

# Tumor Necrosis Factor (TNF)- $\alpha$ Gene +489 Polymorphisms: Association with Psoriatic Arthritis

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## Abstract

**Objective of this work was to investigate the role of single nucleotide polymorphisms (SNPs) at position +489 of the tumor necrosis factor (TNF)- $\alpha$  gene in genetic susceptibility and severity of psoriatic arthritis (PsA). Fifty-seven Caucasian PsA patients diagnosed according to CASPAR criteria and 155 healthy matched controls were studied. PASI score, DAS28 and Disability Index HAQ were calculated. Genomic DNA was extracted from peripheral blood samples and SNPs +489 G>A (rs 80267959) were amplified by PCR. The SNP +489 genotype was significantly associated with PsA susceptibility ( $p=0.0136$ ) and severity of clinical and laboratory parameters ( $p$  values ranging from 0.016 to  $2.908 \times 10^{-12}$ ). The difference in severity was accounted for by the differences between the AA and GA genotypes with respect to the GG genotype. These findings suggest that TNF- $\alpha$  gene polymorphisms may influence PsA susceptibility and severity.**

**Psoriatic arthritis (PsA) is a complex immune-mediated disease that results from the interplay between multiple genetic and environmental factors [1]. Although the pathogenesis of PsA remains elusive, there is evidence that genetic factors may contribute to the etiology of the disease [2]. It has been estimated that at least one third of the genetic contribution to PsA resides in the major histocompatibility complex (MHC) region [2]. The tumor necrosis factor (TNF)- $\alpha$  gene, which is located in the short arm of chromosome 6 in the MHC class III region between the HLA-B and HLA-DR genes, has been proposed as a major candidate gene in PsA [3]. This hypothesis is supported by studies which**

**have found high serum, synovial fluid and synovial membrane TNF- $\alpha$  levels in patients with PsA [4,5].**

**Several single nucleotide polymorphisms (SNPs) have been identified in the TNF- $\alpha$  gene promoter [6]. In particular, two common polymorphisms, namely G to A substitutions at positions -238 and -308 have been studied in patients with PsA. However, association studies of these two TNF- $\alpha$  polymorphisms and genetic susceptibility to PsA have lead to conflicting results [7-12]. Previous studies have indicated the potential role of the SNP at +489 position in the first intron of the TNF- $\alpha$  gene in the susceptibility to some rheumatic autoimmune diseases like rheumatoid arthritis [13], systemic lupus erythematosus [14] and systemic sclerosis [15]. However, to our present knowledge, studies on the association of +489 polymorphism with PsA susceptibility and response to TNF- $\alpha$  inhibitors are not reported in the literature. In this study we investigated the role of SNPs at +489 within the TNF- $\alpha$  gene in PsA susceptibility and severity.**

## Materials and Methods

### Patients and treatment

Fifty-seven Caucasian unrelated patients with PsA and 155 unrelated healthy Caucasian controls similar for sex, age and the Italian geographical origin were enrolled. The study was approved by the local Hospital Ethics Committee. Inclusion criteria were: age above 18 years, PsA diagnosed according to the CASPAR criteria [16]. PsA was classified according to the pattern of peripheral joint disease as oligoarticular ( $\leq 5$  joints ever involved) or polyarticular ( $>5$  joints ever involved). Plain radiographs of the hands and feet were obtained and the distal interphalangeal, proximal interphalangeal (PIP), metacarpophalangeal and carpal joints in the hand and the PIP and metatarsophalangeal joints in the feet were examined. The presence of erosions and periostitis was recorded. Spinal involvement was defined as a history of inflammatory back pain or imaging evidence of

sacroiliitis or spondylitis. Psoriasis was classified as type 1 (onset at age <40 years) or type 2 (onset at age  $\geq$ 40 years). The Psoriasis Area and Severity Index (PASI) score, the Disease Activity Score (DAS) 28 and the Disability Index Health Assessment Questionnaire (HAQ) were also calculated. [17,18].

### Molecular analysis

Genomic DNA was extracted from peripheral blood samples according to standard protocols. The polymorphisms of SNP +489 G>A (rs80267959), located in intron 1 of TNF- $\alpha$  gene, were investigated. The genomic region of TNF- $\alpha$  including this SNP was amplified by polymerase chain reaction (PCR) by standard protocol and polymorphisms were genotyped by direct sequencing. Sequencing reactions were performed using BigDye Terminator Sequencing Kit v3.1 (Applied Biosystems), according to the manufacturer's instructions, and resolved on an ABI 3100 - Avant automated sequencer (Applied Biosystems, Foster City, CA). Hardy-Weinberg equilibrium was assessed in the sample series to identify potential genotyping errors. No deviation from Hardy-Weinberg expectation was found on both populations.

### Statistical analysis

The hypothesis of genotypic and allelic associations between the SNP and the disease were tested by Pearson's chi-squared test with 1df and Yates's continuity correction or by Fisher's exact test as appropriate. The statistical significance level was set at  $p = 0.05$ . Associations between SNP+489 genotypes and clinical parameters regarded as quantitative traits were calculated by the Wald test implemented in PLINK [19]. The differences between the means among the genotypes have been confirmed by ANOVA and posthoc internal comparisons by the Kruskal-Wallis non parametric test. The clinical traits regarding the number of joints affected have also been dichotomized as equal or below 5 and above 5, and the comparison of allelic distributions among the two groups has been carried out by Fisher's exact test.

## Results

### Patients population

Patients population included 57 patients (25 males and 32 females) with a mean age of  $50 \pm 7$  years and a mean disease duration of  $12 \pm 2$  years (Table 1).

Table 1. Baseline characteristics of patients. Values are \*number and percentage or \*\*mean  $\pm$  SD

Male/Female	25 (44%) / 32 (56%)*
Age	$50 \pm 7^{**}$
Disease duration (years)	$12 \pm 2$

Psoriasis: type 1/type 2	11 (19%) / 46 (81%)
Oligoarticular ( $\leq$ 5 joints)	20 (35%)
Polyarticular (> 5 joints)	37 (65%)
Swollen joint count	$12 \pm 7$
Tender joint count	$16 \pm 9$
Erythrocyte sedimentation rate (mm/1 <sup>st</sup> h)	$57 \pm 13$
C-reactive protein (mg/dl)	$14 \pm 4$
Disease Activity Score (DAS)28	$5.60 \pm 0.45$
Psoriasis Area and Severity Index (PASI) score	$11.27 \pm 3.26$
Visual analogic scale (VAS) of pain	$48 \pm 9.26$
Physician's global assessment of disease activity (PhGADA)	$56 \pm 10.33$
Patient's global assessment of disease activity (PGADA)	$54 \pm 9.04$
Disability Index Health Assessment Questionnaire (HAQ)	$2 \pm 0.74$

Baseline characteristic of patients indicate that the majority of them was affected by type 2 psoriasis, by polyarticular involvement and by active disease as shown by the elevated ESR, PCR, DAS28 and PASI mean levels as well as by VAS, PhGADA, PGADA and HAQ mean values (Table 1). Plain radiographs of hands and feet were obtained in 42 patients. Thirty-three of them (78.6%) had joint erosions of hands and feet, and 13 (31%) had radiographic signs of periostitis. Magnetic resonance imaging demonstrated sacroiliitis in 8 (14%) and spondylitis in 5 (9%) patients. Associations between TNF- $\alpha$  gene polymorphisms, PsA susceptibility and disease severity. The A allele of the SNP +489, was significantly associated with PsA susceptibility ( $p = 0.0136$ ) (Table 2).

Table 2. SNP +489 alleles in controls and patients. MAF= minor allele frequency (namely the frequency of the A allele). \*Significance of chi-square test with Yate's correction for the allelic association to the disease (case-control comparison) and Fisher test (two tailed) p-value for the allelic association in the subgroups comparisons.

	Alleles		MAF	p-value*
	G N (%)	A N (%)		
Controls	267 (86)	43 (22)	0.139	
Patients	86 (75)	28 (25)	0.246	0.0136

The SNP +489 genotype was also strongly associated with disease severity as shown by its highly significant association ( $p < 10^{-7}$ ) with most clinical and laboratory parameters and, although to a lesser degree, with DAS28 ( $p = 0.01$ ). In particular, the most significant associations were those with HAQ and PASI ( $p = 2.91 \times 10^{-12}$  and  $p = 4.9 \times 10^{-12}$ , respectively) (Table 3). The significance of the associations was maintained for all parameters but DAS28 after correcting for the number of tests performed. These results were confirmed by ANOVA as well as by the non parametric Kruskal Wallis test (data

not shown). For each parameter analyzed, the difference in severity was accounted for by the differences between the AA and GA genotypes with respect to the GG genotype, while no differences in severity could be detected between individuals carrying the GA and the AA genotype. Dichotomizing the number of swollen and tender joints, based on the cutoff of minor or equal of 5 and above 5, yield to a similar association, as expected (data not shown). Finally, the SNP +489 A allele was also significantly associated with the presence of joint erosions, periostitis, sacroiliitis and spondylitis (Table 4).

Collectively, these data support the role of the SNP +489 A allele not only in disease susceptibility but also in disease severity.

Table 3. Associations between clinical and laboratory parameters and SNP +489. \*See Table 1 for abbreviations. \*\* Values are mean  $\pm$  SD \*\*\* Significance of the association between the parameters and SNP +489 genotype. Statistical analysis was performed by Wald test (based on t-distribution) asymptotic two tailed p-value (p-values in italics are those significant after Bonferroni's correction).

Parameters*	SNP +489 genotype			p-value***
	GG (N.=34)	GA (N.=18)	AA (N.=5)	
ESR	49.80 $\pm$ 8.00**	65.70 $\pm$ 10.02	72.20 $\pm$ 15.40	<i>1.15 x 10<sup>-8</sup></i>
CRP	10.90 $\pm$ 1.80	18.10 $\pm$ 3.90	17.40 $\pm$ 4.00	<i>1.12 x 10<sup>-9</sup></i>
Swollen joint count	7.20 $\pm$ 4.00	18.40 $\pm$ 5.60	20.20 $\pm$ 4.60	<i>3.27 x 10<sup>-11</sup></i>
Tender joint count	10.20 $\pm$ 6.60	24.20 $\pm$ 6.50	25.00 $\pm$ 3.90	<i>2.84 x 10<sup>-9</sup></i>
VAS	42.40 $\pm$ 5.10	56.70 $\pm$ 6.90	54.00 $\pm$ 11.40	<i>7.36 x 10<sup>-8</sup></i>
PhGADA	49.90 $\pm$ 5.20	63.90 $\pm$ 9.90	66.00 $\pm$ 11.40	<i>2.68 x 10<sup>-8</sup></i>
PGADA	48.20 $\pm$ 4.90	62.50 $\pm$ 6.50	63.00 $\pm$ 7.60	<i>1.53 x 10<sup>-10</sup></i>
HAQ	1.20 $\pm$ 0.20	2.30 $\pm$ 0.50	2.30 $\pm$ 0.30	<i>2.91 x 10<sup>-12</sup></i>
DAS28	5.60 $\pm$ 0.50	6.10 $\pm$ 1.00	6.20 $\pm$ 0.90	<i>0.01 x 10<sup>-2</sup></i>
PASI	8.80 $\pm$ 1.00	13.30 $\pm$ 2.40	13.40 $\pm$ 1.00	<i>4.90 x 10<sup>-12</sup></i>

Table 4. SNP +489 alleles in patients' subgroups. MAF= minor allele frequency (namely the frequency of the A allele). \*Significance of chisquare test with Yate's correction for the allelic association to the disease (case-control comparison) and Fisher test (two tailed) p-value for the allelic association in the subgroups comparisons.

Patients' subgroups	Alleles		MAF	p-values*
	G N (%)	A N (%)		
Joint erosions				
Presence	39 (59)	27 (41)	0.409	<i>4.23x10<sup>-7</sup></i>
Absence	47 (98)	1 (2)	0.021	
Periostitis				
Presence	10 (38)	16 (62)	0.615	<i>3.48x10<sup>-6</sup></i>
Absence	56 (82)	12 (18)	0.136	
Sacroiliitis				
Presence	6 (37)	10 (63)	0.625	<i>5.42x10<sup>-4</sup></i>
Absence	80 (82)	18 (18)	0.184	
Spondylitis				
Presence	3 (30)	7 (70)	0.700	<i>1.96x10<sup>-3</sup></i>
Absence	83 (80)	21 (20)	0.202	

## Discussion

PsA has a proved inheritability, as reflected by a relative risk of 55 among first degree relatives with the disease [20]. The TNF- $\alpha$  gene has long been considered a major susceptibility gene in immune-mediated diseases including PsA [2], however several population studies designed to evaluate the association between some TNF- $\alpha$  gene

polymorphisms and PsA lead to conflicting results [7-12, 21-24].

The present study analyzed the SNP at the position +489 in the first intron of the TNF- $\alpha$  gene and demonstrated the strong association between the SNP +489 A allele with both PsA susceptibility and severity. Indeed, the presence and progression of joint erosions of the hands and feet as well as the presence of periostitis, sacroiliitis and

spondylitis were associated with the SNP +489 A allele. Mullighan et al. [25] reported that the SNP +489 A allele may be associated with increased TNF- $\alpha$  levels, suggesting that a genetic predisposition to produce higher levels of TNF- $\alpha$  is an important factor in the development of erosions. Significantly increased levels of TNF- $\alpha$  have been found in PsA serum, synovial fluid and synovial membrane [4,5] and our study suggests that the SNP +489 A allele, directly or in association with other polymorphisms, could influence the events that lead to the development and progression of joint erosions.

In conclusion, our findings suggest that the SNP +489 A allele, alone or in association with other polymorphisms in the same region, could influence the genetic susceptibility to PsA and the severity of the disease.

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