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Association between polymorphisms of interleukin 12 and rheumatoid arthritis associated biomarkers in a Chinese population

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ABSTRACT

Introduction: The aim of the present study was to examine the association between polymorphisms of interleukin 12 (IL-12) and rheumatoid arthritis (RA) associated biomarkers in a Chinese population.
Materials and methods: We studied IL-12A rs2243115 T/G and IL-12B rs3212227 A/C polymorphisms in 615 RA patients and 839 controls in a Chinese population. Genotyping was done by a custom-by-design 48-Plex SNPscan™ Kit. The plasma level of IL-12 was measured by an enzyme-linked immune-sorbent assay in 90 RA patients and 90 controls. Clinical data with other potential diagnostic value were provided by the physicians.
Results: A significantly increased risk for RA associated with the IL-12A rs2243115 GG (GG versus TT: OR = 4.81, 95% CI 1.33–17.36, $P = 0.017$; and GG versus TG + TT: OR = 4.55, 95% CI 1.27–16.36, $P = 0.020$) genotype was evident among rheumatoid factor (RF) negative patients, and with the IL-12B rs3212227 AC (AC versus AA) and AC + CC (AC + CC versus AA) genotypes were evident among older patients (OR = 1.48, 95% CI 1.06–2.06, $P = 0.020$), RF positive patients (OR = 1.35, 95% CI 1.04–1.75, $P = 0.026$) and anti-cyclic citrullinated peptide antibodies (ACPA) negative patients (OR = 1.53, 95% CI 1.11–2.10, $P = 0.009$). The plasma level of IL-12 was significantly higher in RA patients ($P < 0.001$). IL-12 plasma level of IL-12A rs2243115 TT ($P < 0.001$) and IL-12B rs3212227 C allele ($P < 0.001$) were significantly higher in RA patients than controls respectively. The plasma level of IL-12 of RF positive RA patients was significantly higher than RF negative patients ($P = 0.008$), especially in rs3212227 AC patients ($P = 0.01$).
Conclusions: These findings suggested that the functional single nucleotide polymorphism (SNP) IL-12A rs2243115 GG genotype may increase the risk of RA in RF negative patients, and the IL-12B rs3212227 AC and AC + CC genotypes are associated with RA risk in older patients, RF positive patients and ACPA negative patients. The IL-12A rs2243115 T/G and IL-12B rs3212227 A/C allele might also impact the inflammatory reaction of IL-12 in patients with RA.

1. Introduction

Rheumatoid arthritis (RA) is characterized by synovitis, progressive erosions, and cartilage destruction [1]. Although the etiology and mechanisms of RA is not clear, it is considered that genetic factors such as single nucleotide polymorphisms (SNPs) play important roles in RA pathogenesis [2,3–5]. Genetic variation is an important factor causing RA. The risk of RA can be influenced by genetic variation in the inflammatory agents, including microRNA-146a [3], CYB5A [4], Interleukin-17A (IL-17A) [6], IL-23 receptor [7], and IL-6 [8].

IL-12 is expressed by infiltrating macrophages and synovial lining cells in rheumatoid arthritis [9]. It is a proinflammatory cytokine produced by different antigen presenting cells. IL-12 has been shown to exert a critical role in inducing T helper 1 (Th1) phenotype, thus initiating cell-mediated immune responses [10], and plays a central role in promoting the differentiation of naive CD4+ T cells into mature interferon-γ (IFN-γ) producing Th1 effector cells [11]. It is also a potent stimulus of natural killer and CD8+ T cells to produce IFN-γ [12]. In response to microbial stimulation, IL-12 can be produced primarily by activated dendritic cells and macrophages

Abbreviations: CI, confidence interval; IL-12, interleukin 12; LD, linkage disequilibrium; OR, odds ratio; SNP, single nucleotide polymorphism..

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[13]. McIntyre et al. showed that reduced incidence and severity of collagen-induced arthritis (CIA) in interleukin-12-deficient mice [14]. The levels of IL-12 in synovium fluid from RA patients were higher than those in sera from both RA patients and healthy individuals [15]. A research demonstrated that IL-12 levels reflect RA disease activity and are involved in the production of proinflammatory cytokines. An IL-12 blockade could be useful for the treatment of RA [16]. The IL-12 family members are heterodimeric cytokines that share subunits and have important roles in autoimmunity. In RA, leukocyte migration, bone erosions and angiogenesis are modulated by an IL-23–IL-17 cascade, which can be negated in part by IL-12 function. However, the IL-12 family members are a relatively new area of research and data have been generated mostly at the preclinical stage [17]. IL-12A gene is located on Chromosome 3 while IL-12B gene is located on Chromosome 5. The associations between IL-12 gene SNPs and diseases have been reported recently. Some researchers studied SNPs of IL-12A rs2243115 T/G and IL-12B rs3212227 A/C. They found that polymorphisms of IL-12B rs3212227 A/C, but not IL-12A rs2243115 T/G, might be a potential biomarker for gastric cardiac adenocarcinoma [18], cervical [19] and nasopharyngeal [20] cancers among Asian populations. Today, a causal relationship between inflammation and innate immunity of cancer is more widely accepted [21]. Researchers studied the association between rs3212227 A/C SNPs in RA patients and controls. They found lack of association of the IL-12B 3'-untranslated region (UTR) SNP, rs3212227, with RA in case-control studies of Greeks, British and Spanish [22–24]. In a recent study, Chang et al. also found lack of association between rs3212227 and RA in American white subjects [13]. No association of IL-12A rs2243115 T/G SNPs and RA risk has been studied till now.

Genetic polymorphisms often vary between ethnic groups. Functional variations in IL-12 gene may contribute to the development with RA etiology, and act as risk or protect factors of RA in other ethnic groups. We therefore undertook genotyping in a hospital-based case-control study in a cohort of 615 patients with RA and 839 controls, aiming to examine the association between polymorphisms of IL-12 and RA associated biomarkers in a Chinese population.

2. Materials and methods

2.1. Study subjects

We obtained approval of the study protocol from the Ethics Committee of Nanjing Medical University (Nanjing, China). All patients provided written informed consent to be included in the study.

Six hundred and fifteen RA patients who fulfilled the criteria for RA set by the American College of Rheumatology classification in 1987 [25] 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; most of the controls were admitted to the hospitals for the treatment of trauma.

To obtain information on demographic data and related risk factors for RA, each patient was interviewed by trained personnel using a pre-tested questionnaire. After the interview, from each subject, 2 mL of peripheral blood was collected.

Blood samples were collected using vacutainers and transferred to test tubes containing ethylenediamine tetra-acetic acid (EDTA). Using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The SNP genotyping work was performed using a custom-by-design 48-Plex SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described [26].

The blood level of IL-12 in 90 RA patients and 90 randomly selected controls was measured using an enzyme-linked immuno-sorbent assay kit (ELISA) (Boster, Wuhan, China). All analytical steps were performed in accordance with the manufacturer's recommendations. The concentration of IL-12 was calculated by referring to a standard curve, according to the manufacturer's instructions. Clinical data with potential diagnostic value, such as age, sex, age at onset, disease duration, treatment duration, RA disease activity score (DAS28), Functional class, levels of rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (ACPA), C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR), were provided by the physicians. In all cases, values above 20 IU/mL were considered positive for RF, values above 10 mg/L were considered positive for CRP, values above 10 mg/L were considered positive for CRP, values above 25 mm/60 min were considered positive for ESR, and values above 3.0 RU/mL were considered positive for ACPA.

2.2. Statistical analyses

Differences in demographics, variables, and genotypes of the IL-12A rs2243115 T/G and IL-12B rs3212227 A/C polymorphism variants were evaluated using a chi-squared test. The associations between IL-12A rs2243115 T/G and IL-12B rs3212227 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) was tested by a goodness-of-fit chi-squared test to compare the observed genotype frequencies to the expected frequencies among controls. Differences in IL-12 polymorphism and IL-12 blood levels were evaluated using the Student's *t*-test. All statistical analyses were done with SAS software (version 9.1.3; SAS Institute, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

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 age and sex ($P = 0.170$ and 0.566, respectively). The genotype distributions of IL-12A rs2243115 T/G and IL-12B rs3212227 A/C in all subjects are illustrated in Table 2. The observed genotype frequencies for the polymorphisms in controls were in HWE for IL-12A rs2243115 T/G ($P = 0.500$), IL-12B rs3212227 A/C ($P = 0.217$).

3.2. Associations between IL-12A rs2243115 T/G and IL-12B rs3212227 A/C polymorphisms and the risk of RA

Logistic regression analyses revealed that IL-12A rs2243115 T/G and *IL-12B* rs3212227 A/C polymorphisms were not associated with the risk for RA (Table 2).

3.3. Stratification analyses of IL-12A rs2243115 T/G and IL-12B rs3212227 A/C polymorphisms and the risk for RA

Stratification analyses were done according to age, sex, CRPstatus, RF, ESR, DAS28, functional class and ACPA status (tables were not provided in this article). After stratification of IL-12A rs2243115 T/G, logistic regression analyses revealed that the significantly increased risk for RA associated with the IL-12A rs2243115 GG genotype was evident among RF-negative patients (GG versus TT: OR = 4.81, 95% CI 1.33–17.36, $P = 0.017$; GG versus