EXTENDED REPORT

Susceptibility to ankylosing spondylitis: no evidence for the involvement of transforming growth factor β1 (*TGFB1*) gene polymorphisms

M van der Paardt, J B A Crusius, M A García-González, B A C Dijkmans, A S Peña, I E van der Horst-Bruinsma

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See end of article for authors' affiliations

Correspondence to: Dr I E van der Horst-Bruinsma, VU University Medical Centre, Department of Rheumatology, Room 4A-42, PO Box 7057, 1007 MB Amsterdam, Netherlands; secr.reumatologie@vumc.nl

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Background: Genetic factors are thought to be crucial in the pathogenesis of ankylosing spondylitis. Transforming growth factor β1 (TGFβ1) is a multifunctional cytokine that plays a key role in inflammation. Two functional single nucleotide polymorphisms (SNPs) in the TGFB1 gene have been described: *TGFB1* T869C and *TGFB1* G915C.

Objective: To determine whether these SNPs contribute to ankylosing spondylitis susceptibility or its disease characteristics.

Methods: Genomic DNA was isolated from the peripheral blood of 134 patients with ankylosing spondylitis and 194 healthy blood donors. All subjects were unrelated and of white Dutch ethnicity. The diagnosis of ankylosing spondylitis was made according to the modified New York criteria. The *TGFB1* T869C and *TGFB1* G915C SNPs were genotyped by a polymerase chain reaction–single strand conformation polymorphism haplotyping method.

Results: No significant differences were found between patients and controls in genotype, allele, and haplotype frequencies or in the carrier rate of the rare alleles of the *TGFB1* T869C and *TGFB1* G915C SNPs.

Conclusions: *TGFB1* T869C and *TGFB1* G915C SNPs are not major factors in the susceptibility to ankylosing spondylitis or its disease characteristics.

Ankylosing spondylitis (AS) is a common familial rheumatic disorder. The disease is characterised by chronic inflammation of the sacroiliac joints and the vertebral column. The inflammatory process leads gradually to fibrosis and calcification of the spine and sacroiliac joints. Ultimately, ossification, interosseous bridging, and ankylosis can occur. Spinal osteoporosis is often observed in patients with active disease and is probably induced by regulatory cytokines.[1 2]

The precise aetiology of the disorder remains unclear, though heritability plays a major role. Twin studies suggest that up to 97% of the susceptibility to AS can be attributed to genetic factors.[3] For over 30 years the main known genetic component has been human leucocyte antigen (HLA) B27. More than 95% of white patients with primary AS and at least 50% of those with AS associated with psoriasis or inflammatory bowel disease are HLA-B27 positive, whereas HLA-B27 is present in only 8% of the general Dutch population.[4]

However, family studies have shown that the major histocompatibility complex (MHC), including HLA-B27, contributes less than 40% to the recurrence risk ratio in AS.[5] Apart from the MHC, Laval *et al* identified six other loci—on chromosomes 1p, 2q, 9q, 10q, 16q, and 19q—that are linked to AS. The peak of linkage on chromosome 19 was observed with the marker D19S420 (LOD score 3.58).[5]

The *TGFB1* gene encoding the human transforming growth factor β1 (TGFβ1) is located 1.8 Mb from marker D19S420 on chromosome 19q13.[6] TGFβ1 is a multifunctional cytokine involved in the regulation and proliferation of cells. This cytokine promotes differentiation of leucocytes, but has inhibitory effects on proliferation of T lymphocytes and activation of macrophages, suggesting a regulatory role in inflammatory states.[7] TGFβ1 is known to be able to control the production of many components of the extracellular matrix and to be a potent regulator of osteoblast proliferation and differentiation.[8] In general, TGFβ stimulates cartilage and bone formation, but the precise effects are complex, depending on the concentration of TGFβ, whether the bone is growing or damaged, the state of activation of the cells, and the presence of other factors, including hormones.[9] Moreover, TGFβ is required to switch B cells into production of IgA,[10 11] which is often increased in AS patients.[12] Therefore, TGFβ is a good candidate for a key cytokine in an inflammatory disease such as AS, in which new bone formation and ultimately ankylosis of the sacroiliac joints and the vertebral column are major symptoms and fibrosis is a related phenomenon.[13–15]

Recently, the single nucleotide polymorphisms (SNPs) at positions +869 (T→C) and +915 (G→C) in the signal protein sequence of the *TGFB1* gene, which change codon 10 (Leu10Pro) and codon 25 (Arg25Pro), respectively,[16] have been reported to be related to variations in the production of TGFβ1, both in vitro[17] and at the serum level.[18]

In the present study we investigated whether the *TGFB1* T869C and *TGFB1* G915C SNPs or their haplotypes are associated with susceptibility to or disease characteristics of AS.

METHODS

Subjects

After informed consent, 134 AS patients were recruited from the outpatient department of rheumatology of the Jan van

Abbreviations: HLA, human leucocyte antigen; HWE, Hardy–Weinberg equilibrium; MHC, major histocompatibility complex; SNP, single nucleotide polymorphism; TGFβ1, transforming growth factor β1

Table 1 Demographic and clinical characteristics of patients with ankylosing spondylitis (n = 134)

Characteristic	Frequency
Age (years)*	50.9 (12.9) (19 to 79)
Age at first complaints (years)†	23.0 (19.0 to 31.5)
Age at diagnosis (years)*	34.7 (10.6) (16 to 63)
Years between first complaints and diagnosis†	7.0 (2.0 to 12.5)
Women	18.7%
HLA-B27 positive	94%
Iridocyclitis	43%
Peripheral arthritis	41%
First degree relatives with AS	28%

*Mean (SD) (range).
†Median (interquartile range).
AS, ankylosing spondylitis.

Breemen Institute. They all fulfilled the diagnostic requirements of AS according to the modified New York criteria.[19] Controls were 194 randomly selected healthy blood donors from the Amsterdam region. All subjects were unrelated and of white Dutch ethnicity.

PCR-SSCP analysis

A polymerase chain reaction–single strand conformation polymorphism (PCR-SSCP) method was optimised for the simultaneous detection of the SNPs at positions +869 and +915 in the *TGFB1* gene and their haplotypes. The method has been described previously by García-González *et al*.[20] In that study in a Dutch population, the alleles were shown to form three haplotypes, denoted as haplotype 1 (*TGFB1*+869T−*TGFB1*+915G), haplotype 2 (*TGFB1*+869C−*TGFB1*+915G), and haplotype 3 (*TGFB1*+869C−*TGFB1*+915C). In brief, the region containing the SNP at position +869 (NCBI SNP CLUSTER ID: rs1982073) and the SNP at position +915 of the *TGFB1* gene (NCBI SNP CLUSTER ID: rs1800471) was amplified by PCR in a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer, Norwalk, Connecticut, USA) according to the following parameters: 97℃ for 90 seconds, 61℃ for 90 seconds, and 72℃ for 60 seconds for three cycles, followed by 32 cycles of 97℃ for 30 seconds, 61℃ for 60 seconds, and 72℃ for 60 seconds, with a final elongation at 72℃ for 10 minutes.

PCR products diluted twofold in 99% formamide were heated at 95℃ for three minutes and placed on ice. Electrophoresis on a pre-cast non-denaturing 20% polyacrylamide PhastGel at 20℃ and silver staining were done semi-automatically on the PhastSystem™ (Amersham Pharmacia, LKB Biotechnology AB, Uppsala, Sweden).

Statistical analysis

Allele and genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) by the χ^2 test. To compare frequencies χ^2 testing was used or, when appropriate, Fisher's exact test. A two sided p value of <0.05 was considered significant. The magnitude of association was expressed as the odds ratio (OR) with 95% confidence intervals (CI). The statistical analyses were undertaken using SPSS 10.0 for Windows.

RESULTS

Characteristics of the 134 AS patients are summarised in table 1. Genotype and distribution of allele frequencies in AS patients and controls for the *TGFB1* T869C and *TGFB1* G915C polymorphism are shown in table 2.

The data for the controls were published previously by Schrijver *et al*.[21] The genotype frequencies in the control group did not deviate from HWE equilibrium. No significant differences were observed between AS patients and controls in the frequencies of the carriership of the allele *TGFB1* 869C (OR = 0.96 (95% CI, 0.69 to 1.33), p = 0.81) and the allele *TGFB1* 915C (OR = 1.62 (0.93 to 2.84), p = 0.09) (table 2); neither were significant differences observed between AS patients and controls in the frequencies of the carriership of the three *TGFB1* haplotypes (table 3).

No significant associations were found between carriage of either of the alleles *TGFB1* 869C and *TGFB1* 915C, or their haplotypes, and sex, past or present peripheral arthritis or acute anterior uveitis, age at first complaints, years between these first complaints and the diagnosis of AS, and the number of patients with at least one first degree family member with AS (data not shown).

DISCUSSION

In this study the *TGFB1* T869C and *TGFB1* G915C SNPs or their haplotypes were not significantly associated with susceptibility to AS or its manifestations in a white Dutch population. Genotype and allele frequencies of the *TGFB1* T869C and *TGFB1* G915C SNPs in our control population were similar to those reported in some European studies undertaken in healthy controls from Northern Ireland and France[16] and from the United Kingdom,[17 22 23] as summarised by García-González *et al*.[20]

Eight polymorphisms have been identified in *TGFB1*: three in the upstream region of the gene at positions −988, −800, and −509 from the transcription start site; one in the non-translated region at position +72; two non-synonymous coding SNPs in the signal peptide sequence: *TGFB1* T869C which changes codon 10 (Leu^{10}→Pro), and *TGFB1* G915C which changes codon 25 (Arg^{25}→Pro); one SNP in the region of the gene coding for the precursor part of the protein is not present in the active form: *TGFB1* C1632T which changes codon 263 (Thr^{263}→Ile); and a one base pair deletion in intron 4 (713-8delC).[16 24] The *TGFB1* promoter polymorphisms and the T869C variant account for most of the diversity at this locus and a relatively small increase in diversity is

Table 2 *TGFB1* genotype and allele frequencies in patients with ankylosing spondylitis and controls

	Controls (n = 194)		Patients (n = 134)		
Genotype	n (%)	AF	n (%)	AF	OR (95% CI)
***TGFB1* T869C**					
TT	80 (41.2)	65.5	57 (42.5)	64.6	
TC	94 (48.5)		59 (44.0)		0.96 (0.69 to 1.33) (p = 0.81)
CC	20 (10.3)	34.5	18 (13.4)	35.4	
***TGFB1* G915C**					
GG	168 (86.6)	93.3	107 (79.9)	89.6	
GC	26 (13.4)		26 (19.4)		1.63 (0.93 to 2.84) (p = 0.09)
CC	0	6.7	1 (0.7)	10.4	

AF, allele frequency; CI, confidence interval; OR, odds ratio.

Table 3 Genotype, phenotype, and haplotype frequencies of the *TGFB1* polymorphisms in patients with ankylosing spondylitis and controls

	Controls (n = 194)			Patients (n = 134)			
TGFB1 haplotype	n (%)	PF, n (%)	HF (%)	n (%)	PF, n (%)	HF (%)	OR* (95% CI)
1.1	80 (41.2)	174 (89.7)	65.5	57 (42.5)	116 (86.6)	64.6	
1.2	76 (39.2)			40 (29.9)			1.35 (0.69 to 2.66)
1.3	18 (9.3)			19 (14.2)			
2.2	12 (6.2)	96 (49.5)	27.8	10 (7.5)	57 (42.5)	25.0	
2.3	8 (4.1)			7 (5.2)			1.32 (0.85 to 2.06)
3.3	0	26 (13.4)	6.7	1 (0.7)	27 (20.1)	10.4	0.61 (0.34 to 1.11)

*Overall odds ratios for carriership of haplotypes 1–3.
Haplotype 1: *TGFB1*+869T−*TGFB1*+915G; haplotype 2: *TGFB1*+869C−*TGFB1*+915G; haplotype 3: *TGFB1*+869C−*TGFB1*+915C.
CI, confidence interval; HF, haplotype frequency; n, number of individuals; OR, odds ratio; PF, phenotype frequency.

attributable to the rare variants *TGFB1* C1632T and *TGFB1* G915C.[25]

The *TGFB1* polymorphism 713-8delC is more frequent in patients with osteoporosis than in normal controls and seems to be associated with very low bone mass in osteoporotic women and with low bone mass and increased bone turnover in both osteoporotic and healthy women.[24] As bone mineral density is often decreased in AS patients, *TGFB1* polymorphisms seem to be of even more interest in this disease.

In a Japanese study, the frequency of the allele *TGFB1* 869T was found to be significantly higher in subjects with osteoporosis than in healthy individuals.[26] On the other hand, the *TGFB1* 869C allele was associated with an increase in osteoporosis in white Australian women.[27] The *TGFB1* 869C allele has also been associated with ossification of the posterior longitudinal ligament in the cervical spine and with spinal osteophytosis in Japanese patients.[28 29] The development of spinal osteophytes is also a characteristic feature of AS.

The *TGFB1* 915G allele is strongly associated with fibrotic lung disease.[17] Apical pulmonary fibrosis is rare but well recognised in patients with AS.[13] No association between Crohn's disease or ulcerative colitis, which are clinically related to AS, and *TGFB1* SNPs had been found in previous studies.[20 30]

The genotype *TGFB1* 915GG has been related to higher serum concentrations of TGFβ1 in a previous study.[17] A combined study from Finland and the United Kingdom found a weak association between age of symptom onset of AS and the *TGFB1* G915C SNP.[25] Furthermore, this study noted a weak positive association of the rare allele *TGFB1* 1632T with AS and also with a younger age at symptom onset of AS. These investigators concluded from their study that ''the polymorphisms (G–800A, C–509T, T+869C, G+915C and C+1632T) in the *TGFB1* gene play at most a small role in AS and that other genes on chromosome 19 are involved in the susceptibility to AS''.[25]

The results from our study do not show an association between the *TGFB1* G915C SNP and AS, although the frequency of allele *TGFB1* 915G was greater in AS patients than in controls, but this difference did not reach significance ($p = 0.09$). In our group of AS patients no association between the *TGFB1* G915C SNP and a younger age of symptom onset of AS could be found.

As TGFβ may play a crucial role in the pathogenesis of AS, especially in new bone formation, further research is necessary to elucidate the role of TGFβ1 in this disease, and possibly open the way towards new pharmacological approaches for preventing the most disabling phenomenon of this disorder, ankylosis.

Authors' affiliations
M van der Paardt, The Jan van Breemen Institute, VU University Medical Centre, Amsterdam, Netherlands
J B A Crusius, M A García-González, Laboratory of Immunogenetics, VU University Medical Centre
A S Peña, Department of Gastroenterology, VU University Medical Centre
B A C Dijkmans, I E van der Horst-Bruinsma, Department of Rheumatology, VU University Medical Centre

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