Immunology Letters 216 (2019) 106–113

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

PTPN22 1858 C/T polymorphism is associated with alteration of cytokine profiles as a potential pathogenic mechanism in rheumatoid arthritis

Khodayar Ghorban[a], Rana Ezzeddini[b], Majid Eslami[c], Bahman Yousefi[d], Bizhan Sadighi Moghaddam[d], Mohammad-Taher Tahoori[e], Maryam Dadmanesh[f], Amir Salek Farrokhi[d,*]

[a] Department of Immunology, Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran
[b] Department of Clinical Biochemistry, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department of Bacteriology and Virology, Semnan University of Medical Sciences, Semnan, Iran
[d] Department of Immunology, School of Medical Sciences, Semnan University of Medical Sciences, Semnan, Iran
[e] Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[f] Department of Infectious Diseases, Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
PTPN22 gene polymorphism
Rheumatoid arthritis
Cytokine
T cells

ABSTRACT

Introduction: Rheumatoid arthritis (RA) is one of the most common prevalent autoimmune diseases. The 1858 C/T (rs2476601) single nucleotide polymorphism (SNP) within the *PTPN22* gene has been associated with susceptibility to inflammatory based diseases in several populations. It is implicated that altered cytokine production has a potential pathogenic role in the development of RA. The aim of this work was to analyze the association of 1858 C/T *PTPN22* polymorphism in RA patients with cytokine profiles.

Materials and Methods: This study was performed on 120 RA patients who were referred to the Rheumatology Research Centre, Shariati Hospital (Tehran, Iran), and 120 healthy controls. Genomic DNA was extracted and genotyped for 1858 C/T PTPN22 gene SNP using the PCR-RFLP technique. Serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ as well as Anti−CCP and RF was measured by ELISA method.

Results: Results showed that 1858 C/T PTPN22 SNP significantly ($P = 0.007$, $OR = 2.321$, 95% $CI = 1.063–5.067$) associated with RA. The 1858 T allele frequency was also significantly increased in RA patients in comparison to the controls ($P = 0.008$, $OR = 3.583$, 95% $CI = 1.3–9.878$). Our data demonstrated a significant reduction of IL-4 and IL-10 in PTPN22 1858C/T compared to 1858C/C RA patients. In addition, upregulation of IL-6, IFN-γ, and TNF-α was observed in PTPN22 1858C/T vs. 1858C/C RA patients.

Discussion: Our findings implicate altered cytokine profiles as a possible pathogenic mechanism by which the 1858 T allele may contribute to the progress of RA.

1. Introduction

Rheumatoid arthritis (RA), as one of the most common prevalent autoimmune disease, is characterized by the destruction of joint cartilage and bone via induction of inflammation in the synovium and also pathological infiltration of lymphocytes in the target tissues. Synovitis might be resulted from both cell-mediated and humoral immune responses. In RA, the synovial membrane is infiltrated by T lymphocytes, which are the main responsible cells to induce inflammation, and damages the tissues. Loss of physiological tolerance to self-antigens is the main reason for the onset of RA disease [1]. It can be associated with progressive joint destruction and severe disability [2]. RA might be complex with regard to the genetic background, and it involves many genes, which encode proteins with important roles in the regulation of immune response [1,3]. In addition, the secretion of pro-inflammatory cytokines can cause joint destruction and disability [4,5]. The precise basis of RA has not been recognized so far although several investigates indicated that pro-inflammatory cytokines such as

Abbreviations: RA, Rheumatoid arthritis; SNP, Single nucleotide polymorphism; PTPN22, Protein tyrosine phosphatase non-receptor type 22; RF, Rheumatoid factor; Anti−CCP, Anti-Cyclic Citrullinated Peptide; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-γ, Interferon gamma; LYP, lymphoid protein tyrosine phosphatase; TCR, T cell receptor; P1, First proline-rich domain; CSK, C-terminal Src kinase; ESR, Erythrocyte sedimentation rate; Arg, Arginine; Trp, Tryptophan

* Corresponding author.
E-mail address: A.salek@semums.ac.ir (A. Salek Farrokhi).

https://doi.org/10.1016/j.imlet.2019.10.010
Received 27 July 2019; Received in revised form 12 October 2019; Accepted 20 October 2019
Available online 24 October 2019

interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α have an essential role in bone and cartilage destruction [6].

Protein tyrosine phosphatase non-receptor type 22 (PTPN22) molecules play a vital role in negative control of T lymphocytes activation and development. Human *PTPN22* gene is located on the chromosome 1p13 and encodes a lymphoid protein tyrosine phosphatase (LYP), which is involved in the preventing spontaneous T lymphocytes activation by dephosphorylation and inactivation of T cell receptor (TCR)-associated kinases and their substrates [1].

Accordingly, it has been reported that the substitution of arginine for tryptophan at codon 620 (R620 W) of the mature protein is due to the 1858 C/T single nucleotide polymorphism (SNP) in the *PTPN22* gene, which is located in the first proline-rich domain (P1) of the LYP protein [2,7]. Although, the 1858 C/T (rs2476601) SNP in the *PTPN22* gene has been associated with susceptibility to multiple autoimmune diseases [2,8], the roles that the SNP plays on the *PTPN22* functions is controversial [9]. However, some researches indicated that the 1858 T allele is a hipomorphic allele with a diminished function and consequently can be associated with uncontrolled TCR signaling. It has been hypothesized that this impaired LYP function may result in hyper-responsive T and B-lymphocytes breaking tolerance, leading to the progress of the autoimmune diseases [10,11].

PTPN22 as a tyrosine phosphatase could downregulate Src and Syk family kinase (SFK) activity downstream of immune-receptor signaling pathways [12]. It has been demonstrated that numerous pathways in various cell types including the B-cell receptor [13], T-cell receptor [14], dectin-1 [15], as well as integrins [16] and Toll-Like Receptor (TLR) signaling pathways [17–20] regulated with PTPN22. Although, it has been established that the PTPN22 R620 W variant related to autoimmunity exhibits decreased binding to the negative regulatory tyrosine kinase Csk due to a missense mutation in the P1 domain [12,21].

The inhibitory effect of Lyp on TCR transduction is increased following its cooperation with the tyrosine kinase CSK [12]. This relation takes place by Lyp, which includes the PTPN22 R620 W polymorphism. Polymorphism at this location may be estimated to change the suppressive function on TCR signal transduction mediated by this complex.

It has been suggested that the 1858 T allele cooperated less strongly with C-terminal Src kinase (CSK). Hence, it was concluded that the PTPN22 1858 T allele could result in a failure of TCR inhibition and T cell hyper-responsiveness [22]. The *PTPN22* gene association with lymphocyte signaling pathways and the relationship of the 1858 T allele with multiple autoimmune diseases propose that they may lead to the autoimmunity progression. Therefore, recognizing the efficient consequences of the PTPN22 1858 T allele might explain a general immune alteration relating to the pathogenesis of these diseases. In this regard, the aim of this study was to analyze the association of 1858 C/T PTPN22 polymorphism with cytokine profiles in RA patients and also evaluate the relationship between the clinical and laboratory parameters with the status of PTPN22 1858 C/T allele in RA patients.

2. Materials and methods

2.1. Patients and controls

This study was performed on 120 RA patients (25 men and 95 women, with ages ranging from 19 to 77 years old) who were referred to the Rheumatology Research Centre at Shariati Hospital (Tehran, Iran). All samples were collected from the same race of Iranian people that examined from April 2015 to October 2015. A group of rheumatologists were selected patients based on American College of Rheumatology criteria (ACR 2010) including joints swelling, duration of disease (≥6 weeks of disease onset), upregulation of acute phase reactants; ESR and CRP, and presence of RF and anti-CCP autoantibodies [23]. Exclusion criteria for the patients of this study were acute infections, endocrine disorders, diabetes, alcohol consumption, emphysema, smoking habit, pregnancy and other immune diseases. A series of laboratory tests, including rheumatoid factor (RF) and erythrocyte sedimentation rate (ESR), were done (78.2% were RF positive and the mean for ESR was 28.5 ± 15.6) for all 120 RA patients. Age and sex matched 120 control subjects were selected from individuals referring to Shariati Hospital laboratory without any immunological and autoimmune disease (35 men and 85 women, age range 18 to 65 years). All samples were gathered from the same race of Iranian people. The medical ethics committees of Aja University of Medical Sciences and Tehran University of Medical Sciences approved research protocols. The study was explained to the subjects and an informed consent was obtained from all participants prior to enrollment.

2.2. Autoantibodies measurement

The level of RF was measured by ELISA method according to the manufacturer's instructions (Abcam, USA). Moreover, anti-CCP antibody was determined by use of ELISA method (Anti-CCP, Eurodiagnostica, Sweden).

2.3. Genomic DNA extraction

Whole blood samples were taken from the participants. RBCs were excluded using RBC lysing buffer and then a QIAamp DNA Blood Mini Kit (Qiagen, Germany) was used to extract genomic DNA from the leukocyte sediments according to the manufacturer's procedure. Genomic DNA was used as a PCR template.

2.4. 1858 C/T PTPN22 gene polymorphism genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was employed to genotype 1858 C/T SNP (rs2476601). To perform PCR reaction, the following primers were used: Forward: 5′-ATGTTGCTTCAACGGAATTT-3′ and Reverse: 5′-CATGCTGCTATTGCTCTGCT-3. The 25 μL PCR reaction mixture contained 1 μg gDNA, 3 μM of each primers, 1 units Taq DNA polymerase (Amplicon, UK), supplied buffer enzyme 1 × 3 mM $MgCl_2$ and 2.5 mM of each dNTPs (Amplicon, UK). Thermal cycling was performed with an initial activation step at 95 °C for 2 min, and then 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 2 min. An amplified fragment of 412 bp analyzed on a 2% agarose gel (Sigma, USA) stained with GelRed (Biotium, USA) was resulted as the PCR product. Then, this fragment was incubated with 3 units of *Xcm I* restriction enzyme (New England BioLabs, USA) for 1 h at 37 °C. Subsequently, the digested fragment was run on a GelRed stained 3% agarose gel and analyzed as follow: The wild-type genotype (CC) corresponds to a 412 bp fragment, heterozygote genotype (CT) is represented by 412, 246 and 166 bp fragments and homozygote polymorphic genotype (TT) corresponds to 246 and 166 bp fragments (Fig. 1).

2.5. Cytokines measurement

A blood sample was obtained from patient and control subjects; centrifuged at 3000 rpm for 5 min. Serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ were measured using the ELISA kits (All from eBioscience, USA) according to the instruction of the manufacturer. Briefly, ELISA plates (Nunc, Denmark) were coated overnight with 100 μl of capture antibody at 4 °C. After washing, the plates were incubated with blocking buffer for 1 h at room temperature. The standards and samples were loaded and incubated for 2 h at room temperature. The wells were washed and then biotinylated antibodies of each kit was used as the detection antibody followed by Streptavidin-HRP. At last, the substrate 3,3′,5,5′-Tetramethylbenzidine (TMB) (BD, USA) was added to the wells and the reaction stopped using sulfuric acid. The absorbance of the wells was read at 450 nm within 30 min.

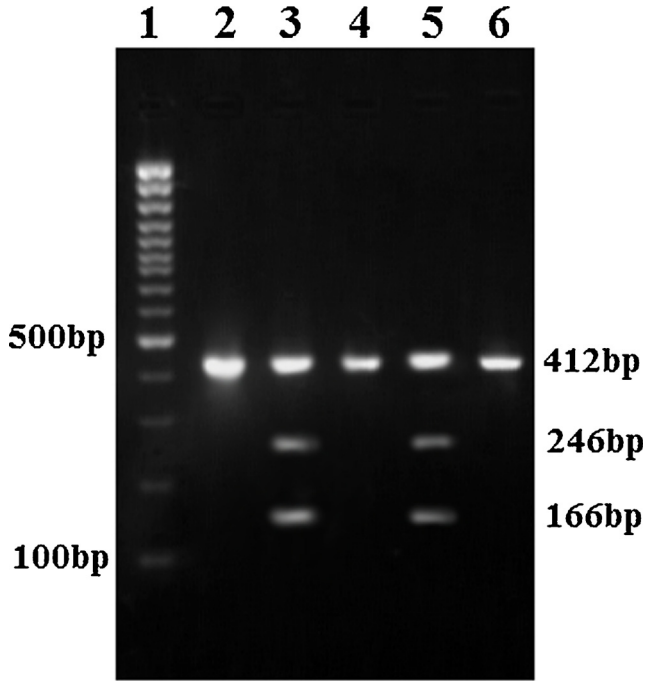

Fig. 1. PTPN22 +1858C/T genotypes. 1) 100bp size marker; 2, 4 and 6) homozygous wild-type genotype (CC); 3 and 5) heterozygote genotype (CT).

2.6. Statistical analysis

Chi-square statistical test, with a 2 × 2 contingency table and one degree of freedom, was used to measure the allele, genotype and haplotype frequencies of case and control groups. SPSS statistical software (Version 20.0) was employed to calculate two-sided *p*-values or Fisher's exact test, odds ratio (*OR*) and 95% confidence interval (*CI*).Furthermore, association of RA with RF and ESR was evaluated using *chi*-square and *t*-test, respectively. Cytokines levels between RA patients and controls as well as two distinct genotypes were analyzed by *t*-test. A multivariate regression analysis of the variables was performed. A *p* value less than 0.05 was considered statistically significant.

3. Results

3.1. Population characteristics

Relevant information, including the characteristics of the cases and control subjects, demographic and clinical data of the RA patients are depicted in Table 1. The mean age of the RA cases was 47 ± 10 years, and 37 ± 9 years for the controls. RA patients showed significantly higher levels of acute phase reactant ESR, RF and anti−CCP with regard to the laboratory assessment compared to the controls (Table 2).

Table 1
Demographic and clinical characteristics of the RA patients.

RA patients (n = 120)	
Demographic	
Age (years)	47 ± 10 (19–77)
Sex (females/males)	95/25
Clinical	
Disease duration (years)	8.1 ± 5.9 (1–32)
Deformity positive	38.2%
Stiffness	< 1h = 26.3% 1-3h = 49.3% > 3 = 16% Negative = 8.4%
Activity positive	54%
Anti-CCP Ab positive	83.5%
RF positive	78.2%
ESR	28.5 ± 15.6 (4-78)

Table 2
Clinical and laboratory parameters in RA patients and controls.

Parameters	RA patients	Control	*P* value
ESR (mm/h) RA: n: 120, C: n: 120	28.5 ± 15.6	7.9 ± 5.1	< 0.001*
Anti-CCP (u/ml) RA: n: 120, C: n: 120	73.5 ± 18.9	1.73 ± 1.09	< 0.001*

The mean difference between groups was analyzed using *t*-test. Statistical significance was set at $P < 0.05$. The values represent the mean ± standard deviation. ESR, erythrocyte sedimentation rate; Anti-CCP, anti-cyclic citrullinated peptide antibodies.*The Anti-CCP and ESR values were significant between RA patients and controls.

3.2. Genotype and allele frequencies of the 1858 C/T PTPN22 gene polymorphism

As shown in Table 3, allele frequencies and genotype distributions for +1858 C/T PTPN22 in the RA cases and control subjects have been compared. The results showed that the PTPN22 C/T genotype (14.1% vs. 4.1%) frequency was significantly higher in RA patients in comparison to the controls (P = 0.007, OR = 2.321, 95%CI = 1.063–5.067). Also, the +1858 T allele frequency was significantly increased in RA patients compared to the controls (P = 0.008, OR = 3.583, 95%CI = 1.3–9.878) (Table 4).

Our results demonstrated that genotype distribution in both patient and control groups for 1858 C/T PTPN22 polymorphism was not concordant with Hardy-Weinberg equilibrium (HWE).

3.3. Clinical parameters and laboratory evaluation of RA patients, according to the 1858 C/T PTPN22 gene polymorphism

According to the 1858 C/T *PTPN22* gene SNP genotypes in the RA patients, clinical parameters of RA patients and control subjects were significantly different as shown in Tables 5 and 6. Accordingly, there were significant differences between C/T and C/C genotypes regarding deformity, duration of the disease and ESR (Table 5 and 6).

3.4. The PTPN22 C/T genotype associated with alteration in serum cytokines production

To address the effects of the PTPN22 C/T genotype on the cytokines balance in serum, we examined IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α cytokines concentration in control and RA patient subjects. Our data showed that IL-4 and IL-10 levels significantly decreased in RA patients compared to controls, whereas upregulated levels of IL-6, IFN-γ, and TNF-α were observed in RA patients. When the results were examined based on genotype, reduced IL-4 and IL-10 were found in PTPN22 1858C/T relative to 1858C/C RA patients. In addition, IL-6, IFN-γ, and TNF-α were significantly increased in PTPN22 1858C/T compared to 1858C/C RA patients (Fig. 2).

In addition, multivariate regression analysis were employed for predictors of cytokines levels in RA patients (Table 7).

4. Discussion

RA is a chronic pro-inflammatory based disease, which affects 1% of the adult populations, and it is characterized by inflammation of the synovial tissue of multiple joints, leading to pain, deformities and a reduced quality of life. However, little is known about the etiology of this disease [24]. Similarly, a growing number of established susceptibility loci have been recognized for RA and other complex diseases

Table 3
Distribution of the 1858 C/T PTPN22 genotype frequency in RA patients and controls.

Polymorphism	Genotype	RA Patients $N = 120$ (%)	Control Subjects $N = 120$ (%)	*OR* (CI)	*P* value
PTPN22	C/C	103 (85.8)	115 (95.8)	–	–
	C/T	17 (14.1)	5 (4.1)	2.321 (1.063-5.067)	0.007[a]
	T/T	0	0	–	–

The values are presented as frequency in percentage and number of genotypes. The frequencies comparison between groups were analyzed by *Chi*-Square test. Statistical significance was set at $P < 0.05$.

[a] There was statistically significant difference in the distributions of PTPN22 gene polymorphism between RA patients and controls.

during the last decades [25]. It has been demonstrated that 1858 C/T *PTPN22* gene SNP results in a significant genetic contribution and increases the risk of the RA by 40–80% in Caucasian populations [25]. Also, the importance of the *PTPN22* gene in susceptibility to RA has been emphasized by Taib and colleagues [26]. In addition, other studies showed that this allele is not related with psoriasis [27] or multiple sclerosis (MS) [28], while it raises the risk of numerous others, such as Hashimoto's thyroiditis [29], rheumatoid arthritis [8,30], type 1 diabetes (T1D) [31–33], vitiligo [34], systemic lupus erythematosus (SLE) [35] and Grave's disease [36]. Overall, the incidence of the 1858 C/T *PTPN22* gene SNP in general population is low, although in certain regions, such as Scandinavia, it can reach ~15% [8,37]. Investigation of this polymorphism frequency in various populations could clear that what makes certain ethnic groups more susceptible to specific autoimmune diseases. Hence, surveying the role of 1858 C/T PTPN22 polymorphisms across ethnic groups will proceed our perceptive of the geo-environmental reinforcements that play a role in autoimmunity appearance. Although, the correlation between the 1858 C/T PTPN22 SNP and RA have been shown in other studies [22,38–41], here we evaluate the association of the 1858 C/T PTPN22 SNP with cytokine profiles in the RA patients. Our results confirm the previous investigations regarding the important roles played by 1858 C/T PTPN22 SNP, and especially C/T genotype and T allele in the pathogenesis of RA. Thus, it may be concluded that 1858 T allele and 1858C/T PTPN22 genotype can be considered as the risk factors for RA development. Generally, different results were obtained in our study between the 1858 C/T *PTPN22* gene SNP genotypes and some of the demographic and clinical characteristics of the RA patients. These results showed that the polymorphism in this gene not only increases the risk of the RA but also is associated with deformity of the tissue and also duration of the disease. Collectively, due to the results it appears that 1858 C/T PTPN22 genotype and T allele are the risk factors for RA and they may affect the pathogenesis and severity of RA.

The previous data that confirmed the association of RA and 1858 C/T SNP as a relationship with RF positive RA patients, indicated the possibility of PTPN22 1858 T allele influences in autoantibody production [22]. These data are inconsistent with our results that showed no significant differences in RF factor between PTPN22C/T and PTPN22C/C RA patients. In addition, there were not any significant differences in anti-CCP levels between PTPN22C/T and PTPN22C/C RA patients according to our findings.

It has been suggested that the Lyp 620 W variant interacted less strongly with CSK. Consequently, it was assumed that the PTPN22 1858 T isoform could cause a deficiency in TCR suppression and T cell hyper-responsiveness [22], although, recent studies using transfected Jurkat cells, have demonstrated that the Lyp 620 W variant has a superior suppression effect on TCR signaling [42]. Nevertheless, it has been recommended that Lyp could play in autoimmunity through other mechanisms beyond T cells, because B and other hemopoietic cells can express Lyp, therefore, this protein possibly could influence on the immune response by other uncharacterized manners. The obvious association among lymphocyte signaling pathways and PTPN22, and the relationship of the 1858 T variant with various autoimmune diseases propose that PTPN22 polymorphism could influence a crucial and general pathway, leading to the progress of autoimmunity. Discovering the efficient effects of the PTPN22 1858 T variant could be useful to reveal pathogenesis mechanisms of these diseases.

On the other hand, it has been suggested that RA is a Th1-type disorder with abnormal T lymphocytes activation [43], which could cause a Th1/Th2 cytokine imbalance [44] and associates with disease activity [45]. In addition, the 1858 T allele of PTPN22 has been related with susceptibility to diverse autoimmune diseases, including RA that has an essential role in T lymphocytes activation [46,47]. Moreover, recent in vitro researches have indicated that PTPN22 is dispensable for Th1 induction in response to CD3 and CD28 stimulation [48]. Therefore, we decided to evaluate the association of cytokine profiles in RA

Table 5
Clinical and laboratory parameters in RA patients.

Genotype	RF (*P* value)	Deformity (*P* value)	Activity (*P* value)	Anti-CCP (*P* value)
PTPN22C/C PTPN22C/T	0.347	0.001[a]	0.186	0.152

[a] There was statistically significant difference in the deformity of patients in C/T genotype compared to C/C. *P*-values were calculated by *chi*-square test with 2 × 2 contingency Table.

Table 6
Clinical and laboratory parameters in RA patients.

Genotype	Duration (*P* value)	ESR (*P* value)	Erosion (*P* value)
PTPN22C/C PTPN22C/T	0.001*	0.025*	0.862

The mean differences between groups were analyzed using *t*- test. Statistical significance was set at $P < 0.05$.

* The duration and ESR were significant between two genotypes in the RA patients.

Table 4
Allele frequencies of PTPN22 SNP in RA patients and controls.

Polymorphism	Allele	RA Patients $N = 120$ (%)	Control Subjects $N = 120$ (%)	*OR* (CI)	*P* value
PTPN22	PTPN22C PTPN22T	223 (93) 17 (7)	235 (98) 5 (2)	3.583 (1.3-9.878)	0.008*

* PTPN22 T allele significantly increased in the RA patients compared to controls (*P*-value < 0.05).

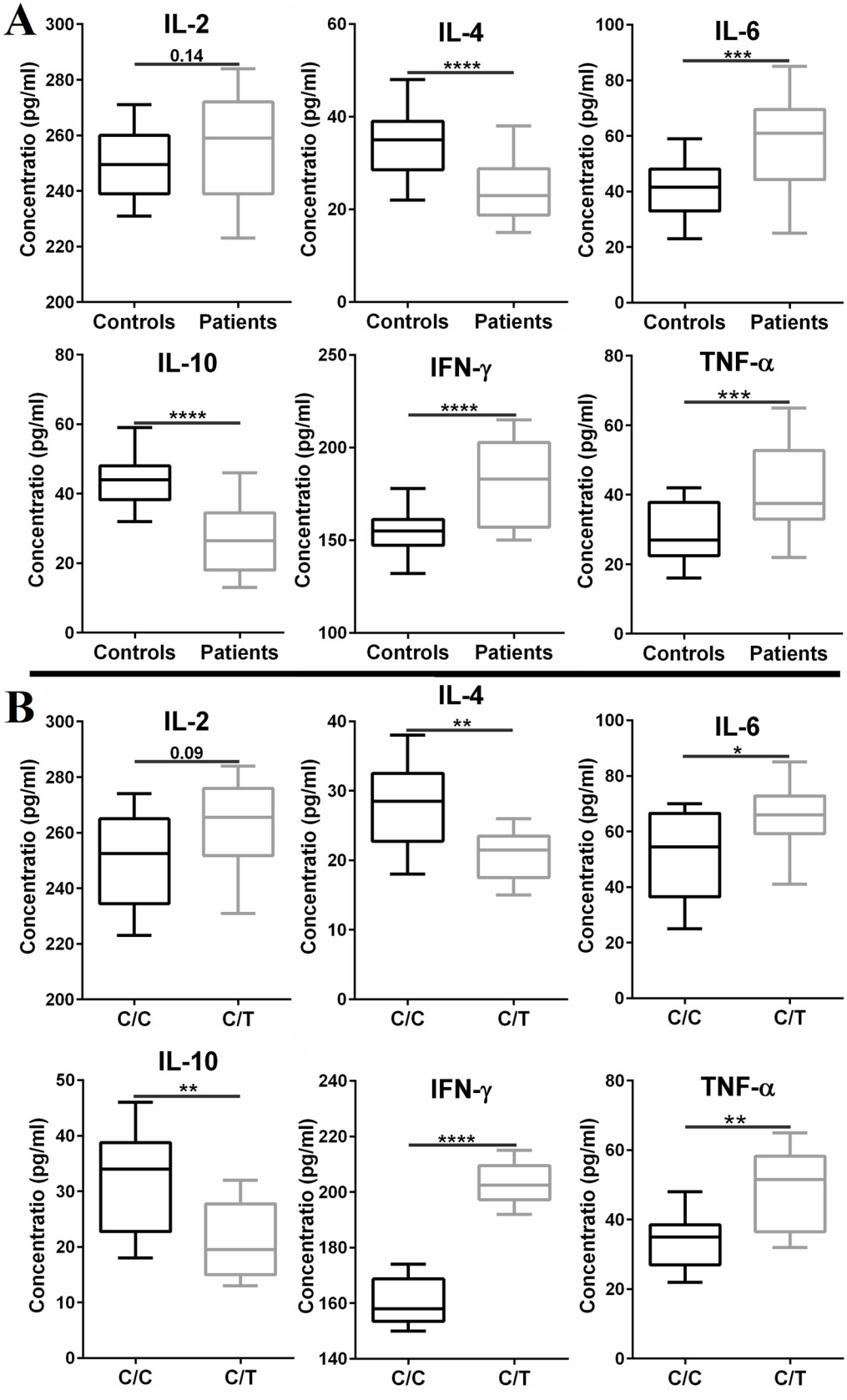

Fig. 2. Cytokines profile in RA patients as well as with and without 1858 T allele. To address the effects of the PTPN22 1858 T allele on the cytokines profile in serum, we examined IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α cytokines concentration in control and RA patient subjects. A blood sample was obtained from patient and control subjects; centrifuged at 3000 rpm for 5 min. Serum levels of intended cytokines was measured using the ELISA method. We found that IL-4 and IL-10 levels significantly reduced in RA patients compared to controls, while IL-6, IFN-γ, and TNF-α significantly increased in RA patients (A). When the results were examined based on genotype, we indicated that IL-4 and IL-10 was significantly decreased in PTPN22 1858C/T compared to 1858C/C RA patients. In addition, IL-6, IFN-γ, and TNF-α was significantly increased in PTPN22 1858C/T compared to 1858C/C RA patients (B). The data presented here are mean ± SD. *, **, *** and **** means p value less than 0.05, 0.01, 0.001 and 0.0001 respectively.

patients with PTPN22 1858 C/T genotype and we observed an altered balance of Th1/Th2 cytokines with up-regulated Th1-type cytokines (IL-2 and IFN-γ) and down-expressed Th2 cytokines (IL-4 and IL-10). Moreover, our results showed that pro-inflammatory cytokines (IL-6 and TNF-α) significantly increased in RA patients with PTPN22 1858 C/T genotype. Consequently, in line with previous studies, our data indicated that RA is a Th1-dominant disease.

The *PTPN22* gene is an essential inhibitor of T lymphocytes

Table 7
Multivariate regression analysis for predictors of cytokines levels in RA patients.

Dependent variables	Parameters	β	t	p
IFN-γ	Anti-ccp	0.473	6.099	0.000
	Duration	0.252	2.197	0.03
	Deformity	0.282	3.12	0.002
	Activity	−0.147	−2.046	0.043
	Erosion	0.015	0.213	0.831
	RF	0.496	6.159	0.000
IL-2	Anti-ccp	0.08	1.191	0.041
	Duration	0.182	1.29	0.2
	Deformity	0.221	1.7	0.092
	Activity	0.082	0.928	0.356
	Erosion	0.068	0.758	0.45
	RF	0.334	2.882	0.005
IL-4	Anti-ccp	−0.189	−2.144	0.034
	Duration	−0.494	−3.79	0.000
	Deformity	−0.015	−0.126	0.9
	Activity	0.085	1.036	0.302
	Erosion	−0.148	−1.79	0.076
	RF	−0.107	−0.998	0.32
IL-6	Anti-ccp	0.215	2.49	0.014
	Duration	0.146	1.14	0.254
	Deformity	0.274	2.334	0.021
	Activity	−0.012	−0.148	0.882
	Erosion	0.05	0.618	0.538
	RF	0.065	0.624	0.534
IL-10	Anti-ccp	−0.142	−1.7	0.092
	Duration	−0.115	−0.926	0.357
	Deformity	−0.356	−3.122	0.002
	Activity	0.09	1.155	0.25
	Erosion	−0.024	−0.31	0.757
	RF	−0.096	−0.946	0.346
TNF-α	Anti-ccp	0.343	4.559	0.000
	Duration	0.053	0.479	0.633
	Deformity	0.42	0.49	0.01
	Activity	−0.004	−0.051	0.96
	Erosion	0.081	1.153	0.251
	RF	0.396	4.346	0.000

activation by its physical connection with Csk [12]. Csk could suppress the kinases Fyn and Lck, which mediate T- lymphocytes activation through binding to Lyp. Substitution of Arg (allele 1858C) by Trp at amino acid residue 620 (allele 1858 T) could dramatically reduce binding of Lyp to Csk [12,21], which might disrupt the function of Csk as an inhibitor of T lymphocytes activation. Therefore, dissociation of Lyp-Csk complex may possibly cause hyper-reactivity of T lymphocytes and more likely increased risk of autoimmunity.

In line with our findings, Vang et al. indicated altered cytokine profiles as a possible pathogenic mechanism, by which the 1858 T allele could contribute to the progress of autoimmunity [49].

In addition, another study by Gerli et al. showed that patients with active RA had dominance of Th1 activity, whereas those patients with early onset RA mainly expressed IL-4 as a Th2 activity cytokine [50].

Moreover, it has been reported that production of IL-10 increased in the 1858 C/T healthy subjects since memory T cells can produce high levels of cytokines, mainly IL-10 [51].

Vang et al. [42] demonstrated a decreased secretion of IL-2 by T1D patients CD4 T cells with 1858 T variant, whereas Rieck et al. detected a tendency to diminished IL-2 production in healthy subjects with the 1858 T variant [51]. Knockout or defect of IL-10 cytokine in mice model could develop autoimmunity risk because of amplification in homeostatic proliferation of the auto-reactive cells and induction of Th1 profile [49,52]. Thus, a decreased production or defects of IL-10 are able to cause the regulation failure, a trend toward autoimmunity among individuals with the PTPN22 1858 C/T variant and expansion of the memory T cell. This conclusion is supported by our findings that IL-10 serum level was diminished in the 1858C/T patients, whereas pro-inflammatory cytokines such as IFN-γ and TNF-α were increased.

Conversely, other study by Talaat RM et al. demonstrated an elevated levels of serum IL-4 and reduced levels of serum IFN-γ in RA patients [53]. Altogether, those findings demonstrated that RA disease is a Th1 type disorder, while in the early stage of disease a Th2 response is observed [50]. The explanation for this inconsistency might have two reasons: (1) the majority of researchers investigated RA patients with different levels of disease activity and (2) the Th1/Th2 balance can alter in RA patients with various disease activities [43]. Moreover, some drugs such as corticosteroids and hydroxychloroquine were used to alleviate clinical symptoms in RA patients, which could influence cytokines production. According to these conflicting findings, it could be concluded that Th1 and Th2 responses mutually play essential roles in the pathogenesis of RA patient's tissue damage.

Our results showed that serum level of IFNγ was increased in RA patients with 1858 C/T genotype compared to 1858 C/C genotype. In consistent with our data, Vang et al. demonstrated that antigen stimulation of $CD4^+$ T cells from healthy subjects with 1858 T allele could cause higher levels secretion of IFN-γ and TNF-α compared to wild-type allele subjects [54]. Moreover, incompatible with our results, Ruiz-Noa et al. reported that RA patients with 1858 C/T genotype have a significant reduce in IFN-γ but interestingly, in healthy subjects it is related with a raise in IFN-γ levels [55]. In addition, Capasso et al. observed that T cell proliferation and IFN-γ production reduced in a type 1 diabetic patient with 1858 T/T PTPN22 genotype [56]. According to these findings, we could suggest that the discrepancy that was observed between these works and our results could be due to the investigation of this polymorphism effects in the RA patients in our study. Possibly, in the RA patients, secretion of other cytokines might be altered through this polymorphism.

Our findings implicate altered cytokine profiles as a possible pathogenic mechanism by which the 1858 C/T genotype may contribute to the progress of RA. Skewing of serum cytokine profiles toward Th1 and related pro-inflammatory cytokines in RA patients suggests that PTPN22 1858 C/T genotype may utilize some of autoimmunity risk via these changes in other autoimmune diseases. Further investigation of the cytokine profiles in the other autoimmune diseases associated with PTPN22 will probably improve our knowledge about the underlying mechanisms in autoimmune phenomena.

Ethics

All procedures performed in this study, involving human participants, were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Funding

This work was supported by Aja University of Medical Sciences (Grant number: 930819).

Declaration of Competing Interest

The authors have no financial conflict of interest.

Acknowledgments

The authors are grateful to Shariati Hospital and Tehran University of Medical Sciences for sample collections. The authors would like to acknowledge financial support of Aja University of Medical Sciences (grant number: 930819).

References

[1] D. Zhebrun, Y. Kudryashova, A. Babenko, A. Maslyansky, N. Kunitskaya, D. Popcova, A. Klushina, E. Grineva, A. Kostareva, E. Shlyakhto, Association of

PTPN22 1858T/T genotype with type 1 diabetes, Graves' disease but not with rheumatoid arthritis in Russian population, Aging (Albany NY) 3 (4) (2011) 368.
[2] V. Goëb, P. Dieudé, R. Daveau, M. Thomas-L'Otellier, F. Jouen, F. Hau, P. Boumier, F. Tron, D. Gilbert, P. Fardellone, Contribution of PTPN22 1858T, TNFRII 196R and HLA-shared epitope alleles with rheumatoid factor and anti-citrullinated protein antibodies to very early rheumatoid arthritis diagnosis, Rheumatology 47 (8) (2008) 1208–1212.
[3] J.J. Goronzy, C.M. Weyand, T-cell regulation in rheumatoid arthritis, Curr. Opin. Rheumatol. 16 (3) (2004) 212–217.
[4] J.S. Smolen, D. Aletaha, I.B. McInnes, Rheumatoid arthritis, Lancet 388 (10055) (2016) 2023–2038.
[5] V. Picerno, F. Ferro, A. Adinolfi, E. Valentini, C. Tani, A. Alunno, One year in review: the pathogenesis of rheumatoid arthritis, Clin. Exp. Rheumatol. 33 (4) (2015) 551–558.
[6] A. Alghasham, Z. Rasheed, Therapeutic targets for rheumatoid arthritis: progress and promises, Autoimmunity 47 (2) (2014) 77–94.
[7] N. Bottini, L. Musumeci, A. Alonso, S. Rahmouni, K. Nika, M. Rostamkhani, J. MacMurray, G.F. Meloni, P. Lucarelli, M. Pellecchia, A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes, Nat. Genet. 36 (4) (2004) 337–338.
[8] M.C. Totaro, B. Tolusso, V. Napolioni, F. Faustini, S. Canestri, A. Mannocci, E. Gremese, S.L. Bosello, S. Alivernini, G. Ferraccioli, PTPN22 1858C& T polymorphism distribution in Europe and association with rheumatoid arthritis: case-control study and meta-analysis, PLoS One 6 (9) (2011) e24292.
[9] T. Vang, A.V. Miletic, N. Bottini, T. Mustelin, Protein tyrosine phosphatase PTPN22 in human autoimmunity, Autoimmunity 40 (6) (2007) 453–461.
[10] J. Zikherman, M. Hermiston, D. Steiner, K. Hasegawa, A. Chan, A. Weiss, PTPN22 deficiency cooperates with the CD45 E613R allele to break tolerance on a non-autoimmune background, J. Immunol. 182 (7) (2009) 4093–4106.
[11] J. Zhang, N. Zahir, Q. Jiang, H. Miliotis, S. Heyraud, X. Meng, B. Dong, G. Xie, F. Qiu, Z. Hao, The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness, Nat. Genet. 43 (9) (2011) 902–907.
[12] J.-F. Cloutier, A. Veillette, Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase, J. Exp. Med. 189 (1) (1999) 111–121.
[13] X. Dai, R.G. James, T. Habib, S. Singh, S. Jackson, S. Khim, R.T. Moon, D. Liggitt, A. Wolf-Yadlin, J.H. Buckner, A disease-associated PTPN22 variant promotes systemic autoimmunity in murine models, J. Clin. Invest. 123 (5) (2013) 2024–2036.
[14] K. Hasegawa, F. Martin, G. Huang, D. Tumas, L. Diehl, A.C. Chan, PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells, Science 303 (5658) (2004) 685–689.
[15] H.A. Purvis, F. Clarke, C.K. Jordan, C.S. Blanco, G.H. Cornish, X. Dai, D.J. Rawlings, R. Zamoyska, A.P. Cope, Protein tyrosine phosphatase PTPN22 regulates IL-1β dependent Th17 responses by modulating dectin-1 signaling in mice, Eur. J. Immunol. 48 (2) (2018) 306–315.
[16] G.L. Burn, G.H. Cornish, K. Potrzebowska, M. Samuelsson, J. Griffié, S. Minoughan, M. Yates, G. Ashdown, N. Pernodet, V.L. Morrison, Superresolution imaging of the cytoplasmic phosphatase PTPN22 links integrin-mediated T cell adhesion with autoimmunity, Sci. Signal. 9 (448) (2016) ra99-ra99.
[17] Y. Wang, D. Ewart, J.N. Crabtree, A. Yamamoto, E.C. Baechler, P. Fazeli, E.J. Peterson, PTPN22 variant R620W is associated with reduced toll-like receptor 7–Induced type I interferon in systemic lupus erythematosus, Arthritis Rheumatol. 67 (9) (2015) 2403–2414.
[18] D.A. Holmes, E. Suto, W.P. Lee, Q. Ou, Q. Gong, H.R. Smith, P. Caplazi, A.C. Chan, Autoimmunity-associated protein tyrosine phosphatase PEP negatively regulates IFN-α receptor signaling, J. Exp. Med. 212 (7) (2015) 1081–1093.
[19] Y. Wang, I. Shaked, S.M. Stanford, W. Zhou, J.M. Curtsinger, Z. Mikulski, Z.R. Shaheen, G. Cheng, K. Sawatzke, A.M. Campbell, The autoimmunity-associated gene PTPN22 potentiates toll-like receptor-driven, type 1 interferon-dependent immunity, Immunity 39 (1) (2013) 111–122.
[20] J. Zhang, N. Zahir, Q. Jiang, H. Miliotis, S. Heyraud, X. Meng, B. Dong, G. Xie, F. Qiu, Z. Hao, The autoimmune disease–associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness, Nat. Genet. 43 (9) (2011) 902.
[21] E. Fiorillo, V. Orru, S.M. Stanford, Y. Liu, M. Salek, N. Rapini, A.D. Schenone, P. Saccucci, L.G. Delogu, F. Angelini, Autoimmune-associated PTPN22 R620W variation reduces phosphorylation of lymphoid phosphatase on an inhibitory tyrosine residue, J. Biol. Chem. 285 (34) (2010) 26506–26518.
[22] A.B. Begovich, V.E. Carlton, L.A. Honigberg, S.J. Schrodi, A.P. Chokkalingam, H.C. Alexander, K.G. Ardlie, Q. Huang, A.M. Smith, J.M. Spoerke, A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis, Am. J. Hum. Genet. 75 (2) (2004) 330–337.
[23] D. Aletaha, T. Neogi, A.J. Silman, J. Funovits, D.T. Felson, C.O. Bingham III, N.S. Birnbaum, G.R. Burmester, V.P. Bykerk, M.D. Cohen, 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative, Arthritis Rheum. 62 (9) (2010) 2569–2581.
[24] M.J. Coenen, G. Trynka, S. Heskamp, B. Franke, C.C. van Diemen, J. Smolonska, M. van Leeuwen, E. Brouwer, M.H. Boezen, D.S. Postma, Common and different genetic background for rheumatoid arthritis and coeliac disease, Hum. Mol. Genet. 18 (21) (2009) 4195–4203.
[25] A. Barton, J. Worthington, Genetic susceptibility to rheumatoid arthritis: an emerging picture, Arthritis Care & Res. 61 (10) (2009) 1441–1446.
[26] W.R.W. Taib, D.J. Smyth, M.E. Merriman, N. Dalbeth, P.J. Gow, A.A. Harrison, J. Highton, P.B. Jones, L. Stamp, S. Steer, The PTPN22 locus and rheumatoid arthritis: no evidence for an effect on risk independent of Arg620Trp, PLoS One 5 (10) (2010).
[27] I. Nistor, R.P. Nair, P. Stuart, R. Hiremagalore, R.A. Thompson, S. Jenisch, M. Weichenthal, G.R. Abecasis, Z.S. Qin, E. Christophers, Protein tyrosine phosphatase gene PTPN22 polymorphism in psoriasis: lack of evidence for association, J. Gen. Intern. Med. 20 (5) (2005) 395–396.
[28] A.B. Begovich, S.J. Caillier, H.C. Alexander, J.M. Penko, S.L. Hauser, L.F. Barcellos, J.R. Oksenberg, The R620W polymorphism of the protein tyrosine phosphatase PTPN22 is not associated with multiple sclerosis, Am. J. Hum. Genet. 76 (1) (2005) 184.
[29] G. Dultz, N. Matheis, M. Dittmar, B. Röhrig, K. Bender, G.J. Kahaly, The protein tyrosine phosphatase non-receptor type 22 C1858T polymorphism is a joint susceptibility locus for immunthyroiditis and autoimmune diabetes, Thyroid 19 (2) (2009) 143–148.
[30] M. Van Oene, R.F. Wintle, X. Liu, M. Yazdanpanah, X. Gu, B. Newman, A. Kwan, B. Johnson, J. Owen, W. Greer, Association of the lymphoid tyrosine phosphatase R620W variant with rheumatoid arthritis, but not Crohn's disease, in Canadian populations, Arthritis Rheum. 52 (7) (2005) 1993–1998.
[31] M. Zoledziewska, C. Perra, V. Orrù, L. Moi, P. Frongia, M. Congia, N. Bottini, F. Cucca, Further evidence of a primary, causal association of the PTPN22 620W variant with type 1 diabetes, Diabetes 57 (1) (2008) 229–234.
[32] A. Long, J.H. Buckner, Intersection between genetic polymorphisms and immune deviation in type 1 diabetes, Current Opinion in Endocrinology, Diabetes and Obesity 20 (4) (2013) 285–291.
[33] D. Smyth, J.D. Cooper, J.E. Collins, J.M. Heward, J.A. Franklyn, J.M. Howson, A. Vella, S. Nutland, H.E. Rance, L. Maier, Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus, Diabetes 53 (11) (2004) 3020–3023.
[34] I. Canton, S. Akhtar, N. Gavalas, D. Gawkrodger, A. Blomhoff, P. Watson, A. Weetman, E. Kemp, A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo, Genes Immun. 6 (7) (2005) 584.
[35] H. Wu, R.M. Cantor, D.S.C. Graham, C.M. Lingren, L. Farwell, P.L.D. Jager, N. Bottini, J.M. Grossman, D.J. Wallace, B.H. Hahn, Association analysis of the R620W polymorphism of protein tyrosine phosphatase PTPN22 in systemic lupus erythematosus families: increased T allele frequency in systemic lupus erythematosus patients with autoimmune thyroid disease, Arthritis & Rheumatism: Off. J. Am Coll. Rheumatol. 52 (8) (2005) 2396–2402.
[36] M. Velaga, V. Wilson, C. Jennings, C. Owen, S. Herington, P. Donaldson, S. Ball, R. James, R. Quinton, P. Perros, The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease, J. Clin. Endocrinol. Metab. 89 (11) (2004) 5862–5865.
[37] G.L. Burn, L. Svensson, C. Sanchez-Blanco, M. Saini, A.P. Cope, Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? FEBS Lett. 585 (23) (2011) 3689–3698.
[38] V.E. Carlton, X. Hu, A.P. Chokkalingam, S.J. Schrodi, R. Brandon, H.C. Alexander, M. Chang, J.J. Catanese, D.U. Leong, K.G. Ardlie, PTPN22 genetic variation: evidence for multiple variants associated with rheumatoid arthritis, Am. J. Hum. Genet. 77 (4) (2005) 567–581.
[39] M. Viken, S. Amundsen, T. Kvien, K. Boberg, I. Gilboe, V. Lilleby, L. Sollid, Ø. Førre, E. Thorsby, A. Smerdel, Association analysis of the 1858C& T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases, Genes Immun. 6 (3) (2005) 271–273.
[40] M. Pierer, S. Kaltenhauser, S. Arnold, M. Wahle, C. Baerwald, H. Hantzschel, U. Wagner, Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: higher frequency of the risk allele in male compared to female patients, Arthritis Res. Ther. 8 (3) (2006) R75.
[41] J. Wesoly, X. Hu, M. Thabet, M. Chang, H. Uh, C. Allaart, R. Toes, J. Houwing-Duistermaat, A. Begovich, T. Huizinga, The 620W allele is the PTPN22 genetic variant conferring susceptibility to RA in a Dutch population, Rheumatology 46 (4) (2007) 617–621.
[42] T. Vang, M. Congia, M.D. Macis, L. Musumeci, V. Orrú, P. Zavattari, K. Nika, L. Tautz, K. Taskén, F. Cucca, Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant, Nat. Genet. 37 (12) (2005) 1317.
[43] K. Yudoh, H. Matsuno, F. Nakazawa, T. Yonezawa, T. Kimura, Reduced expression of the regulatory CD4+ T cell subset is related to Th1/Th2 balance and disease severity in rheumatoid arthritis, Arthritis & Rheumatism: Off. J. Am Coll. Rheumatol. 43 (3) (2000) 617–627.
[44] R.J. Dolhain, A.N. van der Heiden, N.T. Ter Haar, F.C. Breedveld, A.M. Miltenburg, Shift toward T lymphocytes with a T helper 1 cytokine-secretion profile in the joints of patients with rheumatoid arthritis, Arthritis Rheum. 39 (12) (1996) 1961–1969.
[45] K. Stark, J. Rovensky, S. Blazickova, H. Grosse-Wilde, S. Ferencik, C. Hengstenberg, R.H. Straub, Association of common polymorphisms in known susceptibility genes with rheumatoid arthritis in a Slovak population using osteoarthritis patients as controls, Arthritis Res. Ther. 11 (3) (2009) 70.
[46] P. Harrison, J. Pointon, C. Farrar, M. Brown, B. Wordsworth, Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients, Rheumatology 45 (8) (2006) 1009–1011.
[47] A. Hinks, A. Barton, S. John, I. Bruce, C. Hawkins, C.E.M. Griffiths, R. Donn, W. Thomson, A. Silman, J. Worthington, Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene, Arthritis Rheum. 52 (6) (2005) 1694–1699.
[48] G. Fousteri, T. Jofra, I. Debernardis, S. Stanford, A. Laurenzi, N. Bottini,

M. Battaglia, The protein tyrosine phosphatase PTPN 22 controls forkhead box protein 3 T regulatory cell induction but is dispensable for T helper type 1 cell polarization, Clin. Exp. Immunol. 178 (1) (2014) 178–189.
[49] A.C. Anderson, J. Reddy, R. Nazareno, R.A. Sobel, L.B. Nicholson, V.K. Kuchroo, IL-10 plays an important role in the homeostatic regulation of the autoreactive repertoire in naive mice, J. Immunol. 173 (2) (2004) 828–834.
[50] R. Gerli, O. Bistoni, A. Russano, S. Fiorucci, L. Borgato, M. Cesarotti, C. Lunardi, In vivo activated T cells in rheumatoid synovitis. Analysis of Th1-and Th2-type cytokine production at clonal level in different stages of disease, Clin. Exp. Immunol. 129 (3) (2002) 549–555.
[51] M. Rieck, A. Arechiga, S. Onengut-Gumuscu, C. Greenbaum, P. Concannon, J.H. Buckner, Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes, J. Immunol. 179 (7) (2007) 4704–4710.
[52] N.J. Davidson, M.W. Leach, M.M. Fort, L. Thompson-Snipes, R. Kühn, W. Müller, D.J. Berg, D.M. Rennick, T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice, J. Exp. Med. 184 (1) (1996) 241–251.
[53] R.M. Talaat, S.F. Mohamed, I.H. Bassyouni, A.A. Raouf, Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: correlation with disease activity, Cytokine 72 (2) (2015) 146–153.
[54] T. Vang, J. Landskron, M.K. Viken, N. Oberprieler, K.M. Torgersen, T. Mustelin, K. Tasken, L. Tautz, R.C. Rickert, B.A. Lie, The autoimmune-predisposing variant of lymphoid tyrosine phosphatase favors T helper 1 responses, Hum. Immunol. 74 (5) (2013) 574–585.
[55] Y. Ruiz-Noa, J. Hernández-Bello, M.A. Llamas-Covarrubias, C.A. Palafox-Sánchez, E. Oregon-Romero, P.E. Sánchez-Hernández, M.G. Ramírez-Dueñas, I. Parra-Rojas, J.F. Muñoz-Valle, PTPN22 1858C& T polymorphism is associated with increased CD154 expression and higher CD4+ T cells percentage in rheumatoid arthritis patients, J. Clin. Lab. Anal. 33 (3) (2019) e22710.
[56] F. Capasso, N. Rapini, G. Di Matteo, M. Testi, S. Arcano, R. Lidano, A. Petrelli, P. Rossi, S. Piccinini, M.L. Manca Bitti, A variable degree of autoimmunity in the pedigree of a patient with type 1 diabetes homozygous for the PTPN 22 1858 T variant, Pediatr. Diabetes 14 (4) (2013) 304–310.