International Journal of Dermatology

Medical genetics

Tumor necrosis factor alpha gene promoter −238G/A polymorphism increases the risk of psoriasis vulgaris in Indian patients

Deepa Rajesh[1], MSc, Rajesh Gurumurthy[2], MD, A. V. Moideen Kutty[1], PhD, and Sharath Balakrishna[1], PhD

[1]Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, India, and [2]Department of Dermatology, Sri Devaraj Urs Medical College, Kolar, India

Correspondence
Sharath Balakrishna, PHD
Department of Cell Biology and Molecular Genetics
Sri Devaraj Urs Academy of Higher Education and Research
Tamaka
Kolar 563103, Karnataka
India
E-mail: genomelab@sduu.ac.in

Funding: None.

Conflicts of interest: None.

doi: 10.1111/ijd.13482

Abstract

Background Tumor necrosis factor alpha (*TNF*α) gene −238G/A polymorphism (rs361525) is associated with psoriasis in several populations worldwide. To the best of our knowledge, there is no information about this polymorphism in Indian psoriatic patients. This study was undertaken to fill the gap in knowledge.

Methods This case–control study involved 72 patients with psoriasis vulgaris (PsV) and 72 age and gender matched healthy individuals. *TNF*α −238G/A polymorphism was genotyped by PCR-RFLP method.

Results *TNF*α −238A allele was 5 times commoner in PsV patients than in the control group ($P = 4.1 \times 10^{-7}$; odds ratio [OR] = 6.5 [0.95 CI: 2.9–14.6]). Distribution of the genotypes in the two groups showed statistically significant difference in dominant genetic model ($P = 2.3 \times 10^{-7}$) and not in recessive genetic model ($P = 2.5 \times 10^{-1}$). Odds ratio for the occurrence of −238A genotype in PsV patients was 8.8 (0.95 CI: 3.5–20.2). The association showed no major difference when PsV patients were subgrouped into type I and type II categories and tested separately. Subgroup analysis on the basis of disease severity showed higher association with the moderate-severe subgroup ($P = 2.4 \times 10^{-9}$, OR 15.4 [0.95 CI: 5.8–41.0]) than with mild subgroup ($P = 1.3 \times 10^{-2}$, OR 3.8 [0.95 CI: 1.3–10.9]).

Conclusions Our results indicate that *TNF*α gene −238G/A polymorphism increases the risk of developing psoriasis vulgaris among Indians. Also, the data show that severity and not the type affects the strength of association in this population.

Introduction

Psoriasis is a chronic, proliferative, T lymphocyte-mediated inflammatory disorder characterized by raised and erythematous scaly plaques on the body.[1] Recent estimates indicate that prevalence of psoriasis is about 0.4–2.8% in the Indian population.[2] Psoriasis can be classified into two types: type I, with an age of onset of less than 40 years, and type II, with an age of onset of more than 40 years.[3] Of the various clinical forms of psoriasis, psoriasis vulgaris (PsV) is the predominant form seen in up to 80% of the patients.[1]

Precise mechanism for the pathogenesis of psoriasis is still unclear. It is a multifactorial disorder involving interplay between immunological and genetic as well as environmental factors. At the immunological level, inflammation involves interaction between different cell types releasing cytokines.[4] The cutaneous and systemic overexpression of several pro-inflammatory cytokines, such as tumor necrosis factor (*TNF*)α, interleukins 2, 6, 8, 12, and interferon γ has been suggested to be responsible for initiation, maintenance, and recurrence of skin lesions. On the other hand, relatively low expression of the anti-inflammatory cytokines IL1, IL4, and IL10 suggests an insufficient counter-regulatory capacity of the immunological system in psoriasis.[5,6]

Tumor necrosis factor is a pro-inflammatory cytokine which has been shown to be elevated in the serum of PsV patients.[7] *TNF*α gene is located on chromosome 6p21.3 in the MHC region between MHC class I and II genes. The common promoter single nucleotide polymorphisms (SNPs) that affect the expression level of *TNF*α gene are −238G/A (rs361525) and −308G/A (rs1800629). These SNPs have been associated with various inflammatory disorders involving TNFα elevation. It has been suggested that the functional polymorphisms in the −238 and −308 sites are associated with the development of psoriasis.[8–10] Several studies have consistently showed that presence of *TNF*α-238A allele in Caucasians is about 3–5 times higher among patients with PsV than among healthy individuals.[9,11,12] Positive association has also been noticed in the Chinese population.[13] However, studies in the Japanese population have not found any evidence for the association.[14] Most of the reported

studies involve patients who are ethnically Caucasian in descent.[15] To the best of our knowledge, there is no information on the association between PsV and *TNFα* −238G/A SNP in the Indian population. In order to bridge the gap, we performed this case–control study to evaluate the distribution of *TNFα* −238G/A (rs361525) genotypes and their association with risk of developing PsV in an Indian population.

Materials and methods

Study design and participants

A prospective case–control design was adopted for the study. A total of 72 PsV patients and 72 age and gender matched healthy individuals with no history of psoriasis and other diseases like diabetes, rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, and Crohn's disease were enrolled in this study. Participants were enrolled from the Department of Dermatology, R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka, India, from March 2014 to March 2016. The study was approved by the University Ethics Committee. Patients visiting the outpatient unit of the Dermatology Department who were diagnosed to have PsV based on history and clinical examination were enrolled in the study after informed consent. Patients with PsV were considered to have early-onset disease if skin symptoms occurred before 40 years of age and late-onset disease if age of onset was more than 40 years. Disease severity was assessed at study entry by determination of the psoriatic area and severity index.[16]

Sample collection and DNA isolation

About 3 mL of peripheral venous blood was collected in EDTA vacutainer and stored at 4 °C until processing. DNA was isolated from the peripheral blood lymphocytes using salting out method. Purity of the sample was determined by UV spectrophotometry (Perkin Elmer model Lambda 35, Waltham, MA, USA).

Genotyping −238G/A *TNFα*

Genomic DNA was amplified by polymerase chain reaction on Bio Rad C1000 Touch Thermal Cycler. The primer pairs used were 5′-AGAAGACCCCCCTCGGAACC-3′ (forward) 5′-ATCTGGAGGAAGCGGTAGTG-3′ (reverse). Twenty-five microliters of reaction mixture included 1x assay buffer, 150 ng genomic DNA, 0.2 mM dNTP, 1 picomole of each primer, 1.5 mM $MgCl_2$, and 1 unit *Taq* DNA polymerase (Bangalore Genei, India). The program comprised of an initial denaturation at 95 °C for 3 min followed by 36 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s; final extension involved 10 min at 72 °C. The PCR product was analyzed on 2% agarose gel. The 152 bp amplicon was subjected to restriction digestion with 20 units of Msp1 (New England Biolabs, Ipswich, MA, USA) at 37 °C and analyzed on 3% agarose gel with ethidium bromide staining. After digestion, three genotypes of *TNFα* polymorphism could be identified; homozygote G/G had one band of 133 and one of 19 base pairs (bp), homozygote A/A had only one band of the 152 bp, and heterozygotes G/A had two bands of 152 and 133 bp fragments. The 19 bp fragment was not visible on the gel because of its small size. Ten percent of the samples were randomly selected for confirmation, and the results were 100% concordant. Sanger sequenced samples were used as positive controls.

Statistical analysis

Sample size and power of the study were calculated using OpenEpi web tool using 95% confidence interval.[17] Normal approximation with continuity correction was considered for power calculation. Statistical analysis was done using the Statistical Packages for Social Sciences software (SPSS version 22; SPSS Inc., Chicago, IL, USA). Allele and genotype frequencies of the two groups were compared using relevant contingency tables. Difference between the groups was determined by calculating two-tailed *P* value using Fisher's exact test. The study population was tested for conformity to Hardy–Weinberg equilibrium using the web program by Rodriguez and coworkers.[18] *P*-values <0.05 were considered as statistically significant. All statistical tests were two-tailed.

Results

Clinical profile of the 72 PsV patients is given in Table 1. Allele and genotype frequencies in patient and control groups are summarized in Table 2. The distribution of the genotypes in both case and control groups was in agreement with Hardy–Weinberg equilibrium.

Allele frequency of −238A among PsV patients was 27.8%, which is five times higher than its frequency in the control group (5.5%). The difference in the distribution profile was found to be statistically significant ($P = 4.1 \times 10^{-7}$). The odds ratio (OR) for the occurrence of −238A allele compared to −238G allele among PsV patients was 6.5 (0.95 CI: 2.9–14.6). Heterozygous −238A genotype was 4.3 times more common among PsV patients (47.2%) than among controls (11.1%). Frequency of homozygous A genotype was very low (4.2%). Also, homozygous −238A

Table 1 Clinical characteristics of the study population

Parameter	Patients	Controls
Number of patients		
Male	52 (72%)	54 (75%)
Female	20 (28%)	18 (25%)
Age group (years)	22–78	20–70
Clinical type		
Type I	39 (54.2%)	
Type II	33 (45.8%)	
Severity		
Mild	31 (43%)	
Moderate-severe	41 (56.9%)	

Table 2 Distribution of genotype and allele frequencies in the study population

Genotype/ allele	Patients	Controls	*P* value	
			Dominant model GG vs. GA + AA	Recessive model GG + GA vs. AA
GG	35 (48.61%)	64 (88%)	2.3×10^{-7}	2.5×10^{-1}
AG	34 (47.2%)	8 (11.1%)	OR = 8.8 (0.95 CI: 3.5–20.2)	
AA	3 (4.2%)	0		
G	104 (72.2%)	136 (94.4%)	4.1×10^{-7}	
A	40 (27.8%)	8 (5.5%)	OR = 6.5 (0.95 CI: 2.9–14.6)	

Table 3 Comparison of genotype frequencies in dominant genetic model on the basis of disease type and severity

Genotype	Control (*n* = 72)	Cases (*n* = 72)	Type I (*n* = 35)	Type II (*n* = 37)	Mild (*n* = 31)	Moderate-severe (*n* = 41)
GG	64	35	18	17	10	27
GA + AA	8	37	21	16	21	14
P value	–	2.3×10^{-7}	2.0×10^{-6}	5.0×10^{-5}	1.3×10^{-2}	2.4×10^{-9}
Odds ratio (95% CI)	–	8.5 (3.5–20.1)	9.3 (3.5–24.6)	7.5 (2.8–20.5)	3.8 (1.3–10.9)	15.4 (5.8–41.0)

CI, confidence interval.

genotype was not observed among controls. The two groups showed statistically significant difference in dominant genetic model and not in recessive genetic model (Table 2). The power of the study was 99.9%. The OR for the occurrence of −238A allele in a dominant genotype in comparison to −238G allele among PsV patients was 8.8 (0.95 CI: 3.5–20.2).

The distribution of the genotypes was stratified on the basis of type of PsV and analyzed (Table 3). The two subgroups did not show any major difference when they were tested separately. This indicates that the association between PsV and *TNFα* −238G/A SNP is not affected by the disease type. Next, we stratified the distribution of the genotypes on the basis of severity of PsV and subjected the profile to statistical analysis. Significant association was found with both mild and moderate-severe subgroups. However, the association was found to be stronger with the moderate-severe subgroup than with the mild subgroup (Table 3). *P* value for association with the moderate-severe subgroup was higher than that for the mild subgroup by an order of 7. Also, the OR for association with the moderate-severe subgroup was higher than that for the mild subgroup by 4.05 times. This indicates that the association of PsV and *TNFα* −238G/A SNP is affected by the severity of the disease. In this context, it is interesting to note that the small fraction of PsV patients who showed homozygous −238A genotypes were with moderate-severe form of PsV.

We saw familial incidence of PsV in two cases. Thus, the frequency of familial incidence was ~2.8%. Both the patients were male and had one affected sibling. Both the patients showed type I and moderate form of PsV. Furthermore, both the patients showed heterozygosity for the −238G/A genotype.

Discussion

Elevation of serum TNFα level in PsV is well established, but information on the causative mechanism is still nebulous. Elevated serum level of TNFα is linked to SNPs in the promoter region of *TNFα* gene. Common SNPs in this region are −238G/A, −308G/A, −857C/T, and −1031T/C. A number of studies have examined the status of *TNFα* gene promoter sequence polymorphism in psoriasis. Data from the various reported association studies have been subjected to at least two meta-analyses.[15,19] In both the studies, the authors found that −238A allele increased the risk of psoriasis (OR = 2.6, 2.5), while −308A reduced the risk of psoriasis (OR = 0.59, 0.57). The two polymorphisms are speculated to manifest through different mechanisms. The −238A allele putatively promotes psoriasis by increased gene expression of *TNFα*. Reduced gene expression caused by −308G potentially impairs clearance of skin infections with *Candida* or streptococci and thus predisposes individuals to develop psoriasis.[8] Thus far, variants in HLA-C (Cw6 allele) and CARD14 (gain-of-function mutations) genes are the two known genetic signatures that predispose the patient to psoriasis in a Mendelian fashion.[20,21] However, these variants show incomplete penetrance. For instance, psoriasis is seen to develop only in about 10% of HLA-Cw6-positive individuals. This suggests the involvement of modifier genes and/or environmental triggers.[22,23] Some studies have indicated that the association between −238G/A and psoriasis is probably because of a strong linkage disequilibrium between −238A allele and HLA Cw6 allele.[24]

Most of the genetic association studies on *TNFα* gene are based in populations derived from Caucasian or Mongoloid

ancestry.[15] Association of −308G/A polymorphism with psoriasis in Indian patients has been reported.[25] To the best of our knowledge, this is the first study to report the association between −238 G/A and PsV in the Indian population. Our results indicate that *TNFα* −238A allele increases the risk of developing PsV in the Indian population as it occurs more frequently in PsV patients than in the control group.

It is interesting to note that the frequency with which the disease associated −238A allele occurs under homozygous condition is usually low. Frequency in the range of 0.4–8.0% has been observed in previous studies.[12,26–28] The frequency of 4.2% observed in the present study appears to be on the higher side. The homozygous genotype was totally absent in some studies.[29,30] Low frequency of the disease allele homozygosity is consistent with the elevated pathogenicity that it can potentially impart to the patient. In corroboration, we noticed that −238 AA genotype was seen only in patients with moderate to severe PsV and not in patients with mild PsV.

Patients with type I and II PsV are known to have different genetic backgrounds. The association between PsV and HLA Cw6 is relatively stronger in case of type I rather than type II psoriasis.[31] It is not clear if the association with *TNFα* −238A is dependent on the disease type.[15,19] In the present study, distribution of the genotype frequencies under dominant genetic model do not show any significant difference between type I and type II PsV subgroups (Table 3). This encourages us to conclude that −238A allele may not be affected by the type of PsV in the Indian population.

We compared the distribution of the genotypes after stratification of disease severity as −238G/A was linked to severity of the disease in the Brazilian Caucasoid population.[32] The association of −238A allele with the moderate-severe subgroup was higher than with the mild group. This indicates that disease manifestation is affected by −238G/A polymorphism. The variation between the subgroups also compels us to assume that −238A allele plays a secondary role in the pathogenesis of PsV. It is possible that HLA Cw6 genotype or CARD14 mutations initiate the development of PsV, after which the course of the disease is affected by −238G/A polymorphism.

In conclusion, our study presents evidence to show that *TNFα* −238A allele is associated with the development of PsV in the Indian population. The results of the study also show that the distribution of *TNFα* −238A allele is not dependant on the type but on the severity of the disease.

References

1 Dogra S, Yadav S. Psoriasis in India: prevalence and pattern. *Indian J Dermatol Venereol Leprol* 2010; **76**: 595–601.

2 Song QH, Shen Z, Xing XJ, *et al.* An association study of single nucleotide polymorphisms of the FOXP3 intron-1 and the risk of Psoriasis vulgaris. *Indian J Biochem Biophys* 2012; **49**: 25–35.

3 Cheng H, Li Y, Zuo XB, *et al.* Identification of a missense variant in LNPEP that confers psoriasis risk. *J Invest Dermatol* 2014; **134**: 359–65.

4 Radtke S, Wüller S, Yang XP, *et al.* Cross-regulation of cytokine signalling: pro-inflammatory cytokines restrict IL-6 signalling through receptor internalisation and degradation. *J Cell Sci* 2010; **123**: 947–59.

5 krueger G, Ellis CN. Psoriasis – recent advances in understanding its pathogenesis and treatment. *J Am Acad Dermatol* 2005; **53**: 94–100.

6 Ozawa M, Aiba S. Immunopathogenesis of psoriasis. *Curr Drug Targets Inflamm Allergy* 2004; **3**: 137–44.

7 Kyriakou A, Patsatsi A, Vyzantiadis TA, *et al.* Serum levels of TNF-α, IL-12/23p40, and IL-17 in plaque psoriasis and their correlation with disease severity. *J Immunol Res* 2014;**000**:467541.

8 Kaluza W, Reuss E, Grossmann S, *et al.* Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. *J Invest Dermatol* 2000; **114**: 1180–3.

9 Arias AI, Giles B, Eiermann TH, *et al.* Tumor necrosis factor-alpha gene polymorphism in psoriasis. *Exp Clin Immunogenet* 1997; **14**: 118–22.

10 Baran W, Szepietowski JC, Mazur G, *et al.* A – 308 promoter polymorphism of tumor necrosis factor alpha gene does not associate with the susceptibility to psoriasis vulgaris. No difference either between psoriasis type I and type II patients. *Acta Dermatovenerol Alp Pannonica Adriat* 2006; **15**: 113–8.

11 Hohler T, Kruger A, Schneider PM, *et al.* A TNF-α promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. *J Invest Dermatol* 1997; **109**: 562–5.

12 Reich K, Westphal G, Schulz T, *et al.* Combined analysis of polymorphisms of the tumor necrosis factor-α and interleukin-10 promoter regions and polymorphic xenobiotic metabolizing enzymes in psoriasis. *J Invest Dermatol* 1999; **113**: 214–20.

13 Long F, Sun C, Deng D, *et al.* TNF-238A is associated with juvenile onset psoriasis in patients of Han population in Southwest China. *J Dermatol Sci* 2004; **36**: 109–11.

14 Tsunemi Y, Nishibu A, Saeki H, *et al.* Lack ofassociation between the promoter polymorphisms at positions -308 and -238 of the tumor necrosis factor alpha gene and psoriasis vulgaris in Japanese patients. *Dermatology* 2003; **207**: 371–4.

15 Li C, Wang G, Gao Y, *et al.* TNF-alpha gene promoter -238G>A and -308G>A polymorphisms alter risk of psoriasis vulgaris: a meta-analysis. *J Invest Dermatol* 2007; **127**: 1886–92.

16 Fredriksson T, Pettersson U. Severe psoriasis – oral therapy with a new retinoid. *Dermatologica* 1978; **157**: 238–44.

17 Dean AG, Sullivan KM, Soe MM. *OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version*. 2013. . www.OpenEpi.com Accessed June 23, 2016.

18 Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009; **169**: 505–14.

19 Zhu J, Qu H, Chen X, *et al.* Single nucleotide polymorphisms in the tumor necrosis factor-alpha gene promoter region alter the risk of psoriasis vulgaris and psoriatic arthritis: a meta-analysis. *PLoS ONE* 2013; **8**: e64376.

20 Jordan CT, Cao L, Roberson EDO, *et al.* PSORS2 is due to mutations in CARD14. *Am J Hum Genet* 2012; **90**: 784–95.

21 Jordan CT, Cao L, Roberson ED, *et al.* Rare and common variants in CARD14, encoding an epidermal regulator of NF-kappaB, in psoriasis. *Am J Hum Genet* 2012; **90**: 796–808.

22 Bhalerao J, Bowcock AM. The genetics of psoriasis: a complex disorder of the skin and immune system. *Hum Mol Genet* 1998; **7**: 1537–45.
23 Bowcock AM, Barker JN. Genetics of psoriasis: the potential impact on new therapies. *J Am Acad Dermatol* 2003; **49**: S51–6.
24 Gudjonsson JE, Karason A, Antonsdottir A, *et al.* Psoriasis patients who are homozygous for the HLA-Cw*0602 allele have a 2.5-fold increased risk of developing psoriasis compared with Cw6 heterozygotes. *Br J Dermatol* 2003; **148**: 233–5.
25 Moorchung NN, Vasudevan B, Chatterjee M, *et al.* A comprehensive study of tumor necrosis factor-alpha genetic polymorphisms, its expression in skin and relation to histopathological features in psoriasis. *Indian J Dermatol* 2015; **60**: 345–50.
26 Reich K, Mossner R, Konig IR, *et al.* promoter polymorphisms of the genes encoding tumor necrosis factor-α and interleukin-1β are associated with different subtypes of psoriasis characterized by early and late disease onset. *J Invest Dermatol* 2002; **118**: 155–63.
27 Mössner R, Kingo K, Kleensang A, *et al.* Association of TNF -238 and -308 promoter polymorphisms with psoriasis vulgaris and psoriatic arthritis but not with pustulosis palmoplantaris. *J Invest Dermatol* 2005; **124**: 282–4.
28 Höhler T, Kruger A, Schneider PM, *et al.* A TNF-alpha promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. *J Invest Dermatol* 1997; **109**: 562–5.
29 Tsunemi Y, Nishibu A, Saeki H, *et al.* Lack of association between the promoter polymorphisms at positions –308 and –238 of the tumor necrosis factor alpha gene and psoriasis vulgaris in Japanese patients. *Dermatology* 2003; **207**: 371–4.
30 Jacob N, Rüschendorf F, Schmitt-Egenolf M, *et al.* Promoter polymorphism at -238 of the tumor necrosis factor alpha gene is not associated with early onset psoriasis when tested by the transmission disequilibrium test. *J Invest Dermatol* 1999; **112**: 514–6.
31 Ikäheimo I, Tiilikainen A, Karvonen J, *et al.* HLA risk haplotype Cw6, DR7, DQA1*0201 and HLA-Cw6 with reference to the clinical picture of psoriasis vulgaris. *Arch Dermatol Res* 1996; **288**: 363–5.
32 Magalhaes RF, Biral AC, Pancoto JA, *et al.* Human leukocyte antigen (HLA) and single nucleotide polymorphisms (SNPs) tumor necrosis factor (TNF)-alpha -238 and -308 as genetic markers of susceptibility to psoriasis and severity of the disease in a long-term follow-up Brazilian study. *Int J Dermatol* 2010; **49**: 1133–40.

