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BRIEF COMMUNICATION

PTPN22 C1858T polymorphism in Colombian patients with autoimmune diseases

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A functional single nucleotide polymorphism (SNP) C1858T in the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene encoding an intracellular phosphatase with negative regulatory effects on T-cell activation is associated with some autoimmune diseases in Caucasians. Taking into account firstly, that SNP frequencies may vary across populations and, secondly, that replication studies are important to confirm previous associations, we examined the influence of PTPN22 polymorphism in 621 Colombian patients with four autoimmune diseases. Accordingly, 298 patients with rheumatoid arthritis (RA), 143 with systemic lupus erythematosus (SLE), 70 with primary Sjogren's syndrome (pSS) and 110 with Type 1 diabetes (T1D) were studied. The control group consisted of 308 matched healthy individuals. Genotyping of PTPN22 was performed by the real-time polymerase chain reaction technology, using the TaqMan 5′-allele discrimination assay. The 1858 T allele was found to be a risk factor for pSS (odds ratio (OR) = 2.42), SLE (OR = 2.56), and T1D (OR = 1.83). A lower but nonsignificant trend was observed for RA (OR = 1.26). These results confirm the influence of PTPN22 in autoimmunity and indicate that autoimmune phenotypes could represent pleiotropic outcomes of nonspecific disease genes that underlie similar immunogenetic mechanisms.
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Introduction

Autoimmune diseases are characterized by a loss of self-tolerance causing immune-mediated tissue destruction. Approximately 5–6% of the population is affected by autoimmune diseases, and examples of common autoimmune diseases are rheumatoid arthritis (RA), Type 1 diabetes (T1D), primary Sjögren's syndrome (pSS), and systemic lupus erythematosus (SLE).[1]

Autoimmune diseases are multifactorial and are caused by an interaction of both genetic and environmental factors. Clinically different autoimmune phenotypes may share common susceptibility genes, which may act as risk factors for autoimmunity. Evidence for shared susceptibility genes is obtained from the observation that several autoimmune diseases tend to cluster in the same families[1,2] and also that the chromosomal regions showing linkage to autoimmune diseases tend to overlap.[3,4]

One of the key points in the pathogenesis of autoimmune diseases is the regulation of the T-cell response.[5,6] Protein tyrosine phosphorylation, a crucial mechanism of cellular signal transduction, is regulated by the action of both protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Owing to their potential etiologic and pathogenic roles in human disease, genes coding for PTPs are considered as good candidates for the study of autoimmune diseases.

The *PTPN22* (protein tyrosine phosphatases nonreceptor 22) gene maps to chromosome 1p13.3–p13.1, in a region linked with RA and SLE,[7,8] and encodes a lymphoid-specific phosphatase known as Lyp. Lyp is an intracellular PTP that contains an N-terminal catalytic domain and noncatalytic C-terminus with four proline-rich domains.[9] Lyp dephosphorylates the kinases Lck, Fyn, and Zap-70, all known to be important in T-cell signalling. An additional function of Lyp is to down-regulate activation of T cells by binding to C-terminal Src tyrosine kinase (Csk). Csk is an essential suppressor of kinases that mediate T-cell activation.[9] In addition, Lyp binds to the adaptor molecule Grb2 (growth factor receptor-bound protein 2) and this interaction is

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thought to play a negative regulatory function in T-cell signalling.[10]

Recent findings have demonstrated that a single nucleotide polymorphism (SNP) of *PTPN22* (C1858T; dsSNP no. rs2476601, R620W), placed at the P1 motif, disrupts the interaction between Lyp and Csk, avoiding the formation of the complex and therefore the suppression of the T-cell activation.[11] In Caucasians, *PTPN22* T allele is a risk factor for multiple autoimmune diseases.[12] Taking into account firstly that SNP frequencies may vary across populations, and secondly that replication studies are important to confirm previous associations, we examined the influence of *PTPN22* polymorphism in Colombian patients with autoimmune diseases.

Results and discussion

We simultaneously examined *PTPN22* C1858T polymorphism in 298 patients with RA, 143 with SLE, 70 with pSS, and 110 with T1D. The control group consisted of 308 matched healthy individuals. The genotypic frequencies are shown in Table 1. Both patients and controls were in Hardy–Weinberg equilibrium. The homozygous genotype (T/T) was absent in the control population as well as in pSS patients, but was observed in a frequency of 0.6, 2, and 0.9% in RA, SLE, and T1D, respectively.

For comparisons of genotype frequencies in RA ($P=0.35$), SLE ($P=0.001$), pSS ($P=0.01$), T1D ($P=0.07$), and controls, we used a 2×3 contingency table. We analyzed 621 consecutive patients with autoimmune diseases. Their clinical and immunological characteristics were systematically recorded as previously reported.[13,14] The study included 263 women and 35 men with RA,[15] their mean age$\pm$s.d. was 48.5 ± 14.2 years, the mean duration of disease was 11 ± 8.1 years, and 80% tested positive for RF. Extra-articular manifestations were registered in 35% of cases, and comorbidity in 70%. There were 138 women and five men with SLE,[16] their mean age was 34 ± 12 years, the mean duration of disease was 7.5 ± 7 years, and antinuclear antibodies and anti-DNA antibodies were positive in 99 and 74%, respectively. All patients with primary SS[17] were women, their mean age was 52.5 ± 13.5 years, and the mean duration of pSS was 6.4 ± 5.2 years. During the course of the disease, all patients presented with exocrine involvement, 63% had vasculatory-inflammatory involvement, and 16% immune-mediated manifestations. All pSS patients disclosed a lymphocytic infiltrate on minor salivary glands with a focus score >1, and anti-Ro and anti-La antibodies were detected in 74 and 32%, respectively. There were 56 girls and 54 boys with T1D[18] whose mean age was 8.8 ± 6.3 years; anti-GAD, anti-IA2, and anti-insulin antibodies were present in 45, 40, and 69%, respectively. The control group was composed of 308 individuals without a history of chronic inflammatory autoimmune or infectious diseases; they were matched to patient groups by gender, ethnicity, and socioeconomic status, and were unrelated to patients. Their mean age was 47 ± 9 years, and 90% were women.

Genomic DNA was extracted from a 10 ml of EDTA-anticoagulated blood sample using the standard salting-out technique. Genotyping for the *PTPN22* SNP C1858T was by TaqMan 5′-allele discrimination Assay-By-Design method (Applied Biosystems, Foster City, CA, USA). The primer sequences were 5′-CCAGCTTCCTCAACCA CAATAAATG-3′(sense) and 5′-CAACTGCTCCAAGGA TAGATGATGA-3′ (antisense). The Taqman minor groove binder probe sequences were 5′-caggtgtccatacagg-3′, and 5′-caggtgtccgtacagg-3′; the probes were labelled with the fluorescent dyes VIC and FAM, respectively. The polymerase chain reaction (PCR) was carried out in a total volume of 12.5 µl using the following amplification protocol: 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. After the PCR, the genotype of each sample was automatically attributed by measuring the allele-specific fluorescence in the ABI Prism 7000 Sequence Detection System, using SDS 1.1 software for allele discrimination (Applied Biosystems). To confirm the genotype obtained by the Taqman 5′-allele discrimination assay, PCR-restriction fragment length polymorphism was performed on representative samples from each genotype as previously described.[11] Briefly, we used the forward primer 5′-TCACCA GCTTCCTCAACCACA-3′ and the reverse primer 5′-GATAATGTTGCTTCAACGGAATTTA-3′. The genotypes were identified by *Xcm*I restriction endonuclease digestion, which recognizes its target sequence only when the *PTPN22* 1858T allele is present. Digestion products were resolved on 3% agarose gels.

Data were managed and stored using the SPSS program (V9.05 for Windows, Chicago, IL, USA). Allele and genotype frequencies of *PTPN22* C1858T were obtained by direct counting. Differences between allele and genotype frequencies were determined using two-tailed χ^2 and Fisher's exact test as appropriate. The influence of *PTPN22* SNP on the clinical and immunological characteristics of patients was analyzed by logistic regression. Crude OR was calculated with 95% confidence intervals (CI). A P-value <0.05 was considered as statistically significant.

Table 2 shows the *PTPN22* C1858T allele frequencies. We observed a similar T-allele frequency to that reported in Caucasians.[12] An association between T allele and pSS (OR = 2.42) and SLE (OR = 2.56) was clearly seen, while for T1D the association was less strong (OR = 1.83). Accordingly, T allele is a high risk factor for pSS and SLE and might be a lower risk factor for T1D in our

Table 1 Genotype frequencies of the *PTPN22* C1858T

Genotype	*RA* $n=298$ *(%)*	*SLE* $n=143$ *(%)*	*PSS* $n=70$ *(%)*	*T1D* $n=110$ *(%)*	*CTRs* $n=308$ *(%)*
CC	266 (89)	116 (81)	56 (80)	94 (85.1)	281 (91.2)
CT	31 (10.4)	24 (17)	14 (20)	15 (14)	27 (8.8)
TT	1 (0.6)	3 (2)	0 (0)	1 (0.9)	0 (0)

Table 2 Allele frequencies of *PTPN22* C1858T

Alelle	*RA* $n=596$ (%)	*SLE* $n=286$ (%)	*PSS* $n=140$ (%)	*T1D* $n=220$ (%)	*CTRs* $n=616$ (%)
C	563 (94)	256 (90)	126 (90)	203 (92)	589 (96)
T	33 (6)[a]	30 (10)[b]	14 (10)[c]	17 (8)[d]	27 (4)

Comparisons were carried out between each patients group and healthy controls
[a]OR 1.26, 95% CI 0.64–2.44, $P=0.5$.
[b]OR 2.56, 95% CI 1.49–4.39, $P=0.001$.
[c]OR 2.42, 95% CI 1.24–4.75, $P=0.01$.
[d]OR 1.83, 95% CI 0.98–3.42, $P=0.06$.

population. There were no significant differences in genotypes or alleles with regard to sex and age. In addition, no influence of *PTPN22* SNP on clinical and immunologic characteristics of patients was found (data not shown).

Replication studies are important because they strengthen the evidence of the studied association. Lack of replication across different ethnic/racial subgroups or across different environmental settings may represent valid observations reflecting different background genes and gene–environment interactions.[19]

Signaling aberration and abnormalities in tyrosine phosphorylation might act as a central defect in the pathogenesis of SLE.[20,21] Our results confirm previous findings in independent SLE cohorts[12,22,23] and suggest that downregulation of T-cell activation plays an important role in T-cell-mediated response in disease.

The observed association between *PTPN22* polymorphism and pSS is new. Two previous negative results in Caucasian patients with pSS have been published. The first was carried out by Criswell *et al*[12] in families with 16 affected subjects. Recently, Itaah *et al*[24] reported a lack of association in 183 French patients with pSS and 172 healthy controls. Differences in ethnicity and patient selection may explain the results. Nevertheless, based upon animal models, *PTPN22* might play a role in diseases characterized by lymphoproliferation such as pSS. *PTPN22* knockout mice display rather subtle changes in a number of immune parameters, such as enlargement of the spleen and the lymph nodes and is accompanied by spontaneous formation of germinal centers and higher levels of antibodies that appeared to be largely secondary to lower thresholds for signaling in T cells.[25] Similar to the above-summarized murine model, an increased germinal center formation is observed in autoimmune models of pSS, SLE, and T1D.[26,27]

As previously shown by others,[11,28–33] our results disclosed that T allele is a risk factor for T1D (OR = 1.83), although this difference was in the threshold of significance ($P=0.06$).

In the case of RA, previous studies have shown that *PTPN22* polymorphism is a low risk for disease (OR < 2)[12,22] and others have reported an association only with rheumatoid factor (RF)–positive disease.[34] We found a very low minor allele frequency of the T allele in our population (6%) compared with that found in the Caucasian population (10%). The genetic heterogeneity between populations is clearly present in this case, since *PTPN22* T allele and genotype frequencies are significantly different between the Colombian and the Caucasian populations, which may account for the failure to replicate the *PTPN22* association with RA. However, the ORs obtained in our study are similar to prior ORs reported in other studies in which *PTPN22* 1858T allele has been associated with genetic predisposition to RA and T1D.[11,22,29] Considering the lower minor allele frequency observed in our patient's population, a larger sample will be required to obtain a similar trend of association to that observed in Caucasians. On the other hand, our lack of replication may also reflect the clinical heterogeneity present in RA patients across populations.[19]

In conclusion, we have evaluated the influence of *PTPN22* C1858T polymorphism in Colombians with autoimmune diseases. Our results confirm the influence of *PTPN22* on autoimmunity and add further evidence supporting the common origin (immunogenetic) to diverse autoimmune diseases.[1,12,14]

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