Genetic polymorphisms in the surfactant proteins in systemic sclerosis in Japanese: T/T genotype at 1580 C/T (Thr131Ile) in the SP-B gene reduces the risk of interstitial lung disease

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Introduction
SSc is a generalized connective tissue disorder and affects at least three components: microcirculation, the immune system, and fibroblasts. Fibroblasts obtained from SSc patients present an activated phenotype synthesizing increased amounts of collagen [1]. Although patients with SSc exhibit common symptoms, such as Raynaud’s phenomenon, autoantibody production and skin fibrosis, the disease expression and prognosis are different in patients. SSc is clinically divided into two major subtypes: dcSSc and lcSSc [1]. The dcSSc subtype is associated with visceral fibrosis, which affects the lungs, heart and gastrointestinal tract with high frequency and is characterized by the possession of anti-Scl-70 (topoisomerase I) antibody. In lcSSc, the dominant clinical feature is vascular manifestation and the representative antibody is anti-centromere.

In visceral involvements of SSc, interstitial lung disease (ILD) is one of the most important complications because it occurs in over 50% of patients and is a major cause of death [2]. Although ILD has been associated with possession of anti-Scl-70 antibody [3] and disease subtype dcSSc, we speculated that some genetic factors other than clinical parameters might influence individual susceptibility to ILD in SSc patients.

Surfactant proteins (SPs), including SP-A, -B, -C and -D, are specifically produced from alveolar type II cells, contribute to lowering of the surface tension of the alveolus, and act as protective agents from pulmonary injury [4]. Deficiency of SPs or derangement of surfactant activity can cause severe respiratory disorders such as congenital alveolar proteinosis [5]. The SP genes are polymorphic [6], and some genetic variants, especially within the SP-A and -B genes, have been associated with individual variability in susceptibility to various pulmonary diseases [7, 8].

In this study, we investigated whether genetic polymorphisms in the SP-A and -B genes influenced the presence or absence of ILD in Japanese SSc patients. Investigated single-nucleotide polymorphisms (SNPs) were nucleotide (nt) 1580 C/T (Thr131Ile) in the SP-B gene and C/T within amino acid (aa) 219 (Arg219Trp) in the SP-A1 gene, which characterizes the 6A4 haplotype of the SP-A1 gene.

Patients and methods

Patients
We studied 127 SSc patients (115 females, 12 males) who were followed at the Institute of Rheumatology, Tokyo Women’s Medical University, and 206 healthy controls (150 females, 56 males) with their informed consent. All the subjects were Japanese, and all the patients met the American College of Rheumatology criteria for the diagnosis of SSc [9]. The clinical subtypes of dcSSc and lcSSc were defined according to the classification of LeRoy and Medsger [1]. ILD was diagnosed according to findings of high-resolution computed tomography of the chest.

The study was approved by the Genome-Ethics Committee of Tokyo Women’s Medical University. All subjects gave their written informed consent before participating.

Determination of the SP-A and -B gene polymorphisms

Genotypes for both C/T within aa 219 of the SP-A1 gene (CGG/Arg to TGG/Trp change, rs4253527) and C/T at nt 1580 (within aa 131, ACT/Thr to ATT/Ile change, rs1130866) for the SP-B gene were determined by the TaqMan method using specific primers and fluorescently labelled probes designed by Applied Biosystems (Foster City, CA, USA). Primers for the SP-A1 and -B genes were determined by the TaqMan method using specific primers and fluorescently labelled probes designed by Applied Biosystems (Foster City, CA, USA). Primers for the SP-A1 and -B genes were determined by the TaqMan method using specific primers and fluorescently labelled probes designed by Applied Biosystems (Foster City, CA, USA).
genes were as follows: SP-A1 forward 5'-TGGAGACTTCCGCCGCT ACTCAGA-3'; SP-A1 reverse 5'-CAGTCGTGTTGATGCACA TCTC-3'; SP-B forward 5'-CCCTGTCATGCTCACTTTC CA-3' and SP-B reverse 5'-GCAGAGTGACGTTGGCA-3'. Probes for the SP-A1 and -B genes were as follows: SP-A1 allele C/T 39 (24.7) 30 (31.2) 16 (25) 14 (50) 22 (24.4) 16 (34.8) C 119 (85.4) 66 (68.8) NS 48 (75) 14 (50) 0.029 68 (75.5) 30 (65.2) NS T/T 2 (2.5) 8 (16.7) 0 5 (35.7) 1 (2.2) 4 (17.4) T/T 2 (2.5) 8 (16.7) 0 5 (35.7) 1 (2.2) 4 (17.4) C/T 35 (44.3) 14 (29.2) 0.009 16 (50) 4 (28.6) 0.0032 20 (44.3) 8 (34.8) NS C/C 42 (53.2) 26 (54.2) 16 (50) 5 (35.7) 2 (17.6) 12 (63.8) 12 (64.3) 0.0001 24 (53.3) 11 (47.8) NS

SSc patients were then divided into two categories, according to the presence or absence of ILD, and the frequencies of genotypes were compared. In the SP-A1 gene (Table 1), the distributions of three genotypes were significantly different \((P = 0.015)\) between the two groups. However, this difference disappeared when the patients were sorted by the presence of anti-Scl-70 antibody or disease subtype of dcSSc (data not shown). There was no significant skewing for a certain genotype in the SP-A1 gene.

In the SP-B gene (Table 2), the distributions of three genotypes were also significantly different \((P = 0.0091)\) between the two groups, with or without ILD. Furthermore, the frequency of the T/T genotype appeared to be significantly lower in the ILD-positive group compared with that in the ILD-negative group (2.5% vs 16.7%; \(P = 0.0062\), OR = 0.13, 95% CI = 0.026, 0.64). Therefore the T/T genotype of the SNP in the SP-B gene was inversely associated with ILD. When the patients were sorted by the possession of anti-Scl-70 antibody \((n = 46)\), none of the 32 patients with ILD had the T/T genotype, whereas 5 (35.7%) of the 14 patients without ILD carried the T/T genotype, and the difference was statistically significant \((P = 0.0015\), OR = 0.027, 95% CI = 0.0013, 0.53). Next, the patients were sorted by disease subset of dcSSc \((n = 68)\), and the frequencies were compared. The frequency of T/T genotype was 2.2% in ILD-positive patients and 17.4% in ILD-negative patients. Although the difference between the two groups in dcSSc patients was statistically significant \((P = 0.041)\), the OR of 0.11 would be controversial \((95% CI = 0.011, 1.03)\).

Furthermore, in the anti-Scl-70 antibody-positive group, the frequency of the T allele was significantly lower in patients with ILD \((16 of 64 chromosomes, 25\%)\) than in those without ILD \((14 of 28 chromosomes, 50\%; P = 0.029, OR = 0.33, 95% CI = 0.13, 0.85)\) (Table 2).

**Discussion**

In this study, we have demonstrated that the T/T genotype at nt 1580 in the SP-B gene was inversely correlated with the complication of ILD in SSc. Our findings suggest that the SP-B protein with the T/T genotype could protect against the development of ILD in patients with SSc. Interestingly, this phenomenon was statistically emphasized in the examination of SSc patients with anti-Scl-70 antibody. Being limited in anti-Scl-70 antibody-positive patients, not only the T/T genotype, but the T allele was also inversely correlated with susceptibility to ILD.
Possession of anti-Scl-70 antibody and disease subtype dSSc was associated with susceptibility to ILD in SSc, whereas anti-centromere antibody reduced the risk [10]. Several recent studies have indicated that genetic polymorphisms in the genes, such as IL-1α [11] or β [12], which are susceptible genes for SSc, influence the predisposition to ILD in SSc. This study showed that genetic polymorphisms that can modulate pulmonary defense also influence susceptibility to ILD in SSc. We sometimes encountered patients who had not been complicated with ILD for a long time, even though they had anti-Scl-70 antibody. Genetic polymorphisms in SPs might explain some parts of this discrepancy. Additionally, by logistic regression analysis by the stepwise method, both T/T genotype in the SP-B gene and possession of anti-centromere antibody were independently associated with the absence of ILD in this study.

This is a case-control study, and the sample size is relatively small. Furthermore, the frequency of T/T genotype in the SP-B gene, the protective genotype from ILD, is low in Japanese (6.3% in the healthy population). These factors would reduce the power of statistical analysis of this study. Another cohort study with a larger sample size or from a different ethnicity is required to overcome these limitations. Interestingly, our results were consistent with previous reports of other pulmonary diseases among different ethnicities [7, 8, 13]. The observation of the advantage of the T allele and disadvantage of the C allele was conserved among various lung diseases from different ethnicities.

SPs do not only have surface tension-lowering characteristics in the alveolus, but also influence the activity of alveolar macrophages, modulating local immune responses. For example, SPs suppress production of inflammatory cytokines from lipopolysaccharide-activated alveolar macrophages [14]. In SSc, alveolar macrophages are phenotypically altered and play an important role in the fibrotic process [15]. Therefore, genetic polymorphisms that alter protein function of SPs can influence the individual susceptibility to ILD in SSc. Because the SNP nt 1580 C/T at the end of exon 4 of the SP-B gene was associated with various lung diseases [7, 8, 13], functional meaning of the SNP has been studied. The C>T substitution causes a Thr→Ile change at aa 131, which can block potential N-linked glycosylation sites, Asn at aa 129 [16]. Wang et al. [17] showed that a stable transfecant with C allele was indeed glycosylated at Asn129,Gln-Thr131, while that of the C allele was not. N-linked glycosylation would interfere with SP-B processing, secretion and folding, resulting in modulating protein levels or functions of SP-B protein. In healthy men, however, there was no association between individual pulmonary function and genotype at nt 1580 C/T in the SP-B gene [18]. We speculate that the SNP in the SP-B gene influences immune response in the lung under certain disease conditions such as SSc.

There are considerable ethnic differences in allele frequency of nt 1580 C/T of the SP-B gene. The frequency of the C allele is reported to be ~0.3 in black subjects and around 0.5 in white and Hispanic subjects [19]. Our data showed that the frequency of the C allele was 0.73 (~203) in the Japanese population, higher than in other ethnicities. Taking the functional significance of the SNP into consideration, it is plausible that Japanese people are at a genetic disadvantage for pulmonary defense.

The human SP-A locus includes two linked functional genes, SP-A1 and -A2 [20]. The SP-A1 gene has five haplotypes (6A1, 6A2, 6A3, 6A4 and 6A5), all of which are composed of combinations of SNPs located in the coding sequences [6]. In idiopathic pulmonary fibrosis in adults, 6A4 was associated with disease risk in smokers [8]. Haplotype 6A4 is characterized by SNP within aa 219, and the Arg→Trp substitution is suggested to alter SP-A protein function [8]. In the present study group, although the genotype distributions of the SP-A1 gene were different between the patients with and without ILD, the contribution of a certain genotype to susceptibility to ILD was not determined.

In conclusion, carrying the T/T genotype at SP-B gene would provide a beneficial role, reducing the risk of ILD in Japanese SSc patients.

### Rheumatology key message

- The T/T genotype at the nt 1580 of the SP-B gene reduced the risk of ILD in Japanese SSc patients.

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### References