Meta Gene 18 (2018) 149–152

Contents lists available at ScienceDirect

Meta Gene

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

IL27 gene single nucleotide polymorphisms confer susceptibility to rheumatoid arthritis in Iranian population

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ARTICLE INFO

Keywords:
Rheumatoid arthritis
Polymorphism
Interleukin 27

ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune inflammatory disease where it seems that different polymorphisms in cytokine genes are involved in the pathological process of this diseases. Interleukin 27 (IL-27) is a cytokine, which different studies have shown that it is similar to the double-edged sword, it means that it can play an anti- or pro-inflammatory role in chronic inflammatory diseases. The aim of this study is to identify the role of polymorphism in the *IL27* gene and its possible association with susceptibility to RA.
Methods: Seven hundred patients with RA and 700 healthy individuals were genotyped for *IL27* gene rs181206 polymorphism using PCR-RFLP method.
Result: Frequencies of the CT and CC + CT genotypes and the C allele were statistically higher in RA patients compared with the healthy control group ($P = 0.012$, $P = 0.007$ and $P = 0.011$ respectively). In RA patients, clinical manifestations, such as ESR ($P = 0.697$), CRP ($P = 0.664$), RF ($P = 0.852$) and Anti-CCP ($P = 0.968$) did not show any significant difference between different rs181206 genotypes.
Conclusion: Present findings indicated that *IL27* rs181206 polymorphism was involved in susceptibility to RA in the Iranian population.

1. Introduction

Rheumatoid arthritis (RA) is a complex heterogeneous chronic, inflammatory, autoimmune disease that most importantly affects the joints. RA is characterized by erosion of joints, symmetrical polyarthritis, and the systemic extra-articular advent, affecting approximately 0.5% to 1% of the adult population worldwide (Li et al., 2014; Smolen et al., 2018; Trajkov et al., 2009). The etiology is still obscure but it seems that RA, as a multifactorial disease, is caused by an interplay between environmental and genetic factors (Paradowska & Łącki, 2018). The influence of genetic contributing factors to RA susceptibility has been shown in familial studies and genome-wide linkage scans (Pope & Shahrara, 2013a). Hence, more knowledge about the genetic root of RA would be useful to explain the pathogenesis of the destructive process of the disease as well as RA prognosis.

Previous studies have demonstrated that there are different kind of genes that can lead to susceptibility to RA (Korczowska, 2014). Among the genes involved in the immunopathogenesis of RA, genetic polymorphism of cytokines may be particularly important in the onset and progression of the disease (Soroka et al., 2010). The interleukin (IL)-12 family members, such as IL-12, IL-23, IL-27, and IL-35 are type I cytokines that have substantial roles in determining differentiation of T-cell (Pope & Shahrara, 2013a). IL-27 through activation of signal transducer and activator of transcription 1 (STAT1), finally resulting in enhancement of interferon (IFN)-γ production, promotes the initial step of T Helper (TH)-1 differentiation (Pope & Shahrara, 2013a). In proteoglycan-induced arthritis (PGIA), a TH1-mediated arthritis model, IL-27 Receptor (IL-27R) - knockout mice developed less severe arthritis in comparison to wild-type mice as a result of a reduced number of IFN-γ producing T-cells (Cao et al., 2008).

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people (Syvanen, 2001). Previous studies have shown that *IL27* polymorphisms were associated with susceptibility to some autoimmune-based diseases like systemic lupus

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https://doi.org/10.1016/j.mgene.2018.09.002
Received 14 June 2018; Received in revised form 4 September 2018; Accepted 5 September 2018

erythematous (SLE) (Paradowska-Gorycka et al., 2016) and inflammatory bowel disease (Trajkov et al., 2009). When we put all the pieces of the puzzle together, *IL27* gene in different populations may be an intriguing candidate for susceptibility to RA.

Although the association of *IL27* gene polymorphisms with RA has been studied by some other researchers (Paradowska-Gorycka et al., 2014; Yan et al., 2015), its relation with RA in Iranian population remains unknown. In this study, we enrolled 700 Iranian RA patients and 700 healthy volunteers to explore the role of *IL27* gene rs181206 SNP in susceptibility to RA.

2. Material and method

2.1. Patients and controls

In this study, 1400 individuals were included for genotyping. The case group contained 700 RA patients, who had no history of other autoimmune or rheumatologic disorders. All patients were selected from outpatient rheumatology clinic of Shariati hospital. Moreover, 700 age and sex matched healthy volunteers were also recruited. All participants fulfilled informed consent with complete satisfaction. The study was approved by the Ethics Committee of Tehran University of Medical Sciences. About 5 ml of peripheral blood was collected from each individual in EDTA-anticoagulated tubes using venipuncture.

2.2. RFLP genotyping

The phenol-chloroform method used for extraction of genomic DNA from whole blood. The storage temperature for extracted DNA was −20 °c. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for determining of allele and genotype frequencies of *IL27* gene rs181206 SNP.

PCR-RFLP on *IL27* rs181206 was performed with 100 ng of the genomic DNA in 36 μl reaction volume containing 3.5 μl of 10 pmol/ml forward primer, 3.5 μl of 10 pmol/ml reverse primer, 16.5μl of 10× PCR Master mix, and 6.5 μl of water. The primers used for amplification of the rs181206 containing fragment were forward 5′- GCTTCAGCCCTTCCATGCCC-3′ and revers 5′-TCTACCTGGAAGCGGAGGTGCC-3′. PCR was performed under the following conditions: initial denaturation at 94 °C 1.5 min followed by 34 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s, and extension at 72 °C for 30s. A final extension at 72 °C for 5 min was carried out. The 132 bp PCR product was digested with 0.25 μl of FuaI R0651 (New England Biolabs, USA) at 55 °C for one hour and was resolved on a 4% agarose gel with Gel Green DNA Staining (Fig. 1).

Through digestion pattern of the product, the genotype of each sample was assigned as below: 132 bp product implied for T allele and 112 and 20 bp for C allele; in case of one single 132 bp product, the genotype was determined as TT, two 112 and 20 bp products demonstrated homozygous CC genotype, and finally three different bands of 132, 112, and 20 bp implied to heterozygous CT genotype.

Table 1
Demographic and Clinical data of RA patients and healthy controls.

Characteristic	RA patients (N = 700) N (%)	Healthy controls (N = 700) N (%)
Gender		
Male	156 (22.2%)	94 (13.4%)
Female	544 (77.8%)	606 (86.6)
Smoking		
Positive	–	34 (4.8%)
Negative	–	664 (95.2%)
CRP		
Positive	322 (46%)	428 (61.1%)
Negative	378 (54%)	272 (38.9%)
ESR	20.64 ± 17.14	10.27 ± 7.95
Age	50.32 ± 11.27	48.44 ± 12.37
RF	85.5 ± 7.63	–
Anti-CCP	138.7 ± 18.96	–

RA; rheumatoid arthritis, ESR; erythrocyte sedimentation rate, RF; rheumatoid factor, Anti-CCP; anti- cyclic citrullinated peptide, CRP; C-reactive protein.

2.3. Statistical analysis

SPSS version 22 (SPSS Inc., Chicago, IL, USA) was applied for data analysis. Through Pearson's chi-square test, the polymorphism association with the disease risk was assessed. Moreover, odds ratio (OR) with 95% confidence intervals (95% CI) was calculated. Genotype frequency in control group were evaluated for deviation from Hardy-Weinberg equilibrium (HWE). Clinicopathological data were also evaluated in association with the genotypes of the SNP using analysis of variance (ANOVA). A *P* less than 0.05 was regarded as statistically significant level.

3. Result

In this study 700 Iranian patients with RA (F/M: 544/156, mean age 50.32 ± 11.27 years,) and 700 healthy control individuals (F/M: 606/94, mean age 48.44 ± 12.37 years) were included (Table 1). Genotype and allelic frequencies of the *IL27* gene rs181206 SNP, are shown in Table 2.

Deviation from Hardy-Weinberg equilibrium was not found in the matter of genotypes distribution in the healthy control group (P = 0.05).

For *IL27* gene rs181206 SNP, the T allele was considered as the reference allele because it was more frequent, and minor allele

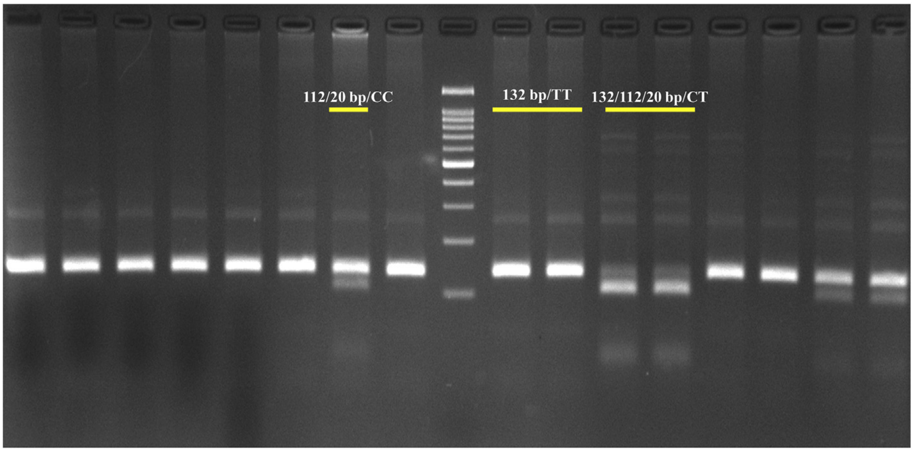

Fig. 1. Digestion pattern of the amplification of *IL27* gene rs181206 SNP product by FuaI R0651, the genotype of each sample was assigned as below: the fragment size for T allele is 132 bp and digested 112 and 20 bp bands for C allele; and the product with single 132 bp length, shows the TT genotype, and two 112 and 20 bp products demonstrates homozygous CC genotype, and finally three different bands of 132, 112, and 20 bp implied to heterozygous CT genotype.