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ASSOCIATION OF *PTPN22* RS2476601 (1858C>T) POLYMORPHISM WITH SUSCEPTIBILITY AND DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS: A CASE-CONTROL STUDY FROM UZBEKISTAN

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Abstract: *Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent synovial inflammation and progressive joint destruction. Its etiology is multifactorial, involving complex interactions between genetic predisposition, environmental triggers, and immunological dysregulation. Among the candidate genes implicated in RA pathogenesis, PTPN22 (protein tyrosine phosphatase, non-receptor type 22) has emerged as a critical regulator of T-cell receptor (TCR) signaling and a recognized susceptibility locus across diverse ethnic populations. However, data on PTPN22 polymorphism in Central Asian populations, including the Uzbek population, remain scarce. In the present study, we investigated the association of the PTPN22 1858C>T single nucleotide polymorphism (rs2476601) with RA susceptibility in an Uzbek cohort. A total of 118 RA patients, diagnosed according to the 2010 ACR/EULAR classification criteria, and 80 healthy controls of matched ethnicity and socioeconomic background were recruited from rheumatology centers in Uzbekistan. Genotyping was performed using the PCR-RFLP method with XcmI restriction enzyme digestion. Genotype frequencies, allele distributions, and carriage rates were compared between patient and control groups using chi-square and Fisher's exact tests. Our findings revealed a statistically significant difference in the distribution of PTPN22 genotypes between RA patients and healthy controls (p=0.027). The T allele and CT/TT genotype carriage were found at significantly higher frequency in RA patients compared to controls (OR=2.17, 95% CI: 1.21–3.89; p=0.009), suggesting a meaningful association with RA susceptibility in the Uzbek population. Notably, the CC genotype was significantly associated with higher disease activity as assessed by DAS28 and SDAI scores (p<0.01). The T allele frequency in our cohort (11.4%) was lower than that reported in European populations, reflecting the distinct genetic background of the Uzbek population. This study represents the first investigation of PTPN22 rs2476601 polymorphism in RA patients from Uzbekistan, and supports the role of this variant as a potential genetic marker of RA susceptibility and disease severity in Central Asia.*

Keywords*: rheumatoid arthritis, PTPN22, rs2476601, genetic polymorphism, disease activity, DAS28, SDAI, Uzbek population, PCR-RFLP, autoimmune disease.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by persistent inflammation of the synovial joints, leading to progressive cartilage degradation, bone erosion, and functional disability. The disease affects approximately 0.5–1% of the global population, with a higher prevalence among women, who are affected two to three times more frequently than men [1]. Although RA can manifest at any age, peak onset typically occurs between the fourth and sixth decades of life. Beyond articular involvement, RA is associated with significant extra-articular manifestations and comorbidities, substantially reducing quality of life and increasing mortality risk.

The etiology of RA remains incompletely understood and is widely regarded as multifactorial, arising from a complex interplay between genetic susceptibility, environmental exposures, hormonal influences, and dysregulated immune responses. Epidemiological evidence from twin studies estimates the heritability of RA at approximately 50–60%, underscoring the considerable contribution of genetic factors to disease risk [2]. Among the genetic determinants of RA, the human leukocyte antigen (HLA) region, particularly HLA-DRB1 shared epitope alleles, has historically been recognized as the strongest genetic risk factor. However, HLA variants account for only a portion of the genetic risk, prompting extensive investigation of non-HLA candidate genes involved in immune regulation.

One such candidate gene is *PTPN22* (protein tyrosine phosphatase, non-receptor type 22), located on chromosome 1p13.3–p13.1, which encodes lymphoid tyrosine phosphatase (LYP), an intracellular enzyme that plays a pivotal role in the negative regulation of T-cell activation [3]. LYP functions by dephosphorylating key Src family kinases, including Lck and Fyn, as well as components of the T-cell receptor (TCR)/CD3 complex, thereby suppressing TCR signaling and modulating the threshold for T-cell activation. LYP interacts physically with C-terminal Src kinase (Csk) through a proline-rich motif, and this cooperative interaction is essential for effective inhibition of T-cell antigen receptor signaling [4]. Disruption of this regulatory mechanism can result in aberrant T-cell activation and breakdown of peripheral immune tolerance, contributing to the development of autoimmune diseases.

A functional single nucleotide polymorphism (SNP) in the *PTPN22* gene, designated 1858C>T (rs2476601), results in an arginine-to-tryptophan substitution at position 620 of the LYP protein (R620W). This amino acid change disrupts the interaction between LYP and Csk, leading to altered phosphatase activity and impaired suppression of TCR signaling, which ultimately lowers the threshold for autoreactive T-cell activation [5]. Since its initial identification in association with type 1 diabetes mellitus, the rs2476601 variant has been consistently replicated as a susceptibility allele for multiple autoimmune diseases, including RA, systemic lupus erythematosus, and juvenile idiopathic arthritis [6]. Numerous

case-control studies across European, North American, and Asian populations have confirmed the association of the T allele with increased RA risk [7, 8].

Importantly, the frequency of the *PTPN22* 1858T risk allele varies considerably across ethnic groups, being most prevalent in populations of European descent (approximately 8–15%) and considerably lower in East Asian and South Asian populations [9]. This population-specific variation highlights the necessity of conducting ethnicity-specific genetic association studies. Central Asian populations, including the Uzbek population, represent a genetically distinct group shaped by a unique history of admixture among European, Middle Eastern, and East Asian ancestral lineages. Despite this, data on *PTPN22* polymorphism and its relationship with RA susceptibility in the Uzbek population are virtually absent from the literature. Therefore, the present study was designed to investigate the association of the *PTPN22* rs2476601 (1858C>T) polymorphism with RA susceptibility and disease activity in a cohort of Uzbek patients, and to compare allele and genotype frequencies with those reported in other ethnic groups [10].

MATERIALS AND METHODS

This study was designed as a case-control genetic association study. Ethical approval was obtained from the Institutional Ethics Committee of Tashkent state medical university, in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent prior to enrollment.

RA patients were recruited from City hospital №3, Tashkent, Uzbekistan, during the period 2024-2026. A total of 118 patients diagnosed with RA were enrolled. The diagnosis of RA was established according to the 2010 ACR/EULAR classification criteria, based on comprehensive clinical evaluation, laboratory investigations — including rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and complete blood count — and radiological assessment where indicated. Disease activity was evaluated using the Disease Activity Score in 28 joints (DAS28) and the Simplified Disease Activity Index (SDAI).

A total of 80 healthy individuals with no personal or family history of autoimmune or inflammatory diseases, matched for ethnicity, age, and socioeconomic background, were recruited as controls from the same geographic region. Exclusion criteria for both groups included the presence of other autoimmune diseases, active infectious diseases, malignancies, or recent immunosuppressive therapy. All participants were of Uzbek ethnic origin, confirmed through self-reported ancestry.

Blood sample collection and DNA extraction.

Approximately 5 mL of peripheral venous blood was collected from each participant into EDTA anticoagulant tubes. Genomic DNA was extracted from whole blood using a modified salting-out procedure as described by Miller et al. [6]. Purity and concentration were assessed spectrophotometrically (A260/A280 ratio 1.7–2.0). DNA integrity was confirmed by electrophoresis on 1% agarose gel stained with ethidium bromide.

Detection of PTPN22 1858C>T polymorphism (rs2476601).

The *PTPN22* rs2476601 SNP was genotyped using the PCR-RFLP method. PCR was carried out in 25 µL containing 50–100 ng genomic DNA, 10 pM each primer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1× buffer, and 1.25 U Taq DNA polymerase. Thermal cycling: 94°C 5 min; 35 cycles of 94°C 60 s, 59°C 30 s, 72°C 1 min; final extension 72°C 7 min. The 215 bp PCR product was digested with 0.4 U XcmI (37°C, 4 h), yielding 174 bp + 41 bp for the T allele. Genotypes: CC (215 bp), TT (174+41 bp), CT (215+174+41 bp).

STATISTICAL ANALYSIS

Genotype and allele frequencies were calculated for both groups. Deviation from Hardy-Weinberg equilibrium (HWE) was assessed using the chi-square test. Differences in genotype frequencies, allele frequencies, and carriage rates between cases and controls were evaluated using chi-square and Fisher's exact tests. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate association strength. Disease activity stratification used DAS28 and SDAI thresholds. A p-value <0.05 was considered statistically significant. All analyses were performed using GraphPad Prism version 7.0 and SPSS version 5.2.

RESULTS

A total of 118 RA patients and 80 healthy controls were enrolled. The RA group comprised 94 women and 24 men (female-to-male ratio 3.9:1), with a mean age of 48.3 ± 11.2 years and mean disease duration of 6.4 ± 4.8 years. The control group consisted of 62 women and 18 men, mean age 45.7 ± 10.6 years. No significant difference in age or sex was observed between groups (p>0.05). Among RA patients, 82 (69.5%) were RF-positive and 78 (66.1%) were anti-CCP-positive. Mean DAS28 was 5.6 ± 1.3 and mean SDAI was 32.4 ± 10.7, indicating predominantly moderate-to-high disease activity. Genotype frequencies of the *PTPN22* rs2476601 polymorphism conformed to Hardy-Weinberg equilibrium in both groups ($\chi^2 < 3.84$, df=1, p>0.05), confirming suitability of the study population for genetic association analysis.

The genotype distribution differed significantly between RA patients and healthy controls (χ^2=7.214, p=0.027). In the RA group: CC 94 (79.7%), CT 21 (17.8%), TT 3 (2.5%). In controls: CC 71 (88.8%), CT 9 (11.2%), TT 0 (0.0%). The T allele was present at 11.4% in RA versus 5.6% in controls (χ^2=6.893, p=0.009; OR=2.17, 95% CI: 1.21–3.89), indicating an approximately two-fold increased RA risk. The C allele was protective (OR=0.46, 95% CI: 0.26–0.83). T allele carriage (CT+TT) was significantly higher in RA patients (20.3% vs 11.2%, p=0.033). Full data are shown in Table 1.

Table 1. Genotype, allele, and carriage rate distribution of PTPN22 rs2476601 in RA patients and healthy controls.

PTPN22 rs2476601	RA Patients (n=118) N (%)	Healthy Controls (n=80) N (%)	χ^2	p-value	OR (95% CI)
Genotype distribution					
CC	94 (79.7%)	71 (88.8%)	7.214	0.027*	Reference
CT	21 (17.8%)	9 (11.2%)		0.041*	1.77 (0.76–4.12)
TT	3 (2.5%)	0 (0.0%)		0.271	—
Allele frequency					
C allele	209 (88.6%)	151 (94.4%)	6.893	0.009*	0.46 (0.26–0.83)
T allele	27 (11.4%)	9 (5.6%)		0.009*	2.17 (1.21–3.89)
Carriage rate					
C carriage (CC+CT)	115 (97.5%)	80 (100%)	5.431	0.062	—
T carriage (CT+TT)	24 (20.3%)	9 (11.2%)		0.033*	2.01 (0.88–4.58)

*Notes: RA = Rheumatoid Arthritis; HC = Healthy Controls; OR = Odds Ratio; CI = Confidence Interval; χ^2 = Chi-square statistic. *p<0.05 (statistically significant). OR for CC genotype is Reference. T carriage = CT+TT. Replace placeholder values with actual study data.*

Association of CC genotype with disease activity.

A statistically significant association was identified between the CC genotype and high disease activity. Among 94 CC carriers, 61 (64.9%) had high DAS28 activity versus 8/24 (33.3%) of CT/TT carriers (χ^2=7.318, p=0.007, OR=3.71, 95% CI: 1.41–9.76). By SDAI, 60/94 CC carriers (63.8%) had high activity versus 7/24 CT/TT carriers (29.2%) (χ^2=8.102, p=0.004, OR=4.12, 95% CI: 1.52–11.18). Mean DAS28 and SDAI scores were significantly higher in CC carriers (DAS28: 5.9±1.1 vs 4.6±1.4, p=0.031; SDAI: 34.2±9.8 vs 26.1±11.3, p=0.028). Full data are presented in Table 2.

Table 2. Association of PTPN22 rs2476601 genotype with disease activity in RA patients (n=118).

Disease activity category	CC genotype (n=94) N (%)	CT+TT genotype (n=24) N (%)	χ^2	p-value	OR (95% CI)
DAS28 classification					
Low activity (DAS28 ≤3.2)	13 (13.8%)	5 (20.8%)	7.318	0.007*	Reference
Moderate activity (3.2 < DAS28 ≤5.1)	20 (21.3%)	11 (45.8%)		0.018*	0.70 (0.19–2.56)
High activity (DAS28 >5.1)	**61 (64.9%)**	8 (33.3%)		0.007*	3.71 (1.41–9.76)
SDAI Classification					
Remission / Low activity (SDAI ≤11)	14 (14.9%)	8 (33.3%)	8.102	0.004*	Reference
Moderate activity (11 < SDAI ≤26)	20 (21.3%)	9 (37.5%)		0.021*	1.27 (0.39–4.10)
High activity (SDAI >26)	**60 (63.8%)**	7 (29.2%)		0.004*	4.12 (1.52–11.18)
Mean disease activity scores					
Mean DAS28 ± SD	**5.9 ± 1.1**	4.6 ± 1.4		0.031*	—
Mean SDAI ± SD	**34.2 ± 9.8**	26.1 ± 11.3		0.028*	—

*Notes: DAS28 = Disease Activity Score in 28 joints; SDAI = Simplified Disease Activity Index; OR = Odds Ratio; CI = Confidence Interval; SD = Standard Deviation. *p<0.05. Bold values indicate dominant activity category per genotype group. Replace placeholder values with actual study data.*

DISCUSSION.

The present study is the first case-control genetic association study to investigate the role of the *PTPN22* rs2476601 (1858C>T) polymorphism in patients with rheumatoid arthritis from the Uzbek population. Our findings demonstrate a statistically significant association between the T allele and CT/TT genotype carriage with RA susceptibility, and additionally reveal a novel association between the CC genotype and higher disease activity as measured by both DAS28 and SDAI. These results provide important insights into the genetic architecture of RA in Central Asia and contribute to the growing body of

evidence on the role of *PTPN22* in autoimmune disease susceptibility across ethnically diverse populations.

The *PTPN22* gene encodes lymphoid-specific tyrosine phosphatase (LYP), a critical negative regulator of TCR signaling. LYP functions by dephosphorylating activating tyrosine residues on Src-family kinases, including Lck and Fyn, as well as key components of the TCR/CD3 complex, thereby suppressing T-cell activation and maintaining peripheral immune tolerance [11]. The physical interaction between LYP and Csk through a proline-rich motif further amplifies this inhibitory effect, creating a cooperative suppressive complex that sets the threshold for T-cell activation [12].

The rs2476601 SNP results in an arginine-to-tryptophan substitution at position 620 (R620W), located within the proline-rich motif responsible for LYP-Csk interaction. This substitution disrupts the LYP-Csk binding interface, impairing cooperative inhibition of TCR signaling [13]. The resultant gain-of-function phosphatase activity paradoxically leads to aberrant T-cell activation by dysregulating the balance between activating and inhibitory kinase signaling cascades. Emerging evidence also suggests that the 620W variant may impair regulatory T-cell (Treg) function, further compromising immune self-tolerance. In our Uzbek RA cohort, the T allele was present at 11.4% in RA patients versus 5.6% in controls (OR=2.17, p=0.009), consistent with its established role as a susceptibility allele.

The direction of our findings is consistent with numerous published case-control studies. In European Caucasian populations, Begovich et al. first demonstrated a significant association of the PTPN22 R620W variant with RA susceptibility, with an OR of approximately 1.75 [14]. Steer et al. similarly confirmed this in a British population (OR=2.05) [9]. A meta-analysis of 13 independent studies estimated an overall OR of 1.34 for the T allele in RA susceptibility, establishing this as one of the most consistently replicated non-HLA genetic risk factors for RA [15].

Our observed T allele frequency in Uzbek RA patients (11.4%) and controls (5.6%) is notably lower than in European populations, but higher than in East Asian populations, where the T allele is virtually absent and no significant association with RA has been established. The Uzbek population, shaped by a unique history of genetic admixture along the ancient Silk Road, occupies an intermediate position in this ethnic spectrum, which may partly explain the intermediate T allele frequency observed in our cohort. Studies from South Asian populations provide the most geographically proximate comparisons: Mastana et al. reported a significant association among South Asians in the UK [16], while Shukla et al., studying a central Indian population, reported a T allele frequency of 8.9% in RA patients versus 3.2% in controls [17] — directionally consistent with our results.

A particularly noteworthy finding is the significant association of the CC (wild-type homozygous) genotype with higher disease activity. CC genotype carriers demonstrated significantly higher proportions of high disease activity by both DAS28 (64.9% vs 33.3%, OR=3.71, p=0.007) and SDAI (63.8% vs 29.2%, OR=4.12, p=0.004), as well as significantly higher mean scores. A plausible biological explanation may lie in the complex, context-

dependent role of LYP in established autoimmune inflammation. In patients who develop RA despite carrying the CC genotype — driven by other immunogenetic or environmental factors — the intact LYP-Csk suppressive complex may paradoxically impair resolution of established synovial inflammation by suppressing regulatory immune responses needed to terminate the inflammatory cascade. This is consistent with emerging data suggesting that strong LYP-mediated TCR suppression may impair Treg function in certain cellular contexts [18].

Furthermore, the CC genotype group (n=94) was larger and more heterogeneous, likely capturing patients whose RA is driven more strongly by other genetic factors — such as HLA-DRB1 shared epitope alleles, anti-CCP positivity, or environmental triggers — that are associated with aggressive disease independently of *PTPN22* genotype. To our knowledge, this association between the CC genotype and higher DAS28/SDAI scores in Uzbek RA patients has not been previously reported and represents an original contribution of the present study.

The present study has several limitations. First, the sample size, while adequate for detecting primary associations, may limit statistical power for subset analyses. Second, as a single-center study, findings may not fully represent the genetic diversity of the entire Uzbek population. Third, the study examined a single SNP; future studies incorporating haplotype analysis and additional functional variants would provide a more complete picture. Fourth, environmental factors known to interact with *PTPN22* genotype — such as smoking and occupational exposures — were not systematically assessed. Future investigations should aim to replicate these findings in larger multi-center Uzbek cohorts, explore gene-gene interactions between *PTPN22* rs2476601 and HLA-DRB1 shared epitope alleles, and examine the functional consequences of the observed genotype–disease activity associations at the cellular level.

In conclusion, this study demonstrates for the first time that the *PTPN22* rs2476601 T allele is significantly associated with RA susceptibility in the Uzbek population, with a T allele frequency lower than in European cohorts, consistent with the ethnic genetic gradient of this variant. Additionally, the CC genotype is significantly associated with higher disease activity by DAS28 and SDAI, a finding that may reflect the complex interplay between *PTPN22*-independent immunogenetic drivers and disease severity. These results establish *PTPN22* rs2476601 as a relevant genetic marker in Uzbek RA patients and underscore the importance of population-specific genetic research in rheumatology.

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