Cellular Immunology 290 (2014) 62–65

Contents lists available at ScienceDirect

Cellular Immunology

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

Protein tyrosine phosphatase non-receptor type 22 *(PTPN22) +1858 C>T* gene polymorphism in Egyptian cases with rheumatoid arthritis

Afrah Salama [a], Rami Elshazli [a,*], Afaf Elsaid [b], Ahmad Settin [b]

[a] *Department of Biochemistry, Faculty of Science, Tanta University, Tanta, Egypt*
[b] *Genetics Unit, Children Hospital, Mansoura University, Mansoura, Egypt*

ARTICLE INFO

Article history:
Received 19 March 2014
Accepted 9 May 2014
Available online 17 May 2014

Keywords:
Rheumatoid arthritis
Polymorphism
PTPN22
Anti-CCP
Gene

ABSTRACT

Background: Rheumatoid arthritis (RA) is a common autoimmune disease with a complex genetic background. The gene encoding protein tyrosine phosphatase non-receptor type 22 (PTPN22) has been reported to be associated with RA in several populations.
Objectives: This work aimed at assessing the association of *PTPN22 +1858 C>T* gene polymorphism with the susceptibility, activity and severity of RA in Egyptian subjects.
Subjects and methods: This study included 112 unrelated RA patients who were compared to 122 healthy unrelated individuals taken from the same locality. For all subjects, DNA was genotyped for *PTPN22 +1858 C>T (rs2476601)* polymorphism using the PCR-RFLP technique. Antibodies to cyclic citrullinated peptides (anti-CCP) were measured by enzyme-linked immunosorbent assay (ELISA).
Results: Cases showed significantly higher *PTPN22 +1858 T* allele carriage rate (CT + TT genotypes) compared to controls (34.8% *vs.* 8.2%, OR = 5.98, 95% CI = 2.81–12.73, $p < 0.001$). Also the frequency of the *PTPN22 +1858 T* allele was significantly higher among cases compared to controls (18.7% *vs.* 4.5%, OR = 4.89; 95% CI = 2.45–9.76, $p < 0.001$). Cases positive to the *PTPN22 T* allele (CT + TT genotypes) showed no significant difference from those with the CC genotype regarding clinical and immune parameters. Nonetheless, they showed a more functional disability presented in their significantly higher health assessment questionnaire (HAQ) score ($p = 0.04$).
Conclusions: This study is a confirmatory evidence of the association of the *PTPN22* +1858 T allele with susceptibility and functional disability of RA in Egyptian subjects.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder affecting 1% of the adult population. The disease is characterized by an inflammation of the synovial tissue of multiple joints leading to pain, deformities and a reduced quality of life [1,2]. Although the etiology of RA is unknown, both genetic and environmental factors have been shown to play a role in its development. Genetic factors were thought to be responsible for up to 50–60% of the predisposition to RA. The genetic background of RA is complex and is likely involving multiple genes which encode proteins with significant functions in the regulation of immune response [3,4].

The rheumatoid factor (RF), which is an immunoglobulin directed against the Fc portion of IgG, is found in over 70% of patients with RA and is used as a serological marker of RA; high RF might confer a worse prognosis [5,6]. On the other hand, anti-cyclic citrullinated peptide (anti-CCP) antibodies had emerged as a sensitive and specific serological marker of RA, being present before the onset of symptoms, hence providing a valuable diagnostic test early in the course of the disease [6].

The protein tyrosine phosphatase non-receptor type 22 (PTPN22) belongs to a family of protein tyrosine phosphatase that is involved in preventing spontaneous T cell activation through dephosphorylation and inactivation of T cell receptor (TCR)-associated kinases and their substrates [7,8]. The human PTPN22 gene, is located on chromosome 1p13, and encodes a lymphoid specific phosphatase (LYP), which is important in the negative control of T cell activation and development [9]. The presence of the *+1858T* allele of *PTPN22* gene, affects LYP function, resulting in a loss of function protein, that are unable to regulate the T cell activation, hence, is thought to predispose to multiple autoimmune diseases [4,9]. Moreover, in vitro experiments suggested that T cells that were expressing the T allele might be hyperresponsive and therefore prone to autoimmunity [10,11]. Knowing that the allelic frequencies of genes often differ substantially between populations; we were interested to test the association of this

* Corresponding author.
E-mail address: Biolab100@gmail.com (R. Elshazli).

http://dx.doi.org/10.1016/j.cellimm.2014.05.003
0008-8749/

seemingly important *PTPN22 +1858 C>T* polymorphism with susceptibility and clinical pattern of RA in Egyptian cases.

2. Subjects and methods

This study included 112 RA patients (93 females and 19 males); all fulfilled the criteria for RA according to the classification of the American College of Rheumatology [12]. Their age mean ± SD was 47.6 ± 10.1 years. They were recruited from the outpatient clinics of Rheumatology and Rehabilitation Department; Mansoura University Hospitals, Egypt. Exclusion criteria had included all patients with RA having abnormal renal or hepatic functions, history of malignancy, alcohol abuse, pregnancy and lactation and/or association with other autoimmune disorders. For comparison, another control sample was taken in the form of 122 healthy unrelated blood donors (99 females and 23 males) from the same locality. Their age mean ± SD was 42.3 ± 10.3 years.

Disease activity was determined on the basis of multiple defined parameters including the swollen joint count (SJC), tender joint count (TJC), disease activity score 28 (DAS28) [13], C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Functional disability and disease severity were determined on the basis of parameters defined by the health assessment questionnaire (HAQ) [14]. The HAQ score is a well evaluated instrument for measuring the functional disability of RA patients [15]. The HAQ score comprised eight general categories including: dressing and grooming, arising, eating, walking, hygiene, reach, grip and other activities. Each of which consists of one or more specific questions. Each question is scored either (0): "without any difficulty", (1): "with some difficulty", (2): "with much difficulty" and (3): "unable to do" with a summed score ranging 0–24 [14].

C-reactive protein (CRP) and rheumatoid factor (RF) were estimated by semi-quantitative latex agglutination with AVITEX-CRP and AVITEX-RF kits (Omega Diagnostics, Alva, Scotland, UK). Serum was considered positive when the titer is 6.0 mg/L for CRP and 8.0 IU/ml for RF. Anti-CCP was detected in serum samples using enzyme-linked immunosorbent assay (ELIZA) kit (Orgentec Diagnostics, Mainz, Germany). A concentration $\geqslant$ 20 U/ml was considered positive.

Genotyping of the *PTPN22 +1858 C>T* gene SNP (rs2476601) was performed via the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, using the forward (5′-ACT GAT AAT GTT GCT TCA ACG G-3′) and reverse (5′-TCA CCA GCT TCC TCA ACC AC-3′) primers [16]. PCR mixture contained 100 ng DNA, 10 pmol of each primer, 50 μm of dNTPs, 1× PCR buffer with $MgCl_2$, 1 U Taq DNA polymerase and H_2O up to 25 μl. PCR amplification protocol consisted of initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, and final extension cycle at 74 °C for 2 min. The amplified product was digested with FastDigest RsaI restriction enzyme (Fermentas, Germany). The digestion products were applied on a 2% agarose gel stained with ethidium bromide permitting the differentiation of the following restriction pattern: the C allele was cut into 176 and 42 bps fragments while the T allele was not digested; so the TT genotype appeared as a single band at 218 bps; the CC genotype appeared as single band at 176 bps (42 bps fragment was too small to be detected) while the CT genotype appeared as two bands; one at 218 bps, and the other at 176 bps.

2.1. Ethical approval and informed consent

The study was commenced on after obtaining an approval from the university ethical and scientific committees. In addition, an informed consent was obtained from all participants before their enrollment into the study.

2.2. Statistical analysis

Data were processed and analyzed using the statistical package of social science (SPSS, version 17.0). The frequencies of studied genotypic and allelic polymorphisms among cases were compared to those of controls using Fisher's exact test and odds ratio (OR) with the 95% confidence interval (95% CI). Quantitative traits were compared using the Student *t*-test while nominal traits were compared using the Chi square test. Conformity with the Hardy Weinberg law of genetic equilibrium (HWE) was assured by a non-significant Chi square test comparing the observed *vs.* the expected genotypes among studied cases and controls. A minimum level of statistical significance was considered at a p level of <0.05.

3. Results

The demographic and clinical data of the RA patients are shown in Table 1. It was noted that 38 cases (33.9%) had a positive family history of rheumatoid arthritis and 50 cases (44.6%) had a positive consanguinity. Analysis of laboratory markers revealed that 105 cases (93.8%) were positive for anti-cyclic citrullinated peptide (anti-CCP) while 83 cases (74.1%) were positive for rheumatoid factor (RF).

The genotypic and allelic frequencies of the *PTPN22 +1858 C>T* gene SNP in RA patients and controls are shown in Table 2. The frequencies of PTPN22 genotypes were in agreement with Hardy–Weinberg equilibrium in both RA patients and control groups ($p > 0.05$). Testing for the dominant model of inheritance (CT + TT *vs.* CC) showed that cases had a significantly higher frequency of the T allele carriers (CT + TT genotypes) compared to controls (34.8% *vs.* 8.2%, OR = 5.98, 95% CI = 2.81–12.73, $p < 0.001$). As the frequency of the TT genotype is low among cases and controls, this significance is almost due to the higher frequency of the CT genotype in RA cases compared to the controls (32.1% *vs.* 7.4%, OR = 6.14, 95% CI = 2.79–13.49, $p < 0.001$). Regarding allelic frequencies, cases showed significantly higher frequency of the *PTPN22 +1858 T* allele compared to controls (18.7% *vs.* 4.5%, OR = 4.89; 95% CI = 2.45–9.76, $p < 0.001$) (Table 2).

Table 1
The main demographic and clinical characteristics of patients of rheumatoid arthritis compared to controls.

Variable*	Cases $n = 112$	Controls $n = 122$
Age, years, M ± SD	47.6 ± 10.1	42.3 ± 10.3
Females/males, *n* (%)	93/19 (83/17)	99/23 (81.1/18.9)
Age of onset, years, M ± SD	37.1 ± 9.9	–
Disease duration, years, M ± SD	10.6 ± 6.6	–
Positive consanguinity, *n* (%)	50 (44.6)	–
Positive family history, *n* (%)	38 (33.9)	–
Positive rheumatoid nodule, *n* (%)	46 (41.1)	–
Positive rheumatoid deformity, *n* (%)	19 (17)	–
RF - positive, *n* (%)	83 (74.1)	–
Anti-CCP - positive, *n* (%)	105 (93.8)	–
CRP - positive, *n* (%)	77 (68.8)	–
ESR, mm/h	36.7 ± 21.4	–
DAS28 score, M ± SD	4.6 ± 1.3	–
Swollen joint number, M ± SD	8.9 ± 6.7	–
Tender joint number, M ± SD	14.9 ± 8.9	–
VAS score, M ± SD	6.0 ± 2.1	–
HAQ score, M ± SD	13.2 ± 6.5	–

* RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide (anti-ccp); CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: RA disease activity score 28, VAS: visual analogue scale and HAQ: health assessment questionnaire.