The role of tumor necrosis factor (TNF)-α and TNF receptor polymorphisms in susceptibility to ankylosing spondylitis

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ABSTRACT

Objective. *Ankylosing spondylitis (AS) is a chronic inflammatory disease affecting the sacroiliac joint and vertebral column. Tumor necrosis factor α (TNF-α), a cytokine that acts via two tumor necrosis factor receptors (TNFR1 and TNFR2), may be implicated in the pathogenesis of AS. The aim of the present study was to examine the role of the polymorphisms 36A>G (TNFR1), 676T>G (TNFR2), -857C>T (TNF-α), -308G>A (TNF-α), and -238G>A (TNF-α) in AS susceptibility.*

Methods. *Forty-nine AS patients and 68 randomly chosen healthy volunteers were enrolled in the study. Polymerase chain reaction coupled with a restriction fragment length polymorphism assay was performed in the genotype analysis of each variant.*

Results. *The polymorphisms 36A>G (TNFR1) and -238G>A (TNF-α) were not found to be in Hardy-Weinberg equilibrium in the patient group and therefore were excluded from the statistical analysis. A marginally statistically significant difference was observed in the distribution of 676T>G (TNFR2) genotypes between AS patients and controls (p=0.054) and was revealed to be more significant in the 676T>G allele distribution between the two groups (p=0.031). The complex genotype TNFR2 676TG/ TNF-α -857CC (p=0.041) was also differently distributed between AS patients and controls.*

Conclusion. *The TNFR2 676T allele is reported here for first time to be differently distributed between AS patients and controls. The higher frequency of the wild type TNFR2 676T allele in AS patients suggests the functional ability of TNFR2 to support increased TNF-α mediated immunoactivity.*

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterized by axial skeletal ankylosis, peripheral arthritis, and inflammation at the insertion of the tendons (enthesitis) (1, 2). The histocompatibilty antigen HLA-B27 has been found to be strongly associated with AS susceptibility (1, 3). In Caucasians the HLA-B27 antigen is present in approximately 8% of healthy individuals, while it is found in more than 90% of AS patients. However, it has been determined that less than 2% of HLA-B27 positive subjects will develop AS, and therefore the identification of other genetic factors in AS manifestation is under investigation (4).

Genome-wide scans have implicated regions on chromosomes 1p, 2q, 3q, 6p (the major histocompatibility complex-MHC), 6q, 9q, 10q, 11q, 16q, 17q, and 19q in the aetiology of AS (5-7). Apart from the HLA-B27 antigen, other MHC genes that have been linked to AS are the MHC class I chain-related gene A (MICA), the MHC class II HLA-DRB1 alleles, the transporter associated with antigen processing (TAP), and the low molecular weight proteosome (LMP) (7). Non-MHC candidate gene analyses have also pointed to interleukin (IL)-1, IL-6, IL-10, cytochrome P450 gene debrisoquine 4-hydroxylase (CYP2D6), and transforming growth factor beta-1 (TGF-β1) in AS susceptibility (8).

Tumour necrosis factor-α (TNF-α), a proinflammatory cytokine produced mainly by macrophages and T cells, has recently been implicated in AS susceptibility (9). TNF-α acts as a mediator of resistance in parasitic, bacterial and viral infections and therefore is important in host defence. Specifically, TNF-α exerts its effects via the binding of cell surface tumour necrosis factor receptors (TNFR1 and TNFR2), inducing signal transduction pathways that activate nuclear factor-κB (NF-κB) (10). Subsequently, activated NF-κB enters the cell nuclei and induces the transcription of genes that code proteins associated with inflammatory and immuno-modulatory reactions (10).

Consequently, single nucleotide polymorphisms in TNF-α, TNFR1, and TNFR2 gene sequences that alter gene transcription or the encoded protein function could account for the variability in the pathogenesis of autoimmune diseases (10-12). To date the majority of studies on the role of TNF-α in AS susceptibility have focused on the polymorphisms -308G>A, and -238G>A of the promoter of TNF-α gene (13-24). However, the role of these genetic variants in AS susceptibility remains the

Table I. Characteristics of the patients with ankylosing spondylitis.

Total number of patients	49
Male	46 (94%)
Mean age ± SD (years)	46.08 ± 10.52
Mean disease duration ± SD (years)	18.18 ± 9.73
HLA-B27 positivity	41 (84%)

subject of debate (13-24). No effect of -238G>A polymorphism on TNF-α gene transcription has been reported, while the role of the variant -308G>A in TNF-α gene transcription remains uncertain (16, 25-28).

The role of TNF-α -857C>T polymorphism in susceptibility to inflammatory diseases has also been examined, since it is reported to affect TNF-α expression levels (18, 29, 30). We therefore decided to examine the TNF-α polymorphisms -308G>A and -238G>A and additionally the genetic variant -857C>T in AS susceptibility. Furthermore, we expanded the association study to TNFR, studying the polymorphisms 36A>G (exon 1 of TNFR1) and 676T>G (exon 6 of TNFR2; Met196Arg). This represents the first expanded study of the role of TNF-α, TNFR1, and TNFR2 gene polymorphisms in AS.

Patients and methods

Forty-nine patients who met the modified New York criteria for AS (31) were enrolled in this study. The demographic characteristics of the patients are shown in Table I. In addition, 68 ethnically matched random controls with neither symptoms nor any previous diagnosis of systemic diseases were recruited (41% males, mean age ± SD: 42.05±9.81 years, HLA-B27 positivity 6%). All subjects gave their informed written consent and the local ethics committee granted approval for the study.

Genomic DNA was extracted from peripheral blood lymphocytes according to the standard salt extraction procedure. Polymorphisms 36A>G (TNFR1), 676T>G (TNFR2), -857C>T (TNF-α), -308G>A (TNF-α), and -238G>A (TNF-α) were amplified using primers already described in the literature (32-34). Subsequently, restriction assays were employed using the restriction endonucleases MspA1 I, Nla III, Hinc II, Nco I, and Msp I, respectively.

Arlequin software was used for the statistical analysis of the polymorphisms in order to determine the Hardy-Weinberg equilibrium in the AS and control groups. The chi-square test provided by the SPSS statistical package was used to test differences in the polymorphism distribution between AS patients and controls. Furthermore, the odds ratio (OR) with a confidence interval (CI) of 95% was calculated. A difference at $p \leq 0.05$ was considered to be statistically significant.

Results

The distribution of genotype polymorphism in the AS patients and controls is shown in Table II. The polymorphisms 36A>G (TNFR1) and -238G>A (TNF-α) were not found to be in Hardy-Weinberg equilibrium in the AS group and therefore were excluded from the analysis. A marginally statistically significant difference was observed in the distribution of 676T>G (TNFR2) genotypes between AS patients and controls (p=0.054, Table II). However, the difference in 676T>G alleles between the two groups was found to be more statistically significant (p=0.031, OR= 1.99; CI: 1.06-3.72, Table II).

In addition the complex genotype TNFR2 676TG/ TNF-α -857CC was found to be distributed differently between AS patients and controls (AS n=7 vs controls n=17, p=0.041, OR=0.50, CI: 0.19-1.32).

Discussion

TNF-α is located in the class III region of the major histocompatibility complex near the HLA-B locus (35). TNF-α acts as a pro-inflammatory cytokine and therefore the role of TNF-α gene polymorphisms in AS susceptibility has been widely investigated. The majority of studies have focused on two polymorphisms – -308G>A and -238G>A of the promoter of TNF-α gene – with contradictory results in terms of their role in AS among various ethnic groups (17). In addition, the TNF-α polymorphism -857C>T has been thoroughly studied in other autoimmune diseases and therefore investigating its role in AS susceptibility has been suggested to

Table II. Distribution of 36A>G (TNFR1), 676T>G (TNFR2), -857C>T (TNF-α), -308G>A (TNF-α), and -238G>A (TNF-α) genotypes in ankylosing spondylitis (AS) patients and controls. Polymorphisms 36A>G and -238G>A were not found in Hardy-Weinberg equilibrium in the AS group and therefore no comparison was made with the control group.

	36A>G (TNFR1)			676T>G (TNFR2)			-857C>T (TNF-α)			-308G>A (TNF-α)			-238G>A (TNF-α)		
	AA	GG	AG	TT	GG	TG	CC	TT	CT	GG	AA	GA	GG	AA	GA
AS n=49 (%)	16 (32.7)	17 (34.7)	16 (32.7)	34 (69.4)	3 (6.1)	12 (24.5)	33 (67.4)	3 (6.1)	13 (26.5)	44 (89.8)	0 (0.0)	5 (10.2)	47 (95.9)	1 (2.0)	1 (2.0)
Controls n=68 (%)	15 (22.1)	26 (38.2)	27 (39.7)	32 (47.1)	6 (8.8)	30 (44.1)	40 (58.8)	5 (7.4)	23 (33.8)	54 (79.4)	0 (0.0)	14 (20.6)	61 (89.7)	0 (0.0)	7 (10.3)
		–			p=0.054			p=0.642				p=0.133		–	
	Alleles														
	A		G	T		G	C		T	G		A	G		A
AS	48		50	80		18	79		19	93		5	95		3
Controls	57		79	94		42	103		33	122		14	129		7
		–			p=0.031			p=0.376			p=0.151			–	

be important (18). However, in a previous study we showed that the response to anti-TNF-α treatment in RA patients could be predicted by the combined determination of TNF-α and TNF-α receptor polymorphisms (36). Therefore, in the present study 36A>G (TNFR1) and 676T>G (TNFR2) polymorphisms were examined in addition to the aforementioned TNFα gene variants.

A statistically significant difference was observed in the TNFR2 676T>G allele distribution between AS patients and controls. This genetic variant is a non-conservative amino acid substitution of argine (196R) by methionine (196M) that has not been studied previously in relation to AS susceptibility. It has been implicated in susceptibility to RA and systemic lupus erythematosus (37-41), but in many other studies such an association was not replicated (42-45).

The association of 676T>G with inflammatory diseases may be attributable to the reported functional alterations on TNFR2 (39, 46). The 676G allele was revealed to increase the production of IL-1 and cell death in transfected HeLa cells, probably because it has a significantly lower ability to induce direct NF-κB signaling via TNFR2 and to enhance TNFR1-dependent TNF-α-induced apoptosis (39, 46). However, if we take into account that the TNFR1 gene is expressed in all nucleated cells, whereas TNFR2 is restricted primarily to haematopoietic cells (47), the role of TNFR1 and TNFR2 polymorphisms in inflammatory disease susceptibility seems to be further complicated.

Therefore, the increased presence of the wild type allele TNFR2 676T in our AS patients could indicate that other TNF-α related inflammatory pathways induce disease rather than the suggested reduced NF-κB signaling via TNFR2 and the increased TNFR1-dependent TNF-α-induced apoptosis in the case of the TNFR2 676G allele (39, 46). Furthermore, the increased presence of the wild type allele TNFR2 676T demonstrated in AS patients could point to the functional ability of TNFR2 to support increased TNF-α mediated immunoactivity.

Additionally, in our previous work the complex genotype of wild type TNFR2 676T>G and TNFα -857C>T was found to be correlated to a good response of RA patients to anti-TNF-α therapy (36). When we studied the complex genotype 676T>G (TNFR2) and -857C>T (TNF-α), the difference in distribution between AS patients and controls continued to exist, but its statistical significance was lower. This fact can be explained since in the present study the complex genotype TNFR2 676TG/ TNF-α -857CC implicated the wild type allele of TNF-α polymorphism -857C>T, whereas previously the non-wild type -857T was related to higher TNF-α transcriptional activity and AS susceptibility (18, 29, 30, 48).

In conclusion, the TNFR2 676T>G alleles are reported here for first time to be differently distributed between AS patients and controls. The higher presence of the wild type TNFR2 676T in AS patients could confirm an increased TNFα-mediated immunoactivity. Further studies in other ethnic groups involving larger numbers of AS patients are needed in order to confirm the suggested association and to direct future studies of TNF-α-implicated pathways in AS susceptibility and therapy.

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