A study on tumor necrosis factor-α single nucleotide polymorphisms and psoriasis vulgaris in Vietnam

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Abstract

This study aims to evaluate the association between tumor necrosis factor-a (TNF–α) single nucleotide polymorphisms and psoriasis vulgaris. This cross-sectional study involved 140 Vietnamese patients of Kinh ethnicity diagnosed with psoriasis vulgaris. The diagnosis of psoriasis vulgaris was based on clinical signs and symptoms. We used Sanger sequencing to analyze two single nucleotide polymorphisms (SNPs), rs1799964 and rs1799724. Data were analyzed by SPSS 25. SNP rs1799964 has the highest rate of TT genotype at 62.1%, more than double the heterozygous TC genotype at 30%, CC genotype has the lowest rate at 7.9%. CC genotype of SNP rs1799724 accounted for 90%, and no homozygous genotype TT was detected. No statistically significant association was found between both SNPs and clinical features (p >0.05). The Psoriasis Area and Severity Index (PASI) was significantly lower in patients with variant alleles (*P* =0.021). Our data show a significant negative association between SNP variant alleles and the disease's severity. Studies with larger sample sizes and more biochemical indices may help identify reliably predictive markers for these SNPs.

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Introduction

Psoriasis is a chronic inflammatory immune-mediated skin disease that not only negatively affects the skin but can also have detrimental effects on joints to the extent of causing disability.[1] According to a systematic review of worldwide epidemiology, the prevalence of psoriasis in adults ranges from 0.51% to 11.43%.[2] Psoriasis pathomechanism is characterized by systemic inflammation and epidermal proliferation specified by many different immunological biomarkers.[3] Among these cytokines, tumor necrosis factor-a (TNF-α) is an important proinflammatory mediator, and its high-level expression has been found within the psoriatic lesions.[4,5] Along with the improvement of science in molecular biology and genetics, single nucleotide polymorphism (SNP) has been chosen for research in genetically complex diseases like psoriasis.[6] SNP is defined as a substitution of a single nucleotide at a specific position in the genome that is present with the fraction of considered population equal or more than 1%.[7] SNP -1031 and -857 are located in the promoter region of the TNF–α gene and both are missense substitutions.[8] Moreover, these SNPs were suggested to be related to a higher rate of TNF-α production.[9] Recently, a meta-analysis study investigated the association between the TNF-α gene SNPs and the risk of psoriasis in 10 countries around the world.[10] The results of this correlation among countries are heterogeneous due to racial and geographic differences.[10] Therefore this study aims to evaluate the association between SNPs TNF-α and psoriasis vulgaris in Vietnam.

Materials and Methods

This cross-sectional study was conducted at Ho Chi Minh City [Ho Chi Minh City (HCMC)] Hospital of Dermato-Venereology from December 2022 to April 2023. It was reviewed and approved by the Ethics Committee of Pham Ngoc Thach University of Medicine (No: 738/TĐHYKPNT-HĐĐĐ). Patient selection and data collection followed the study protocol and national ethics disciplines. We collected the written informed consent form

[Informed Consent Form (ICF)] from each patient involved in the study.

- Patients who were under 18 years old;
- Patients who were not Vietnamese and not of Kinh ethnicity;
- Patients who received systemic or biologic treatment in the past three months.

According to a previous study,[11,12] we calculated the minimum sample size needed for each group using the formula as follows:

$$n = \frac{Z^2_{1-a/2}.p.(1-p)}{d^2}, \text{ with } Z_{1-a/2} = 1.96, \text{ d} \approx 6.5\%.$$

By that, we intended to enroll 140 psoriasis patients in this study.

The diagnosis of psoriasis was based on clinical signs and symptoms, and we used the PASI score to evaluate the severity of psoriasis. The clinical features were assessed directly by experienced dermatologists. The main outcome of the study was the SNP detection; 2 ml of venous blood samples were collected and put into [Ethylene Diamine Tetra Acetic (EDTA)]-coated tubes to prepare for DNA extraction. DNA separation using the silica adsorption technique is conducted to extract DNA from blood samples. The DNA extracted samples were stored at -20^{0}C, ready for the Sanger sequencing afterward with the following primer set (Table 1):

We used Microsoft Excel and SPSS software to process data and perform statistical analyses. The associations between SNPs and other variables were assessed by the Chi-squared test (or Fisher's exact test). The comparison of continuous variables was demonstrated by Student's t-test. Statistical significance was defined by p-value <0.05.

Results

In this study, the median age of patients was 44 years, with an interquartile range of 25 years. Male patients accounted for a proportion of 65.7%. The proportion of patients without comorbidity was higher than that of the comorbidity group (63.6% versus 36.4%). Clinical severity of psoriasis was assessed using the PASI scale: the median PASI score was 14.35 points. At the same time, nail damage due to psoriasis was assessed by the [Nail Psoriasis Severity Index (NAPSI)] index and had a median value of 47 points. Table 2 shows the genotype frequency of rs1799964 and rs1799724.

Table 3 recorded the associations between SNP rs1799964 and rs1799724 with clinical characteristics. We did not identify a statistically significant difference between the gene morphologies of SNP rs1799964 and rs1799724 based on the clinical features.

Table 1. The primer set and location of SNPs.

SNP	Location	Sequence (5'-3')
rs1799964 (-1031 T→C)	chr6:31574531 (GRCh38.p14)	Forward primer: GAGCTTCAGGGATATGTGATGG
rs1799724 (-857 C→T)	chr6:31574705 (GRCh38.p14)	Reverse primer: TTGGCTTCCAAGGAACTCTG

Table 2. Genotype frequencies in TNF-α rs1799964 and rs1799724.

SNP	Genotype	n	%
rs1799964	TT	87	62.1
	TC	42	30.0
	CC	11	7.9
rs1799724	CC	126	90.0
	CT	14	10.0
	TT	0	0

Table 3. Associations between SNP rs1799964 and rs1799724 with clinical characteristics.

Clinical characteristics		rs1799964			P-value	rs1799724		P-value
		TT (n=87)	TC (n=42)	CC (n=11)		CC (n=126)	CT (n=14)	
Family history	Yes	17 (19.5%)	7 (16.7%)	3 (27.3%)	0.726[a]	25 (19.8%)	2 (14.3%)	1.000[b]
	No	70 (80.5%)	35 (83.3%)	8 (72.7%)		101 (80.2%)	12 (85.7%)	
Age of onset	<40	59 (67.8%)	26 (61.9%)	4 (36.4%)	0.120[a]	79 (62.7%)	10 (71.4%)	0.520[a]
	≥ 40	28 (32.2%)	16 (38.1%)	7 (63.6%)		47 (37.3%)	4 (28.6%)	
Psoriatic arthritis	Yes	28 (32.2%)	11 (26.2%)	2 (18.2%)	0.548[a]	37 (29.4%)	4 (28.6%)	1.000[b]
	No	59 (67.8%)	31 (73.8%)	9 (81.8%)		89 (70.6%)	10 (71.4%)	
Disease severity scale (PASI)	Mild	27 (31.0%)	19 (45.2%)	3 (27.3%)	0.195[a]	42 (33.3%)	7 (50.0%)	0.466[b]
	Moderate	26 (29.9%)	15 (35.7%)	4 (36.4 %)		42 (33.3%)	3 (21.4%)	
	Severe	34 (39.1%)	8 (19.0%)	4 (36.4%)		42 (33.3%)	4 (28.6%)	
Nail severity scale (NAPSI)		48 (48)	37 (43)	56 (71)	0.152[c]	48 (47.25)	40 (51)	0.436[d]

[a]Chi-squared test; [b]Fisher's exact test; [c]Kruskal Wallis test; [d]Mann-Whitney U test.

Table 4. Associations between variant alleles and clinical characteristics.

Clinical characteristics		Presence of variant alleles Yes (n=64)	No (n=76)	P
Family history	Yes	12 (18.8%)	15 (19.7%)	1.000[a]
	No	52 (81.2%)	61 (80.3%)	
Age of onset	<40	37 (57.8%)	52 (68.4%)	0.220[a]
	≥ 40	27 (42.2%)	24 (31.6%)	
Psoriatic arthritis	Yes	15 (23.4%)	26 (34.2%)	0.194[a]
	No	49 (76.6%)	50 (65.8%)	
Disease severity (PASI) (Median/IQR)		11.65 (6.45-20.15)	16.90 (9.20-23.18)	0.021[b]
Nail severity scale (NAPSI) (Median/IQR)		40 (18.50-64)	49 (28-76)	0.078[b]

[a]Chi-squared test; [b]Mann-Whitney U test

However, when we evaluated patients for the presence of TNF-a polymorphisms, we discovered a significant difference in the PASI score between the two groups (P=0.021).

Discussion

Psoriasis is a disease with a complex pathogenesis characterized by the interaction between both genome-specific factors and environmental factors. This complicated interaction leads to clinical and paraclinical characteristics that differ between individuals and races.[13] The results of analyzing SNP rs179964 showed that the homozygous TT genotype had the highest frequency of up to 62.1%, more than double in comparison to the heterozygous TC genotype of 30%, whereas the CC genotype had the lowest frequency of 7.9%. These results are consistent with the results of genetic analysis on Caucasian psoriasis patients in Spain conducted by Gallo et al. (2012)[14] and Cabaleiro et al. (2013)[15] in terms of genotype and allele frequencies. Moreover, similar results were also observed in German Caucasian plaque psoriasis patients in a case-control study with a sample size of up to 1126 individuals by Reich et al.[12] Most recently, in 2022, a study on Caucasian Italian patients with psoriasis also demonstrated the same results regarding both genotype and allele frequencies.[16] However, the results of genetic analysis of SNP rs1799724 did not find any cases of homozygous variant allele T and homozygous CC genotype accounted for the majority of surveyed cases up to 90%, while the heterozygous genotype only accounts for 10%. We found the T-variant allele frequency of rs1799724 to be very low, at only 5%. In contrast to the similarity in genotype and allele frequencies in rs1799964, the sequencing results of rs1799724 have statistically significant differences compared to two studies on Caucasian patient populations in Spain by Cabaleiro et al.[15] and Gallo et al.[14] However, there was a statistically significant difference in the genotype and allele frequencies in the study of Aslihan Gulel, et al. on Turkish Caucasian patients.[15,16] This shows that geographical and anthropological factors affect the distribution of TNF-a SNPs, thereby confirming the importance of in-depth genomic studies on each specific ethnic group.

A study by Higuchi, et al. on Japanese people showed that variant alleles of both SNPs of the TNF-α gene rs1799964 and rs1799724 nearly double the amount of TNF-α produced in the body when stimulated by different agents[9] (Table 4). TNF-α is a pro-inflammatory cytokine that amplifies inflammation through Th1, Th17 cells, cytokines such as [Interleukin (IL)]-12, IL-23, IL-8, and especially the positive feedback with the nuclear factor kappa B (NF-κB). Furthermore, the concentration of TNF-α in the blood, as well as the expression level of mRNA of the TNF-α gene have been shown to be closely related to the pathogenesis of psoriasis and the severity of the disease.[17-21] When analyzing rs1799964 and rs1799724 separately, we did not find any significant associations with clinical features of psoriasis. Our results are similar to the study of Daprà, et al. on Italian Caucasian patients with psoriasis.[16] However, in terms of the presence of any variant alleles, we discovered that the PASI score was significantly higher in the non-SNP group. The result indicated that the SNP may affect the production of TNF-a, which in turn aggregates the severity of psoriasis. We also hypothesize that patients with SNPs may not adapt effectively with TNF-a inhibitors. Due to the limited resources, we could not evaluate the serum TNF-a level in the study patients to prove our hypothesis. We propose other studies with larger sample sizes and involving laboratory data to elicit the link between SNP and clinical features and treatment.

Conclusions

The proportion of SNPs on TNF-encoded genes, *i.e.* rs1799964 and rs1799724, was relatively high. The SNPs seemed to affect the severity of psoriasis. Though the study could not conclude the association between the SNPs and their expression profiles, we still propose hypotheses for future studies to move forward and fill the gap between gene and clinical contexts of psoriasis.

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