Human Immunology 78 (2017) 547–552

Contents lists available at ScienceDirect

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

Copy number variations of the IL-22 gene are associated with ankylosing spondylitis: A case-control study in Chinese Han population

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ARTICLE INFO

Article history:
Received 13 April 2017
Revised 28 June 2017
Accepted 11 July 2017
Available online 14 July 2017

Keywords:
Ankylosing spondylitis
IL-22
Gene
Copy number variation
CNV

ABSTRACT

IL-22 provides a new insight into the mechanisms of autoimmunity, and copy number variations (CNVs) are associated with autoimmune diseases. This study aims to explore the association of IL-22 gene CNVs with ankylosing spondylitis (AS) susceptibility. The copy numbers of IL-22 gene (2 fragments: IL-22_1, IL-22_2) were examined by AccuCopy™ methods in a cohort of 649 AS patients and 628 controls. Association of IL-22 CNVs and AS susceptibility was analyzed, and AS risk was estimated by Odds Ratio (ORs) and 95% confidence intervals (CIs), and the Benjamini-Hochberg method was applied to regulate the false discovery rate (FDR). We found one copy of IL-22 gene was significantly associated with AS [OR = 0.345, 95%CI (0.144, 0.827), $P = 0.013$, $P_{FDR} = 0.026$] in the IL-22_2 fragment, and this association still exist after adjustment of age and sex [OR = 0.344, 95%CI (0.143, 0.825), $P = 0.017$, $P_{FDR} = 0.034$]. In the stratification analysis by gender, the statistical difference was detected in males in the IL-22_2 fragment [OR = 0.306, 95%CI (0.121, 0.778), $P = 0.009$, $P_{FDR} = 0.018$; adjusted OR = 0.306, 95%CI (0.120, 0.777), $P = 0.013$, $P_{FDR} = 0.026$]. We suggest that IL-22 CNVs are associated with AS and that lower copy number might be a protective factor for AS, especially in male patients.

1. Introduction

Ankylosing spondylitis (AS) is a complex inflammatory autoimmune disease. AS mainly invades the axial and sacroiliac joints, but neither exact pathogenesis nor effective treatments are available for it. Therefore, it is essential to explore the underlying mechanisms of AS. In the past few decades, numerous studies suggested that immunologic factors and genetic predispositions play vital roles in the development and progression of AS [1].

Interleukin-22 (IL-22), belongs together with IL-10, IL-19, IL-20, IL-24 and IL-26, is a member of IL-10 cytokine family that is mainly from activated T cells of Th17 and Th22 subsets [2]. IL-22 controls tissue responses to inflammation through the activation of proliferation and inhibition of apoptosis. Previous research has found that the expression levels of IL-22 and the number of IL-22 producing NKp44 NK cells are elevated in the gut of AS patients [3], and another study suggested that the increasing percentages of Th22 cells and the elevation of plasma IL-22 levels may be involved in the pathogenesis of AS [4]. Nevertheless, the major causes of AS heritability remain to be identified. In genetic research of AS, the central aim is to understand the inherited basis of phenotypic variation in human populations and to clarify the development and progression of disease [5,6]. Single nucleotide polymorphisms

Abbreviations: AS, ankylosing spondylitis; BH, Benjamini-Hochberg; BASFI, Bath Ankylosing Spondylitis Functional Index; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CNV, copy number variation; CNP, copy number polymorphism; CI, confidence intervals; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ELISA, enzyme linked immunosorbent assay; FDR, false discovery rate; IL-22, Interleukin-22; IQR, interquartile range; OR, odds ratios; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; SLE, systemic lupus erythematosus; SD, standard deviation.

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http://dx.doi.org/10.1016/j.humimm.2017.07.006
0198-8859/

(SNPs) have been widely used in the research of complex diseases. IL-22 gene is located on chromosome 12q15 and contains five exons, and its variations are associated with various autoimmune diseases. For example, SNP of IL-22-429C/T is a risk factor of ulcerative colitis [7]. This finding has been facilitated by validation of millions of SNPs by the International HapMap project [8]. In the last few years, a DNA structure variation known as copy number variation (CNV), in which a DNA segment that is one kb or larger in size presents a variable copy number in comparison with a reference genome [9], has attracted much attention because of its potential in the pathogenesis of autoimmune diseases. In 2006, Redon and colleagues found 1447 CNV regions by constructing a CNV map of the human genome and the CNV regions encompassed hundreds of genes, disease-associated loci, functional elements and segmental duplications. More importantly, these CNV regions contain more nucleotide content per genome than SNPs do, making CNV important in genetic diversity and evolution [10].

CNVs, also known as copy number polymorphisms (CNPs), are widespread human genomic regions and are a significant source of genetic variation accounting for various diseases [11,12] Recently, a genome-wide association study in AS has identified deletion CNVs in nine regions. Among these, five regions (1q32.2, 2q31.2, 6p21.32, 13q13.1 and 16p13.3) were related to an increased risk and four regions (1p34.2, 11q22.1, 14q24.2 and 22q11.1) played a protective role in AS susceptibility [13]. Another study revealed that aberrant copy numbers of the HLA-DQA1 gene were significantly associated with AS susceptibility [14]. Additionally, our previous studies also found that DEFB4 gene CNVs were associated with both susceptibility and progression of AS [15], and that a lower copy number ($\leq$3) of FCGR3A and FCGR3B genes was a risk factor of AS [16]. These results contributed to a better understanding of the pathogeny of AS and prompted us to further research gene CNVs. The associations between IL-22 gene CNVs and autoimmune diseases have also been reported. For example, the mRNA expression of IL-22 gene in systemic lupus erythematosus (SLE) patients was significantly higher in patients with >2 copies of IL-22 gene than in those with 2 copies [11]. Moreover, another study suggested that genetic variations in IL-22 copy numbers contribute to the risk of early-onset psoriasis vulgaris (PsV) [2]. AS is an immune-related disease that shares some genetic overlaps and pathogenic mechanisms with SLE and PsV [17]. However, the effect of IL-22 gene CNVs on AS has not been explored yet. Combining the evidence that plasma IL-22 levels are elevated in AS [4] and IL-22 CNVs can influence IL-22 expression [11], we hypothesized that the increased IL-22 expression in AS is caused by IL-22 CNVs. Thus, we aimed to clarify the relationship between IL-22 gene CNVs and AS susceptibility in Chinese Han population using a case-control study.

2. Materials and methods

2.1. Subjects

A total of 689 unrelated AS patients and 668 unrelated healthy controls matched by age, gender and ethnicity were enrolled in this study. Of these 1357 participants, 1277 (649 patients and 628 controls) were evaluated for IL-22 gene CNV, and 80 (40 patients and 40 controls) were evaluated for serum IL-22 related cytokines levels. AS patients were collected from the First Affiliated Hospital of Anhui Medical University, and healthy volunteers were recruited from the local community hospitals. The diagnosis of AS has been made by qualified rheumatologists according to the modified New York criteria [18]. Written informed consent was signed by each participant after a verbal explanation of the study. This research was approved by the ethics committee of Anhui Medical University. According to manufacturer's instructions, DNA samples were extracted from the peripheral venous blood of AS patients and healthy controls, and were stored at minus 20 °C before CNV genotypes detection. All patients were requested to fill out a questionnaire, and all procedures performed in this study were in accordance with the Declaration of Helsinki.

2.2. Determination of IL-22 copy numbers

We designed two probes to examine the copy numbers of the first (IL-22_2 fragment) and last (IL-22_1 fragment) exons of the IL22 gene, and these CNVs were measured using Multiplex AccuCopy™ method (Genesky Biotechnologies Inc., Shanghai, China) which is based on a multiplex fluorescence competitive polymerase chain reaction (PCR) assay [19]. PCR primer sequences are provided in Table 1 and the genetic structure of the IL-22 gene is presented in Fig. 1. PCR condition was same as previously described [15].

2.3. Measurement of serum IL-22 related cytokines levels

Our previous study found that the serum IL-17 and IL-23 levels were significantly elevated in AS patients [20]. In consideration of the correlation between IL-17, IL-22 and IL-23 cytokines, four IL-22-related cytokines (IL-10, IL-15, TGF-β and TNF-α) serum levels were further measured using Enzyme Linked Immunosorbent Assay (ELISA) kits according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA), and the plasma levels of IL-10, IL-15, TGF-β and TNF-α were calculated by referring to a standard curve.

2.4. Statistical analysis

Data were described as either mean ± standard deviation (SD) or median (interquartile range, IQR) based on the distribution of data. The differences in age and gender between two groups were compared using independent-samples *t*-test and Pearson's chi-square test, respectively. All subjects were divided into different groups according to the IL-22 copy numbers. The distribution of the IL-22 gene CNVs between patients and controls were estimated using Pearson's chi-square test (or Fisher exact test) and unconditional logistic regression model. Associations between IL-22 CNVs and clinical indicators of AS in the patients group, including Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), were compared using a linear regression model. The differences of serum levels of IL-22-related cytokines among different copies of IL-22 gene were analyzed using Mann-Whitney *U* test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated, and *P*-values less than 0.05 (two-side) were considered as statistically significant. Considering the multiple comparisons, Benjamini-Hochberg (BH) method was applied to regulate the false discovery rate (FDR) [21]. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL) software.

3. Results

3.1. Clinical and demographic characteristics

A total of 649 AS patients (the ratio of males to females is 5.01:1) and 628 healthy controls (ratio: 4.74:1) were included in this study. The mean age ± standard deviation was 28.25 ± 8.89 in patients and 27.83 ± 7.58 in controls. There were no statistically significant differences in age ($t = 0.883$, $P = 0.377$) and gender ($\chi^2 = 0.135$, $P = 0.713$) between the two groups. Of the 649 patients, the HLA-B27 positive rate was 93.8% and the results of ESR, CRP, BASFI and BASDAI were reported as median and interquartile range (Table 2).