Association between PTPN22 C1858T polymorphism and alopecia areata risk

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Abstract. Alopecia areata (AA) is a skin condition in which hair is lost from certain or all areas of the body. This condition has been described as an immune-mediated complex genetic disease, characterized by the presence of lymphocytes that are directed to the hair follicles in the anagen phase. The gene encoding the protein tyrosine phosphatase, non-receptor type 22 (PTPN22), which is exclusively expressed in immune cells, has been considered as a risk factor associated with a number of autoimmune diseases. In AA, the single nucleotide polymorphism, rs2476601, has been identified as a risk factor in several populations. The aim of the present study was to investigate the effect of PTPN22 C1858T inherited genetic polymorphism on the predisposition to severe forms of AA, in a case-control study on individuals. The study included 64 unrelated patients diagnosed with several types of AA, as well as 225 healthy unrelated subjects. The DNA samples were genotyped for PTPN22 C1858T polymorphism using the polymerase chain reaction-restriction fragment length polymorphism technique. Causal associations were determined by χ^2 test and their respective odds ratio (OR) was assessed in a 2x2 contingency table. The results demonstrated a significant association of the T allele [P=0.040; OR=3.196; 95% confidence interval (CI), 0.094-10.279] and the CT genotype (P=0.038; OR=3.313; 95% CI, 1.008-10.892) with patchy AA. In conclusion, the results of the present study suggested the possible involvement of the T allele of the PTPN22 C1858T SNP as a genetic risk factor for this type of AA in the population studied.

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Introduction

Alopecia areata (AA) is a condition of the skin in which hair is lost from certain or all areas of the body, typically from certain areas of the scalp. AA can appear at any age, although the condition more frequently occurs in young patients (1), and is characterized by circular or oval bald spots. According to the disease progression, the condition can spread to the entire scalp (alopecia totalis) or the entire body (alopecia universalis). Well-defined pattern hair loss is localized to the sides and lower back of the scalp (alopecia ophiasis) (2).

According to a previous study, it is estimated that AA accounts for 2% of the dermatologist consultations (1). The average prevalence of AA in the general population is 1.7%, ranking between 0.1 and 6.9% depending on the ethnic group studied (3,4). Generally, 60% of patients develop their first bald spot at the age of 20 years, and ~60% of cases occur between the ages of 10 and 25 years (4).

AA has been described as a immune-mediated complex genetic disease, characterized by the presence of lymphocytes that are directed to hair follicles in the anagen phase (1). Skin-resident T cells are critical for immune surveillance; however, they may also be pathogenic in dermatological diseases, including AA, psoriasis and allergic contact dermatitis (5). The presence of infiltrating $CD4^+$ and $CD8^+$ lymphocytes has been described in the intrafollicular and perifollicular region of the hair follicle, which supports the hypothesis of an autoimmune origin of this disease (2,4).

Different research groups have described the involvement of various genes that confer susceptibility to the development of AA (6-9). The gene encoding for the protein tyrosine phosphatase, non-receptor type 22 (PTPN22), is exclusively expressed in immune cells, and has been considered as an important risk factor out of the major histocompatibility complex

(MHC) molecules. In particular, its non-synonymous C1858T substitution in codon 620 [R620W variant; single-nucleotide polymorphism (SNP), rs2476601] is associated with a number of autoimmune diseases (10). It has been suggested that the arginine (R) to tryptophan (W) amino acid change leads to the rapid degradation of the phosphatase, resulting in increased capacity response in the T cells and dendritic cells (11). The impaired function of PTPN22 in T cells may induce the production of autoantibodies by the hyper-responsive B-cells and the development of autoimmune diseases (12).

In AA, the SNP rs2476601 has been identified as a risk factor in several populations including: i) In Belgian-German patients with a family history of AA or early onset (13); ii) in Egyptian patients, in which the predisposition to severe forms of the disease and the response to treatment were investigated (14); and iii) in North American patients with severe AA forms (15). However, only a small number of studies have been conducted on the PTPN22 gene in Mexico and they are limited to other immune pathologies (16,17).

The aim of the present hospital case-control study was to investigate the effect of the PTPN22 C1858T genetic polymorphism on the predisposition to severe forms of AA in Mexican patients from Northeast Mexico.

Patients and methods

Selection of subjects and controls. The present study included 64 Mexican patients diagnosed with AA at the Dermatology Department of the University Hospital 'Dr Jose Eleuterio González' (Monterrey, Mexico), recruited between February 2009 and December 2009. Prior to participation, written informed consent was obtained from the patients. Ethical approval was provided by the Institutional Review Board of the University Hospital 'Dr Jose Eleuterio González', and the study was registered under the code DE09-001. Details regarding the clinical and demographic characteristics, AA type and distribution, family history of the disease and other immune diseases for each patient were obtained through interview, a questionnaire, review of medical records and clinical evaluation.

The control subjects consisted of 225 healthy unrelated Mexican individuals. Although typically a patient:control subject ratio of 1:1 or 1:2 is used, certain studies have indicated that a ratio of 1:4 produces the maximum gain in the study in relation to its power (18,19). Peripheral blood samples were obtained during a previous study (unpublished data) realized in the population of Northeast Mexico at the blood bank of the University Hospital 'Dr Jose Eleuterio González', where a negative medical family history for autoimmune/inflammatory diseases was assessed. For the purpose of the present study, individuals classified as Mexican were those born in Mexico and whose last three ascending generations were also born in Mexico.

DNA isolation. Genomic DNA was isolated from the peripheral venous blood using the salting-out method and ethanol precipitation (20). DNA was resuspended in Tris-EDTA (pH 7.8) at a final concentration of 0.1-1.0 μg/μl prior to use.

PTPN22 C1858T genotyping. Genotyping was performed using a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay, modified from the method presented by Harrison *et al* (21). PCR was performed using an MJ Research PTC-100 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA), as follows: The total amount of the PCR mixture consisted of 250 ng genomic DNA, 0.5 μM of each PTPN22 primer (forward, 5'-ATGTTGCTTCAACGG AATTT-3', and reverse, 5'-CATGCTGCTATTGCTCTG CT-3'), 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase (Promega Corporation, Madison, WI, USA). PCR was performed at 94˚C for 1 min, 58˚C for 1 min and 72˚C for 1 min (33 cycles). Next, the 400 base-pairs (bp) PCR amplicons (~1 μg) were digested overnight with *Xcm*I (New England Biolabs, Ipswich, MA, USA) at 37˚C, and the generated fragments were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide and analyzed in a gel documentation system (UVP model M-26; UVP, LLC, Upland, CA, USA). The products obtained during this process were the 238 and 162 bp fragments for the mutant T allele, and 400 bp (non-digested PCR product) for the wild-type C allele.

Statistical analysis. Data collected from the patients were organized in a database and statistical calculations were performed using the SPSS version 17.0 software for Windows (SPSS, Inc., Chicago, IL, USA) and the Epi-INFO™ 7 statistical software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Hardy-Weinberg equilibrium (HWE) for the alleles was analyzed using a Fisher's exact test, Pearson's χ^2 (degree of freedom = 1) and log-likelihood ratio χ^2 (degree of freedom = 1). Genotypic dependence between patients and controls was determined by the χ^2 test, and odds ratio (OR) was calculated from 2x2 contingency tables. In addition, Mann-Whitney U test and Spearman's rho test were employed to determine the correlation of age, gender, immune diseases and genotype for each study group. Yates' correction was applied when the value of an expected frequency was <5. Differences were considered to be statistically significant when $P<0.05$.

Results

Clinical characteristics of the participants. The present study analyzed the genotype distribution and allele frequency for the C1858T polymorphism located within the gene coding region of PTPN22. A total of 289 subjects were included, which were divided into two groups categorized based on the presence (patient group, n=64; female, 38; male, 26) or absence of AA (control group, n=225; female, 131; male, 94). The mean age of the patients at the time of consultation was 30.73±15.63 years, with a median of 30 years, while the mean age of the controls was 24.58±8.23 years, with a median of 23 years. The main clinical characteristics of participants and their family history of AA are summarized in Table I.

Allele and genotype analysis. In order to assess the HWE, several tests were performed (control group: Pearson test P=0.81, likelihood-ratio (Llr) test P=0.74, Fisher exact test P=1.00; case group: Pearson test P=0.75, Llr test P=0.65, Fisher exact test P=1.00). No statistically significant differences were observed in all these tests (P>0.05), indicating that the rs2476601 genotype was in HWE in AA patients and

Table I. General characteristics of AA patients.

Alopecia type	Gender, n (%)		Autoimmune diseases, n (%)	Family history, n (%)	
	Female	Male		AA	Autoimmune disease
Patchy AA					
One	9 (14.06)	8 (12.50)	2 (3.13)	1 (1.56)	12 (18.75)
Multiple	23 (35.94)	12 (18.75)	8 (12.50)	2 (3.13)	26 (40.63)
Ophiasis	4 (6.25)	2 (3.13)	2 (3.13)	-	5 (7.81)
Totalis	1 (1.56)	-	-	-	1 (1.56)
Universalis	1 (1.56)	4 (6.25)	-	-	1 (1.56)

AA, alopecia areata.

Table II. Frequency of PTPN22 C1858T genotypes and alleles in AA patients and healthy control individuals from the Northeastern Mexican population.

A, All AA types

Parameter	AA patients, n (%)	Controls, n (%)	χ^2	OR	95% CI	P-value
Genotype						
CC	59 (92.19)	218 (96.89)	2.7673	0.3789	0.1161-1.2370	0.0962
CT	5 (7.81)	7 (3.11)	2.7673	2.6392	0.8084-8.6162	0.0962
TT	0 (0.00)	0 (0.00)				
Alleles						
C	123 (96.09)	443 (98.44)	2.7086	0.3887	0.1213-1.2461	0.0998
T	5 (3.91)	7 (1.56)	2.7086	2.5726	0.8025-8.2468	0.0998

B, Patchy AA

Parameter	AA patients, n (%)	Controls, n (%)	χ^2	OR	95% CI	P-value
Genotype						
CC	47 (90.38)	218 (96.89)	4.3116	0.3018	0.0918-0.9923	0.0379
CT	5 (9.62)	7 (3.11)	4.3116	3.3131	1.0077-10.892	0.0379
TT	0 (0.00)	0 (0.00)				
Alleles						
C	99 (95.16)	443 (98.38)	4.2161	0.3129	0.0973-1.0062	0.0400
T	5 (4.84)	7 (1.62)	4.2161	3.1962	0.9938-10.279	0.0400

PTPN22, protein tyrosine phosphatase, non-receptor type 22; AA, alopecia areata; OR, odds ratio; CI, confidence interval.

controls. The PTPN22 rs2476601 allele and genotype frequencies in patients with different AA types and in the control subjects, as well as the genotype and allele association with the development of AA, are shown in Table II. In total, 92.19% of the AA patients showed the homozygous CC genotype and the remaining 7.81% presented the heterozygous CT genotype. The homozygous TT genotype was not identified in the patient and control groups.

Association of genotypes in patients with AA. When the PTPN22 C1858T genotypes and allele frequencies were compared between the entire cohort of cases (all AA types) and controls, the CC genotype was more frequently detected in the controls compared with all AA patients. By contrast, the CT genotype was more frequently detected in all AA patients and patchy AA patients. However, no evidence of association was observed in the T allele of all the AA patients analyzed and the presence of this condition (Table II). A statistically significant association was observed in the T allele when comparing the patchy AA group and controls [P=0.040; OR=3.196; 95% confidence interval (CI), 0.994-10.279] and CT genotype in the same patients (P=0.038; OR=3.313; 95% CI, 1.008-10.8921; Table II).

Table III. Clinical characteristics of AA patients with normal and heterozygous PTPN22 genotype.

Characteristics	CC genotype (n=59)	CT genotype (n=5)	P-value
Age range, years	3-73	7-23	
Mean age, years	32.15±15.39	14.00±5.83	0.0003[a]
Immune diseases	12/59	0/5	0.6016
DM	30/59	0/5	0.0853
AH	19/59	0/5	0.3156
Vitiligo	3/59	0/5	0.5583
Alopecia	3/59	0/5	0.5583
Positive family history	41/59	2/5	0.3939

[a]P<0.05 indicates a statistically significant difference. Data are presented as a range, mean ± standard deviation or number/total number. AA, alopecia areata; PTPN22, protein tyrosine phosphatase, non-receptor type 22; DM, diabetes mellitus; AH, arterial hypertension.

Regarding the personal and family history of autoimmune diseases in the group of patients analyzed, diseases frequently present included diabetes mellitus (DM), arterial hypertension (AH), vitiligo and alopecia (Table III). When considering the association of the genotypes with the age and other patient characteristics, such as AA development and a family history of immune diseases, an association was only detected between the different genotypes and the patient age (P<0.05). An early age of disease development was observed in patients with the polymorphic CT genotype (P=0.0003, for patients <23-years-old). Furthermore, a tendency toward an association between the presence of DM and the development of alopecia was observed in the group with the normal homozygous CC genotype (P=0.0853). By contrast, no association was identified between the presence of the CT genotype and the presence of autoimmune diseases, and other analyzed clinical variables in these AA patients (Table III).

Discussion

AA, a condition that results in hair loss, is non-scarring telogenic alopecia of autoimmune etiology (22). Although hair loss as single or multiple patches is the most common presentation, other clinical presentations may be observed, such as alopecia universalis and alopecia totalis (22).

The pathogenetic basis and the etiology of AA are not yet fully understood. Multiple trigger factors have been implicated in the development of AA, including stress (22), viral, bacterial or fungal infections (23,24), certain drugs or therapies (25), endocrine and autoimmune disorders, which may act independently or all together (26), and other environmental factors. However, these factors were relevant only in certain patients in the present study. It is believed that this disease exhibits a non-Mendelian pattern of genetic predisposition due to multiple phenotypes that cannot be attributed to a single gene locus (27).

In an attempt to determine the genetic distribution in AA, a number of association studies for candidate genes have been conducted in participating genes, including those associated with the immune response (28), death pathways (29) and cytokine production (30). A significant association between AA and the human leukocyte antigen polymorphism has been described in studies conducted within the last two decades, indicating the involvement of immune mechanisms in the development of this disease (9,31-33).

Several reports have proposed an autoimmune origin of this pathology due to the frequent association of AA with diseases such as atopy (27), atopic dermatitis, DM, rheumatic arthritis (33), thyroiditis and vitiligo, among other immune diseases (34). In the population of the current study, diseases including DM (4.7%), AH (29.7%), previous episodes of hair loss (4.7%) and vitiligo (4.7%) were detected. These results are observed at higher rates compared with those in an Egyptian population, as described in a previous study (14). In addition, we observed a tendency toward an association of DM and the development of alopecia in the group with normal homozygous genotype (CC genotype; Table III).

Since in various genetic case-control studies (28,35,36) the MHC loci have been associated with the development of AA, the identification of non-MHC loci associated with this pathology and other immune diseases is important. The PTPN22 locus is one of the most critical non-MHC genetic risk factors associated with autoimmune diseases, with the missense R620W polymorphism in the PTPN22 gene considered as a risk factor (37-40). However, data on the association of this gene with AA in the Mexican population are limited.

The PTPN22 gene encodes a lymphoid protein tyrosine phosphatase (LYP) which acts as a negative regulator of T cell signaling. The presence of the T allele of the PTPN22 C1858T SNP results in a variant protein that is unable to form a LYP1/CSK complex, resulting in a loss of functional proteins that are unable to regulate T cell activation, leading to a hyper-responsive phenotype of T, B and dendritic cells (16,41). This effect may have a role in the immunopathogenesis of AA. This observation is supported by studies describing hair follicle autoantigens, such as the melanogenesis-associated peptides generated during the anagen phase of hair growth (42-44). These are potential key targeted by auto-reactive cytotoxic T cells, which may be associated with the local inflammatory responses of the hair loss region (44).

In the present study, the T allele of the PTPN22 C1858T SNP frequency in all AA types (3.91%) was found to be similar to that reported in Mexican patients with lupus erythematosus (3.4%) (17). Furthermore, the patchy AA T allele frequency

was similar to that reported for rheumatoid arthritis in the Mexican population (4.8 and 6%, respectively) (16). In the two previous studies (16,17), the T allele frequency reported in control subjects (1-2%) was similar to that reported in the present study (1.56%).

Only a limited number of worldwide studies have investigated the association between PTPN22 C1858T polymorphism and the development of AA, conferring susceptibility to the development of AA in English (45), Belgian-German (13), Egyptian (14) and North American Caucasian and non-Caucasian populations (46). The PTPN22 C1858T genotype frequency varies based on the ethnicity, ranking between 1.43% (in the Chinese population) and 21.3% (in the North American population) (15). Nevertheless, the PTPN22 C1858T polymorphism has not been previously assessed for AA in other American populations.

Although the present study analyzed the presence of this polymorphism in all types of AA, an association of the T allele with all patchy AA (P=0.040, OR=3.196; 95% CI, 0.994-10.279) and the CT genotype in these AA patients was detected (P=0.0045; OR=4.98; 95% CI, 1.48-16.68). In conclusion, our data suggest the involvement of the PTPN22 1858T allele as a genetic risk factor for susceptibility to patchy AA in a Mexican population, supporting the evidence of the possible involvement of the immune response against the hair follicles in the pathogenesis of this form of alopecia.

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