RESEARCH ARTICLE

Association of IL1R polymorphism with HLA-B27 positive in Iranian patients with ankylosing spondylitis

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ABSTRACT. ***Objective.*** **Ankylosing spondylitis (AS) is one of the most common causes of inflammatory arthritis, with an estimated prevalence of 0.1-0.9%. Genetic factors have been strongly implicated in its aetiology, and heritability as assessed by twin studies has been estimated to be >90%. HLA- B27 is almost essential for inheritance of AS; it is not merely sufficient for explaining the pattern of familial recurrence of the disease. This study's purpose is to investigate the association of ankylosing spondylitis with single-nucleotide polymorphisms (SNPs) in the IL-1 family: *IL-1a* (-889C/T) rs1800587, *IL-1b* (-511C/T) rs16944, *IL-1b* (+3962C/T) rs1143634, *IL-1R* (Pst-1 1970C/T) rs2234650 and *IL-1RA* (Mspa-1 11100C/T) rs315952.** ***Methods.*** **99 unrelated Iranian AS patients and 217 healthy control subjects were selected. Cytokine typing was performed by the polymerase chain reaction with sequence-specific primers assay. The allele and genotype frequencies of the polymorphisms were determined:** ***Results.*** **The *IL1*α rs1800587, *IL1*β rs16944 and *IL1*β rs1143634 were not significantly associated with AS. Genotype frequencies at *IL1R* rs2234650 differed between cases and controls (χ^2=8.85; p=0.01); the *IL1R* rs2234650 C/T and T/T genotypes were less common in AS patients than controls. The *IL1R* rs2234650 C/T genotype was inversely associated with AS comparing with the *IL1R* rs2234650 C/C genotype (OR=0.48; p=0.005). *IL1R* rs2234650 C/T genotype was less common in patients than controls (OR=0.37; p=0.02).Furthermore *IL1R* rs2234650 T allele was strongly associated with HLA-B2702 patients rather than HLA-B2705 but was not associated with HLA-B27 negative patients (OR=0.33; p=0.01).** ***Conclusion.*** **Polymorphisms of *IL1*α rs1800587, *IL1*β rs16944 and *IL1*β rs1143634 were not significantly associated with ankylosing spondylitis but inversely in this study *IL1R* rs2234650 was significantly associated and carriage of T allele in *IL1R* rs2234650 seems to be protective, while carriage of C allele result in two fold higher risk of developing AS.**

Key words: ankylosing spondylitis, gene polymorphism, IL1 gene cluster, andHLA-B27 subtypes

INTRODUCTION

Ankylosing spondylitis (AS) is a common inflammatory arthropathy with the highest prevalence after rheumatoid arthritis [1]. Prevalence of ankylosing spondylitis is about 0.1-0.9% [2]. In people suffering AS, axial skeleton including the spine and sacroiliac joints is affected and they feel pain, stiffness and bony ankylosis [1]. Some genetic factors are associated with the aetiology and heritability of this disease. One of the genetic factors is the HLA-B27 carriage. This factor is present in >95% of white and 69.7% of Iranian individuals with AS [1, 3, 4].

According to recent genome-wide association studies the MHC locus was the largest cause of susceptibility to AS and six non-MHC regions were related to AS. These regions were located on chromosomes 1p, 2q, 9q, 10q, 16q and 19q [5]. Interleukin 1 (IL-1) gene family is located in these regions [5].

The IL-1 gene family contains IL-1A, IL-1B and IL-1RN and these proteins act via IL-1R [6]. IL-1A and IL-1B are pro-inflammatory cytokines that are synthesized and released against infection. These cytokines act via binding to their receptor (IL-1R). IL-1Ra is an anti-inflammatory molecule encoded by *IL-1RN* and competes with IL-1A and IL-1B for binding to IL-1R [1].

According to the role of IL-1 family as a pro-inflammatory cytokine and their role in autoimmune diseases [7, 8] we investigated some SNPs in these genes in AS patients and healthy controls. Our data demonstrated the IL-1R is associated with susceptibility to AS but it is not the same for IL-1 family in Iranian patients.

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PATIENTS AND METHODS

Subjects

Ninety-nine unrelated ankylosing spondylitis patients (88 males and 11 females), mixed ethnic origins, and residents of Iran, were recruited randomly from Iranian Ankylosing Spondylitis Association and Rheumatology Research Center (RRC) during 2010-2011. The diagnosis of AS in these patients were performed according to the Modified New York Criteria (MNYC) [9]. Two hundred and seventeen unrelated healthy control individuals were also selected from blood donors at Iranian blood transfusion organizations in Tehran [10]. All control subjects were healthy, unrelated, and randomly selected from this region. The Ethical Committee of Tehran University of Medical Sciences approved this project. Written informed consent was obtained from all subjects before sampling.

Genotyping

Cytokine gene typing was performed by Polymerase Chain Reaction (PCR) with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany) [10]. Amplification was carried out using a thermal cycler TechneFlexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on an ultraviolet trans-illuminator, and photography for interpretation and documentation was performed. Each of the primer mixes contained a control primer pair that amplified either a part of the β-globin gene or a part of the C-reactive protein gene. The β-globin control primers produce an 89-bp fragment, while the primer pairs amplifying the CRP gene produced a 440 bp amplicon. The allele and genotype frequencies of the following pro-inflammatory cytokine genes were determined: *IL-1*α (C/T -889), *IL-1*β (C/T -511), *IL-1* β (C/T +3962), *IL-1R* (C/T Pst-I 1970), and *IL-1RA* (C/T Mspa-I 11100) (*table 1*).

Analysis

Genotypes from 99 patients (23 HLAB27 negative, 27 HLAB2702 and 49 HLAB2705) and 217 controls (all of them HLAB27 negative) were examined for those with and without AS in Iranian population. For each locus, the chi-square (χ^2) statistic (or Fisher's exact test statistic for cell expected frequency <5) was calculated to compare the distribution of genotypes in patients and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression, a parametric method that allows adjustment for confounders and analysis of potential interactions in diseases with complex genetic aetiologies [11, 12], to compare genotypes with variant allele with the homozygous wild-type genotype. We did not adjust for multiple comparisons.

Single locus regression models were run to estimate the effects of each of the five polymorphisms: for each locus we examined each genotype and carriage of the variant allele (either homozygous or heterozygous) compared to the homozygous wild-type genotype.

A multivariable logistic regression model was developed including all five loci, to adjust for potential confounding by linkage disequilibrium between adjacent polymorphisms. Only the main effects at each locus were examined.

To assess the effects of each polymorphism stratified by presence or absence of HLA-B27 and its subtypes in AS patients, single locus models were run at each locus separately. Also independent association of each locus was estimated in a multiple logistic regression model containing all five, thereby taking into account potential confounding by adjacent polymorphisms.

When sparse or zero samples occurred, the logistic regression using Firth's bias reduction [13] was employed. All statistical analyses were performed in statistical package R [14].

RESULTS

Table 2 shows the frequency of *IL1*α, *IL1*β, *IL1R* and *IL1Ra* genotypes in patients suffering from AS and healthy controls. Genotype frequencies were in Hardy-Weinberg equilibrium. The *IL1*α rs1800587, *IL1*β rs16944 and *IL1*β rs1143634 were not significantly associated with AS. Genotype frequencies at *IL1R* rs2234650 were different in patients comparing with controls (χ^2=8.85; p=0.01). The *IL1R* rs2234650C/T genotype was inversely associated with AS compared to the *IL1R* rs2234650C/C genotype (OR=0.48; p=0.005). Carriage of the *IL1R* rs2234650T allele was inversely associated with AS in single locus model (OR=0.48; p=0.003). The apparent inverse association was pronounced after adjusting for confounding effects of other polymorphisms (OR=0.47; p=0.004). Viewed as a protective factor, the *IL1R* rs2234650C/C genotype was associated with about two-fold increased risk of AS compared with carriage of the *IL1R* rs2234650T allele (OR=2.13,95%CI 1.28 to 3.57; p=0.004). There was no significant effect for number of copies of IL1R rs2234650T allele. The *IL1Ra* rs315952 was not significantly associated with AS.

Table 1
Markers genotyped

RS number	Name	Gene	Location/exon	Variant	Chromosome 2 Position
rs1800587	IL1α -889	*IL1A*	5'UTR	C/T	113542960
rs16944	IL1β -511	*IL1B*	Promotor	C/T	113594867
rs1143634	IL1β +3962	*IL1B*	5	C/T	113590390
rs2234650	IL1R pst 1970	*IL1R1*	promotor	C/T	102758327
rs315952	IL1Ra mspa -11100	*IL1RA*	7	C/T	113890304

Position are given from NCBI 2011 (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp&cmd=search&term=)

Table 2
Frequencies of *IL1*α, *IL1*β, *IL1R*, *IL1RA* genotypes and associations with AS *

Genotypes	Cases (n=99) n (%)	Controls (n=217) n (%)	OR (95% CI)†
*IL1*α rs1800587			
C/C	48 (48.5)	95 (44.6)	1.0 (referent)
C/T	41 (41.4)	97 (45.5)	0.84 (0.50 to 1.38)
T/T	10 (10.1)	21 (9.9)	0.94 (0.41 to 2.16)
χ^2 (2df)‡	0.49, p=0.79		
C/T or T/T vs. C/C (single locus model)			0.85 (0.53 to 1.38)
C/T or T/T vs. C/C (five locus model)			1.07 (0.62 to 1.85)
*IL1*β rs16944			
C/C	29 (29.3)	49 (22.7)	1.0 (referent)
C/T	53 (53.5)	137 (63.4)	0.65 (0.37 to 1.14)
T/T	17 (17.2)	30 (13.9)	0.96 (0.45 to 2.03)
χ^2 (2df)	2.79, p=0.25		
C/T or T/T vs. C/C (single locus model)			0.71 (0.41 to 1.21)
C/T or T/T vs. C/C (five locus model)			0.72 (0.41 to 1.28)
*IL1*β rs1143634			
C/C	55 (55.6)	103 (47.5)	1.0 (referent)
C/T	34 (34.3)	99 (45.6)	0.64 (0.39 to 1.07)
T/T	10 (10.1)	15 (6.9)	1.25 (0.53 to 2.96)
χ^2 (2df)	3.82, p=0.15		
C/T or T/T vs. C/C (single locus model)			0.72 (0.45 to 1.16)
C/T or T/T vs. C/C (five locus model)			0.61 (0.35 to 1.06)
IL1R rs2234650			
C/C	57 (57.6)	86 (39.6)	1.0 (referent)
C/T	34 (34.3)	107 (49.3)	0.48 (0.29 to 0.80) [a]
T/T	8 (8.1)	24 (11.1)	0.50 (0.21 to 1.20)
χ^2 (2df)	8.8, p=0.01		
C/T or T/T vs. C/C (single locus model)			0.48 (0.30 to 0.78) [b]
C/T or T/T vs. C/C (five locus model)			0.47 (0.28 to 0.78) [c]
IL1Ra rs315952			
T/T	64 (64.6)	119 (55.1)	1.0 (referent)
C/T	35 (35.4)	97 (44.9)	0.67 (0.41 to 1.10)
χ^2 (1df)	2.5, p=0.11		
C/T or C/C vs. T/T (five locus model)			0.72 (0.43 to 1.20)

*Frequencies based on total number shown except as follows: *IL-1*α rs1800587based on 213 controls, *IL-1*β rs16944 based on 216 controls and *IL-1Ra* rs315952based on 216 controls.
†OR (odds ratios) and 95% CI (confidence intervals) were estimated using logistic regression models for each locus separately (single locus model) and adjusting for genotypes at all five loci (five locus model) as indicated.
‡ χ^2 statistic shown.
[a]p=0.005, [b]p=0.003, [c]p=0.004

Also the frequencies of *IL1*α, *IL1*β, *IL1R* and *IL1Ra* genotypes in patients with HLA-B2705, B2702 and B27 negative were analyzed (results were not shown). None of the polymorphisms were associated with HLA-B27.

Table 3 shows the frequency of *IL1*α, *IL1*β, *IL1R* and *IL1Ra* genotypes in patients stratified according to HLA-B27 subtypes and controls. Genotype frequencies at *IL1R* rs2234650 differed among cases with *HLA-B2702* and controls (χ^2=7.36; p=0.02); we observed that the *IL1R* rs2234650C/T genotype were less common in these patients than controls (OR=0.37; p=0.02).Carriage of the *IL1R* rs2234650T allele was inversely associated with *HLA-B2702* AS in single locus model (OR=0.33; p=0.01). The significant inverse association was pronounced after

Table 3
Frequencies of IL 1α, IL 1β, IL 1R, IL 1RA genotypes and associations with AS (stratified cases according to the HLA-B27)

	Cases (n=99)			Controls (n=217)	OR (95%CI)†			
Genotype	***HLA-B2705*** **(n=49)** **n (%)**	***HLA-B270 2*** **(n=27)** **n (%)**	**HLA-B27 neg (n=23)** **n (%)**	**n (%)**	***HLA-B2705*** ***vs*** **controls**	***HLA-B2702*** ***vs*** **controls**	**HLA-B27 pos** ***vs*** **controls**	**HLA-B27 neg** ***vs*** **controls**
*IL1*α rs1800587								
C/C	22 (44.9)	13 (48.1)	13 (56.5)	95 (44.6)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
C/T	22 (44.9)	10 (37.0)	9 (39.1)	97 (45.5)	0.98 (0.51 to 1.89)	0.75 (0.31 to 1.80)	0.89 (0.51 to 1.56)	0.69 (0.28 to 1.64)
T/T	5 (10.2)	4 (14.8)	1 (4.3)	21 (9.9)	1.03 (0.35 to 3.03)	1.39 (0.41 to 4.70)	1.16 (0.49 to 2.78)	0.49 (0.05 to 2.20)
χ^2 (2df)‡					0.01, p=0.99	1.02, p=0.60	0.39, p=0.83	1.51, p=0.47
C/T or T/T *vs* C/C (single locus model)					0.99 (0.53 to 1.84)	0.87 (0.39 to 1.93)	0.94 (0.56 to 1.60)	0.62 (0.26 to 1.47)
C/T or T/T *vs* C/C (five locus model					1.10 (0.53 to 2.28)	1.02 (0.41 to 2.52)	1.10 (0.60 to 2.02)	0.87 (0.33 to 2.29)
*IL1*β rs16944								
C/C	15 (30.6)	6 (22.2)	8 (34.8)	49 (22.7)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
C/T	23 (46.9)	19 (70.4)	11 (47.8)	137 (63.4)	0.55 (0.26 to 1.13)	1.08 (0.44 to 2.99)	0.71 (0.39 to 1.33)	0.49 (0.19 to 1.29)
T/T	11 (22.4)	2 (7.4)	4 (17.4)	30 (13.9)	1.20 (0.49 to 2.95)	0.62 (0.11 to 2.63)	1.01 (0.44 to 2.31)	0.82 (0.23 to 2.95)
χ^2 (2df)					4.73, p=0.09	0.95, p=0.62	1.58, p=0.45	2.27, p=0.32
C/T or T/T vs. C/C (single locus model)					0.66 (0.33 to 1.32)	1.03 (0.41 to 2.92)	0.77 (0.42 to 1.39)	0.55 (0.22 to 1.37)
C/T or T/T vs. C/C (five locus model)					0.67 (0.33 to 1.38)	1.06 (0.39 to 2.90)	0.81 (0.43 to 1.52)	0.44 (0.16 to 1.20)
*IL1*β rs1143634								
C/C	25 (51.0)	15 (55.6)	15 (65.2)	103 (47.5)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
C/T	21 (42.9)	8 (29.6)	5 (21.7)	99 (45.6)	0.87 (0.46 to 1.66)	0.55 (0.22 to 1.37)	0.75 (0.43 to 1.31)	0.35 (0.12 to 0.99) [i]
T/T	3 (6.1)	4 (14.8)	3 (13.0)	15 (6.9)	0.82 (0.22 to 3.07)	1.83 (0.54 to 1.37)	1.20 (0.46 to 3.17)	1.37 (0.35 to 5.31)
χ^2 (2df)					0.21, p=0.90	3.65, p=0.16	1.42, p=0.49	5.11,, p=0.08
C/T or T/T vs. C/C (single locus model)					0.87 (0.47 to 1.61)	0.72 (0.32 to 1.62)	0.81 (0.48 to 1.37)	0.48 (0.20 to 1.18)
C/T or T/T vs. C/C (five locus model)					0.75 (0.36 to 1.58)	0.57 (0.22 to 1.44)	0.69 (0.37 to 1.27)	0.39 (0.13 to 1.14)
IL1R pst rs2234650								
C/C	27 (55.1)	18 (66.7)	12 (52.2)	86 (39.6)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
C/T	17 (34.7)	8 (29.6)	9 (39.1)	107 (49.3)	0.51 (0.26 to 0.99) [a]	0.37 (0.15 to 0.85) [c]	0.45 (0.25 to 0.79) [f]	0.61 (0.24 to 1.48)
T/T	5 (10.2)	1 (3.7)	2 (8.7)	24 (11.1)	0.66 (0.23 to 1.91)	0.29 (0.03 to 1.23)	0.48 (0.18 to 1.25)	0.71 (0.13 to 2.58)
χ^2 (2df)					4.11 ,p=0.13	7.36 ,p=0.02	8.74 ,p=0.01	1.35 ,p=0.51
C/T or T/T vs. C/C (single locus model)					0.53 (0.29 to 0.99) [b]	0.33 (0.14 to 0.75) [d]	0.45 (0.27 to 0.77) [g]	0.60 (0.25 to 1.43)

Table 3
(*Continued*)

	Cases (n=99)			Controls (n=217)	OR (95%CI)†			
Genotype	***HLA-B2705*** **(n=49) n (%)**	***HLA-B270 2*** **(n=27) n (%)**	**HLA-B27 neg (n=23) n (%)**	**n (%)**	***HLA-B2705*** ***vs*** **controls**	***HLA-B2702*** ***vs*** **controls**	**HLA-B27 pos *vs* controls**	**HLA-B27 neg *vs* controls**
C/T or T/T vs. C/C (five locus model)					0.56 (0.29 to 1.07)	0.30 (0.12 to 0.71) [e]	0.45 (0.26 to 0.78) [h]	0.52 (0.21 to 1.31)
IL1Ra rs315952								
T/T	32 (65.3)	15 (55.6)	17 (73.9)	119 (55.1)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
C/T	17 (34.7)	12 (44.4)	6 (26.1)	97 (44.9)	0.65 (0.34 to 1.24)	0.98 (0.44 to 2.19)	0.76 (0.44 to 1.29)	0.43 (0.16 to 1.14)
χ^2 (1df)					1.70, p=0.19	0.00, p=0.96	1.04, p=0.31	3.00, p=0.08
C/T or C/C vs. T/T (five locus model)					0.70 (0.36 to 1.36)	1.04 (0.45 to 2.38)	0.82 (0.47 to 1.42)	0.51 (0.19 to 1.38)

†OR (odds ratios) and 95% CI (confidence intervals) were derived from logistic regression models comparing the common homozygous genotype with other genotypes. Values are derived from unconditional logistic models.When sparse or zero samples occurred, the logistic regression using Firth's bias reduction was employed.
[a]p=0.046, [b] p=0.05, [c] p= 0.02, [d] p=0.01, [e] p=0.006, p=0.005, p=0.003, p=0.004, p=0.048

adjusting for confounding effects of other polymorphisms (OR=0.30; p=0.02).Viewed as a protective factor, the *IL1R* rs2234650C/C genotype increased risk of HLA-B2702 AS about three-fold, compared to carriage of the *IL1R* rs2234650T allele (OR=3.33; 95%CI 1.41 to 8.33; p=0.02). There was no significant effect for number of copies of *IL1R* rs2234650T allele.

The *IL1R* rs2234650C/T genotype was also less common in cases with *HLA-B2705* than controls (OR=0.51; p=0.046) but overall, there was not significant association between the *IL1R* rs2234650 and AS with *HLA-B2705*. Carriage of the *IL1R* rs2234650T allele was inversely associated with *HLA-B2705* AS in single locus model (OR=0.53; p=0.05) but the inverse association did not pronounce after adjusting for confounding effects of other polymorphisms (OR=0.56; p=0.08).

When we pooled the genotype frequency of *HLA-B2705* and *B2702* cases, again the distribution of *IL1R* rs2234650 was different in HLA-B27 positive cases compared to healthy controls (χ^2=8.74; p=0.01).The *IL1R* rs2234650C/T genotype was inversely associated with HLA-B27 positive AS compared with the *IL1R* rs2234650C/C genotype (OR=0.45; p=0.005). Carriage of the *IL1R* rs2234650T allele was inversely associated with HLA-B27-positive AS in both single (OR=0.45; p=0.003) and five (OR=0.45; p=0.004) locus model. Viewed as a protective factor, the *IL1R* rs2234650C/C genotype increased the risk of HLA-B27-positive AS about two-fold, compared with carriage of the *IL1R* rs2234650T allele (OR=2.22,95%CI 1.28 to 3.85; p=0.004). There was no significant effect for number of copies of *IL1R* rs2234650T allele.

The *IL1*β rs1143634C/T genotype was significantly less common in HLA-B27 negative cases than controls (OR=0.35; p=0.048), but there was no overall association between *IL1*β rs1143634 genotypes with HLA-B27 negative AS (χ^2=5.11; p=0.08).

DISCUSSION

As far as we know this is the first study simultaneously examining these five loci with respect to ankylosing spondylitis and to report an association between allelic variations at *IL1R* rs2234650 and ankylosing spondylitis. *IL1R* rs2234650C/C genotype was associated with a two-fold higher risk of ankylosing spondylitis than carriage of T allele. We did not observe a dose effect for the number of copies of *IL1R* rs2234650T allele. However, repeating this study with larger sample size would provide greater power to detect such correlation. No study was performed on association of *IL1R* and ankylosing spondylitis till now. The *IL-1*α rs1800457, *IL1*β rs16944, *IL1*β rs1143634 and *IL1Ra* rs315952 polymorphisms was not significantly associated with ankylosing spondylitis.

In a study performed on IL1 gene cluster and its association with AS by Maksyowych *et al.* (2006, Canada) association of several SNPs with *IL-1A* and *IL-1B* genes and AS was reported, while no correlation between rs114360, rs1800587 and rs1143627 SNPs and the disease were observed [2]. However, another study in England demonstrated significant association between AS and rs1800587 and rs16944 SNPs [15] on the other hand, a survey on Taiwanese-Chinese population showed no correlation between s16944 and the disease [16].

In the second part of this study the frequency of *IL1A IL1B* and *ILRN* genotypes in patients stratified according to HLA-B27 subtypes and controls. *IL1R* rs2234650C/C genotype was three times more associated with risk of *HLA-B2702* ankylosing spondylitis compared to carriage of rs2234650T allele. We did not observe a dose effect for the number of copies of IL1R rs2234650T allele. This occurred again when we pooled genotype frequency of *HLA-B2705* and *B2702* (HLA-B27 positive) cases.

Association of *IL1B*, *IL1A* and *IL1RN* polymorphism and *HLA-B2702* and *HLA-B2705* ankylosing spondylitis has been studied for the first time. Correlation between

rs2234650 in IL1R and HLA-B27 and its subtypes were observed clearly in this population.

90% of patients suffering from ankylosing spondylitis are HLA-B27 positive, it is 70% about Iranian population, However about 2% of the world's population are HLA-B27 positive and only 1-5% of these people suffer from ankylosing spondylitis [17].

These statistics show that other genetic factors interfere the process, the roles of which are much more obvious in Iranian patients. These candidate genes according to previously performed studies are *ERAP-1*, *IL-1R2*, *IL-1R1, IL-1Ra*, *IL-23R* and *KIR* respectively, which is based on significances of various SNPs of these genes in different populations [18, 19].

CONCLUSIONS

The observed associations with ankylosing Spondylitis and HLA-B27 subtypes support the hypothesis that genetic variation in *IL1R* rs2234650 is involved in the aetiology of Ankylosing spondylitis and merit further investigation in other cytokine and candidate SNPs for determination of their association with HLA-B27 positive and negative AS patients.

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