

## KIR3DL1/S1 allotypes contribute differentially to the development of Behçet Disease

Petrushkin, Harry; Norman, Paul J; Lougee, Emma ; Parham, Peter; Wallace, Graham; Stanford, Miles R; Fortune, Farida

DOI:

[10.4049/jimmunol.1801178](https://doi.org/10.4049/jimmunol.1801178)

License:

Other (please specify with Rights Statement)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Petrushkin, H, Norman, PJ, Lougee, E, Parham, P, Wallace, G, Stanford, MR & Fortune, F 2019, 'KIR3DL1/S1 allotypes contribute differentially to the development of Behçet Disease', *Journal of Immunology*, vol. 203, no. 8, j1801178, pp. 1629-1635. <https://doi.org/10.4049/jimmunol.1801178>

[Link to publication on Research at Birmingham portal](#)

### **Publisher Rights Statement:**

H. Petrushkin, P. J. Norman, E. Lougee, P. Parham, G. R. Wallace, M. R. Stanford, F. Fortune, (2019) KIR3DL1/S1 Allotypes Contribute Differentially to the Development of Behçet Disease, The Journal of Immunology, volume 203, issue 8, article no. j1801178, DOI: 10.4049/jimmunol.1801178

This article is distributed under The American Association of Immunologists, Inc., Reuse Terms and Conditions for Author Choice articles. The definitive published version can be viewed at <https://www.jimmunol.org/content/early/2019/08/09/jimmunol.1801178>

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

# KIR3DL1/S1 Allotypes Contribute Differentially to the Development of Behçet Disease

Harry Petrushkin,<sup>\*,†</sup> Paul J. Norman,<sup>‡</sup> Emma Lougee,<sup>§</sup> Peter Parham,<sup>¶</sup> Graham R. Wallace,<sup>||</sup> Miles R. Stanford,<sup>#</sup> and Farida Fortune<sup>†</sup>

Behçet disease is a chronic, relapsing-remitting autoinflammatory syndrome with a strong *HLA-B\*51* association. In this paper, we describe a human cohort of 267 individuals with Behçet disease and 445 matched controls from a tertiary referral center in the U.K. *HLA-B\*51* was confirmed as a genetic risk factor in this group ( $p = 0.0006$ , Bonferroni–Dunn correction for multiple testing [ $P_c$ ] = 0.0192, odds ratio [OR] 1.92, 95% confidence interval [CI] 1.33–2.76). KIR3DL1/S1 allele-level analysis indicated that low-expressing KIR3DL1/S1 alleles in combination with KIR3DS1 increased the risk of developing Behçet disease (KIR3DL1<sup>LOW</sup>/KIR3DS1:  $p = 0.0004$ ,  $P_c = 0.0040$ , OR 2.47, 95% CI 1.43–4.25), whereas high-expressing KIR3DL1/S1 alleles in combination with a null-expressing KIR3DL1 reduced the risk of disease (KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup>:  $p = 0.0035$ ,  $P_c = 0.0350$ , OR 0.53, 95% CI 0.33–0.87). Behçet disease can manifest as a purely mucocutaneous disease or can involve other organ systems such as the eyes. In the U.K. cohort studied in this study, KIR3DL1<sup>LOW</sup>/KIR3DS1 increased the risk of ophthalmic disease ( $p = 1.2 \times 10^{-5}$ , OR 3.92, 95% CI 2.06–7.47), whereas KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup> reduced the risk of having purely mucocutaneous disease ( $p = 0.0048$ , OR 0.45, 95% CI 0.25–0.81). To our knowledge, this is the first analysis of KIR3DL1/S1 allelic variation in Behçet disease and may provide insight into the pathogenic role of *HLA-B\*51* and its interaction with KIR3DL1/S1. *The Journal of Immunology*, 2019, 203: 000–000.

**B**ehçet disease (BD) is a chronic autoinflammatory, relapsing-remitting, multisystemic syndrome. It is characterized by orogenital ulcers, papulopustular and erythema nodosum-like skin lesions, uveitis, retinal vasculitis, thrombophlebitis, arterial aneurysms, and arthritis. BD is found primarily along the ancient Silk Route from the Mediterranean Basin across Asia to Japan, but cohorts have also been reported in Northern Europe, North America, and South America (1). The

prevalence of BD across the Middle East and Asia is 20–400 per 100,000 (2–4). Recent data from the U.K. BD Centres of Excellence in 2017 suggest the incidence in the United Kingdom is likely to be at least 2 per 100,000 (H. Petrushkin and F. Fortune, unpublished observations), an increase on previous estimates (5), suggesting the impact of this disease is underrecognized in individuals of European extraction.

BD has a genetic basis, with the strongest association loci residing within the *HLA-B* region on chromosome 6 and with *HLA-B\*51* in particular. Evidence for this association was first described in 1982, when Ohno et al. observed that HLA-Bw51 (now *HLA-B\*51*) was associated with BD in Japanese patients. Recent meta-analyses have shown *HLA-B\*51* carriage increases the risk of BD by a factor of six. Eight genome-wide association studies (GWAS) investigating BD have been carried out that support single nucleotide polymorphisms in noncoding regions located between the *HLA-A–HLA-F* loci, with considerable interstudy heterogeneity regarding other reported associations (6, 7). These GWAS have mainly been carried out in patients from regions of the world where BD is most prevalent (Turkey, Japan, China, Korea, Italy, and Iran), although in 2015, a mixed population of patients with BD from the Netherlands was reported (6, 8–14).

To date, the mechanistic association between *HLA-B\*51* and disease pathogenesis remains unclear. HLA class I molecules present Ag to CD8 cytotoxic T cells; however, there is little evidence for a role of these cells in BD. Certain HLA class I molecules are also ligands for the killer Ig-like receptor (KIR) molecules expressed on NK cells. KIR3DL1, a polymorphic inhibitory receptor, interacts with *HLA-B\*51* via the Bw4 epitope, a region spanning residues 77–83 on the  $\alpha 1$  helix of certain *HLA-A* and *-B* allotypes (15, 16). KIR3DL1 is an inhibitory receptor encoded by multiple alleles of the *KIR3DL1/S1* gene, whereas KIR3DS1 is a conserved, activating receptor encoded at the same locus. KIR3DS1 is primarily thought to interact with *HLA-F* (17–20). The *KIR* gene cluster has only recently been defined at

\*Moorfields Eye Hospital National Health Service Foundation Trust, Medical Retina Department, London EC1V 2PD, United Kingdom; †Clinical and Diagnostic Oral Sciences, Queen Mary University of London, Blizard Institute, E1 2AT London, United Kingdom; ‡Division of Biomedical Informatics and Personalized Medicine, Department of Microbiology and Immunology, University of Colorado, Aurora, CO 80045; §Viapath, Clinical Transplantation Laboratory, Guy's Hospital, Guy's and St Thomas' National Health Service Foundation Trust, London SE1 9RT, United Kingdom; ¶Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305; ||Centre for Translational Inflammation Research, University of Birmingham Research Laboratories, Queen Elizabeth Hospital, University of Birmingham, Birmingham B15 2TT, United Kingdom; and #Medical Eye Unit, St Thomas' Hospital, Guy's and St Thomas' National Health Service Foundation Trust, London SE1 7EH, United Kingdom

ORCID: 0000-0001-8370-7703 (P.J.N.); 0000-0003-2054-0509 (G.R.W.); 0000-0001-5930-1973 (F.F.).

Received for publication October 3, 2018. Accepted for publication July 8, 2019.

This work was supported by The T.F.C. Frost Charitable Trust (20121210) (Grant PET2014), The Royal College of Ophthalmology/Pfizer Fellowship (20131310), and Bart's Charity (G-001005). P.J.N. and P.P. were supported by National Institutes of Health (U01AI090905).

Address correspondence and reprint requests to Prof. Farida Fortune, Queen Mary University of London, Blizard Building, 4 Newark Street, London E1 9RT, U.K. E-mail address: f.fortune@qmul.ac.uk

The online version of this article contains supplemental material.

Abbreviations used in this article: BD, Behçet disease; CI, confidence interval; ERAP-1, endoplasmic reticulum aminopeptidase-1; GWAS, genome-wide association study; HC, healthy control; KIR, killer Ig-like receptor; MC, mucocutaneous; OR, odds ratio;  $P_c$ , Bonferroni–Dunn correction for multiple testing.

This article is distributed under The American Association of Immunologists, Inc., [Reuse Terms and Conditions for Author Choice articles](#).

Copyright © 2019 by The American Association of Immunologists, Inc. 0022-1767/19/\$37.50

a high resolution, and disease-specific associations with KIR allotypes are limited (21–24).

Previously, Middleton et al. (25) examined Turkish patients with BD for the presence/absence of *KIR3DL1/S1* but found no association after controlling for the effects of *HLA-B\*51*. Erer et al. (26) studied a large, carefully compiled dataset of 1799 Turkish BD patients and reported an association between *KIR3DS1* and ocular disease, but no effect of *KIR3DL1/S1* overall on disease risk. More recently, Mohammad-Ebrahim et al. (27) examined 397 Iranian patients with BD but found no specific *KIR* gene to be associated with BD. At present, there are no studies that analyze *KIR3DL1/S1* allelic variation or their effect in BD. In this study, we set out to characterize *HLA-A*, *-B*, and *KIR3DL1/S1* genotype frequencies in a cohort of BD patients from the United Kingdom.

## Materials and Methods

### Patients

Two hundred sixty-seven individuals with BD and four hundred forty-five demographically matched healthy controls (HC) were recruited from the BD Centre of Excellence at the Royal London Hospital of Bart's Health National Health Service Trust (London, U.K.). All patients gave written consent, and the project was approved by the local research ethics service of the City and East London (P/03/122). The study conformed to the Declaration of Helsinki. All patients fulfilled the Behçet Disease International Study Group diagnostic criteria (28). Samples were collected between 2013 and 2015. Individuals in the HC group were not related to individuals in the BD group, and every effort was made to match the two groups for sex and ethnicity. To avoid recruiting controls with undiagnosed BD, all HC were over 40 y old because it is uncommon for BD to manifest past the fourth decade.

### Genotyping

Blood samples were collected in 10 ml EDTA tubes, and DNA was extracted with QIAamp DNA Blood Maxi Kits (QIAGEN, Hilden, Germany). DNA was eluted in AE buffer and stored at  $-80^{\circ}\text{C}$  until use. *HLA-B* and *KIR3DL1/S1* genes were sequenced using a Fluidigm library preparation (Fluidigm, South San Francisco, CA). Amplicons were designed to span the coding DNA sequence of *HLA-B* and *KIR3DL1/S1* (primers available upon request). Sequencing was carried out using a MiSeq platform (Illumina, San Diego, CA) at the Bart's and the London Genome Centre (Bart's and the London Medical School, Charterhouse Square, London, U.K.). *HLA-B* alleles were assigned using NGSengine software (GenDx, Utrecht, the Netherlands), and *KIR3DL1/S1* alleles were assigned using the Pushing Immunogenetics to the Next Generation pipeline (29).

### HLA-A typing

*HLA-A* genotyping was carried out using sequence-specific oligonucleotide probes (Immucor, Peachtree Corners, GA) on a Luminex platform (Luminex, Austin, TX) at the Viapath Clinical Transplantation Laboratory at Guys Hospital (London, U.K.).

### Statistical analysis

Probabilities for categorical data were calculated using a two-tailed Fisher exact test. Nonparametric, ordinal data were analyzed with a Mann-Whitney *U* test. Differences between groups were calculated using a two-way ANOVA. The Bonferroni–Dunn correction for multiple testing (*P*<sub>c</sub>) was applied (GraphPad Prism 7, GraphPad Software, La Jolla, CA). Where the frequency of an outcome was 0, the Haldane correction was used to estimate the odds ratio (OR) and 95% confidence intervals (CI). The magnitude of the effect was estimated using the OR and 95% CI.

For 80% power with an  $\alpha = 0.05$ , assuming a 1:2 case/control recruitment, we aimed to collect at least 199 cases and 398 controls. This was based on the population prevalence of *KIR3DL1\*001* in the United Kingdom of 15% (30). As the exact *KIR3DL1/S1* allele frequencies within a United Kingdom-based BD cohort have not been explored, we collected as many samples as possible to allow for patients withdrawing from the study and technical problems with samples.

A *p* value  $\leq 0.05$  was considered to be statistically significant but only in the context of a 95% CI that did not cross 1.00. If *p* < 0.05 but *P*<sub>c</sub> > 0.05 and the 95% OR did not cross 1, the result was considered relevant for discussion.

## Results

The group demographics, including self-reported ethnicity, are presented in Table I. There are no significant differences between the sex or ethnicity of the cases and controls. The control group was, on average, 13 y older than the BD group. The population analyzed in this paper is of mixed ethnicity with 67.4% being of self-reported Northern European ancestry. Clinical manifestations along with the ethnicity of the patient groups are given in Fig. 1. After quality control, *HLA-A* genotypes were obtained for 260 (97.38%) cases and 426 (95.73%) controls, *HLA-B* genotypes were obtained for 266 (99.63%) cases and 443 (99.55%) controls, and *KIR3DL1/S1* genotypes were obtained for 256 (95.88%) cases and 433 (97.30%) controls.

### Association of *HLA-B\*51* with BD is replicated in the U.K. cohort

*HLA-B\*51* was associated with BD (*p* = 0.0006, *P*<sub>c</sub> = 0.0192, OR 1.92, 95% CI 1.33–2.76). A smaller effect was also identified for *HLA-B\*08* (*p* = 0.0238, *P*<sub>c</sub> = NS, OR 1.49, 95% CI 1.06–2.11), *HLA-A\*25* (*p* = 0.0121, *P*<sub>c</sub> = NS, OR 2.51, 95% CI 1.17–5.41), and *HLA-A\*26* (*p* = 0.0291, *P*<sub>c</sub> = NS, OR 1.76, 95% CI 1.00–3.10). A suggestion of protective effects was found for *HLA-B\*58* (*p* = 0.0248, *P*<sub>c</sub> = NS, OR 0.21, 95% CI 0.05–0.90), and *HLA-A\*33* (*p* = 0.0131, *P*<sub>c</sub> = NS, OR 0.45, 95% CI 0.22–0.92) (Table II).

The HLA class I data were analyzed for the presence/absence of Bw4, and the gene dosage of Bw4 encoding alleles was calculated (i.e., an individual may have a maximum of four Bw4 encoding alleles if both alleles of *HLA-A* and *-B* encode HLA allotypes with the Bw4 epitope such as *HLA-A\*23/A\*24* and *HLA-B\*51/B\*44*). The results indicate that there was no disease association between the presence/absence or gene dosage of Bw4 encoding alleles and BD (data not shown).

### *KIR3DL1/S1* “functional genotypes” are associated with BD

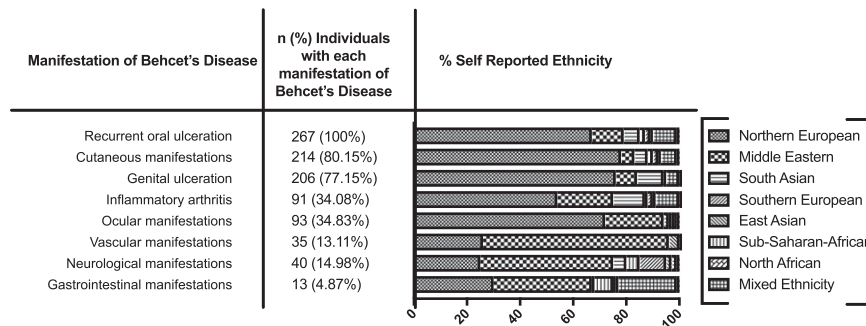
Having established that *HLA-B\*51* conferred a risk of BD in this cohort of patients, we investigated the effects of *KIR3DL1/S1* allelic variation. *KIR3DL1* was present in 96% of individuals in both groups. There was no difference in the frequency of *KIR3DL1/S1* heterozygotes between the groups and no association between the *KIR3DL1/S1* presence/absence, and BD was identified. Nor was there any departure from Hardy–Weinberg

Table I. Patient and HC demographics

	Control (n)	BD (n)	<i>p</i> Value
Participants	445	267	—
Sex			
Male	191	108	NS
Female	254	159	NS
Age	54 (IQ-range 54–58)	41 (IQ-range 36–43)	<i>p</i> < 0.0001
Ethnicity (self-reported)			
Northern European	302	180	NS
Middle Eastern	39	31	NS
South Asian	40	15	NS
Sub-Saharan-African	2	6	NS
Southern European	13	6	NS
East Asian	12	4	NS
Mixed ethnicity	31	23	NS
North African	6	2	NS

The cases and controls were matched for gender and ethnicity. The control group was selected to be over 50 y of age as BD usually manifests in the first five decades of life. This was done to ensure no individuals with an unmasked autoinflammatory disease were incorrectly labeled as healthy.

IQ, interquartile.



**FIGURE 1.** The diagnosis of BD was made in a tertiary referral center by a multidisciplinary team, with experts from the following fields: oral medicine, rheumatology, ophthalmology, neurology, immunology, and psychology. Percentage of the self-reported ethnicity can be seen next to each manifestation of BD. The majority of patients presenting with MC disease, arthritis, and ocular disease were of Northern European ethnicity, in keeping with the overall makeup of the cohort. Of note, increased percentages of Middle Eastern patients had vascular, neurologic, and gastrointestinal manifestations.

Equilibrium between the BD and HC groups (Supplemental Table I). We then analyzed our disease cohorts to ascertain whether any individual *KIR3DL1/S1* allele increased or decreased the risk of developing BD (Supplemental Table III); *KIR3DL1\*005* and *KIR3DS1\*013* were found to contribute toward the risk of disease, but the association did not survive correction for multiple testing.

We grouped *KIR3DL1/S1* allotypes by their expression phenotype based on previously published data (31–36), to create functional genotypes. For those allotypes in which functional data were not available, we assigned a presumptive expression phenotype based on the nearest *KIR3DL1/S1* allotypes for which there is data (Supplemental Table II). We found no association between high-expressing *KIR3DL1* (*KIR3DL1<sup>HIGH</sup>*), low-expressing *KIR3DL1* (*KIR3DL1<sup>LOW</sup>*), *KIR3DL1* retained intracellularly (*KIR3DL1<sup>NULL</sup>*), or the activating allele, *KIR3DS1* (data not shown). In contrast, we found the combination of *KIR3DL1<sup>LOW</sup>/KIR3DS1* was associated with BD ( $p = 0.0004$ ,  $P_c = 0.0040$ , OR 2.47, 95% CI 1.43–4.25), whereas the combination of *KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup>* was associated with protection from disease ( $p = 0.0035$ ,  $P_c = 0.0350$ , OR 0.53, 95% CI 0.33–0.87) (Table III). The increased and decreased risk conferred by *KIR3DL1<sup>LOW</sup>/KIR3DS1* and *KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup>*, respectively, remained when we removed those alleles without direct evidence of their expression level (Supplemental Table IV). These results indicate that the *KIR3DL1<sup>LOW</sup>/KIR3DS1* functional genotype is associated with the risk of developing BD, whereas the *KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup>* functional genotype is associated with protection from BD.

We then analyzed the allelic composition of the implicated functional genotypes. There were two genotypes associated with BD: *KIR3DL1\*0050101<sup>LOW</sup>/KIR3DS1\*0130101* ( $p = 0.0210$ , OR 1.91, 95% CI 1.07–3.43) and *KIR3DL1\*0070101<sup>LOW</sup>/KIR3DS1\*0130101*

( $p = 0.0008$ , OR 15.22, 95% CI 1.92–120.87). Conversely, *KIR3DL1\*0010101<sup>HIGH</sup>/KIR3DL1\*00401<sup>NULL</sup>* ( $p = 0.0172$ , OR 0.36, 95% CI 0.15–0.89) was found to be protective (Table IV).

To examine whether the *KIR3DL1/S1* functional genotypes conferring risk and protection are associated with BD or merely the presence of *Bw4/HLA-B\*51*, we repeated the above analysis, controlling for these variables. We found the effect of possessing a *KIR3DL1<sup>LOW</sup>/KIR3DS1* risk genotype to be significant in all groups except the *HLA-B\*51<sup>+</sup>* individuals. The protective effects of the *KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup>* genotype were only significant in individuals possessing *Bw4* (Fig. 2).

#### Differential associations between *KIR3DL1/S1* functional genotypes and clinical manifestations of BD

The BD population was then split into two subgroups: those with purely mucocutaneous (MC) disease ( $n = 166$ ) and those with ophthalmic involvement ( $n = 91$ ). The MC and ophthalmic groups did not significantly differ with regard to their ethnic makeup ( $p = \text{NS}$ ) (Fig. 1). The two groups were then compared with the HC group. *HLA-B\*51* remained associated with BD in both groups (MC group:  $p = 0.0009$ , OR 4.14, 95% CI 2.72–6.32; ophthalmic group:  $p = 0.0367$ , OR 1.75, 95% CI 1.05–2.92), and there was no difference between the groups with regard to *KIR3DL1/S1* presence/absence (data not shown). We were unable to replicate the association between an increase in *KIR3DS1* alleles and ophthalmic involvement as was previously reported (26).

Examining *KIR3DL1/S1* allele combinations in the MC group revealed an increase in *KIR3DL1\*00701/KIR3DS1\*013* compared with HCs ( $p = 0.0071$ , OR 13.04, 95% CI 1.51–112.49). Despite this, none of the functional genotypes were associated with MC disease. Conversely, no specific combination of *KIR3DL1/S1* alleles conferred protection to MC disease, but the functional

Table II. *HLA-A* and *-B* associations in the U.K. cohort

HLA Type	U.K. Cohort (HC $n = 445$ , BD $n = 267$ )							
	HC ( $n$ )	HC (%)	BD ( $n$ )	BD (%)	$p$ Value	$P_c$	OR	95% CI
Increased risk								
<i>HLA-B*51</i>	62	6.97	67	12.55	<b>0.0006</b>	<b>0.019</b>	1.92	1.33–2.76
<i>HLA-B*08</i>	78	8.76	67	12.55	<b>0.0238</b>	NS	1.49	1.06–2.11
<i>HLA-A*25</i>	11	1.2	17	3.1	<b>0.0121</b>	NS	2.51	1.17–5.41
<i>HLA-A*26</i>	24	3.2	26	5.1	<b>0.0291</b>	NS	1.76	1.00–3.10
Reduced risk								
<i>HLA-B*58</i>	16	1.80	2	0.37	<b>0.0248</b>	NS	0.21	0.05–0.90
<i>HLA-A*33</i>	36	4.3	10	2.3	<b>0.0131</b>	NS	0.45	0.22–0.92

Those in bold are significant ( $p < 0.05$ ) results in which the 95% CI does not cross 1.



Table III. The effects of the functional genotype *KIR3DL1/S1* combinations

<i>KIR3DL1/S1</i> allele		HC n=433, BD n=256							
Allele 1	Allele 2	HC (n)	HC (%)	BD (n)	BD (%)	P	P <sub>c</sub>	OR	95% CI
<i>3DL1</i> <sup>HIGH</sup>	<i>3DL1</i> <sup>HIGH</sup>	85	19.63	60	23.44	NS	NS	1.19	0.82-1.74
<i>3DL1</i> <sup>HIGH</sup>	<i>3DL1</i> <sup>LOW</sup>	71	16.40	36	14.06	NS	NS	0.86	0.56-1.32
<i>3DL1</i> <sup>HIGH</sup>	<i>3DL1</i> <sup>NULL</sup>	76	17.55	24	9.38	<b>0.0035</b>	<b>0.0350</b>	0.53	0.33-0.87
<i>3DL1</i> <sup>HIGH</sup>	<i>3DS1</i>	83	19.17	39	15.23	NS	NS	0.79	0.52-1.21
<i>3DL1</i> <sup>LOW</sup>	<i>3DL1</i> <sup>LOW</sup>	17	3.93	7	2.73	NS	NS	0.70	0.28-1.7
<i>3DL1</i> <sup>LOW</sup>	<i>3DL1</i> <sup>NULL</sup>	23	5.31	16	6.26	NS	NS	1.18	0.61-2.27
<i>3DL1</i> <sup>LOW</sup>	<i>3DS1</i>	24	5.54	35	13.67	<b>0.0004</b>	<b>0.0040</b>	2.47	1.43-4.25
<i>3DL1</i> <sup>NULL</sup>	<i>3DL1</i> <sup>NULL</sup>	10	2.31	7	2.73	NS	NS	1.18	0.45-3.15
<i>3DL1</i> <sup>NULL</sup>	<i>3DS1</i>	30	6.93	23	8.98	NS	NS	1.30	0.74-2.29
<i>3DS1</i>	<i>3DS1</i>	14	3.23	9	3.52	NS	NS	1.09	0.46-2.55

*KIR3DL1*<sup>HIGH</sup>–*KIR3DL1* alleles are highly expressed and confer strong inhibition to cell-mediated lysis. *KIR3DL1*<sup>LOW</sup>–*KIR3DL1* alleles are expressed at a low level and confer weak inhibition to cell-mediated lysis. *KIR3DL1*<sup>NULL</sup>–*KIR3DL1*\*004 is mostly retained intracellularly and has minimal inhibitory potential. *KIR3DS1* activates *KIR* allele. Those in bold signify significant ( $p < 0.05$ ) results in which the 95% CI does not cross 1. Red indicates an inhibitory *KIR3DL1* allotype expressed at high levels on the cell surface, pink indicates an inhibitory *KIR3DL1* allotype expressed at low levels on the cell surface, yellow indicates a null *KIR3DL1* allotype not expressed on the cell surface, and green indicates an activating *KIR3DL1* allotype.

genotype *KIR3DL1*<sup>HIGH</sup>/*KIR3DL1*<sup>NULL</sup> reduced the risk of developing purely MC disease ( $p = 0.0048$ , OR 0.45, 95% CI 0.25–0.81).

The ophthalmic group had an increased frequency of *KIR3DL1*\*00501/*KIR3DS1*\*013 ( $p = 0.0003$ , OR 3.27, 95% CI 1.65–6.48) and *KIR3DL1*\*00701/*KIR3DS1*\*013 ( $p = 0.0039$ , OR 18.83, 95% CI 2.08–170.47) compared with controls. Of note, the functional genotype *KIR3DL1*<sup>LOW</sup>/*KIR3DS1* conferred significant risk ( $p = 1.2 \times 10^{-5}$ , OR 3.92, 95% CI 2.06–7.47) in these individuals. There were no protective effects observed in this group (Fig. 3).

## Discussion

*KIR* expression is variable on individual NK cells, and this can have a major influence on the response to immune challenge (37). We have examined the risk alleles associated with BD in a large cohort of patients in the United Kingdom, the majority of whom are of Northern European heritage. As expected, the strongest risk association was found with *HLA-B*\*51 with weaker associations from *HLA-B*\*08, *HLA-A*\*25, and *HLA-A*\*26. Protective effects were identified from *HLA-B*\*58 and *HLA-A*\*33, confirming previous studies in European and Korean patients (38, 39).

As stated, *KIR3DL1/S1* recognizes the Bw4 epitope at the C terminus of the  $\alpha$ -chains forming the peptide binding groove in a subset of MHC class I molecules. As such, polymorphisms within *KIR3DL1/S1* can alter the strength of binding and thus the inhibitory potential of the *KIR*–*HLA* interaction.

Because of small sample numbers, some specific allele associations did not remain significant after multiple testing [both *KIR3DL1*\*00501 and *KIR3DS1*\*01301 showed a trend toward an association with BD (Supplemental Table III)] but nevertheless

deserve further attention with a wider cohort and functional studies.

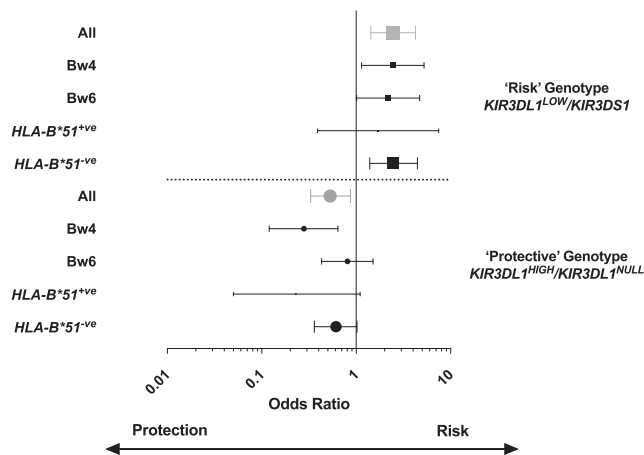
The functional genotype results show an association between a risk genotype made up of a *KIR3DL1*<sup>LOW</sup> allele (\*00501 or \*00701) and the activating allele *KIR3DS1*\*013 (*KIR3DL1*<sup>LOW</sup>/*KIR3DS1*) and protective effects from the genotype made up of *KIR3DL1*\*0010101 and \*00401 (*KIR3DL1*<sup>HIGH</sup>/*KIR3DL1*<sup>NULL</sup>). In 2018, Martin et al. (40) described a single variant in *KIR3DL1* that significantly modified progression of HIV-1 due to the protective response from *HLA-B*\*57, supporting the concept of *KIR*/*HLA* influencing disease outcome.

Despite many structural similarities between *KIR3DL1* and *KIR3DS1*, it remains contentious as to whether Bw4 is a ligand for *KIR3DS1*. There has been one report of *KIR3DS1* binding *HLA-B*\*57 (41) in the context of HIV-1 infection. More recently, *HLA-F* was identified as a high-affinity ligand for *KIR3DS1* (18). *HLA-F* neither binds peptide nor requires  $\beta$ 2-microglobulin to fold stably. It is upregulated during cell stress (42) and has also been implicated in NK cell education (19). *HLA-F* also binds allotypes of *KIR3DL1* but with a lower affinity. In BD, *KIR3DS1* may recognize *HLA-F* that has been upregulated on lymphocytes because of cell stress, leading to a persistent proinflammatory state, which could be exacerbated by an immune “trigger” such as a viral infection or mechanical trauma, downregulation of cell-surface *HLA*, and increased cell-mediated cytotoxicity. This, in conjunction with a *KIR3DL1*<sup>LOW</sup> allotype, may result in NK cells that are more likely to degranulate than those without an inhibitory *KIR3DL1* or indeed an activating receptor. *KIR3DS1* has also been linked to a number of other diseases without a Bw4 association (43–45), suggesting that the effects of *KIR3DS1* can be

Table IV. *KIR3DL1* alleles that make up the risk and protective functional genotypes

<i>KIR3DL1/S1</i> allele		UK Cohort (HC n=445, BD n=267)							
		HC (n)	HC (%)	BD (n)	BD (%)	P	P <sub>c</sub>	OR	95% CI
Increased Risk									
<i>3DL1</i> *0050101	<i>3DS1</i> *01301	23	5.16	26	9.73	<b>0.0210</b>	NS	1.91	1.07-3.43
<i>3DL1</i> *0070101	<i>3DS1</i> *01301	1	0.23	9	3.37	<b>0.0008</b>	<b>0.0307</b>	15.22	1.92-120.87
Decreased Risk									
<i>3DL1</i> *0010101	<i>3DL1</i> *004	28	6.29	6	2.24	<b>0.0172</b>	NS	0.36	0.15-0.89

A combination of the weakly expressed *KIR3DL1*\*005 and 007 were the *KIR3DL1* alleles implicated in the risk genotype in conjunction with *KIR3DS1*\*013. The protective genotype was made up of highly expressed *KIR3DL1*\*001 and *KIR3DL1*\*004, a null-expressing allele. Those in bold signify significant ( $p < 0.05$ ) results in which the 95% CI does not cross 1. Red indicates an inhibitory *KIR3DL1* allotype expressed at high levels on the cell surface, pink indicates an inhibitory *KIR3DL1* allotype expressed at low levels on the cell surface, yellow indicates a null *KIR3DL1* allotype not expressed on the cell surface, and green indicates an activating *KIR3DL1* allotype.



**FIGURE 2.** Square markers represent “risk” genotypes, and circular markers represent “protective” genotypes. The size of the markers are proportional to the size of the subgroups. Points above the dotted line represent the OR and 95% CIs for  $KIR3DL1^{LOW}/KIR3DS1$  risk group. Data are shown (in descending order) for all individuals and then only those that possess Bw4 (one or two copies), Bw6 (two copies), and *HLA-B\*51* (one or two copies) and those that lack *HLA-B\*51*. The points below the dotted line represent the OR and 95% CIs for the protective genotype  $KIR3DL1^{HIGH}/KIR3DL1^{NULL}$  with the same data groups.

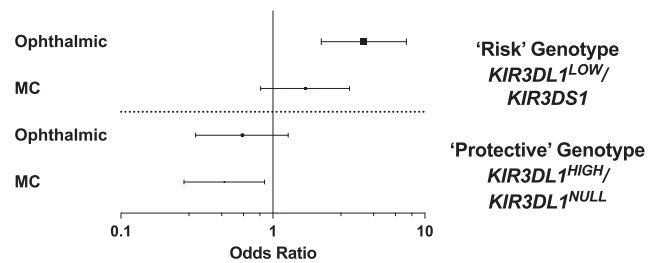
independent of Bw4. Similarly, the  $KIR3DL1^{LOW}/KIR3DS1$  functional genotype described in this study was found to be significant in the Bw6 subgroup in addition to the Bw4 subgroup.

The protective  $KIR3DL1^{HIGH}/KIR3DL1^{NULL}$  genotype has no activating KIR3D and thus relies on other activating receptors to initiate degranulation. Why  $KIR3DL1^{HIGH}/KIR3DL1^{NULL}$  should result in protection from disease whereas  $KIR3DL1^{HIGH}/KIR3DL1^{LOW/HIGH}$  does not remains unclear. It may be that a specific degree of KIR3DL1 surface tuning is required to provide protective effects and that only KIR3DL1\*004 is able to provide this. It is also possible that only a small fraction of total KIR3DL1\*004 is required to interact with Bw4 to lead to downstream effects as previously suggested by the protective effects of KIR3DL1\*004 in HIV progression (46).

The role of KIR3DL1\*004 in disease is of current interest. Whereas KIR3DL1\*004 was shown to be retained intracellularly and not contribute to the inhibitory response (47), Taner et al. (46) demonstrated that a small portion is correctly folded and cell surface expressed. There is evidence to suggest that possession of Bw4 and KIR3DL1\*004 slows progression of HIV infection to AIDS (48). Whether KIR3DL1\*004 can be considered ‘null’ in the context of disease association is a subject for further investigation.

Given the number of genetic risk factors uncovered by recent GWAS, there are probably many different pathways, each with small effects, that lead to the development of BD. The HLA–KIR interaction may be a feasible biological explanation, but it fails to explain the role of KIR3DL1/S1 in Bw4<sup>+</sup> patients with BD. Whether there is an increased role for CD8 T cells–KIR interactions in these individuals remains to be seen (49).

The putative hypothesis driving this project was that KIR3DL1 allotypes interact with Bw4 (and therefore HLA-B\*51) at different levels (31, 47) and thus may be implicated in disease pathogenesis. However, despite a strong *HLA-B\*51* association, there was little evidence to suggest that the  $KIR3DL1/S1$  genotype associations found in this study are mediated either by Bw4 or *HLA-B\*51* (Fig. 2). There are two possible explanations for this finding; first, the mechanisms by which the  $KIR3DL1^{LOW}/KIR3DS1$  functional genotype contribute toward disease risk are not directly due to an



**FIGURE 3.** Square markers represent risk genotypes, and circular markers represent protective genotypes. The size of the markers are proportional to the size of the subgroups. The protective  $KIR3DL1^{HIGH}/KIR3DL1^{NULL}$  genotype was associated with a reduced risk of MC disease, whereas the risk  $KIR3DL1^{LOW}/KIR3DS1$  genotype was associated with an increased risk of ophthalmic disease.

inhibitory interaction between KIR3DL1–Bw4 but rather by indirect mechanisms. Whereas protective effects conferred from the  $KIR3DL1^{HIGH}/KIR3DL1^{NULL}$  functional genotype are mediated via a direct KIR3DL1–Bw4 interaction. Second, it may be that the KIR3DL1–Bw4 interaction is integral to both risk and protective effects but that there is a lack of power to detect the risk effects within the *HLA-B\*51*<sup>+</sup> group. It should be noted that the number of *HLA-B\*51*<sup>+</sup> individuals with either the risk or protective functional genotype was small, and this study is unlikely to be sufficiently powered to exclude involvement of *HLA-B\*51* in KIR3DL1/S1 interactions. However, it is possible that KIR3DL1/S1 is affecting disease susceptibility in ways that are independent of Bw4. KIR3DL1–Bw4 interactions have been extensively investigated and found to be highly variable, depending both on the allotype of KIR3DL1 and on effects outside the Bw4 binding site (50). These interactions are complex and not easily studied in a project designed to analyze the allelic variation of *KIR3DL1*.

BD is a heterogeneous condition. Many patients will only ever experience orogenital ulceration, whereas others progress to develop blinding uveitis or inflammatory brain disease. In this study, two low-expressing *KIR3DL1* alleles (\*00501 and 00701) were both found to confer risk in combination with *KIR3DS1*. In the MC group, there was no effect from *KIR3DL1*\*005, whereas in the ophthalmic group, both alleles contributed to disease susceptibility. *KIR3DL1*\*007 was found less frequently in both groups, but its effect size was between 5 and 10 times greater than *KIR3DL1*\*005. *KIR3DL1* comprises three ancestral lineages: *KIR3DS1*\*01301, *KIR3DL1*\*005, and *KIR3DL1*\*015 (47). From these lineages, selection pressures have led to an abundance of *KIR3DL1* allotypes. The *KIR3DL1*\*015 lineage is most diverse in Africans, whereas the *KIR3DL1*\*005 lineage is more common in Caucasians (30). Whereas *KIR3DL1*\*005 has remained stable for more than three million years, *KIR3DL1*\*007 segregated as a clade from *KIR3DL1*\*015 more recently. It is notable in our cohort that *KIR3DL1*\*015 does not associate with BD, whereas *KIR3DL1*\*007 with its narrow binding pattern and *KIR3DL1*\*005 with its broader specificity do. Despite these biological differences, both receptors are expressed at low levels on the cell surface and confer weak inhibition of target cell lysis (51, 52).

Although this is the first study, to our knowledge, to examine *KIR3DL1/S1* alleles in BD, our findings correlate with published data on other inflammatory diseases. In 2016, Ahn et al. examined the frequency of KIR3DL1 allotypes in 203 patients with psoriasis and 111 HC of European descent. Their results suggested that the presence of a weakly inhibitory KIR3DL1 allotype increased the risk of developing psoriasis, whereas *KIR3DL1*\*004 was protective (22). Similarly, Díaz-Peña et al. (53) found that the

combination of *KIR3DS1\*013* and *KIR3DL1\*004* protects against ankylosing spondylitis.

Several other factors are involved in HLA-B\*51 activity, including endoplasmic reticulum aminopeptidase-1 (ERAP-1), which cleaves peptides to fit into class I molecules. Polymorphisms in ERAP-1 have been associated with BD, and ERAP-1 variants influence the binding of a low affinity peptidome, which may be relevant in BD (54, 55). Moreover, NK cells require only a few degranulation events (around 10% of the total lytic granules) to kill target cells. Such killing was dependent on the size of granules and the speed at which they are released (56). Activating and inhibitory KIR have been shown to have distinct nanocluster formation on the cell surface controlled by their transmembrane sequence. KIR2DS1 was found to have larger clusters than KIR2DL1 and induce greater downstream signaling (57). Whether KIR allotypes would influence these processes is a possible mechanism to be investigated.

To conclude, this is the first study, to our knowledge, to analyze the effect of KIR3DL1 allotypes in BD. We found *HLA-B\*51* to be associated with BD in a large United Kingdom-based cohort and confirm that *HLA-A\*26* is a risk allele in this population (58) and found *HLA-B\*08* and *A\*25* are also implicated. We also confirm that *HLA-B\*58* and *HLA-A\*33* are protective (38, 39). *KIR3DL1* allele-level analysis revealed the *KIR3DL1<sup>LOW</sup>/KIR3DS1* functional genotype to be implicated in disease pathogenesis and *KIR3DL1<sup>HIGH</sup>/KIR3DL1<sup>NULL</sup>* to be protective.

## Acknowledgments

We thank the patients and HCs who contributed to this project. We would also like to acknowledge Steven Marsh (Anthony Nolan, London, U.K.) for advice regarding HLA and KIR typing, Robert Vaughan and Elli Kondeatis (Viapath, Clinical Transplantation Laboratory, Guy's Hospital, London, U.K.) for facilitating HLA-A typing, and the staff of the Behçet's Syndrome Centre of Excellence for help and support.

## Disclosures

The authors have no financial conflicts of interest.

## References

- Verity, D. H., J. E. Marr, S. Ohno, G. R. Wallace, and M. R. Stanford. 1999. Behçet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. *Tissue Antigens* 54: 213–220.
- Papoutsis, N. G., M. B. Abdel-Naser, A. Altenburg, H. Orawa, I. Kötter, L. Krause, U. Pleyer, D. Djawari, R. Stadler, U. Wollina, et al. 2006. Prevalence of Adamantiades-Behçet's disease in Germany and the municipality of Berlin: results of a nationwide survey. *Clin. Exp. Rheumatol.* 24(5, Suppl. 42): S125.
- Azizlerli, G., A. A. Köse, R. Sarica, A. Gül, I. T. Tutkun, M. Kulaç, R. Tunç, M. Urgancıoğlu, and R. Dişi. 2003. Prevalence of Behçet's disease in Istanbul, Turkey. *Int. J. Dermatol.* 42: 803–806.
- Al-Araji, A., K. Sharquie, and Z. Al-Rawi. 2003. Prevalence and patterns of neurological involvement in Behçet's disease: a prospective study from Iraq. *J. Neurol. Neurosurg. Psychiatry* 74: 608–613.
- Azizerli, H., M. Aksoy, and F. Alagöl. 1988. Acute myeloblastic leukemia after propyl-thio-uracil, a simple coincidence? *Acta Haematol.* 79: 229.
- Mizuki, N., A. Meguro, M. Ota, S. Ohno, T. Shiota, T. Kawagoe, N. Ito, J. Kera, E. Okada, K. Yatsu, et al. 2010. Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci. *Nat. Genet.* 42: 703–706.
- Meguro, A., H. Inoko, M. Ota, Y. Katsuyama, A. Oka, E. Okada, R. Yamakawa, T. Yuasa, T. Fujioka, S. Ohno, et al. 2010. Genetics of Behçet disease inside and outside the MHC. *Ann. Rheum. Dis.* 69: 747–754.
- Xavier, J. M., F. Shahram, I. Sousa, F. Davatchi, M. Matos, B. S. Abdollahi, J. Sobral, A. Nadji, M. Oliveira, F. Ghaderibarm, et al. 2015. FUT2: filling the gap between genes and environment in Behçet's disease? *Ann. Rheum. Dis.* 74: 618–624.
- Kappen, J. H., C. Medina-Gomez, P. M. van Hagen, L. Stolk, K. Estrada, F. Rivadeneira, A. G. Uitterlinden, M. R. Stanford, E. Ben-Chetrit, G. R. Wallace, et al. 2015. Genome-wide association study in an admixed case series reveals IL12A as a new candidate in Behçet disease. *PLoS One* 10: e0119085.
- Lee, Y. J., Y. Horie, G. R. Wallace, Y. S. Choi, J. A. Park, J. Y. Choi, R. Song, Y. M. Kang, S. W. Kang, H. J. Baek, et al. 2013. Genome-wide association study identifies GIMAP as a novel susceptibility locus for Behçet's disease. *Ann. Rheum. Dis.* 72: 1510–1516.
- Kirino, Y., G. Bertias, Y. Ishigatsubo, N. Mizuki, I. Tugal-Tutkun, E. Seyahi, Y. Ozyazgan, F. S. Sacli, B. Erer, H. Inoko, et al. 2013. Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between HLA-B\*51 and ERAP1. *Nat. Genet.* 45: 202–207.
- Hou, S., Z. Yang, L. Du, Z. Jiang, Q. Shu, Y. Chen, F. Li, Q. Zhou, S. Ohno, R. Chen, et al. 2012. Identification of a susceptibility locus in STAT4 for Behçet's disease in Han Chinese in a genome-wide association study. *Arthritis Rheum.* 64: 4104–4113.
- Remmers, E. F., F. Cosan, Y. Kirino, M. J. Ombrello, N. Abaci, C. Satorius, J. M. Le, B. Yang, B. D. Korman, A. Cakiris, et al. 2010. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. *Nat. Genet.* 42: 698–702.
- Fei, Y., R. Webb, B. L. Cobb, H. Direskeneli, G. Saruhan-Direskeneli, and A. H. Sawalha. 2009. Identification of novel genetic susceptibility loci for Behçet's disease using a genome-wide association study. *Arthritis Res. Ther.* 11: R66.
- Vivian, J. P., R. C. Duncan, R. Berry, G. M. O'Connor, H. H. Reid, T. Beddoe, S. Gras, P. M. Saunders, M. A. Olshina, J. M. Widjaja, et al. 2011. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. *Nature* 479: 401–405.
- Wan, A. M., P. Ennis, P. Parham, and N. Holmes. 1986. The primary structure of HLA-A32 suggests a region involved in formation of the Bw4/Bw6 epitopes. *J. Immunol.* 137: 3671–3674.
- Burian, A., K. L. Wang, K. A. Finton, N. Lee, A. Ishitani, R. K. Strong, and D. E. Geraghty. 2016. HLA-F and MHC-I open conformers bind natural killer cell Ig-like receptor KIR3DS1. *PLoS One* 11: e0163297.
- Garcia-Beltran, W. F., A. Hölzemer, G. Martus, A. W. Chung, Y. Pacheco, C. R. Simoneau, M. Rucevic, P. A. Lamothe-Molina, T. Pertel, T. E. Kim, et al. 2016. Open conformers of HLA-F are high-affinity ligands of the activating NK-cell receptor KIR3DS1. *Nat. Immunol.* 17: 1067–1074.
- Carlomagno, S., M. Falco, M. Bono, C. Alicata, L. Garbarino, M. Mazzocco, L. Moretta, A. Moretta, and S. Sivori. 2017. KIR3DS1-Mediated recognition of HLA-B\*51: modulation of KIR3DS1 responsiveness by self HLA-B allotypes and effect on NK cell licensing. *Front. Immunol.* 8: 581.
- Dulberger, C. L., C. P. McMurtrey, A. Holzemer, K. E. Neu, V. Liu, A. M. Steinbach, W. F. Garcia-Beltran, M. Sulak, B. Jabri, V. J. Lynch, et al. 2017. Human leukocyte antigen F presents peptides and regulates immunity through interactions with NK cell receptors. *Immunity* 46: 1018–1029.e7.
- Hollenbach, J. A., M. J. Pando, S. J. Caillier, P. A. Gourraud, and J. R. Oksenberg. 2016. The killer immunoglobulin-like receptor KIR3DL1 in combination with HLA-Bw4 is protective against multiple sclerosis in African Americans. *Genes Immun.* 17: 199–202.
- Ahn, R. S., H. Moslehi, M. P. Martin, M. Abad-Santos, A. M. Bowcock, M. Carrington, and W. Liao. 2016. Inhibitory KIR3DL1 alleles are associated with psoriasis. *Br. J. Dermatol.* 174: 449–451.
- McLaren, P. J., and M. Carrington. 2015. The impact of host genetic variation on infection with HIV-1. *Nat. Immunol.* 16: 577–583.
- Mancusi, A., L. Ruggeri, E. Urbani, A. Pierini, M. S. Massei, A. Carotti, A. Terenzi, F. Falzetti, A. Tosti, F. Topini, et al. 2015. Haploidentical hematopoietic transplantation from KIR ligand-mismatched donors with activating KIRs reduces nonrelapse mortality. *Blood* 125: 3173–3182.
- Middleton, D., A. Meenagh, C. Sleator, P. A. Gourraud, T. Ayna, H. Tozkir, A. A. Köse, G. Azizlerli, and A. S. Diler. 2007. No association of KIR genes with Behçet's disease. *Tissue Antigens* 70: 435–438.
- Erer, B., M. Takeuchi, D. Ustek, I. Tugal-Tutkun, E. Seyahi, Y. Ozyazgan, J. Duymaz-Tozkir, A. Gül, D. L. Kastner, E. F. Remmers, and M. J. Ombrello. 2016. Evaluation of KIR3DL1/KIR3DS1 polymorphism in Behçet's disease. *Genes Immun.* 17: 396–399.
- Mohammad-Ebrahim, H., E. Kamali-Sarvestani, M. Mahmoudi, M. Beigy, J. Karami, N. Ahmadzadeh, and F. Shahram. 2018. Association of killer cell immunoglobulin-like receptor (KIR) genes and their HLA ligands with susceptibility to Behçet's disease. *Scand. J. Rheumatol.* 47: 155–163.
- International Study Group for Behçet's Disease. 1990. Criteria for diagnosis of Behçet's disease. *Lancet* 335: 1078–1080.
- Norman, P. J., J. A. Hollenbach, N. Nemat-Gorgani, W. M. Marin, S. J. Norberg, E. Ashouri, J. Jayaraman, E. E. Wroblewski, J. Trowsdale, R. Rajalingam, et al. 2016. Defining KIR and HLA class I genotypes at highest resolution via high-throughput sequencing. *Am. J. Hum. Genet.* 99: 375–391.
- Norman, P. J., L. Abi-Rached, K. Gendzekhadze, D. Korbel, M. Gleimer, D. Rowley, D. Bruno, C. V. Carrington, D. Chandanayingyong, Y. H. Chang, et al. 2007. Unusual selection on the KIR3DL1/S1 natural killer cell receptor in Africans. *Nat. Genet.* 39: 1092–1099.
- Saunders, P. M., P. Pymm, G. Pietra, V. A. Hughes, C. Hitchen, G. M. O'Connor, F. Loiacono, J. Widjaja, D. A. Price, M. Falco, et al. 2016. Killer cell immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of HLA class I recognition. *J. Exp. Med.* 213: 791–807.
- Tao, S. D., Y. M. He, Y. L. Ying, J. He, F. M. Zhu, and H. J. Lv. 2014. KIR3DL1 genetic diversity and phenotypic variation in the Chinese Han population. *Genes Immun.* 15: 8–15.
- Sanjanwala, B., M. Draghi, P. J. Norman, L. A. Guethlein, and P. Parham. 2008. Polymorphic sites away from the Bw4 epitope that affect interaction of Bw4+ HLA-B with KIR3DL1. *J. Immunol.* 181: 6293–6300.
- Sharma, D., K. Bastard, L. A. Guethlein, P. J. Norman, N. Yawata, M. Yawata, M. Pando, H. Thananchai, T. Dong, S. Rowland-Jones, et al. 2009. Dimorphic motifs in D0 and D1+D2 domains of killer cell Ig-like receptor 3DL1 combine to

- form receptors with high, moderate, and no avidity for the complex of a peptide derived from HIV and HLA-A\*2402. *J. Immunol.* 183: 4569–4582.
35. Thomas, R., E. Yamada, G. Alter, M. P. Martin, A. A. Bashirova, P. J. Norman, M. Altfeld, P. Parham, S. K. Anderson, D. W. McVicar, and M. Carrington. 2008. Novel KIR3DL1 alleles and their expression levels on NK cells: convergent evolution of KIR3DL1 phenotype variation? *J. Immunol.* 180: 6743–6750.
  36. Pelak, K., A. C. Need, J. Fellay, K. V. Shianna, S. Feng, T. J. Urban, D. Ge, A. De Luca, J. Martinez-Picado, S. M. Wolinsky, et al; NIAID Center for HIV/AIDS Vaccine Immunology. 2011. Copy number variation of KIR genes influences HIV-1 control. [Published erratum appears in 2011 *PLoS Biol.* 9.] *PLoS Biol.* 9: e1001208.
  37. Boudreau, J. E., T. J. Mulrooney, J. B. Le Luque, E. Barker, and K. C. Hsu. 2016. KIR3DL1 and HLA-B density and binding calibrate NK education and response to HIV. *J. Immunol.* 196: 3398–3410.
  38. Kang, E. H., J. Y. Kim, F. Takeuchi, J. W. Kim, K. Shin, E. Y. Lee, Y. J. Lee, E. B. Lee, M. H. Park, and Y. W. Song. 2011. Associations between the HLA-A polymorphism and the clinical manifestations of Behçet's disease. *Arthritis Res. Ther.* 13: R49.
  39. Montes-Cano, M. A., M. Conde-Jaldón, J. R. García-Lozano, L. Ortiz-Fernández, N. Ortego-Centeno, M. J. Castillo-Palma, G. Espinosa, G. Graña-Gil, M. A. González-Gay, A. C. Barnosí-Marín, et al. 2013. HLA and non-HLA genes in Behçet's disease: a multicentric study in the Spanish population. *Arthritis Res. Ther.* 15: R145.
  40. Martin, M. P., V. Naranbhai, P. R. Shea, Y. Qi, V. Ramsuran, N. Vince, X. Gao, R. Thomas, Z. L. Brumme, J. M. Carlson, et al. 2018. Killer cell immunoglobulin-like receptor 3DL1 variation modifies HLA-B\*57 protection against HIV-1. *J. Clin. Invest.* 128: 1903–1912.
  41. O'Connor, G. M., J. P. Vivian, E. Gostick, P. Pymm, B. A. Lafont, D. A. Price, J. Rossjohn, A. G. Brooks, and D. W. McVicar. 2015. Peptide-dependent recognition of HLA-B\*57:01 by KIR3DS1. *J. Virol.* 89: 5213–5221.
  42. Goodridge, J. P., A. Burian, N. Lee, and D. E. Geraghty. 2010. HLA-F complex without peptide binds to MHC class I protein in the open conformer form. *J. Immunol.* 184: 6199–6208.
  43. Trydzinska, H., K. Juerchott, N. Lachmann, K. Kotsch, K. Kunert, B. Weist, C. Schönemann, R. Schindler, P. Nickel, M. F. Melzig, et al. 2013. The genetic predisposition of natural killer cell to BK virus-associated nephropathy in renal transplant patients. *Kidney Int.* 84: 359–365.
  44. Besson, C., S. Roetenck, F. Williams, L. Orsi, C. Amiel, C. Lependevan, G. Antoni, O. Hermine, P. Brice, C. Ferme, et al. 2007. Association of killer cell immunoglobulin-like receptor genes with Hodgkin's lymphoma in a familial study. *PLoS One* 2: e406.
  45. Venstrom, J. M., T. A. Gooley, S. Spellman, J. Pring, M. Malkki, B. Dupont, E. Petersdorf, and K. C. Hsu. 2010. Donor activating KIR3DS1 is associated with decreased acute GVHD in unrelated allogeneic hematopoietic stem cell transplantation. *Blood* 115: 3162–3165.
  46. Taner, S. B., M. J. Pando, A. Roberts, J. Schellekens, S. G. Marsh, K. J. Malmberg, P. Parham, and F. M. Brodsky. 2011. Interactions of NK cell receptor KIR3DL1\*004 with chaperones and conformation-specific antibody reveal a functional folded state as well as predominant intracellular retention. *J. Immunol.* 186: 62–72.
  47. Parham, P., P. J. Norman, L. Abi-Rached, and L. A. Guethlein. 2011. Variable NK cell receptors exemplified by human KIR3DL1/S1. *J. Immunol.* 187: 11–19.
  48. Martin, M. P., Y. Qi, X. Gao, E. Yamada, J. N. Martin, F. Pereyra, S. Colombo, E. E. Brown, W. L. Shupert, J. Phair, et al. 2007. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* 39: 733–740.
  49. Iraolagoitia, X. L., R. G. Spallanzani, N. I. Torres, R. E. Araya, A. Ziblat, C. I. Domaica, J. M. Sierra, S. Y. Nuñez, F. Secchiari, T. F. Gajewski, et al. 2016. NK cells restrain spontaneous antitumor CD8+ T cell priming through PD-1/PD-L1 interactions with dendritic cells. *J. Immunol.* 197: 953–961.
  50. Saunders, P. M., J. P. Vivian, N. Baschuk, T. Beddoe, J. Widjaja, G. M. O'Connor, C. Hitchen, P. Pymm, D. M. Andrews, S. Gras, et al. 2015. The interaction of KIR3DL1\*001 with HLA class I molecules is dependent upon molecular microarchitecture within the Bw4 epitope. *J. Immunol.* 194: 781–789.
  51. Gumperz, J. E., L. D. Barber, N. M. Valiante, L. Percival, J. H. Phillips, L. L. Lanier, and P. Parham. 1997. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NK1 killer cell-inhibitory receptor. *J. Immunol.* 158: 5237–5241.
  52. Gumperz, J. E., N. M. Valiante, P. Parham, L. L. Lanier, and D. Tyan. 1996. Heterogeneous phenotypes of expression of the NK1 natural killer cell class I receptor among individuals of different human histocompatibility leukocyte antigens types appear genetically regulated, but not linked to major histocompatibility complex haplotype. *J. Exp. Med.* 183: 1817–1827.
  53. Díaz-Peña, R., J. R. Vidal-Castñeira, R. Alonso-Arias, B. Suarez-Alvarez, J. L. Vicario, R. Solana, E. Collantes, A. López-Vázquez, J. Martínez-Borra, and C. López-Larrea. 2010. Association of the KIR3DS1\*013 and KIR3DL1\*004 alleles with susceptibility to ankylosing spondylitis. *Arthritis Rheum.* 62: 1000–1006.
  54. Guasp, P., E. Barnea, M. F. González-Escribano, A. Jiménez-Reinoso, J. R. Regueiro, A. Admon, and J. A. López de Castro. 2017. The Behçet's disease-associated variant of the aminopeptidase ERAP1 shapes a low-affinity HLA-B\*51 peptidome by differential subpeptidome processing. *J. Biol. Chem.* 292: 9680–9689.
  55. Guasp, P., C. Alvarez-Navarro, P. Gomez-Molina, A. Martín-Esteban, M. Marcilla, E. Barnea, A. Admon, and J. A. López de Castro. 2016. The peptidome of Behçet's disease-associated HLA-B\*51:01 includes two sub-peptidomes differentially shaped by endoplasmic reticulum aminopeptidase 1. *Arthritis Rheumatol.* 68: 505–515.
  56. Gwalani, L. A., and J. S. Orange. 2018. Single degranulations in NK cells can mediate target cell killing. *J. Immunol.* 200: 3231–3243.
  57. Oszmiana, A., D. J. Williamson, S. P. Cordoba, D. J. Morgan, P. R. Kennedy, K. Stacey, and D. M. Davis. 2016. The size of activating and inhibitory killer Ig-like receptor nanoclusters is controlled by the transmembrane sequence and affects signaling. *Cell Rep.* 15: 1957–1972.
  58. Kuranov, A. B., I. Kötter, J. C. Henes, S. T. Abisheva, I. Steiert, F. Riewerts, K. T. Momynaliev, and C. A. Müller. 2014. Behçet's disease in HLA-B\*51 negative Germans and Turks shows association with HLA-Bw4-80I. *Arthritis Res. Ther.* 16: R116.