

# The Genetics of Cytokines in Ankylosing Spondylitis



In this issue of *The Journal* Gonzalez, *et al* perform a case-control evaluation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) promoter polymorphisms in ankylosing spondylitis (AS)<sup>1</sup>. Several polymorphisms of the TNF gene have been described. The 2 variants most widely studied are at positions -238 and -308 (Figure 1). Both these variants are located in the putative transcription regulatory region and involve G to A transitions, where 238.1(G) and 308.1(G) are the wild-type (common alleles) and 238.2(A) and 308.2(A) are the variant (uncommon alleles). It has been suggested that these alterations may affect TNF- $\alpha$  production and as a result fundamentally alter the cytokine network in various rheumatic diseases such as AS, reactive arthritis (ReA), and rheumatoid arthritis (RA).

In the Gonzalez study the patient population consisted of individuals with primary AS (no inflammatory bowel disease or psoriasis). AS patients who were B27 negative had a higher frequency of peripheral arthritis and lower frequency of uveitis compared with patients who were B27 positive. It is unclear whether the B27 negative patients may have represented in part an overlap with other clinical phenotypes such as reactive arthritis. Clinical stratification in the spondyloarthropathies (SpA) becomes critical to our interpretation of TNF promoter polymorphism frequencies in particular, and to genetic studies in general. In addition it is not clear whether the control patients were examined to exclude an unrecognized SpA, an important concern in view of the frequency of sacroiliac abnormalities in B27 positive "healthy" controls.

In their study, the TNF 308.1 allele was found to be overrepresented in B27 positive AS patients. This was explained by linkage disequilibrium with B27, as had been reported by this group<sup>2</sup>. In addition, they found that the TNF 238.2 allele was overrepresented in B27 negative AS patients compared to B27 positive AS patients (OR = 4.33) and to B27 negative healthy controls (OR = 5.9). It is known that TNF promoter polymorphisms are in linkage disequilibrium with HLA Class II genes. HLA-DR1 has been shown to be asso-

ciated with primary AS<sup>3</sup> and HLA-DRB1\*0103 allele with AS complicating inflammatory bowel disease<sup>4</sup>. However, the present study lacked sufficient numbers to address whether the association with 238.2 in their patient population was independent of HLA Class II.

## ROLE OF MHC VS NON-MHC GENES IN AS

The heritability of AS has been estimated at > 90% on the basis of twin studies<sup>5</sup>. Although the association with B27 is strong, with almost 90% of patients carrying the gene, only a small proportion of B27 positive individuals ever develop the disease, suggesting there are other factors involved in the pathogenesis, as recently reviewed<sup>6</sup>. A genome-wide screen has confirmed the importance of the MHC in susceptibility, yet may account for only 30% of the genetic contribution to AS<sup>6</sup>. In fact, 30% may be an overestimation of the contribution<sup>6</sup>. The TNF locus is located within the MHC only 250 kb centromeric from the class I locus. This has prompted several investigators of rheumatic diseases to undertake a search for other susceptibility genes in this region. Such genes may either function independently of HLA Class I or II genes, or may function as genetic cofactors with the HLA genes.

## PRIOR STUDIES ON TNF PROMOTER POLYMORPHISMS (TABLE 1)

There have been conflicting reports with respect to the reported frequencies of the TNF promoter polymorphisms. AS patients in southern Germany and western Scotland were reported to have a decreased frequency of the -308.2 allele<sup>7-9</sup>. However, studies from Holland and Spain have not reported any such association<sup>8,10</sup>. The frequency of 238.2 allele was shown to be increased in psoriasis<sup>9</sup> and juvenile psoriasis and in AS<sup>11</sup>, but the latter was not confirmed in more recent studies<sup>2,10</sup>.

Interpretation of the discrepancies between earlier case-control studies<sup>8</sup> reporting lack of association of the TNF promoter polymorphisms with AS is difficult, as these

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*See TNF-238 alpha promoter polymorphism contributes to susceptibility to AS in HLA-B27 negative patients, page 1288*

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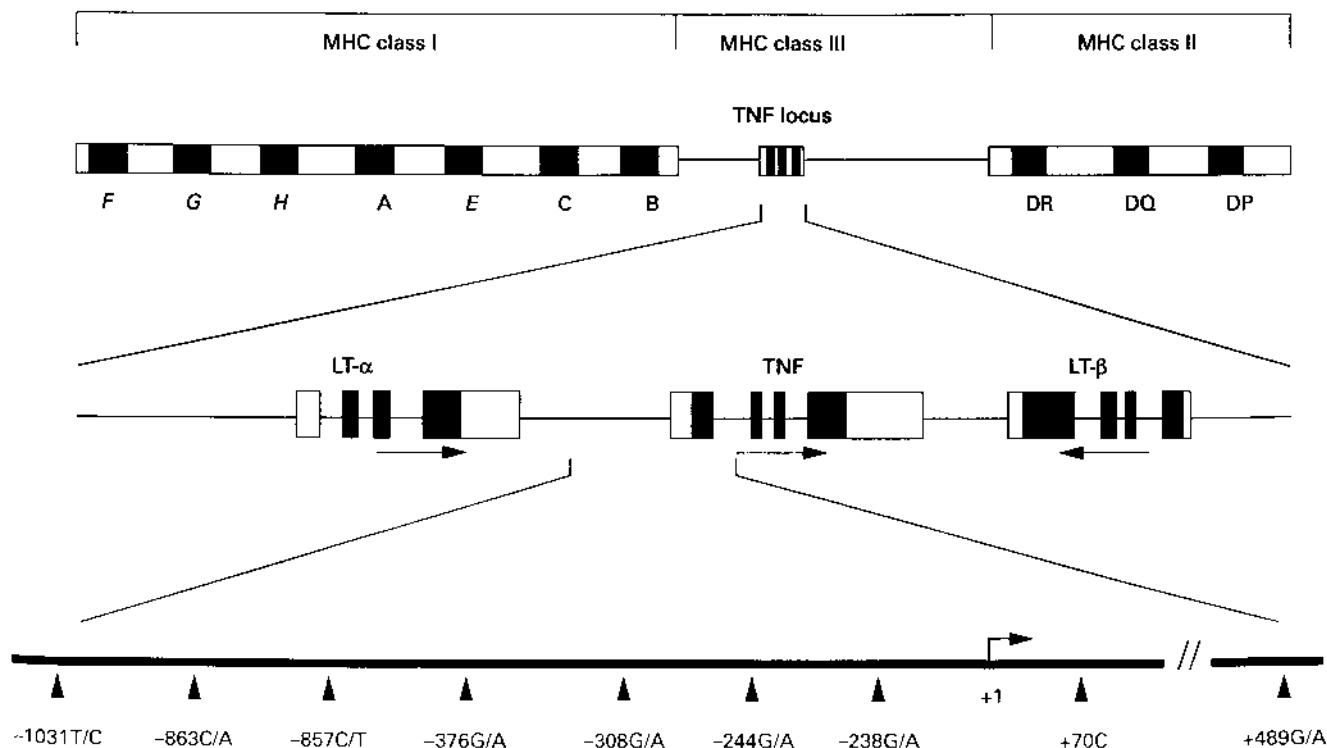


Figure 1. The location of the TNF gene within the major histocompatibility complex. The middle row represents the intron/exon organization of the TNF and LT genes. Arrows indicate the transcriptional orientation of the TNF and LT genes. At the bottom, the 5' region of the gene including the transcription initiation site is depicted. The position of the single nucleotide polymorphism within this part of the gene is indicated. From Verweij CL. Ann Rheum Dis 1999;58 Suppl 1:120-6; with permission.

Table 1. TNF promoter allele frequencies.

Allele	Association		No Association	
	Population	Reference	Population	Reference
↑308.1*	AS B27+ vs B27+/- controls	6	AS B27+ vs B27+/- controls	10
↓308.2†	AS B27+ vs B27+/- controls	8	AS B27+ vs B27- controls	11
			AS B27+ vs B27- controls	13
↓238.2†	AS B27+ vs B27+/- controls	9	AS B27+ vs B27+/- controls	10
↑238.2†	Ps, PsA vs controls	8	AS B27+ vs B27- controls	11

\*Wild type allele; †variant allele.

studies had adequate power to detect only large genetic effects. This is in contrast to the recently published study by the Oxford group<sup>10</sup>. They contrasted the -238 and -308 polymorphisms in 306 English AS cases versus 204 ethnically matched B27 positive controls, and in 96 German AS cases versus 58 B27 positive and 251 B27 negative controls. Their study had sufficient power to detect small effects. In the southern German AS patients a significant reduction in the TNF -308.2 alleles was seen compared with B27 positive controls (OR = 0.4,  $p = 0.03$ ), confirming prior findings

in this population, but no difference in allele frequencies was observed at TNF -238. In contrast, no significant association between AS and either TNF -238 or -308 was observed in the English patients. Such results have led to the conclusion among many investigators that these polymorphisms alone do not influence susceptibility to AS but that other genes in linkage disequilibrium with these loci may do so. However, further studies of haplotypes across the MHC addressing particularly the area centromeric of TNF will be required to determine the precise nature of such genes.

**FUNCTIONAL SIGNIFICANCE OF  
POLYMORPHISMS FOR TNF- $\alpha$  PRODUCTION  
(TABLE 2)**

There is controversy surrounding the functional significance of the TNF promoter polymorphisms. Several groups have observed higher transcriptional activity for the TNF -308 allele associated with inducible levels of TNF- $\alpha$ <sup>14-17</sup>, but this has not been replicated by other groups<sup>18-20</sup>. The functional significance of the -238 allele also remains unclear, as one group has shown a decreased TNF-producing phenotype<sup>21</sup>, but this has not been confirmed by others<sup>12,15,18</sup>.

**RELEVANCE OF POLYMORPHISMS TO DISEASE  
PATHOGENESIS**

TNF is a pleomorphic cytokine that has many important roles in both health and disease. It is produced primarily in macrophages and is an important cytokine during the course of infection, where it has been shown to have a bimodal role depending on the phase of the infectious process. Using TNF receptor knockout mice we have determined that in the early phase of infection with *Yersinia enterocolitica*, TNF- $\alpha$  exerts primarily a deleterious effect on host defense and mediates apoptosis of CD4<sup>+</sup> T cells<sup>22</sup>. However, in the chronic phase of *Yersinia* infection, TNF plays a protective role in host defense and figures critically in macrophage microbicidal effects on the pathogen<sup>23</sup>. Mice deficient in TNF- $\alpha$  responses have not only increased mortality, but also more extensive joint damage in the chronic ReA observed.

TNF promoter polymorphisms have been associated with the clinical course of human infections such as cerebral malaria and cutaneous leishmaniasis<sup>18,24</sup>. Individuals who are homozygous for TNF 308.2 have an increased risk of developing cerebral malaria (RR 4.0) and of dying from the infection (RR 7.7). This risk is independent of any association with HLA alleles, suggesting that TNF-308 is a dominant genetic susceptibility factor for cerebral malaria. Susceptibility for mucocutaneous leishmaniasis has also been associated with the -308.2 allele.

In ReA, where there is evidence of an infectious etiology, it has been shown that TNF- $\alpha$  levels are low in synovial fluid<sup>20,25</sup>. Among patients with ReA followed prospectively, those with a more chronic disease course have lower TNF- $\alpha$  levels than patients with self-limited disease<sup>26</sup>. In contrast

to ReA, a pathogenic role for bacteria in AS is less clear, and as yet no distinct cytokine pattern has been clearly defined for AS. Recent reports suggest that in AS there is a *decrease* in TNF- $\alpha$  and interferon- $\gamma$  compared to RA<sup>19,27</sup>. On the other hand, TNF- $\alpha$  mRNA has been detected in sacroiliac joint biopsies of patients with AS<sup>16</sup>. In addition, there has been recent experience that monoclonal antibody to TNF successfully controls spinal inflammation in refractory AS<sup>28-30</sup>. In our recent experience treating 21 AS patients with infliximab, there has been consistent improvement in functional indices, markers of inflammation, and in magnetic resonance imaging measurements of activity of disease<sup>25</sup>. While this provides strong circumstantial evidence for TNF- $\alpha$  playing a role in axial inflammation, its role in the ankylosis that characterizes the chronic disease is undefined, and the role of TNF blocking therapies in the longterm management of the disease awaits further trials.

**FUTURE DIRECTIONS**

Clearly there is a role for genes other than HLA-B27 in the pathogenesis of AS. There is evidence in favor of TNF- $\alpha$  playing some role in the disease process. To date, there is conflicting evidence from association studies on the frequency and functional significance of TNF promoter polymorphisms in AS. Family studies in progress in North America and Europe will help elucidate the situation. Meanwhile, studies in pharmacogenetics to define the biologic basis of differential responses to the new biologic agents such as infliximab will also be fruitful in further determining the genetic basis for AS.

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Table 2. Functional significance of TNF promoter alleles.

Allele	TNF- $\alpha$ Secretion	Reference
308.2 <sup>†</sup>	Increased	14, 16, 17
	No alteration	13, 17-19
238.2 <sup>†</sup>	Decreased	21
	No alteration	15,18

<sup>†</sup> Variant allele.

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