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SHORT COMMUNICATION

Association analysis of the PTPN22 gene in childhood-onset systemic lupus erythematosus in Mexican population

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Several studies have identified a functional single nucleotide polymorphism 1858C/T in the PTPN22 gene to be associated with several autoimmune diseases. Association studies of this polymorphism with familial and sporadic systemic lupus erythematosus (SLE) have shown some discrepancies. To our knowledge, this is the first study that includes only pediatric-onset SLE patients. We performed a case–control association study in 250 unrelated Mexican patients with childhood-onset SLE consisting of 228 cases with sporadic SLE and 22 cases with familial SLE and 355 healthy controls. We observed a statistically significant difference in the frequency of the PTPN22 1858T allele between SLE patients (3.4%) and healthy controls (1.1%) (P = 0.0062, odds ratio (OR) 3.09 (95% confidence interval 1.32–7.21)). The association was also observed when only sporadic cases were analyzed (OR = 3.19). Our results support the association of the PTPN22 1858T allele with sporadic childhood-onset SLE in Mexican population.
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Introduction

Human autoimmune diseases often cluster within families; in systemic lupus erythematosus (SLE), there is familial aggregation of SLE, rheumatoid arthritis and other autoimmune diseases.[1] Autoimmune diseases are thought to develop through a complex interaction of genetic and environmental factors. Genome-wide linkage studies have shown overlapping of susceptibility loci between different human autoimmune diseases, making it highly probable that they are controlled by a common set of genetic factors.[2] Recently, several studies have identified a functional single nucleotide polymorphism (SNP) 1858C/T (rs2476601) in the protein tyrosine phosphatase non-receptor type 22 (*PTPN22)* gene as a disease risk allele in type I diabetes,[3,4] rheumatoid arthritis,[5,6] SLE,[7–10] autoimmune thyroid diseases[11,12] and juvenile idiopathic arthritis.[13,14]

The tyrosine phosphatase encoded by *PTPN22*, also known as lymphocyte phosphatase (Lyp), is a hematopoietic tissue-specific protein that is thought to inhibit T-cell activation through its association with the Csk tyrosine kinase.[15] The 1858C/T SNP results in an amino-acid substitution of a highly conserved arginine to thryptophan in codon 620 (R620W) in the SH3-binding domain of *PTPN22* that disrupts the interaction with CsK.[3,5] Therefore, this polymorphism could lead to the hyperactivity of T cells observed in many autoimmune diseases, suggesting that different autoimmune diseases may share some common pathogenic mechanisms.

Despite the wealth of evidence to support the involvement of the *PTPN22* 1858T allele in rheumatoid arthritis,[16] the association studies of this polymorphism with SLE have shown some discrepancies. Kyogoku *et al*.[7] reported for the first time the association of the *PTPN22* 1858T allele with both familial and sporadic SLE in European Americans. This association was further confirmed in sporadic SLE in Caucasian Spanish,[8] Swedish[9] and Colombian patients.[10] In contrast, Wu *et al*.[17] did not find association of the *PTPN22* 1858T polymorphism with sporadic SLE in Caucasian individuals from northern America, the UK and Finland, neither in familial SLE cases from northern America and Finland. However, Kaufman *et al*.[18] recently reported that this polymorphism is associated with familial but not with sporadic SLE in European American patients. The previous studies included mainly adult-onset SLE patients, and to our knowledge there are no studies that include only pediatric-onset SLE patients. Therefore, the aim of this study was to investigate the possible

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involvement of the 1858C/T SNP in the *PTPN22* gene in Mexican patients with childhood-onset SLE.

Results and discussion

We performed a case–control association study in 250 unrelated patients with childhood-onset SLE recruited from Mexico City. The SLE cohort consisted of 228 cases with sporadic SLE and 22 cases with familial SLE. All patients were <16 years of age at onset of disease and fulfilled the American College of Rheumatology (ACR) criteria for SLE.[19] Of these, 214 were female (85.6%) and 36 were male (14.4%), with a mean ± s.d. age at onset of 11.62 ± 2.46 years in the whole group. Additionally, 355 ethnically and sex-matched blood bank donors were included as healthy control group. This study was approved by the respective local ethics and research committees and all parents/patients when appropriate, provided signed, informed consent.

Genotype frequencies were in Hardy–Weinberg equilibrium in patients and controls. When *PTPN22* 1858C/T genotypes and allele frequencies were compared between the whole cohort of cases and controls, a strong evidence of association was observed (Table 1). The genotype CT was more frequent in patients with SLE than in controls ($P = 0.0057$). The homozygous TT genotype was absent both in cases and controls. The frequency of the *PTPN22* 1858T allele in our SLE patients was significantly lower (3.4%) than that reported in Spanish,[8] Swedish,[9] Colombians[10] and European Americans SLE patients[7] (range: 9.8–16.5%). However, the association of the T allele with childhood-onset SLE susceptibility was stronger (odds ratio (OR) 3.09, 95% confidence interval (95% CI) 1.32–7.21), than that reported in previous studies including mainly adult-onset SLE (ORs ranging from 1.42 to 2.56).[7–10,18] Although the relative lower effect of this polymorphism on adult-onset SLE compared with our pediatric SLE population may be explained by differences in the genetic background between the different ethnic populations, it also could be that some polymorphisms may have different effect in childhood-onset and adult-onset SLE. Actually, Wu *et al*.[17] observed a younger age at diagnosis in one of their cohorts when stratified by CT/TT versus CC genotype. Furthermore, although pediatric SLE is phenotypically similar to adult-onset SLE, in childhood-onset the initial symptoms tend to be more severe and it has a more aggressive clinical course.

When we analyzed only the 228 sporadic SLE cases, the *PTPN22* 1858T allele still showed a significant association with childhood-onset susceptibility (OR = 3.19, 95% CI 1.35–7.52) (Table 1). Kaufman *et al*.[18] also observed an association of the *PTPN22* 1858T allele with sporadic SLE in 98 Hispanics who reside in Texas, Mexico, and the Caribbean, when compared with 172 Hispanic controls (OR = 2.06, 95% CI 1.04–4.07). Nevertheless, compared with our population they found a higher frequency of the risk allele both in their cases (3.5 versus 9.6%) and controls (1.1 versus 4.9%). However, the term 'Hispanic' describe a common language and cultural heritage rather than a race, uniform ethnicity or a common genetic background. Hispanics are genetically complex and comprised of various proportions of Native American, African and European genetic origins.[20] Therefore, this may explain why they found a higher frequency of the T allele compared with our population.

Table 1 Frequency of *PTPN22* 1858C/T genotypes and alleles in childhood onset SLE patients and healthy controls

Genotype[a]	*SLE patients no. (%)*	*Controls no. (%)*	χ^2	P-*value*	OR	*95% CI*
All SLE patients						
No. of genotypes	250	355				
CC	233 (93.2)	347 (97.7)				
CT	17 (6.8)	8 (2.3)	7.65	0.0057	3.17	1.34–7.45
TT	0 (0)	0 (0)				
No. of alleles	500	710				
C	483 (96.6)	702 (98.9)				
T	17 (3.4)	8 (1.1)	7.49	0.0062	3.09	1.32–7.21
Sporadic SLE						
No. of genotypes	228	355				
CC	212 (93)	347 (97.7)				
CT	16 (7)	8 (2.3)	7.98	0.0047	3.27	1.38–7.78
TT	0 (0)	0 (0)				
No. of alleles	456	710				
C	440 (96.5)	702 (98.9)				
T	16 (3.5)	8 (1.1)	7.82	0.0052	3.19	1.35–7.52

[a]Genotyping of the *PTPN22* 1858C/T SNP was performed using the TaqMan system 5′-allele discrimination Assay-By-Design method (Applied Biosystems, Foster City, CA, USA) on the ABI 7900 analyzer. The PCR primer sequences were 5′-CCAGCTTCCTCAACCACAA TAAATG-3′ (forward) and 5′-CAACTGCTCCAAGGATAGATGATGA-3′ (reverse). The TaqMan minor groove binder probe sequences were 5′-VIC-TCAGGTGTCCATACAGG-3, and 5′-FAM-TCAGGTGTCCGTACAGG-3′. The polymerase chain reaction was performed as follows: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and annealing and extension at 60°C for 1 min. The assay reproducibility was 100% in a set of samples from 138 cases and 120 controls, including both heterozygous and homozygous genotypes. Genotype and allele frequencies were compared between cases and controls by χ^2 test that combined the 2×2 contingency tables. Also, genotype (CT versus CC) and allele frequencies for cases and controls were used to calculate the odds ratio (OR) and the 95% confidence interval (95% CI). Genotype distributions in patients and controls were evaluated for departure from Hardy–Weinberg equilibrium by using a contingency table χ^2 test. *P*-values less than or equal to 0.05 were considered significant.

In conclusion, our results suggest the involvement of the *PTPN22* 1858T allele as a genetic risk factor for susceptibility in childhood-onset SLE in Mexican population. Our data also support the association of this SNP with sporadic SLE reported in other populations. Nevertheless, recognizing the possible genetic confounding effects owing to population stratification in case–control designs, family-based association studies are needed to confirm our case–control findings. Although we are collecting trios, given the low frequency of the minor allele in our population the current trio collection is underpowered to detect the effect by transmission disequilibrium test.

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