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Activating killer immunoglobulin-like receptors genes are associated with increased susceptibility to ankylosing spondylitis

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Summary

The aim of this study was to analyse the association of specific killer cell immunoglobulin-like receptors (KIR) genes and haplotypes with susceptibility to ankylosing spondylitis (AS) and its different clinical manifestations in a Spanish population. The presence or absence of all KIR genes was studied for their association with AS. A total of 176 patients with AS and 435 healthy control subjects were selected for this study based on clinical criteria. The commercial KIR-sequence-specific oligonucleotides (SSO) typing kit was used to investigate KIR typing. Frequencies of KIR2DS1 and KIR3DS1 genes were increased significantly in patients compared with healthy controls [52·8 *versus* 38·2%, $P_{Bonf} < 0{\cdot}01$, odds ratio (OR) = 1·81 (1·28–2·59); 51·7 *versus* 37·5%, $P_{Bonf} < 0{\cdot}01$, OR = 1·79 (1·25–2·54)]. Moreover, the frequency of activating genotypes in the AS patient group was significantly higher than in the healthy control group ($P < 0{\cdot}05$). KIR2DS1 and KIR3DS1, in addition to human leucocyte antigen (HLA)-B27, may play an important role in the pathogenesis of AS. However, we show that the contribution of the KIR genes to AS susceptibility extends beyond the association with individual KIRs, with an imbalance between activating and inhibitory KIR genes seeming to influence the susceptibility to AS.

Keywords: ankylosing spondylitis, HLA-B27, KIR, susceptibility

Introduction

Ankylosing spondylitis (AS) is the prototypic spondyloarthropathy, a group of diseases involving the axial skeleton. Particularly, AS can involve peripheral joints or extra-articular sites such as the uvea and tendon insertions. Like many other autoimmune diseases, AS is a complex disease where genetic variants, environmental factors and random events interact to trigger pathological pathways. It has long been known that AS is highly heritable (> 90%), and this heritability is also significant with respect to the clinical manifestations of the disease [1,2]. Heritabilities for radiographic disease severity, age of symptom onset and for disease activity measured by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Index (BASFI) questionnaires have been estimated (40, 62, 51 and 76%, respectively) [2].

The association between human leucocyte antigen (HLA)-B27 and AS is unequivocal. It has been demonstrated worldwide, and evidence for the role of HLA-B27 in AS comes from linkage and association studies and transgenic animal models [2–4]. Nevertheless, HLA-B27 accounts for only about 16% of the total genetic risk for the disease [1]. AS is a complex genetic trait, and HLA-B27 is only one of a number of genes implicated. The challenge, therefore, should be to identify genetic variants involved in the different clinical manifestations and AS-associated features.

Linkage results have shown that some genes on chromosome 19q could contribute to the development of AS [5]. The killer immunoglobulin-like receptor (KIR) gene family is located on human chromosome 19 within the leucocyte receptor complex (LRC) in region 19q13.4 encoding receptors with distinctive immunoglobulin-like extracellular domains. The KIR gene family currently consists of 15 genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3 and KIR3DS1) and two pseudogenes (KIR2DP1 and KIR3DP1), and they have been classified into two types. One type consists of activating KIRs (KIR2DS and KIR3DS), and the other type consists of inhibitory KIRs

(KIR2DL and KIR3DL), some of which recognize HLA class I molecules with varying affinities and modulate the activation and inhibition of natural killer (NK) cells [6]. Haplotypic variability exists due to the different number and nature of KIR genes, two basic groups, haplotypes A and B, being recognized. Haplotype A contains only one activating KIR gene (KIR2DS4), whereas haplotype B contains various combinations of activating KIR genes: KIR2DS1, 2DS2, 2DS3, 2DS5, 3DS1 and 2DS4. The extensive polymorphism of KIR genes results in different signalling potentials towards NK and T cells [6], creating a further level of complexity.

Different associations of KIR genes with spondyloarthropathies and other autoimmune disorders have been described [7,8]. Regarding AS, genetic associations in distinct populations have been noted, although no reported association has been replicated widely. Two different studies showed increases in the frequency of the KIR3DS1 and KIR2DL5 genes in East Asian populations [9,10]. In white Europeans we have reported genetic evidence for an association of the KIR3DL1/3DS1 locus in AS susceptibility [11,12], taking into account HLA-B27-positive populations. However, the absence of an association of KIR variants with AS in a British cohort has been reported [13,14]. A study in a Chinese HLA-B27-positive population also reported an increased gene frequency of KIR2DL1 and KIR2DL5 [15]. Moreover, the role of the HLA-B27 heavy chain homodimers and their recognition by KIR receptors in the pathogenesis of spondyloarthropathies has been studied [16]. The impact of KIR genes involved in the AS-associated features has not yet been investigated widely. In this study, we analysed the possible association of specific KIR genes and haplotypes with the susceptibility to AS and different clinical manifestations in a Spanish population.

Methods

A total of 176 patients with AS diagnosed following New York criteria [17] were enrolled into the study, and 435 unrelated healthy individuals were enrolled as controls. All patients and controls gave written informed consent prior to enrolment. The patients were diagnosed at the Rheumatology Units of the Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain, and at the Rheumatology Unit of the Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain. All patients completed a questionnaire that included date of AS diagnosis, BASDAI, BASFI and the occurrence of uveitis, psoriasis and inflammatory bowel disease (IBD), verified in the corresponding medical units. The protocol was approved by the ethics committees of our hospitals and conducted according to the Declaration of Helsinki.

For KIR typing, the commercial KIR sequence-specific oligonucleotides (SSO) typing kit from Luminex (Tepnel Lifecodes, Stamford, CT, USA) was used, according to the manufacturer's instructions. KIR3DL1 and KIR3DS1 were considered as alleles from the same locus. The same was true for KIR2DL2 and KIR2DL3. The KIR genotypes were deduced from KIR profiles, as described previously [8,9]. Thus, the genetic profile of this region was distributed into two haplotypes, A and B, based on the presence or absence of the activating KIRs. Many activating genes are present in the B haplotype, and individuals with either one (AB heterozygotes) or two (BB homozygotes) activating haplotypes were given the genotype designation of Bx.

Data were analysed using SPSS version 15 software (SPSS, Inc., Chicago, IL, USA). The significance of the associations was determined using the χ^2 test with Yates's correction or Fisher's exact test. The 95% confidence interval (95% CI) of the calculated odds ratio (OR) was estimated. The χ^2 test was used to test for Hardy–Weinberg equilibrium (HWE) by comparing the observed number of subjects for each of the genotypes with the expected number of subjects, assuming the existence of HWE. Unconditional logistic regression was used to compute the odds ratios (ORs) and their 95% CIs, with adjustments for several covariates found to be associated with AS risk.

Results

The frequency of each KIR gene in AS patients and controls is shown in Table 1. Two framework genes (KIR3DL2 and KIR3DL3) are present in each of the AS cases and controls. KIR gene frequencies of KIR2DS1 and KIR3DS1 were increased significantly in patients compared with healthy controls (52·8 *versus* 38·2%, $P_{BONF} < 0{\cdot}01$, OR = 1·81, 95% CI = 1·28–2·59; 51·7 *versus* 37·5%, $P_{BONF} < 0{\cdot}01$, OR = 1·79, 95% CI = 1·25–2·54). We also analysed the frequencies of AA and Bx genotypes in AS patients and healthy controls (Table 1). While the frequency of the AA genotype of the study group was significantly lower than in the healthy control group, the rate of Bx genotypes was significantly higher in the AS patients ($P < 0{\cdot}05$).

We also tested whether the presence or absence of particular clinical manifestations of AS might have different genetic backgrounds with respect to KIR genes (Table 1). KIR2DL5 gene frequency was increased in AS patients presenting uveitis episodes compared to AS patients without uveitis episodes (82·4 *versus* 64·1%, $P < 0{\cdot}05$, OR = 2·62, 95% CI = 1·02–6·74). We also compared KIR gene frequencies in the patients with AS (with or without uveitis) with the control subjects. There was a trend towards increased frequencies of the KIR genes KIR3DS1 and KIR2DS1 in the AS patients presenting and not presenting uveitis episodes compared with the control subjects. Again, KIR2DL5 reached statistical significance when we compared AS patients presenting uveitis episodes and healthy controls (82·4 *versus* 57·7%, $P_c < 0{\cdot}01$, OR = 3·42, 95% CI = 1·39–8·43). A trend was observed with the age of onset of AS:

Table 1. Distribution of killer cell immunoglobulin-like receptor (KIR) gene frequencies and KIR genotypes, among ankylosing spondylitis (AS) patients compared to healthy controls.

			AS patients	
KIR genes	Controls, % ($n = 435$)	AS patients, % ($n = 176$)	Uveitis-negative ($n = 142$)	Uveitis-positive ($n = 34$)
2DL1	423 (97·2)	170 (96·6)	136 (95·8)	32 (94·1)
2DL2	265 (60·9)	122 (69·3)	92 (64·8)	22 (64·7)
2DL3	358 (82·3)	143 (81·3)	106 (74·7)	25 (73·5)
2DL4	434 (99·7)	176 (100)	142 (100)	34 (100)
2DL5[§]	251 (57·7)	120 (68·2)	91 (64·1)	28 (82·4)
3DL1	407 (93·6)	163 (92·6)	131 (92·3)	32 (94·1)
2DS1*	166 (38·2)	93 (52·8)	67 (47·2)	19 (55·9)
2DS2	260 (59·8)	110 (62·5)	84 (59·2)	23 (67·6)
2DS3	154 (35·4)	55 (31·3)	39 (27·5)	13 (38·2)
2DS4	407 (93·6)	157 (89·2)	131 (92·3)	32 (94·1)
2DS5	155 (35·6)	73 (41·5)	58 (40·8)	11 (32·4)
3DS1[†]	163 (37·5)	91 (51·7)	65 (45·8)	19 (55·9)
KIR genotypes				
AA	118 (27·1)	33 (18·8)	29 (20·4)	5 (14·7)
Bx[‡]	317 (72·9)	143 (81·2)	113 (79·6)	29 (85·3)

Values are the number (%). *Controls *versus* AS patients: $P_B < 0{\cdot}01$, odds ratio (OR) = 1·81, 95% confidence interval (CI) = 1·28–2·59. [†]Controls *versus* AS patients: $P_B < 0{\cdot}05$, OR = 1·79, 95% CI = 1·25–2·54. [‡]Controls *versus* AS patients: $P < 0{\cdot}05$, OR = 1·61, 95% CI = 1·05–2·49. [§]Uveitis-negative AS patients *versus* uveitis-positive AS patients: $P_c < 0{\cdot}05$, OR = 2·62, 95% CI = 1·02–6·74. Controls *versus* uveitis-positive AS patients: $P_c < 0{\cdot}01$, OR = 3·42, 95% CI = 1·39–8·43. AS = ankylosing spondylitis; P = one-tailed Fisher's exact test; Pc = two-tailed Fisher's exact test; P_B = Bonferroni-corrected P.

KIR2DL5 gene frequency was increased in AS patients whose age at AS diagnosis was ≤17 years compared to AS patients diagnosed at age ≥18 years (84·6 *versus* 67·5%) (data not shown). No associations were found between KIR genes and the simultaneous presence of psoriasis or/and IBD, BASDAI and BASFI.

Discussion

Given the class I specificity and immunomodulatory functions of KIRs, disease studies have been conducted showing their associations with spondyloarthropathies and other autoimmune disorders [7,8]. These studies have proposed a model in which KIRs synergize with HLAs to generate genotypes that provide different levels of activation and inhibition of NK or T cells. Regarding AS, the hypothesis that KIR is associated with AS-related pathogenesis is still controversial. Genetic associations in distinct populations have been described, although no reported association has been widely replicated [9–12]. A meta-analysis carried out in order to investigate the impact of the KIR genes on susceptibility to AS indicated that the KIR2DL1, KIR2DS4, KIR2DS5 and KIR3DS1 genes might be associated closely with susceptibility to AS [18]. However, few association studies of candidate genes and clinical manifestations in AS have been performed.

In this study we found a significantly higher frequency of the Bx genotype in our AS patient group and a higher frequency of the AA genotype in the control group. It is known that diseases can be modified by specific KIR–ligand interactions by direct binding of KIR molecules to their putative HLA ligands [19,20]. Because of the biological significance of the A/B haplotype difference, it is conceivable to infer that combinations of B haplotypes can influence AS susceptibility. Indeed, the present study has confirmed that such a situation occurs in this set of patients. Individuals diagnosed with AS possessing greater numbers of activating KIR genes might exhibit increased transmission of activating signals to NK or T cells, enhancing their genetic susceptibility to AS.

Our results suggest that two activating genes, KIR2DS1 and KIR3DS1, seem to increase the risk of developing AS, confirming earlier results in East Asian populations [9,10]. A previous study showed elevated expressions of KIR2DS1 and KIR3DS1 in AS patients compared to healthy controls [21], suggesting a role for excessive or inappropriate NK cell activation through the KIR/HLA system, and revealing that KIR2DS1 and KIR3DS1 might be largely responsible for the enhancement of the activity of NK cells in AS patients. In fact, the activation of NK or T cells via the KIR3DS1 receptor may be considered as one of the critical events in AS development [22]. We also report here that the inhibitory KIR2DL5 is associated with an increased risk of AS in patients presenting uveitis episodes compared to patients without uveitis episodes. This study is the first to show that a KIR gene is associated with clinical

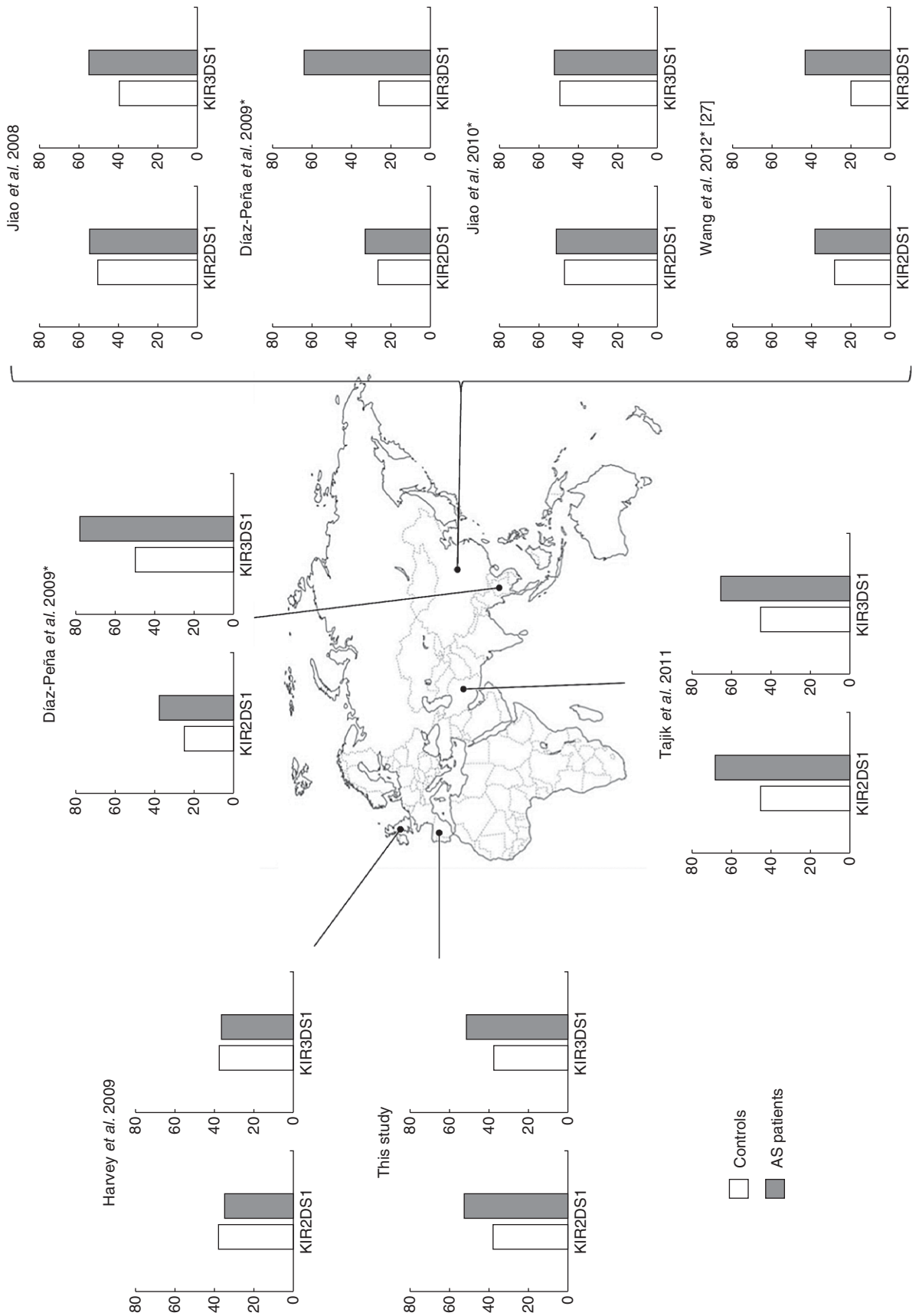

Fig. 1. Killer immunoglobulin-like receptor (KIR)2DS1 and KIR3DS1 gene frequencies in ankylosing spondylitis (AS) patients and controls from published studies. *Human leucocyte antigen (HLA)-B27-positive populations (AS patients and healthy controls).

manifestations of AS. Although the KIR2DL5 ligand is still undetermined, its influence in psoriasis vulgaris, coeliac disease, systemic lupus erythematosus, multiple sclerosis and ankylosing spondylitis has been reported [9,10,23–26]. Discovering its interactions will be essential to understand the role of KIR2DL5 in immunity. Future studies should address the functional characterization of the ligand and correlate its presence with the frequency distribution of the KIR2DL5 gene in disease.

We have searched through all studies carried out to evaluate the association of KIR genes and AS, finding that the KIR gene frequency distributions were completely different across populations, except for KIR2DS1 and KIR3DS1 (Fig. 1). In all studies performed, KIR2DS1 and KIR3DS1 frequencies showed a similar trend; KIR frequencies increased in AS patients compared to controls (except the study conducted by Harvey *et al.* [13] in a British population). In conclusion, we show that the contribution of the KIR genes to AS susceptibility clearly extends beyond an association with individual KIRs. These findings emphasize the need for comprehensive and detailed case–control studies in which all KIR genes should be analysed for association with AS, including a complete HLA class I typing and a rigorous clinical characterization incorporating patient response to treatments. For this purpose, international collaborations are necessary in order to include large patient cohorts.

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Disclosure

No conflicts of interest are reported by any of the authors.

References

1 Brown MA, Kennedy LG, MacGregor AJ *et al.* Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997; **40**:1823–8.
2 Thomas GP, Brown MA. Genetics and genomics of ankylosing spondylitis. Immunol Rev 2010; **233**:162–80.
3 Gonzalez-Roces S, Alvarez MV, Gonzalez S *et al.* HLA-B27 polymorphism and worldwide susceptibility to ankylosing spondylitis. Tissue Antigens 1997; **49**:116–23.
4 DeLay ML, Turner MJ, Klenk EI, Smith JA, Sowders DP, Colbert RA. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. Arthritis Rheum 2009; **60**:2633–43.
5 Laval SH, Timms A, Edwards S *et al.* Whole-genome screening in ankylosing spondylitis: evidence of non-MHC genetic-susceptibility loci. Am J Hum Genet 2001; **68**:918–26.
6 Khakoo SI, Carrington M. KIR and disease: a model system or system of models? Immunol Rev 2006; **214**:186–201.
7 Díaz-Peña R, Blanco-Gelaz MA, López-Larrea C. KIR genes and their role in spondyloarthropathies. Adv Exp Med Biol 2009; **649**:286–99.
8 Espeli M, Niederer HA, Traherne JA, Trowsdale J, Smith KG. Genetic variation, Fcγ receptors, KIRs and infection: the evolution of autoimmunity. Curr Opin Immunol 2010; **22**:715–22.
9 Díaz-Peña R, Blanco-Gelaz MA, Suárez-Alvarez B *et al.* Activating KIR genes are associated with ankylosing spondylitis in Asian populations. Hum Immunol 2008; **69**:437–42.
10 Jiao YL, Ma CY, Wang LC *et al.* Polymorphisms of KIRs gene and HLA-C alleles in patients with ankylosing spondylitis: possible association with susceptibility to the disease. J Clin Immunol 2008; **28**:343–9.
11 Lopez-Larrea C, Blanco-Gelaz MA, Torre-Alonso JC *et al.* Contribution of KIR3DL1/3DS1 to ankylosing spondylitis in human leukocyte antigen-B27 Caucasian populations. Arthritis Res Ther 2006; **8**:R101.
12 Díaz-Peña R, Vidal-Castiñeira JR, Alonso-Arias R *et al.* Association of the KIR3DS1*013 and KIR3DL1*004 alleles with susceptibility to ankylosing spondylitis. Arthritis Rheum 2010; **62**:1000–6.
13 Harvey D, Pointon JJ, Sleator C *et al.* Analysis of killer immunoglobulin-like receptor genes in ankylosing spondylitis. Ann Rheum Dis 2009; **68**:595–8.
14 McCappin J, Harvey D, Wordsworth BP, Middleton D. No association of KIR3DL1 or KIR3DS1 or their alleles with ankylosing spondylitis. Tissue Antigens 2010; **75**:68–73.
15 Jiao YL, Zhang BC, You L *et al.* Polymorphisms of KIR gene and HLA-C alleles: possible association with susceptibility to HLA-B27-positive patients with ankylosing spondylitis. J Clin Immunol 2010; **30**:840–4.
16 Kollnberger S, Bowness P. The role of B27 heavy chain dimer immune receptor interactions in spondyloarthritis. Adv Exp Med Biol 2009; **649**:277–85.
17 Van de Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984; **27**:361–8.
18 Zuo HN, Wang ZL, Cui DR, Xin DJ. Genetic variations in the KIR gene family may contribute to susceptibility to ankylosing spondylitis: a meta-analysis. Mol Biol Rep 2014; **41**:5311–19.
19 Yu J, Heller G, Chewning J, Kim S, Yokoyama WM, Hsu KC. Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands. J Immunol 2007; **179**:5977–89.
20 Martin MP, Nelson G, Lee JH *et al.* Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles. J Immunol 2002; **169**:2818–22.
21 Tajik N, Shahsavar F, Poormoghim H, Radjabzadeh MF, Mousavi T, Jalali A. KIR3DL1+HLA-B Bw4Ile80 and KIR2DS1+HLA-C2 combinations are both associated with ankylosing spondylitis in the Iranian population. Int J Immunogenet 2011; **38**:403–9.
22 Zvyagin IV, Mamedov IZ, Britanova OV *et al.* Contribution of functional KIR3DL1 to ankylosing spondylitis. Cell Mol Immunol 2010; **7**:471–6.

23 Suzuki Y, Hamamoto Y, Ogasawara Y *et al*. Genetic polymorphisms of killer cell immunoglobulin-like receptors are associated with susceptibility to psoriasis vulgaris. J Invest Dermatol 2004; **122**:1133–6.
24 Santin I, Castellanos-Rubio A, Perez de Nanclares G, Vitoria JC, Castaño L, Bilbao JR. Association of KIR2DL5B gene with celiac disease supports the susceptibility locus on 19q13.4. Genes Immun 2007; **8**:171–6.
25 Kimoto Y, Horiuchi T, Tsukamoto H *et al*. Association of killer cell immunoglobulin-like receptor 2DL5 with systemic lupus erythematosus and accompanying infections. Rheumatology (Oxf) 2010; **49**:1346–53.
26 García-León JA, Pinto-Medel MJ, García-Trujillo L *et al*. Killer cell immunoglobulin-like receptor genes in Spanish multiple sclerosis patients. Mol Immunol 2011; **48**:1896–902.
27 Wang S, Li G, Ge R *et al*. Association of KIR genotype with susceptibility to HLA-B27-positive ankylosing spondylitis. Mod Rheumatol 2013; **23**:538–41.