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Rs4676410 and Rs2531875 are Associated with the Risk of Ankylosing Spondylitis in the Han Chinese Population

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Abstract

Objectives: Although the exact cause of ankylosing spondylitis (AS) is unknown, genetics play a key role in AS. A recent genome-wide association study identified new immune-related susceptibility loci for AS in East Asians, and these need to be validated in the Chinese population.

Methods: We enrolled 848 patients who met the modified New York criteria for AS and 1123 healthy normal controls in the present study. High-resolution melting analysis accompanied with sequencing was carried out to genotype five polymorphisms: rs12615545, rs4676410, rs1250550, rs2531875, and rs7282490. Distributions of genotypes and alleles were compared between AS patients and healthy controls, and among AS patients stratified by clinical parameters, age, and gender.

Results: rs4676410 (p=0.0237; odds ratio (OR): 1.109; 95% confidence interval (CI): 1.014–1.213) and rs2531875 (p=0.0308; OR: 1.117; 95% CI: 1.01–1.234) were both associated with the risk of AS. However, no association was found between the studied polymorphisms and AS severity. An association between the rs4676410T allele and iridocyclitis was observed (p=0.0142; OR: 1.403; 95% CI: 1.11–1.774).

Conclusion: rs4676410 and rs2531875 are associated with AS susceptibility in the Han Chinese population. The rs4676410T allele might be correlated with iridocyclitis.

Keywords: Ankylosing spondylitis; Single nucleotide polymorphism; Association study; rs4676410; rs2531875

Introduction

Ankylosing spondylitis (AS), the prototype disease in the spectrum of spondyloarthritides, is a chronic, systemic, inflammatory disease with a strong predilection for the axial skeleton [1]. AS is estimated to affect 0.1%–0.3% of the population and thus constitutes a significant health problem worldwide [2]. Currently, the pathogenesis of AS is poorly understood, but recent large-scale genetics and gene-expression profiling studies have identified some of the underlying mechanisms and pathways contributing to this disease [3,4].

A recent study identified multiple risk variants for AS through high-density genotyping of immune-related loci [5]. In that study, new loci associated with AS at genome-wide significance were observed in both in European and East Asian cohorts. These loci included rs12615545 (in the UBE2E3 gene), rs4676410 (in the GPR35 gene), rs1250550 (in the ZMIZ1 gene), rs2531875 (in the NOS2 gene), and rs7282490 (in the ICOSLG gene). These single-nucleotide polymorphisms (SNPs) showed diverse allele frequencies among people from different cohorts. In that study, the East Asian cohort consisted of 1,550 cases and 1,567 controls from the Chinese, Taiwanese, and Korean populations [5]. In the present study, we determined whether these susceptibility loci were associated with the risk of AS in the Han Chinese population [6-9].

Materials and Methods

Patient selection and clinical characteristics of AS in the study cohort

We recruited 848 Han Chinese patients who fulfilled the 1984 modified New York criteria for AS [10]. Among them, 660 patients were male, and 188 were female. AS was diagnosed by a qualified rheumatologist and sacroiliitis was confirmed by a qualified radiologist. These patients were consecutively recruited at Peking University Shenzhen Hospital, Shenzhen, Guangdong, China. The detailed clinical history included extraspinal manifestations, age at symptom onset, and family history of AS. A total of 1123 healthy controls were recruited from people undergoing medical examinations at our hospital. The study was approved by the Peking University Health Science Center, and the study design and final report conform to the Declaration of Helsinki. All the subjects gave written consent.

Bath AS indices

BASDAI and BASFI were applied to evaluate disease activity and physical function, respectively. In all patients, the BASDAI and BASFI scores were evaluated at the active stage of the disease. The modified Chinese versions of BASDAI and BASFI have good intra-class correlation and Cronbach's alpha [11].

Genotyping

Genomic DNA from the AS patients and healthy controls was isolated from peripheral blood cells using the Innogent genomic DNA extraction kit (Innogent, Shenzhen, Guangdong, China), according to the manufacturer's protocol. The genotypes of rs12615545, rs4676410, rs1250550, rs2531875, and rs7282490 were directly genotyped by HRMA using Light Cycler software (release 1.5.0, Roche, Indianapolis, IN, USA) with high sensitivity detection and auto-grouping after polymerase chain reaction (PCR) amplification. The sequences of the primers used in the current study are listed in Supplementary Table 1. PCR was performed in a volume of 10 μl containing 5 μl Ssofast^{TM} Evagreen R Supermix (BioRad, US), 0.5 μl of forward primer (10 μM), 0.5 μl of reverse primer (10 μM), 3 μl water, and 1 μl DNA (40 ng/μl). The auto-grouping and sequencing of the targeted SNPs are illustrated in figure 1.

SNP	Forward	Reverse
rs12615545	TCACAATAATTTCTTTCTACACT	CTTCCCACTTCACACTCAAG
rs4676410	GTCCTTCCTGCCCTTCCTC	ATGGCCCATGGAGACTGG
rs1250550	TCCCTGACCCCAATGATTCC	GCAGTTTGGGTGTAGAGGGA
rs2531875	TGCACTGCATATCACTTCTACC	GCTTGGAGGCCAGCAATT
rs7282490	CCGCGCTAAAATCCCATTCA	CCCTAGGAAGTGTCAGATCCC

Table 1: Sequences of primer sets used for SNP genotyping.

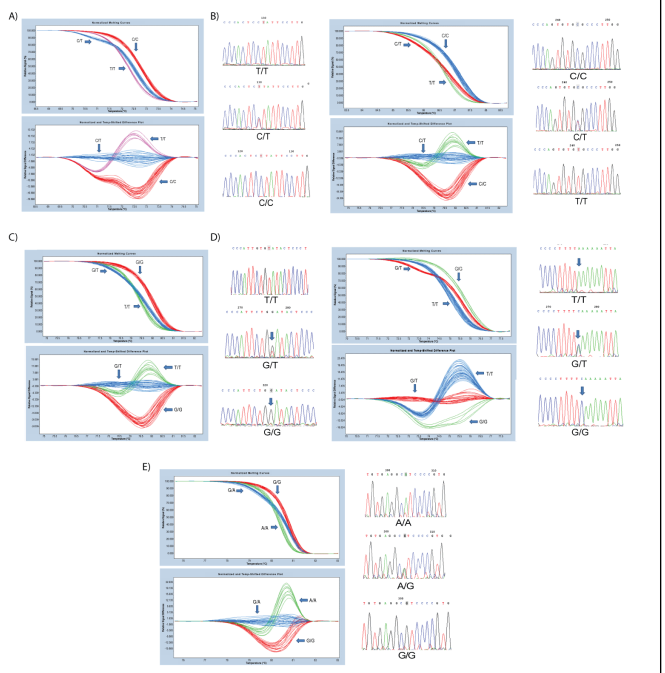

Figure 1: Auto-grouping of the indicated SNPs using LightCycler software (version 1.5.0) and the results of verification by DNA sequencing.

Statistical analysis

The Hardy–Weinberg equilibrium was assessed by the chi-square test with one degree of freedom. Differences in genotype and allele frequencies between the patient and control groups were analyzed by the chi-square test. Analysis of variance was used to compare the means of continuous variables (BASDAI, BASFI, and Bath Ankylosing Spondylitis Patient Global score) among AS patients with different genotypes. Multiple regression analysis was used to adjust for age, sex, and disease duration. The Bonferroni test was used to correct for multiple tests. P values less than 0.05 was considered significant.

Results

Association between rs4676410/rs2531875 genetic polymorphisms and AS susceptibility

As shown in Table 1, the results of the Hardy–Weinberg equilibrium tests showed that allele and genotype frequencies of all the indicated polymorphisms in the studied population were stable. Among the five studied SNPs, the rs4676410T allele (p=0.0237; odds ratio (OR): 1.109; 95% confidence interval (CI): 1.014–1.213) and rs2531875G allele (p=0.0308; OR: 1.117; 95% CI: 1.01–1.234) were found to be associated with AS susceptibility. A significant association between the rs30187T allele and the risk of AS was also observed. Age at onset and gender had no influence on the allele frequencies of the selected polymorphisms (Tables 2 and 3).

		Case (%)	Control (%)		Case (%)	Control (%)	Genotypic	Allelic
	Genotype	(n=848)	(n=1123)	**Allele**	(n=848)	(n=1123)	P value	P value
rs12615545	**CC**	388 (45.8)	487 (43.4)	**C**	1134 (66.9)	1492 (66.4)	0.1936	0.7748
	CT	358 (42.2)	518 (46.1)	**T**	562 (33.1)	754 (33.6)		

	TT	102 (12.0)	118 (10.5)					
Hardy-Weinberg test P value		0.3874	0.5188		OR (%95 CI): 1.007 (0.963~1.052)			
rs4676410	**TT**	108 (12.7)	106 (9.4)	**T**	588 (34.7)	702 (31.3)	0.0435	0.0237
	CT	373 (44.0)	490 (43.7)	**C**	1108 (65.3)	1544 (68.7)		
	CC	367 (43.3)	527 (46.9)					
Hardy-Weinberg test P value		0.6867	0.8759		OR (95% CI): 1.109 (1.014–1.213)			
rs1250550	**GG**	314 (37.0)	377 (33.6)	**G**	1032 (60.8)	1316 (59.8)	0.2789	0.1530
	GT	404 (47.6)	562 (50.0)	**T**	664 (39.2)	930 (40.2)		
	TT	130 (15.4)	184 (16.4)					
Hardy-Weinberg test P value		0.9999	0.5758		OR (95% CI): 1.039 (0.986–1.096)			
rs2531875	**GG**	77 (9.1)	78 (7.0)	**G**	506 (29.8)	600 (26.7)	0.0889	0.0308
	GT	352 (41.5)	444 (39.5)	**T**	1190 (70.2)	1646 (73.3)		
	TT	419 (49.4)	601 (53.5)					
Hardy-Weinberg test P value		0.9695	0.9481		OR (95% CI): 1.117 (1.01–1.234)			
rs7282490	**AA**	184 (21.7)	206 (18.4)	**A**	778 (45.9)	968 (43.1)	0.1632	0.0826
	GA	410 (48.3)	556 (49.5)	**G**	918 (54.1)	1278 (56.9)		
	GG	254 (30.0)	361 (32.1)					
Hardy-Weinberg test P value		0.7444	0.9512		OR (95% CI): 1.064 (0.992–1.142)			
rs30187	**TT**	235 (27.7)	281 (25.0)	**T**	906 (53.4)	1108 (49.3)	0.0169	0.0110
	CT	436 (51.4)	546 (48.6)	**C**	790 (46.6)	1138 (50.7)		
	CC	177 (20.9)	296 (26.4)					
Hardy-Weinberg test P value		0.6278	0.6554		OR (95% CI): 1.083 (1.019–1.151)			

Table 1: Genotype and allele frequencies of indicated polymorphisms in controls and patients with AS; AS: Ankylosing Spondylitis; OR: Odds Ratio; CI: Confidence Interval.

		Male (%)	**Female (%)**		**Male (%)**	**Female (%)**	**Genotypic**	**Allelic**
	Genotype	(n=660)	(n=188)	**Allele**	(n=660)	(n=188)	P value	P value
rs4676410	**TT**	82 (12.4)	26 (13.8)	**T**	460 (34.8)	129 (34.3)	0.6252	0.7972
	CT	296 (44.9)	77 (41.0)	**C**	860 (65.2)	247 (65.7)		
	CC	282 (42.7)	85 (45.2)					
rs2531875	**GG**	62 (9.4)	15 (8.0)	**G**	395 (29.9)	111 (29.5)	0.7873	0.8802
	GT	271 (411)	81 (43.1)	**T**	925 (70.1)	265 (70.5)		
	TT	327 (49.5)	92 (48.9)					

Table 2: Genotype and allele frequencies of indicated polymorphisms in male or female AS patients.

		Adult (%)	**Juvenile (%)**		**Adult (%)**	**Juvenile (%)**	**Genotypic**	**Allelic**
	Genotype	(n=691)	(n=157)	**Allele**	(n=691)	(n=157)	P value	P value
rs4676410	**TT**	84 (12.2)	24 (15.3)	**T**	470 (34.0)	119 (37.9)	0.4295	0.1913

	CT	302 (44.9)	71 (41.2)	**C**	912 (66.0)	195 (62.1)		
	CC	305 (42.9)	62 (39.5)					
	GG	66 (9.6)	11 (7.0)	**G**	423 (30.6)	83 (26.4)	0.3478	0.1444
rs2531875	**GT**	291 (42.1)	61 (38.9)	**T**	959 (69.4)	231 (73.6)		
	TT	334 (48.3)	85 (54.1)					

Table 3: Genotype and allele frequencies of indicated polymorphisms in juvenile (<18) or adult (18) onset AS patients.

Association between rs4676410 and iridocyclitis in AS patients

We further investigated whether the above two genetic polymorphisms were associated with the complications (uveitis and IBD) and clinical phenotypes (BASDAI and BASFI) of AS. As shown in Table 4, an association was found between the GPR35 polymorphism rs4676410 and **iridocyclitis**. We did not find any association between rs4676410/rs2531875 polymorphisms and IBD (Tables 5). When the combinatorial effects were considered using a two-locus model (rs4676410 and rs2531875), a borderline association (p=0.0459) was obtained between the polymorphisms and BASDAI scores (Table 6).

		Iridocyclitis (%)	**No iridocyclitis (%)**		**Iridocyclitis (%)**	**No iridocyclitis (%)**	**Genotypic**	**Allelic**
	Genotype	(n=42)	(n=806)	**Allele**	(n=42)	(n=806)	P value	P value
	TT	12 (28.6)	96 (11.5)	**T**	40 (47.6)	547 (33.6)	**0.0065**	**0.0142**
rs4676410	**CT**	16 (38.1)	357 (44.0)	**C**	44 (52.4)	1065 (66.4)		
	CC	14 (33.3)	353 (44.5)		**OR (95% CI): 1.403 (1.11–1.774)**			
	GG	4 (9.5)	73 (9.1)	**G**	28 (33.3)	478 (29.7)	0.6733	0.5508
rs2531875	**GT**	20 (47.6)	332 (41.2)	**T**	56 (66.7)	1134 (70.3)		
	TT	18 (42.9)	401 (49.7)					

Table 4: Genotype and allele frequencies of rs4676410 and rs2531875 in AS patients with or without iridocyclitis; AS: Ankylosing Spondylitis; OR: Odds Ratio; CI: Confidence Interval.

		IBD (%)	**No IBD (%)**		**IBD (%)**	**No IBD (%)**	**Genotypic**	**Allelic**
	Genotype	(n=53)	(n=795)	**Allele**	(n=53)	(n=795)	P value	P value
	TT	11 (20.8)	97 (12.2)	**T**	45 (42.5)	544 (34.2)	0.1669	0.1748
rs4676410	**CT**	23 (43.4)	350 (44.0)	**C**	61 (57.5)	1046 (65.8)		
	CC	19 (35.8)	348 (43.8)					
	GG	5 (9.4)	72 (9.1)	**G**	32 (30.2)	474 (29.8)	0.9954	0.9344
rs2531875	**GT**	22 (41.5)	330 (41.5)	**T**	74 (69.8)	1116 (70.2)		
	TT	26 (49.1)	393 (49.4)					

Table 5: Genotype and allele frequencies of rs4552569 in AS patients with or without IBD; AS: Ankylosing Spondylitis; IBD: Inflammatory Bowel Disease; OR: Odds Ratio; CI: Confidence Interval.

	rs4676410			**rs2531875**			**Two-locus model**	
Genotype	**TT**	**CT**	**CC**	**GG**	**GT**	**TT**	**High risk groupδ**	**Low risk groupδ**
Case no.	108	373	367	77	352	419		
BASDAI	4.98 ± 1.55	5.18 ± 1.36	4.91 ± 1.45	5.17 ± 1.38	5.11 ± 1.23	4.88 ± 1.57	5.14 ± 1.41	4.89 ± 1.55
Unadjusted P value	0.2258 (TT + CT VS. CC)			0.1679 (GG + GT VS. TT)			0.0474φ	

Adjusted P value	0.2174φ (TT + CT VS. CC)			0.1492φ (GG + GT VS. TT)			0.0459φ	
BASFI	3.71 ± 2.50	3.98 ± 2.90	3.83 ± 2.24	3.98 ± 2.34	3.74 ± 2.31	3.98 ± 2.81	3.95 ± 2.65	3.89 ± 2.72
Unadjusted P value	0.7128 (TT + CT VS. CC)			0.8718 (GG + GT VS. TT)			0.7382φ	
Adjusted P value	0.7318φ (TT + CT VS. CC)			0.8201φ (GG + GT VS. TT)			0.7197φ	

Data represent means ± S.D.

δAS patients who had the TT genotype of rs4676410, GG genotype of rs2531875, or were heterozygous at both loci were classified into the high-risk group, and the others were classified into the low-risk group.

φAdjusted for age and sex.

Table 6: Difference in BASDAI and BASFI scores among AS patients stratified by rs4676410 and rs2531875 genotypes.

No associations of the selected genetic polymorphisms with HLA-B27 (+) AS patients

We compared HLA-B27 (+) subjects and normal controls in terms of the indicated genetic variants, but did not observe a significant association (Table 7). In HLA-B27 (+) AS patients, we examined whether the complications of AS were associated with rs4676410 and rs2531875; our results showed no statistically significant association after Bonferroni correction (data not shown here). Our results also revealed no association of the rs4676410 and rs2531875 genotypes with the BASDAI and BASFI scores among HLA-B27 (+) AS patients (Table 8).

		HLA-B27 (+) (%)	HLA-B27 (-) (%)		HLA-B27 (+) (%)	HLA-B27 (-) (%)	Genotypic	Allelic
	Genotype	(n=772)	(n=76)	**Allele**	(n=772)	(n=76)	P value	P value
rs12615545	**CC**	354 (45.9)	34 (44.7)	**C**	1030 (66.7)	104 (68.4)	0.4211	0.6689
	CT	322 (41.7)	36 (46.6)	**T**	514 (33.3)	48 (31.6)		
	TT	96 (12.4)	6 (7.9)					
rs4676410	**TT**	100 (12.5)	8 (10.5)	**T**	543 (34.7)	46 (30.3)	0.4543	0.2255
	CT	343 (44.2)	30 (39.5)	**C**	1001 (65.3)	106 (69.7)		
	CC	329 (43.3)	38 (50.0)					
rs1250550	**GG**	286 (37.0)	28 (36.8)	**G**	938 (60.8)	94 (61.8)	0.8373	0.7926
	GT	366 (47.4)	38 (50.0)	**T**	606 (39.2)	58 (38.2)		
	TT	120 (15.6)	10 (13.2)					
rs2531875	**GG**	71 (9.2)	6 (7.9)	**G**	460 (29.8)	46 (30.3)	0.8150	0.9037
	GT	318 (41.2)	34 (44.7)	**T**	1084 (70.2)	106 (69.7)		
	TT	383 (49.6)	36 (46.6)					
rs7282490	**AA**	170 (22.0)	14 (18.4)	**A**	708 (45.9)	70 (46.1)	0.4477	0.9628
	AG	368 (47.7)	42 (55.3)	**G**	836 (54.1)	82 (53.9)		
	GG	234 (30.3)	20 (26.3)					

AS: Ankylosing Spondylitis

Table 7: Genotype and allele frequencies of the indicated polymorphisms in AS patients who were positive or negative for HLA-B27.

	rs4676410			**rs2531875**			**Two-locus model**	
Genotype	**TT**	**CT**	**CC**	**GG**	**GT**	**TT**	**High-risk groupδ**	**Low-risk groupδ**
Case no.	100	343	329	71	318	383		
BASDAI	4.94 ± 1.52	5.14 ± 1.33	4.93 ± 1.51	5.13 ± 1.37	5.08 ± 1.29	4.96 ± 1.49	5.11 ± 1.38	4.91 ± 1.56

Unadjusted P value	0.2258 (TT + CT VS. CC)			0.1679 (GG + GT VS. TT)			0.0571	
Adjusted P value	0.2174φ (TT + CT VS. CC)			0.1492φ (GG + GT VS. TT)			0.0558φ	
BASFI	3.74 ± 2.46	3.93 ± 2.82	3.87 ± 2.27	3.96 ± 2.29	3.81 ± 2.35	3.94 ± 2.78	3.94 ± 2.69	3.91 ± 2.45
Unadjusted P value	0.7454 (TT + CT VS. CC)			0.8318 (GG + GT VS. TT)			0.8138	
Adjusted P value	0.7681φ (TT + CT VS. CC)			0.8297φ (GG + GT VS. TT)			0.8062φ	

Data represent means ± S.D.

δAS patients who had the TT genotype of rs4676410, GG genotype of rs2531875, or were heterozygous at both loci were classified into the high-risk group; other patients were classified into the low-risk group.

φAdjusted for age and sex.

Table 8: Difference in the BASDAI and BASFI scores among HLA-B27–positive AS patients stratified by rs4676410 and rs2531875 genotypes.

Discussion

There are distinct genotypic distributions, linkage disequilibria, and haplotype blocks between Caucasians and Asians. Replication studies are needed to validate the association of newly identified susceptibility loci with the risk of AS in different populations. Here we first found the associations between rs4676410/rs2531875 polymorphisms and risk of AS in Han Chinese population. Furthermore, we observed an association between rs4676410 polymorphism and **iridocyclitis**, which was also a novel finding. The allele frequencies of rs12615545, rs4676410, rs1250550, and rs2531875 were similar to those reported in an East Asian cohort (Table 9) [5], while our data of rs7282490 had a marked deviation from that report. This difference requires further validation in different East Asian populations.

Single nucleotide polymorphism	**rs12615545**	**rs4676410**	**rs1250550**	**rs2531875**	**rs7282490**
Risk allele/Ref	**C/T**	**T/C**	**G/T**	**G/T**	**A/G**
	case/control	**case/control**	**case/control**	**case/control**	**case/control**
Europeansa	0.451/0.421	0.232/0.209	0.678/0.652	0.396/0.367	0.411/0.390
East Asiansa	0.710/0.673	0.346/0.312	0.583/0.539	0.296/0.256	0.581/0.543
Current Study	0.669/0.664	0.347/0.313	0.608/0.598	0.298/0.267	0.459/0.431

Table 9: Comparison of allele frequencies of indicated polymorphisms in different studies; a. International Genetics of Ankylosing Spondylitis C (Cortes A et al.)

A previous study also showed that rs4676410 was associated with early-onset IBD susceptibility [12], while it was found to be assoicated with uveitis in AS patients in the present study. rs4676410 lies within the *GPR35* (G protein-coupled receptor 35) gene, which encodes a G protein-coupled receptor. High expression of *GPR35* is found in immune and gastrointestinal tissues. In human invariant natural killer T cells, the specific activation of *GPR35* by selective receptor agonists is functionally correlated with a significant reduction in interleukin-4 (IL-4) release [13]. IL-4 is a cytokine that induces the differentiation of naive helper T cells to Th2 cells and is significantly elevated in AS patients, as compared with that in controls [14]. Furthermore, another SNP in *GPR35* is associated with primary sclerosing cholangitis and ulcerative colitis [15]. Other genes in the LD block of rs4676410 include *CAPN10*, *KIF1A*, and *RNPEPL1*. The effect of the rs4676410 genotype on the expression of these genes should be examined in the future.

The SNP rs2531875 (17q11) is located in the *NOS2* (nitric oxide synthase 2) gene, which encodes a nitric oxide synthase expressed in the liver and is inducible by a combination of lipopolysaccharide and certain cytokines. Inducible nitric oxide synthase (iNOS) activation and increased NO production have been found to contribute to the pathogenesis of osteoporosis in AS patients [16]. Furthermore, lymphocytic infiltration and iNOS expression and activity were detected in duodenal and colonic mucosa from patients with AS [17]. Whether the different genotypes of rs2531875 have an impact on the expression or activity of *NOS2* needs to be determined.

We also performed an association study between rs4676410/rs2531875 and disease severity. Although no association was found between these two polymorphisms and BASDAI/BASFI scores, we found increased BASDAI scores in the high-risk group, which was a combination of AS patients with the TT genotype of rs4676410, GG genotype of rs2531875, or heterozygous genotypes at both loci. These further indicated the involvement of these two polymorphisms in the development of AS.

In summary, the rs4676410 and rs2531875 genetic polymorphisms may be susceptibility factors for AS development in Han Chinese. An association between the rs4676410 T allele and **iridocyclitis** was also observed.

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References

1. Slobodin G, Rosner I, Rimar D, Boulman N, Rozenbaum M, et al. (2012) Ankylosing spondylitis: field in progress. Isr Med Assoc J 14: 763-767.
2. Dillon CF, Hirsch R (2011) The United States National Health and Nutrition Examination Survey and the epidemiology of ankylosing spondylitis. Am J Med Sci 341: 281-283.
3. Australo-Anglo-American Spondyloarthritis Consortium (TASC), Reveille JD, Sims AM, Danoy P, Evans DM, et al. (2010) Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 42: 123-127.
4. Brown MA, Pile KD, Kennedy LG, Campbell D, Andrew L, et al. (1998) A genome-wide screen for susceptibility loci in ankylosing spondylitis. Arthritis Rheum 41: 588-595.
5. International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes A, Hadler J, Pointon JP, Robinson PC, et al. (2013) Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nat Genet 45: 730-738.
6. Pimentel-Santos FM, Ligeiro D, Matos M, Mourão AF, Sousa E, et al. (2009) Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. Clin Exp Rheumatol 27: 800-806.
7. Chen R, Yao L, Meng T, Xu W (2012) The association between seven ERAP1 polymorphisms and ankylosing spondylitis susceptibility: a meta-analysis involving 8,530 cases and 12,449 controls. Rheumatol Int 32: 909-914.
8. Davidson SI, Wu X, Liu Y, Wei M, Danoy PA, et al. (2009) Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population. Arthritis Rheum 60: 3263-3268.
9. Wang CM, Ho HH, Chang SW, Wu YJ, Lin JC, et al. (2012) ERAP1 genetic variations associated with HLA-B27 interaction and disease severity of syndesmophytes formation in Taiwanese ankylosing spondylitis. Arthritis Res Ther 14: R125.
10. 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.
11. Wei JC, Wong RH, Huang JH, Yu CT, Chou CT, et al. (2007) Evaluation of internal consistency and re-test reliability of Bath ankylosing spondylitis indices in a large cohort of adult and juvenile spondylitis patients in Taiwan. Clin Rheumatol 26: 1685-1691.
12. Imielinski M, Baldassano RN, Griffiths A, Russell RK, Annese V, et al. (2009) Common variants at five new loci associated with early-onset inflammatory bowel disease. Nat Genet 41: 1335-1340.
13. Fallarini S, Magliulo L, Paoletti T, de Lalla C, Lombardi G (2010) Expression of functional GPR35 in human iNKT cells. Biochem Biophys Res Commun 398: 420-425.
14. Li GX, Wang S, Duan ZH, Zeng Z, Pan FM (2013) Serum levels of IL-33 and its receptor ST2 are elevated in patients with ankylosing spondylitis. Scand J Rheumatol 42: 226-231.
15. Ellinghaus D, Folseraas T, Holm K, Ellinghaus E, Melum E, et al. (2013) Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4. Hepatology 58: 1074-1083.
16. Armour KE, Van'T Hof RJ, Grabowski PS, Reid DM, Ralston SH (1999) Evidence for a pathogenic role of nitric oxide in inflammation-induced osteoporosis. J Bone Miner Res 14: 2137-2142.
17. Lamarque D, Nhieu JT, Breban M, Bernardeau C, Martin-Garcia N, et al. (2003) Lymphocytic infiltration and expression of inducible nitric oxide synthase in human duodenal and colonic mucosa is a characteristic feature of ankylosing spondylitis. J Rheumatol 30: 2428-2436.

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