NSIP. It would be of interest for future studies to investigate the association of *MUC5B* with UIP vs NSIP in patients with SSc.

There are radiographic and histologic similarities between IIP and SSc-IP; both are progressive and often fatal. However, there are differences in these conditions to explain our results. In contrast to IIP, SSc-IP occurs more frequently in blacks,[6] involves a mixture of classic UIP and NSIP findings on HRCT scan, and often is associated with a stereotypic immune response (antitopoisomerase I, anti-U3 RNP, anti-U11/U12 RNP, anti-Th/To, and antihistone autoantibodies). Also, SSc-IP is observed more frequently in women and typically occurs in the third to fifth decades of life, whereas idiopathic pulmonary fibrosis is more common in men, with a typical age of onset after the fifth decade.[3,23-26] Thus, when considering the genetic factors that increase the risk of developing SSc-IP, it is not surprising that SSc-IP appears to be distinct from the idiopathic forms of IP. In fact, based on our findings, we would predict that the immunologic causes of IP do not involve the *MUC5B* promoter polymorphism. Different genetic causes of

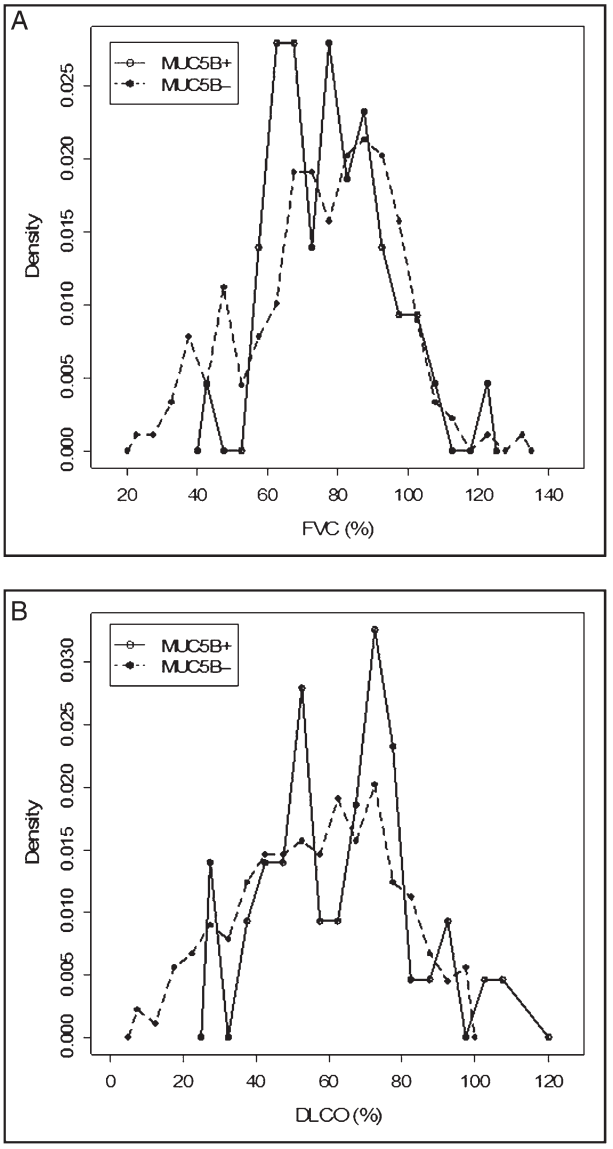

Figure 1. A, Distribution of FVC by *MUC5B* carrier status ($P = .42$). B, Distribution of DLCO by *MUC5B* carrier status ($P = .06$). DLCO = diffusing capacity of the lung for carbon monoxide.

Table 2—*HRCT Scan and PFT Results in Subjects With SSc by* MUC5B *Carrier Status*

Result	MUC5B$^+$	MUC5B$^-$	*P* Value
HRCT scan			
IP			.93
No	23 (52)	99 (53)	
Yes	21 (48)	88 (47)	
Honeycombing			.83
No	35 (80)	154 (82)	
Yes	9 (20)	33 (18)	
Reticulation			.88
No	25 (57)	106 (57)	
Yes	19 (43)	81 (43)	
Ground glass			.60
No	27 (61)	104 (56)	
Yes	17 (39)	83 (44)	
PFT			
FVC % pred, mean (SD)	78.6 (15.8)	76.3 (20.2)	.42
Dlco % pred, mean (SD)	64.0 (19.4)	57.4 (21.1)	.06

Data are presented as No. (%) unless otherwise noted. Dlco = diffusing capacity of the lung for carbon monoxide; PFT = pulmonary function test; pred = predicted. See Table 1 legend for expansion of other abbreviations.

SSc-IP vs IIP may lead to the apparent differences in pathogenesis. SSc-IP is a systemic disease that involves injury to lung endothelial cells, whereas IIP is nonsystemic and is characterized by injury to the alveolar epithelium.

We cannot exclude the possibility that expression of MUC5B is increased in SSc-IP as a consequence of scleroderma lung disease, as is observed in IIP.[19] It is possible that although there was no evidence of the *MUC5B* promoter polymorphism influencing SSc-IP, other epigenetic-driven or transcriptional changes may affect *MUC5B* expression in subjects with SSc-IP. However, if MUC5B is important in SSc-IP, we would still expect to see some overrepresentation of the *MUC5B* polymorphism among the subjects with SSc with IP. Since the frequencies of the *MUC5B* polymorphism were essentially the same among subjects with SSc with and without IP (10.6%, 9.4%), this would suggest that *MUC5B* expression is not a factor in the development of SSc-associated IP. We also cannot exclude the possibility that our study was underpowered to detect an association with the MUC5B promoter polymorphism with a small effect. The availability of HRCT scans for study subjects limited the sample size for testing. With the allelic association test, we had approximately 60% power to detect association for an OR of 2.0. Evaluating *MUC5B* expression in lung biopsies from patients with SSc-IP might shed further light on this question.

Acknowledgments

Author contributions: Dr Peljto is the guarantor of the manuscript.
Dr Peljto: contributed to the study conception and design; analysis and interpretation of data; and the drafting, review, and final approval of the manuscript.
Dr Steele: contributed to the study conception and design, acquisition of data, and the review and final approval of the manuscript.
Dr Fingerlin: contributed to the analysis and interpretation of data and the review and final approval of the manuscript.
Dr Hinchcliff: contributed to the acquisition of data and the review and final approval of the manuscript.
Ms Murphy: contributed to the acquisition of data and the review and final approval of the manuscript.
Ms Podlusky: contributed to the acquisition of data and the review and final approval of the manuscript.
Ms Carns: contributed to the acquisition of data and review and final approval of the manuscript.
Dr Schwarz: contributed to the study conception and design, interpretation of data, and the review and final approval of the manuscript.
Dr Varga: contributed to the study conception and design, acquisition of data, interpretation of data, and the drafting, review, and final approval of the manuscript.
Dr Schwartz: contributed to the study conception and design, acquisition of data, interpretation of data, and the drafting, review, and final approval of the manuscript.
Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.
Other contributions: This work was performed at University of Colorado Denver, Northwestern University, and Duke University.

References

1. Radstake TR, Gorlova O, Rueda B, et al; Spanish Scleroderma Group. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet*. 2010;42(5):426-429.
2. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest*. 2007;117(3):557-567.
3. Ferri C, Valentini G, Cozzi F, et al; Systemic Sclerosis Study Group of the Italian Society of Rheumatology (SIR-GSSSc). Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. *Medicine (Baltimore)*. 2002;81(2):139-153.
4. Bouros D, Wells AU, Nicholson AG, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am J Respir Crit Care Med*. 2002;165(12):1581-1586.
5. Wells AU, Cullinan P, Hansell DM, et al. Fibrosing alveolitis associated with systemic sclerosis has a better prognosis than lone cryptogenic fibrosing alveolitis. *Am J Respir Crit Care Med*. 1994;149(6):1583-1590.
6. McNearney TA, Reveille JD, Fischbach M, et al. Pulmonary involvement in systemic sclerosis: associations with genetic, serologic, sociodemographic, and behavioral factors. *Arthritis Rheum*. 2007;57(2):318-326.
7. Okano Y, Steen VD, Medsger TA Jr. Autoantibody reactive with RNA polymerase III in systemic sclerosis. *Ann Intern Med*. 1993;119(10):1005-1013.
8. Steen VD. Autoantibodies in systemic sclerosis. *Semin Arthritis Rheum*. 2005;35(1):35-42.
9. Steen VD, Powell DL, Medsger TA Jr. Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. *Arthritis Rheum*. 1988;31(2):196-203.
10. Sacks DG, Okano Y, Steen VD, Curtiss E, Shapiro LS, Medsger TA Jr. Isolated pulmonary hypertension in systemic sclerosis with diffuse cutaneous involvement: association with serum anti-U3RNP antibody. *J Rheumatol*. 1996;23(4):639-642.

11. Wallace DJ, Lin HC, Shen GQ, Peter JB. Antibodies to histone (H2A-H2B)-DNA complexes in the absence of antibodies to double-stranded DNA or to (H2A-H2B) complexes are more sensitive and specific for scleroderma-related disorders than for lupus. *Arthritis Rheum*. 1994;37(12):1795-1797.
12. Greidinger EL, Flaherty KT, White B, Rosen A, Wigley FM, Wise RA. African-American race and antibodies to topoisomerase I are associated with increased severity of scleroderma lung disease. *Chest*. 1998;114(3):801-807.
13. Fertig N, Domsic RT, Rodriguez-Reyna T, et al. Anti-U11/U12 RNP antibodies in systemic sclerosis: a new serologic marker associated with pulmonary fibrosis. *Arthritis Rheum*. 2009; 61(7):958-965.
14. Kane GC, Varga J, Conant EF, Spirn PW, Jimenez S, Fish JE. Lung involvement in systemic sclerosis (scleroderma): relation to classification based on extent of skin involvement or autoantibody status. *Respir Med*. 1996;90(4):223-230.
15. Dieudé P, Guedj M, Wipff J, et al. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. *Arthritis Rheum*. 2009;60(1):225-233.
16. Dieudé P, Guedj M, Wipff J, et al. STAT4 is a genetic risk factor for systemic sclerosis having additive effects with IRF5 on disease susceptibility and related pulmonary fibrosis. *Arthritis Rheum*. 2009;60(8):2472-2479.
17. Dieudé P, Guedj M, Wipff J, et al. NLRP1 influences the systemic sclerosis phenotype: a new clue for the contribution of innate immunity in systemic sclerosis-related fibrosing alveolitis pathogenesis. *Ann Rheum Dis*. 2011;70(4):668-674.
18. Gladman DD, Kung TN, Siannis F, Pellett F, Farewell VT, Lee P. HLA markers for susceptibility and expression in scleroderma. *J Rheumatol*. 2005;32(8):1481-1487.
19. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-1512.
20. Zhang Y, Noth I, Garcia JG, Kaminski N. A variant in the promoter of MUC5B and idiopathic pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1576-1577.
21. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol*. 1988;15(2):202-205.
22. Miller MR, Hankinson J, Brusasco V, et al; ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J*. 2005; 26(2):319-338.
23. Coultas DB, Zumwalt RE, Black WC, Sobonya RE. The epidemiology of interstitial lung diseases. *Am J Respir Crit Care Med*. 1994;150(4):967-972.
24. Scussel-Lonzetti L, Joyal F, Raynauld JP, et al. Predicting mortality in systemic sclerosis: analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. *Medicine (Baltimore)*. 2002;81(2):154-167.
25. King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med*. 2001;164(7):1171-1181.
26. Strollo D, Goldin J. Imaging lung disease in systemic sclerosis. *Curr Rheumatol Rep*. 2010;12(2):156-161.