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.

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ABSTRACT

Background: Genes encoded within the MHC complex on chromosome 6 are thought to play an important role in the pathogenesis of psoriasis. A potential candidate is tumor necrosis factor alpha (TNF-α) gene. Psoriasis features an increased activity of the proinflammatory cytokine TNF-α in affected lesions. TNF-α promoter region contains several polymorphisms, including G/A transition at position -308, which influences transcriptional activity of TNF-α. This study was performed to investigate the association between TNF-α single nucleotide polymorphism and susceptibility for psoriasis vulgaris.

Material and methods: DNA from 78 patients with psoriasis vulgaris and 74 healthy volunteers with no personal and family history of psoriasis was investigated. TNF-α promoter gene single nucleotide polymorphism (SNP) in position – 308 was evaluated by PCR-SSP. The results were compared between group of psoriatic patients, divided into early onset of psoriasis (type I) and late onset of psoriasis (type II) subgroups, and healthy subjects.

Results: There were no significant differences in the polymorphism of TNF-α promoter -308 (genotype distribution, allele frequencies) between psoriasis patients and healthy controls. Similar results were obtained analyzing subgroups of psoriasis patients (type I and type II of psoriasis) and gender groups.

Conclusion: TNF-α promoter single nucleotide polymorphism (-308) is not associated with susceptibility to psoriasis vulgaris.

KEY WORDS

psoriasis, TNF α, A-308 promotor polymorphism, no association

Introduction

Psoriasis is a chronic inflammatory disease affecting about 1-3% of Caucasian population (1). This disorder has a heterogeneous genetic background (2) and one of the most consistent associations exist with the genes of the major histocompatibility complex (MHC), which is located on the short arm of chromosome 6 (3). Many of the human leukocyteantigens (HLA) associated with psoriasis are in linkage disequilibrium and possible ex-

Table 1. Distribution of TNFα (-308 G/A) genotypes and allele frequencies among 78 patients and 74 controls.

TNF-α-308					
Genotype	**Psoriasis**		**Controls**		**p**
	n	f	n	f	
GG	62	0.79	57	0.77	$p>0.8 p_{adj}>0.9$
GA	16	0.21	16	0.22	
AA	0	0	1	0.01	
Carriage	n	f	N	f	$p=0.9 p_{adj}=0.95$
G	78	0.83	74	0.82	
A	16	0.17	16	0.18	
Allele	n	f	N	f	$p=0.6 p_{adj}>0.7$
G	140	0.9	130	0.88	
A	16	0.1	18	0.12	

n – number, f – relative frequency, G – guanine, A – adenine

planation of this could be, that other genes encoded within the MHC are involved in the pathogenesis of psoriasis. One such candidate is the tumor necrosis factor α (TNF-α) gene (2,4). TNF-α is an important inflammatory mediator and its expression has been shown to be increased in psoriatic lesions (5). Monocytes of psoriatic patients produce higher amount of TNF-α than in controls (6). TNF-α stimulates the release of interleukin 8 (IL-8) by keratinocytes and fibroblasts (7,8) and transforming growth factor α(TGF-α) by keratinocytes. Both IL-8 and TGF-α may be involved in autocrine stimulation of keratinocytes proliferation in psoriatic lesions (9).

TNF-α is encoded within the HLA class III region of the MHC between HLA-B and HLA-DR (10,11). Single nucleotide conversions from guanine (G) to adenine (A) at position -308 and -238 are the most common in white populations (12,13). These transitions have been shown to influence TNF-α expression, a position -308 allele A is associated with a 6-7 fold increased transcriptional activity (14) and higher constitutive and inducible levels of TNF-α (15). Although an association of polymorphisms in the TNF-α promoter region with psoriasis susceptibility has been reported in few previous studies, this problem remains still a matter for further research (16). In this study, we evaluated the association between TNF-α polymorphism at position – 308 and the predisposition to psoriasis vulgaris in a Polish cohort.

Table 2. Distribution of TNFα (-308 G/A) genotypes and allele frequencies between type I and type II psoriatic patients.

TNF-α-308					
Genotype	**Type I**		**Type II**		**p**
	n	f	n	f	
GG	62	0.79	57	0.77	$p>0.8 p_{adj}>0.9$
GG	45	0.83	17	0.71	$p>0.2 p_{adj}>0.3$
GA	9	0.17	7	0.29	
AA	0	0	0	0	
Carriage	n	f	n	f	$p>0.3 p_{adj}>0.4$
G	54	0.86	24	0.78	
A	9	0.14	7	0.22	
Allele	n	f	n	f	$p>0.2 p_{adj}>0.3$
G	99	0.92	41	0.86	
A	9	0.08	7	0.14	

n – number, f – relative frequency, G – guanine, A – adenine

Table 3. Distribution of TNFα (-308 G/A) allelic frequencies in the different populations in other studies.

	present study	Tsunemi et al. (17)	Kim et al. (16)	Nishibu et al. (18)	Reich et al. (1)	Hohler et al. (4)
GG	79%	98.8%	96%	97.3%	74.8%	87%
GA	21%	1.2%	6.8%	0%	25.2%	11%
AA	0%	0%	0%	2,7%	0%	2%

G – guanine, A - adenine

Material and methods

Patients and controls

Seventy eight patients with psoriasis vulgaris (37 females (47.43%) and 41 males (52.57%)) were included into the study. They were divided into two groups: early onset psoriasis (type I - onset not later than at the age of 40 years and positive family history of psoriasis) and type II psoriasis (onset after the age of 40 years and negative family history of psoriasis). The mean age of the type I group was 44.12±11.80 years (range 19-67 years) and it included 28 females (51.85%) and 26 males (48.15%). The type II group with a mean age of 61.37±11.21 years consisted of 9 females (37.5%) and 15 males (62.5%). The control cohort included 74 healthy, unrelated subjects (33 females and 41 males) with no family history of psoriasis. The study was approved by the Commission of Bioethics at Wroclaw Medical University (KB 359/2003).

TNF-α genotyping

DNA was isolated from the whole peripherial blood taken on EDTA with the use of Qiagen DNA Isolation Kit (Qiagen GmbH, Hilden,Germany). Biallelic polymorphism within the promoter region of TNF-α gene at position -308 was determined by PCR-SSP technique employing commercial primers (One Lambda, Inc. Canoga Park, CA, USA): sense, 5'- AGGCAATAGG-TTTTGAGGGCCAT-3', antisense, 5'- GAGCGTCTGCT-GGCTGGGTG-3'. The use of this kit (due to number of primer mix combination) allows to assess the presence of particular TNF-α haplotypes (ie. high – AA, AG and low producers - GG). For each polymorphic site one PCR reaction was carried out on DNA template with a pair of specific primers, the additional control primers, reaction mix (provided by a manufacturer), and Taq polymerase (Invitrogen, USA) in a total volume of 10μl. Amplifications were performed in MJ Research Apparatus (Watertown, MA, USA). PCR cycling conditions were as follows: 96°C for 130s, 63°C for 60s, followed by nine cycles of 96°C for 10s, 63°C for 60s, and followed by 20 cycles of 96°C for 10s, 59°C for 50s, 72°C for 30s, ending with 4°C. PCR products were analyzed electrophoretically in 2% agarose gel and visualized under UV.

Evaluation and statistical analysis

Genotype and allele frequencies were compared between the study groups by the Chi^2-test with Yates correction or Fisher's exact test when necessary. *P* values less than 0.05 were considered statistically significant.

Results

There were no significant differences in the distribution of the polymorphism of TNF-α promoter -308 between psoriasis patients and healthy controls. Frequencies in genotypes were similar between patients and control group (GG 0.79 vs 0.77 and GA 0.21 vs 0.22; p>0.8, p_{adj}>0.9 respectively) (Table 1). Homozygosity for the TNF-α – 308A allele was present only in one patient, in control group. Analyzing subgroups of psoriasis patients separately, no statistically significant differences between type I - type II psoriasis and gender groups were found (GG 0.83 vs 0.71 and GA 0.17 vs 0.29; p>0.2, p_{adj}>0.3 respectively). Table 2.

Similarly comparing the alleles carriage no significant differences were found between psoriatic patients and healthy controls (G carriage 0.83 vs 0.82, A carriage 0.17 vs 0.18; p=0.9, p_{adj}=0.95) and between type I - type II psoriatic patients subgroups (G carriage 0.86 vs 0.78, A carriage 0.14 vs 0.22; p>0.3, p_{adj}>0.4). The total numbers of alleles were also similar in all investigated groups with no statistical differences - psoriasis vs healthy controls (G alleles 140 vs 130, A alleles 16 vs 18; p=0.6, p_{adj}>0.7) and psoriatic patients type I vs psoriatic patients type II (G alleles 99 vs 41, A alleles 9 vs 7; p>0.2, p_{adj}>0.3).

Discussion

Results of the present study showed no association between TNF-α promoter polymorphism (-308) and psoriasis vulgaris in a Polish population. This study con-

firms the finding of the previous studies, reporting the lack of association between TNF-α – 308 promoter polymorphism and susceptibility to psoriasis. Our results concerning frequency of TNF-α polymorphism in psoriatic patients are similar to that published earlier by other authors (1,4,16-18). The most commonly observed genotype (both in patients and control group) was GG (low TNF-α producer), AA homozygosity (high TNF-α producer) was only accidentally present in population. The results of the present study were compared to other studies in table 3.

Comparing our results to that of Kim at al. (16) who investigated only Korean population, and Nishibu et al. (18), Tsunemi et al. (17) who evaluated TNF-α polymorphism in Japanese patients, it seems that relationship between psoriasis and TNF-α -308 polymorphism does not depend on racial differences. Both in Caucasians, which was confirmed by the present study and earlier one reported by Reich et al. (1), and in Asians TNF-α -308 single nucleotide polymorphism did not associate with psoriasis vulgaris. It is very interesting that in Asians genotype GA seems to be more rare than in Caucasians, as presented in table 3. It is possible that -308 polymorphism does not itself regulate the production of TNF-α, but is in linkage disequlibrium with TNF-α – 238 (A) alleles, which were found to be increased in early onset of psoriasis (1).

In conclusion, the results based on the examination of a -308 promoter polymorphism of tumor necrosis factor alpha gene suggest that there is no direct link between genotype distribution, alleles carriage and overall number of alleles and susceptibility for psoriasis vulgaris. However, further studies are required to determine completely the molecular basis of the susceptibility to psoriasis which could be different for races and will definitely broaden our knowledge on the pathogenesis of this disorder.

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