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The +1858C/T *PTPN22* gene polymorphism confers genetic susceptibility to rheumatoid arthritis in Mexican population from the Western Mexico

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a common autoimmune disease with a complex genetic background. The *PTPN22* gene encodes lymphoid tyrosine phosphatase LYP, a potent negative regulator of T cell activation. Polymorphic variants of this gene have previously been associated with various autoimmune disorders. The +1858C/T single-nucleotide polymorphism (SNP) (rs2476601), in the exon 14 of the *PTPN22* gene has been associated with susceptibility to RA in several population.
Objective: The aim of this work was to investigate whether the +1858C/T of the *PTPN22* gene is associated with susceptibility to RA in Western Mexico population.
Methods: A total of 309 unrelated RA patients, classified according to American College of Rheumatology (ACR) 1987 criteria, as well as 347 controls residents from Western Mexico were recruited for this study. The DNA samples were genotyped for +1858C/T *PTPN22* gene SNP using the PCR-RFLP technique. Antibodies to cyclic citrullinated peptides (anti-CCP) were measured by enzyme-linked immunosorbent assay (ELISA).
Results: The frequency of +1858T risk allele was significantly increased in patients with RA compared with controls ($p = 0.001$, OR = 2.83, 95%CI = 1.50–5.32). To confirm this results we established a comparison between subjects carrying of CT + TT genotypes versus those carrying CC genotype, between both groups ($p = 0.004$, OR = 2.65, 95%CI = 1.33–5.36). Nevertheless, we not observed association of the +1858C/T *PTPN22* gene SNP with clinical activity and functional disability in RA patients. Likewise, the +1858T variant in RA patients seropositive for anti-CCP antibodies, increased the risk for RA ($p = 0.008$, OR = 2.5, 95%CI = 1.3–5.0) when we compared with controls; however, in the group of seronegative patients, no was found significant difference ($p = 0.1$, OR = 2.5, 95%CI = 0.9–7.2).
Conclusions: Our results support the association of the +1858T risk allele of the +1858C/T *PTPN22* polymorphism with susceptibility to RA and confirm that, in combination with anti-CCP antibodies, this SNP influence the autoimmune processes towards a development of RA in Mexican population.
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1. Introduction

Autoimmune diseases are a clinically diverse group of complex disorders that affect to 5–7% of the world population; these diseases occur as a result of the loss of physiological tolerance to self-antigens and are characterized by persistent activation of immune cells, leading to tissue damage [1]. Among all the systemic autoimmune diseases, the rheumatoid arthritis (RA) is the most common affecting between 0.3% and 1% of the adult population worldwide [2,3], and can lead to progressive joint

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destruction and severe disability [3]. The genetic background of the systemic autoimmune diseases such as RA is complex and likely involves many genes which encoding proteins with significant functions in the regulation of immune response [1,4]. Likewise, loss of tolerance to self-antigens, which leads to stimulation of lymphocytes and other immune cells, release of cytokines, activation of complement and the production of autoantibodies, contributes to the pathogenesis of the RA [5]. Genetic factors are thought to be responsible for up 50–60% of the predisposition to RA [6]. The +1858C/T (rs2476601) single-nucleotide polymorphism (SNP) in the protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene has been associated with susceptibility to multiple autoimmune diseases [3,6]. The human *PTPN22* gene, is located on chromosome 1p13, and encodes a lymphoid protein tyrosine phosphatase (LYP), which is important in negative control of T cell activation and in T cell development. *PTPN22* gene belongs to a family of protein tyrosine phosphatase involved in preventing spontaneous T cell activation by dephosphorylation and inactivation of T cell receptor (TCR)-associated kinases and their substrates [1].

The +1858C/T SNP in the *PTPN22* gene is located within the first proline-rich domain (P1) of the LYP protein and results in the substitution of arginine for tryptophan at codon 620 (R620W) of the mature protein [3,7]. Encountered results are discussed by many authors about the functional significance of this SNP. One group show that LYP-W620 is a gain-of-function phosphatase that produce a more efficient inhibition of TCR signaling in activated T cell [8], whereas other authors demonstrated that the +1858T allele is a hipomorphic variant, which means that the polymorphic variant of the protein has a diminished function and cannot inhibit the TCR signaling in T cells correctly; therefore LYP-620W is considered a loss-of-function protein. This impaired LYP function, in T cells, might drive to hyperresponsive B cells breaking tolerance in the periphery and producing autoantibodies, leading to a develop of autoimmune diseases [9]. This results were confirmed recently by Zhang et al., who demonstrate that levels of the phosphatase were dramatically reduced by degradation mediated by calpain-1, in carriers of +1858T allele, and because of this LYP-W620 are unable to regulate the activation of lymphocytes. Thus, B-cell, T cell and dendritic cell hyperresponsiveness represent a mechanism whereby LYP-W620 may increase risk for autoimmune diseases [10].

The association between the +1858C/T *PTPN22* gene SNP and susceptibility to RA has been confirmed in several ethnically different populations [3,11–26]. However, previous studies have been reported an inter-population variability in autoimmune diseases for +1858C/T *PTPN22* SNP [1]. One of the frequent characteristics of RA is the presence of antibodies to citrullinated proteins/peptides (ACPAs). ACPAs comprise a group of antibodies that are highly specific for RA and are observed in around 70% patients; among these are the anti-cyclic citrullinated peptide (anti-CCP) antibodies. The presence of anti-CCP antibodies at early diagnosis predicts more pronounced radiographic progression, as demonstrated by many studies showing a strong association between anti-CCP positivity and the development of bone erosions [27,28]. Furthermore, the production of anti-CCP antibodies has been associated, in several studies, with *PTPN22* +1858T allele [25,29,30] and Johansson et al., in 2006 demonstrated that the combination of *PTPN22* +1858T risk allele and anti-CCP positivity gave a 100% specificity for the disease and strongly predicted the future onset of RA [29].

Considering this facts, the aim of this study was to analyze the association of +1858C/T *PTPN22* polymorphism with RA and evaluated the relationship between anti-CCP status and *PTPN22* +1858C/T SNP for susceptibility to RA in population from Western Mexico.

2. Materials and methods

2.1. Subjects

In this study we recruited a total of 309 RA patients (286 women and 23 men, mean age 48 ± 14 years, range 18–86 years) residents from Western Mexico, which were classified according to the ACR criteria [31] and 347 controls (200 women and 147 men, mean age 36 ± 12 years, range 16–74 years) which were unrelated individuals from the same Mexican population. Ethnically, both patients and controls were classified as Mestizos, who are defined as those individuals born in Mexico having a Spanish derived last name, with Mexican ancestors at least back to the third generation. Mestizos are the result of 500 years of admixture between Spaniards (53.2%), Amerindians (30.8%), and Africans (15.9%) individuals, and they currently represent most of the Mexican population (≥90%) [32,33]. The RA patients were enrolled from the Rheumatology Departments of the Hospital Civil "Fray Antonio Alcalde", Guadalajara, Jalisco, Mexico and Hospital "Valentín Gómez Farías", ISSSTE, Zapopan, Jalisco, Mexico. Spanish HAQ-DI (Spanish version of the health assessment questionnaire disability index) [34] and DAS28 (disease activity score using 28 joint counts) [35] scores were applied to the RA patients in order to measure the functional disability and clinical activity, respectively. Informed written consent was obtained from all subjects before their enrollment to the study. The investigation was performed according to the ethical guidelines of the 2008 Declaration of Helsinki and was approved by both the ethical investigation and biosecurity committee of the University Center of Health Sciences of the University of Guadalajara and ethical committee of the Hospital Civil "Fray Antonio Alcalde".

2.2. Laboratory assessment

Erythrocyte sedimentation rate (ESR), white blood cell count (WBC), platelet count (PLT) (CELL-DYN 3700, Abbott Diagnostics, North Chicago, Illinois) and C-reactive protein (CRP) (IMMAGE™ Immunochemistry Systems, Beckman Coulter Inc., Fullerton, CA), were determined in all subjects included in the study.

2.3. Auto-antibodies measurement

Rheumatoid factor (RF) was measured by nephelometry according to the manufacturer's instructions (IMMAGE™ Immunochemistry Systems, Beckman Coulter Inc., Fullerton, CA). Individuals with values >20 IU/mL were regarded as RF positive. The presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies was determined using a commercially available second-generation enzyme-linked immunosorbent assay (ELISA) (Anti-CCP, DIASTAT™, Axis-Shield Diagnostics Limited, Dundee, Scotland, UK). A cut off of >5 U/mL was used as a stringent criterion for anti-CCP positive.

2.4. Genotyping of the +1858C/T PTPN22 gene polymorphism

The genomic DNA (gDNA) from the patients and controls were obtained from peripheral blood leukocytes according to standard procedures. Genotyping of the +1858C/T *PTPN22* gene SNP (rs2476601) was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, using the following sense primer (5′-ATGTTGCTTCAACGGAATTT-3′) and antisense primer (5′-CATGCTGCTATTGCTCTGCT-3′) [23]. The 25 μL PCR reactions contained 1 μg of gDNA, 3 μM of each primer, 0.625 units *Taq* DNA polymerase (Invitrogen™ Life Technologies, Carlsbad, CA), supplied buffer enzyme 1×, 3 mM $MgCl_2$ and 2.5 mM of each dNTP (Invitrogen™ life technologies). Thermal cycling was performed with an initial activation step at 95 °C

for 2 min, 33 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 2 min. The PCR product resulted in an amplified fragment of 412 bp analyzed on a 2% agarose gel (Invitrogen™ life technologies) stained with ethidium bromide. The amplified fragment was then incubated with 3 unit of *Xcm I* restriction enzyme (New England BioLabs, Beverly, Massachusetts) for 1 h in a heat bath at 37 °C. Subsequently, the digestion product was applied on a 3% agarose gel (Invitrogen™ life technologies) stained with ethidium bromide permitting the differentiation of the following restriction pattern: the wild-type genotype (CC) corresponds to a 412 bp fragment, heterozygote genotype (CT) is represent by 412, 246 and 166 bp fragments and homozygote polymorphic genotype (TT) corresponds to 246 and 166 bp fragments (data not shown). The genotyping technique for the +1858C/T *PTPN22* gene SNP was confirmed by DNA sequencing of a subset of samples, randomly selected, using a DNA Genetic Analyzer ABI Prism 310 (Applied Biosystems, Foster, California) (data not shown).

2.5. Statistical analysis

Hardy–Weinberg equilibrium and genotypic and allelic frequencies were tested using Chi-square test on 2×3 or 2×2 contingency tables (Epi Info Statistical Software 3.3.2, Atlanta, GA). For other comparisons, we used non-parametric Kruskal–Wallis test, Mann–Whitney *U* test and Spearman's correlation test (SPSS Statistical Software 18.0, Chicago, IL, USA). Results were given as mean values, standard deviation and minimum and maximum scores. In each test, a *p* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical features

The demographic and clinical data of the RA patients are shown in Table 1. All RA patients presented a disease moderate clinical activity and some functional disability degree, defined by DAS28 (4.71 ± 1.46 score) and Spanish HAQ-DI (0.73 ± 0.63 score) indexes, respectively. In addition, the RA patients had a disease average duration of 10 ± 9 years and they were treated mainly with disease modifying antirheumatic drugs (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs). Moreover, of the 304 RA patients tested for anti-CCP antibodies, the 83% were positives to these antibodies and of the 204 RA patients analyzed for RF the 92% were positives. Respect to the laboratory assessment, the RA patients showed significantly higher levels of acute phase reactants (ESR and CRP), RF, anti-CCP, WBC and PLT counts in comparison with controls ($p < 0.001$; Table 2).

3.2. Genotype and allele frequencies of the +1858C/T PTPN22 gene polymorphism

The genotypic and allelic frequencies of the +1858C/T *PTPN22* gene SNP in RA patients and controls is shown in Table 3. Our population were in agreement with the Hardy–Weinberg equilibrium ($p = 0.927$; data not shown). When we compared the genotypic frequencies between RA patients and controls, we observed significant differences ($p = 0.01$, OR = 2.40, 95%CI = 1.24–4.64). Moreover, carriage of the +1858T allele (CT + TT combination) was significantly increased in patients with RA compared with controls (CT + TT genotype versus CC genotype, $p = 0.004$, OR = 2.65, 95%CI = 1.33–5.36). In addition, the +1858T allele frequency was also significantly increased in RA patients compared with controls ($p = 0.001$, OR = 2.83, 95%CI = 1.50–5.32) (Table 3).

Table 1
Demographic and clinical characteristics of the RA patients.

	RA patients ($n = 309$)
Demographic	
Age (years)	48 ± 14 (18–86)
Sex (females/males)	286/23
Clinical	
Disease duration (years)	10 ± 9 (0.11–52)
Painful joints (28 counts)	7 ± 7 (0–28)
Swollen joints (28 counts)	6 ± 6 (0–28)
Patient's global assessment of disease status (0–10 VAS)	5 ± 3 (0–10)
DAS28 score	4.71 ± 1.46 (1.13–8.42)
Spanish HAQ-DI score	0.73 ± 0.63 (0.00–2.83)
Anti-CCP Ab positive (number anti-CCP Ab positive/total number of patients)	251/304 (83%)
Anti-CCP Ab negative (number anti-CCP Ab negative/total number of patients)	53/304 (17%)
RF positive (number RF positive/total number of patients)	188/204 (92%)
RF negative (number RF negative/total number of patients)	16/204 (8%)
Drug treatment	
Steroids	74/309
Prednisone <8.5 mg/day	48/74
DMARDs	240/309
Methotrexate	192/240
Chloroquine	111/240
Hydroxichloroquine	46/240
Azulfidine	92/240
Azathioprine	8/240
Leflunomide	6/240
Biologics	7/309
Etanercept	6/7
Infliximab	1/7
NSAIDs	224/309

The data are presented as the mean ± standard deviation, minimum and maximum scores. RA, rheumatoid arthritis; CS, control subjects; VAS, visual analogue scale; DAS28, disease activity score using 28 joint counts; Spanish HAQ-DI, Spanish version of the Health Assessment Questionnaire Disability Index; Anti-CCP, anti-cyclic citrullinated peptide antibodies; Ab, antibody; RF, rheumatoid factor; DMARDs, disease modifying anti-rheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs.

3.3. Demographic and clinical characteristic and laboratorial assessment in RA patients according to the +1858C/T PTPN22 gene polymorphism

The demographic and clinical characteristics of RA patients according to the +1858C/T *PTPN22* gene SNP genotypes did not show significant differences, however, a slight tendency to an increased number of painful joints, and DAS28 score in RA patients carriers of the CT genotype was observed ($p > 0.05$; data not shown). In addition, we stratified the RA patients according to anti-CCP status (Table 4). In the group of patients seropositive for anti-CCP antibodies, carrying the +1858T variant increased the risk for RA ($p = 0.008$, OR = 2.5, 95%CI = 1.3–5.0) when we compared with controls; however, in the group of seronegative patients, we no found significant difference ($p = 0.1$, OR = 2.5, 95%CI = 0.9–7.2). Furthermore, we analyzed the relationship between the +1858T allele (CT + TT) and the presence or absence of autoantibodies (anti-CCP) in RA patients, however, there was no significant differences between the RA patients subgroups ($p = 0.7$; data not shown).

4. Discussion

Rheumatoid arthritis is a chronic inflammatory disorder affecting 1% of the adult populations. The disease is characterized by inflammation of the synovial tissue of multiple joints leading pain, deformities and a reduced quality of life. The etiology of rheumatoid arthritis is complex and largely unknown [36]. During the last decades, there have been an increasing number of confirmed

Table 2
Clinical and hematological parameters in RA patients and controls.

Parameters	RA patients	CS	*p* value
ESR (mm/h) (RA: *n* = 309; CS: *n* = 347)	36.88 ± 13.70 (5–67)	16.28 ± 11.29 (0–50)	<0.001
CRP (mg/dL) (RA: *n* = 309; CS: *n* = 347)	12.72 ± 27.92 (0.10–168.80)	1.66 ± 2.42 (0.06–17.00)	<0.001
RF (UI/mL) (RA: *n* = 204; CS: *n* = 210)	350.60 ± 634.82 (1.4–4280)	13.97 ± 8.45 (0.00–42.0)	<0.001
Anti-CCP Ab (U/mL) (RA: *n* = 304; CS: *n* = 266)	129.00 ± 135.06 (0.90–621.40)	1.61 ± 0.49 (0.88–4.66)	<0.001
WBC (κ/μL) (RA: *n* = 309; CS: *n* = 347)	7.20 ± 2.40 (2.23–16.80)	6.46 ± 1.72 (3.26–12.30)	<0.001
PLT (κ/μL) (RA: *n* = 309; CS: *n* = 347)	327.90 ± 107.89 (108–839)	267.17 ± 68.65 (91–584)	<0.001

The values represent the mean ± standard deviation, minimum and maximum scores. RA, rheumatoid arthritis; CS, control subjects; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptide antibodies; Ab, antibody; WBC, white blood cells count; PLT, platelet count. The means difference between groups was analyzed using Mann–Whitney *U* test. Statistical significance was at $p < 0.05$.

Table 3
Distribution of the +1858C/T *PTPN22* gene polymorphism frequency in RA patients and controls.

	RA patients (*n* = 309)		CS (*n* = 347)		*p* value	OR	95%CI
	%	(*n*)	%	(*n*)			
Genotype							
CC	90	(278)	96	(333)			
CT	9	(28)	4	(14)	0.01	2.40	1.24–4.64
TT	1	(3)	0	(0)	0.16[a]	8.4	0.4–163.0
CT + TT	10	(31)	4	(14)	0.004[b]	2.65	1.33–5.36
Allele							
C	94	(584)	98	(680)			
T	6	(34)	2	(14)	0.001	2.83	1.50–5.32

The values are presented as frequency in percentage and number of genotypes or alleles. The frequencies comparison between groups was analyzed by Chi-Square test. Statistical significance was at $p < 0.05$.

[a] Fisher's Exact Test (2 tailed) with a hypothetical CS positive for +1858T variant.

[b] As compared with the RA patients and CS carriers of the CC genotypes. RA, rheumatoid arthritis; CS, control subjects; OR, odds ratio; CI, confidence interval.

susceptibility loci identified for RA and other complex diseases [37]. One of the first known genetic loci responsible for susceptibility to RA was found within the major histocompatibility complex, namely immune response genes in the human leukocyte antigen (HLA) class II region. However, recently genome-wide association studies (GWAS) have confirmed known and identified new genetic determinants of RA [11]. These studies showed that the second important genetic contribution comes from +1858C/T *PTPN22* gene SNP, which increases the risk of the RA by 40–80% in Caucasian populations [37]. Likewise, this GWAS have emphasized the importance of the *PTPN22* gene in susceptibility to rheumatoid arthritis [38].

The association between the +1858C/T *PTPN22* SNP and RA has been documented in several studies [12,22,24,39,40]. This is the first study that analyzes the association of the +1858C/T *PTPN22* SNP with RA in Mexican population and in accordance with other authors we demonstrated a strong significant association between the +1858C/T *PTPN22* SNP with RA, since the +1858T risk allele was significantly more frequent in RA patients than controls and confirm that +1858T allele behaves as a dominant variant, conferring increased risk of disease already when is present in a single copy [41]. However, in the case of homozygous genotype (TT) for the *PTPN22* +1858C/T SNP, we no found significant difference, maybe due to a low frequency of this genotype in our population; but, none control subject was homozygous for the polymorphic variant of this SNP.

Likewise, although the frequencies of the +1858C/T *PTPN22* gene polymorphism in RA patients and controls in our population are statistically different, they are not dramatically different, and this is probably due to the low frequency of the +1858T risk allele. However, our data are very close to previous reports in other Latin-American populations like Colombian [13], maybe due to the similar ancestry of both populations. The genotypic and allelic frequencies reported for Spanish [18], North American [17] and Dutch [24] populations are similar to ours, but those reported in others European populations are higher, demonstrating the allelic variation pattern observed for the +1858T risk allele in Caucasian populations, since generally is increased its frequency from southern to northern Europe [8,41]. Taken together, our data support the previously reported association of the +1858T risk allele with RA in ethnically different populations as well as the role of the *PTPN22* gene as a susceptibility genetic marker for RA.

On the other hand, at present, the effect of the +1858C/T *PTPN22* SNP on the disease severity and clinical activity in RA is unclear, due to that the studies that analyzed the effect of the +1858T risk allele on these clinical characteristics have not found any association [14,22,23]. In our study not significant differences were observed between the +1858C/T *PTPN22* gene SNP genotypes and the demographic and clinical characteristics of the RA patients. In our study we evaluated the relationship between anti-CCP status and +1858C/T *PTPN22* SNP for susceptibility to RA in population from Western Mexico and confirm that in patients who were

Table 4
Frequencies of +1858C/T *PTPN22* genotypes in rheumatoid arthritis patients stratified according to anti-CCP antibodies status.

	CC % (*n*)	CT + TT % (*n*)	RA versus all CS		
			p value	OR	95% IC
Controls (*n* = 347)	96 (333)	4 (14)			
Anti-CCP Ab-positive[a] (*n* = 251)	90 (227)	10 (24)	0.008	2.5	1.3–5.0
Anti-CCP Ab-negative[a] (*n* = 53)	91 (48)	9 (5)	0.1	2.5	0.9–7.2

[a] Referred as subgroups of rheumatoid arthritis patients; OR, odds ratio; CI, confidence interval; anti-CCP, anti-cyclic citrullinated peptide; Ab, antibody; RA, rheumatoid arthritis.

seropositive for anti-CCP antibodies, the presence of the +1858T allele (CT+TT) increase a risk for developing RA; but not in RA patients who were seronegative for this autoantibody. None of the control subject were anti-CCP positive in our sample. Our results are in agreement with others previous reports [25,29,42].

The present results, together with data from other groups [17,20–23] suggest that the polymorphic variant of the +1858C/T *PTPN22* SNP is implicated in the pathogenesis of RA through a mechanism that involves the previous production of antibodies against citrullinated peptide/proteins [25,27,29]. The *PTPN22* gene encodes a phosphatase called LYP, a negative regulator of the T cells signaling [20]. LYP is also expressed in other cells types like B cells, monocytes, neutrophils and dendritic cells [12]. The presence of the +1858T allele, affects LYP function, resulting in a loss of function protein [1,5,9,10], that are unable to regulates the T cell activation leading to a hyperresponsiveness phenotype of T, B and also dendritic cells [10]. Under these conditions, B lymphocytes could produces increasing amounts of anti-CCP antibodies, leading to a vicious circle of inflammation and conditioning an immunologic milieu favoring the emergence of autoimmune diseases like RA [10,27].

5. Conclusions

In conclusion, this study shown that the +1858T allele in the *PTPN22* gene is a genetic risk factor for susceptibility to RA and confirm that, in combination with anti-CCP antibodies, this SNP influence the autoimmune processes towards a development of RA in Mexican population.

Conflict of interest statement

The authors declare that they have no conflicts of interest related to the publication of this manuscript.

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References

[1] Zhebrun D, Kudryashova Y, Babenko A, Maslyansky A, Kunitskaya N, Popcova D, et al. Association of PTPN22 1858T/T genotype with type 1 diabetes, Graves' disease but not with rheumatoid arthritis in Russian population. Aging (Albany, NY) 2011;3:368–73.
[2] Ikari K, Momohara S, Inoue E, Tomatsu T, Hara M, Yamanaka H, et al. Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population. Rheumatology (Oxford) 2006;45:1345–8.
[3] Goëb V, Dieudé P, Daveau R, Thomas-L'otellier M, Jouen F, Hau F, et al. Contribution of PTPN22 1858T, TNFRII 196R and HLA-shared epitope alleles with rheumatoid factor and anti-citrullinated protein antibodies to very early rheumatoid arthritis diagnosis. Rheumatology (Oxford) 2008;47:1208–12.
[4] Goronzy JJ, Weyand CM. T-cell regulation in rheumatoid arthritis. Curr Opin Rheumatol 2004;16:212–7.
[5] Suarez-Gestal M, Calaza M, Dieguez-Gonzalez R, Perez-Pampin E, Pablos JL, Navarro F, et al. Rheumatoid arthritis does not share most of the newly identified systemic lupus erythematosus genetic factors. Arthritis Rheum 2009;60:2558–64.
[6] Totaro MC, Tolusso B, Napolioni V, Faustini F, Canestri S, Mannocci A, et al. PTPN22 1858C>T polymorphism distribution in Europe and association with rheumatoid arthritis: case–control study and meta-analysis. PLoS One 2011;6:e24292.
[7] Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 2004;36:337–8.
[8] Vang T, Miletic AV, Bottini N, Mustelin T. Protein tyrosine phosphatase PTPN22 in human autoimmunity. Autoimmunity 2007;40:453–61.
[9] Zikherman J, Hermiston M, Steiner D, Hasegawa K, Chan A, Weiss A. PTPN22 deficiency cooperates with the CD45 E613R allele to break tolerance on a non-autoimmune background. J Immunol 2009;182:4093–106.
[10] Zhang J, Zahir N, Jiang Q, Miliotis H, Heyraud S, Meng X, et al. The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. Nat Genet 2011;43:902–7.
[11] Stark K, Rovenský J, Blazicková S, Grosse-Wilde H, Ferencik S, Hengstenberg C, et al. Association of common polymorphisms in known susceptibility genes with rheumatoid arthritis in a Slovak population using osteoarthritis patients as controls. Arthritis Res Ther 2009;11:R70.
[12] Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330–7.
[13] Gomez LM, Anaya JM, Gonzalez CI, Pineda-Tamayo R, Otero W, Arango A, et al. PTPN22 C1858T polymorphism in Colombian patients with autoimmune diseases. Genes Immun 2005;6:628–31.
[14] van Oene M, Wintle RF, Liu X, Yazdanpanah M, Gu X, Newman B, et al. Association of the lymphoid tyrosine phosphatase R620W variant with rheumatoid arthritis, but not Crohn's disease, in Canadian populations. Arthritis Rheum 2005;52:1993–8.
[15] Hinks A, Barton A, John S, Bruce I, Hawkins C, Griffiths CE, et al. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. Arthritis Rheum 2005;52:1694–9.
[16] Simkins HM, Merriman ME, Highton J, Chapman PT, O'Donnell JL, Jones PB, et al. Association of the PTPN22 locus with rheumatoid arthritis in a New Zealand Caucasian cohort. Arthritis Rheum 2005;52:2222–5.
[17] Lee AT, Li W, Liew A, Bombardier C, Weisman M, Massarotti EM, et al. The PTPN22 R620W polymorphism associates with RF positive rheumatoid arthritis in a dose-dependent manner but not with HLA-SE status. Genes Immun 2005;6:129–33.
[18] Orozco G, Sánchez E, González-Gay MA, López-Nevot MA, Torres B, Cáliz R, et al. Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Rheum 2005;52:219–24.
[19] Steer S, Lad B, Grumley JA, Kingsley GH, Fisher SA. Association of R602W in a protein tyrosine phosphatase gene with a high risk of rheumatoid arthritis in a British population: evidence for an early onset/disease severity effect. Arthritis Rheum 2005;52:358–60.
[20] Dieudé P, Garnier S, Michou L, Petit-Teixeira E, Glikmans E, Pierlot C, et al. Rheumatoid arthritis seropositive for the rheumatoid factor is linked to the protein tyrosine phosphatase nonreceptor 22-620W allele. Arthritis Res Ther 2005;7:R1200–7.
[21] Wesoly J, van der Helm-van Mil AH, Toes RE, Chokkalingam AP, Carlton VE, Begovich AB, et al. Association of the PTPN22 C1858T single-nucleotide polymorphism with rheumatoid arthritis phenotypes in an inception cohort. Arthritis Rheum 2005;52:2948–50.
[22] Pierer M, Kaltenhäuser S, Arnold S, Wahle M, Baerwald C, Häntzschel H, et al. Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: higher frequency of the risk allele in male compared to female patients. Arthritis Res Ther 2006;8:R75–81.
[23] Harrison P, Pointon JJ, Farrar C, Brown MA, Wordsworth BP. Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients. Rheumatology (Oxford) 2006;45:1009–11.
[24] Wesoly J, Hu X, Thabet MM, Chang M, Uh H, Allaart CF, et al. The 620W allele is the PTPN22 genetic variant conferring susceptibility to RA in a Dutch population. Rheumatology (Oxford) 2007;46:617–21.
[25] Kokkonen H, Johansson M, Innala L, Jidell E, Rantapää-Dahlqvist S. The PTPN22 1858C/T polymorphism is associated with anti-cyclic citrullinated peptide antibody-positive early rheumatoid arthritis in northern Sweden. Arthritis Res Ther 2007;9:R56–62.
[26] Majorczyk E, Jasek M, Płoski R, Wagner M, Kosior A, Pawlik A, et al. Association of PTPN22 single nucleotide polymorphism with rheumatoid arthritis but not with allergic asthma. Eur J Hum Genet 2007;15:1043–8.
[27] van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. Arthritis Rheum 2004;50:709–15.
[28] Van der Helm-van Mil AH, Le Cessie S, van Dongen H, Breedveld FC, Toes RE, Huizinga TW. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. Arthritis Rheum 2007;56:433–40.
[29] Johansson M, Arlestig L, Hallmans G, Rantapää-Dahlqvist S. PTPN22 polymorphism and anti-cyclic citrullinated peptide antibodies in combination strongly predicts future onset of rheumatoid arthritis and has a specificity of 100% for the disease. Arthritis Res Ther 2006;8:R19.
[30] Feitsma AL, Toes RE, Begovich AB, Chokkalingam AP, de Vries RR, Huizinga TW, et al. Risk of progression from undifferentiated arthritis to rheumatoid arthritis: the effect of the PTPN22 1858T-allele in anti-citrullinated peptide antibody positive patients. Rheumatology (Oxford) 2007;46:1092–5.
[31] Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
[32] Rangel-Villalobos H, Muñoz-Valle JF, González-Martín A, Gorostiza A, Magaña MT, Páez-Riberos LA. Genetic admixture, relatedness and structure patterns

among Mexican populations revealed by the Y-chromosome. Am J Phys Anthropol 2008;135(4):448–61.
[33] Rubi-Castellanos R, Martínez-Cortés G, Muñoz-Valle JF, González-Martín A, Cerda-Flores RM, Anaya-Palafox M, et al. Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. Am J Phys Anthropol 2009;139(3):284–94.
[34] Cardiel MH, Abello-Banfi M, Ruiz-Mercado R, Alarcon-Segovia D. How to measure health status in rheumatoid arthritis in non-English speaking patients: validation of a Spanish version of the Health Assessment Questionnaire Disability Index (Spanish HAQ-DI). Clin Exp Rheumatol 1993;11:117–21.
[35] Leeb BF, Andel I, Sautner J, Bogdan M, Maktari A, Nothnagl T, et al. Disease activity measurement of rheumatoid arthritis: comparison of the simplified disease activity index (SDAI) and the disease activity score including 28 joints (DAS28) in daily routine. Arthritis Rheum 2005;53:56–60.
[36] Coenen MJ, Trynka G, Heskamp S, Franke B, van Diemen CC, Smolonska J, et al. Common and different genetic background for rheumatoid arthritis and coeliac disease. Hum Mol Genet 2009;18:4195–203.
[37] Barton A, Worthington J. Genetic susceptibility to rheumatoid arthritis: an emerging picture. Arthritis Rheum 2009;61:1441–6.
[38] Wan Taib WR, Smyth DJ, Merriman ME, Dalbeth N, Gow PJ, Harrison AA, et al. The PTPN22 locus and rheumatoid arthritis: no evidence for an effect on risk independent of Arg620Trp. PLoS One 2010;5:e13544.
[39] Viken MK, Amundsen SS, Kvien TK, Boberg KM, Gilboe IM, Lilleby V, et al. Association analysis of the 1858C>T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. Genes Immun 2005;6:271–3.
[40] Carlton VE, Hu X, Chokkalingam AP, Schrodi SJ, Brandon R, Alexander HC, et al. PTPN22 genetic variation: evidence for multiple variants associated with rheumatoid arthritis. Am J Hum Genet 2005;77:567–81.
[41] Gregersen PK, Lee HS, Batliwalla F, Begovich AB. PTPN22: setting thresholds for autoimmunity. Semin Immunol 2006;18:214–23.
[42] Orozco G, Pascual-Salcedo D, López-Nevot MA, Cobo T, Cabezón A, Martín-Mola E, et al. Auto-antibodies, HLA and PTPN22: susceptibility markers for rheumatoid arthritis. Rheumatology (Oxford) 2008;47:138–41.