Published in final edited form as:

Genes Immun. 2016 December ; 17(7): 396–399. doi:10.1038/gene.2016.36.

Evaluation of KIR3DL1/KIR3DS1 polymorphism in Behçet's disease

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Abstract

The Behçet's disease (BD)-associated HLA allele, HLA-B*51 (B*51), encodes a ligand for a pair of allelic killer immunoglobulin-like receptors (KIR) present on cytotoxic cells — KIR3DL1, which inhibits their cytotoxicity, and KIR3DS1, which activates their cytotoxic activity. We tested whether KIR-regulated mechanisms contribute to BD by testing for association of KIR3DL1/KIR3DS1 genotypes with disease in 1799 BD patients and 1710 healthy controls from Turkey, as well as in different subsets of individuals with HLA-type-defined ligands for the KIR3D receptors. HLA types were imputed from SNP genotypes determined with the Immunochip. The presence of inhibitory KIR3DL1 or activating KIR3DS1 alleles did not differ significantly between cases and controls (KIR3DL1: 92.9% vs 93.4%, $P_{dominant}$ = 0.55; KIR3DS1: 42.7% vs 41.0%, $P_{dominant}$ = 0.29). The KIR3DL1/KIR3DS1 alleles were also present at similar frequencies among cases and controls bearing HLA-B with a Bw4 motif; HLA-B with a Bw4 motif with isoleucine at position 80; and HLA-B*51. Our results suggest that pathogenic mechanisms associated with HLA-B*51 do not primarily involve differential interactions with KIR3DL1 and KIR3DS1 receptors. However, due to the complexity of this locus (i.e., sequence variation, copy number variation), we cannot exclude a role for other types of KIR variation in the pathogenesis of BD.

Introduction

Behçet's disease (BD) is a chronic, multisystem disorder characterized by recurrent self-limited inflammatory episodes affecting the mucocutaneous tissues, eyes, all types and sizes

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Conflict of Interest

The authors have no competing financial interest or other conflicts of interest to disclose with respect to the work described.

of blood vessels, and several other organs and tissues including the joints, lungs, and central nervous and gastrointestinal systems[1]. Although the etiopathogenesis of BD is unknown, it has long been assumed that immunological abnormalities, which may be induced by exogenous or endogenous pathogenic triggers in genetically susceptible individuals, are important in its pathogenesis.[2, 3] Data obtained from observational studies of familial cases suggest a genetic contribution to the tendency to develop BD.[4] A relatively high sibling recurrence risk ratio (λ_s value between 11.4 and 52.5) also supports a contribution of genetic factors[5] and recent genetic studies show a polygenic contribution to disease risk.[6–9] More importantly, association studies have identified the major histocompatibility complex (MHC) class I allele HLA-B5 (B*51) as the strongest genetic susceptibility factor for BD, a result that has been independently replicated in multiple different ethnic groups.[9–12]

Although it is the strongest risk factor for BD, the mechanism by which HLA-B*51 contributes to disease risk remains unknown. A recent study identified multiple disease-associated polymorphic amino acid residues in the MHC Class I HLA-B and HLA-A proteins at positions important to both peptide binding and to their interactions with killer immunoglobulin-like receptors (KIR), which may lead to increased BD risk through the regulation of both innate natural killer (NK) cells and adaptive cytotoxic T lymphocytes.[13]

KIRs are a diverse family of activating and inhibitory receptors expressed on human NK cells and a subset of T cells.[14] They interact with specific class I MHC molecules to regulate the activity and function of NK cells and cytotoxic CD8-positive T cells. Recently, KIR receptors have also been identified on a subset of CD4-positive T cells that are a major source of interleukin-17.[15] The human KIR family genes are located on a region of human chromosome 19q13.4 that is highly polymorphic and varies among individuals in gene number and proportion of genes encoding activating and inhibitory KIRs.[16] Based on differential gene content alone, there are more than 37 different haplotypes, and sequence data reveals multiple alleles of each of the KIR genes.[17] The complex structure of the KIR genomic region has hampered its analysis in SNP-based genome-wide association studies, and therefore a targeted approach is required to evaluate the association of genetically encoded KIR variation with disease.

KIRs are named according to the number of extra-cellular immunoglobulin-like domains (2D or 3D) and the length of the cytoplasmic tail [short (S) or long (L)].[18] The binding of long-tailed KIR (L) proteins by specific class I MHC molecules almost always produces inhibitory signals, whereas engagement of short-tailed KIRs (S) transmits activating signals.[14] Activating and inhibitory signals work in concert to produce an effective and appropriate immune response to infection, while limiting its duration and reducing collateral damage.

The BD-associated HLA-B*51 protein is one of several Class I proteins with the Bw4 epitope that have been shown to interact with KIR3DL1, and presumably with KIR3DS1, which shares nearly identical sequence in the ligand-binding extracellular portion of the molecule.[19] Although not originally appreciated, KIR3DL1 and KIR3DS1 are now recognized as alternate alleles of a single gene.[16] In this study we sought evidence that KIR-regulated signaling contributes to BD by testing for association of the KIR3DL1/KIR3DS1

cytoplasmic tail length variation with BD in a very large and carefully assembled collection of Turkish BD patients and geographically matched, healthy Turkish individuals along with stratification for HLA-Bw4 epitopes and HLA-B*51.

Results

The estimation of classical HLA types was highly accurate. HLA-B*51 genotypes were determined with posterior probability greater than 0.9 in 96.5% of the samples and the concordance rate of imputed HLA-B*51 alleles and directly ascertained HLA-B*51 alleles was 97.6% in the 2213 members of the study population in whom directly ascertained data were available. Allelic inhibitory KIR3DL1 and activating KIR3DS1 polymorphism genotypes were determined for 1799 BD cases and 1710 control subjects. Although rare haplotypes with neither a KIR3DL1 nor a KIR3DS1 allele have been reported[17], none of the 3509 genotyped Turkish DNA samples possessed this null genotype.

Association tests for KIR3DL1/KIR3DS1 were performed in the full case-control collection, as well as in HLA type-defined subsets under additive, dominant, and recessive models. In the full collection, neither the presence of inhibitory KIR3DL1 alleles nor activating KIR3DS1 alleles differed significantly between cases and controls (KIR3DL1 present in 92.9% of cases vs 93.4% of controls, $P_{dominant} = 0.55$; KIR3DS1 present in 42.7% of cases vs 41.0% of controls, $P_{dominant} = 0.29$; Table 1). Furthermore, the alleles were not significantly associated with disease under any of the genotypic models tested. The KIR3DL1/KIR3DS1 polymorphism also did not appear to contribute to disease risk by interacting with HLA-B alleles bearing Bw4 epitopes, which are KIR3DL1/KIR3DS1 receptor ligands (Table 2); with the Bw4 epitope with isoleucine at position 80, which are strongly interacting ligands (Table 3); or even with the HLA-B*51 type, which is the BD-associated type and is one of the Bw4-I80 types (Table 4). Moreover, we did not observe an enrichment of the Bw4-I80 motif among HLA-B*51 negative cases compared with B*51 negative controls (found in 19.8% of cases vs 22.5% of controls, P = 0.155).

To evaluate whether KIR3DL1/KIR3DS1 alleles influenced specific clinical presentations, we examined KIR3DL1 and KIR3DS1 alleles in subsets of the cases with and without specific characteristics (Table 5). This analysis revealed an increased frequency of KIR3DS1 alleles in the subset of cases with ocular disease compared to those without ocular disease ($P_{additive} = 0.0006$; Supplementary Table 1). Given that HLA-B*51 is known to predispose to the ocular manifestations of BD, we repeated this analysis in the HLA-B*51 positive and negative case subsets to evaluate whether the effect of KIR3DS1 on ocular disease was dependent on HLA-B*51. However there was no difference in the effect of KIR3DS1 alleles between the HLA-B*51 positive ($OR_{additive} = 1.3$) and HLA-B*51 negative ($OR_{additive} = 1.3$) subsets. (Supplementary Table 2). Aside from ocular involvement, this analysis identified no association between KIR3DL1/KIR3DS1 alleles and any other clinical characteristic (Supplementary Table 1).

Discussion

In the current study, which comprised a very large and meticulously assembled collection of Turkish BD patients and geographically matched, healthy Turkish individuals with HLA-B type data, we found no association of the activating KIR3DS1 or inhibitory KIR3DL1 alleles with BD, even in an analysis limited to individuals bearing the disease-associated HLA-B*51, a known high avidity ligand for KIR3DL1/KIR3DS1. This result suggests that HLA-B*51 does not influence BD risk through differential interactions with KIR3D1 on the basis of tail length (KIR3DL1 vs. KIR3DS1). Additionally, in the subset of cases with ocular disease, we observed an increase in activating KIR3DS1 alleles among both HLA-B*51 positive and HLA-B*51 negative individuals. These findings suggest that although HLA-B*51 is a known ligand for KIR3DL1, the KIR3DS1 association is not dependent on the presence of HLA-B*51 to increase the risk of ocular involvement, thus supporting a ligand-independent role for this KIR Nonetheless, given mounting evidence of allele-specific differential binding of KIRs by class I MHC molecules,[20] this study does not exclude the possibility that HLA-B*51 may influence BD risk through differential interaction with specific KIR3DL1 and/or KIR3DS1 allotypes.

KIR3DS1 alleles have been associated with response to HIV infection and with ankylosing spondylitis. The activating KIR3DS1 allele is associated with delayed progression to AIDS and protection from opportunistic infections in individuals bearing a Class I Bw4-I80 epitope,[21, 22] presumably because the activating allele promotes efficient killing of virus infected cells. This subset of Bw4 epitopes was tested for association because Class I molecules with Bw4 epitopes with isoleucine at position 80 have a higher avidity for the KIR3D receptors than those with threonine at position 80.[23] The ankylosing spondylitis-KIR3DS1 allele association is less clear. Ankylosing spondylitis is strongly associated with HLA-B*27. Most HLA-B*27 alleles bear the Bw4 epitope, but with threonine at position 80. Two studies reported association of the KIR3DS1 allele with ankylosing spondylitis in HLA-B*27-positive individuals from Spain, the Azores, China, and Thailand,[24, 25] however this association was not found in HLA-B*27-positive individuals from Ireland or the United Kingdom.[26, 27] A recent study suggests a role for a different KIR, KIR3DL2, in HLA-B*27-positive ankylosing spondylitis,[28] raising the question whether a different KIR may also contribute to Behçet's disease.

All of the functional HLA-B*51 subtypes encode the high avidity Bw4 allele with isoleucine at position 80, suggesting a favorable binding affinity in interactions with the KIR3DL1 or KIR3DS1 receptors. In a smaller collection of BD cases and controls Middleton et al[29] failed to identify a disease association of KIR3DL1 or KIR3DS1 in HLA-B*51 positive individuals (93 cases and 43 controls). The large number of individuals included in our study provided us greater power to test for epistasis between HLA-B alleles and KIR3DL1/KIR3DS1 alleles in individuals bearing an HLA-B Bw4 epitope ligand (1523 cases and 1263 controls), in individuals bearing the high avidity Bw4-I80 ligand (1216 cases and 744 controls), and in individuals bearing HLA-B*51 (1072 cases and 463 controls), but we did not find evidence of any such an interaction. Recently, Kuranov and colleagues found an increase of the Bw4-I80 motif HLA types among HLA-B*51-negative BD cases compared with matched controls.[30] In the current and much larger study, we did not observe any

increase in the frequency of individuals bearing the Bw4-I80 epitope among HLA-B*51-negative individuals.

In conclusion, our data for the first time reveal a potential role for activating KIR3DS1 alleles in ocular manifestations of BD in an HLA-B*51 independent manner. This finding suggests that the pathogenic mechanism of KIR3DS1 in ocular involvement in BD does not involve HLA-B*51. Similarly, the lack of an association between KIR3DL1/KIR3DS1 and BD as a whole suggests that HLA-B*51, the strongest BD susceptibility factor, does not primarily mediate BD risk through interactions with the KIR3DL1/KIR3DS1 receptors. However we cannot exclude a role for other types of KIR variation in the pathogenesis of BD. Further studies of KIR3DL1/KIR3DS1 allotypes and copy number variants, as well as that of other KIRs, are warranted to clarify the role of KIRs in the pathogenesis of BD and its clinical manifestations.

Materials and Methods

Patients

We studied 1,900 Turkish BD cases and 1,779 genetically matched control subjects used in previous GWAS studies.[9] GWAS samples were collected at the Behçet's Disease Clinics of Istanbul Faculty of Medicine and Cerrahpasa Faculty of Medicine, Divisions of Rheumatology. All of the patients fulfilled the International Study Group diagnostic criteria for BD.[31] Healthy control subjects were selected from healthy blood bank donors or volunteers to match the geographic birthplace of the patients. Prospective control subjects were excluded if a family history of BD, or a personal or family history of recurrent oral aphthous ulcers or other BD–related manifestations were identified in an initial interview. All study participants provided written informed consent, and the study was approved by the ethics committee of Istanbul University, Istanbul Faculty of Medicine.

Blood samples were collected from all participants, and genomic DNA was isolated using the MagNA Pure Instrument with the MagNA Pure Compact DNA Isolation Kit for 1 ml volume (Roche).

Genotyping

KIR locus typing was performed by using two sequence-specific primer–polymerase chain reactions (PCR-SSP) that each specifically amplify a short DNA fragment, collectively detecting detecting the presence or absence of both 3DL1 or 3DS1.[32] This SSP assay also utilizes a second pair of PCR primers that amplify a universally present DNA fragment. This internal positive control (IPC) allows discrimination between a negative PCR result indicating the absence of the allele and PCR failure.

The optimal concentrations of the KIR-specific primers were as described.[33] Briefly, we dissolved all lyophilized primers at 100 μM in 10 mM Tris–HCl pH 8.0 and we used 1.05 μL of each of the KIR-specific primers and 0.42 μL of each of the IPC-specific primers in each 10 μl reaction. Each reaction included 3.8 μL of dH2O, 1.0 μL of 10X Taq polymerase buffer (containing 15 mM MgCl2), 0.10 μL of 50 mM MgCl2, 0.05 μL of dNTPs (with 20uM each nucleotide), 0.064 μL of 5U/ μL Hotstar Taq (Qiagen, USA), and 1 μL of 25 ng per μL DNA.

PCR was performed using a PTC 225 Thermal cycler (MJ Research Inc., USA). PCR conditions were as follows: initial denaturation for 15 min at 95°C, then 10 cycles of 10 s at 94°C and 40 s at 65°C; and 20 cycles of 20 s at 94°C, 20 s at 61°C, and 30 s at 72°C. PCR amplicons were visualized by agarose gel electrophoresis [E-Gel 96, 2% (wt/vol) agarose, Invitrogen].

HLA type imputation

All DNA samples were genotyped using the Infinium assay on the Immunochip (Illumina). Genotypes of 6,994 SNPs from the HLA region were used to impute the individuals' HLA types using SNP2HLA and a reference panel of 5,225 European individuals.[34] All types with posterior probability greater than 0.9 were included in the analyses.

HLA-B types of 2213 samples were determined from DNA samples using a reverse sequence-specific oligonucleotide method (One Lambda) and a Bio-Plex 200 suspension array system (BioRad).

Statistical analysis

Association tests were performed with SNP and Variation Suite 7.5.2 software (Golden Helix) to evaluate the contribution of KIR3DS1/KIR3DL1 alleles to disease susceptibility. The correlation/trend test under the additive, dominant, and recessive models was applied to the full collection, as well as to the subsets of Bw4 positive individuals, Bw4-I80 positive individuals, and HLA-B*51 positive individuals. For this analysis, a P value less than 0.0042 (0.05/12) was considered significant after a Bonferroni correction for multiple comparisons. Additionally, KIR3DS1/KIR3DL1 alleles were evaluated in ten subsets of patients defined by specific characteristics: genital ulcers, erythema nodosum, folliculitis, pathergy, arthritis, ocular involvement, vascular involvement, neurologic involvement, intestinal involvement, and positive family history. For this analysis, a P value less than 0.0019 (0.05/27) was considered significant after a Bonferroni correction for multiple comparisons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Intramural Research Programs of the National Institute of Arthritis and Muscuoskeletal and Skin Diseases (Z01-AR041198) and the National Human Genome Research Institute (Z01-HG200370) of the National Institutes of Health.

References

1. Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. The New England journal of medicine. 1999; 341(17):1284–91. [PubMed: 10528040]
2. Lehner T. Immunopathogenesis of Behcet's disease. Annales de medecine interne. 1999; 150(6): 483–7. [PubMed: 10615534]
3. Sakane T. New perspective on Behcet's disease. International reviews of immunology. 1997; 14(1): 89–96. [PubMed: 9203029]

4. Fietta P. Behcet's disease: familial clustering and immunogenetics. Clinical and experimental rheumatology. 2005; 23(4 Suppl 38):S96–105. [PubMed: 16273774]

5. Gul A, Inanc M, Ocal L, Aral O, Konice M. Familial aggregation of Behcet's disease in Turkey. Annals of the rheumatic diseases. 2000; 59(8):622–5. [PubMed: 10913059]

6. Kirino Y, Bertsias G, Ishigatsubo Y, Mizuki N, Tugal-Tutkun I, Seyahi E, et al. Genome-wide association analysis identifies new susceptibility loci for Behcet's disease and epistasis between HLA-B*51 and ERAP1. Nature genetics. 2013; 45(2):202–7. [PubMed: 23291587]

7. Kirino Y, Zhou Q, Ishigatsubo Y, Mizuki N, Tugal-Tutkun I, Seyahi E, et al. Targeted resequencing implicates the familial Mediterranean fever gene MEFV and the toll-like receptor 4 gene TLR4 in Behcet disease. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(20):8134–9. [PubMed: 23633568]

8. Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, et al. Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behcet's disease susceptibility loci. Nat Genet. 2010; 42(8): 703–6. [PubMed: 20622879]

9. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behcet's disease. Nature genetics. 2010; 42(8):698–702. [PubMed: 20622878]

10. de Menthon M, Lavalley MP, Maldini C, Guillevin L, Mahr A. HLA-B51/B5 and the risk of Behcet's disease: a systematic review and meta-analysis of case-control genetic association studies. Arthritis and rheumatism. 2009; 61(10):1287–96. [PubMed: 19790126]

11. Ohno S, Aoki K, Sugiura S, Nakayama E, Itakura K. Letter: HL-A5 and Behcet's disease. Lancet. 1973; 2(7842):1383–4.

12. Ohno S, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behcet's disease. Archives of ophthalmology. 1982; 100(9):1455–8. [PubMed: 6956266]

13. Ombrello MJ, Kirino Y, de Bakker PI, Gul A, Kastner DL, Remmers EF. Behcet disease-associated MHC class I residues implicate antigen binding and regulation of cell-mediated cytotoxicity. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(24): 8867–72. [PubMed: 24821759]

14. Williams AP, Bateman AR, Khakoo SI. Hanging in the balance. KIR and their role in disease. Molecular interventions. 2005; 5(4):226–40. [PubMed: 16123537]

15. Bowness P, Ridley A, Shaw J, Chan AT, Wong-Baeza I, Fleming M, et al. Th17 cells expressing KIR3DL2+ and responsive to HLA-B27 homodimers are increased in ankylosing spondylitis. Journal of immunology. 2011; 186(4):2672–80.

16. Kelley J, Walter L, Trowsdale J. Comparative genomics of natural killer cell receptor gene clusters. PLoS genetics. 2005; 1(2):129–39. [PubMed: 16132082]

17. Pyo CW, Wang R, Vu Q, Cereb N, Yang SY, Duh FM, et al. Recombinant structures expand and contract inter and intragenic diversification at the KIR locus. BMC genomics. 2013; 14:89. [PubMed: 23394822]

18. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D, et al. Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. Tissue antigens. 2003; 62(1):79–86. [PubMed: 12859599]

19. Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, Yawata M, et al. Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. Journal of immunology. 2007; 178(1):33–7.

20. Saunders PM, Pymm P, Pietra G, Hughes VA, Hitchen C, O'Connor GM, et al. Killer cell immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of HLA class I recognition. J Exp Med. 2016

21. Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nature genetics. 2002; 31(4):429–34. [PubMed: 12134147]

22. Qi Y, Martin MP, Gao X, Jacobson L, Goedert JJ, Buchbinder S, et al. KIR/HLA pleiotropism: protection against both HIV and opportunistic infections. PLoS pathogens. 2006; 2(8):e79. [PubMed: 16933987]

23. Gumperz JE, Barber LD, Valiante NM, Percival L, Phillips JH, Lanier LL, et al. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. Journal of immunology. 1997; 158(11):5237–41.
24. Diaz-Pena R, Blanco-Gelaz MA, Suarez-Alvarez B, Martinez-Borra J, Lopez-Vazquez A, Alonso-Arias R, et al. Activating KIR genes are associated with ankylosing spondylitis in Asian populations. Human immunology. 2008; 69(7):437–42. [PubMed: 18638658]
25. Lopez-Larrea C, Blanco-Gelaz MA, Torre-Alonso JC, Bruges Armas J, Suarez-Alvarez B, Pruneda L, et al. Contribution of KIR3DL1/3DS1 to ankylosing spondylitis in human leukocyte antigen-B27 Caucasian populations. Arthritis research & therapy. 2006; 8(4):R101. [PubMed: 16805919]
26. Harvey D, Pointon JJ, Sleator C, Meenagh A, Farrar C, Sun JY, et al. Analysis of killer immunoglobulin-like receptor genes in ankylosing spondylitis. Annals of the rheumatic diseases. 2009; 68(4):595–8. [PubMed: 19019897]
27. McCappin J, Harvey D, Wordsworth BP, Middleton D. No association of KIR3DL1 or KIR3DS1 or their alleles with ankylosing spondylitis. Tissue antigens. 2010; 75(1):68–73. [PubMed: 19874556]
28. Wong-Baeza I, Ridley A, Shaw J, Hatano H, Rysnik O, McHugh K, et al. KIR3DL2 binds to HLA-B27 dimers and free H chains more strongly than other HLA class I and promotes the expansion of T cells in ankylosing spondylitis. Journal of immunology. 2013; 190(7):3216–24.
29. Middleton D, Meenagh A, Sleator C, Gourraud PA, Ayna T, Tozkir H, et al. No association of KIR genes with Behcet's disease. Tissue antigens. 2007; 70(5):435–8. [PubMed: 17868255]
30. Kuranov AB, Kotter I, Henes JC, Abisheva ST, Steiert I, Riewerts F, et al. Behcet's disease in HLA-B*51 negative Germans and Turks shows association with HLA-Bw4-80I. Arthritis research & therapy. 2014; 16(3):R116. [PubMed: 24887019]
31. Disease ISGfBs. Criteria for diagnosis of Behcet's disease. Lancet. 1990; 335(8697):1078–80. [PubMed: 1970380]
32. Vilches C, Castano J, Gomez-Lozano N, Estefania E. Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue antigens. 2007; 70(5):415–22. [PubMed: 17854430]
33. Ordonez D, Moraru M, Gomez-Lozano N, Cisneros E, Vilches C. KIR typing by non-sequencing methods: polymerase-chain reaction with sequence-specific primers. Methods in molecular biology. 2012; 882:415–30. [PubMed: 22665248]
34. Yin J, Zheng G, Jia X, Zhang Z, Zhang W, Song Y, et al. A Bmi1-miRNAs cross-talk modulates chemotherapy response to 5-fluorouracil in breast cancer cells. PLoS One. 2013; 8(9):e73268. [PubMed: 24039897]

Table 1

KIR3DL1/KIR3DS1 genotyping results from all samples.

Genotype	Cases count (freq)	Controls count (freq)	P values for KIR3DS1*		
			Additive	Dominant	Recessive
DL1/DL1	1030 (0.573)	1009 (0.590)	0.281	0.293	0.549
DL1/DS1	642 (0.357)	589 (0.344)			
DS1/DS1	127 (0.071)	112 (0.065)			
total	1799	1710			

*The association test for KIR3DL1 under the recessive model is equivalent to association of KIR3DS1 under the dominant model and that for KIR3DL1 under the dominant model is equivalent to that of KIR3DS1 under the recessive model.

Table 2

KIR3DL1/KIR3DS1 genotyping results from HLA-Bw4 positive subset.

Genotype	Cases count (freq)	Controls count (freq)	P values for KIR3DS1		
			Additive	Dominant	Recessive
DL1/DL1	868 (0.569)	744 (0.589)	0.156	0.308	0.131
DL1/DS1	544 (0.357)	445 (0.352)			
DS1/DS1	111 (0.072)	74 (0.058)			
total	1523	1263			

Table 3

KIR3DL1/KIR3DS1 genotyping results from HLA-Bw4-I80 positive subset.

Genotype	Cases count (freq)	Controls count (freq)	P values for KIR3DS1		
			Additive	Dominant	Recessive
DL1/DL1	685 (0.563)	436 (0.586)	0.120	0.324	0.059
DL1/DS1	435 (0.358)	266 (0.358)			
DS1/DS1	96 (0.079)	42 (0.056)			
total	1216	744			

Table 4

KIR3DL1/KIR3DS1 genotyping results from HLA-B*51 positive subset.

Genotype	Cases count (freq)	Controls count (freq)	P values for KIR3DS1		
			Additive	Dominant	Recessive
DL1/DL1	597 (0.556)	264 (0.570)	0.650	0.630	0.853
DL1/DS1	391 (0.364)	164 (0.354)			
DS1/DS1	84 (0.078)	35 (0.075)			
total	1072	463			

Table 5

Clinical characteristics of BD study patients.

Clinical characteristic	Frequency (%)
Recurrent oral aphthous ulcers	100.0
Genital ulcers	77.0
Folliculitis	77.2
Erythema nodosum	54.0
Pathergy reaction	56.4
Uveitis	59.9
Arthritis	44.7
Vascular involvement	22.8
Neurologic involvement	7.4
Intestinal involvement	1.4
Positive family history	19.0