

Genetic Susceptibility to Interstitial Lung Disease Associated with Systemic Sclerosis

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Supplementary Issue: Current Developments in Interstitial Lung Disease

ABSTRACT: Systemic sclerosis (SSc) is a connective tissue disease that is characterized by tissue fibrosis, microvasculopathy, and autoimmunity. Interstitial lung disease (ILD) is a common complication of SSc and is one of the frequent causes of mortality in SSc. Although the exact etiology of SSc remains unknown, clinical and experimental investigations have suggested that genetic and environmental factors are relevant to the pathogenesis of SSc and SSc-ILD. More than 30 genes have been identified as susceptibility loci for SSc, most of which are involved in immune regulation and inflammation. It is thought that the key pathogenesis of SSc-ILD is caused by the release of profibrotic mediators such as transforming growth factor β 1 and connective tissue growth factor from lung cells induced by a persistent damage. This review presents the genetic susceptibility to SSc-ILD, including human leukocyte antigen and non-human leukocyte antigen genes, especially focusing on connective tissue growth factor.

KEYWORDS: systemic sclerosis, scleroderma, interstitial lung disease, genetics, CTGF

SUPPLEMENT: Current Developments in Interstitial Lung Disease

CITATION: Tochimoto and Kawaguchi. Genetic Susceptibility to Interstitial Lung Disease Associated with Systemic Sclerosis. *Clinical Medicine Insights: Circulatory, Respiratory and Pulmonary Medicine* 2015;9(S1) 135–140 doi: 10.4137/CCRP.M.S23312.

TYPE: Review

RECEIVED: June 29, 2015. **RESUBMITTED:** December 14, 2015. **ACCEPTED FOR PUBLICATION:** January 14, 2016.

ACADEMIC EDITOR: Hussein D. Foda, Editor in Chief

PEER REVIEW: Five peer reviewers contributed to the peer review report. Reviewers' reports totaled 551 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Paper subject to independent expert blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

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Introduction

Systemic sclerosis (SSc) is a chronic multisystem autoimmune disease that is characterized by autoimmunity, tissue fibrosis, and vasculopathy. Clinical and experimental investigations have suggested that genetic factors and environmental factors are relevant to the pathogenesis of SSc.¹ Recently, various genetic studies have shown that candidate genes and single nucleotide polymorphisms (SNPs) may be risk factors for the development of SSc.²

Interstitial lung disease (ILD) is a life-threatening complication and is one of the frequent causes of mortality in SSc, along with pulmonary arterial hypertension. The key pathogenesis of SSc-ILD is thought to be initiated by a persistent injury to lung cells that induces profibrotic stimuli, which leads to fibroblast activation and myofibroblast transition. Lung injury results in the release of profibrotic mediators such as transforming growth factor β 1 (TGF- β 1) and connective tissue growth factor (CTGF); CTGF causes the additional recruitment of myofibroblasts and the overproduction and accumulation of extracellular matrix.^{3–5}

Several studies show that an SNP in the *CTGF* gene plays a crucial role in SSc and SSc-ILD.^{6,7} CTGF is increased in the circulation, skin, and fibroblasts of patients with SSc. CTGF regulates the proliferation of fibroblasts and the

production of extracellular matrix.^{8–10} Our aim in this review is to present the genetic susceptibility to SSc-ILD.

Genetics and SSc

The incidence of SSc is 1.5%–1.7% in people with a family history of SSc, while it is 0.026% in the general population. SSc is not inherited in a Mendelian fashion; however, having family members who have SSc or another autoimmune disease increases the relative risk. Having a sibling with SSc results in a 15- to 19-fold increased risk, and first-degree relatives have a 13- to 15-fold increased risk of having SSc compared with the general population.¹¹

Observation studies among Caucasian and African-American patients have suggested that African-Americans have a genetic tendency to develop SSc and also have a higher morbidity and mortality.^{12,13} Early genetic studies indicated that SSc was associated with the human leukocyte antigen (HLA) region.¹⁴ Moreover, many candidate gene studies have identified multiple novel polymorphisms that increase susceptibility to SSc. Explorative genetic association studies later discovered significant associations between SSc and SNPs within the immune regulatory genes, including *STAT4*,^{15–17} *TNFSF4*,^{18,19} *TBX21*,¹⁷ *NLR family pyrin domain containing 1 (NLRP1)*,²⁰ *BLK*,^{21–23} *BANK1*,^{24,25} *CD247*,^{26,27}



PTPN22,^{28–30} *IL23R*,^{31,32} *CD226*,³³ *PLD4*,³⁴ and *TLR2*,³⁵ and the inflammatory genes, including *IL1A*,³⁶ *interleukin-1 receptor-associated kinase 1 (IRAK1)*,^{37,38} *interferon regulatory factor 5 (IRF5)*,^{39–41} *TNFAIP3*,^{42,43} *CTGF*,^{6,7} *CXCL8*,⁴⁴ and *CCR6*.⁴⁵

Genome-wide association studies (GWASs) can be used for a comprehensive identification of novel loci. In 2010, a large GWAS reconfirmed the associations of SSc with HLA, *IRF5*, and *STAT4* and identified *CD247*, *IRF8*, *GRB10*, and *SOX5*.^{26,46} Other GWASs in a French cohort recognized three new loci, namely, *TNIP1*, *PSORS1C1*, and *RHOB*, that were associated with SSc.⁴⁷

Genetic Association in SSc-ILD

HLA-dependent genes. Associations among HLA alleles, susceptibility to SSc and specific autoantibodies, and clinical features of SSc have been identified. A study in Canada among Caucasian patients with SSc revealed that *HLA-Cw*0602* is associated with patients with pulmonary fibrosis.⁴⁸ In South Africans, *DRB1*11* is associated with pulmonary fibrosis.⁴⁹ Another study conducted in Japan suggested that the *HLA-DRB5*01:05* allele was a risk factor for ILD patients with SSc (Table 1).⁵⁰

Non-HLA genes. As mentioned earlier, numerous genes have been identified as susceptibility loci for SSc. Some of them revealed an association with SSc-ILD. *CTGF* was significantly associated with the presence of ILD, and the details are presented below. The *CD226* gene encodes DNAX accessory molecule-1, which is involved in the adhesion and activation of T-cells. The nonsynonymous *CD226* rs763361 SNP (Gly307Ser) may affect the expression of the CD226 molecule. In European Caucasians, the rs763361 T allele was associated with a diffuse cutaneous SSc subtype (dcSSc), an antitopoisomerase I antibody, and SSc-ILD subsets.³³ *IRF5* is a crucial component of type I IFN regulation. A large case-control study in French Caucasians discovered an association between the *IRF5* rs2004640 T allele and SSc. Additional associations between T allele and fibrosing alveolitis were found.³⁹

NLRP1 performs as an endogenous microbial product and a metabolic stress sensor and consequently activates the innate immune system in conjunction with inflammasomes. These cytoplasmic proteins promote the inflammatory cytokines such as IL-1 β , IL-18, and IL-33. IL-1 β has been suggested to have an association with SSc fibroblasts. In European Caucasians, the *NLRP1* rs8182352 variant was associated with antitopoisomerase I antibody and SSc-ILD.⁵¹

Table 1. Genetic association in SSc-ILD: HLA dependent genes.

HLA	ALLELES	POPULATION	REFERENCES
HLA-C	Cw*0602	Caucasian	48
HLA-DR	DRB1*1102	South African	49
	DRB5*01:05	Japanese	50

IRAK1, which is located on the X chromosome, regulates T-cell receptor signaling pathways and Toll-like receptor activation. A study in European Caucasians discovered the notable association between the *IRAK1* rs1059702 TT genotype and dcSSc, the antitopoisomerase I antibody, and SSc-ILD.³⁷ Another study in five independent Caucasian cohorts showed that the association of *IRAK1* rs1059702 with dcSSc was interpreted by the nearby SNP of *MECP2* rs17435, but the association of *IRAK1* rs1059702 with pulmonary fibrosis was still present.³⁸

Matrix metalloproteinase-12 (MMP-12) is one of the proteins of the MMP family. An association between serum MMP-12 levels and severity of skin and pulmonary fibrosis and peripheral vascular damage in SSc patients has been reported.⁵² In an Italian cohort, a significant difference was observed in *MMP12* rs2276109 genotype distribution between patients with SSc and a control group. In particular, the homozygosity for the A allele was significantly associated with dcSSc, antitopoisomerase I antibody, and ILD.⁵³

Interleukin-1 α (IL-1 α) stimulates dermal fibroblasts to synthesize several types of cytokines and growth factors that activate fibroblasts to produce the extracellular matrix. It was shown that SSc fibroblasts constitutively produced high levels of pro-IL-1 α .⁵⁴ The association among three SNPs at positions -889, +4729, and +4845 of the *IL1A* gene and SSc in Japanese patients has been reported. The three SNPs were in linkage disequilibrium, and the CTG haplotype frequency was significantly higher in patients with SSc than in healthy control subjects. The frequency of the diplotype CTG/CTG was significantly associated with SSc-ILD patients.³⁶

Hepatocyte growth factor (HGF) is an antifibrotic factor and acts against the profibrotic actions of TGF β . However, SSc patients have high levels of HGF in circulation. These findings suggest that increased HGF levels are insufficient to inhibit fibrotic progression.⁵⁵ In Japanese patients, the distribution of *HGF* rs3735520 SNP was not significantly different between patients with SSc and healthy controls. However, the SNP was significantly associated with patients with end-stage lung disease.⁵⁶

Pulmonary surfactant protein B (SP-B) acts as a protective substance against lung injury. A study in Japanese patients showed that the frequency of the TT genotype in the *SFTPB* rs1130866 was not different between SSc patients and healthy controls but was significantly lower in patients with SSc-ILD than in those without ILD. This result suggested that the TT genotype in SP-B was associated with a lower risk of SSc-ILD (Table 2).⁵⁷

Gene Polymorphisms of CTGF Associated with SSc-ILD

CTGF was found to have increased expression in the circulation, skin, and fibroblasts of patients with SSc. Moreover,

Table 2. Genetic association in SSc-ILD: non-HLA genes.

GENE	SNP	RISK ALLELE/ GENOTYPE/DIPLTYPE	POPULATION	N (SSc-ILD, CONTROL)	OR (95%CI)	REFERENCES
CTGF	rs6918698	GG genotype	UK Caucasian	207, 500	3.1 (1.9–5.0)	6
		G allele	Japanese	188, 269	2.0 (1.5–2.6)	7
CD226	rs763361	T allele	European Caucasian	662, 1642	1.27 (1.12–1.45)	33
IRF5	rs2004640	T allele	French Caucasian	280, 730	1.44 (1.19–1.76)	39
NLRP1	rs8182352	CC genotype	European Caucasian	674, 1587*	1.43 (1.10–1.84)	20
IRAK1	rs1059702	TT genotype	European Caucasian	604, 2217	2.09 (1.35–3.24)	37
		T allele	Caucasian	461, 2043	1.30 (1.07–1.58)	38
MMP12	rs2276109	AA genotype	Italian Caucasian	74, 176*	2.94 (1.25–6.95)	51
IL1A	rs1800587 (–889C/T)	CTG/CTG diplotype	Japanese	42, 70	–	36
	rs2856841 (+4729T/C)		Japanese			
	rs17561 (+4845G/T)		Japanese			
HGF	rs3735520	TT genotype	Japanese	17**, 126***	8.1 (2.5–26.0)	55
SFTPB	rs1130866	TT genotype	Japanese	79, 48*	0.13 (0.03–0.64)	56

Notes: *SSc without ILD, **SSc with end-stage lung disease (ESLD), ***SSc without ESLD.

Abbreviation: 95%CI, 95% confidence interval.

CTGF regulates proliferation of fibroblasts and production of extracellular matrix.^{8–10,58} Studies in the UK and Japan reported that an SNP in the promoter region of *CTGF* rs6918698 (–945C/G) was significantly associated with SSc.^{6,7}

In a UK cohort study by Fonseca et al, the GG genotype of *CTGF* rs6918698 was significantly more common in patients with SSc than in control subjects. The study showed a notable association between the GG genotype and the presence of antitopoisomerase I and anticentromere antibodies. Additionally, a logistic regression analysis was performed among the clinical phenotypes, which revealed a significant association between the G allele and fibrosing alveolitis. The study also showed that the presence of C allele at position –945 is critical for transcriptional suppression of the *CTGF* gene through Sp3 binding. The result of this suppression would reduce *CTGF* production. A previous study reported that Sp1 often acts as a stronger activator than Sp3 and that Sp3 also often acts as a repressor in the *CTGF* promoter. Fonseca et al showed that the ratio of Sp1 to Sp3 binding in lung fibroblasts was approximately three times as high for GG homozygote cells as for CC homozygote, a finding that was in agreement with higher transcriptional activity from the G allele. Moreover, they showed that SSc fibroblasts had a generally higher ratio of Sp1 to Sp3 binding than control cells. This functional explanation would be reasonable for the observation that the G allele is associated with SSc. *CTGF* is constitutively overexpressed by fibroblasts in fibrotic skin lesions as well as in lung fibroblasts of SSc patients.⁵⁹ Furthermore, higher levels of *CTGF* are seen in bronchoalveolar lavage fluid from active SSc-ILD patients.⁵⁸ Such a functional explanation would be in agreement with

the observation that the G allele is associated with SSc-ILD. Therefore, increased *CTGF* expression is comprehensible in the observation of functional analysis.⁶

A study in a Japanese cohort by Kawaguchi et al confirmed the association between the frequency of the G allele in *CTGF* rs6918698 and the susceptibility to SSc. The study also confirmed a significant association between the frequency of the G allele and the presence of dcSSc, ILD, and antitopoisomerase I antibody in SSc. However, the authors did not indicate any association between the SNP and the presence of an anticentromere antibody in SSc. They discovered a significant association between the G allele and the GG genotype and patients with limited cutaneous SSc and ILD. Therefore, these observations suggest that the G allele and the GG genotype of *CTGF* rs6918698 may be susceptible to SSc-ILD, independent of skin fibrosis.⁷

A replication study of the *CTGF* gene in 1311 North American patients (white Americans, African-Americans, and Hispanic Americans) did not validate susceptibility to any form of SSc.⁶⁰ A multicenter study in seven independent case–control sets, including Spanish, Dutch, German, French, British, Swedish, and North American groups, also failed to confirm an association between SNP and SSc.⁶¹ Two other replication studies in France and Thailand also could not confirm this association.^{62,63} Zhang et al performed a meta-analysis of six published case–control studies including 3335 cases and 3589 controls. The study showed that the CC and CG genotypes of *CTGF* rs6918698 in Asians were significantly associated with a reduced SSc risk. In contrast, no association was revealed in Caucasians or the overall population. This meta-analysis did not assess any clinical

**Table 3.** Summary of studies on association of the *CTGF* rs6918698 polymorphism with SSc.

AUTHOR	PUBLICATION YEAR	POPULATION	N (SSc, CONTROL)	OR (95%CI)	P VALUE	REFERENCES
Fonseca	2007	Caucasian	500, 500	2.2 (1.5–3.2)	<0.001	6
Gourth	2008	Caucasian, African American, Hispanic American	994, 668	1.02 (0.9–1.2)	0.83	59
Kawaguchi	2009	Asian (Japanese)	395, 269	1.5 (1.2–1.9)	0.002	7
Rueda	2009	Caucasian	1180, 1784	1.12 (0.99–1.25)	0.06	60
Granel	2010	Caucasian	241, 269	N.D.	0.61	61
Louthrenoo	2011	Asian (Thai)	50, 99	N.D.	0.6	62

Note: N.D., not determined.

Abbreviation: 95%CI, 95% confidence interval.

phenotypes or antibodies because some of the studies lacked detailed data.⁶⁴

Discrepancies in the genetic association studies of different races have been reported.^{65–67} One of the possible explanations for this discrepancy between Asians and Caucasians could be a result of different gene–environment interactions among populations. A second explanation might be because of the linkage disequilibrium or correlation with a different SNP. The French study by Granel et al showed that *CTGF* rs6918698 was not correlated with another SNP (rs9399005). However, in the HapMap reference populations, the two markers were correlated.⁶² Thus, these genetic and environmental factors affect the susceptibility to SSc and complicate these replication analyses (Table 3).

The Difference of Susceptible Genes Among SSc-ILD and Idiopathic Pulmonary Fibrosis

Genetic factors contribute to the susceptibility of idiopathic pulmonary fibrosis (IPF) also. In familial IPF cases, strong associations have been identified with rare mutations in the surfactant protein A or C genes. Several reports revealed that the genetic variants in the genes within the human telomerase components are associated with familial and sporadic IPF. These mutations result in telomere shortening and a consequent apoptosis of cells, including the alveolar epithelial cells. Recently, two GWASs reported that a common SNP of the *MUC5B* gene, which encodes airway epithelial mucin glycoprotein, is associated with the risk of IPF. These studies also identified other IPF-associated loci, including the *Toll-interacting protein (TOLLIP)*, which negatively interacts with Toll-like receptor signaling. The *MUC5B* promoter and *TOLLIP* variants have been related to survival in IPF.

Interestingly, except HLA, none of the known genetic variants, including the *MUC5B* gene linked to IPF, have been associated with SSc-ILD.^{68,69}

This discrepancy between SSc-ILD and IPF may be explained by the difference of the prominent pattern of the radiological and pathological patterns. The prominent pattern is nonspecific interstitial pneumonia in SSc-ILD. On the other hand, usual interstitial pneumonia is predominant in

IPF. These findings suggest that the pathogenesis of SSc-ILD is different from IPF.

Conclusions

The pathogenesis of SSc is not fully understood. In this article, we reviewed the genetic association with SSc-ILD, especially the SNP in the *CTGF* gene that is involved in the development of SSc and SSc-ILD. SSc-ILD does not have an effective treatment and results in increased mortality in patients with SSc. Investigation of the pathogenesis, including genetic analysis, may result in the development of a novel therapy.

Author Contributions

Conceived and designed the experiments: AT, YK, HY. Analyzed the data: AT, YK. Wrote the first draft of the manuscript: AT. Contributed to the writing of the manuscript: AT, YK. Agree with manuscript results and conclusions: AT, YK, HY. Jointly developed the structure and arguments for the paper: AT, YK, HY. Made critical revisions and approved final version: AT, YK, HY. All authors reviewed and approved of the final manuscript.

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