Joint Bone Spine 82 (2015) 182–186

Available online at ScienceDirect www.sciencedirect.com

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Original article

Association between polymorphisms of interleukin 10 with inflammatory biomarkers in East Chinese Han patients with rheumatoid arthritis

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ARTICLE INFO

Article history:
Accepted 26 November 2014
Available online 23 January 2015

Keywords:
Interleukin 10
Polymorphisms
Rheumatoid arthritis
Molecular epidemiology

ABSTRACT

Objectives: The aim of the present study was to examine the association between polymorphisms of IL-10 with inflammatory biomarkers in East Chinese Han patients with rheumatoid arthritis (RA).
Methods: We examined *IL-10* rs1800872 A/C polymorphisms in 615 RA patients, and 839 controls, in an East Chinese Han population. Genotyping was performed using a custom-by-design 48-Plex SNP scan TM Kit. The blood plasma concentration of IL-10 was measured using an Iodine [^{125}I] IL-10 Radioimmunoassay Kit, in 90 RA patients and 90 controls.
Results: IL-10 rs1800872 A/C polymorphisms were associated with risk of RA. Following stratified analysis, an increased risk of RA was associated with the CC genotype among male, older, C-reactive protein-positive, anti-cyclic citrullinated peptide antibody-positive, and rheumatoid factor-positive-patients, and among patients with a DAS28 of $\geq$ 3.20 or an erythrocyte sedimentation rate of $\geq$ 25, and in functional class I and II patients. The average plasma concentration of IL-10 was significantly higher in RA patients compared with controls. RA patients positive for the homozygote CC were characterized by significantly higher levels of IL-10 compared with patients with the heterozygote AC. We also found that there were significant relationships between the single nucleotide polymorphisms in the human *IL-10* rs1800872 A/C and production of IL-10.
Conclusions: Our results suggest that the *IL-10* rs1800872 A/C allele might increase the risk of RA. The *IL-10* rs1800872 A/C allele might also impact the inflammatory reaction of IL-10 in patients with RA.

1. Introduction

Rheumatoid arthritis (RA) involves synovial inflammation resulting from an interaction between genetic and environmental factors [1]. Single nucleotide polymorphisms (SNPs) play an important role in RA pathogenesis [2].

IL-10, a type-II cytokine, is secreted by B lymphocytes, monocytes, and activated T-cells in RA patients [3,4]. IL-10 has received attention since originally being characterized as a cytokine synthesis inhibition factor several years ago [5]. IL-10 is an intrinsic anti-inflammatory peptide produced by helper T (Th)2 cells during immune diseases [6]. It can also inhibit the production of a number of cytokines, including IL-2, IL-3, interferon (IFN)-γ, granulocyte macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF)-α, and is categorized as a Th2 cytokine [7,8]. IL-10 is a major inhibitor of Th1 function [9]; IL-10–deficient mice develop a form of inflammatory bowel disease similar to Crohn's disease [10].

The *IL-10* gene contains five exons, and is located on chromosome 1 in humans [11]. It has an open reading frame of 178 amino acids; the mature protein is 18 kDa.

RA results from a persistent imbalance between pro- and anti-inflammatory immune mechanisms, leading to chronic synovitis inflammation. A recent research discovered a correlation between

Abbreviations: CI, confidence interval; IL-10, interleukin 10; LD, linkage disequilibrium; OR, odds ratio; SNP, single nucleotide polymorphism.

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http://dx.doi.org/10.1016/j.jbspin.2014.11.007
1297-319X/

the concentration of serum levels of IL-17 and synovial hypertrophy measured by BM score in RA [12]. However, IL-10 is a very important anti-inflammatory factor. IL-10 production is markedly higher in RA patients compared with controls [3]. The serum IL-10 levels of RA patients are negatively correlated ($P < 0.01$) with disease activity scores (DAS 28) [13].

A study of Polish patients demonstrated that *IL-10* rs1800872 A/C polymorphisms might be genetic risk factors for RA susceptibility and severity [14]; however, another study provided contrary findings [15]. In a study of RA in a Dutch population, the *IL-10* genotype was not associated with RA incidence, but the -2849 IL-10-promoter polymorphism was associated with autoantibody production and subsequent joint damage [16]. Studies using Asian populations suggest an association between *IL-10* rs1800872 A/C polymorphism and RA [17–19].

Genetic polymorphisms frequently vary according to ethnic group and environment. Functional variations in the *IL-10* gene might contribute to RA development, and serve as either a risk or protective factor for RA in different ethnic groups and environments. We performed genotyping of 615 RA patients and 839 controls in a hospital-based case-control study of East Chinese Han individuals.

2. Patients and methods

2.1. Study subjects

We obtained approval for the study protocol from the Ethics Committee of Nanjing Medical University (Nanjing, China). All patients provided written informed consent prior to their participation.

Six hundred and fifteen RA patients who fulfilled the criteria for RA set by the American College of Rheumatology (1987) [20] were consecutively recruited from the Changzhou Second Hospital-Affiliated Hospital of Nanjing Medical University, the Changzhou First Hospital, and the Changzhou Traditional Chinese Medical Hospital, between September 2010 and October 2013. The controls were patients without RA, matched for age (± 5 years) and sex, and recruited from the same institutions during the same time period; the majority of the controls were admitted to the hospitals for the treatment of trauma.

To obtain demographic and RA risk factor data, each patient was interviewed by trained personnel using a pre-validated questionnaire. Following the interview, 2 mL of peripheral blood were collected from each patient.

Blood samples were collected using vacutainers, and transferred to test tubes containing ethylenediaminetetraacetic acid (EDTA), using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). SNP genotyping was performed using a custom-by-design 48-Plex SNP scan TM Kit (Genesky Biotechnologies Inc., Shanghai, China) as described previously [21].

The blood plasma concentration of IL-10 in 90 RA patients and 90 randomly selected controls were measured using an Iodine [^{125}I] IL-10 Radioimmunoassay Kit (North China Institute of Bio-technology, Beijing, China). All analytical steps were performed in accordance with the manufacturer's recommendations. The assays used can detect the biologically active form of the protein. The concentration of IL-10 was calculated by referring to a standard curve, according to the manufacturer's instructions.

2.2. Statistical analyses

Differences in the demographic characteristics, variables, and the genotypes of the *IL-10* rs1800872 A/C polymorphism variants were evaluated using a chi-squared test. Associations between *IL-10* rs1800872 A/C genotypes and risk of RA were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression analyses. The Hardy–Weinberg equilibrium (HWE) principle was tested by a goodness-of-fit chi-squared test, to compare the observed and expected genotype frequencies among controls. Differences in *IL-10* polymorphism and IL-10 blood plasma concentrations were evaluated using the Student's *t*-test. All statistical analyses were performed using the SAS software package (ver. 9.1.3; SAS Institute, Cary, NC, USA).

3. Results

3.1. Characteristics of the study population

The demographic and clinical characteristics of all subjects are summarized in Table 1. Subjects were adequately matched for

Table 1
Patient demographics and risk factors in rheumatoid arthritis, all subjects.

Variable	Cases ($n = 615$)	Controls ($n = 839$)	P
Age (years)	54.51 (± 15.19)	55.44 (± 10.80)	0.170
Female/male	472/143	633/206	0.566
Age at onset, years, mean ± SD	46.06 (± 13.24)	–	–
Disease duration, years, mean ± SD	8.52 (± 9.24)	–	–
Treatment duration, years, mean ± SD	7.30 (± 7.91)	–	–
RF-positive, no. (%)	486 (79.02%)	–	–
ACPA positive, no. (%)	321 (52.20%)	–	–
CRP-positive, no. (%)	165 (26.83%)	–	–
ESR, mm/h	35.79 (± 28.70)	–	–
DAS28	4.46 (± 1.50)	–	–
Functional class, no. (%)		–	–
I	78 (12.68%)	–	–
II	281 (45.69%)	–	–
III	220 (35.77%)	–	–
IV	36 (5.85%)	–	–
IL-10 levels[a] (pg/mL)	126.51 (± 50.46)	86.78 (± 35.53)	**< 0.001**

RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibodies; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: RA disease activity score.
[a] IL-10 levels were available in 90 controls (TT: 39; TG: 42; GG: 9) and 90 RA cases (TT: 40; TG: 34; GG: 16), bold values are statistically significant ($P < 0.05$).