Association of IL-12B Genetic Polymorphism with the Susceptibility and Disease Severity of Ankylosing Spondylitis

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ABSTRACT. ***Objective.*** Interleukin 23 (IL-23) stimulates the differentiation of T helper 17 (Th17) cells, which are involved in the pathogenesis of ankylosing spondylitis (AS). Binding of IL-23 to the IL-23 receptor complex activates Janus kinases 2 and tyrosine kinase 2, which phosphorylate IL-23R and subsequently promote the transcription of the *IL-17* gene. *IL-12B* encodes a p40 subunit common to IL-12 and IL-23. We evaluated the effects of *IL-12B* and *IL-23R* genotype on the occurrence and clinical features of AS.

Methods. A total of 362 patients with AS and 362 healthy controls were enrolled in the study. Genotypes of *IL-12B* A1188C (rs3212227) and *IL-23R* C2370A (rs10889677) were identified by polymerase chain reaction/restriction fragment-length polymorphism. Disease activity and functional status were assessed by Bath AS indices.

Results. Subjects carrying *IL-12B* CC [matched relative risk (RR_m) 1.93, 95% CI 1.23–3.03] and *IL-12B* AC (RR_m 1.73, 95% CI 1.21–2.46) genotypes had a significantly greater risk of developing AS than subjects with the *IL-12B* AA genotype. Subjects carrying both *IL-12B* CC and *IL-23R* AA genotypes also had a significantly higher risk (RR_m 2.98, 95% CI 1.51–5.89) of developing AS compared to those with *IL-12B* AA and *IL-23R* CC/CA genotypes, and this interaction between *IL-12B* and *IL-23R* was significant. Patients with AS who had *IL-12B* CC and *IL-12B* AC genotypes had an obviously increased Bath Ankylosing Spondylitis Disease Activity Index score compared to those who carried the *IL-12B* AA genotype (4.3 vs 3.7).

Conclusion. The *IL-12B* A1188C genotype was associated with the development and disease severity of AS.
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Key Indexing Terms:
ANKYLOSING SPONDYLITIS GENETIC POLYMORPHISM INTERLEUKIN-12B
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Ankylosing spondylitis (AS) is an autoimmune disease in which the main clinical symptoms are sacroiliitis and extraarticular manifestations[1]. However, the pathogenesis of AS is presently unclear. *HLA-B27* gene is strongly associated with AS, but accounts for only 16% of the genetic variability seen in AS patients[2,3].

It has been suggested that T helper 17 (Th17) cells are involved in AS pathogenesis[4], and are also thought to be involved in psoriasis, Crohn's disease, and inflammatory arthritis[5,6,7]. Increased expression of serum interleukin 17 (IL-17) and IL-23 was observed in patients with AS compared to healthy controls[8]. IL-23 is an IL-12-related cytokine, which might stimulate the differentiation and proliferation of Th17 cells[9]. However, the effect of IL-12 on Th17 is controversial. IL-12 might be involved in pathogenesis, as it affects the expansion of IL-23R+CD4+ T cells, and preferentially stimulates these cells to secrete predominately IL-17[10]. Binding of IL-23 to the IL-23 receptor complex, which is composed of an IL-23R and IL-12Rß1[11], leads to the activation of Janus kinases 2 (Jak2) and tyrosine kinase 2 (Tyk2), which can phosphorylate IL-23R, which increases binding of the STAT proteins. The STAT are then phosphorylated by the Jaks, resulting in their translocation to the nucleus and subsequently transcriptional activation of *IL-17*[11,12].

Formation and secretion of IL-12 requires that the

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IL-12p35 (IL-12α) and IL-12p40 (IL-12ß) subunit be bound to one another[13]. The *IL-12B* gene is located in the region of chromosome 5q31-33[14]. *IL-12B* encodes a common p40 subunit to IL-12 and IL-23[15]. It was also noted that IL-12 promotes the development of naive T cells, while IL-23 mediates chronic inflammation[16]. Several intronic polymorphisms and a polymorphic TaqI site (+16974 A/C) in the 3' untranslated regions (3'-UTR) have been identified; however, no polymorphisms were found that resulted in amino acid substitutions[17]. Importantly, a single-nucleotide polymorphism (SNP) at position 1188 in the *IL-12B* gene 3'-UTR (rs3212227) causes an A to C transition and results in increased IL-12 secretion[18]. Subjects with the *IL-12B* A1188C CC genotype also have significantly higher IL-12 expression from lipopolysaccharide-stimulated peripheral blood mononuclear cells[19].

A genomewide association study in a population of white European ancestry found that the *IL-23R* gene may also be involved in the development of AS[20]. The *IL-23R* gene is located on chromosome 1p31[11]. Several SNP of *IL-23R* have been identified, but the biological influence of these variants on the expression and functionality on IL-23R is currently unknown. A population study in Hungarians also revealed that a polymorphism (*IL-23R* C2370A) in the 3'-UTR (rs10889677) in *IL-23* might be associated with the risk of AS[21]. However, the influence of carrying both *IL-12B* A1188C and *IL-23R* C2370A polymorphisms on the development of AS has not been evaluated in other populations.

Genetic factors affect not only AS development but also contribute to the severity of the disease[22,23]. Using a case-control study, we investigated the relationship of the *IL-12B* and *IL-23R* genotypes with the development and clinical characteristics of AS in Taiwanese patients.

MATERIALS AND METHODS

Study subjects. Our study recruited 362 patients with AS from the arthritis clinic of Chung Shan Medical University Hospital (CSMU; Taichung, Taiwan). All patients were diagnosed using the modified New York criteria, by qualified rheumatologists[24]. Patients were ≥ 18 years of age and gave their informed consent to participate.

At the beginning of our study, physicians recorded detailed clinical histories, including age at initial symptoms, medication history, family history of AS, and extraspinal manifestations. Age at initial symptom was defined as the time when the first symptom developed, whether it was an axial symptom, peripheral arthritis, uveitis, or enthesitis. Delayed diagnosis was defined as the interval between the onset of first symptom and the correct diagnosis of AS. The use of nonsteroidal antiinflammatory drugs and disease-modifying antirheumatic drugs was defined as administration for > 3 months. Family history was defined as first-degree relative with AS, inflammatory bowel disease (IBD), reactive arthritis, or psoriatic arthritis. Peripheral arthritis was diagnosed if there was at least 1 swollen joint. IBD was defined as presence of the inflammatory condition of the colon and small intestine, including ulcerative colitis and Crohn's disease. Uveitis was defined as the presence of inflammation of the middle layer of the eye involving unilateral, bilateral, or alternative patterns. The above symptoms were ascertained by a rheumatologist, ophthalmologist, and gastroenterologist.

Healthy controls were matched 1:1 with AS patients by age (± 5 years) and sex. A total of 362 potential controls were randomly selected from subjects with regular health examinations in the center, and they had no rheumatic or autoimmune symptoms. The study conformed to the Declaration of Helsinki, and the design of the work and final report were approved by the appropriate ethics committees of CSMU Hospital.

Bath AS indices. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Bath Ankylosing Spondylitis Global (BAS-G) were applied to evaluate the disease activity, physical function, and global well-being, respectively. The modified Chinese versions of BASDAI, BASFI, and BAS-G have good intraclass correlations and Cronbach's alpha measures[25].

Laboratory analyses. Peripheral blood was collected and centrifuged to separate serum and cells. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and IgA were measured. Carriage of *HLA-B27* was assessed by flow cytometry[26].

Genomic DNA was extracted from peripheral blood using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen Scientific, Union City, CA, USA). The *IL-12B* A1188C (rs3212227)[27] and *IL-23R* C2370A (rs10889677)[28] genotypes were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

The following primers were used for the amplification of the *IL-12B* A1188C genotype: 5'-TTC TAT CTG ATT TGC TTT A-3' and 5'-TGA AAC ATT CCA TAC ATC C-3'. DNA (0.5 μl) was added to a PCR buffer containing a 200 ng mix of primers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.1% bovine serum albumin in a final volume of 50 μl. Amplification was carried out with the following PCR conditions: the denaturing step at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min. PCR products were digested with TaqI for 16 h at 65°C. Homozygous AA individuals exhibited a product fragment of 234 bp, whereas homozygous CC individuals resulted in 165 bp and 69 bp fragments, and heterozygous AC individuals had all 3 fragments. Primers for amplification of the *IL-23R* C2370A genotype were 5'-ATC GTG AAT GAG GAG TTG CC-3' and 5'-TGT GCC TGT ATG TGT GAC CA-3'. Amplification occurred under the following PCR conditions: the denaturing step at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min. PCR products were digested with MnlI for 16 h at 37°C. Homozygous AA showed 2 fragments of 286 bp and 185 bp, homozygous CC showed 3 fragments of 225 bp, 185 bp and 61 bp, and heterozygous CA individuals had all 4 fragments.

For each assay, a nontemplate control was also added to monitor reagent contamination. All genotypic analyses of case and control samples were performed blinded. To validate the results, about 20% of the samples in each genotype group were randomly selected for repeated PCR analysis.

Statistical analyses. Hardy-Weinberg equilibrium was performed to test *IL-12B* A1188C and *IL-23R* C2370A genotypes for goodness-of-fit. Demographic data and clinical features of AS patients and controls are presented by number (%) and mean ± SD. Both genotypic and allelic frequencies were compared between patients with AS and controls by chi-square tests. The matched relative risks (RR_m) and 95% CI on development of AS were evaluated for the *IL-12B* A1188C and *IL-23R* C2370A genotypes using a conditional logistic regression model. Interaction was further assessed using the likelihood ratio test to calculate chi-square and p values. In the test for interaction, the conditional logistic regression model with only main effects was compared to that with both main effect terms and the interaction term. Interaction effect was defined as the difference of their deviance. The differences among the genotypes and alleles for continuous variables (BASDI, BASFI, BAS-G, ESR, and CRP) were calculated using Student t tests for 2 groups and analysis of variance for more than 2 groups. After adjustment for effects of potential confounding factors, association among BASDAI, BASFI, BAS-G, ESR, and CRP and *IL-12B* and *IL-23R* genotypes was further assessed using a general linear model. All p values were calculated using 2-tailed tests and a value < 0.05 was considered statistically significant. SAS 9.1 for Windows (SAS Inc., Cary, NC, USA) was used for all analysis.

RESULTS

Demographic characteristics and clinical features of patients with AS and healthy controls are shown in Table 1, and geno-

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Table 1. Demographic characteristics and clinical features of patients with AS and healthy controls. Data are mean ± SD unless otherwise indicated.

Variables	AS, n = 362	Controls, n = 362
Age, yrs	41.9 ± 11.0	42.9 ± 11.1
Male (%)	251 (69.3)	251 (69.3)
Family history of AS (%)	158 (43.6)	
Clinical features		
Age at symptom onset, yrs	30.2 ± 12.5	
Disease duration, yrs	11.8 ± 10.1	
Delayed diagnosis, yrs	5.7 ± 7.3	
Peripheral arthritis (%)	174 (48.1)	
Uveitis (%)	98 (27.1)	
Psoriasis (%)	47 (13.0)	
Inflammatory bowel disease (%)	18 (5.0)	
Laboratory results		
HLA-B27-positive	334 (92.3%)	
ESR, mm/h	25.1 ± 19.1	
CRP, mg/dl	1.2 ± 1.8	
IgA, mg/dl	327.1 ± 176.4	
BASDAI, cm	4.1 ± 2.3	
BASFI, cm	2.3 ± 2.3	
BAS-G, cm	4.5 ± 2.8	
Medication history		
NSAID use (%)	192 (53.0)	
DMARD use (%)	107 (29.6)	

AS: ankylosing spondylitis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BAS-G: Bath Ankylosing Spondylitis Global; NSAID: nonsteroidal anti-inflammatory drug; DMARD: disease-modifying antirheumatic drug.

typing and allele frequencies of *IL-12B* A1188C and *IL-23R* C2370A polymorphisms are shown in Table 2. Among the controls, the frequencies of *IL-12B* CC, AC, and AA genotypes were 17.1%, 42.8%, and 40.1%, respectively; and *IL-23R* AA, CA, and CC genotypes were 53.3%, 37.9%, and 8.8%. *IL-12B* A1188C and *IL-23R* C2370A conformed to Hardy-Weinberg equilibrium ($p > 0.05$). Individuals with *IL-12B* CC (RR_m 1.93, 95% CI 1.23–3.03, p = 0.0044) and AC (RR_m 1.73, 95% CI 1.21–2.46, p = 0.0025) genotypes had significantly greater risk of AS development compared to those with the AA genotype. The *IL-12B* C allele had a 1.45-fold risk (95% CI 1.16–1.81, p = 0.0011) for AS development compared to the *IL-12B* A allele. In contrast, the *IL-23R* C2370A genotype was not significantly associated with development of AS.

We further evaluated the interaction of *IL-12B* A1188C and *IL-23R* C2370A genotypes on development of AS (Table 3). Because the number of *IL-23R* CC genotypes was small, *IL-23R* CC and CA genotypes were combined for subsequent analyses. A statistically significant interaction between *IL-12B* and *IL-23R* genotypes on AS development was observed (p = 0.0013). When subjects with *IL-12B* AA and *IL-23R* CC/CA genotypes were selected as the reference, those with *IL-12B* AC and *IL-23R* CC/CA genotypes had a significantly increased risk for AS development (RR_m 1.81, 95% CI 1.05–3.13, p = 0.0329). Subjects with *IL-12B* AC and *IL-23R* AA genotypes also had a 1.83-fold risk (95% CI 1.07–3.12, p = 0.0278) for AS development compared to those with *IL-12B* AA and *IL-23R* CC/CA genotypes. Subjects with *IL-12B* CC and *IL-23R* AA genotypes had the highest risk for AS development (RR_m 2.98, 95% CI 1.51–5.89, p = 0.0017). Individuals with *IL-12B* CC and *IL-23R* CC/CA genotypes and those with *IL-12B* AA and *IL-23R* AA genotypes also had elevated risks for AS development compared to those with *IL-12B* AA and *IL-23R* CC/CA genotypes, but these were not statistically significant.

Subjects with the *IL-12B* C allele might have higher expression of IL-12[11], thus *IL-12B* CC and AC genotypes were combined in order to increase statistical power. As shown in Table 4, AS patients with *IL-12B* CC/AC genotypes had a significantly increased BASDAI score compared to those with *IL-12B* AA genotype (4.3 vs 3.7, respectively; p = 0.032). Patients with the *IL-23R* AA and CA genotypes also had increased BASDAI scores compared to those with the *IL-23R* CC genotype (3.9 and 4.5 vs 3.6; p = 0.055). After adjustment for effects of age, sex, disease duration, and med-

Table 2. Genotyping and allele frequency of *IL-12B* A1188C and *IL-23R* C2370A polymorphisms in patients with AS and in healthy controls.

Genotype		AS, n = 362 (%)	Control, n = 362 (%)	p	RR_m (95% CI)
IL-12B	CC	73 (20.2)	62 (17.1)	0.0044	1.93 (1.23–3.03)
	AC	189 (52.2)	155 (42.8)	0.0025	1.73 (1.21–2.46)
	AA	100 (27.6)	145 (40.1)	—	1.00 (reference)
	C	335 (46.3)	279 (38.5)	0.0011	1.45 (1.16–1.81)
	A	389 (53.7)	445 (61.5)	—	1.00 (reference)
IL-23R	AA	204 (56.4)	193 (53.3)	0.9569	1.02 (0.58–1.80)
	CA	125 (34.5)	137 (37.9)	0.2693	0.86 (0.49–1.54)
	CC	33 (9.1)	32 (8.8)	—	1.00 (reference)
	A	533 (73.6)	523 (72.2)	0.5373	1.08 (0.84–1.39)
	C	191 (26.4)	201 (27.8)		1.00 (reference)

RR_m: matched relative risk; AS: ankylosing spondylitis.

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Table 3. Interaction of *IL-12B* A1188C and *IL-23R* C2370A genotypes in the development of AS. Interaction was assessed using the likelihood ratio test.

		IL-23R					
		CC/CA			AA		
		AS	Controls	RR_m (95% CI)	AS	Controls	RR_m (95% CI)
IL-12B	CC	27	36	1.39 (0.70–2.76)	46	26	2.98 (1.51–5.89)
	AC	91	76	1.81 (1.05–3.13)	98	79	1.83 (1.07–3.12)
	AA	40	57	1.00 (reference)	60	88	1.11 (0.63–1.96)
Test for interaction		$\chi^2 = 10.33$ (1 df); p = 0.0013					

RR_m: matched relative risk; AS: ankylosing spondylitis.

Table 4. BASDAI, BASFI, BAS-G, ESR, and CRP levels in AS patients with *IL-12B* A1188C and *IL-23R* C2370A genotypes. Data are mean ± SD.

Genotype		BASDAI	BASFI	BAS-G	ESR	CRP
IL-12B	CC	4.2 ± 2.3	2.3 ± 2.2	4.6 ± 2.9	24.8 ± 17.7	1.2 ± 1.8
	AC	4.3 ± 2.3	2.4 ± 2.2	4.7 ± 2.7	24.9 ± 19.0	1.2 ± 1.7
	AA	3.7 ± 2.5	2.2 ± 2.4	4.0 ± 2.9	25.8 ± 20.4	1.3 ± 1.8
	p	0.101	0.636	0.156	0.915	0.896
	Adjusted p*	0.178	0.835	0.274	0.911	0.765
	CC/AC	4.3 ± 2.3	2.4 ± 2.2	4.7 ± 2.8	24.8 ± 18.6	1.2 ± 1.8
	AA	3.7 ± 2.5	2.2 ± 2.4	4.0 ± 2.9	25.8 ± 20.4	1.3 ± 1.8
	p	0.032	0.371	0.060	0.672	0.695
	Adjusted p*	0.072	0.842	0.184	0.867	0.562
IL-23R	AA	3.9 ± 2.4	2.3 ± 2.2	4.3 ± 2.8	24.3 ± 19.0	1.1 ± 1.4
	CA	4.5 ± 2.3	2.5 ± 2.3	4.9 ± 2.9	26.1 ± 18.8	1.2 ± 1.6
	CC	3.6 ± 2.0	2.0 ± 2.1	4.0 ± 2.3	26.3 ± 21.1	1.1 ± 2.1
	p	0.055	0.471	0.097	0.655	0.772
	Adjusted p*	0.031	0.426	0.061	0.495	0.384
	AA	3.9 ± 2.4	2.3 ± 2.2	4.3 ± 2.8	24.3 ± 19.0	1.1 ± 1.4
	CA/CC	4.3 ± 2.3	2.4 ± 2.3	4.7 ± 2.8	26.2 ± 19.2	1.2 ± 1.7
	p	0.148	0.660	0.151	0.357	0.524
	Adjusted p*	0.086	0.318	0.090	0.238	0.169

* Adjusting the effects of age, sex, disease duration, and medication history. AS: ankylosing spondylitis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BAS-G: Bath Ankylosing Spondylitis Global.

ication history, differences in BASDAI score for patients with AS who had different *IL-12B* genotypes were marginally significant (p = 0.072). No significant differences in BASFI, BAS-G, ESR, or CRP levels were found in patients with AS who had different *IL-12B* and *IL-23R* genotypes. In addition, the proportions of peripheral arthritis, uveitis, and IBD were not different in the *IL-12B* and *IL-23R* genotypes (data not shown).

DISCUSSION

In our study, the *IL-12B* A1188C genotype was associated with the development of AS. Further, there was a significant interaction between *IL-12B* and *IL-23R* genes on development of AS. In patients with AS, the *IL-12B* A1188C genotype was also related to the disease activity indicated by the BASDAI score.

It has been suggested that Th17 cells are involved in the pathogenesis of AS[4]. IL-12, made up of the IL-12p35 (IL-12α) and IL-12p40 subunits, is a proinflammatory cytokine that induces expression of interferon-γ, leading to the differentiation and proliferation of Th1[13]. Elevated expression of Th1 is known to be involved in the pathway of the spondyloarthropathies[29,30]. *IL-12p40* knockout mice, which are defective in both the IL-12 and IL-23 pathways, are not susceptible to experimentally induced autoimmune encephalomyelitis[31], suggesting that IL-12p40 might play a key role in the Th1 and Th17 pathways. However, the effect of IL-12 on Th17 cells is still controversial, and the role of IL-12 in the pathogenesis of AS remains unclear. An A to C polymorphism at position 1188 in the 3'-UTR of the *IL-12B* gene might result in the increased secretion of IL-12[18]. Similar to a recent study[32], we observed that *IL-12B* genetic polymorphism confers susceptibility to AS in our population. This might be due to increased IL-12p40 protein secretion, resulting in enhanced differentiation of Th17 cells. Further, IBD and psoriasis frequently occur in patients with AS[33], and spondyloarthritis is

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common in patients with IBD[32]. *IL-12B* has also been shown to be associated with IBD and psoriasis[32,34]. These findings suggest that differentiation and proliferation of Th17 cells might be a common etiopathogenic pathway for AS, IBD, and psoriasis, and also highlight the involvement of common risk variants across multiple diseases. However, *IL-12B* encodes the p40 subunit common to IL-12 and IL-23[15]. A previous study observed that IL-23 plays a more dominant role than IL-12 in psoriasis[35]. Again, the role of IL-12 in the pathogenesis of AS is rather unclear, thus the effect of the *IL-12B* genotype on increasing production of IL-12 or IL-23 requires careful consideration.

Genomewide association study has identified *IL23R* as having a strong association with AS[20]. But the *IL-23R* C2370A polymorphism (rs10889677) was not associated with AS in our study. This result is similar to previous reports[36,37]. The statistical power of the *IL-23R* C2370A genetic polymorphism in our study was only 6.7%. Thus, we could not observe any statistical association between *IL-23R* C2370A polymorphism and development of AS in our subjects. This SNP is likely to be truly associated with AS, although most likely through linkage disequilibrium with rs11209026 and rs11209032, which are thought to be the causative variants[20].

IL-12 and IL-23 have been proposed to play critical roles in susceptibility to AS and rheumatoid arthritis[38]. Binding of IL-23 to the IL-23 receptor complex leads to overexpression of IL-17[11,12]. We observed a significant interaction of *IL-12B* A1188C and *IL-23R* C2370A genotypes in development of AS. We also found that subjects that carried both *IL-12B* AC and *IL-23R* CC/CA or *IL-12B* AC and *IL-23R* AA genotypes had moderately increased risks of AS development compared to those with both *IL-12B* AA and *IL-23R* CC/CA genotypes. Subjects with both of the *IL-12B* CC and *IL-23R* CC/CA genotypes also had an elevated risk of developing AS, although this was not significant, which may indicate that these polymorphisms have little influence on disease development, or that the study did not have sufficient power to detect a difference. Interestingly, subjects who carried the *IL-12B* CC and *IL-23R* AA genotypes were more likely to experience development of AS than those with *IL-12B* AA and *IL-23R* CC/CA genotypes. These findings suggest that the combination of specific “high-risk” polymorphisms creates a greater risk for AS than either polymorphism alone. IL-12 is mainly expressed by activated macrophages, and acts on T cells and natural killer cells[39]. IL-23, which shares the common p40 subunit with IL-12[15], is an essential initiating cytokine in autoimmune diseases and has been linked to the development and maintenance of Th17 cells. A subunit of the receptor for IL-23A/IL-23 encoded by the *IL-23R* gene accompanies the receptor IL-12RB1 to form a receptor complex enabling the heterodimeric cytokine to confer immune responses associated with IL-23R[40]. Therefore, variants in both genes are very likely to influence signaling activities and to result in susceptibility to AS. Additional functional studies are required to test this hypothesis.

The disease severity of AS may also be associated with genetic characteristics[21,22]. We found obvious differences in the BASDAI in AS patients with *IL-12B* polymorphisms, although the results was marginally significant after adjustment for the effects of potential confounders. Our AS patients with *IL-12B* CC/AC genotypes had higher BASDAI score than those with the AA genotype. This might reflect that overexpression of IL-12p40 leads to increased expression of Th17.

The frequencies of *IL-12B* C (38.5%) and *IL-23R* A alleles (72.2%) in our healthy controls were similar to those reported previously for Han Chinese (*IL-12B* C allele: 41.5%; *IL-23R* A allele: 67.1%; Website: http://www.ncbi.nlm.nih.gov/SNP/) and conformed to Hardy-Weinberg equilibrium. These findings indicate the validity of the genotyping methodology used in this study.

One must be aware of several limitations when interpreting our results. Our study has no confirmation cohort, and the sample size was too small to be robust for primary associations of modest effects. In particular, our finding for gene-gene interaction might have a false-positive result. Larger cohort studies would be necessary to confirm evidence regarding our findings. In our study, only 28 patients with AS were HLA-B27-negative. Thus, it was difficult to evaluate the genetic effects of *IL-12B* A1188C and *IL-23R* C2370A in HLA-B27-negative subjects. Subjects were interviewed retrospectively, raising issues with regard to recall bias. However, the 3 questionnaires (BASDAI, BASFI, and BAS-G) were administered simultaneously. In addition, our patients and controls were matched by age and sex in an attempt to reduce for selection bias.

Our study suggests there is an association of *IL-12B* A1188C with susceptibility of a person for development of AS. In addition, an interaction between *IL-12B* A1188C and *IL-23R* C2370A genotypes appeared to influence development of AS, and indicated that the *IL-12B* A1188C genotype was related to the disease activity of AS.

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