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Lack of association between rheumatoid arthritis and genetic variants rs10889677, rs11209026 and rs2201841 of IL-23R gene

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is an autoimmune diseases, where different genetic variants in cytokine genes may play a pathogenic role. A GWAS in autoimmune diseases highlighted the IL-23R gene as a one of the susceptibility factors. We examined three candidate single nucleotide polymorphisms (SNPs) rs10889677, rs11209026 and rs2201841 of the IL-23R gene, as well as determined their possible association with RA in a Polish population.
Patients and methods: The IL-23R gene polymorphisms were genotyped for 422 RA patients and 348 healthy individuals using TaqMan SNP genotyping assay.
Results: The genotypes frequency did not deviate from HWE in each examined group. A comparison of the allele as well as genotype frequencies of the IL-23R polymorphisms under codominant, dominant and recessive genetic model revealed no significant differences between RA patients and healthy subjects. We also demonstrated that IL-23R rs2201841 and rs11209026 as well as rs11209026 and rs10889677 were in complete linkage disequilibrium ($D' = 1.0$). Our genotype-phenotype analysis demonstrated that in carriers of rs10889677C and/or rs2201841A allele the RF, extra-articular manifestations and erosion were more frequent present than in patients with rs10889677A and/or rs2201841A allele, although this association was not significant.
Discussion: Present findings indicated that the autoimmune disease-associated genetic variants in IL-23R gene are not associated with RA in the Polish population.

Falta de asociación entre la artritis reumatoide y los polimorfismos genéticos rs10889677, rs11209026 y rs2201841 en el gen IL-23R

Palabras clave:
Artritis reumatoide
Polimorfismos del gen de las citocinas
Interleucina
IL-23R
Patogenia

RESUMEN

Introducción: La artritis reumatoide (AR) es una enfermedad autoinmune en la que las diferentes variantes genéticas en los genes de las citocinas pueden desempeñar un papel patogénico. Un GWAS de enfermedades autoinmunes destacó al gen IL-23R como uno de los factores de susceptibilidad. Examinamos tres polimorfismos de nucleótido único candidatos (SNP), rs10889677, rs11209026 y rs2201841 del gen IL-23R, y determinamos su posible asociación con AR en una población de Polonia.
Pacientes y métodos: Se genotipificaron los polimorfismos del gen IL-23R en 422 pacientes de AR y 348 individuos sanos, utilizando la prueba TaqMan de genotipificación de SNP.
Resultados: La frecuencia genotípica no se desvió de HWE en cada grupo examinado. La comparación del alelo, así como las frecuencias genotípicas de los polimorfismos de IL-23R con arreglo al modelo genético

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codomitante, dominante y recesivo no reveló diferencias significativas entre los pacientes de AR y los sujetos sanos. Demostramos también que rs2201841 y rs11209026 de IL-23R, al igual que rs11209026 y rs10889677, se hallaban en desequilibrio completo de ligamiento (D' =1). Nuestro análisis genotipo-fenotipo demostró que en portadores del alelo rs10889677C y/o rs2201841A eran más frecuentes el FR, las manifestaciones extra-articulares y la erosión que en los pacientes portadores del alelo rs10889677A y/o rs2201841A, aunque esta asociación no fue significativa.
Discusión: Los hallazgos presentes demostraron que las variantes genéticas asociadas a las enfermedades autoinmunes en el gen IL-23R no están asociadas a AR en la población polaca.

Introduction

Rheumatoid arthritis (RA) is an complex immune-inflammatory disease characterized by an inappropriate T-cell response leading to joint inflammation, destruction of articular cartilage/bone and synovial hyperplasia.[1,2] Growing evidence indicates that genetic susceptibility plays a key role in the susceptibility to as well as severity of the disease; however, more than 10 years after the completion of the human genome sequencing project and several genome-wide association studies (GWAS), we still do not fully understand the genetic basis of RA.[3] GWAS have exposed more than 100 genetic loci associated with RA with modest effect size, which can explain a small proportion of the RA heritability.[4] Therefore, further case-control studies to find the genetic basis of RA are needed, because genetic factors not only play an important role in the pathogenesis of the RA, but they may also serve as a prognostic tool in the treatment of disease.

Although the precise pathogenesis of RA remains unclear, the current state of knowledge leads to the conclusion that the disease is at least partially driven by T cells, which are important in the modulating of the inflammatory process.[5,6] Among all of the T cells involved in the pathogenesis of RA, T helper (Th) 17 cells are the dominant players in the induction of the autoimmune inflammation.[5,7] Th17 cells current in the rheumatoid joint may generate a positive feedback loop performing to the continuous activation of T cells that is a critical step in the generation of autoimmunity.[8] The expansion of human Th17 cells is maintain by interleukin (IL)-23, which is a central player in the T cell-dependent inflammation.[9] The IL-23-IL-17 axis may play a very important role in the inflammatory processes in rheumatoid joints. Moreover, this axis is not only essential for the onset phase of RA, but also for the destruction phase.[1,10] Furthermore, IL-23 activity is mediated by the IL-23 receptor (IL-23R), which is a heterodimer formed by the products of two different genes such as IL-12Rβ1 and IL-23R, located on chromosome 1 (1p31.2-32.1).[9,11]

A GWAS in autoimmune diseases highlighted the IL-23R gene as a one of the susceptibility factors.[12,13] To test this hypothesis, we examined three candidate single nucleotide polymorphisms (SNPs) in the IL-23R gene, rs10889677, rs11209026 and rs2201841, as well as determined their possible association with RA in a Polish population using a case-control approach.

Materials and Methods

Study group

In total, 422 RA patients (340 female, 82 male, mean age 57.5 ± 12.5 years) and 338 unrelated healthy controls (261 female, 77 male, mean age 60.6 ± 15.4 years) without history of immunological diseases were included in this study. Both RA patients and healthy controls were from the same geographical area and they had the same socioeconomic status and ethnicity. The demographical and clinical characteristics of RA patients are presented in Table 1. For the identification part of our study, we used two Polish cohorts. RA patients were recruited from the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw and Pomeranian Medical University in Szczecin, Poland. All RA patients had been diagnosed with RA according to the criteria of the American College of Rheumatology/European League against Rheumatism.[14] Disease activity was determined on the basis of DAS28 score. Those patients with DAS28 ≤ 2.5 were classified as subjects in remission of disease symptoms, while those with DAS28 > 2.5 were classified as subjects with an active form of RA.[15,16] All RA patients included in the study were treated with a regimen of oral methotrexate at a dosage from 7.5 mg to 25 mg weekly and glucocorticosteroids.

Our study was approved by the Research Ethics Committee of the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw and of the Pomeranian Medical University in Szczecin. All RA patients and healthy subjects gave written informed consent, obtained according to the Declaration of Helsinki as revised in 2000.

Genotyping

Genomic DNA was extracted from whole blood collected in EDTA tubes using the standard isothiocyanate guanidine extraction method and/or the QIAamp DNA Blood Mini Kit (Qiagen). DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. To the identify the all examined IL-23R gene polymorphisms we used the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA): C_11283764_10 (rs10889677), C___1272298_10 (rs11209026) and C___1272302_10 (rs2201841). The reaction was performed in 10ul volume on StepOne Real-Time PCR system in Quant Studio 5 Real-Time machine (Applied Biosystems, Foster City, CA, USA) with the fallowing amplification protocol: denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s, and annealing and extension at 60 °C for 1 min. The genotypes obtained in this study were subsequently validated and confirmed by sequencing the PCR products using an ABI PRISM Sequencer (Applied Biosystems).

Table 1
Clinical and demographic parameters of patients with rheumatoid arthritis (RA).

Parameter	RA (n = 422)
Sex [F/M]	340/82
Age (years) [mean ± SD]	57.47 ± 12.45
Disease duration [mean ± SD]	10.07 ± 8.32
Age at disease onset [mean ± SD]	47.40 ± 13.22
Rheumatoid factor [positive]	75.36%
Erosive RA	80.09%
Extra-articular manifestations (ExA)	17.06%
DAS28 [mean ± SD]	3.45 ± 2.39

N: number of patients; F: number of females; M: number of males.

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