Lack of Association between PTPN22 (+1858 C>T) rs2476601 polymorphism and susceptibility to rheumatoid arthritis (RA) in Northeast of Iran

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Abstract

Background and objectives: Rheumatoid arthritis (RA) is an autoimmune disease with a complex genetic background. The protein tyrosine phosphatase non-receptor type 22 (PTPN22) is a lymphoid specific protein tyrosine phosphatase which is involved in negative regulation of T cell response. Several studies have assessed the association between PTPN22 single nucleotide polymorphisms (SNPs) with RA susceptibility. Here, we aimed to assess the association of PTPN22 (1858 C>T) variant with the susceptibility to RA in northeast of Iran.

Methods: A total of 127 RA patients and 119 age- and sex- matched healthy donors were enrolled. The polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) technique (PCR-RFLP) was applied to detect PTPN22 (1858 C>T) SNP. SPSS 22.0 software was used to analyze data using relevant statistical tests.

Results: Comparison of allele and genotype frequencies of PTPN22 (1858 C>T) SNP in RA patients and healthy donors revealed no significant association with RA susceptibility.

Conclusion: The present study suggests that the PTPN22 (1858 C>T) genetic variants are not associated with RA susceptibility and disease activity. While this is the first report from northeast of Iran, further studies are needed to confirm these findings.

Keywords: Protein tyrosine phosphatase non-receptor type 22 (PTPN22); Rheumatoid arthritis; rs2476601
Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease in which the immune response is deregulated (1). The inflammatory involvement of synovial joints in RA may lead to a usually symmetrical and deteriorative destruction of affected organs (2). RA is considered as a growing health burden by affecting approximately up to 1% of the adult population worldwide (3). It has been introduced as a multifactorial disorder in which both genetic and environmental components could be involved and determine the susceptibility of the disease (4). The genetic factors contribute to approximately 60% of RA pathogenesis. The latest reports have revealed that several inheritable risk alleles and genetic loci could be involved in predisposing individuals to develop RA (5). Human leukocyte antigen (HLA) class II molecules including HLA_DRB1 locus have been introduced as the major genetic determinants for RA (6). However, various potential susceptibility loci outside the HLA region including cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) (7), STAT4 (8), peptidyl arginine deiminase type IV (PADI4) (9) and several inflammatory cytokines (10) and chemokines (11) have been proposed according to genome-wide association studies (GWAS) to evaluate genetic susceptibilities and associations with RA.

The protein tyrosine phosphatase non-receptor type 22 (PTPN22) is a lymphoid specific (Lyp) protein tyrosine phosphatase (PTP) (located on human chromosome 1p13.3-13.1; a linkage region for RA) which is involved in negative regulation of T cell response by binding to the SH3 domain of C-Src tyrosine kinase or C-terminal Src kinase (Csk kinase) through its proline-rich motif (12). Therefore, mutations in PTPN22 gene may promote T cell activation and induce autoimmunity. Several studies have addressed the association between PTPN22 single nucleotide polymorphisms (SNPs) and possible variants with the susceptibility to RA (6, 12, 13). However, the previous studies between PTPN22 genetic variants and RA have been controversial in different populations. Moreover, there is lack of straightforward association studies between PTPN22 genetic variants and RA susceptibility in Iran. In the present case-control study, we aimed to assess the association of PTPN22 (+1858 C>T) rs2476601 genetic variants with the susceptibility to RA in Northeast of Iran.

Materials and Methods

Patients and healthy subjects

A total of 127 patients fulfilling the revised criteria of the American College of Rheumatology (ACR) for RA (1) and 119 age- and sex-matched healthy subjects were enrolled in this study from Rheumatology Research Center, Sayyad Shirazi educational hospital, Gorgan, Iran. An expert rheumatologist confirmed the primary diagnosis and clinical manifestations and calculated Disease Activity Scale (DAS) 28a. All participants signed a written informed consent following the declaration of Helsinki (14). A total volume of 5mL whole blood was taken from all individuals. Plasma was isolated immediately and Ficoll-Paque (Baharafshan, Tehran, Iran) gradient centrifugation was used to separate PBMCs, as previously described (15).
DNA extraction and genotyping

Genomic DNA was extracted from PBMCs using a DNA isolation kit (Dena Zist, Iran) and stored at -20 °C until use. The polymerase chain reaction (PCR) was used to amplify the polymorphic regions and restriction fragment length polymorphism (RFLP) technique was applied to detect PTPN22 (1858 C>T) rs2476601 polymorphism. The PCR was performed in a final volume of 25 mL reaction mixture containing 50 ng of template DNA, 2X PCR buffer (Yekta Tajhiz, Iran), 0.5 mM forward primer (5’-TCACCAGCTTCCTCAACCACA -3’), 0.5 mM reverse primers (5’-GATAATGTTGCTTCAACGGAATTTA -3’) and 1.5 U Taq polymerase (Yekta Tajhiz, Iran) as follows: initial denaturation at 95 °C for 5 minutes, 35 cycles of 95 °C for 20 seconds, 60 °C for 30 seconds, 72 °C for 60 seconds and final extension at 72 °C for 5 minutes. RFLP digestion was performed in a 25 ml reaction mixture containing 5 U of RsaI (Fermentase, USA) incubated at 37 °C for 16-18 hours followed by 2.5% agarose gel electrophoresis. The undigested PCR product with 215 bp represented T allele. The presence of C allele was confirmed by visualizing two fragments of digested PCR product with 173bp and 42 bp.

Statistical analyses

The Hardy-Weinberg equilibrium was checked by Pearson’s goodness of fit test. SPSS 22.0 (SPSS, Chicago, USA) software was used to analyze data statistically. The odds ratio (OR) and 95% confidence interval (CI) were determined to evaluate case-control study associations. Chi square test or Fisher’s exact test was used to compare the frequencies of genotypes between groups. The one-Way ANOVA with Tukey’s post hoc test or nonparametric Kruskal-Wallis with Dunn-Bonferroni post hoc tests were used to compare the means of multiple samples. P-values lower than 0.05 were considered as statistically significant.

Results

The distribution of alleles and genotypes were in Hardy-Weinberg equilibrium (HWE) among RA patients and normal subjects. Setting CC as the reference genotype under co-dominant inheritance model showed no significant association in comparison to CT [OR = 2.01, 95% CI (0.91–4.42), P-value = 0.21] and TT [OR = 1.16, 95% CI (0.07–18.82), P-value = 0.21] genotypes. These findings were also confirmed under dominant [OR = 1.94, 95% CI (0.9–4.16), P-value = 0.085] and recessive [OR = 1.07, 95% CI (0.07–17.27), P-value = 0.96] models. Moreover, comparison of allele frequencies in patients and healthy donors revealed no significant association of T or C allele with RA susceptibility [OR = 1.75, 95% CI (0.86–3.54), P-value = 0.11] (Table 1).

We also evaluated the association of PTPN22 rs2476601 genotypes with the clinical and laboratory characteristics including age, disease activity, ESR, age of onset, anti-CCP and family history of RA patients and found no significant association (Table 2).
### Table 1. The genotype and allele frequencies of PTPN22 (C/T) rs2476601 in RA patients and healthy subjects under different inheritance models

<table>
<thead>
<tr>
<th>Genotypes and alleles</th>
<th>RA patients (n=127)</th>
<th>Healthy subjects (n=119)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2476601 C</td>
<td>241 (95%)</td>
<td>217 (91%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T 13 (5%)</td>
<td>21 (9%)</td>
<td>1.75 (0.86-3.54)</td>
<td>0.11</td>
</tr>
<tr>
<td>Co-dominant model</td>
<td>CC 115 (90.5%)</td>
<td>99 (83.2%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT 11 (8.7%)</td>
<td>19 (16%)</td>
<td>2.01 (0.91-4.42)</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>TT 1 (0.8%)</td>
<td>1 (0.8%)</td>
<td>1.16 (0.07-18.82)</td>
<td>0.21</td>
</tr>
<tr>
<td>Dominant model</td>
<td>CC 115 (90.6%)</td>
<td>99 (83.2%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT+TT 12 (9.4%)</td>
<td>20 (16.8%)</td>
<td>1.94 (0.90-4.16)</td>
<td>0.079</td>
</tr>
<tr>
<td>Recessive model</td>
<td>CC+CT 126 (99.2%)</td>
<td>118 (99.2%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT 1 (0.8%)</td>
<td>1 (0.8%)</td>
<td>1.07 (0.07-17.27)</td>
<td>0.96</td>
</tr>
<tr>
<td>Over-dominant model</td>
<td>CC+TT 116 (91.3%)</td>
<td>100 (84%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT 11 (8.7%)</td>
<td>19 (16%)</td>
<td>2.00 (0.91-4.41)</td>
<td>0.079</td>
</tr>
<tr>
<td>X² HWE* (P-value)</td>
<td>0.21 (0.65)</td>
<td>0.14 (0.71)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*X²-values lower than 0.05 are considered as statistically significant. Significant associations are also shown in Bold. Sex and age adjustment was performed to standardize the risk assessment. Exact test for Hardy-Weinberg equilibrium was also conducted.*

### Table 2. Clinical and laboratory characteristics of RA patients regarding PTPN22 (C/T) rs2476601 genotypes

<table>
<thead>
<tr>
<th>Characteristics and genotypes (N=127)</th>
<th>CC</th>
<th>TT</th>
<th>CT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46.74±1.01</td>
<td>-</td>
<td>47.89±3.05</td>
<td>0.812</td>
</tr>
<tr>
<td>DAS28a</td>
<td>2.43±0.23</td>
<td>-</td>
<td>2.19±1.34</td>
<td>0.737</td>
</tr>
<tr>
<td>ESR</td>
<td>25.63±2.25</td>
<td>-</td>
<td>26.11±4.01</td>
<td>0.739</td>
</tr>
<tr>
<td>Age of onset</td>
<td>42.00±1.96</td>
<td>-</td>
<td>53.33±2.85</td>
<td>0.580</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>Positive</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>91.5%</td>
<td>0.6%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Family history</td>
<td>Yes</td>
<td>96.6%</td>
<td>0%</td>
<td>3.4%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>86%</td>
<td>2.3%</td>
<td>11.6%</td>
</tr>
</tbody>
</table>

Data are presented as means±SE (standard error) for continuous variables and percentages of positive patients (%) for categorical variables. ESR: erythrocyte sedimentation rate; DAS28a: disease activity score 28a; Anti-ccp: anti-cyclic citrullinated peptide. Significant associations are also shown in Bold. *P*-value <0.05
Discussion

Both genetic and environmental components have been introduced to be involved in the initiation and propagation of RA (16). Several genetic loci have been introduced to be associated with RA susceptibility including HLA-DRB1, CTLA4, STAT4, PADI4, cytokines and chemokines (6-9). The PTPN22 is a lymphoid specific protein tyrosine phosphatase (PTP) which is involved in negative regulation of T cell response and plays a critical role in RA pathogenesis. Binding PTPN22 to the SH3 domain of C-Src tyrosine kinase or C-terminal Src kinase (Csk kinase) through its proline-rich motif results in modulation of T cell response and mutations in PTPN22 gene may promote T cell activation and induce autoimmunity (6, 12, 13). Although various studies have studied the association between PTPN22 genetic variants and RA susceptibility (17, 18), there is still lack of reports in Iran.

In the present study, we assessed the association of PTPN22 (1858 C>T) rs2476601 genetic variants with the susceptibility to RA in an Iranian population in comparison to normal subjects. This functional genetic variant results in a change from arginine to tryptophan in codon 620 (R620W) and disrupts the interaction between Lyp and Csk which inhibits complex formation and suppresses T-cell modulation. Auto-reactive T cells would be enabled by reduced T-cell signaling and escape deletion during thymic selection. These cells persist in the circulation and become activated at a later stage (6, 12, 19). Previous studies have also shown that T allele confers susceptibility to several autoimmune disorders including RA, systemic lupus erythematosus (SLE), Grave’s disease (GD), and juvenile idiopathic arthritis (JIA) (20-23). The initial reports on the association of PTPN22 (1858 C>T) polymorphism and increased risk of RA were presented in European and North American populations (20, 24). However, the effect of T allele on RA susceptibility and severity and activity in other populations is not clear. Two recent studies revealed no significant association between the T allele and the rate of joint destruction and structural damage in Caucasian populations (20, 24). The results from Asian populations are more conflicting while some studies suggested that the T variant acts as a susceptibility allele for RA and others reported no significant association (18, 25, 26).

The results of a recent meta-analysis of 33 studies including 27205 cases and 27677 controls revealed that the PTPN22 1858T allele was not associated with an increased or a decreased risk of RA in Asian populations which were in accordance with our findings. However, a statistically significant relationship between the PTPN22 1858T genotype and RA risk was detected in Caucasian populations in the same study {Nabi, 2016 #58}. Therefore, the controversial findings between the relation of RA and the PTPN22 (1858C>T) SNP could be dependent on the prevalence of the PTPN22 polymorphisms in different ethnic groups.

Despite several association studies on PTPN22 (1858C>T) SNP and RA susceptibility worldwide, there is still lack of information in Iran. A most recent study by Abbasi et al. in an Iranian population in Ahvaz, southwest of Iran showed that the frequency of 1858T allele was significantly increased in RA patients with no association with the disease severity (17). However, the sample size of this study was small and was...
not comparable to the present study in a different ethnicity and geographical region. In contrast to our findings, Hashemi et al reported that CT genotype and T allele could be the risk factors for susceptibility to RA in Zahedan, Iran (19). In a more reliable study with a bigger sample size, Ahmadloo et al. reported that rs2476601 plays no role in susceptibility to RA in Iranian population which was confirmed in our study (18).

Conclusion

Our findings revealed that PTPN22 (1858C>T) SNP was associated with the susceptibility to RA and not with the disease activity in a population in Gorgan, northeast of Iran. According to the lack of previous reports about the association of PTPN22 SNPs and RA in Iran, the differences in allele and genotype frequencies and associations may be due to ethnic variations with other geographic regions.

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Declarations

Authors’ contributions

All authors have the same contribution to this work.

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Ethics approvals and consent to participate

Code of Ethics: IR.GOUMS.RES.1396.178

Conflict of interest

We declare that we have no conflicts of interest.

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