Aetiology, Risk Factors, and Biomarkers in Systemic Sclerosis with Interstitial Lung Disease

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ONLINE DATA SUPPLEMENT

Online Data Supplement

Table E1. Statistically Significant Associations Between SSc-ILD and HLA Alleles: Studies with Ssc-ILD Cohorts ≥ 100 Patients (E1). Adapted by permission from Nature: Springer Nature. *Eur J Hum Genet*. Genetic predictors of systemic sclerosis-associated interstitial lung disease: a review of recent literature. Stock CJW, Renzoni EA. COPYRIGHT (2018). This table is excluded from the Open Access license; permission must be obtained from Springer Nature for its reuse.

HLA region	Allele/Serotype	OR and *P* Value for SSc-ILD	Population	Cohort Size
DPB1	301	OR = 3.56 (1.27–10.73)*	Han Chinese	199/78[†]
		P = 0.0069		
	1301	OR = 2.25 (1.4–3.62)[‡]	Han Chinese	199/480[§]
		$P = 3.3 \times 10^{-4}$		
DQB1	501	OR = 5.03[‡]	Han Chinese	134/239[§]
		$P = 6 \times 10^{-7}$		
DRB1	3	OR = 2.47 (1.35–4.52)[‡]	Han Chinese	295/458[§]
		P = 0.0026		

Definition of abbreviations: HLA = human leukocyte antigen; ILD = interstitial lung disease; OR = odds ratio; SSc = systemic sclerosis; SSc-ILD = systemic sclerosis-associated interstitial lung disease.

*Versus SSc-no ILD.

[†]SSc-ILD/SSc-no ILD.

[‡]Versus control.

[§]SSc-ILD/control.

Table E2. Statistically Significant Associations Between SSc-ILD and Non-HLA Genes: Studies with SSc-ILD Cohorts ≥ 100 Patients (E1). Adapted by permission from Nature: Springer Nature. *Eur J Hum Genet*. Genetic predictors of systemic sclerosis-associated interstitial lung disease: a review of recent literature. Stock CJW, Renzoni EA. COPYRIGHT (2018). This table is excluded from the Open Access licence; permission must be obtained from Springer Nature for its reuse.

Gene	Polymorphism	Function	OR and *P* Value for SSc-ILD	Population	Cohort Size
CD226	rs763361:T>A	–	OR = 1.27 (1.12–1.45)* *P* = 2.98 x 10^{-4}	French, German, Italian†	662/1642‡
	Haplotype rs763361:T>A, rs34794968:C>A, rs727088:G>A	Correlates with expression levels in T cells	OR = 1.27 (1.05–1.54)* *P* = 0.032	Spanish, German, Dutch, Italian, Swedish, British, Norwegian†	729/3,966‡
CTGF	rs918698:G>C	Alters ratio of Sp1:Sp3 binding affecting transcriptional activity	OR = 3.1 (1.9–5.0)* *P* =0.001	British	207/500‡
	rs6918698:G>C	See above	OR = 2.0 (1.5–2.6)* *P* = 0.001	Japanese	188/269‡

IRAKI	rs1059702:A>G/ rs1059703:G>A (in complete LD)	Increased NFκ-B activity	OR = 1.37 (1.16–1.62)*	French, Italian, German[†]	604/2,217[‡]
			P = 1.99 x10^{-4}	(Female only)	
	rs1059702:A>G/ rs1059703:G>A (in complete LD)	See above	OR = 1.30 (1.07–1.58)*	Spanish, German, Dutch, British[†]	461/2,043[‡]
			P = 8.46 x10^{-3}	(Female only)	
	rs1059702:A>G/ rs1059703:G>A (in complete LD)[§]	See above	OR = 1.2 (1.05–1.37)[‖]	European descent[†]	1,065/2,237[¶]
			P = 0.007		
IRF5	rs2004640:G>T	Results in transcription of alternative exon 1	OR = 1.44 (1.19–1.76)*	French	280/760[‡]
	rs2004640:G>T	See above	OR = 1.38 (1.1–1.75)*	Han Chinese	502/227[‡]
			P = 0.028		
	Haplotype rs3757385:G>T – rs2004640:G>T – rs10954213:G>A rs4728142:G>A	In LD with 5-bp indel which increases SP1 binding	OR = 0.64 (0.51–0.79)*	French	292/989[‡]
		Associated with lower expression	Mean difference = 2.64 (0.43–4.84)	American Caucasian	914**

			P = 0.019		(Linear regression analysis with FVC % predicted)
	rs2004640:G>T[§]	See above	OR = 1.12 (1.02–1.22)[‖] *P* = 0.014	French, European Caucasian, Han Chinese[†]	1,682/2,806[¶]
NLRP1	rs8182352:T>C	–	OR = 1.19 (1.05–1.36)* *P* = 0.0065	French, German, Italian[†]	674/1,587[‡]
STAT4	rs7574865:T>G	–	OR = 1.42 (1.16–1.73)* *P* = 0.008	French	316/970[‡]
	rs7574865:T>G	–	OR = 1.86 (1.34–2.59)* *P* = 1.2 x 10^{-4}	Han Chinese	237/534[‡]
	rs7574865:T>G[§]	–	OR = 1.259 (1.07–1.47)[‖] *P* = 5.35 x 10^{-3}	French, Spanish, Han Chinese[†]	640/842[¶]
	rs10168266:C>T	–	OR = 1.73 (1.24–2.41) *P* = 7.7 x 10^{-4}	Han Chinese	237/534[‡]
	rs3821236:G>A	–	OR = 1.54 (1.07–2.22)* *P* = 0.015	Han Chinese	237/534[‡]

Unreplicated studies with small cohort sizes	–			European descent	439/399[¶]
ALOX5AP	rs10507391:A>T (NC_000013.11: g_30737959A>T)		OR = 1.45 (1.17–1.79)[‖] *P* = 0.0006		

Definition of abbreviations: ALOX5AP = arachidonate 5-lipoxygenase activating protein; bp = base pairs; CTGF = connective tissue growth factor; FVC = forced vital capacity; CD226 = cluster of differentiation 226; HLA = human leukocyte antigen; ILD = interstitial lung disease; IRAK1= Interleukin-1 receptor-associated kinase 1; IRF5 = interferon Regulatory Factor 5; LD, linkage disequilibrium; NFκβ = nuclear factor κβ; NLRP1 = NLR family pyrin domain containing 1; OR = odds ratio; SSc = systemic sclerosis; STAT4 = signal transducer and activator of transcription 4; SSc-ILD = systemic sclerosis-associated interstitial lung disease.

Corrected *P* values given where available. ORs are shown as OR (95% confidence interval), 517 where available.

*Versus control.

[†]Meta-analysis of the different populations 519 included.

[‡]SSc-ILD/control.

[§]Meta-analysis or previously published studies.

[‖]Versus SSc-no ILD.

[¶]SSc-ILD/SSc-no ILD.

**Total number of SSc patients 518, when SSc-ILD number not given.

Table E3. Levels of Serum Biomarkers in SSc-ILD: Comparison with Healthy Controls, Ssc Without ILD and IPF. Significant Differences Between Study Groups Were Only Seen with Respect to KL-6, SP-D and MMP7 (the Kruskal–Wallis Test was Used to Assess for Differences Across the Four Groups) (E2). Data are presented as median (interquartile range). Reprinted with permission of Mattioli 1885: Kennedy B et al. Biomarkers to identify ILD and predict lung function decline in scleroderma lung disease or idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 2015; 32: 228–236.

	Controls	**SSc w/o ILD**	**SSc-ILD**	**IPF**	***P* Value**
KL-6 (ng/ml)	198 (52–360)	192 (0–525)	836 (431–1303)	633 (492–1,675)	0.0003*
SP-D (ng/ml)	137 (97–284)	169 (137–219)	398 (190–727)	542 (305–577)	0.0012†
MMP7 (ng/ml)	0 (0–0.06)	2.36 (1.2–5.1)	5.4 (2.6–7.25)	2.85 (1.5–3.6)	0.0009‡
TGF-β (pg/ml)	7,251 (5,654–10,034)	2,986 (2,483–4,029)	3,743 (1,855–5,500)	2,388 (1,501–7,367)	0.07
CCL18 (ng/ml)	46.85 (34.6–153.1)	49.1 (43.65–65.05)	62.05 (52.3–137.4)	48.4 (36.8–90.5)	0.58
PDGF-AA (pg/ml)	1,011 (605–2,989)	437 (314.5–649)	554 (328–935)	405 (167.5–1,222)	0.057
TNF-α (pg/ml)	2.73 (2.18–3.39)	2.53 (2.43–3.21)	3.41 (2.24–10.06)	2.78 (1.9–5.3)	0.84
VEGF (pg/ml)	60.32 (23.3–209.6)	22.9 (11.88–29.28)	24.96 (20.5–33.46)	24.14 (11.45–37.28)	0.053
Thrombomodulin (ng/ml)	3.07 (1.84–4.45)	1.36 (1.1–2.57)	1.63 (1.05–3.07)	2.57 (1.72–6.2)	0.054
PAI-1 (ng/ml)	37.2 (26.7–61.35)	21.3 (9.15–41.95)	40.55 (21.55–56.5)	32.7 (15.75–56.2)	0.35
VCAM-1 (ng/ml)	467.5 (397.1–686.6)	700.1 (567–969.5)	706.1 (583.2–801.3)	753.7 (444.5–916.3)	0.12
ICAM-1 (ng/ml)	297.7 (206.5–742.7)	259.5 (210.4–361.8)	431.4 (325.3–504.80)	416 (289.7–569.1)	0.18

P-Selectin (ng/ml)	168.5 (91.35–224.6)	131.3 (110–137.3)	133.9 (115.4–167.1)	119.1 (100.9–170.3)	0.51
L-Selectin (ng/ml)	1,397 (914.3–1,878)	1,385 (1,032–1679)	1329 (818.1–1746)	1,203 (891.4–1,784)	0.9
CCL2 (pg/ml)	84.9 (78.3–121.1)	86.7 (43.85–121.7)	145.2 (118.8–189.5)	159.4 (103.7–180.3)	0.06

CCL = chemokine (C-C motif) ligand; ICAM-1 = Intercellular Adhesion Molecule 1; IL = interleukin; KL-6 = Krebs von den lugen-6; MMP = matrix metalloproteinase; Pal-1 = Plasminogen activator inhibitor-1; PDGF-AA = Platelet Derived Growth Factor AA; SP-A = surfactant protein A; TGF-β = Tumor growth factor beta; TNF-α = tumor necrosis factor alpha ; VCAM-1 = vascular cell adhesion molecule 1; VEGF = vascular endothelial growth factor

Table E4. Comparison of Clinical and Mechanistic Features of SSc-ILD and IPF

Feature of Comparison	SSC-ILD	IPF
Lung involvement	Lung fibrosis occurs in ~80% of patients with SSc, 25–30% of whom develop progressive ILD (E3).	All patients develop characteristic progressive lung fibrosis (E4, E5)
Pulmonary symptoms	Dyspnea on exertion, nonproductive cough and predominantly basal inspiratory crackles on auscultation (E6, E7, E8)	Dyspnea on exertion, non-productive cough and predominantly basal inspiratory crackles on auscultation (E6, E5)
Extra-pulmonary features	Multisystem characteristics of SSc (e.g., vasculopathy, Raynaud's phenomenon, immune dysfunction, skin fibrosis, gastro-esophageal reflux) (E3, E9, E10)	Digital clubbing (E6)
Clinical course	Variable rate of progression (some patients show rapid, early decline; disease course may be stabilized by treatment with immunosuppressants; spontaneous regression can occur [albeit infrequently]); median survival is 5–8 years (E6, E11)	Progressive decline in lung function; spontaneous regression never occurs and the disease is unlikely to respond to immunosuppressant therapy; median survival is 2–3 years (E6, E4)
Disease mechanisms	Repetitive endothelial/epithelial cell injury leads to activation of innate and adaptive immune system, recruitment and activation of fibroblasts, and differentiation of fibroblasts to a myofibroblast phenotype, accumulation of ECM and development of fibrosis (E7, E12–E14). Increased numbers of	Similar to SSc-ILD, fibroblast activation, proliferation and differentiation into myofibroblasts culminates in excess deposition of ECM (E18, E14). However, unlike SSc-ILD, mast cell density is increased versus healthy controls and no increases in CD4+CD25+ regulatory T-cells or IL-22-

	CD4+CD25+ regulatory T-cells and IL-22-producing T-helper cells (E15, E16); mast cell density similar to healthy controls (E17).	producing T-helper cells are observed (E17, E19, E20).
Autoimmune characteristics	Most patients are positive for antinuclear antibodies and other specific autoantibodies (E6).	No clinically relevant levels of autoantibodies (E6)
Radiographic features	NSIP pattern is typical, including ground-glass opacities with areas of subpleural sparing, reticular markings and traction bronchiectasis. UIP observed in a minority of patients, with honeycombing of lower prominence compared with IPF (E6, E22).	UIP pattern with honeycombing; ground-glass opacities not seen (E6, E21).

Definition of abbreviations: ECM, extracellular matrix; IL = interleukin; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; NSIP = nonspecific interstitial pneumonia; SSc = systemic sclerosis; UIP = usual interstitial pneumonia.

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