Ankylosing spondylitis: analysis of gene-gene interactions between $\text{IL-12}\beta$, $\text{JAK2}$, and $\text{STAT3}$ in Han Chinese and Algerian cohorts

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Abstract

Introduction: Association studies have recently identified the importance of new genetic variants for ankylosing spondylitis (AS) in several populations. Our aim was to confirm associations of variants within genes involved in the IL-23 signalling pathway with AS in two ethnically different populations: Han Chinese and Algerian.

Material and methods: Two case-control studies were performed in separate cohorts: Han Chinese (430 AS patients and 580 controls) and Algerian (130 AS patients and 120 controls). We genotyped four single nucleotide polymorphisms (SNPs): rs3212227 (or +1188A/C) and rs6887695 in IL-12$\beta$, rs7857730 in JAK2, and rs2293152 in STAT3, using TaqMan SNP genotyping assays. Gene-gene interaction analyses were also tested by logistic regression and multifactor dimensionality reduction (MDR).

Results: Statistical analysis revealed a difference in allele frequencies between AS patients and controls for rs321222 in the IL-12$\beta$ gene in both the Han Chinese ($p = 0.005$) and the Algerian ($p = 0.031$) cohorts. Two other associations were reported with JAK2 rs7857730 in the Han Chinese (allelic $p = 0.014$) and STAT3 rs2293152 in the Algerian (allelic $p = 0.006$) cohort. Moreover, logistic regression analyses showed a number of significant combinations within the two populations, and the gene-gene epistasis effects in AS were also confirmed by MDR.

Conclusions: Our findings have confirmed the association between genes in IL-23 signalling pathway and the pathogenesis of AS. This association was particularly novel in both Han Chinese and Algerian populations with the 3' untranslated region (3'UTR) variant rs3212227 (or +1188A/C) of IL-12$\beta$. The gene-gene interaction models in this pathway may thus increase the risk of AS in these populations.

Key words: ankylosing spondylitis, interleukin-23 signalling pathway, polymorphisms, gene-gene interaction.
factors encoded by non-major-histocompatibility-complex (non-MHC) genes may contribute to this disease [11, 12]. Accordingly, many previous association studies tagging a number of non-MHC candidate genes have identified significant associations with AS in the Chinese population [13-17]. Recently, a genome-wide association study in Caucasian European populations from Australia, North America, and the United Kingdom provided the first evidence that interleukin-23 (IL-23) is involved in the pathogenesis of ankylosing spondylitis, and variants in several genes involved in the IL-23 pro-inflammatory cytokine pathway have been shown to be associated with the disease [18].

Interleukin-23 and interleukin-12 are critical cytokines that bridge innate and adaptive immunities, although they have different activities. IL-23 promotes a T-cell population characterised by the production of IL-17, IL-17F, TNF, and IL-6 [19]. IL-12β is on chromosome 5q31 and encodes the IL-12 p40 subunit that heterodimerises with the IL-12 p35 subunit (IL-12A gene) to form IL-12 cytokine, or with the IL-23 p19 subunit (IL-23A gene) to form IL-23 cytokine. IL-12pβ is important for the differentiation of naive T cells into IFN-γ-producing T-helper 1 cells (Th1), which is essential for the antimicrobial response [20], and this could explain its role in AS and other inflammatory-mediated processes [21]. The functional roles of IL-12β polymorphisms are still unclear, but most of the case-control studies on inflammatory diseases, including ankylosing spondylitis, have analysed one important variant in the IL-12β gene: the 3' untranslated region (3'UTR) variant rs3212227 (or A1188C) [22-24].

The aim of our work was to confirm whether there is any association between ankylosing spondylitis and four SNPs (single-nucleotide polymorphisms) within three genes (IL-12β, JAK2, and STAT3), all involved in the IL-23 signalling pathway, for two ethnically different populations: Han Chinese and Algerian. In addition, we performed for each population a gene-gene interaction analysis to determine whether these four sequence variants could have any substantial effect increasing the incidence of AS.

Material and methods

Subjects

Two different populations were included in this case-control study, Han Chinese and Algerian. Experiments were carried out separately for the two cohorts in the Institute of Medical Genetics of Shandong University. We recruited 1010 unrelated Chinese individuals (430 patients with ankylosing spondylitis and 580 healthy controls) living in the same urban area and collected from the Department of Rheumatology and Immunology of Qilu Hospital in Shandong, an eastern coastal province in the north of China. We also recruited 250 Algerian individuals (130 unrelated AS patients and 120 healthy controls) from EHS Ben Aknoun, a central hospital in Algiers, Algeria.

Ankylosing spondylitis was diagnosed according to the modified 1984 New York criteria [25]. Magnetic resonance imaging (MRI) and computerised tomography (CT) were used as imaging modalities for sacroiliitis. Diagnosis of AS was confirmed by a qualified rheumatologist. All patients were only affected by ankylosing spondylitis, while those displaying other diseases such as inflammatory bowel disease (IBD) or psoriasis were excluded.

All healthy, unrelated controls recruited for this study were matched for age. Detailed information, clinical characteristics, and original medical institution were obtained for all participants, who gave written informed consent. The study was approved by the relevant Ethics Committee.

SNP selection and genotyping

Four single-nucleotide polymorphisms (SNPs) within three genes were selected for the Han Chinese cohort from the international HapMap project database (http://www.hapmap.org/) with the Chinese Beijing Han population (CHB), namely rs3212227 (A1188C) and rs6887695 for the same gene IL-12β, rs7857730 for JAK2, and rs2293152 for STAT3. All these SNPs are involved in the IL-23 signalling pathway (Fig. 1). These four SNPs were also investigated for the first time in an Algerian cohort, since no previous association studies between AS and the three studied genes were available for the Algerian population.

Genomic DNA from all Chinese subjects was extracted from peripheral blood mononuclear cells (PBMCs; leukocytes) by a standard phenol-chloroform method and was
diluted to 20 mg/ml. QIAamp DNA Mini Kit® was used to extract genomic DNA from peripheral blood cells for all Algerian individuals [26].

Genotyping of the two cohorts involved allelic discrimination by TaqMan® SNP Genotyping Assays with an ABI prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), using four pre-made, double-dye probes (C_2084293_10 for rs3212227, C_1994999_10 for rs6887695, C_3140302_10 for rs2293152, and C_29340600_20 for rs7857730). The endpoint fluorescence was read using the Roche 480 Real-Time PCR System (Hoffmann-La Roche Ltd, Basel City, Switzerland) with 96-well plates. The PCR reaction was run in a total solution volume of 5 μl containing 2.5 μl of 2 × c Premix EX Taq (Takara, Otsu, Shiga, Japan), at least 10 ng of genomic DNA, 2.5 μM of each primer, and 0.05 μl of probe. The PCR amplification conditions were 95°C for 30 sec, 45 cycles of 95°C for 5 sec, 60°C for 20 sec, and 40°C for 30 sec for cooling. Genotyping accuracy in the samples was confirmed by direct sequencing of PCR products for 5% of randomly chosen samples. Only those individuals with 100% genotype success for all markers were included for final analysis.

Statistical analysis

Single nucleotide polymorphisms were assessed for genotypic and allelic association analysis among cases and controls, separately for the Chinese and the Algerian cohorts. The genotypic data of the four SNPs were tested for Hardy-Weinberg equilibrium by comparing genotype frequencies within the two groups (AS patients and controls), using the chi-squared test. The odds ratios (ORs) with 95% confidence intervals (95% CIs) were also calculated for single site analysis to estimate the risk associated with ankylosing spondylitis. Statistical analyses were carried out to calculate p-values using SPSS statistical software (SPSS Statistics 16.0; 2008 SPSS Inc., Chicago, IL).

In order to analyse gene-gene interactions, multiplicative interactions were estimated separately for the two cohorts using SPSS 16.0 software for logistic regression analyses. The multiplicative variable model was defined as disease status (1 is unaffected, 2 is affected) and SNP genotypic values ranging from 0 to 2 indicating the number of risk alleles in an individual subject (0 is zero risk allele, 1 is one risk allele, 2 is two risk alleles). For each SNP, random combinations were constructed to estimate a binary logistic regression model based on the case-control status (dependent variable) and the other SNP variables studied (independent variables or covariates).

Because the effect of any single genetic variation was likely to be dependent on other genetic variations, multi-factor dimensionality reduction (MDR) software analysis (http://sourceforge.net/projects/mdr/) was performed to test various interaction models for one-variable, two-variable, three-variable, and four-variable gene-gene epistasis on ankylosing spondylitis. We analysed all data using 10-fold cross-validation.

Results

Association results for the Han Chinese cohort

Genotypic and allelic association analysis of Chinese cases and controls is shown in Table 1. The frequency distributions of the four SNPs within patients and controls were in Hardy-Weinberg equilibrium (p > 0.05). For the Chinese Han cohort, the SNP IL-12β rs3212227 (or A1188C) showed the strongest association with AS (p genotype = 0.007, p allele = 0.005 [OR 0.77, 95% CI: 0.65-0.93]). Another association with AS was also observed for SNP rs7857730 of the JAK2 gene (genotypic p = 0.029 and allelic p = 0.014). The minor allele frequency results revealed that C and T might be protective alleles for rs3212227 and rs7857730, respectively. Thus, subjects carrying A and G alleles showed increased risk of AS for the same variants. However, no significant differences were observed between AS patients and controls for the two other variants (IL-12β rs6887695 and STAT3 rs2293152) within the Han Chinese cohort.

Association results for the Algerian cohort

Association analysis results of Algerian patients and controls are represented in Table 1. All calculated frequencies were in Hardy-Weinberg equilibrium. Two variants showed associations with AS: rs3212227 of IL-12β (allelic p = 0.031) and rs2293152 of STAT3 (allelic p = 0.006). These results might reveal that A and C represent risk alleles for Algerian AS patients within the IL-12β and STAT3 genes, respectively. For variants IL-12β rs6887695 and JAK2 rs7857730, no significant association was found between AS and Algerian individuals.

Multiplicative interaction analysis results for the two cohorts

The analysis of random combinations of two, three, or four SNPs by binary logistic regression model revealed 11 interactions for each cohort, as summarised in Table 2. Significantly different results between Chinese AS patients and controls were shown by the combinations rs3212227 * rs6887695 (p = 0.008), rs6887695 * rs7857730 * rs2293152 (p = 0.006), and rs3212227 * rs6887695 * rs7857730 * rs2293152 (p = 0.045). For Algerian AS patients and controls, only rs3212227 * rs7857730 * rs2293152 was found as a significant combination (p = 0.035).

MDR analysis results and gene-gene epistasis for the two cohorts

Various interaction models were tested by MDR analysis in order to detect any potential interaction effect of
Table 1. Genotypic and allelic association analysis of the four single-nucleotide polymorphisms in the Han Chinese and Algerian ankylosing spondylitis (AS) cohorts

| Cytogenetic location | Gene | SNPs | Function | Genotype/Allele | Chinese cohort | Algerian cohort |
|---------------------|------|------|----------|-----------------|----------------|----------------|
|                     |      |      |          | Number (%)   | Number (%)     | Genotype/Allele | Number (%)   | Number (%)     | OR (95% CI) | p-value | OR (95% CI) | p-value |
| chr5q33             | *IL-12β* | rs3212227 | UTR 3' (A1188C) | CC | 54 (0.125) | 115 (0.198) | 1.00 | CC | 6 (0.046) | 17 (0.142) | 1.00 |
|                     |      |      |          | CA | 217 (0.505) | 280 (0.482) | 0.61 (0.42-0.88) | CA | 54 (0.415) | 48 (0.400) | 0.31 (0.11-0.86) | 0.029 |
|                     |      |      |          | AA | 159 (0.370) | 185 (0.320) | 0.55 (0.37-0.80) | AA | 70 (0.540) | 55 (0.458) | 0.28 (0.10-0.75) | 0.142 |
|                     |      |      |          | C  | 325 (0.378) | 510 (0.440) | 1.00 | C  | 66 (0.254) | 82 (0.342) | 1.00 |
|                     |      |      |          | A  | 535 (0.622) | 650 (0.560) | 0.77 (0.65-0.93) | A  | 194 (0.746) | 158 (0.658) | 0.66 (0.45-0.96) | 0.031 |
| chr5q33             | *IL-12β* | rs6887695 | Promoter | CC | 60 (0.140) | 92 (0.160) | 1.00 | CC | 23 (0.177) | 28 (0.230) | 1.00 |
|                     |      |      |          | CG | 196 (0.460) | 272 (0.470) | 0.91 (0.62-1.31) | CG | 60 (0.462) | 55 (0.460) | 0.75 (0.39-1.46) | 0.472 |
|                     |      |      |          | GG | 168 (0.400) | 216 (0.370) | 0.84 (0.57-1.23) | GG | 47 (0.361) | 37 (0.310) | 0.65 (0.32-1.30) | 0.351 |
|                     |      |      |          | C  | 316 (0.370) | 456 (0.393) | 1.00 | C  | 106 (0.408) | 111 (0.460) | 1.00 |
|                     |      |      |          | G  | 532 (0.630) | 704 (0.607) | 0.92 (0.76-1.10) | G  | 154 (0.592) | 129 (0.540) | 0.80 (0.56-1.14) | 0.216 |
| chr9p24             | *JAK-2* | rs7857730 | Intron | TT | 80 (0.186) | 147 (0.253) | 1.00 | TT | 56 (0.431) | 42 (0.350) | 1.05 (0.48-2.28) | 1.00 |
|                     |      |      |          | TG | 230 (0.535) | 296 (0.510) | 0.70 (0.51-0.97) | TG | 53 (0.408) | 63 (0.525) | 1.66 (0.78-3.55) | 0.176 |
|                     |      |      |          | GG | 120 (0.279) | 137 (0.237) | 0.62 (0.43-0.90) | GG | 56 (0.431) | 42 (0.350) | 1.05 (0.48-2.28) | 1.00 |
|                     |      |      |          | T  | 390 (0.453) | 590 (0.509) | 1.00 | T  | 95 (0.365) | 93 (0.388) | 1.00 |
|                     |      |      |          | G  | 470 (0.547) | 570 (0.491) | 0.99 (0.83-1.18) | G  | 165 (0.635) | 147 (0.612) | 0.91 (0.63-1.31) | 0.610 |
| chr17q21            | *STAT-3* | rs2293152 | Intron | GG | 102 (0.237) | 124 (0.214) | 1.00 | GG | 8 (0.061) | 18 (0.150) | 1.00 |
|                     |      |      |          | GC | 201 (0.468) | 287 (0.495) | 1.17 (0.85-1.61) | GC | 48 (0.370) | 50 (0.417) | 0.46 (0.18-1.16) | 0.025 |
|                     |      |      |          | CC | 127 (0.295) | 169 (0.291) | 1.09 (0.77-1.55) | CC | 74 (0.569) | 52 (0.433) | 0.31 (0.13-0.77) | 0.176 |
|                     |      |      |          | G  | 405 (0.470) | 535 (0.461) | 1.00 | G  | 64 (0.246) | 86 (0.358) | 1.00 |
|                     |      |      |          | C  | 455 (0.530) | 625 (0.539) | 1.04 (0.87-1.24) | C  | 196 (0.754) | 154 (0.642) | 0.58 (0.40-0.86) | 0.006 |

OR – odds ratio, 95% CI: 95% confidence interval
p values < 0.05 are indicated in bold.
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Table 2. Multiplicative interaction analysis reported by logistic regression for the two populations investigated

| Population       | Chinese                      | Algerian                     |
|------------------|------------------------------|------------------------------|
| Interaction      | χ²  | OR (95% CI) | p value | χ²  | OR (95% CI) | p value |
| rs321227 * rs6887695 | 6.930 | 0.783 (0.652-0.939) | 0.008 | 2.143 | 0.722 (0.143-1.117) | 0.143 |
| rs6887695 * rs7857730 | 0.685 | 1.111 (0.865-1.427) | 0.408 | 0.744 | 0.736 (0.367-1.476) | 0.388 |
| rs321227 * rs7857730 | 1.275 | 1.168 (0.892-1.530) | 0.259 | 0.432 | 0.899 (0.653-1.236) | 0.511 |
| rs321227 * rs7857730 | 0.177 | 1.056 (0.819-1.363) | 0.674 | 1.322 | 1.301 (0.831-2.038) | 0.250 |
| rs321227 * rs2293152 | 1.706 | 1.161 (0.928-1.452) | 0.192 | 0.662 | 1.155 (0.816-1.635) | 0.416 |
| rs6887695 * rs2293152 | 0.034 | 1.016 (0.855-1.208) | 0.853 | 2.192 | 1.408 (0.895-2.126) | 0.139 |
| rs321227 * rs6887695 * rs7857730 | 0.937 | 1.119 (0.892-1.403) | 0.333 | 0.624 | 1.205 (0.758-1.916) | 0.430 |
| rs6887695 * rs7857730 * rs2293152 | 7.431 | 0.801 (0.683-0.940) | 0.006 | 0.096 | 0.949 (0.680-1.324) | 0.757 |
| rs321227 * rs6887695 * rs2293152 | 0.490 | 1.064 (0.895-1.264) | 0.484 | 0.490 | 1.064 (0.895-1.264) | 0.484 |
| rs321227 * rs7857730 * rs2293152 | 0.989 | 1.050 (0.954-1.156) | 0.320 | 4.468 | 1.227 (1.015-1.482) | 0.035 |
| rs321227 * rs6887695 * rs7857730 * rs2293152 | 4.005 | 1.136 (1.003-1.287) | 0.045 | 0.023 | 0.981 (0.765-1.258) | 0.880 |

OR – odds ratio, 95% CI: 95% confidence interval  
*p values < 0.05 are indicated in bold

Table 3. Gene-gene interaction model results of MDR for each population

| Model                          | Training accuracy (%) | Testing accuracy (%) | Cross-validation consistency |
|-------------------------------|-----------------------|----------------------|----------------------------|
|                               | Chinese               |                       |                            |
| IL-12β (rs3212227)           | 53.63                 | 53.63                | 10/10                      |
| IL-12β (rs3212227), JAK2 (rs7857730) | 55.51                 | 53.90                | 10/10                      |
| IL-12β (rs3212227), JAK2 (rs7857730), STAT3 (rs2293152) | 58.02                 | 55.52                | 8/10                       |
| IL-12β (rs3212227), IL-12β (rs6887695), JAK2 (rs7857730), STAT3 (rs2293152) | 61.91                 | 54.80                | 10/10                      |
|                               | Algerian              |                       |                            |
| STAT3 (rs2293152)            | 57.68                 | 53.62                | 8/10                       |
| IL-12β (rs3212227), JAK2 (rs7857730) | 62.63                 | 59.49                | 10/10                      |
| IL-12β (rs3212227), JAK2 (rs7857730), STAT3 (rs2293152) | 66.73                 | 62.44                | 10/10                      |
| IL-12β (rs3212227), IL-12β (rs6887695), JAK2 (rs7857730), STAT3 (rs2293152) | 61.91                 | 54.80                | 10/10                      |

MDR – multifactor dimensionality reduction

Discussion

While the precise molecular aetiology of ankylosing spondylitis remains unclear, a variety of genetic factors have been involved in the development and incidence of the disease. Thus, many studies have reported a number of new non-MHC genes to be involved in the pathogenesis of AS, in particular those involved in the IL-23 pathway [27-29]. In the present study, our data indicated that SNP IL-12β rs3212227 (+1188A/C) was significantly associated with AS in the Han Chinese cohort. This polymorphism

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Gene-gene epistasis represented in Table 3 for the two cohorts. All data were based on the highest testing accuracy and cross-validation consistency. For the Han Chinese cohort, the one-variable model SNP IL-12β rs3212227 showed the highest testing accuracy among the four SNPs. The two-variable gene-gene interaction model (IL-12β rs3212227, JAK2 rs7857730) and the three-variable gene-gene interaction model (IL-12β rs3212227, JAK2 rs7857730, and STAT3 rs2293152) showed the highest testing accuracy as compared to the other two-locus and three-locus interaction models (p = 0.0001 and p < 0.0001; respectively). MDR also showed a high testing accuracy for the four-variable gene-gene interaction model (p < 0.0001) for the Chinese cohort (Fig. 2).

For the Algerian cohort, the one-locus model SNP STAT3 rs2293152 represented the model with highest testing accuracy among the four SNPs. The two-locus (IL-12β rs3212227 and JAK2 rs7857730), the three-locus (IL-12β rs3212227, JAK2 rs7857730, and STAT3 rs2293152), and the four-locus interaction models all showed the highest testing accuracy (p < 0.0001) within the other interaction models (Fig. 3).
Fig. 2. Graphic models determined by multifactor dimensionality reduction (MDR), representing the following different interactions in the Chinese cohort: A) IL-12β rs3212227, B) IL-12β rs3212227 and JAK2 rs7857730, C) IL-12β rs3212227, JAK2 rs7857730, and STAT3 rs2293152, D) IL-12β rs3212227, IL-12β rs6887695, JAK2 rs7857730, and STAT3 rs2293152. SNP genotypic values range from 0 to 2 (0 = zero risk allele, 1 = one risk allele, 2 = two risk alleles). Each cell shows counts of “Class 1 = unaffected” on the left and “Class 2 = affected” on the right, and shades on cells represent the risk degree of the disease (dark cell = high risk, light cell = low risk).
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**Fig. 3.** Graphic models determined by multifactor dimensionality reduction (MDR), representing the following different interactions in the Algerian cohort: A) STAT3 rs2293152, B) IL-12β rs3212227 and JAK2 rs7857730, C) IL-12β rs3212227, JAK2 rs7857730, and STAT3 rs2293152, D) IL-12β rs3212227, IL-12β rs6w887695, JAK2 rs7857730, and STAT3 rs2293152. SNP genotypic values range from 0 to 2 (0 = zero risk allele, 1 = one risk allele, 2 = two risk alleles). Each cell shows counts of “Class 1 = unaffected” on the left and “Class 2 = affected” on the right, and shades on cells represent the risk degree of the disease (dark cell = high risk, light cell = low risk).
was also associated with AS in the Algerian cohort, and it is worth noting that the AA genotype may increase the risk of AS in both Chinese and Algerian subjects. Previously, linkage analysis studies in the Chinese population have considered IL-12B as a candidate gene for AS, and several polymorphisms were tested within this gene, but their results were contentious regarding whether some of these polymorphisms are associated with AS susceptibility and others are associated with disease severity [30, 31]. Nevertheless, in a recent association study of IL-12B polymorphism susceptibility with ankylosing spondylitis in the Mainland Han population, no significant difference was found for IL-12β rs3212227 variant, while only the SNP rs6871626 from the eight polymorphisms studied showed a significant difference in genotype distribution between AS and healthy controls [32]. Another meta-analysis studying the relationship between rs3212227 polymorphism of the IL-12B gene and susceptibility to multiple autoimmune diseases in East Asian populations failed to find any association with Graves’ disease and ankylosing spondylitis [23]. Additionally, the International Genetics of Ankylosing Spondylitis Consortium (IGAS) reported other SNPs within IL-12β that conferred risk of ankylosing spondylitis in Europeans, but the two SNPs analysed in our study were not genotyped [28].

In fact, IL-12β may play a key role in the pathogenesis of several inflammatory diseases because it encodes the p40 subunit of IL-23 and IL-12 cytokines and is thus involved in both the IL-12/Th1 and IL-23/Th17 pathways. Many studies were recently conducted to confirm the susceptibility of polymorphisms in IL-12β, in particular rs3212227 (+1188A/C) polymorphism, to cause ankylosing spondylitis (AS) and/or other autoimmune diseases such as rheumatoid arthritis (RA), type 1 diabetes (TID), Behcet’s disease (BD), Graves’ disease (GD), and multiple sclerosis (MS) [23, 33]. Hence, taken together, our results may accentuate the importance of IL-12β rs3212227 (+1188A/C) as a novel risk polymorphism in the development of ankylosing spondylitis in both Chinese and Algerian subjects. Unfortunately, the second polymorphism in IL-12β rs6887695 was not associated with susceptibility to AS, neither in the Chinese nor in the Algerian individuals. The two polymorphisms rs3212227 and rs6887695 in IL-12β have been previously found to be associated with risk of psoriasis and psoriatic arthritis in the Chinese population [24, 34].

We further analysed the two other polymorphisms rs2293152 in STAT3 and rs7857730 in JAK2, but our results were only partially in agreement with the findings of Chen et al. [14], who reported no difference in the distribution of alleles and genotypes between AS groups and controls in their Chinese cohort in both JAK2 and STAT3 genes. In fact, our findings only showed a significant association between Chinese AS patients and controls for rs7857730 in the JAK2 gene. The difference in these findings is probably due to the difference in the recruitment of Chinese subjects for the two studies. Chen et al. also reported that haplotype analysis revealed an association of haplotype rs1536798/rs10119004/rs7857730-CGT in the JAK2 locus with AS, which is consistent with our results. Therefore, our findings may confirm the association of rs7857730 polymorphism in JAK2 with the susceptibility to AS and may emphasise again the importance of the IL-23/IL-17 axis as a causal pathway of AS in the Chinese population, for which earlier studies have also investigated its role in AS [27] and related diseases such as IBD [35] and psoriasis [36].

Another case-control study, carried out by Davidson et al. [15], in which a number of genes implicated in the pathogenic mechanisms of AS were tagged in the Han Chinese population, showed that SNP rs2293152 in STAT3 had a significant association with AS in their Chinese cohort, a result that corroborates the one previously found in STAT3 rs2293152 variant by the Australo-Anglo-American Spondyloarthritis Consortium (TASC) in Caucasian European populations [18]; however, this was in contrast to our results.

Unlike the Chinese cohort, we reported no significant difference between Algerian AS patients and controls for the variant JAK2 rs7857730, but a significant association for the variant STAT3 rs2293152 was found in this group. This discrepancy in our findings could be explained by differences in the genetic pools between the two populations investigated.

Our analysis also considered different interactions among one-variable, two-variable, three-variable, and four-variable gene-gene epistasis with ankylosing spondylitis, which were analysed to address concerns about DNA sequence variations that could have an interaction effect but not a statistically significant independent main effect on AS. Remarkably, logistic regression analysis showed significant interactions between IL-12β rs6887695 and the three other variables in the Han Chinese cohort, although this SNP itself showed no significant association with AS. MDR analysis also revealed a gene-gene interaction model for the Han Chinese cohort, in which IL-12β rs6887695 interacts with the other variables. This difference for IL-12β rs6887695 between individual association results and multiple interaction findings could be explained by the relatively small sample size. In addition, IL-12β rs3212227 showed the strongest association with AS in the Chinese population, so the significant interaction observed between the two variables of the IL-12β gene (rs3212227 and rs6887695) could confirm once again the important role played by this gene in the IL-23 signalling pathway and the pathogenesis of AS.

Analysis of interaction by logistic regression in the Algerian cohort revealed a significant three-variable interaction between IL-12β (rs3212227), JAK2 (rs7857730), and STAT3 (rs2293152) SNPs for AS susceptibility.
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(p = 0.035). Because both IL-12β and STAT3 polymorphisms individually analysed showed a significant association with AS, this interaction suggests that IL-12β and STAT3 may be involved in the risk of developing AS in Algerian patients. In fact, STAT3 is predominantly activated by IL-23, which signals through IL-12Rβ1 and IL-23R to activate JAK and STAT signalling molecules, and induces IL-17A, IL-17F, and/or IL-22 and stabilises TH17 cells [19]. Thus, the additive interaction effect of the two SNPs in STAT3 and IL-12β may increase the risk of AS for Algerian patients.

In conclusion, we confirmed in our study a significant association between ankylosing spondylitis and the IL-12β gene for the rs3212227 variant in both the Han Chinese and Algerian populations. Also, JAK2 rs7857730 polymorphism showed an association in the Han Chinese population, while rs2293152 polymorphism in the STAT3 gene was associated with AS in the Algerian population. Despite the relatively small sample sizes and the number of SNPs selected for each gene, our findings remain novel. Additionally, the gene-gene interaction analysis through the effect of different SNPs was combined and may therefore provide a better understanding of AS and its pathogenic mechanisms.

The authors declare no conflict of interest.

References

1. Pile KD, Kennedy LG, Calin A, et al. (1996): HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis 55: 268-270.
2. Wei JC, Tsai WC, Lin HS, et al. (2004): HLA-B60 and B61 are strongly associated with ankylosing spondylitis in HLA-B27-negative Taiwan Chinese patients. Rheumatology (Oxford) 43: 839-842.
3. Spencer CC, Pointon JJ, Su Z, et al. (2011): Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet 43: 761-767.
4. Yi L, Wang J, Guo X, Espitia MG, et al. (2013): Profiling of hla-B alleles for association studies with ankylosing spondylitis in the Chinese population. Open Rheumatol J 7: 51-54.
5. Liu Y, Jiang L, Cai Q et al. (2010): Predominant association of HLA-B*2704 with ankylosing spondylitis in Chinese Han patients. Tissue Antigens 75: 61-64.
6. Brown MA, Kennedy LG, MacGregor AJ, et al. (1997): Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 40: 1823-1828.
7. Mustafa KN, Hammoudeh M, Khan MA (2012): HLA-B27 prevalence in Arab populations and among patients with ankylosing spondylitis. H J Rheumatol 39: 1675-1677.
8. Amroun H, Djoudi H, Busson M, et al. (2005): Early-onset ankylosing spondylitis is associated with a functional MICA polymorphism. Hum Immunol 66: 1057-1061.
9. Kchir MM, Hamdi W, Laadhari L, et al. (2010): HLA-B, DR and DQ antigens polymorphism in Tunisian patients with ankylosing spondylitis (a case-control study). Rheumatol Int 30: 933-939.
10. Akassou A, Yacoubi H, Jamil A, et al. (2015): Prevalence of HLA-B27 in Moroccan healthy subjects and patients with ankylosing spondylitis and mapping construction of several factors influencing AS diagnosis by using multiple correspondence analysis. Rheumatol Int 35: 1889-1894.
11. Reveille JD (2009): Recent studies on the genetic basis of ankylosing spondylitis. Curr Rheumatol 11: 340-348.
12. Reveille JD (2012): Genetics of spondyloarthritis: beyond the MHC. Nat Rev Rheumatol 8: 296-304.
13. Davidson SI, Wu X, Liu Y, et al. (2009): Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population. Arthritis Rheum 60: 3263-3268.
14. Chen C, Zhang XS and Wang Y, et al. (2010): Analysis of JAK2 and STAT3 polymorphisms in patients with ankylosing spondylitis in Chinese Han population. Clin Immunol 136: 442-446.
15. Davidson SI, Liu Y, Danoy PA, et al. (2011): Association of STAT3 and TNFRSF1A with ankylosing spondylitis in Han Chinese. Ann Rheum 70: 289-292.
16. Liu YC, Zhang H, Li J, et al. (2013): Association of common variants in KIF21B and ankylosing spondylitis in a Chinese Han population: a replication study. Immunogenetics 65: 835-839.
17. Shan S, Dang J, Li JX, et al. (2014): ETS1 variants confer susceptibility to ankylosing spondylitis in Han Chinese. Arthritis Res Ther 16: R87.
18. Reveille JD, Sims AM, Danoy P, et al. (2010): Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 42: 123-127.
19. Parham C, Chirica M, Timans J, et al. (2002): A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. J Immunol 168: 5699-5708.
20. O’Garra A, Arai N (2000): The molecular basis of T helper 1 and T helper 2 cell differentiation. Trends Cell Biol 10: 542-550.
21. Windsor L, Morahan G, Huang D, et al. (2004): Alleles of the IL12B 3’UTR associate with late onset of type 1 diabetes. Hum Immunol 65: 1432-1436.
22. Tsunemi Y, Saeki H, Nakamura K, et al. (2002): Interleukin-12 p40 gene (IL12B) 30-untranslated region polymorphism is associated with susceptibility to atopic dermatitis and psoriasis vulgaris. J Dermatol Sci 30: 161-166.
23. Zhang C, Wang JB, Chen SS, et al. (2016): Relationship between the IL12B (rs3212227) gene polymorphism and susceptibility to multiple autoimmune diseases: a meta-analysis. Mod Rheumatol 26: 1-27.
24. Zhu K, Zhu CY, Shi G, Fan YM (2013): Meta-analysis of IL12B polymorphisms (rs3212227, rs6887695) with psoriasis and psoriatic arthritis. Rheumatol Int 33: 1785-1790.
25. Van der Linden S, Valkenburg HA, Cats A (1984): Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. Arthritis Rheum 27: 361-368.
26. QiAamp® DNA Mini and Blood Mini Handbook “Third Edition” (2010). DNA purification from blood or body fluids (spin protocol), pages: 26-29.
27. Burton PR, Clayton DG, Cardon LR, et al. (2007): Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 39: 1329-1337.
28. International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes A, Hadler J, Pointon JP, et al. (2013): Iden-
Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nat Genet 45: 730-738.
29. Zhai J, Rong J, Li J, et al. (2013): Immunogenetic study in Chinese population with ankylosing spondylitis: are there specific genes recently disclosed? Clin Develop Immunol 2013: 1-6 (ID 419357).
30. Zhou L, Zhang JY, Liu Y, et al. (2013): Novel association between IL-12B gene polymorphism and risk of ankylosing spondylitis in Ningxia population. Int J Lab Med 34: 1372-1375.
31. Wong RH, Wei JC, Huang CH, et al. (2012): Association of IL-12B genetic polymorphism with the susceptibility and disease severity of ankylosing spondylitis. J Rheumatol 39: 135-140.
32. Zhang L, Fan D, Liu L, et al. (2015): Association study of IL-12B polymorphisms susceptibility with ankylosing spondylitis in Mainland Han population. PLoS One 10: 1-9.
33. Huang J, Yang Y, Zhou F, et al. (2016): Meta-analysis of the IL23R and IL12B polymorphisms in multiple sclerosis. Int J Neurosci 126: 205-212.
34. Zheng HF, Zuo XB, Lu WS, et al. (2011): Variants in MHC, LCE and IL12B have epistatic effects on psoriasis risk in Chinese population. J Dermatol Sci 61: 124-128.
35. Duerr RH, Taylor KD, Brant SR, et al. (2006): A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 314: 1461-1463.
36. Cargill M, Schrodij SJ, Chang M, et al. (2007): A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet 2007; 80: 273-290.