Lymphoid tyrosine phosphatase R620W variant and inflammatory bowel disease in Tunisia

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AIM: To assess the possible association between PTPN22 (R620W) gene polymorphism and inflammatory bowel disease (IBD).

METHODS: One hundred and sixty-four patients with IBD [105 Crohn's disease (CD) and 59 ulcerative colitis (UC)] and 100 healthy controls were recruited. Genotyping of the PTPN22 gene 1858C→T polymorphism was performed by restriction fragment length polymorphism-polymerase chain reaction with RsaI digestion.

RESULTS: The genotypic and allelic frequencies of (R620W) PTPN22 gene polymorphism reveal a significant association of the PTPN22 620-W allele with IBD, compared to the healthy control group (OR: 17.81, 95% CI: 4.18-21.86, \( P = 0.00001 \)). Nevertheless, no difference in this polymorphism was found between CD and UC patients. No significant association was found between the frequencies of genotypes of the PTPN22 gene with either the clinical features such as sex, age, age at disease onset, and extent of colitis, or the production of serological markers (anti-Saccharomyces cerevisiae antibody in CD and perinuclear anti-neutrophil cytoplasmic antibody in UC).

CONCLUSION: These observations confirm the association of IBD susceptibility with the PTPN22 1858T (620-W) allele in Tunisian patients.

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Key words: Inflammatory bowel disease; PTPN22; Genetic polymorphism; Genetic susceptibility

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory bowel diseases (IBDs) that are characterized clinically by periods of well being punctuated by episodes of clinical disease activity that involve various sites in the gastrointestinal tract[1]. Several environmental, microbial, immunological, genetic, and life-style factors have been suggested to play a role in disease initiation[2]. Heterogeneity is observed in terms of disease location,
behavior, age of onset and serologic markers. There is some support for the concept that this heterogeneity may be in large measure genetically determined. Genomewide scans performed in patients with IBD have failed to find a major unique susceptibility locus and have prompted the general agreement that these diseases are polygenic entities in which several genes may contribute to susceptibility. Significant linkages in chromosomes 1, 3, 6, 7, 12, 14, 16, and 19 have been reported.

The PTPN22 gene, located on chromosome 1p13, encodes a lymphoid-specific protein tyrosine phosphatase (LYP), a member of a family of proteins involved in suppressing spontaneous T-cell activation via a negative regulatory C-terminal Src kinase (Csk). Indeed, LYP is implicated in maintaining the resting phenotype of lymphocytes and in controlling signals caused by an antigen, co-stimulation and cytokines. A functional C1858T single nucleotide polymorphism (SNP), which encodes an arginine to tryptophan substitution at residue 620 (R620W), is located in the P1 proline-rich motif of PTPN22, which binds with high affinity to the Src homology 3 (SH3) domain of the tyrosine kinase. Thus, it is plausible that this genetic discrepancy in PTPN22 influences a range of diseases in which the phenotypic spectrum includes an aberrant or hyperactive immune response. An association of PTPN22 (R620W) polymorphism was reported first with type 1 diabetes and later also with myasthenia gravis, systemic lupus erythematosus, and rheumatoid arthritis. However, there are also some inflammatory diseases such as psoriasis, multiple sclerosis and Behçet’s disease without an association with this polymorphism.

In this study, we analyzed the PTPN22 (R620W) polymorphism in Tunisian patients with CD and UC, to evaluate the contribution of the C1858T SNP to IBD susceptibility.

**MATERIALS AND METHODS**

**Patients and controls**

Blood samples were obtained from 164 subjects with IBD. There were 105 patients with CD (50 men, 55 women) with a mean age of 36.07 years (range: 23-60 years), and 59 patients with UC (17 men, 42 women) with a mean age of 37.89 years (range: 25-74 years). All subjects were unrelated Tunisians treated at the Department of Gastroenterology of Charles Nicolle and La Rabta Hospitals in Tunis. The diagnosis of CD and UC was determined in accordance with the standardized set of clinical, endoscopic and/or radiological and histological criteria provided by International Organization for the study of IBD. Data obtained from each patient included age at diagnosis, disease location and extent, disease characteristics, and extra-intestinal manifestations (especially peripheral arthritis, ankylosing spondylitis in CD disease and primary sclerosing cholangitis in UC), which were used to group the patients according to the Vienna classification (Table 1).

| CD patients | n = 105 |
|-------------|---------|
| Sex         | 55 men and 50 women |
| Mean age (yr) | 36 ± 10.2 |
| Age at diagnosis (yr) | 31.2 ± 12.8 |
| Disease location         |  |
| Ileitis (%) | 27 (25.7) |
| Ileocolitis (%) | 54 (51.5) |
| Colitis (%) | 24 (22.8) |
| Anal involvement (%) | 44 (42) |
| Extra-intestinal manifestations (%) | 20 (19.04) |

CD: Crohn’s disease; UC: Ulcerative colitis; ASCA: Anti-Saccharomyces cerevisiae antibodies; p-ANCA: Perinuclear anti-neutrophil cytoplasmic antibodies.

In the CD sample group, 47 sera out of 105 (44.8%) were anti-Saccharomyces cerevisiae antibodies (ASCA)-positive, and in UC sample group, 16 sera out 59 (27.1%) were perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA)-positive.

A total of 100 unrelated healthy subjects (52 men and 48 women) matched for age, sex and ethnic origin were used as the control population. None of the healthy controls had any evidence of autoimmune diseases such as IBD or diabetes.

All patients and controls gave informed consent to participate in this study, which was approved by the Ethics Committee of Charles Nicolle Hospital in Tunis.

**Methods**

Genomic DNA isolated from EDTA-anticoagulated peripheral blood samples of unrelated healthy blood donors and IBD patients was extracted by a salting-out process. Genotyping was performed using the restriction fragment length polymorphism-polymerase chain reaction method. The PCR reactions were performed in 10-μL final volume using 10 pmol of each primer: 5’-TGCCCCATGCC ACATTTAT-3’, forward primer and 5’-ACCTCCTGGGTTTGATACCTA-3’, reverse primer, and contained 50 ng extracted DNA, 1 U Taq polymerase (Promega, Madison, WI, USA), 1.5 mmol/L MgCl₂ and 0.2 mmol/L dNTP. After an initial denaturing time of 15 min at 95°C, PCR reactions were run for 35 cycles including 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, with a final extension at 72°C for 10 min. The PCR product was digested by 1 U of the enzyme Rsal (Promega) at 37°C for 1.5 h, subjected to electrophoresis in 3% agarose gel, and stained with

**Table 1 Clinical characteristics and serological markers of CD and UC**

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Allele frequency

R/R 0.010
R/W 0.152
W/W 0.889

Table 2  Genotype and allele frequencies of PTPN22 polymorphism in controls and patients

| Groups          | Genotype frequency | Allele frequency | P       |
|-----------------|--------------------|------------------|---------|
|                 | n                  | R/R              | W/W     |
| Controls (%)    | 100                | 98 (98)          | 2 (2)   | 0 (0)   | 0.990 | 0.010 | P/C: 0.00001 (OR: 17.81 CI 95%: 4.18-21.86) |
| IBD patients (%)| 164                | 114 (69.5)       | 50 (30.5)| 0 (0)| 0.848 | 0.152 |
| CD (%)          | 105                | 68 (64.8)        | 37 (35.2)| 0 (0) | 0.823 | 0.177 |
| UC (%)          | 59                 | 46 (78)          | 13 (22) | 0 (0) | 0.889 | 0.111 |

P: Patients; C: Controls; NS: Not significant.

Statistical analysis

All statistical analyses were performed with SPSS version 13.0 (Chicago, IL, USA). The Hardy-Weinberg equilibrium was assessed by the goodness-of-fit test for biallelic markers. Calculation was done using internet programs (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Statistical power was calculated using a web power calculator (http://calculators.stat.ucla.edu/powercalc/). Allelic and genotypic frequencies were evaluated by direct counting. Statistical comparisons were performed, between patients and controls, by Pearson's χ² test calculated on 2 × 2 contingency tables. Fisher's exact test was used when an expected cell value was < 5. P < 0.05 was considered to be statistically significant. The strength of the association of PTPN22 R620/W genotypes as a genetic factor with the frequency of an IBD symptom was estimated by the calculation of OR (≥1: protective and 95% CI, using the same software. For groups of equal variance, the correlation between age at onset of symptoms and PTPN22 genotype was followed by two-sample Student's t test, to compare the means of the individual groups.

RESULTS

The allele frequencies were in Hardy-Weinberg equilibrium both in the patients and controls. When comparing IBD patients with the control group, the frequency of the PTPN22 620-W allele was found to be significantly higher in IBD patients than in controls (Pc: 0.00001; OR: 17.81, 95% CI: 4.18-21.86). Nevertheless, no difference in this polymorphism was found between CD and UC patients (Table 2). Indeed, analysis of IBD patients according to clinical behavior revealed no difference between those carrying or not carrying the PTPN22 (R620/W) allele (data not shown), in both CD and UC patients.

When stratifying IBD patients according to the Vienna classification, we did not find a statistically significant association between the frequencies of genotypes of the PTPN22 gene and the clinical features such as sex, age, age of disease onset, or extent of colitis. Moreover, no correlation was found between polymorphism studied and the production of p-ANCA and ASCA in UC or CD patients, respectively.

DISCUSSION

Autoimmune diseases represent a different set of associated phenotypes that are supposed to have common underlying mechanisms and thus, some degree of common genetic predisposition[20]. IBD is a chronic inflammatory condition of the gastrointestinal tract that manifests as UC or CD. Notwithstanding intensive research, the etiology of this condition remains unknown[24]. However, it is thought to result from a combination of genetic predisposition and environmental factors that may be channeled through an abnormality in gut-barrier function, with a loss of antigen tolerance. Some genetic markers that predispose to inflammatory disease have been identified (alleles DRB1*0103, DBB*12, and mutations in the NOD2/Card15 gene on chromosome 16). Nevertheless, the CARD15 variations are not sufficient to explain the entire risk for predisposition to IBD[25]. Other polymorphisms in genes that code for proteins involved in the immune response also appear to exert an influence on the immunological mechanisms that lead to loss of tolerance to commensal microflora.

A specific role of PTPN22 in T-cell regulation has been confirmed by the results of knocking out the murine homologue of PTPN22, which results in lowered thresholds for T-cell-receptor signaling in these animals. Recent findings have revealed that the PTPN risk-associated variant, W620, results in a gain of PTPN22 phosphatase activity in T cells, which opens up new approaches for exploring disease mechanisms[26].

Initially identified as a susceptibility allele for type 1 diabetes[17], the PTPN22 variant has now been implicated in the genetic etiology of rheumatoid arthritis[18], Hashimoto thyroiditis, juvenile idiopathic arthritis[27], Graves' disease[28] and most recently, systemic lupus erythematosus[19]. Despite the association of PTPN22 C1858T SNP with several different autoimmune disorders, a role for this polymorphism in susceptibility to IBD does not appear so clear. Wagenleiter et al[29] have revealed no association of the 620-W allele with CD in 146 patients of Northern German origin. Additionally, they have not found any difference in PTPN22 allele frequencies between CARD15+ and CARD15- patients. These results are in agreement with previous studies of Criswell et al[30].
et al,)[34], Martin et al,[31] in Spanish subjects, Prescott et al,[32] in British subjects, and van Oene et al,[33] in Canadian patients. However, given the relatively small sample size of the patient and control cohort in these studies, a false-negative result cannot be ruled out.

Our data showed the association of IBD susceptibility with the PTPN22 1858T (620-W) allele in Tunisian patients. Similar findings were detected in Takayasu’s arteritis in which PTPN22 R620W polymorphism revealed a wide variation in allele frequencies among different populations; the polymorphic allele being present most in Scandinavia, but absent in Asian and African populations.[34,35]

No explanations have been presented for the discrepancy between positive findings, like ours, and the negative findings of others. The most plausible is the known genetic diversity of the different populations at the haplotype level. The reason for this divergence is not clear but might reflect an ethnic difference in the contribution of genetic factors.

Kyogoku et al,[36] have suggested that the PTPN22 C1858T variant predisposes persons to autoimmune diseases by assisting the production of certain disease-associated antibodies, thereby contributing to disease development. IBD is thought to be primarily a T-cell mediated disease, currently, a serology panel including p-ANCA and ASCA is used for auxiliary diagnosis of IBD. However, relying exclusively on serum antibodies for IBD diagnosis is not justified yet, because the available sero-immunological markers are not sensitive and specific enough, and their role in disease pathogenesis and progression is not well established.[36] In this study, we showed an association of IBD susceptibility with the PTPN22 1858T (620-W) allele in Tunisian patients but no significant association was found between the frequencies of genotypes of the PTPN22 gene and the production of ASCA in CD or p-ANCA in UC.

PTPN22 encodes LYP, which dephosphorylates the kinases LCK, Fyn and Zap-70, all known to be important in T-cell signaling.[37] An additional function of LYP is to downregulate activation of T cells by binding to Csk,[38] an important suppressor of kinases that mediate T-cell activation. Furthermore, LYP has been demonstrated to bind to the adaptor molecule growth factor receptor-bound protein 2, and this interaction is thought to play a negative regulatory role in T-cell signaling.[39,40] The PTPN22 1858 C/T SNP changes the amino acid at position 620 from an arginine (R) to a tryptophan (W), disturbs the interaction between LYP and Csk, which avoids formation of the complex, and therefore, the suppression of T-cell activation.

It remains to be determined precisely how the PTPN22 (620-W) allele influences the progression of IBD, especially since our study indicates that the analyzed polymorphism of the PTPN22 gene do not appear to be involved in the severity of CD or UC, as defined-by the need for surgery (data not shown).

In conclusion, our data showed the association of IBD susceptibility with the PTPN22 1858T (620-W) allele in Tunisian patients. However, no correlation was found between this PTPN22 polymorphism and the clinical or biological characteristics of CD or UC. Further studies are needed to confirm this association in more subjects and to determine the mechanisms by which this polymorphism affects the pathogenesis of this disease.

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**COMMENTS**

**Background**

In recent years, a few studies have been published that have addressed the question of where and under which conditions PTPN22 is produced in the gut in the normal and neoplastic situation. Some of these studies have considerably influenced our view of the role of the PTPN22 system. That is why it has appeared necessary to analyze the PTPN22 1858 C/T SNP in unrelated Tunisian patients with Crohn’s disease (CD) and ulcerative colitis (UC) to evaluate the contribution of the CD95 gene to genetic susceptibility to inflammatory bowel disease (IBD).

**Research frontiers**

Recent findings have revealed that the PTPN risk-associated variant, W620, results in a gain of PTPN22 phosphatase activity in T cells, which has opened new approaches for exploring disease mechanisms.

**Innovations and breakthroughs**

The relationship between PTPN22 polymorphism and IBD has not been reported yet. This is probably the first report on the association of PTPN22 polymorphisms in Tunisian IBD patients. However, this polymorphism was associated with the development of CD and UC, which provides strong support for an IBD susceptibility gene in the region surrounding PTPN22.

**Applications**

By understanding how the PTPN22 polymorphism is associated with the development of CD and UC, this study may indicate a future strategy for therapeutic intervention in patients with IBD.

**Peer review**

This clinical study focused upon the frequency of polymorphism in a specific gene in a small group of individuals with IBD. The results are of great interest and relevance to understanding the pathogenesis of IBD.
