Transforming Growth Factor ß1 Gene Polymorphism in Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To determine whether transforming growth factor ß1 (TGFß1) gene DNApolymorphism is associated with pathogenesis in the fibrosis of patients with systemic sclerosis (SSc).

Methods. Eighty-seven Japanese patients with SSc including 30 with diffuse type and 57 with limited type together with 110 unrelated controls were investigated. Pulmonary fibrosis was determined in 34 SSc patients using high-resolution chest computed tomography. TGFß1 genetic polymorphisms were analyzed in 2 loci; T869C (Leu10Pro) in codon 10 at exon 1, and C-509T in the promoter region using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results. Neither the genotype of T/C polymorphism in T869C nor C/T polymorphism in C-509T revealed any difference in distribution between SSc and controls. In the group of SSc patients with pulmonary fibrosis, a weak but significantly high frequency (p = 0.05) of TC+CC (the presence of C allele) in T869C, and CT+TT (the presence of T allele) in C-509T was found. Compared with controls, the pulmonary fibrosis group showed no difference in the highly frequent alleles.

Conclusion. Our results suggest that TGFß1 polymorphisms do not play a role in the pathogenesis of SSc, even though there remains the possibility of a risk factor for genetic susceptibility to pulmonary fibrosis. (J Rheumatol 2003;30:1520–3)

Key Indexing Terms:
TRANSFORMING GROWTH FACTOR DNAPOLYMORPHISM SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by cutaneous and visceral fibrosis, prominent small vessel damage, and an autoimmune phenomenon. Several cytokines and chemokines are known to induce the secretion of collagen and the formation of fibrosis in SSc. Transforming growth factor ß (TGFß) is a multifunctional cytokine which controls cell differentiation and proliferation. In mammals, there are 3 isoforms, TGFß1, ß2 and ß3 that possess nearly identical biological properties. The overexpression of TGFß1 is increased in patients with idiopathic pulmonary fibrosis and autoimmune hepatic fibrosis, as well as in patients with SSc. Immunohistological localization of TGFß1 expression was detected in the alveolar epithelium and interstitium in the lungs of patients with SSc[1]. Using skin biopsies from patients with an inflammatory and sclerotic stage of SSc, mRNA for TGFß1, 2 and 3 were detected in inflammatory skin areas, but not in sclerotic or healthy skin[2]. In addition, the blockade of TGFß signaling with TGFß antibodies or a TGFß1 antisense oligonucleotide inhibited the increased mRNAexpression in SSc fibroblasts[3]. TGFß1 was predicted to be one of the key cytokines in the pathogenesis of SSc.

The genetic polymorphisms of TGFß1 regulate its expression, and may have a role in predisposing patients to fibrotic disease, especially SSc.

In a UK Caucasian population study by Susol, *et al*[4], several microsatellite markers including TGFß1, 2 and 3 and a tissue inhibitor of metalloproteinase-1 (TIMP1) were studied. They demonstrated that there was no association between microsatellite markers for TGFß1 and SSc. However, another recent report showed a positive association in a UK population[5]. The SSc patients in that study had a higher frequency of C allele at codon 10 than controls. Thus, the association of TGFß1 polymorphism was ambiguous. We investigated whether TGFß1 DNA polymorphism is linked to the pathogenesis of fibrosis in SSc patients.

MATERIALS AND METHODS

Patients. We studied 87 Japanese patients with SSc, as well as 110 Japanese controls in the same geographic area. All patients fulfilled the criteria of the American College of Rheumatology for SSc. All patients and controls provided written informed consent. Thirty SSc patients had the diffuse type, and the other 57 had the limited type classified as defined by Leroy, *et al*[6]. Pulmonary fibrosis was seen using high-resolution chest computed tomography (CT) scans in 34 SSc patients. No patients showed clinical evidence of renal failure suggesting renal sclerosis.

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Genotype analysis. Genomic DNAfrom peripheral blood was isolated. We analyzed 2 TGFß1 polymorphisms: T869C (Leu10Pro) in codon 10 (exon1) in the signal peptide sequence region, and C-509Tin the promoter region. The genotypes of these 2 loci were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as described[7] (Figure 1). Genotype distributions in SSc patients and controls were analyzed with Fisher's exact test. A p value < 0.05 was considered statistically significant.

RESULTS

There was no significant difference in the distribution among SSc patients and controls of either T869C or C-509T. When comparing TC+CC (the presence of C allele) with TT homozygous genotype in T869C, we found no significant difference between them (Table 1). Moreover, there was no difference between CT+TT (the presence of T allele) and CC homozygous in C-509T.

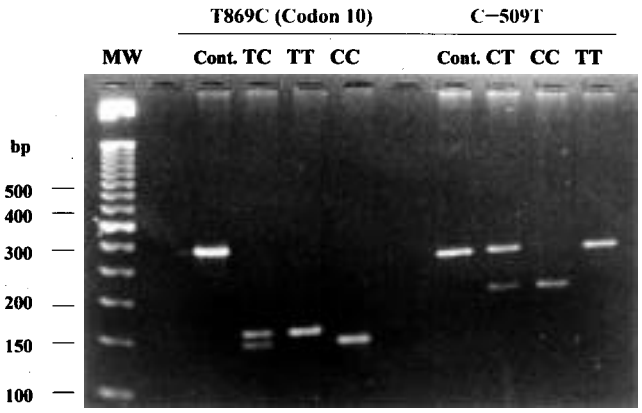

Figure 1. Ethidium bromide-stained 4% agarose gel showing the representative determination of TGFß1 polymorphism of T869C in codon 10, and C-509T in the promoter region using PCR-RFLP. After digestion of PCR products (294 bp) by MspA1 in T869C (codon 10), T allele and C allele were characterized by a 161 bp and 149 bp fragment, respectively. After digestion of PCR products (265 bp) by Bsu36 in C-509T, the C allele was represented by a 195 bp fragment, and undigested T allele was shown at 265 bp. Three genotypes in T869C (codon 10) were determined, i.e., homozygous for alleles TT and CC, and heterozygous for TC. The polymorphism in C-509Twas also determined, i.e., homozygous for the alleles CC and TT, and heterozygous for CT. MW: 50 bp ladder of molecular size markers; Cont.: undigested amplicon.

The distributions of these polymorphisms according to the clinical characteristics of SSc patients are summarized in Table 2. We found no difference in the distribution of either the T869C or C-509T polymorphism between the diffuse and limited type of SSc. However, the TC+CC genotype (the presence of C allele) in T869C, and CT+TT (the presence of T allele) in C-509T was more frequently found in the diffuse type, but this trend was not significant.

Among 87 SSc patients, 34 patients also had pulmonary fibrosis. A significantly higher proportion of polymorphisms in T869C was recognized among SSc patients with pulmonary fibrosis carrying the TC+CC genotype (the presence of C allele) compared to the TT homozygous genotype (the absence of C allele) (p = 0.05). Similarly, the CT+TT genotype (the presence of T allele) in C-509T polymorphism with pulmonary fibrosis was significantly more frequent than the CC genotype (the absence of T allele) (p = 0.05). However, neither the presence of C allele in T869C or of T allele in C-509T revealed any significant difference compared to controls.

DISCUSSION

TGFß1 has been identified as containing 7 polymorphisms: 3 in the promoter regions at positions -988, -800, and -509; one at position +72 in a non-translated region; 2 in the signal sequence at +869 (Leu10Pro) and +915 (Arg25Pro); and one in the coding region for the precursor part not present in the active form, Ile263Thr. The promoter polymorphism, C-509T, has been recognized in linkage disequilibrium with T869C[8,9]. We observed that 192 (98.5%) of 195 subjects including SSc and controls showed a linkage between the T869C and T-509C genotypes. Two polymorphisms between -509 and -800 also showed the linkage disequilibrium[9]. In other regions at -988 and codon 263, no difference in the genetic alteration was observed[9]. Another signal sequence at codon 25, G915C, was shown to have a significant association with myocardial infarction, hypertension[10], and fibrotic lung[8] in Caucasian populations. However, we did not detect this genetic alteration of codon 25. In the Japanese population described previously[11], nearly all indi-

Table 1. Distribution of TGFß1 polymorphism at T869C and C-509Tin patients with systemic sclerosis (SSc) and controls.

	n	Age Mean ± SD	Women/Men	TT n (%)	TC n (%)	CC n (%)	TT n (%)	TC+CC n (%)
T869C								
Controls	110	44.5 ± 18.9	68/42	24 (21.8)	53 (48.2)	33 (30.0)	24 (21.8)	86 (78.2)
SSc	87	56.0 ± 11.7	74/13	23 (26.4)	38 (43.7)	26 (29.9)	23 (26.4)	64 (73.6)
	n	Age Mean ± SD	Women/Men	CC	CT	TT	CC	CT+CT
C-509T								
Controls	110	44.5 ± 18.9	68/42	23 (20.9)	54 (49.1)	33 (30.0)	23 (20.9)	87 (79.1)
SSc	87	56.0 ± 11.7	74/13	23 (26.4)	38 (43.7)	26 (29.9)	23 (26.4)	64 (73.6)

Table 2. TGFß1 polymorphism between diffuse and limited SSc, or between presence or absence of pulmonary fibrosis.

	n	Age Mean ± SD	Women/Men	TT (%)	TC (%)	CC (%)	TT(%)	TC+CC (%)
T869C								
Diffuse SSc	30	55.3 ± 13.1	22/8	6 (20.0)	14 (46.7)	10 (33.3)	6 (20.0)	24 (80.0)
Limited SSc	57	56.3 ± 11.0	52/5	17 (29.9)	24 (42.1)	16 (28.0)	17 (29.8)	40 (70.2)
Pulmonary fibrosis								
Presence	34	55.6 ± 12.3	27/7	5 (14.7)	18 (52.9)	11 (32.4)	5 (14.7)	29 (85.3) *
Absence	53	56.2 ± 11.5	47/6	18 (34.0)	20 (32.7)	15 (28.3)	18 (34.0)	35 (66.0) *
	n	Age Mean ± SD	Women/Men	CC (%)	CT(%)	TT(%)	CC (%)	CT+CT (%)
C-509T								
Diffuse SSc	30	55.3 ± 13.1	22/8	6 (20.0)	13 (43.3)	11 (36.7)	6 (20.0)	24 (80.0)
Limited SSc	57	56.3 ± 11.0	52/5	17 (29.8)	25 (43.9)	15 (26.3)	17 (29.8)	40 (70.2)
Pulmonary fibrosis								
Presence	34	55.6 ± 12.3	27/7	5 (14.7)	17 (50.0)	12 (35.3)	5 (14.7)	29 (85.3) *
Absence	53	56.2 ± 11.5	47/6	18 (34.0)	21 (39.6)	14 (26.4)	18 (34.0)	35 (66.0) *

* p = 0.05

viduals were detected as GG genotype, so they could not be used for the polymorphism study.

We failed to detect a difference in the distribution of TGFß1 polymorphism at T869C and C-509T between SSc patients and controls in a Japanese cohort. These negative associations of TGFß1 polymorphism confirm 2 previous investigations[4,12]. One was a small population study by Zhou, *et al*[12] showing a negative association of TGFß1, in the Oklahoma Choctaw native Americans, exhibiting a high frequency of SSc. This study has been used for several genetic analyses of SSc. The second was a large population study in 191 SSc Caucasian patients by Susol, *et al*[4]. That study found associations indicating a possible role for TGFß3, ß2, and TIMP1 in genetic susceptibility to SSc and for TGFß3 in determining the degree of cutaneous fibrosis. Other than the above-reported negative associations of TGFß1, a positive association was found by Crilly, *et al*[5] in 89 SSc patients in a UK population. In their study, there was no difference between diffuse and limited SSc, similar to our results.

T869C at codon 10 and C-509T promoter polymorphism were thought to be associated with TGFß1 protein expression. In the Japanese population, the serum concentration of TGFß1 was significantly higher in the C allele than in the T allele at codon 10 in a study of genetic susceptibility to osteoporosis[13] or myocardial infarction[11]. The C allele at codon 10 was more frequent in black than white Americans with hypertension, and this allele was associated with higher levels of TGFß1 protein and mRNA[14]. Otherwise, the C allele at codon 10 was associated with lower TGFß1 protein expression[15] in patients with cystic fibrosis. Although these studies have limitations regarding the association with TGFß1 protein expression, if the hypothesis is correct that the C allele at codon 10 and the T allele at C-509T are high producer alleles, our results are not incompatible with the idea that pulmonary fibrosis is found at significantly higher frequencies in these alleles. Such alleles may be a possible risk factor for genetic susceptibility following overexpression of TGFß protein in SSc patients with pulmonary fibrosis. In contrast to the findings by Crilly *et al*[5] in the UK population, the negative associations in our Japanese cohort could be due to the heterogeneity of SSc, or perhaps to some racial difference. Otherwise, the TGFß3 gene may be the more likely candidate for genetic susceptibility to SSc than the TGFß1 gene[4]. Further studies should be undertaken on a much larger cohort with accurate characterization of clinical features including skin, lung, renal, or gastro-intestinal fibrosis in SSc patients.

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