Ankylosing spondylitis: analysis of gene-gene interactions between *IL-12*β, *JAK2,* and *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-12β, 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-12β 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 $p = 0.014$) cohort 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-12β. 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.*

Introduction

Ankylosing spondylitis (AS) is a common inflammatory arthritis characterised by acute and chronic inflammation of the axial skeleton, causing pain, stiffness, and progressive joint ankylosis. AS has been strongly associated with human leukocyte antigen-B27 (HLA-B27), and its contribution to the genetics of AS is best characterised at present in various populations [1-4]. For instance, HLA-B27 is found in more than 80% of Chinese patients with AS [5], while only 1-5% of HLA-B27 carriers develop AS in the Chinese population [6]. However, the reported prevalence of AS ranges between 2% and 5% among major Arab populations [7]. Moreover, association studies on AS in the Maghreb population have mainly focused on the implication of the *HLA-B27* gene in disease susceptibility [8-10], thus showing a weaker association between AS and *HLA-B27* for Algerian patients as compared to European patients (63% vs. 80-90%, respectively) [8].

Although the contribution of *HLA-B27* to the genetics of AS is best characterised at present, the pathogenesis of AS remains poorly understood, and other genetic

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-12β* 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
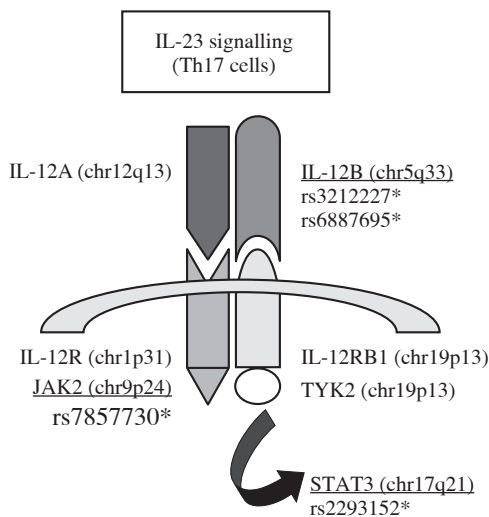
Fig. 1. Schematic representation of the three candidate genes and the four tag SNPs selected for this study in the *IL-23* signalling pathway (SNPs rs3212227 and rs6887695 in *IL-12β*, SNP rs7857730 in *JAK2*, and SNP rs2293152 in STAT3)

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_1994992_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) by 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				Genotype/ Allele	Algerian cohort			
					Number (%) AS patients	Number (%) Controls	OR (95% CI)	p-value		Number (%) AS patients	Number (%) Controls	OR (95% CI)	p-value
chr5q33	IL-12β	rs3212227 (A1188C)	UTR 3'	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)	**0.007**	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)	
				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)	**0.005**	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)	0.650	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)	
				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)	0.351	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		GG	21 (0.161)	15 (0.125)	1.00	
				TG	230 (0.535)	296 (0.510)	0.70 (0.51-0.97)	**0.029**	GT	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)		TT	56 (0.431)	42 (0.350)	1.05 (0.48-2.28)	
				T	390 (0.453)	590 (0.509)	1.00		G	95 (0.365)	93 (0.388)	1.00	
				G	470 (0.547)	570 (0.491)	0.99 (0.83-1.18)	**0.014**	T	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)	0.606	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)	
				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)	0.664	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.

Table 2. Multiplicative interaction analysis reported by logistic regression for the two populations investigated

Population	Chinese			Algerian		
Interaction	χ^2	OR (95% CI)	*p* value	χ^2	OR (95% CI)	*p* value
rs3212227 * 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
rs3212227 * rs7857730	1.275	1.168 (0.892-1.530)	0.259	0.432	0.899 (0.653-1.236)	0.511
rs3212227 * rs7857730	0.177	1.056 (0.819-1.363)	0.674	1.322	1.301 (0.831-2.038)	0.250
rs3212227 * 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 (0.895-2.216)	0.139
rs3212227 * 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
rs3212227 * rs6887695 * rs2293152	0.490	1.064 (0.895-1.264)	0.484	0.490	1.064 (0.895-1.264)	0.484
rs3212227 * rs7857730 * rs2293152	0.989	1.050 (0.954-1.156)	0.320	4.468	1.227 (1.015-1.482)	**0.035**
rs3212227 * 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

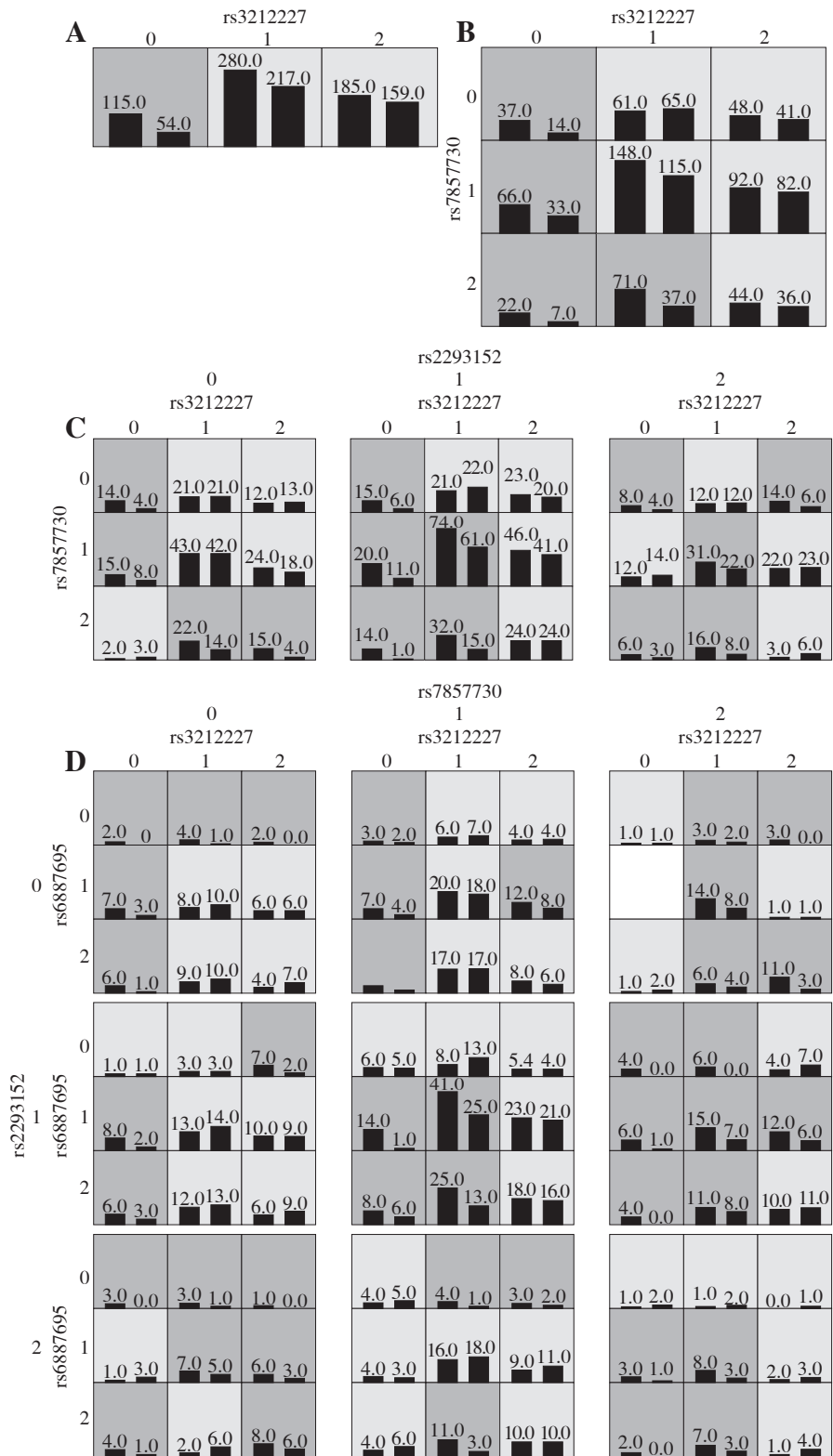*MDR – multifactor dimensionality reduction*

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).

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

Fig. 2. Graphic models determined by multifactor dimensionality reduction (MDR), representing the following different interactions in the Chinese cohort: **A)** *IL-12*β rs3212227, **B)** *IL-12*β rs3212227 and *JAK2* rs7857730, **C)** *IL-12*β rs3212227, *JAK2* rs7857730, and *STAT3* rs2293152, **D)** *IL-12*β 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)
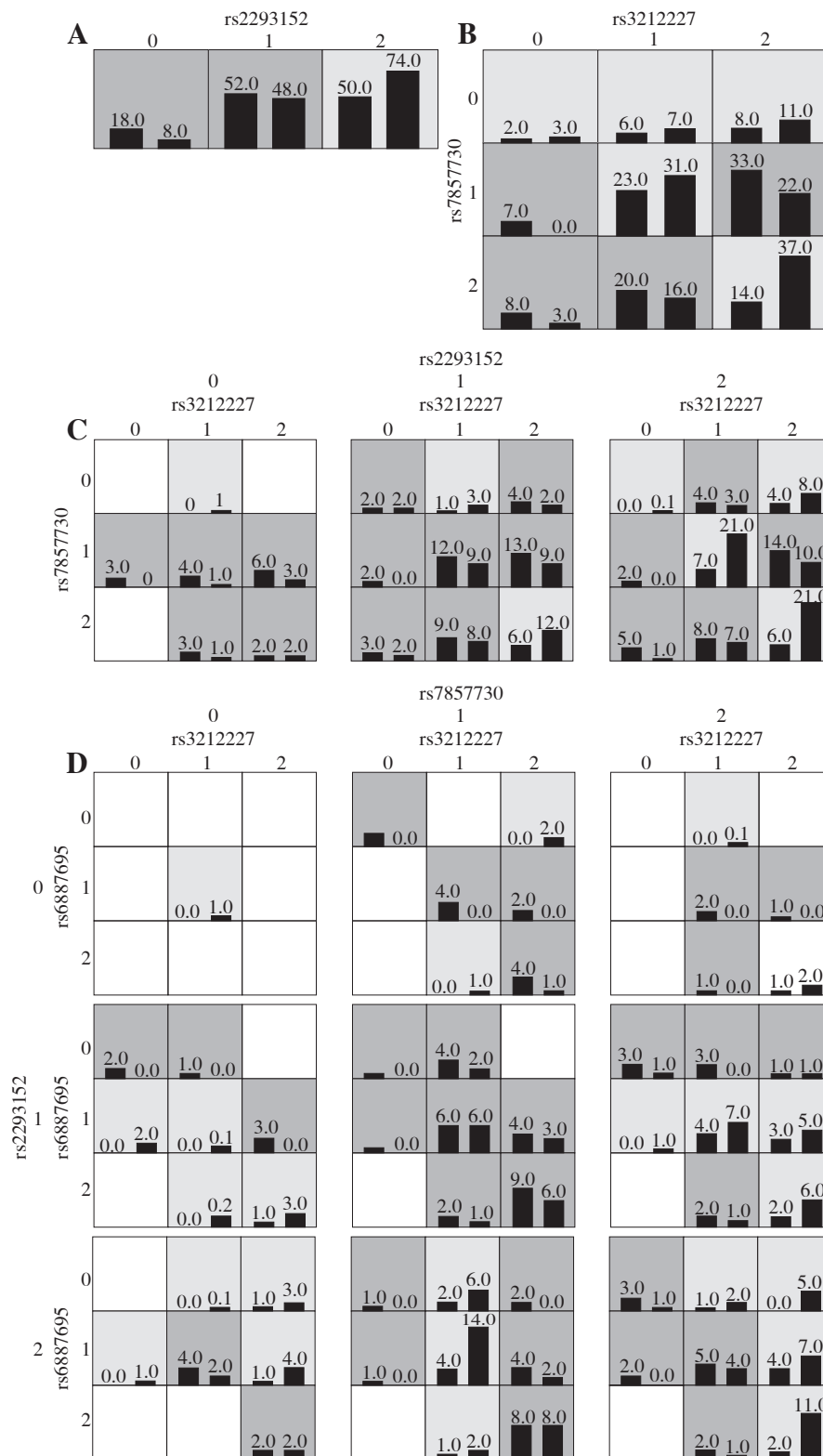
Fig. 3. Graphic models determined by multifactor dimensionality reduction (MDR), representing the following different interactions in the Algerian cohort: **A**) STAT3 rs2293152, **B**) IL-12β rs3212227 and *JAK2* rs7857730, **C**) IL-12β rs3212227, JAK2 rs7857730, and STAT3 rs2293152, **D**) IL-12β 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 (T1D), 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 find-

ings 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 S*TAT3* 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

(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.

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