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Innate partnership of *HLA-B* and *KIR3DL1* subtypes against HIV-1

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Abstract

Allotypes of the natural killer (NK) cell receptor KIR3DL1 vary in both NK cell expression patterns and inhibitory capacity upon binding to their ligands, HLA-B Bw4 molecules, present on target cells. Using a sample size of over 1,500 human immunodeficiency virus (HIV)[+] individuals, we show that various distinct allelic combinations of the *KIR3DL1* and *HLA-B* loci significantly and strongly influence both AIDS progression and plasma HIV RNA abundance in a consistent manner. These genetic data correlate very well with previously defined functional differences that distinguish KIR3DL1 allotypes. The various epistatic effects observed here for common, distinct *KIR3DL1* and *HLA-B Bw4* combinations are unprecedented with regard to any pair of genetic loci in human disease, and indicate that NK cells may have a critical role in the natural history of HIV infection.

NK cells are critical components of the innate immune system that have direct involvement in the antiviral immune response[1]. NK cells are controlled by many activating and inhibitory receptors[2,3], including members of the killer cell immunoglobulin-like receptor (KIR) family. Similarly to other NK cell receptors, KIRs are expressed on T cells as well as on NK cells, affirming their role in both innate and adaptive immunity, but they are distinct from other NK cell receptors in that they are exceptionally diverse and rapidly evolving[4]. Each *KIR* locus encodes either an inhibitory or an activating receptor, except for the *KIR3DL1* gene, which encodes one common activating allotype, KIR3DS1, and several inhibitory allotypes. The ligands for the inhibitory KIR3DL1 allotypes are HLA-B molecules that contain the Bw4 motif at positions 77–83 (ref. 5), particularly the subset of Bw4 allotypes containing isoleucine at position 80 (Bw4-80I) as opposed to those with threonine (Bw4-80T) at this position[5–7]. The ligands for KIR3DS1 are not known, but this molecule shares 97% sequence similarity with KIR3DL1 allotypes and may recognize a similar or overlapping set of ligands.

KIRs are expressed in a variegated manner on the NK cell population of a given individual[8]: only a certain percentage of NK cells express the product of a given *KIR* gene. Further, mean fluorescence intensity measurements show subtypes of KIR3DL1 to be expressed at different abundances on NK cells[9]. Recently, it was shown that NK cell inhibitory capacities of individual KIR3DL1 allotypes are closely linked to their abundances on NK cells and to the percentages of cells expressing these molecules within the NK cell population of a given individual[10]. The functional repercussions of variability at the *KIR3DL1* locus provide a logical, operative grouping system for the alleles of this locus: alleles encoding high-expression allotypes (*KIR3DL1*h*), low-expression allotypes (*KIR3DL1*l*), and no cell surface expression (*004*). *KIR3DL1*004* represents an unusual allele as it encodes a protein that is retained within the cell[11], a characteristic that may have functional importance. Variation in the affinity of individual KIR3DL1 allotypes for various HLA-B Bw4 allotypes (controlling for expression levels) has also been shown to a limited extent[6,10]: the KIR3DL1 high-expression allotypes that have been tested show higher affinity for Bw4-80I allotypes than for Bw4-80T, which results in greater inhibition through Bw4-80I recognition.

The most protective *HLA* class I alleles in terms of HIV disease progression belong to the group of *HLA-B Bw4* alleles. In particular, two of these *Bw4* alleles, *B*27* and *B*57* (*HLA-B*

alleles being represented hereinafter by the allele names standing alone), show the greatest protection in several genetic and functional studies (reviewed in ref. 12), the basis of which stems, at least in part, from the immuno-dominant HIV peptides recognized by these allotypes in acquired immune responses[13]. However, many of the other members of the *Bw4* group also tend toward protection relative to the alternative group of *HLA-B* alleles, *Bw6* (ref. 14). As individual allotypes belonging to the Bw4 group recognize distinct peptide motifs[15], it is unlikely that common peptide recognition explains the protection conferred by Bw4 as a group; rather, overall Bw4 protection probably has to do with the function of these molecules in the innate immune response as ligands for KIR3DL1 and/or KIR3DS1 (KIR3DL1;3DS1) (ref. 16). Indeed, we have previously shown that the activating *KIR3DS1* allele in combination with *Bw4-80I* associates with protection against HIV disease progression[17], as well as against opportunistic infections in HIV+ individuals[18]. These data raise the possibility that KIR3DS1 may bind one or more of the HLA-B Bw4-80I allotypes on cells infected with HIV-1, directly mediating effector cell killing of the infected target, a parsimonious model in which NK cell activation renders protection against HIV. Given the functional diversity of the KIR3DL1 subtypes in terms of the degree of inhibition they confer, it is possible that they may also differentially affect HIV pathogenesis (apart from the protection conferred by *KIR3DS1 + Bw4-80I*).

Here we tested the effects of inhibitory *KIR3DL1* subtypes in combination with *HLA-B* allelic groups on HIV disease progression and viral load. These data illustrate a primary role for various distinct combinations of *KIR3DL1* and *HLA-Bw4* in the innate immune response against HIV. Of note, the highly expressed, highly inhibitory *KIR3DL1*h* alleles strongly enhance protection conferred by *Bw4-80I* alleles, including *B*57* specifically, an unexpected result given the protective effect of a putatively activating genotype, *KIR3DS1 + Bw4-80I*, against AIDS progression[17]. These seemingly contrasting results can be explained logically by a model in which greater dependency on the expression of specific KIR3DL1 + Bw4 receptor-ligand pairs for NK cell inhibition in the resting state (where *KIR3DL1*h > KIR3DL1*l*) results in more pronounced NK cell responses when that inhibition is abrogated in the face of infection. This model is consistent with recent studies implicating inhibitory receptors for MHC class I in the education and eventual killing capacity of NK cells[19–21].

RESULTS

KIR3DL1*004 + Bw4 slows progression to AIDS

We compared the frequencies of each *KIR3DL1* allele in our African American and European American subjects, and the most notable racial differences observed were with *KIR3DL1*015* (a highly expressed allele), which was by far the most common allele in African Americans, and, as reported previously, *KIR3DS1* (ref. 17) (Fig. 1). As was the case for the *KIR3DS1 + Bw4-80I* effect[17,18], the genetic effects described here were consistent across the two main ethnic groups based on analyses stratified by ancestry (see Methods). All statistics provided in this report were derived from analyses stratified by race.

The influence of individual *KIR3DL1* alleles on AIDS progression (see Methods for a description of the AIDS outcomes) was tested in a cohort of 915 HIV+ individuals whose date of seroconversion was known within a period of 6 months on average (seroconverters).

In order to eliminate confounding by the protective effect of *KIR3DS1 + Bw4-80I*, individuals with this compound genotype were removed from all analyses described herein. (Our previous study showing protection with this compound genotype[17] was performed in this same cohort). HLA-B Bw4 (and not HLA-B Bw6) allotypes serve as ligands for KIR3DL1 subtypes[5,7]. In *Bw6/Bw6* individuals, it does not matter which *KIR3DL1* subtype is present because the ligand is absent, so the KIR3DL1 molecule is nonfunctional in these individuals. As the *Bw6/Bw6* group does not contain any *KIR3DL1 + HLA-B* genotype combinations to confound our analyses, we used this group of individuals as a consistent control group for every analysis of *KIR3DL1* subtypes, except as noted otherwise. Using *Bw6/Bw6* as a consistent reference group allowed direct comparisons of the degrees of effects conferred by distinct *KIR3DL1 + Bw4* subtypes. In the presence of *Bw4*, *KIR3DL1*004* showed the most significant protection relative to all other *KIR3DL1* alleles (relative hazard (RH) = 0.50 and 0.59, P = 0.0001 and 0.001; the first value in each case refers to the AIDS1987 outcome analysis and the second to the CD4$^+$ T-cell count < 200 cells/mm^3 outcome analysis (see Methods), and this convention will apply wherever two statistics are given; Fig. 2 and Supplementary Table 1 online). The q value (the expected proportion of false positives[22]) for this association (q = 0.00005 and 0.0002) indicates the extreme unlikelihood that this is a false positive discovery (Supplementary Table 1). The protective effect of *KIR3DL1*004* was completely dependent on the presence of *Bw4*, as indicated by the lack of any effect in a comparison of individuals with a *Bw6/Bw6* genotype who either had *KIR3DL1*004* (*Bw6/Bw6 + KIR3DL1*004; N* = 86 and 81) or did not (*Bw6/Bw6* + no *KIR3DL1*004; N* = 254 and 246; RH = 1.11 and 0.91, P = 0.56 and 0.59). KIR3DL1*004 is a unique KIR3DL1 allotype in that it is retained within the cell[11], a characteristic that has led to its functionality being called into question. Slow progression to AIDS among individuals carrying *KIR3DL1*004 + Bw4* raises the possibility that the KIR3DL1*004 molecule may actually bind Bw4 molecules intracellularly with functionally relevant consequences, a possibility that will require further investigation.

Effects of KIR3DL1 allelic groups + Bw4 on AIDS progression

The *Bw4* alleles as a group show protection against AIDS progression[14], and at least part of this protection can be attributed to two independent *KIR + HLA* compound genotypes: (i) *KIR3DS1 + Bw4-80I* (ref. 17) and (ii) *KIR3DL1*004 + Bw4* (Supplementary Table 1; Fig. 2). The remaining *KIR3DL1* alleles can be divided into one of two groups: the *KIR3DL1*h* (*001, *002, *008, *015, *009*) or *KIR3DL1*l* (*005, *007*) groupings, which were defined previously by KIR3DL1 expression patterns and, in some cases, corresponding inhibitory capacity[6,9,10]. Furthermore, in terms of intensity of staining and percentage of NK cells expressing the allotype, *h/*h individuals show significant differences from both *h/*l and *l/*l, but *h/*l and *l/*l do not show any observable difference from one another[10]. To test whether the high-expression alleles differ from the low-expression alleles, individuals were divided into one of two groups: (i) individuals who carried *KIR3DL1*h* in the absence of *KIR3DL1*l* (i.e., *KIR3DL1 *h/*h* or *KIR3DL1*h/*004*; abbreviated as *KIR3DL1*h/*y*, where *y = *h* or *004*), and (ii) individuals with at least one copy of *KIR3DL1*l* (*KIR3DL1*l/*l, KIR3DL1*l/*h*, or *KIR3DL1*l/*004;* abbreviated as *KIR3DL1*l/*x*, where *x = *l, *h,* or *004*). (Individuals with *KIR3DL1*004* were not excluded from further analyses because this allele was observed at an equal percentage in the *h* and *l* groups, and

the protection conferred by *KIR3DL1*004 + Bw4* was not significantly different in the presence of **h* versus **l*). Although *KIR3DL1*h/*y + Bw4* and *KIR3DL1*l/*x + Bw4* were each protective compared with *Bw6/Bw6*, *KIR3DL1*h/*y* showed a more significant effect (RH = 0.66 and 0.70, *P* = 0.002 for *KIR3DL1*h/*y + Bw4* and RH = 0.79 and 0.77, *P* = 0.12 and 0.05 for *KIR3DL1*l/*x + Bw4*; Table 1). Thus, a consistent trend showing more pronounced protection proceeded as follows: *KIR3DL1*h/*y + Bw4 > KIR3DL1*l/*x + Bw4 > Bw6/Bw6* (trend *P* = 0.001 and 0.002).

KIR3DL1*h/*y + Bw4-80I protects against AIDS progression

In studies performed to date, HLA-B allotypes with Bw4-80I behave as stronger ligands for KIR3DL1 than do allotypes with Bw4-80T (refs. 6,7). Although this stratification had no effect on *KIR3DL1*004* protection (data not shown), the protection conferred by *KIR3DL1 *h/*y + Bw4* can be attributed primarily to the subset with *KIR3DL1*h/*y + Bw4-80I* (RH = 0.56–0.61, *P* = 0.001; Table 1). In contrast, *KIR3DL1*l/*x* showed no significant protection in the presence of *Bw4-80I*, although this *KIR + HLA* combination did tend toward protection compared with the *Bw6/Bw6* group (RH = 0.79 and 0.89; Table 1 and Fig. 3). Indeed, *KIR3DL1*h/*y + Bw4-80I* was borderline significantly more protective than *KIR3DL1*l/*x + Bw4-80I* in a stringent, direct comparison of the two groups (RH = 0.63 and 0.66, *P* = 0.06 and 0.04; Supplementary Table 2 online). *KIR3DL1*l/*x* did, however, show some protection against CD4$^+$ T-cell count < 200 cells/mm^3 (an earlier outcome than AIDS1987, which includes HIV infection plus an AIDS-defining illness) in the presence of *Bw4-80T* (RH = 0.68, *P* = 0.02; Table 1 and Fig. 3). Overall, these data support a high-affinity ligand-receptor interaction between Bw4-80I and KIR3DL1*h, and raise the possibility of greater affinity of KIR3DL1*l allotypes for ligands containing Bw4-80T than for those containing Bw4-80I. A significant trend for most protective to least protective proceeded as *KIR3DL1*h/*y + Bw4-80I > KIR3DL1*l/*x + Bw4-80I > Bw6/Bw6* (trend *P* = 0.001; Table 1).

Synergism between specific Bw4 alleles and KIR3DL1*h and *l

HLA-B*57 and *B*27* are the two most protective alleles in our cohorts of HIV-infected individuals[23,24], and a sizeable body of literature has indicated that cytotoxic T lymphocytes restricted by these allotypes serve as the basis for their exceptional ability to control HIV (reviewed in ref. 13). All *B*57* allelic subtypes encode members of the Bw4-80I group of allotypes, whereas all of the *B*27* subtypes in our cohorts, with the exception of six subjects with *B*2702*, encoded Bw4-80T allotypes. As *KIR3DL1*h/*y* showed a significant epistatic relationship with *Bw4-80I*, but not with *Bw4-80T*, against HIV disease, we tested the hypothesis that *KIR3DL1*h/*y* would enhance the protective effect of *B*57*, but not *B*27* (with the six individuals with *B*2702* removed from the analyses). Using *Bw6/Bw6* individuals as the control group in each analysis, both the *B*57 + KIR3DL1*h/*y* and the *B*57 + KIR3DL1*l/*x* groups were protective against both AIDS outcomes, but the *B*57* effect was enhanced in the presence of *KIR3DL1*h/*y* as compared with *KIR3DL1*l/*x* (RH = 0.26 and 0.30, *P* = 0.003 and 0.0005 for *B*57 + KIR3DL1*h/*y*; RH = 0.60 and 0.50, *P* = 0.19 and 0.06 for *B*57 + KIR3DL1*l/*x*, Fig. 4, Supplementary Table 3 online). The *B*57 + KIR3DL1*h/*y* genotype conferred stronger protection against AIDS progression

than any other host genotype identified to date. The progressive effect of *B*57* in the presence of distinct *KIR3DL1* groups indicates that the overall protection conferred by *B*57* does not stem solely from its function as a cytotoxic T lymphocyte–restricting element in the acquired immune response, but perhaps also as a mediator of innate immunity.

The group of individuals with *B*27-80T* (*B*2705*, $N = 60$ and *B*2709*, $N = 1$), on the other hand, showed borderline significant protection in the presence of *KIR3DL1*l/*x* and only very moderate, nonsignificant protection in the presence of *KIR3DL1*h/*y* (RH = 0.44 and 0.47, $P = 0.05$ and 0.04 for *B*27* + *KIR3DL1*l/*x*; RH = 0.70 and 0.82, $P = 0.25$ and 0.46 for *B*27* + *KIR3DL1*h/*y*, Fig. 4, Supplementary Table 3). The more prominent protection of *B*57* in the presence of *KIR3DL1*h/*y* than in the presence of *KIR3DL1*l/*x* accurately parallels the interaction between *KIR3DL1*h/*y* and *Bw4-80I* in general, as described above.

Consistent KIR3DL1 + Bw4 effects on plasma HIV RNA

Several *KIR3DL1* + *Bw4* genotypes showed protection against HIV disease progression relative to the control group *Bw6/Bw6*, the strongest and most conclusive of which included the following: *KIR3DL1*h/*y* + *Bw4-80I*, *KIR3DL1*004 + *Bw4*, *KIR3DL1*h/*y* + *B*57*, and to a lesser extent, *KIR3DL1*l/*x* + *B*27*. We tested the potential effect of these and related genotypes on MVL measurements that were available for 891 subjects from five studies, 508 of whom were unique to these analyses and were not included in the disease progression analyses. Based on their mean viral load (MVL, given throughout in RNA copies per ml plasma), each individual was categorized into one of three groups: (i) MVL < 2,000, (ii) MVL = 2,000–10,000 or (iii) MVL > 10,000. We then determined the frequency of each compound genotype within each of these groupings and compared it with that of *Bw6/Bw6*.

First we directly compared the two extreme MVL groupings. The relative frequency of *KIR3DL1*h/*y* + *Bw4-80I* was higher than that of *Bw6/Bw6* in the MVL <2,000 grouping, whereas the opposite was observed in the MVL >10,000 grouping, and these distributions were significantly different (odds ratio (OR) = 0.24 for *KIR3DL1*h/*y* + *Bw4-80I*, $P = 6 \times 10^{-8}$; Table 2). Furthermore, they remained significantly different upon removal of individuals positive for *B*57*, *B*27* and/or *KIR3DL1*004 from the *KIR3DL1*h/*y* + *Bw4-80I* group (OR = 0.29, $P = 5 \times 10^{-4}$). *KIR3DL1*l/*x* + *Bw4-80I* also showed significant protection compared with the *Bw6/Bw6* group (OR = 0.43, $P = 0.007$; Table 2), but the effect was diminished upon removal of *B*57*, *B*27* and/or *KIR3DL1*004 (OR = 0.55, $P = 0.17$). Further, protection conferred by *KIR3DL1*l/*x* + *Bw4-80I* was less than that conferred by *KIR3DL1*h/*y* + *Bw4-80I*, with borderline significance, in a direct comparison of these two *Bw4-80I* groupings (OR = 0.58, $P = 0.08$; Supplementary Table 2). *KIR3DL1*004 + *Bw4* also showed significant protection relative to the *Bw6/Bw6* control group (OR = 0.36, $P = 2 \times 10^{-4}$), and the distributions remained significant upon removal of all individuals with *B*57*, *B*27*, and/or *KIR3DL1*h/*y* + *Bw4-80I* from the *KIR3DL1*004 + *Bw4* group (OR = 0.27, $P = 5 \times 10^{-5}$; Table 2). These viral load data concur well with the disease progression data and strongly support protective roles for *KIR3DL1*h/*y* + *Bw4-80I*

and *KIR3DL1*004 + Bw4*, even when the samples that overlap between these two groups are removed from the analyses (Supplementary Table 4 online).

The individual effects of *B*57* (a *Bw4-80I* allele) and *B*27* (a *Bw4-80T* allele) on HIV MVL closely followed and emphasized the patterns of association observed in the progression analysis (Fig. 4): in comparison with the *Bw6/Bw6* group, *B*57* showed exceptionally robust protection in the presence of *KIR3DL1*h/*y* (OR = 0.1, $P = 7 \times 10^{-11}$; Table 2), an effect that was diminished in the presence of *KIR3DL1*l/*x* (OR = 0.38, $P = 0.04$), whereas *B*27* was more protective in the presence of *KIR3DL1*l/*x* (OR = 0.22, $P = 0.003$) than in the presence of *KIR3DL1*h/*y* (OR = 0.37, $P = 0.05$). A direct comparison between the *B*57 + KIR3DL1*h/*y* and the *B*57 + KIR3DL1*l/*x* effects on viral load indicated a significantly stronger protective effect of *B*57 + KIR3DL1*h/*y* (OR = 0.23, $P = 0.009$; Supplementary Table 2). These data indicate that *KIR3DL1* allotypes can strongly modulate the protection conferred by *B*57* in particular, and imply an important role for *B*57* (and possibly *B*27*) in the innate immune response against HIV.

In a second set of analyses, the differences in frequency trends across the three viral load divisions (MVL < 2,000, MVL = 2,000–10,000, and MVL > 10,000) were compared between individual *KIR3DL1 + Bw4* genotypic groups and the *Bw6/Bw6* group (Table 2, far right columns). In each comparison, the relative strength and significance of these differences reflected that observed in the comparisons of the two extreme groups (MVL < 2,000 and MVL > 10,000).

DISCUSSION

The functional consequences of allelic variability at the *KIR3DL1* locus have been studied in greater detail than that of any other single *KIR* gene[6,10]. In this study, we grouped inhibitory *KIR3DL1* alleles based on distinct functional characteristics and identified differential protective effects of these allelic groups on HIV disease progression and viral load. *KIR3DL1*004* represented the most protective single allele at this locus, and its protection was completely dependent on the presence of *HLA-B* alleles with the *Bw4* motif, the ligands for inhibitory KIR3DL1 allotypes. We found this result surprising, as the KIR3DL1*004 molecule is retained within the cell, offering the possibility that it has a null phenotype. The genetic data presented herein dispel this hypothesis and imply a novel intracellular function for KIR3DL1*004 that directly or indirectly involves the Bw4 ligand. Alternatively, the KIR3DL1*004 molecule may be marking (by linkage disequilibrium) another neighboring locus that confers protection in an additive or synergistic manner with *Bw4*.

Previous data have indicated a functional distinction between the strongly inhibitory *KIR3DL1*h* alleles and the weakly inhibitory *KIR3DL1*l* alleles[9,10], and based on data presented herein these groupings also have differential effects in the HIV disease process. Overall, a pattern emerged indicating relatively strong protection through *KIR3DL1*h + Bw4-80I*, and a weak protective effect through *KIR3DL1*l + Bw4-80T*. These data were reinforced by parallel conclusions when the individual *B*57* (*Bw4-80I*) and *B*27* (*Bw4-80T*) alleles were examined and shown to be most protective in the presence of *KIR3DL1*h/*y* and *KIR3DL1*l/*x*, respectively. Previous functional and genetic studies have also indicated

a stronger relationship of some KIR3DL1 allotypes with Bw4-80I than with Bw4-80T (refs. 6,7, 10,17,25), but our genetic data imply greater complexity in the receptor-ligand interactions between these molecules. An analysis of MVL data determined from a group of 891 individuals, only 383 of whom overlapped with the 915 individuals used in the disease progression analyses, showed protective effects of the various *KIR3DL1 + HLA-B* genotypes strongly consistent with those observed in disease progression analyses. Thus, the influence of these genotypes on disease course occurs early after infection, especially for *B*57 + KIR3DL1*h/*y*, and may continue throughout the chronic phase of infection. The protection against AIDS progression conferred by various combinations of *KIR3DL1 + Bw4* genotypes reported herein represents the first data to support a consequential impact of differential KIR3DL1 allotypic functions on disease outcome of any sort. There was a continuum of synergistic effects of the *KIR3DL1* and *HLA-B* loci (including *KIR3DS1 + Bw4-80I*) in order by degree of protection (disease progression, Fig. 5a; MVL, Fig. 5b; see also Table 2 and Supplementary Table 5 online) emphasizing the complexity and strength of protection conferred by combinations of these two loci. There is no other pair of loci evaluated thus far that together shows the diversity in protective effects against AIDS (or any other disease) of that observed with *KIR3DL1;3DS1* and *HLA-Bw4*.

The activating *KIR3DS1* in the presence of *Bw4-80I* associates with slow progression to AIDS[17], lower viral load[18], and protection against opportunistic infections during HIV infection[18]. Based on these data, we proposed that NK cell and/or CD8^{+} T cell activation through a KIR3DS1–Bw4-80I interaction protects against HIV. Further, activating *KIR* genotypes may protect against HIV infection[26]. This model of effector cell activation leading to protection appears incongruent with the protection conferred by inhibitory *KIR3DL1* subtypes (particularly those that are expressed at high levels and transmit strong inhibitory signals) in the presence of their class I ligands, as shown herein. However, these findings are actually very compatible given our knowledge of the functional development of NK cells and the role of inhibitory receptors in this development. Broadly accepted models of NK cell repertoire development suggest that NK cells continue to acquire inhibitory receptors until sufficient signals are generated to quench autoreactivity through recognition of autologous class I ligands[27–29]. The resultant population of NK cells utilizes various KIR + HLA class I pairings that confer various degrees of inhibition to achieve overall nonresponsiveness to self. Therefore, the larger the contribution of a given receptor-ligand pairing to NK cell inhibition under 'normal' conditions, the more vigorous an effector cell response will be when the interaction between that inhibitory receptor and its ligand is disrupted. The application of this concept to KIR3DL1 subtypes is particularly apropos, as there are clear distinctions between KIR3DL1*h and KIR3DL1*l subtypes in terms of the level of their expression on a per-cell basis[9], the overall frequency of NK cells expressing these subtypes[10] and the ability of HLA-Bw4 to increase the frequency of NK cells expressing KIR3DL1*h to a significantly greater extent than KIR3DL1*l[10]. The activation potential of an NK cell population would also be subject to the affinity between the particular KIR3DL1-Bw4 allotypic pair, an effect implied by the synergism between *KIR3DL1*h* and *B*57* and, alternatively, between *KIR3DL1*l* and *B*27* (Table 2 and Fig. 4).

Notably, this concept of greater inhibition during NK cell development ultimately leading to more vigorous activation under appropriate conditions, such as viral infection, is consistent with data implying inhibitory receptor involvement in the developmental regulation of NK cell responsiveness[8,19–21,30,31]. These data indicate that weak or missing inhibitory signals during NK cell development result in poor (or complete lack of) activating potential by that NK cell, whereas strong inhibitory signals during NK cell development lead to greater potential for NK cell activation upon interaction with an aberrant target cell. Thus, engagement of highly expressed, highly inhibitory KIR3DL1 allotypes with their ligands may ultimately secure stronger NK cell responses in the event of viral infection, such as HIV, when KIR3DL1-mediated inhibition is suspended.

The complexity of *KIR3DL1* is steadily being disentangled by a combination of genetic and functional studies that feed off one another. One allele at this locus, the activating *KIR3DS1*, has been implicated in resistance to both HIV[17] and hepatitis C virus[32], and in susceptibility to cervical cancer[33]. It is now clear that variation among the inhibitory *KIR3DL1* subset of alleles also has an influence on HIV disease, and dissection of their differential influence is worth investigating in other diseases—such as cervical neoplasia, in which the presence of *Bw4* associates with decreased risk of high-grade cervical lesions and cancer[33]. Of the three classical *HLA* class I loci, *HLA-B* is the most rapidly evolving[34] and is also the most consequential in the acquired immune response against HIV from both functional and genetic epidemiological perspectives[23,35]. Notably, however, among *HLA-B* alleles, *Bw4* alleles show greater protection against HIV than *Bw6* alleles, an observation that strongly implicates a defense mechanism involving *KIR3DL1*, as shown herein. Like *HLA-B*, the *KIR3DL1* locus is also evolving rapidly compared with most other *KIR* loci[36], and in our cohorts it is this locus that shows the strongest effects on HIV disease. It is entirely plausible that coordinated coevolution of these two loci[37,38] is driven by pathogens such as HIV, resulting in a primary influence of specific *HLA-B* + *KIR3DL1* allelic combinations in the innate immune response against this virus.

METHODS

Subjects

We used data from a total of 1,496 HIV⁺ individuals in the studies described. For the disease progression studies, 915 HIV-1–infected subjects for whom the dates of seroconversion were known were derived from four cohorts: the Multicenter AIDS Cohort Study (MACS)[39], the Multicenter Hemophilia Cohort Study (MHCS)[40], the San Francisco City Clinic Cohort (SFCCC)[41] and the AIDS Linked to Intravenous Experience (ALIVE)[42]. For the viral load studies, 891 subjects for whom viral load data was available were obtained from five cohorts: the MACS, the Swiss HIV Cohort (http://www.shcs.ch), the Study on the Consequences of Protease Inhibitor Era (SCOPE) cohort[43], the Massachusetts General Hospital (MGH) Controller Cohort, and the NIAID long term nonprogressor cohort[44]. There were 383 individuals whose clinical data were used in both the disease progression and the viral load studies. Thus, 508 were used only in the viral load studies and 532 were used only in the disease progression studies. This study was approved by the protocol review office of

the US National Cancer Institute institutional review board. Informed consent was obtained at the study sites from all individuals.

HLA genotyping and *KIR3DL1* allelic typing

We performed genotyping of the *HLA* locus following the PCR-SSOP (sequence-specific oligonucleotide probing) typing protocol recommended by the 13th International Histocompatibility Workshop (http://www.ihwg.org/components/ssopr.htm). For *KIR3DL1* subtyping, polymorphic exons 3, 4, 5, 7, 8 and 9 were selectively amplified in four PCR reactions using locus-specific primers (a separate PCR for each of exons 3, 4 and 5, and a fourth PCR for exons 7, 8 and 9 combined). The PCR products were blotted on nylon membranes and hybridized with a panel of 56 sequence-specific oligonucleotide (SSO) probes designed to detect unique sequence motifs of known *KIR3DL1* alleles (Supplementary Table 6 online). *KIR3DL1* alleles were assigned by the reaction patterns of the SSO probes based on the known *KIR3DL1* sequences. Ambiguous SSO typing results were resolved by sequencing analysis.

Plasma HIV RNA measurements

Viral load measurements (HIV RNA copies per ml plasma in the absence of any drug therapy) determined previously from 891 HIV[+] individuals were available for analysis. MVL was determined for each individual and categorized into one of three groupings: <2,000, 2,000–10,000, and >10,000. The average number of measurements per person was 7.9 with a range of 1–52 and a total of 7,084 measurements. The ethnic breakdown was as follows: European American ($N = 685$), African American ($N = 136$), other ($N = 53$), unknown ($N = 17$).

Statistical analysis

All statistics provided were derived from analyses stratified by race. Two AIDS-related outcomes were considered in the survival analysis: (i) a CD4^{+} T-cell count of <200 cells/mm^3 and (ii) progression to AIDS according to the 1987 definition of the US Centers for Disease Control, which includes HIV infection plus an AIDS-defining illness[45]. In order to eliminate confounding by the previously described protective effect of *KIR3DS1* + *Bw4-80I*, individuals with this compound genotype were removed from all analyses. With the exception of the comparisons listed in Supplementary Table 2, all test variables were compared with those of the *Bw6/Bw6* control group.

The influence of individual and groups of *KIR3DL1* alleles on AIDS progression was tested using Cox model analyses. We divided *KIR3DL1* alleles into high- and low-expressing groups based on the previously defined expression patterns. The high-expressing alleles (*KIR3DL1*h*) in our cohorts were *001*, *002*, *008*, *015* and *009*, and the low-expressing alleles (*KIR3DL1*l*) were *005* and *007*. KIR3DL1*h/*h individuals show significant differences from both KIR3DL1*h/*l and *l/*l, but KIR3DL1*h/*l and *l/*l do not show any observable differences from one another in terms of intensity of staining and percentage of NK cells expressing the allotype[10]. Furthermore, the high-expression *KIR3DL1* alleles are more common than the low-expression alleles, resulting in a small *KIR3DL1*l/*l* genotypic frequency (13% in our entire dataset, 2.7% of whom had *Bw4-80I* and 4.7% of whom had

Bw4-80T). For these biological and practical reasons, the tests for different effects of high-versus low-expression alleles involved the division of individuals with only high-expression alleles from those with at least one low-expression allele.

We used SAS 9.1 (SAS Institute) for data management and statistical analyses. PROC FREQ was used to compute frequencies on individual variables. PROC LOGISTIC was used for categorical analyses to obtain odds ratios and 95% confidence intervals. PROC LIFETEST and PHREG were used for Kaplan-Meier and Cox model analyses. Statistical significance refers to two-sided *P* values of <0.05. The *q* value shown in Supplementary Table 1 is the false discovery rate. The false discovery rate is the expected proportion of false positives when declaring the significance level equal to *P; q* value estimates were calculated for the effect of individual *KIR3DL1* alleles with *HLA-Bw4* on AIDS progression using the *q* value package, v1.1 in the R statistical computing program[22].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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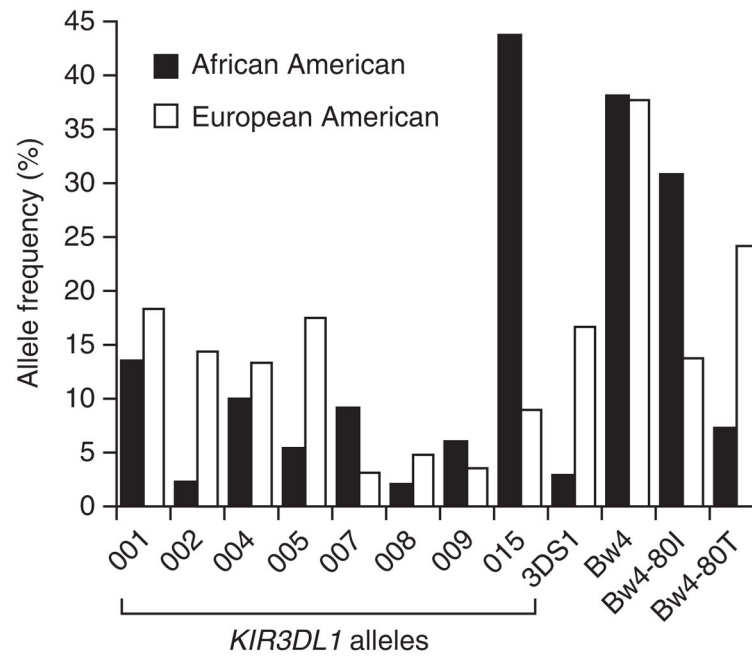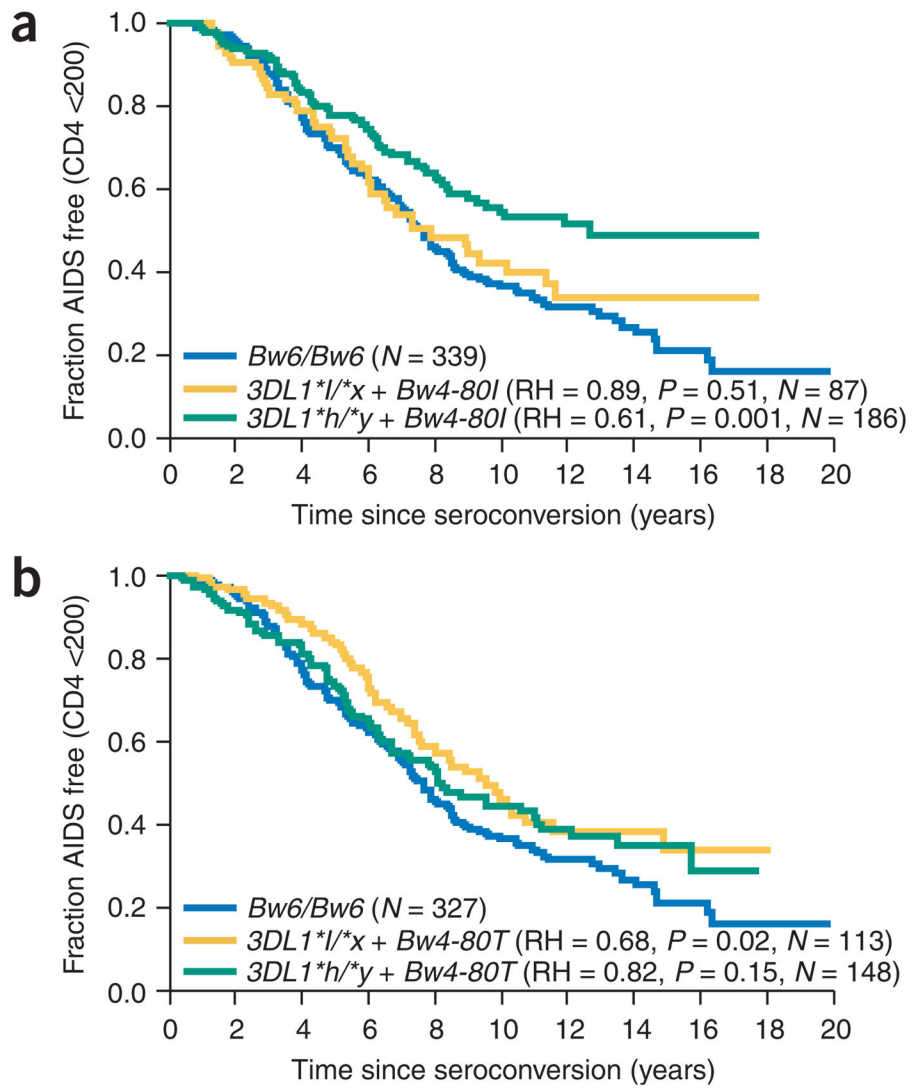
Figure 1.
Allele frequency of *KIR3DL1* alleles and *HLA-B Bw4* subtypes. The frequency of each variable is shown for African Americans and European Americans.
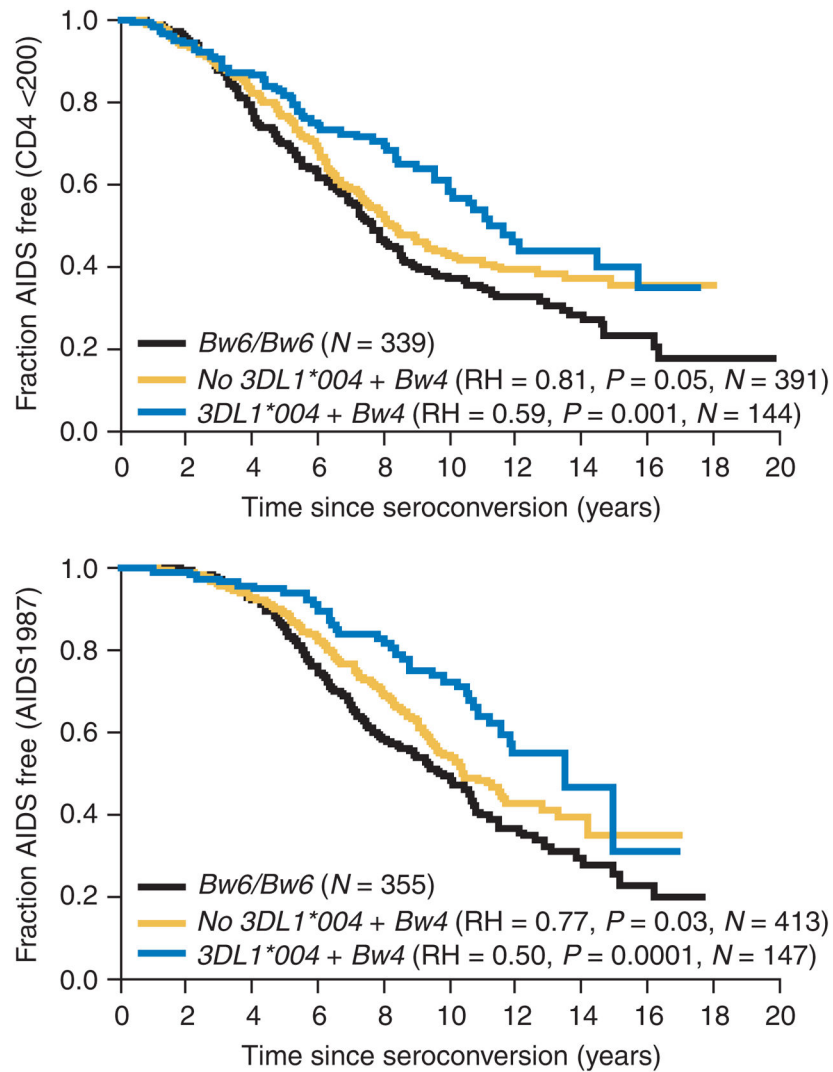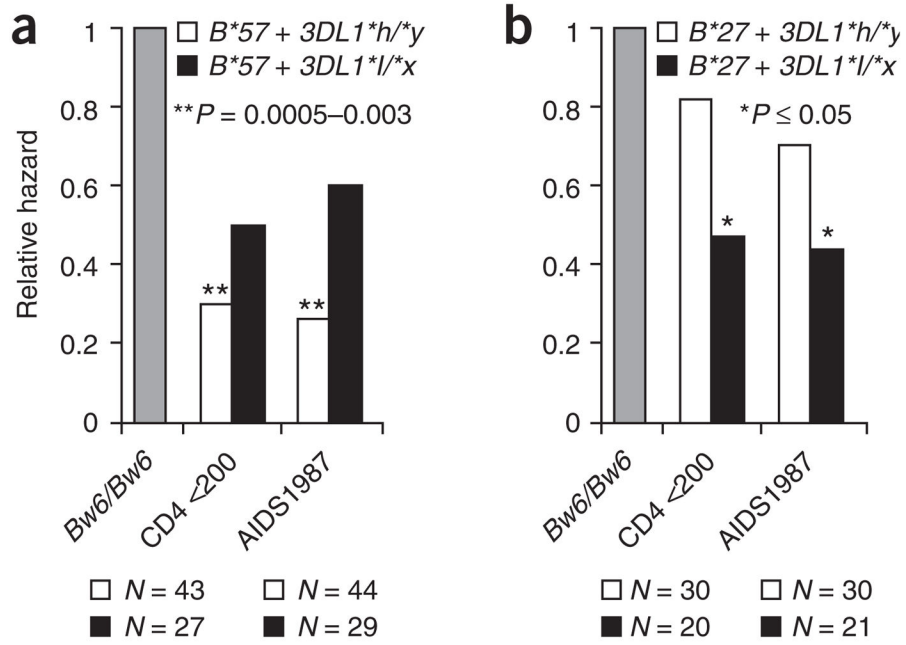
Figure 2.
Effect of *KIR3DL1*004 + HLA-B Bw4* on AIDS progression. Individuals with
KIR3DL1*004 and *Bw4* and those lacking this compound genotype were compared with
individuals homozygous for *HLA-Bw6* for two AIDS outcomes. The subjects were
seroconverters of all racial groups in combined cohorts. Relative hazard (RH) and
significance (*P*) are given for Cox proportional model analyses.

Figure 3.
Effect of *KIR3DL1 + HLA-B Bw4* genotypes on progression to CD4^{+} T-cell count < 200 cells/mm^{3}. (**a**) *KIR3DL1* genotypes with *Bw4-80I*. (**b**) *KIR3DL1* genotypes with *Bw4-80T*. Relative hazard (RH) and *P* values are based on comparisons between the specific genetic variables listed and *Bw6/Bw6*. The *KIR3DL1*h/*y* group includes *KIR3DL1*h/*h* and *KIR3DL1*h/*004* individuals, and the *KIR3DL1*l/*x* group includes *KIR3DL1*l/*l*, *KIR3DL1*l/*h* and *KIR3DL1*l/*004* individuals.

Figure 4.
Synergistic influence of specific *HLA-B* allotypes with *KIR3DL1*h* and *KIR3DL1*l*
genotypes on progression to two AIDS outcomes. (**a**) *HLA-B*57*; (**b**)*HLA-B*27*. *Bw6/Bw6*
individuals were used as the control group in each analysis. The number of individuals in
each category is shown below the bars. The *KIR3DL1*h/*y* group includes *KIR3DL1*h/*h*
and *KIR3DL1*h/*004* individuals, and the *KIR3DL1*l/*x* group includes *KIR3DL1*l/*l*,
*KIR3DL1*l/*h* and *KIR3DL1*l/*004* individuals.
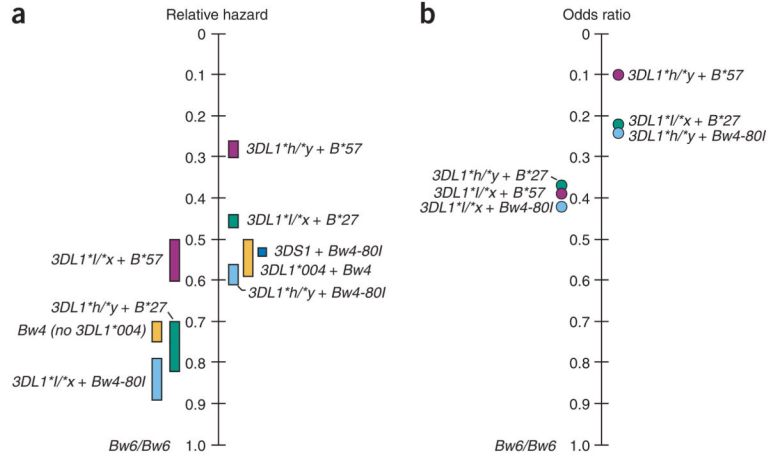
Figure 5.
KIR3DL1 + Bw4 continuum of protection. Genotypes are ordered by degree of protection in terms of (**a**) disease progression and (**b**) control of viral load. The most protective genotypes are shown on the right of the vertical scale and the alternative (less protective) genotypes are shown on the left. (**a**) Relative hazard ranges for the two AIDS outcomes (CD4^{+} T-cell count < 200 cells/mm^{3} and AIDS1987) relative to the *Bw6/Bw6* control group. (**b**) Odds ratios (dots) of protective genotypes relative to the *Bw6/Bw6* control group in terms of their distributions in the < 2,000 versus the > 10,000 MVL groupings. Purple bars and dots, *B*57* with *KIR3DL1* genotypes; green bars and dots, *B*27* with *KIR3DL1* genotypes; light blue bars and dots, *Bw4-80I* with *KIR3DL1* genotypes; dark blue bar, *Bw4-80I+KIR3DS1*; yellow bars, *Bw4* with or without *KIR3DL1*004*.

Table 1

Effect of distinct allelic combinations of *KIR3DL1* and *HLA-Bw4* on AIDS progression

Outcome	Genotype	N	RH	95% CI	P value	Test for trend	N	P for trend
CD4 < 200	3DL1*h/*y+Bw4	306	0.70	0.56–0.88	0.002	3DL1*h/*y+Bw4 versus	306	0.002
	3DL1*l/*x+Bw4	182	0.77	0.59–1.00	0.05	3DL1*l/*x +Bw4 versus	182	
						Bw6/Bw6	327	
	3DL1*h/*y+Bw4-80I	186	0.61	0.45–0.81	0.001	3DL1*h/*y+Bw4-80I versus	186	0.001
	3DL1*l/*x+Bw4-80I	87	0.89	0.64–1.25	0.51	3DL1*l/*x +Bw4-80I versus	87	
						Bw6/Bw6	327	
	3DL1*h/*y+Bw4-80T	148	0.82	0.62–1.08	0.15	3DL1*l/*x +Bw4-80T versus	148	0.01
	3DL1*l/*x+Bw4-80T	113	0.68	0.50–0.94	0.02	3DL1*h/*y+Bw4-80T versus	113	
						Bw6/Bw6	327	
AIDS1987	3DL1*h/*y+Bw4	317	0.66	0.51–0.86	0.002	3DL1*h/*y+Bw4 versus	317	0.001
	3DL1*l/*x+Bw4	194	0.79	0.59–1.06	0.12	3DL1*l/*x +Bw4 versus	194	
						Bw6/Bw6	340	
	3DL1*h/*y+Bw4-80I	194	0.56	0.40–0.80	0.001	3DL1*h/*y+Bw4-80I versus	194	0.001
	3DL1*l/*x+Bw4-80I	94	0.79	0.53–1.19	0.26	3DL1*l/*x +Bw4-80I versus	94	
						Bw6/Bw6	340	
	3DL1*h/*y+Bw4-80T	152	0.76	0.56–1.03	0.08	3DL1*l/*x +Bw4-80T versus	152	0.05
	3DL1*l/*x+Bw4-80T	119	0.73	0.52–1.04	0.08	3DL1*h/*y+Bw4-80T versus	119	
						Bw6/Bw6	340	

P values are based on comparisons between the genotypic variable listed and the *Bw6/Bw6* control group. *3DL1*h/*y*: *KIR3DL1*h/*h* or *KIR3DL1*h/*h**004*; *3DL1*l/*x*: *KIR3DL1*l/*l*, *KIR3DL1*l/*h* or *KIR3DL1*l/**004*. CD4, CD4+ T-cell count (cells/mm^3); CI, confidence interval.

Table 2

Effect of combinations of *KIR3DL1* and *HLA-B* on MVL

	MVL < 2,000	MVL > 10,000	OR	95% CI	P value	Without covariables[a] MVL < 2,000	MVL > 10,000	OR	95% CI	P value	MVL < 2,000	MVL 2,000–10,000	MVL > 10,000	P for trend
	N (%)	N (%)				N (%)	N (%)				N (%)	N (%)	N (%)	
Bw6/Bw6	43 (17.3)	205 (82.7)	1.00			43 (17.3)	205 (82.7)	1.00			43 (16.0)	21 (7.8)	205 (76.2)	
3DL1*h/*y + Bw4-80I	63 (47.7)	69 (52.3)	0.24	0.14–0.40	6×10^{-8}	22 (42.3)	30 (57.7)	0.29	0.15–0.59	5×10^{-4}	63 (42.3)	17 (11.4)	69 (46.3)	3×10^{-8}
3DL1*l/*x + Bw4-80I	25 (32.1)	53 (67.9)	0.43	0.23–0.79	0.007	10 (26.3)	28 (73.7)	0.55	0.24–1.28	0.17	25 (29.4)	7 (8.2)	53 (62.4)	0.007
3DL1*004 + Bw4	40 (34.2)	77 (65.8)	0.36	0.21–0.62	2×10^{-4}	25 (41.0)	36 (59.0)	0.27	0.14–0.50	5×10^{-5}	40 (32.3)	7 (5.7)	77 (62.1)	4×10^{-4}
3DL1*h/*y + B*57	39 (69.6)	17 (30.4)	0.10	0.05–0.19	7×10^{-11}						39 (62.9)	6 (9.7)	17 (27.4)	4×10^{-11}
3DL1*l/*x + B*57	10 (37.0)	17 (63.0)	0.38	0.15–0.95	0.04						10 (33.3)	3 (10.0)	17 (56.7)	0.02
3DL1*h/*y + B*27	8 (33.3)	16 (66.7)	0.37	0.14–1.00	0.05						8 (25.8)	7 (22.6)	16 (51.6)	0.02
3DL1*l/*x + B*27	10 (47.6)	11 (52.4)	0.22	0.08–0.59	0.003						10 (41.7)	3 (12.5)	11 (45.8)	0.002

P values are based on comparisons between the genotypic variable listed and the *Bw6/Bw6* control group. Genotype symbols are as defined in Table 1. CI, confidence interval.

[a]For the *Bw4-80I* groups, all individuals with *KIR3DL1*004*, *B*57* and *B*27* were removed from the analysis. For the *KIR3DL1*004* group, all individuals with *B*57*, *B*27* and *KIR3DL1*h/*y* + *Bw4-80I* were removed from the analysis.