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Association of the PTPN22 R620W polymorphism with increased risk for SLE in the genetically homogeneous population of Crete

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Abstract

Autoimmune diseases affect approximately 5% of the population, but much work remains to define the genetic risk factors and pathogenic mechanisms underlying these conditions. There is accumulating evidence that common genetic factors might predispose to multiple autoimmune disorders. Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are complex autoimmune disorders with multiple susceptibility genes. The functional R620W (C1858T) polymorphism of the protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene, a member of the PTPs that negatively regulate T-cell activation, has been recently associated with susceptibility to various autoimmune diseases. The aim of this study was to assess whether the C1858T polymorphism of *PTPN22* also confers increased risk for SLE and RA in the genetically homogeneous population of Crete. It was found that the minor T allele of the *PTPN22* C1858T SNP was more common in SLE patients than in control individuals (odds ratio [OR] = 1.91, 95% confidence interval [CI] = 1.11 to 3.9, $p = 0.017$). No significant difference was observed in the frequency of this allele when RA patients were compared with controls (OR = 1.14, 95% CI = 0.65 to 1.9, $p = 0.64$). Although the *PTPN22* 1858T allele is found at decreased frequency in Southern Europe, including Crete, an association was found between this allele and SLE in the population studied.

Keywords

polymorphism; *PTPN22* gene; rheumatoid arthritis; systemic lupus erythematosus

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Conflict of interest
None declared.

Introduction

Autoimmune diseases arise from the aberrant immune response to self-antigens, and share a number of characteristics suggesting common etiologic pathways. They are chronic conditions, characterized by varying clinical manifestations. Although autoimmune diseases affect approximately 5% of the population, much work remains to elucidate the genetic risk factors and pathogenic mechanisms involved in these complex diseases. Recent observations support a model of shared genetic risk factors for many of these diseases, pointing to previously unexpected common biologic pathways. The clarification of the role of shared immunological mechanisms in several immune-related disorders will further improve our understanding of the etiology of these diseases and should contribute to the development of preventative and disease-modifying therapeutic protocols. Common genetic loci, including the human leukocyte antigen (HLA)[1] genes and many non-HLA genes, have been implicated in multiple autoimmune diseases in populations of different ethnic or racial origin, such as cytotoxic T-lymphocyte-associated- 4 (*CTLA-4*), protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*) (protein tyrosine phosphatase), signal transducer and activator of transcription-4 (*STAT4*), TNF-receptor associated factor 1 and complement component 5 (*TRAF1/C5*), interleukin-2 receptor A (IL2RA), and interleukin-23 receptor (IL23R).[2]

The lymphoid-specific phosphatase (LYP) encoded by the *PTPN22* gene on chromosome 1p13 was initially thought to be an excellent candidate for autoimmune diseases because it is involved in preventing spontaneous T-cell activation.[3] Indeed, there are data showing that the R620W polymorphism of the *PTPN22* gene is associated with SLE,[4] RA,[5] juvenile idiopathic arthritis (JIA),[6] type 1 diabetes (T1D),[7,8] systemic sclerosis,[9] Wegener's granulomatosis (WG),[10] Graves' disease (GD),[11] vitiligo,[12] Addison's disease,[13] alopecia areata[14] and Hashimoto thyroitidis.[15] This long list of *PTPN22*-associated autoimmune conditions strongly supports the idea that particular genes are capable of predisposing to multiple autoimmune diseases. The *PTPN22* gene encodes for a tyrosine phosphatase, LYP protein, which is involved in immune regulation as a negative regulator of T-cell activation. The *PTPN22* gene polymorphism causing an amino acid change (R620W) at the proline-rich motif of LYP has been shown to affect the protein–protein interaction with tyrosine kinase Csk in T cell activation. Individuals carrying the variant allele of *PTPN22* are thought to have changes in the threshold for thymic selection, with increased numbers of auto-reactive T cells escaping negative selection, thus persisting in the circulation, and are prone to autoimmunity.[16] Additionally, the C1858T variant is associated with changes in cytokine profile in SLE patients in vivo.[17] However, a further elucidation of the molecular mechanism of action is needed, as both gain and loss of function mechanisms have been reported.[18]

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune diseases involving multiple organ systems. SLE is a multifactorial, systemic disease that predominantly affects women, characterized by the production of autoantibodies directed against nuclear components. The etiology of the disease remains elusive, even though it has been intensively studied.[19] Rheumatoid arthritis is a chronic autoimmune disease of unknown etiology, characterized by progressive joint destruction resulting in severe disability.[20] Both SLE and RA result from the combined effects of a large number of genes; each allele contributes mildly and the accumulation of several genes is presumed necessary to significantly increase the risk of the disease.

It has become increasingly clear that there are distinct differences regarding the genetic association between various genes and autoimmune diseases depending upon the geographical ancestry of the population studied. In the case of *PTPN22*, there is a strong

gradient of C1858T allele frequency between Southern and Northern Europe (from 2.1% in Southern Europe up to 15.5% in Northern Europe), whereas it is almost absent in Asian populations.[21] Additionally, the *PTPN22* C1858T polymorphism is almost completely absent in African populations.[22] This heterogeneity of *PTPN22* C1858T allele frequency in different world populations supports the importance of genetic association studies of this allele in multiple ethnic populations. The purpose of this study was to investigate the role of the C1858T SNP polymorphism of *PTPN22*, representing the primary disease-associated genetic variant of the *PTPN22* gene locus, in susceptibility to SLE and RA in the genetically homogeneous population of Crete.

Materials and methods

Study population

DNA was obtained from cohorts of SLE and RA from unrelated families from the island of Crete. The SLE sample set included 328 patients and 427 controls. The RA sample set comprised 378 patients and 430 healthy controls. Control cohorts were age- and sex-matched to each specific disease. Healthy volunteers from the Department of Transfusion Medicine of the University Hospital of Crete served as controls. Patients with SLE and RA were diagnosed by a rheumatologist. All SLE patients met the 1982 ACR revised criteria for the classification of SLE,[23] while RA patients met the American Rheumatism Association 1987 revised criteria.[24] All patients and controls were included if they had no other autoimmune diseases. Ethnic bias within the population studied was minimized by excluding patients who were not of Cretan origin, defined as having four grandparents with Cretan ancestry. The study was approved by the institutional committee of University Hospital of Crete.

DNA extraction and genotyping of rs2476601 *PTPN22* SNP

Whole blood was collected in EDTA-containing tubes, and genomic DNA was isolated from blood leukocytes by using the commercial kit Qiamp DNA Blood Mini kit (QIAGEN Inc., CA, USA). The extracted DNA was stored at −20°C until it was used for genotyping. After we confirmed by preliminary genotyping that the rs2476601 SNP was also polymorphic in Crete, we proceeded to the genotyping of the cohort of patients as well as the proper age- and sex-matched controls. The upstream primer 5′-TCACCAGCTTCCTCAACCACA-3′ and the downstream primer 5′-GATAATGTTGCTTCAACGGAATTT-3′ were used to generate a 220 bp amplicon from the *PTPN22* gene. The amplification was carried out using the Taq PCR Core kit provided by QIAGEN. PCR products were analyzed through electrophoresis on 2.5% agarose gel and ethidium bromide fluorescence in reference to a molecular weight marker. Genotyping for the *PTPN22* rs2476601 PCR product (215 bp) was performed by restriction analysis using the *Xcm*I (New England Biolabs, Ipswich, MA) restriction enzyme, which digests specifically DNA amplified from the minor allele (T).[25] Both undigested and digested PCR products were visualized in 2.5% agarose gel stained with ethidium bromide. Genotypes were scored blindly and analysis of all ambiguous samples was repeated.

Ten percent of the samples were amplified twice to check the accuracy of our genotyping results, and the results were 100% identical to those of the first genotyping attempt. The accuracy of our RFLP genotyping approach was further verified by genotyping half of the RA patients and the control samples from Crete by using the Sequenom® MassArray™ technology, according to the manufacturer's instructions, using iPLEX chemistry (http://www.sequenom.com), and this analysis provided identical results (Darren Plant, University of Manchester, personal communication).

Statistical analysis

In the case–control comparisons, only unrelated cases and controls were used. The *PTPN22* gene variants under investigation were evaluated for deviation from Hardy–Weinberg equilibrium by comparing observed and expected genotype frequencies using Fisher's exact test in the control groups. Fisher's exact test, with one degree of freedom, was used to examine differences of genotype and allele frequencies between patients and controls. OR and CI were calculated according to Rothman. As one polymorphism was being investigated in each disease condition, a p value threshold of 0.05 was defined as significant.

Results

The rs2476601 *PTPN22* C1858T SNP confers susceptibility to SLE

We replicated for the first time in the Greek population the association of the *PTPN22* C1858T variant with potential risk for developing SLE. The study group (N = 328) consisted of 301 women (91.77%) and 27 men (8.23%), while unrelated healthy controls (N = 427) were of similar age and sex. Mean (±SD) age in patients with SLE was 40.2±8.9 years. Allele and genotype frequencies of the analyzed samples of the rs2476601 *PTPN22* C/T polymorphisms are depicted in Table 1. We found an increased frequency of the C/T heterozygosity in SLE patients compared with healthy controls (10.06% versus 5.39%, p = 0.015, OR = 1.96, 95% CI = 1.13–3.41). Homozygosity for the minor allele T was not detected in any group, and thus no information could be obtained regarding the putative involvement of this genotype, and results of an allelic association test were very similar to the genotype test results, as would be expected (p = 0.017, OR = 1.9, 95% CI = 1.1–3.3) (Table 1). These findings clearly support a role for this polymorphism in the development of SLE in Greece, in accordance with previous studies conducted in other ancestral backgrounds. Of note, no difference was observed when the C/T genotype was assessed in relation to lupus glomerulonephritis, but the limited number of patients with this genotype makes the detection of subphenotype association difficult (data not shown). The distribution of genotypes did not show any deviation from Hardy–Weinberg equilibrium in patients (p = 0.92) or controls (p = 0.98).

The rs2476601 *PTPN22* C1858T SNP is not associated with RA

Allele and genotype frequencies of the *PTPN22* C/T polymorphism analyzed in patients and controls are summarized in Table 2. The RA study group consisted of 305 women and 73 men, and unrelated healthy controls (N = 430) were of similar age and sex. The mutated genotype C/T was observed in 6.61% of RA patients and 5.81% of controls, without any statistically significant difference between them. Similar findings were observed for the allele frequencies (C and T) in patients and controls. The minor allele T was observed in 3.31% of RA patients and 2.91% of controls. Statistical analysis showed that this difference was not statistically significant (p = 0.64, OR = 1.14, 95% CI = 0.65–1.9). The distribution of genotypes showed no deviation from Hardy–Weinberg equilibrium (Fisher's exact test, p = 0.98 for controls, p = 0.97 for patients). Notably, when we examined the association between *PTPN22* C/T genotype in RA patients and the presence of rheumatoid factor (RF), no correlation was found (data not shown). It is worthwhile noting that the minor allele T was not found in the homozygous state in any of the Cretan cases or controls in this study.

Discussion

Despite extensive research efforts for more than a decade, the genetic basis of common human autoimmune diseases remains largely unknown. Thus, although there have been some notable successes in both Genome Wide Association Studies (GWAS) and candidate gene studies, much of the heritability of these disorders has not been explained. Moreover,

there is a strong precedent for differences in the genetic factors associated with autoimmunity in different populations. In this context, the aim of the present study was to confirm the role of *PTPN22* rs2476601 gene polymorphism in the predisposition to SLE and RA in a genetically homogeneous Greek population from Crete.

While we confirm the association of the *PTPN22* 1858 T polymorphism with SLE susceptibility in Greece, we cannot confirm the association of this polymorphism with RA in the current dataset. Of note, preliminary results from association studies conducted by using two Greek cohorts of T1D patients did not confirm the well-known association between T1D and *PTPN22* 1858 T polymorphism (data not shown). The present observations are in accordance with inconsistencies concerning previously reported genetic associations in European-derived cohorts and those observed in Cretan cohorts, probably due to the genetic peculiarities of the population of Crete. Crete is the largest island of Greece, with about 0.65 million inhabitants. This population offers advantages for genetic association studies in complex diseases, since its members share the same genetic and cultural background and a common environment. These factors enable genetic studies, given that the validity of genetic association studies is greatly affected by genetic heterogeneity, the low penetrance of individual disease alleles, and the potential for gene–gene and gene–environment interactions.[26]

The observation that the T allele, shown to confer higher risk for RA development in other studies, does not do so in the Greek population is striking, given that this association has been widely replicated in the literature. In terms of strength of association, *PTPN22* is second in importance only to the major histocompatibility complex (MHC) in RA.[2] However, the aforementioned situation does not weaken the validity of our results. In previous studies of European-derived RA cohorts, the *PTPN22* genetic association was specific to the subset of RA patients who had anti-cyclic citrullinated peptide (anti-CCP) antibodies. Anti-CCP antibody testing was not available in our cohort; it is possible that future stratification of the Cretan RA cohort by anti-CCP antibodies may reveal an association confined to the CCP-positive group, similarly to other European cohorts. Recent association studies in autoimmune diseases performed in Greece did not replicate some of the associations reported in other cohorts. For example, the well-established SLE susceptibility rs7574865 SNP of *STAT4* is not associated with increased risk for SLE in the Greek population.[27] Discrepancies could be due to differences in the genetic background and/or small sample sizes. Furthermore, the existence of gene–gene interactions with regard to the development of the autoimmune diseases under study, as well as the importance of the entire genetic background, are concepts that should not be under-estimated, as these interactions may play a crucial role in producing variations in phenotype. However, there is still the possibility that, by increasing the size of the cohort, an association of the rs2476601 *PTPN22* SNP with RA could be found, although the minor variant is relatively rare in Greece. Considering the minor allele frequency of the *PTPN22* variant in Greek control samples (2.7%), more than 5000 patient and control samples would be required to detect even a large effect at this locus (i.e. a 50% increase in risk with 80% power at $p = 0.05$). The minor allele for rs2476601 was observed also at low frequency in patients from Crete in a previous study (2.9%),[27] similar to that found in the Turkish population (3%). Of note, no association with RA or SLE has been detected in Turkey.[28,29]

In conclusion, while studying patients and controls from the island of Crete, we replicated the association between the *PTPN22* T allele and SLE, but not RA, as has been reported in numerous studies. The low prevalence of this polymorphism in Greece and South Europe may account for the lack of association with RA. Our data show that the shared risk factors appear to have unique overlaps between different autoimmune diseases, but their

relationships may also be further complicated by ethnic differences in the frequency and/or risk of particular variants for autoimmune diseases.

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Table 1

Genotypes and allele frequency of the *PTPN22* C1858T polymorphism analyzed in 328 patients with SLE and 427 healthy controls

	SLE patients (total *N* = 328)	Control group (total *N* = 427)	*p* value	OR (95% CI)
Genotype				
C/C	295 (89.94%)	404 (94.61%)	0.015	1.96 (1.13–3.41)
C/T	33 (10.06%)	23 (5.39%)		
T/T	0 (0%)	0 (0%)		
Allele				
C	623 (94.97%)	831 (97.3%)	0.017	1.91 (1.11–3.3)
T	33 (5.03%)	23 (2.69%)		

CI: confidence interval, OR: odds ratio, SLE: systemic lupus erythematosus.

Table 2

Genotypes and allele frequency of the *PTPN22* C1858T polymorphism analyzed in 378 RA patients and 430 healthy controls

	RA patients (total *N* = 378)	Control group (total *N* = 430)	*p* value	OR (95% CI)
Genotype				
C/C	353 (93.39%)	405 (94.19%)	0.6	1.14 (0.64–2.03)
C/T	25 (6.61%)	25 (5.81%)		
T/T	0 (0%)	0 (0%)		
Allele				
C	731 (96.69%)	835 (97.09%)		
T	25 (3.31%)	25 (2.91%)	0.64	1.14 (0.65–1.9)

CI: confidence interval, OR: odds ratio, RA: rheumatoid arthritis.