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SHORT COMMUNICATION

−383 A/C tumor necrosis factor receptor 1 polymorphism and ankylosing spondylitis in Mexicans: a preliminary study

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Abstract The objective of this study was to evaluate the differences in allele and genotype frequencies of −383 tumor necrosis factor receptor 1 (*TNFR1*) polymorphism between ankylosing spondylitis (AS) and controls. Mexican Mestizos with AS were matched by gender, age, and ethnicity with healthy controls and compared in allele and genotype frequencies of the −383 *TNFR1* polymorphism. Polymorphisms were genotyped using PCR–RFLP. The AA genotype occurred at a higher frequency in the AS group (92%) compared with controls (79%, $P = 0.03$). A allele was increased in AS (96% vs. 88%, $P = 0.015$) and was associated with genetic susceptibility for AS (odds ratio = 3.48, 95% CI = 1.23–10.61). This preliminary study is the first assessing the association of the −383 A/C *TNFR1* polymorphism with AS, although it has the limitation of a small sample size. These data are of interest for the genetic epidemiology of AS in the Mexican population, requiring further investigation in other countries.

Keywords Ankylosing spondylitis · Tumor necrosis factor receptor 1 · Genetic polymorphism · Case–control studies

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder of the axial skeleton involving mainly sacroiliac joints and lumbar spine [1]. Tumor necrosis factor-alpha (TNF-α) plays a central role in the pathogenesis of AS, participating in the genesis of the inflammatory process; both TNF-α messenger RNA and its protein have been identified in sacroiliac joints of patients with active disease [2]. TNF-α constitutes a pro-inflammatory cytokine exerting its

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actions through a link with two cellular surface receptors: TNFR1 and TNFR2 [3]. The sequence of events following the activation of TNFR1 is considered to be the dominant pathway in the induction of the inflammatory effects, including macrophage activation, up-regulation of adhesion cell molecules, and NF-κB stimulation [4]. However, conformational changes in the molecular structure of TNFR1 may modify the efficiency of the signaling process or mediate an aberrant signaling [5]. This issue is particularly interesting for identifying whether genetically inherited structural changes can modify the frequency of a particular disease's development.

A polymorphism in the promoter region of the *TNFR1* gene that results in an adenine to cytosine (A/C) transition at position −383 relative to the translation start site (−383 *TNFR1*) was previously described [6]. To date, the −383C allele has been associated with the presentation of type 1 diabetes [7], but no studies have been performed to evaluate the −383 *TNFR1* polymorphism in Mexican Mestizos with AS.

Therefore, we evaluated, in this preliminary study, whether there are differences in the allele and genotype frequencies of the −383 *TNFR1* polymorphism between Mexican patients with AS versus controls of the same ethnic origin.

Patient and methods

Study subjects

The study was performed in a secondary care hospital (Hospital General Regional 110) in Guadalajara, Mexico, from August 2006 to July 2007.

Cases were selected from a cohort of patients with AS attending an outpatient rheumatology clinic. The inclusion criteria were as follows: (1) met the 1984 modified New York criteria for AS [8], (2) Mexican Mestizo ethnicity (defined as individuals who, for three generations including their own, were born in Mexico and were descendants of the original autochthonous inhabitants of the region and of individuals mainly Spaniards) [9], and (3) 18 years of age and older. Only cases with one family member with AS were included (sporadic AS). We excluded also patients who ignored their biologic ascendancy or those who had an overlapping syndrome or inflammatory bowel disease.

A healthy control group was obtained from the general population matched with the cases for gender and age. All the controls were Mexican Mestizos. Controls that ignored their ascendancy or who had a suspected or recognized chronic disease were excluded. Each control was from a different family, and the families had no blood relationship with the cases.

Clinical assessment of AS

The clinical assessment included clinical and demographical characteristics, the evolution of their disease, clinimetric measures and treatments. Disease activity was assessed by BASDAI [10] and functioning by BASFI [11] and HAQ-S [12].

DNA extraction

On the same day of the clinical evaluation, a peripheral venous blood sample was taken in tubes containing EDTA. Genomic DNA was extracted from mononuclear cells of whole blood using the Miller's modified technique [13].

TNFR1 −383 genotyping

Genotyping of the −383 A/C polymorphism was performed using the following primers: forward primer 5′-TTA TTG CCC CTT GGT GTT TGG TTG-3′ and reverse primer 5′-GGA GGG GAA GAG TGA GGC AGT GTT-3′ in order to obtain a PCR fragment amplified that includes a restriction site for the *BglII* enzyme. The resulting fragments were analyzed by phototyping (Kodak *Electrophoresis Documentation and Analysis System* 290) in 4% agarose gels, stained with ethidium bromide.

Statistical analysis

The allele and genotype frequencies of −383 *TNFR1* polymorphism were obtained by direct counting. The Hardy–Weinberg equilibrium was investigated using the χ^2 test. The −383 *TNFR1* genotypic differences between patients and healthy control subjects were evaluated by the Mantel–Haenszel test using the EPI INFO 6 (version 6.04d) statistical program. For allelic differences, we used Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (95% CI) were computed using the junction of the AC or CC genotypes of *TNFR1* as a risk factor for AS. The OR were computed to evaluate the risk for AS conferred by the A allele. All analyses used two-sided tails with $P \leq 0.05$ used as significance criterion.

Ethics

The study protocol was approved by the Ethics and Research Committee of the hospital (approval 2006-1301-33). All patients signed an informed consent form. The study was performed in accordance with the principles of the declaration of Helsinki.

Table 1 Clinical characteristics of the patients with ankylosing spondylitis in this study

Characteristics	AS ($n = 66$)
Age (years)	41 ± 11
Gender (males), *n* (%)	49 (74)
Disease duration (years)	11 ± 7
BASDAI score	4.95 ± 2.15
BASFI score	4.47 ± 2.38
HAQ-S score	0.86 ± 0.55
Morning stiffness (VAS)	48 ± 29
Schober (cm)	3.56 ± 1.77
Occiput to wall (cm)	4.42 ± 5.99

Quantitative variables are expressed as mean ± standard deviation (SD)

VAS visual analogue scale evaluated from 0 to 100 mm

Table 2 Genotype and allele frequencies of −383 tumor necrosis factor receptor 1 (TNFR1) in Mexican patients with AS and controls

Genotype	AS ($n = 66$) (%)	Controls ($n = 108$) (%)	OR (95% CI)	*P*
AA	61 (92)	85 (79)	3.30 (1.11–10.54)	0.03
AC	5 (8)	20 (18)	1	–
CC	0	3 (3)		
Allele				
A	127 (96)	190 (88)	3.48 (1.23–10.61)	0.015
C	5 (4)	26 (12)	1	–

AS Ankylosing spondylitis, *OR* odds ratios, 95% *CI* 95% confidence interval. For genotype, OR was computed using AA as risk factor and AC + CC as referent

Results

We included 66 patients with AS and 108 gender- and age range–matched controls. Table 1 shows the general characteristics of the AS patients. The mean age was 41 years; 49 patients were men (74%), and the AS patients had a mean disease duration of 11 years. Most of the patients had a BASDAI > 4.0 and 17% were receiving anti-TNF therapy.

We evaluated the Hardy–Weinberg equilibrium previous to comparing the allele and genotypic frequencies between patients and controls. The control group was in equilibrium (data not shown).

Table 2 shows the allele and genotype frequencies for −383 *TNFR1* polymorphism. A high frequency of the AA genotype was observed in the AS group compared with the control group (92% vs. 79%, $P = 0.03$). Using AA as a risk factor versus other genotypes, the estimated OR for AA in AS patients was 3.30 (95% CI: 1.11–10.54). The A allele frequency was higher in the AS group compared with controls (96% vs. 88%, $P = 0.015$). This allele was associated with a higher risk of AS (OR = 3.48, 95% CI: 1.23–10.61).

Discussion

The results of the present exploratory study showed that the AA genotype of the −383 *TNFR1* polymorphism is associated with AS in Mexican Mestizo patients. We consider this finding to be a relevant contribution to understanding some differences in the genetic susceptibility of some Mexican patients to the development of this spondyloarthropathy.

TNF-α promotes angiogenesis, cell adhesion molecule expression, lymphocyte activation, fibroblast proliferation, and the synthesis and expression of pro-inflammatory cytokines, chemokines, prostaglandins, and metalloproteinases [14]. TNF-α exerts their pro-inflammatory effects through its binding to two different cell surface receptors: TNFR1 and TNFR2 [5], being TNFR1 the dominant pathway for the induction of macrophage activity, up-regulation of adhesion molecules, and NF-κB stimulation [4]. A genetic polymorphism may produce conformational changes in the molecule of TNFR1, leading to modifications in the signaling induced by TNF-α through mediating an abnormal signaling [5]. Therefore, several polymorphisms in *TNFR1* may influence the development of an abnormal immune response in AS and other autoimmune disorders. Nevertheless, the information regarding the role of TNFR1 polymorphism is still insufficient and controversial in many diseases.

For example, Briddges et al. [15] did not find differences in the allele frequencies in −383 A/C *TNFR1* polymorphism between African-American patients with rheumatoid arthritis and healthy African-American controls. Taking into account the differences in ethnic background, Bridges et al. [15] observed that prevalence of the −383 *TNFR1* polymorphism was dependent on the ethnicity with significant differences in prevalence between healthy Caucasians and healthy African-Americans.

This issue is relevant to interpreting the results of our study, because we exclusively assessed Mexican Mestizos, and further studies should confirm the participation of −383 *TNFR1* polymorphism in AS patients of other ethnic origins.

Polymorphism 36A > G of *TNFR1* were investigated in a small sample of Greeks; unfortunately, they did not found to be in Hardy–Weinberg equilibrium in the AS group and therefore were excluded these results in their analysis [16].

Recently, the Australo-Anglo-American-Spondyloarthritis-Consortium (TASC) through genome-wide association study identified susceptibility loci for AS among European descent from Australia, North America and

Britain, and this study included the assessment of a possible association between the marker rs4149577 in TNFR1 and AS [17]. Although it was a trend for this association, the confirmation of this association on other populations has been only evaluated in one study performed in China where these authors observed a statistical association between rs4149577 in TNFR1 and AS [18]. To date, the original TASC study although included Caucasians from North America did not evaluate other ethnic groups as American Mestizos that represent the major population in Central America or South America. Thus, studies in these populations with other ethnic groups should be conducted in order to confirm similitude of associations between other populations.

A limitation of our exploratory study is a small sample size; nevertheless, although only 66 patients with AS and 108 controls were evaluated, this sample was sufficient to demonstrate significant statistical differences in the genotype and allele frequencies of −383 A/C *TNFR1* polymorphism between AS and controls. This preliminary finding requires further validation in other populations from different ethnic origin and other regions in Mexico.

A final commentary is that although the observed prevalence of −383A allele in our study was higher in AS than in controls, AS is a disease with multifactorial etiology being genetics only one of a multiplicity of factors implicated in its pathogenesis and no single polymorphism can be considered as a unique cause for the development of the disease. Nevertheless, this polymorphism may contribute to the expression of the disease in a subgroup of patients.

In summary, the results of this preliminary study showed a higher prevalence of the −383A allele and −383AA genotype in patients with AS compared with matched controls. This increase in prevalence may indicate an increased susceptibility to AS development in Mexican Mestizo patients carrying this polymorphism. Further studies in other regions of Mexico are required in order to detect whether this association observed in AS remains significant. The role of this polymorphism in other ethnic group remains to be determined.

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Conflict of interest None.

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