Tumor necrosis factor-α -308G/A polymorphism is associated with active vitiligo vulgaris in a northeastern Mexican population

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Abstract. Vitiligo is a skin disease characterized by depigmentation. Its etiopathogenesis is unclear, but it has been associated with autoimmune processes. Gene polymorphisms in the tumor necrosis factor-α (TNF-α) have been associated with several imflammatory diseases. In particular, the -308G/A polymorphism in the gene promoter region has been reported to be associated with increased plasma levels of TNF-α and with an increased risk to develop autoimmune diseases. To date, this polymorphism has not been associated with vitiligo. To assess a possible association between the TNF-α -308G/A and vitiligo vulgaris (VV), 198 vitiligo patients and 395 control subjects were recruited for the study. A complete demographic and clinical profile of each case was registered to analyze the possible risk factors of vitiligo. Genomic DNA isolated from peripheral blood was subjected to PCR-RFLP for genotyping of the TNF-α -308G/A polymorphism. Causal associations were determined by χ^2 test and their respective OR was assessed in a 2x2 contingency table. When population variables of type of vitiligo, gender, age of disease onset, and active disease status were considered, an association between active VV and the TNF-α GA genotype was found (P=0.0295, OR=2.0; 95% CI 1.01-3.93). All other variables were irrelevant to vitiligo. Our data suggest a possible association between the TNF-α -308 GA genotype and the active form of VV in a Mexican population.

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Introduction

Vitiligo is characterized by skin depigmentation due to the lack of melanocytes in the dermis or inability to produce melanin. This depigmentation may be attributed to undifferentiated, non-melanogenic melanocytes or to selective destruction of these cells (1).

Worldwide prevalence of vitiligo is between 0.1 and 2.0%, but it varies among different ethnic groups: 0.14% in Russia, 1.0-2.5% in the US and Japan, 4.0% in Mexico, and 8.8% in India (2,3). Although the age of onset is variable, most of the patients develop symptoms between 10 and 30 years of age, and the disease is evident in almost half of the patients by the age of 30.

The etiology of vitiligo is unknown. Different factors and complex interactions have been linked to this disorder. A family history of the disease accounts for approximately 20% of cases (4,5). Several statistical models have been proposed for the etiology of vitiligo based on family predisposition, age of onset, clinical phenotype, and environmental factors. These factors could also interact with each other to produce different clinical subtypes of this disease.

Several candidate genes have been linked to vitiligo, such as the human leukocyte antigen system (HLA), the cytotoxic T-lymphocyte antigen 4 (CTLA4), the catalase (CAT) gene and others (6,7). Tumor necrosis factor-α (TNF-α) is of particular interest in this regard as it has been previously associated with other autoimmune diseases. This gene resides within 6p21.3, close to the MHC class III locus (8,9). The minor allele -308 A has a prevalence of 0.2 in Caucasians and has been associated with increased risk to develop autoimmune diseases in this ethnic group (10). TNF-α may be a candidate gene for vitiligo, considering its previous association with diseases characterized by tissue inflammation, local metabolic disturbances and destruction (11,12).

A previous report determined the frequency of the -308 TNF-α polymorphism in Mexican populations (13), and it

was strongly correlated with a wide variety of autoimmune and inflammatory pathologies (11,12,14,15). Studies concerning the TNF-α -308G/A polymorphism and its relation to vitiligo have been carried out in Iranian and Turkish populations with contrasting results (16,17); however, no previous research describes the possible involvement of this polymorphism in vitiligo in Mexican patients. Therefore, the aim of this study was to investigate the relationship between the TNF-α -308G/A polymorphism and vitiligo variants in northeastern Mexican patients.

Materials and methods

Subjects. A group of 198 northeastern Mexican patients (states of Coahuila, Nuevo León, San Luis Potosí, Tamaulipas, and Zacatecas) were recruited at the Service of Dermatology of the University Hospital, UANL in Monterrey, Mexico, from November 2009 to May 2010. The University Hospital-UANL IRB approved and registered this study under the code DE08-008. After giving their informed consent, patients were interviewed and evaluated to confirm the diagnosis of vitiligo. The information gathered from the patients included age, gender, birthplace, clinical history of the patient and his or her family, age of disease onset, previous treatment (if any), vitiligo lesions in previously traumatized regions (Koebner's Syndrome), stress level at the time of disease onset (as perceived by the patient), class and distribution of vitiligo in the patient and pedigrees. Classic subtypes of vitiligo were further classified as focal (one or more maculae in an unsegmented pattern); segmented (one or more maculae distributed like a dermatome or zosteriform), vulgaris (symmetric or asymmetric distribution of maculae in one or more areas) and universal (>80% skin depigmentation). The course of the disease was classified as active (progressive, appearance of new maculae in the last 6 months) or stable. The control group consisted of 395 subjects from a general unbiased healthy population recruited at the Blood Bank of the same hospital. For purposes of this study, a Mexican was defined as a person born in Mexico, whose last three ascending generations were also born in the country.

Experimental procedure. Three samples of 3-5 ml of peripheral venous blood were drawn from each patient. One sample was used for thyroid profile laboratory analyses and the second was used for blood cell count. The third sample was centrifuged, the plasma was aliquoted and stored at -70˚C for further analysis, and the buffy-coat was processed for DNA isolation by the salting-out method.

TNF-α -308 genotyping. Genotyping was performed by PCR-RFLP as modified by Chen and Cols (18). PCR was carried out using an MJ Research PTC'100 thermal cycler as follows: a total amount of 250 ng genomic DNA, 0.5 μM primers (TNF-308F, 5'-gggacacacaagcatcaagg-3', TNF-308R, 5'-aataggttttgagggccatg-3'), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 2.5 units of Taq DNA polymerase (Promega); 94˚C/30 sec, 61˚C/30 sec and 72˚C/30 sec (35 cycles). The PCR samples (~0.5 μg) were then digested overnight with *Nco*I (New England Biolabs) at 37˚C. Digested amplicons were analyzed by electrophoresis in 3% agarose gels using ethidium

Table I. Demographic and clinic variables of the vitiligo subjects.

Vitiligo type	Gender		Age (years)	Age of onset		Activity		Family history of vitiligo			Thyroid pathology
	Female n (%)	Male n (%)		<30 years n (%)	≥30 years n (%)	Active n (%)	Stable n (%)	1st and 2nd degree n (%)	3rd and 4th degree n (%)	<30 years n (%)	≥30 years n (%)	n (%)
VV	93 (46.97)	83 (41.92)	27±15.5	106 (53.54)	70 (35.35)	89 (44.90)	87 (43.9)	57 (28.79)	33 (16.66)	62 (31.48)	28 (14.14)	40 (20.20)
UV	2 (1.01)	1 (0.50)	12±12.6	3 (1.52)	0	-	3 (1.5)	2 (1.01)	1 (0.50)	0	0	1 (0.50)
FV	8 (4.04)	10 (5.05)	27±19.5	10 (5.05)	8 (4.04)	6 (3.00)	12 (6.1)	2 (1.01)	2 (1.01)	3 (1.52)	6 (3.03)	3 (1.52)
SV	1 (0.50)	-	3	1 (0.50)	0	1 (0.50)	-	1 (0.50)	0	1 (0.50)	0	0

VV, vulgaris vitiligo; UV, universal vitiligo; FV, focal vitiligo; SV, segmental vitiligo.

Table II. TNF-α -308 genotype distribution and allele frequencies in patients with vitiligo and control samples.

	Vitiligo n (%)	Control n (%)	χ^2 test	P-value	OR	95% CI
TNF-α -308G/A polymorphism						
Genotypes						
GG	177 (89.39)	356 (90.13)	0.08	0.78	0.92	0.51-1.68
GA/AA	21 (10.61)	39 (9.87)	0.08	0.78	1.08	0.60-1.96
TNF-α -308G/A polymorphism						
Alelles						
G	375 (94.7)	748 (94.68)	0	0.99	1.00	0.57-1.78
A	21 (5.3)	42 (5.32)	0	0.99	1.00	0.56-1.76

TNF-α, tumor necrosis factor-α.

bromide staining and documented in a UVP M-26 (Upland, CA, USA).

Detection of TNF-α levels in serum. TNF-α serum levels were determined using the Human TNF-Immunoassay Quantikine® (R&D Systems) following the manufacturer's instructions.

Statistical analysis. Collected data were organized in a database, and statistical calculations were performed with the SPSS v17.0 software (SPSS, Inc. Chicago, IL, USA) and the EPIINFO statistical program v3.5.1 (USD Incorporated 2008, Stone Mountain, GA, USA). Hardy-Weinberg equilibrium was obtained using a goodness-of-fit test. Genotypic dependence between cases was determined by the χ^2 test, and OR was calculated from 2x2 contingency tables. Mann-Whitney U test and Spearman's Rho were used to determine the correlation of age, gender and genotype for each studied group. P-value <0.05 was considered to indicate a statistically significant difference.

Results

The main clinical and demographic features of the participants are illustrated in Table I. Vitiligo vulgaris (VV) was the most common type of disease in our study (88.9%): active and stable forms of this variant represented 50.6 and 49.4% of the cases, respectively. Because of the prevalence of vitiligo types, we decided to focus the analyses of this study on the VV type.

A goodness-of-fit test demonstrated that the TNF-α genotypes in the controls were present in Hardy-Weinberg equilibrium (χ^2 test=3.54, P=0.06, 1 degree of liberty). The TNF-α -308 allele and genotype frequencies among the different types of vitiligo and the control are shown in Table II. A total of 89.39% vitiligo patients were homozygous (GG) and the remaining 10.61% were heterozygous (GA) (10.61%). Homozygous (AA) genotype was absent among the patients. We decided to compute for the load of the allele A by adding the frequencies for the GA and AA (GA/AA) genotypes in both cases and controls. The TNF-α -308 A load was not different between vitiligo patients and the controls (P=0.78, OR=1.08; 95% CI 0.6-1.96).

When considering population variables independently, as gender (data not shown), type of vitiligo, age of disease onset, and active vs. stable disease status, an association was found between the active VV and the allele A load (P=0.0295, OR=2.0; 95% CI 1.01-3.93) (Table III).

Serum level analyses of TNF-α were determined in 5 patients with active VV, 5 patients with stable VV (all TNF-α -308G/A positive), 5 homozygous (GG) undetermined VV patients and 16 control. All analyzed samples showed levels <7.8 pg/ml (reference values, 7.8-500 pg/ml).

Discussion

Melanocyte cell death by immune-mediated apoptosis is considered an important susceptibility factor for vitiligo. Cytokines as TNF-α favor the apoptotic process (19). Accordingly, TNF-α is an important candidate for genetic susceptibility to vitiligo. It has been observed that the TNF-α -308G/A gene polymorphism confers a significant risk for acquiring vitiligo in an Iranian study (16), but it has not been associated with the disease in the Turkish population (17). The TNF-α -308G/A polymorphism has not been previously assessed for vitiligo in populations of the Americas. We did not find an association between vitiligo in general and the studied polymorphism, but an interesting association was found for active VV and the A allele load of the polymorphism (P=0.0295), which conferred an increased OR of 2.0. Despite this association, no differences in TNF-α plasma levels were observed between cases and control. Microenvironment, TNF-α fluctuations, and altered physiological responses may affect circulating levels, while differential uptake of the inflammatory factor in the affected skin areas may be increased, yet this hypothesis requires further analyses.

In conclusion, we report that the TNF-α -308 GA genotype is associated with active VV and conferred an increased OR for this type of skin disorder in a northeastern Mexican population.

Table III. TNF-α -308 genotype distribution and allelic frequencies in the patients with vitiligo vulgaris.

A, Patients with active vitiligo vulgaris (AVV).

	AVV n (%)	Control n (%)	χ^2 test	P-value	OR	95% CI
TNF-α -308G/A polymorphism Genotypes						
GG	73 (82.02)	356 (90.13)	4.74	0.0295	0.50	0.25-0.99
GA/AA	16 (17.98)	39 (9.87)	4.74	0.0295	2.00	1.01-3.93
TNF-α -308G/A polymorphism Alelles						
G	162 (90.9)	748 (94.68)	3.48	0.062	0.57	0.30-1.08
A	16 (9.1)	42 (5.32)	3.48	0.062	1.76	0.92-3.32

B, Patients with stable vitiligo vulgaris (SVV).

	SVV n (%)	Control n (%)	χ^2 test	P-value	OR	95% CI
TNF-α -308G/A polymorphism Genotypes						
GG	82 (94.25)	356 (90.13)	1.46	0.226	1.80	0.65-5.36
GA/AA	5 (5.75)	39 (9.87)	1.46	0.226	0.56	0.19-1.54
TNF-α -308G/A polymorphism Alelles						
G	169 (97.13)	748 (94.68)	1.83	0.176	1.90	0.71-5.54
A	5 (2.87)	42 (5.32)	1.83	0.176	0.53	0.18-1.42

TNF-α, tumor necrosis factor-α.

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