Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients

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Objectives. To confirm the association of a functional single-nucleotide polymorphism (SNP), C1858T (rs2476601), in the PTPN22 gene of British Caucasian rheumatoid arthritis (RA) patients and to evaluate its influence on the RA phenotype.

Methods. A total of 686 RA patients and 566 healthy volunteers, all of British Caucasian origin, were genotyped for C1858T polymorphism by PCR–restriction fragment length polymorphism assay. Data were analysed using SPSS software and the χ2 test as applicable.

Results. The PTPN22 1858T risk allele was more prevalent in the RA patients (13.9%) compared with the healthy controls (10.3%) (P = 0.008, odds ratio 1.4, 95% confidence interval 1.09–1.79). The association of the T allele was restricted to those with rheumatoid factor (RF)-positive disease (n = 524, 76.4%) (P = 0.004, odds ratio 1.5, 95% confidence interval 1.1–1.9).

We found no association between PTPN22 and the presence of the HLA-DRB1 shared epitope or clinical characteristics.

Conclusions. We confirmed the previously reported association of PTPN22 with RF-positive RA, which was independent from the HLA-DRB1 genotype.

Key words: PTPN22, Rheumatoid arthritis, HLA, Risk allele, Rheumatoid factor positive.

Rheumatoid arthritis (RA) is a destructive inflammatory condition affecting about 1% of the UK population. A genetic component of susceptibility to RA is well established and around 30% of it has been attributed to the HLA-DRB1 locus [1, 2]. Association between a functional single-nucleotide polymorphism (SNP) in the coding region of the gene PTPN22 and RA in Caucasians was first described by Begovich et al. and has been replicated by several other groups in the US, UK, Spanish and Dutch RA patient populations [3–8]. PTPN22 encodes the lymphocyte-specific phosphatase (Lyp), which is an important potential factor in modifying signalling through the T-cell receptor (TCR) [9]. The presence of the PTPN22 1858T/T genotype increases the risk of RA more than 2-fold. However, little is known about its effects on severity and co-association with other autoimmune diseases.

We studied the distribution of the PTPN22 C1858T polymorphism in our large sample of British Caucasian RA patients for two reasons: firstly, to confirm the strength of the association with RA; and secondly, to evaluate epistatic interactions with HLA-DRB1 alleles.

Patients and methods

Patients

We studied 686 British Caucasian RA patients (488 female and 198 male), all satisfying the 1987 American College of Rheumatology diagnostic criteria, attending the Nuffield Orthopaedic Centre, Oxford. Written informed consent was obtained from all subjects and the study was approved by the Oxford Research Ethics Committee. DNA samples from 566 British Caucasian healthy blood donors were used as controls.

Methods

A structural questionnaire was used to identify demographic characteristics and clinical features of the disease, including age of onset, treatment, personal or family history of autoimmune diseases, and surgical history. Patients who were positive for rheumatoid factor (RF) by the particle agglutination test at two different time points from the review of the case notes were considered to have seropositive disease. Where these data were not available, RF was measured using nephelometry at the time of recruitment; a titre ≥40 IU/ml was considered positive. The presence of changes consistent with RA was estimated from standard hand radiographs. These were available for 661 patients (96%). Co-association of insulin-dependent diabetes mellitus (IDDM) was present in 10 (1.5%) patients and 53 (7.7%) had autoimmune thyroid pathology. Genomic DNA was extracted from whole blood. Cases and controls were genotyped for C1858T polymorphism by RFLP (restriction fragment length polymorphism analysis) of PCR products. The C → T transition at codon 620 creates in the T allele a restriction site for XcmI (New England Biolabs). For DNA amplification, the forward primer 5'-CAT GCT ATT GCT CTG CT-3' and the reverse primer 5'-ATG TTG CTT CAA CGG AAT TT-3' were used. The PCR conditions were as follows: 95°C for 10 min; 35 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 30 s; and 72°C for 10 min. XcmI digestion products were resolved on 3% agarose gel stained with ethidium bromide and visualized under ultraviolet light. To confirm the genotypes obtained by RFLP, we performed direct sequencing for PTPN22 C1858T variants on representative samples from each genotype (Applied Biosystems 3100, Japan). Limited HLA-DRB1 typing of RA cases using eight group-specific PCR amplification reactions were performed on all RA cases for
DRB1*01–*16 typing [10]. Statistical analysis for association and Hardy–Weinberg equilibrium were evaluated using the $\chi^2$ test. $P$ values less than 0.05 were considered significant. The results were also expressed as odds ratios (OR) with or without 95% confidence intervals (CI). The Bonferroni correction was used to control for multiple comparisons where appropriate. To analyse continuous variables, the software package SPSS 13.0 for Windows was used.

Results

The $PTPN22$ genotypes were in Hardy–Weinberg equilibrium in cases and controls. The genotyping data are shown in Table 1. The risk allele (1858T) was more prevalent in RA patients (14%) than in healthy controls (10%) ($P = 0.008$, OR 1.4, 95% CI 1.1–1.8). Of interest, while the T allele was significantly increased in the RF-positive cases (14%), its prevalence (11%) in the RF-negative cases did not differ significantly from that in controls.

In the RA group overall, 536 (78%) patients had one or more copies of $HLA-DRB1$ shared epitope (SE) alleles. The proportion of patients in the RF-positive subset carrying the SE did not differ from the general RA population (80 and 78%, respectively). There was no association between SE alleles and $PTPN22$ 1858T (data not shown).

The different $PTPN22$ genotypes were equally distributed among female and male patients. The median age of RA onset for the entire group was 48 yr and did not differ significantly between the various $PTPN22$ genotypes.

We extended our study by stratifying RA cases by clinical features (data not shown). No association between $PTPN22$ and the presence of RA nodules or radiographic changes was elicited. Hip or knee joint replacement during the first 15 yr of disease duration was more common in the $PTPN22$ 1858T allele group ($P = 0.04$, OR 1.4, 95% CI 1.0–2.0), although this was not significant after applying Bonferroni correction for the number of comparisons made.

Finally, we compared the effect of this $PTPN22$ polymorphism on the co-occurrence of RA and either IDDM or autoimmune thyroid disease. IDDM was more common in the $PTPN22$ C1858T group ($P = 0.04$, OR 2.7, 95% CI 1.1–6.9), but only a weak (non-significant) trend was seen with autoimmune thyroid disease ($P = 0.2$, OR 1.4, 95% CI 0.8–2.4).

Discussion

Our data confirm previously reported associations of the functional $PTPN22$ 1858T polymorphism with RA, but this effect was limited to RF-positive RA cases. This result is similar to those of other studies conducted on different RA patient groups, although associations with RF-negative RA have also been reported [6, 11].

$PTPN22$ appears to function primarily by modifying T-cell receptor signalling. The presence of a risk allele does not lead to the development of autoimmune disease per se. It has been suggested that subtle changes, especially in T-cell function, might, in part, influence B-cell differentiation and consequently have an effect on autoantibody production and the development of humoral autoimmunity. This is based on the observation that the $PTPN22$ risk allele has been associated with multiple autoimmune diseases, where autoantibodies have been an established feature.

The frequency of the $PTPN22$ 1858T allele varies in different ethnic populations. Begovich et al. [3] found that the risk allele frequency in all control Caucasians studied was 8.7%. The T allele was virtually absent in Han Chinese and Africans, whereas in African Americans and Mexican Americans the risk allele was detected in intermediate frequencies (2.4 and 3.5%, respectively). In our control population, the frequency of the 1858T risk allele was 10.3%, similar to that in another UK study, indicating that this allele is relatively common in Caucasians [6]. This study did not reveal additional effects arising from homozygosity in the $PTPN22$ 1858T risk allele. However, our ability to detect subtle differences was limited by the relatively low frequency of the T allele and the small number of TT homozygotes.

Little is known about the effects of the $PTPN22$ risk allele on the clinical course of RA. Orozco et al. [7] studied 826 Spanish RA patients and suggested differences in extra-articular disease in patients carrying the CC genotype, although these were not robust after correction for multiple comparisons. In our study, $PTPN22$ status did not generally associate with specific clinical characteristics of RA. However, the increased frequency of the 1858T allele in our patients with early hip or knee replacement (within 15 yr of disease onset) is worth further study. While such end-stage arthritis may be considered a marker for more aggressive, destructive inflammation, it would be preferable to confirm this in a prospective study of erosive changes over time.

An excess of various other autoimmune diseases is well recognized in RA patients and their relatives. For example, thyroid dysfunction and IDDM have been found in higher frequencies among RA patients and their family members [12, 13]. Studies have shown that co-association of autoimmune illnesses might be slightly higher in patients carrying the risk allele [14, 15]. In our RA population, the prevalence of thyroid pathology and IDDM was slightly higher than in the population (7.7 vs 1% and 1.5 vs 0.3%, respectively). We found only a weak predisposition to IDDM in the $PTPN22$ C1858T group ($P = 0.04$, OR 2.7, 95% CI 1.1–6.9) and also a weak (non-significant) trend was seen with autoimmune thyroid disease. Since associations have already been described between $PTPN22$ 1858T and IDDM and Graves disease independently of RA, we feel these trends are likely to be relevant but that our study was underpowered to detect them reliably.

Table 1. Genotyping data and allele frequencies

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<thead>
<tr>
<th>Allele frequency</th>
<th>Genotype frequency</th>
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<tbody>
<tr>
<td></td>
<td>C No. (%)</td>
</tr>
<tr>
<td>RA cases (n = 686)</td>
<td>1182 (86.2)</td>
</tr>
<tr>
<td>Controls (n = 566)</td>
<td>1015 (89.7)</td>
</tr>
<tr>
<td>RF− (n = 149)</td>
<td>265 (88.9)</td>
</tr>
<tr>
<td>RF+ (n = 524)*</td>
<td>897 (85.6)</td>
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<tr>
<td>RF+ vs RF−</td>
<td>1.4 (0.9–2.0)</td>
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*Compared with healthy controls.
In summary, we have confirmed the PTPN22 1858T association with RA that is restricted to RF-positive patients and independent of HLA genotype. This genetic association may have importance in helping to understand the pathogenesis of RA and related autoimmune diseases.

The authors have declared no conflicts of interest.

Reference