Ann Dermatol Vol. 27, No. 2, 2015 http://dx.doi.org/10.5021/ad.2015.27.2.128

ORIGINAL ARTICLE

# *TNF*, *IL12B*, and *IFNG* Gene Polymorphisms in Serbian Patients with Psoriasis

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**Background:** Psoriasis is a common chronic inflammatory skin disease with a strong genetic basis. Cytokines such as tumor necrosis factor alpha (TNF-$\alpha$), interleukins (ILs) such are IL-12 and IL-23, and interferon gamma (IFN-$\gamma$) are released from various inflammatory and resident cells, and have been implicated in the initiation/maintenance of inflammation. Certain alleles of the aforementioned cytokines may be associated with disease susceptibility/severity. **Objective:** To investigate the association of three common functional gene polymorphisms, namely *TNF* −308 G/A (rs1800629), *IL12B* (encoding the p40 subunit of IL-12/23) +1188 A/C (rs3212227), and *IFNG* +874 T/A (rs2430561) with psoriasis development and severity in Serbian patients. **Methods:** We genotyped 130 patients with psoriasis (26 of whom also had psoriatic arthritis) and 259 controls; rs1800629 and rs3212227, and rs2430561, by real-time PCR assay. **Results:** The *TNF* GG genotype was detected at a higher frequency in patients with psoriasis compared to control subjects (OR, 1.420; 95% CI, 0.870∼2.403) without statistical significance ($p$=0.191). Lack of the *TNF* G allele was associated with lower psoriasis severity ($p$=0.007). The *IL12B* AC genotype was underrepresented in the patients with psoriatic arthritis compared to healthy subjects (OR, 0.308; 95% CI, 0.090∼1.057; $p$=0.049). The distribution of the rs2430561 allele and genotype frequencies was similar between patients with psoriasis and controls. **Conclusion:** Our study demonstrates an effect of the rs1800629 on psoriasis severity, and a marginal impact of the rs3212227 on susceptibility to psoriatic arthritis. Collectively, our results obtained in a Serbian cohort expand current knowledge regarding individual predisposition to psoriatic disease. **(Ann Dermatol 27(2) 128∼132, 2015)**

**-Keywords-**
Allele frequencies, Interferon gamma, p40 IL-12/23, Psoriasis, Real-time PCR, Tumor necrosis factor-alpha

## INTRODUCTION

Psoriasis vulgaris (PsV) is a common chronic inflammatory skin disease that is characterized by cutaneous inflammation and keratinocyte hyperproliferation, and it is often accompanied by severe complications such as psoriatic arthritis (PsA)[1]. It is believed to result from pathological immune activation in genetically prone individuals. Substantial evidence supports a central role for T helper ($T_H$) cells, such are $T_H1$ and $T_H17$ cells, in the pathogenesis of PsV[1]. A variety of cytokines have also been implicated, including the proinflammatory cytokine tumor necrosis factor alpha (TNF-$\alpha$), the $T_H1$-axis cytokines, interleukin (IL)-12 and interferon gamma (IFN-$\gamma$), and the $T_H17$-axis cytokines IL-23 and IL-17[1].

The importance of single nucleotide polymorphisms (SNPs) that affect cytokine production is well established and has been extensively studied, since SNPs may contribute to the susceptibility, severity, and clinical outcome of various

Received March 27, 2014, Revised June 24, 2014, Accepted for publication June 24, 2014

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immune-mediated chronic inflammatory diseases. One of the most studied SNPs is the polymorphism at position −308 in the promoter region of the *TNF* gene (rs1800629), which represents a G to A substitution[2]. It has been shown that transcriptional activators preferentially bind to the A allele, making AA homozygous individuals high TNF-$\alpha$ producers[3]. A recent meta-analysis identified variant genotypes and alleles of this SNP as protective in PsV[4]. Another functional SNP is the +1188 A/C polymorphism of the *IL12B* gene (rs3212227). The *IL12B* gene encodes the p40 IL-12B subunit that heterodimerizes with the p35 subunit (IL-12A) to form IL-12, or with the p19 subunit (IL-23A) to form IL-23. The rs3212227 SNP is believed to be functional, since the CC genotype confers increased p40 IL-12B expression and mRNA stability[5]. Recent genome wide association studies (GWASs) identified a significant association between this *IL12B* SNP and PsV[6,7]. A third well-defined functional SNP is the polymorphism at +874 T/A in the *IFNG* gene (rs2430561). For this SNP, a T to A change results in disruption of a nuclear factor kappa-B binding site, which leads to lower IFN-$\gamma$ production[8].
Although all three functional SNPs in the genes encoding TNF-$\alpha$, the p40 subunit of IL-12/23, and IFN-$\gamma$ were associated with PsV and/or PsA in several studies[4,9,10], there have also been a considerable number of studies that failed to replicate these results[11-14]. Obviously, the influence of various genetic variants on PsV risk may be very complex due to the existence of stratification factors, such as ethnic variance and subtype of the disease. As there is a lack of comprehensive studies in the Serbian population, our aim was to analyze the association between these three functional SNPs and PsV in Serbian patients.

## MATERIALS AND METHODS

### Patients

This study included 130 patients with PsV who were treated at the Clinic of Dermatovenereology, the Clinical Centre of Serbia. Demographic and clinical data for all patients are shown in Table 1. Patients with PsV were stratified according to their psoriasis activity and severity index (PASI) score, as described previously[15]; a diagnosis of PsA was confirmed in patients with PsV by an experienced rheumatologist. Ethnically matched blood samples from 259 healthy blood donors were obtained from the National Blood Transfusion Institute of Serbia. Informed written consent was obtained from all individuals participating in this study prior to blood sampling, and this study was approved by the ethics committees of the University of Belgrade School of Medicine, the Clinical Centre of Serbia, and the National Blood Transfusion Institute (IRB No. 29/5-15), in accordance with the Declaration of Helsinki.

### *DNA* extraction

Genomic DNA was isolated from peripheral blood that had been sampled in tubes containing ethylenediaminetetraacetic acid, using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Fermentas Thermo Fisher Scientific Inc., Vilnius, Lithuania).

### SNP detection

Detection and analysis of the *TNF* −308 G/A (rs1800629) and *IL12B* +1188 A/C (rs3212227) polymorphisms were performed using real-time PCR with commercial TaqMan probes (Applied Biosystems Inc., Foster City, CA, USA) and Maxima Probe qPCR Master Mix (Fermentas Thermo Fisher Scientific Inc.), and the cycling conditions recommended by the manufacturer of the oligonucleotide mix. The *IFNG* +874 T/A (rs2430561) polymorphism was determined as previously described[16].

### Statistical analysis

Comparisons between genotype and allele frequencies in different populations were performed using Pearson's chi-square test, Fisher's exact test, or the Kruskal-Wallis test, followed by the Mann-Whitney U test, as appropriate. All genotype frequencies were in Hardy-Weinberg equilibrium.

## RESULTS

The results of genotyping analysis for patients with PsV and healthy controls are presented in Table 2. The genotype distribution of each of the three cytokine genes examined was similar in patients with PsV and the control population (Table 3). In addition, there were no significant dif-

**Table 1.** Characteristics of the 130 patients with PsV

| Characteristic | n (%) |
|---|---|
| Gender | |
| Male | 93 (71.5) |
| Female | 37 (28.5) |
| Type of PsV | |
| Type 1 (age at onset <30 y) | 62 (47.7) |
| Type 2 (age at onset >30 y) | 68 (52.3) |
| PASI score | |
| Low (<10) | 12 (9.2) |
| Intermediate (10>PASI>20) | 63 (48.5) |
| High (>20) | 55 (42.3) |
| Psoriatic arthritis | 26 (20.0) |

PsV: psoriasis vulgaris, PASI: psoriasis activity and severity index.

**Table 2.** Allele frequencies and genotype distribution of *TNF*, *IL12B*, and *IFNG* SNPs in patients and controls

| | | Controls | PsV | | | |
|---|---|---|---|---|---|---|
| | | | Total | Type 1 | Type 2 | PsA |
| *TNF* (rs1800629) | | | | | | |
| Genotypes | GG | 196 (0.757) | 106 (0.815) | 50 (0.807) | 56 (0.824) | 22 (0.846) |
| | GA | 62 (0.239) | 23 (0.177) | 11 (0.177) | 12 (0.176) | 4 (0.154) |
| | AA | 1 (0.004) | 1 (0.008) | 1 (0.016) | 0 (0.000) | 0 (0.000) |
| *IL12B* (rs3212227) | | | | | | |
| Genotypes | AA | 173 (0.668) | 96 (0.739) | 47 (0.758) | 49 (0.720) | 22 (0.846) |
| | AC | 77 (0.297) | 32 (0.246) | 14 (0.226) | 18 (0.265) | 3 (0.115) |
| | CC | 9 (0.035) | 2 (0.015) | 1 (0.016) | 1 (0.015) | 1 (0.039) |
| *IFNG* (rs2430561) | | | | | | |
| Genotypes | AA | 74 (0.286) | 39 (0.300) | 14 (0.226) | 25 (0.368) | 8 (0.308) |
| | AT | 128 (0.494) | 61 (0.469) | 31 (0.500) | 30 (0.441) | 10 (0.384) |
| | TT | 57 (0.220) | 30 (0.231) | 17 (0.274) | 13 (0.191) | 8 (0.308) |

Values are presented as number (frequency).
PsV: psoriasis vulgaris, PsA: psoriatic arthritis.

**Table 3.** OR, with 95% CI, and *p*-value for susceptibility to the indicated condition vs. controls associated with *TNF*, *IL12B*, and *IFNG* alleles and genotypes

| | | PsV total | | PsV Type 1 | | PsV Type 2 | | PsA | |
|---|---|---|---|---|---|---|---|---|---|
| | | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) |
| *TNF* (rs1800629) | | | | | | | | | |
| Genotypes | GG | 0.191 | 1.42 (0.87～2.40) | 0.406 | 1.34 (0.67～2.67) | 0.244 | 1.50 (0.76～2.98) | 0.306 | 1.77 (0.59～5.32) |
| | GA | 0.159 | 0.68 (0.40～1.16) | 0.296 | 0.69 (0.34～1.40) | 0.269 | 0.68 (0.34～1.35) | 0.306 | 0.57 (0.19～1.70) |
| | AA | 1.000 | 2.00 (0.12～32.2) | 0.349 | 4.23 (0.26～68.5) | 1.000 | NA | 1.000 | NA |
| *IL12B* (rs3212227) | | | | | | | | | |
| Genotypes | AA | 0.155 | 1.40 (0.88～2.24) | 0.170 | 1.56 (0.82～2.94) | 0.410 | 1.28 (0.71～2.31) | 0.062 | 2.73 (0.91～8.18) |
| | AC | 0.290 | 0.77 (0.48～1.25) | 0.261 | 0.69 (0.36～1.32) | 0.597 | 0.85 (0.47～1.55) | 0.049 | 0.31 (0.09～1.06) |
| | CC | 0.349 | 0.43 (0.09～2.04) | 0.694 | 0.46 (0.06～3.66) | 0.480 | 0.42 (0.05～3.33) | 1.000 | 1.11 (0.14～9.13) |
| *IFNG* (rs2430561) | | | | | | | | | |
| Genotypes | AA | 0.764 | 1.07 (0.68～1.70) | 0.342 | 0.73 (0.38～1.40) | 0.191 | 1.45 (0.83～2.55) | 0.806 | 1.11 (0.46～2.67) |
| | AT | 0.639 | 0.91 (0.59～1.38) | 0.920 | 1.02 (0.59～1.78) | 0.435 | 0.81 (0.47～1.38) | 0.286 | 0.64 (0.28～1.46) |
| | TT | 0.806 | 1.06 (0.64～1.76) | 0.362 | 1.34 (0.71～2.52) | 0.603 | 0.84 (0.43～1.64) | 0.310 | 1.58 (0.65～3.81) |

OR: odds ratio, 95% CI: 95% confidence interval, PsV: psoriasis vulgaris, PsA: psoriatic arthritis, NA: not applicable.

ferences between patients with either early onset (type 1) or late onset (type 2) PsV and the control subjects (Table 3). When controls were compared with only those patients with PsV who also had PsA, a borderline significance was observed for the rs3212227 AC genotype (conferring protection) and AA genotype (conferring risk) of the *IL12B* gene SNP ($p=0.049$ and $p=0.062$, respectively), while genotypes for other gene SNPs did not differ between the groups (Table 3). Furthermore, *TNF*, *IL12B*, and *IFNG* allele carriage analysis did not reveal any significant differences between controls and patients with PsV or PsA, or between the two types of PsV (data not shown). However, a decreased frequency of *TNF* G allele carriers among patients with a low PASI score reinforces the possible risk effect associated with this allele or, alternatively, indicates a protective role of the other allele (A) in PsV (Table 4). None of the *TNF*, *IL12B*, or *IFNG* allele combinations was significantly different in patients compared to control subjects (data not shown).

## DISCUSSION

In this study, we investigated the association of PsV in Serbian patients with polymorphisms in the genes encoding TNF-$\alpha$, the p40 subunit of IL-12/23, and IFN-$\gamma$, which had previously been reported to affect gene expression and to have possible roles in the disease's development and severity. The results of our investigation did not identify any

**Table 4.** *TNF*, *IL12B*, and *IFNG* allele carriage in patients with PsV stratified by psoriasis activity and severity index score

| | Low PASI | Intermediate PASI | High PASI | *p*-value |
|---|---|---|---|---|
| *TNF* (rs1800629) | | | | |
| G (GA+GG) | 11 (91.7) | 63 (100.0)* | 55 (100.0)† | 0.007 |
| A (AA+GA) | 3 (25.0) | 11 (17.5) | 10 (18.2) | 0.826 |
| *IL12B* (rs3212227) | | | | |
| A (AA+AC) | 11 (91.7) | 63 (100.0) | 54 (98.2) | 0.099 |
| C (CC+AC) | 5 (41.7) | 16 (25.0) | 13 (23.6) | 0.431 |
| *IFNG* (rs2430561) | | | | |
| A (AA+AT) | 11 (91.7) | 46 (73.0) | 43 (78.2) | 0.360 |
| T (TT+AT) | 9 (75.0) | 43 (68.3) | 39 (70.9) | 0.881 |

Values are presented as number (%). Percentages refer to the portion of patients within a stratum, according to PASI score. PsV: psoriasis vulgaris, PASI: psoriasis activity and severity index. *$p=0.022$, †$p=0.032$, compared to low PASI group.

significant difference in allele frequencies or genotype distribution between our patients and healthy individuals.

The important role of TNF-$\alpha$ in the pathogenesis of PsV is well established. However, the influences of SNPs located in the *TNF* promoter region, such as rs1800629, on altered PsV and PsA risk are less clear. Published studies show conflicting results, with some authors reporting association of this SNP with disease susceptibility[4] and others failing to find any association[11,14], suggesting that this *TNF* polymorphism may have diverse effects in a population made up of various ethnicities[4]. Thus, our result could reflect ethnic specificity in the Serbian population, although another study with more patients is needed in order to confirm this. In addition, the most recent meta-analysis revealed a significant protective effect of the *TNF* −308 A allele (genotypes AA and AG) in PsV[4]. This is in line with our finding of an association of the G allele with more severe disease, although the number of patients with low PASI score was small in our study.

Data obtained for *IL12B* genotypes and alleles did not differ in our cohort of patients in comparison with disease-free individuals. This is in contrast with several studies from Europe, Asia, and America that demonstrated the protective effect of the rs3212227 C allele in PsV/PsA[10]. Such a discrepancy was probably due to a limited sample size in our study. Indeed, the frequency of the risk-conferring AA genotype in our study was clearly higher among PsV patients than the controls (0.74 vs. 0.67), but the difference did not reach statistical significance. Likewise, associations with the AC and AA genotypes were at the limits of significance when patients with PsA were compared with healthy individuals. Assuming the observed genotype frequencies, a total of more than 570 patients with PsV should have been included in our study in order to reach a power of 80% at a probability of $p=0.05$, which is beyond the number we could gather in our hospital. Interestingly, similar genotype distributions and a lack of significant difference were observed in a recently published study of Spanish patients with PsV[12].

In contrast to *TNF* and *IL12B* SNPs, studies analyzing the effects of the rs2430561 *IFNG* polymorphism on PsV development and severity are scarce, yet our results support previous evidence that this SNP does not directly influence susceptibility to PsV[11]. Lack of association of this *IFNG* polymorphism was also found in a comprehensive GWAS that looked for correlation between 438,670 SNPs in 1,539 PsV cases and 1,400 controls[13]. The only exception among these reports was a study by Baran et al.[9] that demonstrated, albeit in a limited number of individuals, the predominance of the TA heterozygote in a healthy group of individuals compared to patients with PsV.

The main caveat of our study is the relatively small sample size. Therefore, some associations could have been missed due to an insufficient number of patients and controls, as was probably the case for the *IL12B* +1188 A/C SNP. Consequently, our results are in apparent contrast to those from several previously published studies that demonstrated association between some of the SNPs and PsV. We believe that the observed differences are primarily due to the low number of patients in our study, since the odds ratios and confidence intervals obtained in our cohort are comparable to those from most other studies[4,10,12]. On the other hand, ethnic specificity of the Serbian population with regard to some of analyzed SNPs cannot be excluded.

Despite these limitations, our study is relevant because it is the first to analyze the association of gene polymorphisms with PsV development and severity in the Serbian population. It is also one of the rare studies that evaluate the relevance of the *IFNG* +874 T/A polymorphism in patients with PsV. Therefore, the results of this study will expand genetic knowledge regarding the role of three important functional polymorphisms in PsV, which may

prove valuable for future meta-analyses and, ultimately, therapy.

## ACKNOWLEDGMENT

This study was supported by the Fund of The Serbian Ministry of Education and Science (Grant No. 175038).

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