The *PTPN22* C1858T variant as a risk factor for rheumatoid arthritis and systemic lupus erythematosus but not for systemic sclerosis in the Colombian population

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Abstract

Objectives

C1858T single nucleotide polymorphism in PTPN22 *encoding the R620W allele variant of Lyp-PTPN22 (a protein phosphatase negatively regulating T-cell activation) has been associated with autoimmunity. This work has investigated the possible association between PTPN22 C1858T (rs2476601) polymorphism and rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) in a Colombian population.*

Methods

A case-control study included 1,042 samples from 413 RA, 94 SLE and 101 SSc patients and 434 healthy controls. The TaqMan allele discrimination assay was used for genotyping.

Results

The case-control study provided robust evidence of association between allele 1858T and RA (p=5E–05), as well as between 1858T and SLE (p=0.004). These observations were confirmed for both diseases by meta-analysis (p=2E–04, pooled OR 1.9; 1.3–2.7 95% CI for RA; p<0.0001, pooled OR 2.8, 1.8–4.5 95% CI for SLE). No significant association was observed between 1858T and SSc (p=0.98, OR 1.11, 0.46–2.65 95% CI).

Conclusion

The study suggested that the PTPN22 1858T variant influences RA and SLE genetic background but not that of SSc in the Colombian population.

Key words

PTPN22, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis

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Introduction

Autoimmune diseases (ADs) are complex disorders which affect 5% of the population. Although complete AD etiology remains unknown, these diseases are known to feature environmental and genetic factors in their development (1). Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) are ADs whose genetic components are still in the relatively early stages of gene discovery. The genetic variants predisposing towards multiple AD confirm shared common pathogenesis pathways, despite their heterogeneous clinical manifestations (1).

This study was thus aimed at evaluating the association between *PTPN22* C1858T polymorphism in RA and SLE development and clinical manifestations and, for the first time, at evaluating the role of C1858T in the genetic components of SSc in a Colombian population.

Materials and methods

Subjects

Patients were recruited from a Rheumatology Unit in Bogotá and Barranquilla, Colombia. C1858T polymorphism was examined in 394 RA patients (335 females). Median age at onset of disease was 44.7±15.6 and median disease duration was 8.2±7.4 years. All patients met the American College of Rheumatology (ACR) criteria for RA (2). Median age at onset for 94 SLE patients (92 females) was 28.4±11.5 and median disease course lasted 13.7±7.7 years. All of them met 1982 ACR criteria for SLE (3); 101 SSc patients (84 females) met ACR criteria for SSc (4). Median age at SSc onset was 43.5±15.7. Control consisted of 434 DNA samples taken from voluntary blood donors from the same places. All subjects studied were Colombian and provided written informed consent. Protocols were approved by the Universidad Nacional de Colombia Medical School Ethics Committee.

Genotyping

DNA was obtained from patients and controls' peripheral blood using standard methods. Samples were genotyped for the 1858C/T (rs2476601) variant of *PTPN22* by a Taqman 5´- allelic discrimination assay-by-design method (Applied Biosystems, Foster City, CA, USA), as described previously (5). All samples were genotyped in the same laboratory to avoid genotyping inconsistencies. Random samples were genotyped twice for quality control, showing 99% identical genotypes. The genotype obtained in real time was verified in 21 randomly-selected samples by direct sequencing.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested for each case-control set by using FINETI software (http://ihg.gsf.de/cgi-bin/hw/hwa2.pl). Significance was calculated by 2x2 contingency tables and Fisher's exact test. Statcalc software (EpiInfo 2002; Centres for Disease Control and Prevention, Atlanta, GA, USA) was used to obtain *p*-values, odds ratios (OR) and 95% confidence intervals (CI). *p*-values below 0.05 were considered to be statistically significant. A meta-analysis compared the present study's results to the only previous report of the influence of *PTPN22* gene R620W polymorphism on Colombian RA and SLE patients using Stats Direct software. OR homogeneity among cohorts was calculated using Breslow-Day and Cochran's *Q*- test methods (6, 7). The pooled OR was then calculated by using a fixed-effects model (Mantel-Haenszel meta-analysis) or random effects (Der Simonian-Laird) where necessary, according to the degree of inconsistency using Higgins's test (I^2) (7).

Results

The 1858T variant was over-represented in RA patients

PTPN22 C1858T polymorphism genotype frequencies were in Hardy-Weinberg equilibrium in both RA patients (n=394) and control individuals (n=434, Table I). The 2x3 contingency table analysis revealed that, while rare T/T homozygous genotype frequency was no different between RA patients and controls (0.2% *vs*. 0.8%, respectively; *p*=0.27), the heterozygous C/T genotype was over-represented in RA patients compared to controls (13.2% *vs*. 5.5%, respectively, *p*=1.3 E-04) (Table I). Furthermore, allele frequency analysis demonstrated that the 1858T

Table I. *PTPN22* R620W variant genotype and allele frequency in RA patients and healthy controls.

PTPN22 C1858T	RA n=394 (%)	Controls n=434(%)	*p*-value	OR (95% CI)
C/C	339 (86.0)	409 (94.2)	6 E-05	0.39 (0.23–0.62)
C/T	52 (13.2)	24 (5.5)	1.3 E-04	2.54 (1.54–4.19)
T/T	3 (0.8)	1 (0.2)	0.271	2.21 (0.32–5.06)
C	730 (92.6)	842 (97.0)	5 E-05	0.35 (0.19–0.68)
T	58 (7.4)	26 (3.0)	5 E-05	2.57 (1.57–4.03)

Allele and genotype frequencies were compared by using 2x3 and 2x2 contingency tables and Fisher's exact test. $p<0.05$ was predetermined.

Table II. *PTPN22* R620W variant genotype and allele frequencies in SLE patients and healthy controls.

PTPN22 C1858T	SLE n= 94(%)	Controls n=434(%)	*p*-value	OR (95% CI)
C/C	81 (86.2)	409 (94.2)	6 E-03	0.37 (0.18–0.75)
C/T	12 (12.8)	24 (5.5)	0.01	2.57 (1.25–5.3)
T/T	1 (1.1)	1 (0.2)	0.23	4.62 (0.48–44.88)
C	174 (92.6)	842 (97.0)	3.7 E-03	0.37 (0.19–0.72)
T	14 (7.4)	26 (3.0)	3.7 E-03	2.68 (1.38–5.18)

Allele and genotype frequencies were compared by using 2x3 and 2x2 contingency tables and Fisher's exact test. $p<0.05$ was predetermined.

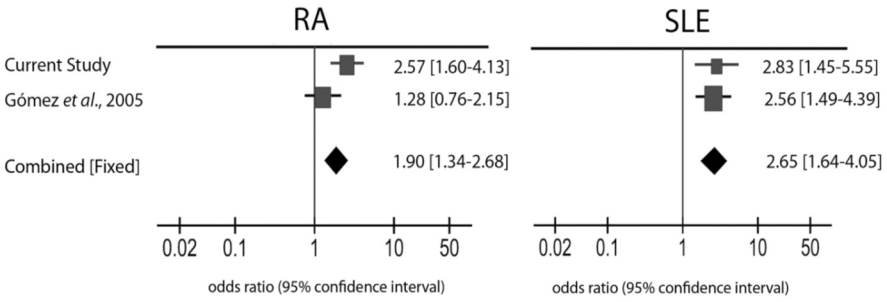

Fig. 1. Forest plot for meta-analysis of T-allele frequency (rs2476601 *PTPN22* polymorphism) in RA and SLE Colombian patients. Common effect size and odds ratios with 95% confidence intervals were calculated by the Mantel-Haenszel method, using a fixed-effect model. The filled squares represent studies in relation to their weighting.

Table III. PTPN22 R620W variant genotype and allele frequency in SSc patients and healthy controls.

PTPN22 C1858T	SSC n= 101(%)	Controls n=434(%)	*p*-value	OR (95% CI)
C/C	95 (94.1)	409 (94.2)	0.94	0.87 (0.36–2.12)
C/T	6 (5.9)	24 (5.5)	0.87	1.20 (0.49–2.93)
T/T	0 (0.0)	1 (0.2)	-	-
C	196 (97.0)	842 (97.0)	0.98	0.90 (0.38–2.16)
T	6 (3.0)	26 (3.0)	0.98	1.11 (0.46–2.65)

Allele and genotype frequencies were compared by using 2x3 and 2x2 contingency tables and Fisher's exact test. $p<0.05$ was predetermined.

allele 620W variant was more frequent in RA patients than in healthy controls (7.4% *vs*. 3.0%; p=5.0 E-05, OR 2.57, 1.57–4.03 95% CI, Table I).

1858T was over-represented in systemic lupus erythematosus patients

Comparing allele frequency between SLE patients (n=94) and healthy controls (n=434) revealed increased rare *PTPN22* T allele prevalence in the SLE group compared to controls (7.4% *vs*. 3.0%; p=3.7 E-03, OR 2.68, 1.38–5.18 95% CI, Table II). This was accompanied by increased heterozygous C/T genotype frequency in SLE patients (12.8%) compared to controls (5.5%, p=0.01, Table II).

The meta-analysis confirmed the findings

A meta-analysis compared the current work to a study reported by Gomez *et al*., (8) to improve statistical power and determine the common effect size of *PTPN22* gene C1858T polymorphism on RA and SLE Colombian patients. Heterogeneity was not observed in *PTPN22* genetic variant and RA meta-analysis (p=0.05, Q=3.79, df=1) or for SLE (p=0.81, Q=0.06, df=1). Combined analysis using fixed and random-effect models showed *PTPN22* T allele association with both diseases; RA (p=3 E-04, pooled OR 1.90, 1.34–2.68 95% CI) and SLE (p=1 E-05, pooled OR 2.65, 1.74–4.05 95% CI) (Fig. 1).

PTPN22 *1858T variant prevalence was not altered in scleroderma patients*

R620W polymorphism was in Hardy-Weinberg equilibrium in scleroderma patients as well as in an independent group of healthy controls. However, R620W variant genotype and allele frequency were similarly distributed in both cohorts (p=0.98, OR 1.11, 0.46–2.65 95% CI) (Table III).

The PTPN22 *1858T variant was not associated with clinical manifestations*

The impact of the 1858T genetic variant on disease status and clinical manifestations was also studied (Tables IV, V). The possible association of the 620W variant was investigated on anti-CCP, positive serum rheumatoid factor anti-dsDNA and ENAS antibody production. No association was found with R620W polymorphism and/or antibody status in RA or SLE (Tables IV, V).

Discussion

PTPN22 has emerged in recent years as a susceptibility gene in multiple autoimmune diseases. Begovich *et al*.

Table IV. *PTPN22* R620W variant genotype and clinical manifestations in RA patients.

Clinical manifestation		CC n (%)	CT n (%)	CT+TT n (%)	*p*-value	OR	95% CI
Extra articular	Yes	62 (80.5)	15 (19.5)	15 (19.5)	0.21	1.75	(0.77–3.98)
Manifestations	No	123 (87.9)	16 (11.4)	17 (12.1)			
ANAS	+	88 (88.9)	11 (11.1)	11 (11.1)	0.99	0.90	(0.34–2.83)
	-	79 (87.8)	10 (11.1)	11 (12.2)			
Ac anti-CCP	+	112 (88.9)	14 (11.1)	14 (11.1)	0.68	1.5	(0.43–5.72)
	-	48 (92.3)	4 (7.7)	4 (7.7)			
RF	+	156 (89.6)	17 (9.8)	18 (10.4)	0.16	0.52	(0.22–1.23)
	-	54 (81.8)	12 (18.2)	12 (18.2)			
Rheumathoid nodules	Yes	11 (91.7)	1 (8.3)	1 (8.3)	0.49	0.55	(0.03–4.41)
	No	176 (85.9)	28 (13.6)	29 (14.1)			
Vasculitis	Yes	2 (66.7)	1 (33.3)	1 (33.3)	0.36	3.19	(0–47.07)
	No	185 (86.4)	28 (13.1)	29 (13.6)			
Joint erosion	Yes	84 (87.5)	12 (12.5)	12 (12.5)	0.55	0.73	(0.31–1.69)
	No	97 (83.6)	18 (15.5)	19 (16.4)			

Clinical manifestations and genotype frequencies were compared by using 2x3 and 2x2 contingency tables and Fisher's exact test. $p<0.05$ was predetermined.

Table V. *PTPN22* R620W variant genotype and clinical manifestations in SLE patients.

Clinical manifestation		CC n (%)	CT n (%)	CT+TT n (%)	*p*-value	OR	95% CI
Nephritis	Yes	26 (83.9)	4 (12.9)	5 (16.1)	0.4	1.83	0.27–15.4
	No	19 (90.5)	2 (9.5)	2 (9.5)			
ANAS	+	51 (86.4)	7 (11.9)	8 (13.6)	0.5	-	
	-	5 (100)	0 (0.0)	0 (0)			
anti-DNA	+	25 (86.3)	3 (10.3)	4 (13.7)	0.39	1.92	0.26–16.87
	-	24 (92.3)	2 (7.7)	2 (7.7)			
Neurological	Yes	8 (88.9)	1 (11.1)	1 (11.1)	0.55	0.61	0.03–6.24
	No	39 (83)	7 (14.9)	8 (17)			
Cutaneous	Yes	34 (89.5)	4 (10.5)	4 (10.5)	0.34	0.51	0.08–3.4
	No	13 (81.3)	2 (12.5)	3 (18.7)			
Vasculitis	Yes	3 (75)	0.00 (0.00)	1 (25)	0.56	1.59	0–21.36
	No	43 (82.7)	9 (17.3)	9 (17.3)			
anti-La	+	6 (85.7)	1 (14.3)	1 (14.3)	0.56	1.61	0–24.36
	-	29 (90.6)	2 (6.3)	3 (9.5)			
anti-Ro	+	18 (94.7)	1 (5.3)	1 (5.3)	0.42	0.41	0.01–5.12
	-	22 (88)	2 (8)	3 (12)			
anti-RNP	+	20 (87)	2 (8.6)	3 (13)	0.38	2.7	0.21–74.03
	-	18 (94.7)	1 (5.3)	1 (5.3)			
anti-SM	+	17 (85)	3 (15)	3 (15)	0.43	1.85	0.21–18.33
	-	21 (91.3)	1 (4.35)	2 (8.7)			

Clinical manifestations and genotype frequencies were compared by using 2x3 and 2x2 contingency tables and Fisher's exact test. $p<0.05$ was predetermined.

have reported an association with rheumatoid arthritis (RA) when studying a wide variety of functional polymorphisms (9). Furthermore, Gomez *et al*. have described an association between the *PTPN22* R620W variant and systemic lupus erythematosus (SLE), T1D and Sjögren primary syndrome, but not for RA in a Colombian population (8). Many studies have validated such association between *PTPN22* polymorphisms RA, SLE and other autoimmune diseases since Bottini *et al*. first described this association between *PTPN22* and disease susceptibility, thereby establishing it as an important genetic variant in human autoimmunity (10, 5).

This study investigated the association between *PTPN22* and RA, SLE and SSc prevalence in two independent Colombian subpopulations. It was reported a strong association between *PTPN22* 1858T polymorphism and RA for the first time in two independent Colombian subpopulations, one from the Northern Caribbean (Barranquilla) and the other from the centre of Colombia (Bogota).

Significant association was also found in SLE prevalence, thereby agreeing with a previously reported study involving a Colombian population (8) and with studies performed worldwide (9, 11, 12-14). The meta-analysis confirmed the RA and SLE findings.

The RA and healthy control case frequencies found in Gomez *et al*.'s study were similar to those found in this study (6% cases, 4% controls; OR 1.26), suggesting a lack of statistical power for finding association due to the low number of cases and healthy controls. The lack of association with SSc was probably due to the low statistical power (56%). A recent case-control study in eight Caucasian populations has shown that the 1858T variant is a risk factor for SSc (15). The authors of this study suggested that the observed effect of 1858T allele magnitude on genetic susceptibility to SSc (OR 1.15) seemed to be weaker than that of other autoimmune diseases; this would indicate that the *PTPN22* gene makes a smaller contribution to SSc genetic susceptibility, thereby making a greater sample necessary size for detecting such effect.

Furthermore, it has been established that *PTPN22* genetic variation is associated with autoimmune diseases characterised by auto-antibody production. The R620W variant results in *PTPN22* function gain within intracellular compartments inducing a misbalance in the signalling cascade downstream of the T-cell receptor (TCR). An association between the R620W allele and autoimmunity could then be explained by Lck deregulation resulting in a loss of negative feedback in TCR signalling.

PTPN22 R620W polymorphism was thus seen to be strongly associated with increased susceptibility to rheumatoid arthritis and SLE development in two independent Colombian communities, thus establishing this polymorphism as a novel RA and SLE biomarker in a Hispanic population (10).

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