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GENES AND DISEASE

Association of PTPN22 1858C→T polymorphism, HLA-DRB1 shared epitope and autoantibodies with rheumatoid arthritis

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Abstract To assess impact of PTPN22 1858C→T polymorphism, HLA shared epitope and autoantibodies on susceptibility and severity of rheumatoid arthritis (RA). A total of 150 RA patients and 150 controls were included in the study. Anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor isotypes (IgG, IgM and IgA) were assayed by ELISA. PTPN22 1858C→T polymorphism was performed by RFLP analysis and HLA-DRB1 genotyping by PCR-SSP analysis. Single-view, anteroposterior radiographs of the hands and feet were obtained on all RA patients. The results showed association of PTPN22 1858 T allele with RA (OR = 2.3, 95 % CI 1.5–3.5) and bone erosion (OR = 2.9, 95 % CI 1.1–7.6). The associations increased with the combination of positive autoantibodies, HLA-DRB1 SE with PTPN22 1858 T allele carriage. The highest association was with the combination with anti-CCP antibodies (OR = 47.3, 95 % CI 10.9–204.4 for RA and OR = 69.4, 95 % CI 15.8–305.5 for erosion $p < 0.001$). Combination of PTPN22 1858 T allele carriage with negative RF isotypes or with absence HLA-DRB1 SE showed no significant association with RA. The presence of PTPN22 1858C→T polymorphism with HLA SE and autoantibodies increases risk of RA development and erosive disease.

Keywords Autoantibodies · PTPN22 gene · Rheumatoid arthritis · Shared epitope

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Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease affecting up to 1 % of the adult population worldwide. It is characterized by progressive systemic arthritis that leads to joints destruction. It is two to three times more prevalent in women than in men. The etiology of RA is multifactorial and mainly caused by interaction of genetic, environmental and hormonal factors [1].

Genetic factors are estimated to account for about 60 % of disease susceptibility and also are considered to be responsible for persistent and destructive polyarthritis [2]. The strongest genetic association is with HLA-DRB1 locus on chromosome 6 [the shared epitope (SE)] and the protein tyrosine phosphatase 22 (PTPN22) gene on chromosome 1p13 [3].

The PTPN22 gene encodes lymphocyte specific phosphatase (Lyp), which is important as a negative control of T cell activation and T cell development. It has potential role in the prevention of spontaneous T cell activation by dephosphorylation and inactivation of T cell receptor-associated kinases and their substrates [4]. Begovich and colleagues [3] were the first to report association between functional single-nucleotide polymorphism (SNP) in the coding region of the gene PTPN22 and RA, and this result

has been replicated in several other studies [5–9]. However, there was no reported significant association between single-nucleotide polymorphism (SNP) within PTPN22 and disease outcome in RA [10].

Our aim is to detect possible association of PTPN22 1858C→T polymorphism with susceptibility and severity of RA and the influence of combining PTPN22 1858C→T polymorphism with HLA SE or autoantibodies on RA.

Patients and methods

The study consisted of 150 rheumatoid arthritis patients diagnosed according to the American College of Rheumatology (ACR) 1987 classification criteria [11]. The power of the study for the association of PTPN22 1858C→T polymorphism with RA was 80 %, α error 0.05 and degree of precision 7 %. Patients were recruited from the outpatient clinic of the Medical Services Unit at National Research Centre and rheumatology clinic of Internal Medicine department at Kasr Al Aini Hospital. Patients with inflammatory arthritis other than RA were excluded from the study. None of the patients was diabetic, and all were free from immune mediated skin disease or any autoimmune disease. One hundred fifty healthy volunteers with negative family history of RA or any autoimmune disease as control group were recruited from workers in Medical Services Unit at National Research Centre and Kasr Al Aini Hospital. They were age and sex matched with the recruited patients. Patients and controls were Egyptian and of the same ethnic origin.

Before enrollment in the study, all participants signed written informed consent and the study was approved by the ethical committee of the National Research Centre.

All participants were subjected to detailed history, thorough clinical examination including musculo-skeletal examination. For each patient, we recorded number of tender and/or swollen joints. Disease activity was assessed using disease activity score in 28 joints (DAS 28) using erythrocyte sedimentation rate [12].

Radiologic examination of patients

Single-view, anteroposterior radiographs of the hands and feet were obtained on all RA patients, and joint damage was assessed by a single skilled radiologist using the Larsen scale according to the modification of Kaarela and Kautiainen [13] Who included 10 metacarpophalangeal joints and wrists, and the second to the fifth MTP joints in the scoring, with a range of 0–100. 0 = intact bony outlines and normal joint space; 1 = erosion <1 mm in diameter or joint space narrowing; 2 = one or several small erosions, diameter >1 mm; 3 = marked erosions; 4 = severe erosions, where there is usually no joint space left, and the original bony outlines are partly preserved; and 5 = mutilating changes, where the original bony outlines have been destroyed. Patients were defined as having erosive disease if they had a Larsen score ≥2 in at least one joint of the hands or feet.

Laboratory methods

Serologic tests

Serum samples were assayed for:

1. Quantitative serum hs-CRP levels by ELISA using commercial kit (Accu-Bind Elisa Microwells, Monobind Inc., USA) [14].
2. Rheumatoid factor of IgM, IgG and IgA isotypes quantitatively using commercially available ELISA (provided by ORGENTEC Diagnostica GmbH, Mainz, Germany) according to Ernst et al. [15]. Rheumatoid factor was considered positive above 20 IU/ml.
3. Anti-cyclic citrullinated peptide (anti-CCP) antibodies using third-generation assays (Quanta Lite™ CCP3 IgG ELISA; Inova Diagnostics, San Diego, CA, USA) according to Bizzaro et al. [16]. Anti-cyclic citrullinated peptide antibody was considered positive above 20 μ/ml.

Genetic studies

Five ml of venous blood on EDTA were used for assessment of HLA-*DRB1 alleles* {HLA-DRB1*01, HLA-DRB1*03 and HLA-DRB1*04} and the PTPN22 1858C→T polymorphism

Genomic DNA was extracted from the peripheral blood mononuclear cells with a QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's guidelines. HLA-DRB1 genotyping was performed by the Dynal AllSet TM PCR-SSP low resolution typing kits according to the manufacturer's instructions (Dynal, UK). Among HLA–DRB1 genes, DRB1*01 and DRB1*04 were defined as "SE genes" [17].

Genotyping of the PTPN22+1858 1858C→T gene SNP (rs2476601) was performed via the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method; Primers used were forward 5′-ACTGATAATGTTGCTTCAACGG-3′ and reverse 5′-TCACCAGCTTCCTCAACCAC-3′. The PCR amplifications were performed according to the following protocol: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, 62 °C for 30 s, extension at 72 °C for 30 s and final extension were carried out at 72 °C for 5 min. Amplified products were digested using RsaI for

PTPN1858C/T. (fermentase fast digest Enzyme) for 5 min at 37 °C. Digested products were electrophoresed on a 3 % agarose gel and visualized by ethidium bromide

Statistical methods

Data entry was carried on excel sheet, and statistical analysis was done using SPSS software program version 18.0. Data were statistically described in terms of mean ± standard deviation (SD), frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using *t* test for comparison between two means. While, when data were not normally distributed, Mann–Whitney *U* test was used when comparing 2 groups. Chi-square was done for qualitative data that presented by numbers and percentages to assess significant association and odds ratio was used to assess the strength of association multivariate logistic analysis was done to predict risk factors significantly associated with rheumatoid arthritis and erosion. *P* value was considered statistically significant when $P < 0.05$ and considered statistically highly significant if $P < 0.01$.

Conformity with the Hardy–Weinberg law of genetic equilibrium (HWE) was assured by a non-significant Chi-square test comparing the observed versus the expected genotypes among studied cases and controls. A minimum level of statistical significance was considered at a *P* level of <0.05.

Results and analysis of the results

The genotype and allele distribution of PTPN22 1858C→T polymorphism were not in agreement with the Hardy–Weinberg equilibrium among the RA patients. The explanation is that patients were coming from several governorates of Egypt, and most of them were from rural areas where the prevalence of consanguinity is high. Also migration in or out of the population is a known feature of the Egyptians with the fact that the loss or addition of people can easily change gene pool frequencies even if there are no other evolutionary mechanisms operating.

Demographic and clinical characteristics of RA patients

One hundred fifty patients with RA and 150 healthy volunteers as controls were enrolled in the study. Mean age of the patients was 46.2 ± 12.2 years; they were 18 males and 132 females. The mean duration of the disease was 8.8 ± 8.1 (range between 1 and 35 years). As most patients were female, only nine patients (6 %) were smoker, 33 females (22 %) received hormonal treatment in the form of contraceptive pills. Twenty-eight patients (18.7 %) reported positive family history of RA. All patients were receiving disease modifying antirheumatic drugs (DMARDs) in the form of methotrexate, sulphasalazin and hydroxychloroquine with or without steroid (dose not exceeding 7.5 mg). None of them was receiving or received biologic therapy. Seventy-six patients (50.7 %) had extra-articular manifestations. Deformity by clinical examination, at the time of the study, was present in 53 patients (35.3 %). Mean disease activity assessed by DAS28 was 5.9 ± 5.3. As the range of the duration of the disease was wide, we divided Larsen score over duration of the disease to estimate disease severity [18]. The mean disease severity was 2.3 ± 3.1. Bone erosion assessed radiologically, using Larsen scale, at the time of the study was present in 121 (80.7) RA patients.

The control group consisted of 150 healthy volunteers, from Cairo and Guiza, with mean age 45.6 ± 11.8, they were 27 males and 123 females, and 14 (9.3 %) of them were smoker.

Autoantibodies in RA patients and controls

Levels of anti-CCP antibodies, RFIgG, RFIgM and RFIgA were significantly higher among patients compared to controls ($P < 0.001$) with odds ratio ranging from 11.5 for RFIgM to 36.9 for anti-CCP (Table 1).

Genotype and allele frequency of PTPN22 1858C→T polymorphism and HLA SE in RA patients and controls

Analysis of the genotype and allele distribution of the PTPN22 1858C/T single-nucleotide polymorphism showed increase prevalence of CC and TT genotype in RA patients compared to controls as well as the PTPN22 1858 T allele that showed positive association with RA (Table 2).

We assessed HLA-DRB1*01, *03 and *04. An individual was considered positive for the shared epitope (SE) if he carried at least one copy of any of HLA-DRB1*01 or *04. The results showed that shared epitope is present in 111 (74 %) patients, while it was present in 48(32 %) of the control group. Thirty-nine (26 %) of RA patients and 102 (68 %) of the controls showed absence of the SE as they were carrying HLA-DRB1*03 without *01 or *04.

Association of demographic data, anti-CCP, RF isotypes and PTPN22 1858C→T polymorphism with demographic data and clinical outcome of RA patients

There was no significant difference in age of diagnosis of RA between PTPN22 1858 T allele carriers and non-carriers (mean 38.6 ± 12.6 vs. 36.6 ± 12.6 year, respectively, $P = 0.3$). Also, mean value of DAS28 did not differ

Table 1 Frequency of anti-CCP and RF isotypes among patients and controls

	Patients *n* (%)	Controls *n* (%)	*P* value	Odds ratio (95 % CI)
Anti-CCP				
>20 μ/ml	84 (56.0)	5 (3.3)	<0.001**	36.9 (14.3–95.2)
≤20 μ/ml	66 (44.0)	145 (96.7)		
RFIgG				
>20 IU/ml	117 (78)	29 (19.3)	<0.001**	14.79 (8.5–25.9)
≤20 IU/ml	33 (22)	121 (80.7)		
RFIgM				
>20 IU/ml	98 (65.3)	21 (14.0)	<0.001**	11.58 (6.5–20.5)
≤20 IU/ml	52 (34.7)	129 (86)		
RFIgA				
>20 IU/ml	80 (53.3)	13 (8.7)	<0.001**	12.04 (6.3–23.1)
≤20 IU/ml	70 (46.7)	137 (91.3)		

Anti-CCP Anti–cyclic citrullinated peptide antibodies, *RF* rheumatoid factor, *CI* confidence interval

** *P* highly significant

Table 2 Genotype and allele frequency of PTPN22 1858C→T polymorphism among patients and controls

	Allele frequency		OR (95 % CI)	Genotype frequency			OR for genotype versus CC	
	C *n* (%)	T *n* (%)		CC *n* (%)	CT *n* (%)	TT *n* (%)	CT	TT
Controls	263 (87.7)	37 (12.3)	®	118 (78.7)	27 (18.0)	5 (3.3)	®	®
RA cases	227 (75.7)	73 (24.3)	2.3 (1.5–3.5)	92 (61.3)	43 (28.7)	15 (10.0)	2.0 (1.2–3.6)	3.9 (1.4–10.9)
			P < 0.001*				*P* = 0.01*	*P* = 0.008*

RA Rheumatoid arthritis, *OR* odds ratio, *CI* confidence interval, ® reference group

* *P* significant

between RA patients carriers of PTPN22 1858 T allele and non-carriers (5.5 ± 1.3 vs. 6.1 ± 6.6, respectively, $P = 0.4$).

Bone erosion at the time of the study was observed in 80.7 % of RA patients. Patients with erosion were older, had longer duration of disease and had higher levels of anti-CCP and RFIgG titer (Table 3). There was no association between gender and PTPN22 1858 T allele carrier in all RA patients and in the subgroup of erosion and non-erosion (Table 4). Erosion was significantly associated with T allele carriers (Table 5).

Moreover, carriers of PTPN22 1858 T allele showed significantly higher disease severity as assessed by Larsen score divided by disease duration compared to non-carrier of PTPN22 1858 T allele (mean ± SD 3.1 ± 4.1 and 1.8 ± 2.1, respectively, $P < 0.001$). These results indicate that PTPN22 1858 T allele is associated with poorer disease outcome.

Since there were many potential confounding variables that might influence erosion, a multivariate logistic analysis was performed, the most significant predictor variables for erosion were anti-CCP, PTPN22 1858 T allele, age of onset of RA and duration of RA (OR 3.9 CI 1.2–7.8, $P = 0.02$, OR 3.2 CI 1.1–9.3, $P = 0.03$ OR 1.0 CI 1.0–1.1, $P = 0.01$; and OR 1.2 CI 1.0 1.3, $P = 0.005$, respectively, Table 6).

Influence of combining PTPN22 1858C→T polymorphism with autoantibodies or with HLA SE on risk of developing RA and erosive disease

The risk of developing RA and erosive disease was higher when combining PTPN22 1858 T allele with positive anti-CCP, RF isotypes or HLA-DRB-1 SE than in absence of PTPN22 1858 T allele with positive anti-CCP, RF isotypes and HLA-DRB1 SE. Moreover, the presence of PTPN22 1858 T allele with negative RF isotypes or absence of

Table 3 Characteristics of RA patients with and without erosion

Variables	RA patients with erosion $n = 121$ mean ± SD	RA patients without erosion $N = 29$ mean ± SD	*P* value
Age (years)	47.7 ± 12.0	38.9 ± 11.2	0.002*
Age of onset (years)	38.1 ± 12.9	34.6 ± 11.0	0.2
Duration (years)	9.6 ± 8.5	5.3 ± 4.2	0.03*
DAS28	5.9 ± 5.8	5.6 ± 1.1	0.4
Tender joint count	11.7 ± 7.9	14.9 ± 7.9	0.05
Swollen joint count	3.8 ± 4.0	3.8 ± 3.4	0.7
CRP (mg/dl)	18.9 ± 18.0	13.8 ± 9.2	0.3
Anti-CCP (μ/ml)	190.0 ± 169.6	102.3 ± 175.0	0.03*
RFIgG (IU/ml)	274.8 ± 506.9	75.6 ± 148.6	0.02*
RFIgM (IU/ml)	159.1 ± 254.4	86.6 ± 137.7	0.3
RFIgA (IU/ml)	132.1 ± 219.3	150.0 ± 27.8	0.1

RA Rheumatoid arthritis, *DAS* disease activity score, *CRP* C-reactive protein, *CCP* cyclic citrullinated peptide, *RF* rheumatoid factor

* *P* significant

Table 4 Association of PTPN22 1858 T allele carriers and gender in controls and RA patients

Subgroups	Controls ($n = 150$)		All RA ($n = 150$)		Erosive RA ($n = 121$)		Non-erosive RA ($n = 29$)	
	CT/TT	CC	CT/TT	CC	CT/TT	CC	CT/TT	CC
Sex								
Females, *n* (%)	26 (21.1)	97 (78.9)	49 (37.1)	83 (62.9)	44 (41.5)	62 (58.5)	5 (19.2)	21 (80.8)
Males, *n* (%)	6 (22.2)	21 (77.8)	9 (50.0)	9 (50.0)	8 (53.3)	7 (46.7)	1 (33.3)	2 (66.7)
P value	0.901		0.293		0.387		0.568	

RA Rheumatoid arthritis

Table 5 Association of PTPN22 1858C→T polymorphism with erosion

PTPN22 1858C→T genotype	Erosion $n = 121$, *n* (%)	No erosion $n = 29$, *n* (%)	OR (95 % CI)	*P*
CC	69 (75)	23 (25)	®	0.03*
CT, TT	52 (89.7)	6 (10.3)	2.89 (1.1–7.61)	

* *P* significant

Table 6 Predictor variables for occurrence of RA and erosion among the studied population

	AOR	95 % CI		*P* value
		Lower	Upper	
PTPN22 1858 T carrier 01	3.2	1.1	9.3	0.03
duration	1.1	1.0	1.3	0.005
Age of diagnosis of RA patients	1.05	1.0	1.1	0.01
Anti-CCP	3.1	1.3	7.8	0.02

Variables entered are: sex, RFIgG1, RFIgM1, RFIgA1, PTPN22 1858, HLA-DRB1SE, DAS28, duration of RA, age of diagnosis of RA, anti-CCP

AOR Adjusted odds ratio

HLA-DRB1 SE showed no significant association with RA (Table 7).

As only 9 (6 %) of patients were smoker, we could not assess the effect of smoking on severity of RA.

Discussion

Genetic factors play an important role in determining susceptibility and outcome of RA. The PTPN22 gene is now considered one of the most robust and reproducible gene outside the HLA locus associated with RA [19].

We report in the present study association of PTPN22 1858C→T polymorphism with susceptibility to RA among Egyptian patients, which is consistent with previous studies done on different ethnic populations such as on Iranian population [20], Italian [21] and Mexican population [22].

A recent meta-analysis confirmed the association of PTPN22 1858 T variant with susceptibility to RA susceptibility in different ethnic groups, especially among

Table 7 Impact of association of PTPN22 1858C→T polymorphism with autoantibodies or HLA SE on risk of RA and erosive disease

Subgroups	Controls ($n = 150$)	All RA ($n = 150$)		Erosive RA ($n = 121$)		Non-erosive RA ($n = 29$)	
		N	OR (95 % CI)	*N*	OR (95 % CI)	*N*	OR (95 % CI)
PTPN22CC + anti-CCP$^-$	115 (76.7)	45 (30.0)	1	29 (64.4)	1	16 (35.6)	1
PTPN22CC + anti-CCP$^+$	3 (2.0)	47 (31.3)	40.0 (11.8–135.2)**	40 (85.1)	52.9 (15.2–183.1)**	7 (14.9)	16.8 (3.9–71.5)**
PTPN22CT + TT + anti-CCP$^-$	30 (20.0)	21 (14.0)	1.8 (0.9–3.4)	17 (81.0)	2.2 (1.1–4.6)*	4 (19.0)	1.0 (0.3–3.1)**
PTPN22CT + TT + anti-CCP$^+$	2 (1.3)	37 (24.7)	47.3 (10.9–204.4)**	35 (94.6)	69.4 (15.8–305.5)**	2 (5.4)	7.1 (0.9–54.6)
PTPN22CC + RFIgM$^-$	103 (68.7)	37 (24.7)	1	28 (75.7)	1	9 (24.3)	1
PTPN22CC + RFIgM$^+$	15 (10.0)	55 (36.7)	10.2 (5.1–20.2)**	41 (74.5)	10.1 (4.9–20.7)**	14 (25.5)	10.8 (3.9–28.9)**
PTPN22CT + TT + RFIgM$^-$	26 (17.3)	15 (10.0)	1.6 (0.8–3.4)	13 (86.7)	1.8 (0.8–4.0)	2 (13.3)	0.8 (0.2–4.3)
PTPN22CT + TT + RFIgM$^+$	6 (4.0)	43 (28.7)	20.0 (7.8–50.7)**	39 (90.7)	23.9 (9.1–62.2)**	4 (9.3)	7.6 (1.8–32.1)**
PTPN22CC + RFIgG$^-$	93 (62.0)	28 (18.7)	1	22 (78.6)	1	6 (21.4)	1
PTPN22CC + RFIgG$^+$	25 (16.7)	64 (42.7)	8.5 (4.5–15.9)**	47 (73.4)	7.9 (4.0–15.6)**	17 (26.6)	10.5 (3.7–29.5)**
PTPN22CT + TT + RFIgG$^-$	28 (18.7)	5 (3.3)	0.6 (0.2–1.7)	2 (40.0)	0.3 (0.1–1.3)	3 (60.0)	1.7 (0.3–7.0)
PTPN22CT + TT + RFIgG$^+$	4 (2.7)	53 (35.3)	44.0 (14.6–132.3)**	50 (94.3)	52.8 (17.2–161.8)**	3 (5.7)	11.1 (2.1–64.2)**
PTPN22CC + RFIgA$^-$	108 (72.0)	49 (32.7)	1	34 (69.4)	1	15 (30.6)	1
PTPN22CC + RFIgA$^+$	10 (6.7)	43 (28.7)	9.5 (4.4–20.4)**	35 (81.4)	11.1 (4.9–24.8)**	8 (18.6)	5.7 (1.9–16.9)**
PTPN22CT + TT + RFIgA$^-$	29 (19.3)	21 (14.0)	1.6 (0.8–3.1)	17 (81.0)	1.8 (0.9–3.8)	4 (19.0)	1.0 (0.3–3.2)
PTPN22CT + TT + RFIgA$^+$	3 (2.0)	37 (24.7)	27.2 (8.0–92.5)**	35 (94.6)	37.1 (10.7–128.1)**	2 (5.4)	4.8 (0.7–31.1)
PTPN22CC + SE$^-$	83 (55.3)	31 (20.7)	1	23 (19)	1	8 (20.7)	1
PTPN22CC + SE$^+$	35 (23.3)	61 (40.7)	4.7 (2.6–8.4)**	46 (38)	4.7 (2.5–8.9)**	15 (51.7)	4.4 (1.7–11.4)**
PTPN22CT + TT + SE$^-$	19 (12.7)	8 (5.3)	1.1 (0.4–2.8)	8 (6.6)	1.5 (0.6–3.9)	0 (0)	Undefined
PTPN22CT + TT + SE$^+$	13 (8.7)	50 (33.3)	10.3 (4.9–21.5)**	44 (36.4)	12.2 (5.6–26.4)**	6 (27.6)	4.7 (1.4–16.0)**

RA Rheumatoid arthritis, *SE* shared epitope

* *P* Significant, ** *P* highly significant

Europeans [23]. Begovich and colleagues also reported strong association of PTPN22 1858 T variant with susceptibility to RA among American Caucasian population [3].

Among Spanish population a contradictory results were obtained. One study showed strong association with susceptibility to RA [8]. In another study, no significant difference in genotype and allele frequency between patients and controls was found; however, in this study, the authors included patients with arthropathy other than RA [24]. On the other hand, other studies failed to find association of 1885C > T polymorphism with RA patients such as in Turkey [25], Russia [26], Japan [27] and china [28].

The frequency of the PTPN22 1858 T allele varies in different ethnic populations. In our study, the frequency was 24.3 % among RA patients and 12.3 % among healthy controls which is higher than in previous studies. In a study done on British Caucasian RA patients, the frequency of the PTPN22 1858 T allele was 13.9 % among RA patients and 10.3 % among healthy controls [4]. Begovich and colleagues found that the frequency of the PTPN22 1858 T allele is 8.7 % in all control Caucasians studied [3]. In African Americans and Mexican Americans, the PTPN22 1858 T allele was detected in intermediate frequencies (2.4 and 3.5 %, respectively), while it was absent in Han Chinese and Africans [3]. A recent study on Indian population revealed the presence of PTPN22 1858 T allele in 16.9 % of RA patients and in 6.4 % of healthy controls. [1].

Radiographic bone erosion is generally accepted as objective measure of articular damage, so we investigated the impact of PTPN22 1858 T allele carriage on bone erosion and we found that PTPN22 1858 T allele carriers are at higher risk to develop bone erosion. Moreover, disease severity, assessed by Larsen score/disease duration, was significantly higher in PTPN22 1858 T allele carriers compared to non-carriers. A longitudinally followed cohort study of 238 Norwegian RA patients reported association

between annual progression rate of Sharp-van der Heijde score and PTPN22 1858 T allele carriers [29]. In this study, radiographic damage of the hands was assessed at baseline and an after 1, 2, 5 and 10 years.

Harrison and colleagues studied 686 British Caucasian RA patients (488 female and 198 male), they could not elicit correlation with radiologic changes; however, they found increased frequency of the PTPN22 1858 T allele in RA patients who required hip or knee replacement within first 15 years of disease onset [4]. A study done in UK on RA patients and controls of white Caucasian ethnicity revealed no association between PTPN22 1858C→T polymorphism with either erosive status or Larsen score by 5 years [10]. Also, in the study done by Morgan and colleagues erosion was observed in 21.5 % of 615 RA patients and was associated with symptom duration and not with PTPN22 C1858→T polymorphism. In their study, the patients were Caucasian of Northern European descent and had symptom duration <24 months [30]. A common feature of these studies is the shorter duration of the disease compared to our study, which may be the cause of contradictory result.

The PTPN22 gene maps to chromosome 1p13, and it encodes lymphoid protein tyrosine phosphatase (Lyp) which is a negative regulator of T cell signaling. Lyp protein is involved in the prevention of spontaneous T cell activation by dephosphorylation and inactivation of T cell receptor-associated kinases and their substrates [31]. The Lyp protein is also expressed in other cell types: B cells, monocytes, neutrophils, dendritic cells and natural killer cells. The presence of PTPN22 1858 T allele variant leads to hyperresponsive phenotype of T, B and dendritic cells with increase number of effector and memory T cells which lead to the development of autoimmune diseases [27]. As synovitis is ultimately dependent on T lymphocytes that regulate the production of proinflammatory monokines and tissue-injurious metalloproteinases [32], so the presence of PTPN22 1858 T variant may be one of the risk factors for increase joint damage.

In the present study, the HLA-DRB1 SE epitope was present in 74 % of RA patients. The HLA-DRB1 SE is among the most potent genetic risk factors for RA as it is associated with susceptibility and severity of RA. A comparable result was obtained by O'Dell and colleagues who observed the presence HLA-DRB1 shared epitope in 74 % of 89 RA patients [33]. In more recent studies, the presence of HLA-DRB1 SE epitope ranged from 63.2 to 67.3 % in RA patients [34–36].

We tested the impact of the interaction of PTPN22 1858C→T polymorphism with autoantibodies or HLA SE on RA development and outcome. We found that the risk of PTPN22 T allele variant for development of RA and erosive disease is dependent on the presence of autoantibodies or HLA SE. On the other hand, the combination of positive antibodies or SE with T allele variant increases the risk of susceptibility to RA and development of erosive disease more than the sum of each separate one. The highest risk was associated with anti-CCP and RF IgG which support previous studies that showed the association of anti-CCP and RF with bone erosion in RA [37, 38]. The observed interaction indicates the existence of a disease mechanism that requires the simultaneous presence of the PTPN22 1858C→T polymorphism and HLA-DRB1 SE alleles and autoantibodies. This supports the effect of PTPN22 in modifying T cell signaling and hence affecting B cell differentiation, leading to the production of autoantibodies and the development of humoral autoimmunity [4].

Conclusion

PTPN22 C1858T polymorphism–HLA SE interaction and PTPN22 1858C→T polymorphism–antibody interaction could increase the risk and worsen the prognosis of RA. However, positive anti-CCP and RF are more important risk factors.

The association of PTPN22 1858C→T polymorphism and autoantibodies especially anti-CCP and RFIgG could identify patients at higher risk of RA and severe disease so they can benefit from more aggressive treatment regimen or immediate biological biotherapy while sparing those least likely to develop severe disease from the potential side effects associated with such drug therapy.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval The study has been approved by the ethical committee of the National Research Centre and has been performed in accordance with the ethical standards of the National Research Centre committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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