

# Association of PTPN22 with Rheumatoid Arthritis Among South Asians in the UK

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**ABSTRACT.** *Objective.* To compare the distribution and assess genetic associations of the *PTPN22* R620W single-nucleotide polymorphism among South Asian (Asiatic Indian) patients with rheumatoid arthritis (RA) and ethnically matched controls.

*Methods.* DNA samples from 133 rheumatoid factor-positive South Asian RA patients and 149 control subjects from the East Midlands of the UK were genotyped for *PTPN22* R620W polymorphism. Genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism method.

*Results.* The *PTPN22* \*T allele frequency was lower than in the Caucasian populations, but the disease association was significant (odds ratio 5.87, 95% confidence interval 1.68–20.52). Similar association was observed for genotypes containing \*T allele.

*Conclusion.* Our results suggest that the T variant acts as a susceptibility allele for autoantibody-positive RA among South Asians. (First Release August 1 2007; J Rheumatol 2007;34:1984–6)

*Key Indexing Terms:*

RHEUMATOID ARTHRITIS

SOUTH ASIAN

INDIAN

*PTPN22* R620W

Rheumatoid arthritis (RA) is a common autoimmune disease with a prevalence of ~1% in different populations<sup>1,2</sup>. It is a chronic systemic disease that affects the joints, bones, muscle, tendons, and fibrous and connective tissues and shows differential sex prevalence. RA has a strong genetic component, although other factors like diet, cigarette smoking, education, and socioeconomic status may also contribute<sup>1-3</sup>. Linkage and association studies have been conducted to identify genes that may contribute to inception, progression, and outcome of RA<sup>1,4,5</sup>.

The *PTPN22* (protein tyrosine phosphatase nonreceptor 22) gene encodes for a lymphoid-specific phosphatase known as Lyp, which is a negative regulator of T cell activation<sup>5,6</sup>. A single-nucleotide substitution (C to T) at position 1858 of the *PTPN22* gene leads to a functional change in the amino acid from arginine to tryptophan at codon 620 (R620W). The *PTPN22* gene is involved in the regulation of T and B cells, natural killer cells, monocytes, and neutrophils<sup>1,5-7</sup>. One possible mechanism for the effect of *PTPN22* in RA is that the protein encoded by the 620W allele has reduced binding to the intracellular kinase Csk. Csk activates the Lyp protein.

Therefore, reduced activity of Csk would decrease the activation of Lyp and this would in turn reduce the negative regulation of T cells, which may lead to increased reactivity in the immune system<sup>1,5-7</sup>. Vang and colleagues<sup>8</sup> proposed a gain of function at *PTPN22* locus as an alternative mechanism for susceptibility/autoimmunity, which needs confirmation of its effect in RA<sup>5,8</sup>. Several autoimmune diseases like type-1 diabetes, lupus, and hypoparathyroidism are associated with *PTPN22*<sup>5-7,9,10</sup>.

The *PTPN22* 620W allele is associated with RA among many Caucasian cohorts (UK, Spain, Canada, France, the Netherlands, Sweden, Germany, and New Zealand); however, no association was observed in Colombian and Japanese RA patients<sup>5,11</sup>. There are no published studies on South Asian RA populations to date.

Our aim was to replicate and extend the reported association of *PTPN22* 1858C/T single nucleotide polymorphism (SNP) with RA in a South Asian (Indian) population in the East Midlands, UK.

## MATERIALS AND METHODS

With approvals by ethical committees and written consent, 133 South Asian patients with RA and 149 ethnically matched control samples were recruited. Patients with RA diagnosed on the basis of the 1987 American Rheumatism Association criteria were recruited by an experienced rheumatologist (AS) from local hospitals. All patients had established, severe RA status and were confirmed to be seropositive. All participants belonged to the migrant Indian population settled in the East Midlands, originally from Northwest India. The majority of participants were born in the Indian subcontinent, although some came to the UK via East Africa.

The *PTPN22* C1858T SNP (rs2476601) was genotyped by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay as described<sup>1</sup>. All samples were genotyped without the knowledge of disease/control status. Two researchers independently scored the genotypes and any ambiguous genotype tests were repeated. Allele and genotype frequencies

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were compared between cases and controls using chi-square statistics and odds ratios.

## RESULTS

The genotypes in both patient and control groups were in Hardy-Weinberg equilibrium (Table 1). Due to the small number of the C/T and T/T genotypes, these were combined for analyses. As shown in Table 1, patients had a 5-fold higher frequency of C/T + T/T susceptibility genotypes compared to controls (10.9% vs 2.1%, respectively), leading to statistically significant odds ratios (OR 5.73, 95% CI 1.61–20.43,  $p = 0.003$ ). A similar effect was observed for \*T allele, which is higher in patients (5.8%) and is significantly associated with the disease (OR 5.87, 95% CI = 1.68–20.52,  $p = 0.002$ ). However, caution is warranted, as the confidence intervals are relatively large, due to small sample size. No differences were found between male and female subjects (chi-square = 0.157,  $p = 0.692$ ).

## DISCUSSION

This is the first study of *PTPN22* polymorphism among South Asian (Indian) RA patients confirming a significant association. An odds ratio of 5.73 for the susceptible genotype and 5.87 for the T allele suggests a significant effect. *PTPN22* 1858T allele association has been replicated in many Caucasian populations<sup>4,5,9,11-14</sup>; however, our results show a higher odds ratio (5.87 in this sample, compared to the range of 1.26–2.13 in other populations<sup>5</sup>), but the lower 95% confidence intervals of this study (1.61 and 1.68) fall well within the range observed in Caucasian populations<sup>4,5,9,11-14</sup>. The small sample size and lower frequency of rare alleles may have led to the higher odds ratio. Our study consisted only of rheumatoid factor (RF)-positive patients belonging to a well characterized homogenous group, which may also have led to the higher OR value. The odds ratios observed in this study are slightly higher than those observed in the RF-positive subgroups in previous studies (T allele OR average ~2, range 1.4–2.97)<sup>4,5,9,11-14</sup>, but caution is warranted due to small sam-

ple size and wide confidence intervals. The other possible reason is that the *PTPN22* 620W allele plays a differential role in the development of RA in individuals of South Asian background than in those of European background, which warrants confirmation with a larger comparative study.

In Europe, there is a geographical heterogeneity and gradient of increasing \*T allele frequency from south to north. The \*T allele frequency is lower in Italian and Sardinian populations (2–3%), increasing to 7–9% in mainland Western European countries. The highest frequencies are in Scandinavian and Finnish populations (12–15%)<sup>5</sup>. This allele is absent in Japanese<sup>15</sup>, Chinese, and Koreans<sup>5</sup>, and its frequency is relatively low in Indian populations — 1.05% in our study, 1.2% in North Indians<sup>10</sup>. There seems to be a west to east decreasing gradient for this allele, which may have implications for disease associations, and this requires further population studies.

Although the association of *PTPN22* gene with RA is well established, this association is not uniform across all populations. The R620W variant is rare in a South Asian (Indian) population; our study has shown an association of this allele with RA.

A limitation of our study is the relatively small sample size, which might be a factor contributing to inflation of the odds ratio and to the observation that the lower limit of the calculated 95% confidence interval at 1.61 overlaps with the range observed in other populations. Notwithstanding this limitation, our findings support the role of *PTPN22* C1858T polymorphism as a genetically determined risk factor for RA in South Asians, and suggest that T variant acts as a susceptibility allele for autoantibody-positive RA. Our study adds to the current base of genetic determinants for RA, and our observations require confirmation through larger studies.

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Table 1. Distribution of genotypes and alleles among South Asian patients with RA and controls and genetic association statistics.

	RA, n = 129	Control, n = 143	OR 95% CI	Chi-square (p)
Genotype, no. (%)				
C/C	115 (89.1)	140 (97.9)	1.00	
C/T	13 (10.1)	3 (2.1)	5.33 (1.48–19.31)	7.9 (0.005)
T/T	1 (0.8)	0	3.68 (0.149–91.23)	1.22 (0.27)
C/T + T/T	14 (10.9)	3 (2.1)	5.73 (1.61–20.43)	8.98 (0.003)
Hardy-Weinberg equilibrium,				
p value	0.37	0.90		
Allele (%)				
*C	243 (94.2)	283 (98.95)		
*T	15 (5.8)	3 (1.05)	5.87 (1.68–20.52)	9.74 (0.002)

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