

# Epistatic interaction between *KIR3DS1* and *HLA-B* delays the progression to AIDS

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Natural killer (NK) cells provide defense in the early stages of the innate immune response against viral infections by producing cytokines and causing cytotoxicity<sup>1</sup>. The killer immunoglobulin-like receptors (KIRs) on NK cells regulate the inhibition and activation of NK-cell responses through recognition of human leukocyte antigen (HLA) class I molecules on target cells<sup>2</sup>. *KIR* and *HLA* loci are both highly polymorphic, and some HLA class I products bind and trigger cell-surface receptors specified by *KIR* genes. Here we report that the activating *KIR* allele *KIR3DS1*, in combination with *HLA-B* alleles that encode molecules with isoleucine at position 80 (*HLA-B Bw4-80Ile*), is associated with delayed progression to AIDS in individuals infected with human immunodeficiency virus type 1 (HIV-1). In the absence of *KIR3DS1*, the *HLA-B Bw4-80Ile* allele was not associated with any of the AIDS outcomes measured. By contrast, in the absence of *HLA-B Bw4-80Ile* alleles, *KIR3DS1* was significantly associated with more rapid progression to AIDS. These observations are strongly suggestive of a model involving an epistatic interaction between the two loci. The strongest synergistic effect of these loci was on progression to depletion of CD4<sup>+</sup> T cells, which suggests that a protective response of NK cells involving *KIR3DS1* and its HLA class I ligands begins soon after HIV-1 infection.

The *KIR* genes, located on chromosome 19q13.4 in the leukocyte receptor complex, encode a group of receptors that are expressed on NK cells and a subset of T cells that recognize major histocompatibility complex (MHC) class I molecules<sup>1</sup>. NK cell-mediated cytotoxicity can be inhibited by the expression of appropriate HLA class I molecules on target cells, whereas activation of NK cells is associated with an absence or decreased expression of these molecules<sup>2</sup>. Several viruses escape MHC class I-restricted cytotoxic T-lymphocyte (CTL) responses by downregulating the expression of class I molecules on the infected cell surface<sup>3</sup>, which justifies the need for a defense system that responds to the absence of self class I molecules. The *nef* gene product of HIV-1 is known to diminish levels of HLA-A and HLA-B on infected cells, thereby mitigating recognition by CTLs<sup>4</sup> but providing a potential stimulus for NK-cell activation.

The *KIR* genes are located in a segment of DNA that has undergone expansion and contraction over time, probably through unequal crossing over. Thus, *KIR* haplotypes vary in the number and type of genes<sup>5</sup>, although a few framework loci, such as the gene *KIR3DL1*, are present on all or nearly all haplotypes<sup>6</sup>. *KIR3DL1* encodes receptors with three extracellular immunoglobulin-like domains—the region of the molecule that determines ligand specificity<sup>7</sup>. High-resolution genotypic analysis of families<sup>8</sup>, segregation

**Table 1 • Bw4/Bw6 motifs and their corresponding alleles**

Serological epitope	Class I type	Amino-acid position						Corresponding alleles <sup>a</sup>
		77	80	81	82	83		
Bw4	HLA-A, HLA-B	Asn	Ile	Ala	Leu	Arg	<i>B*1513, B*1516, B*1517, B*1524, B*2702, B*3801, B*4901, B*51, B*5201, B*5301, B*5302, B*57, B*58, A*23, A*24</i>	
	HLA-B	Asn	Thr	Ala	Leu	Arg	<i>B*13, B*3802, B*44</i>	
	HLA-A	Ser	Ile	Ala	Leu	Arg	<i>A*25, A*32</i>	
	HLA-B	Asp	Thr	Leu	Leu	Arg	<i>B*2705, B*2709, B*3701, B*4701</i>	
Bw6	HLA-B	Ser	Asn	Leu	Arg	Gly	<i>B*07, B*0801, B*14, *1501, B*1503, B*1504, B*1507, B*1509, B*1515, B*1518, B*1522, B*1537, B*1538, B*1545, B*1548, B*18, B*35, B*39, B*40, B*41, B*42, B*4501, B*4601, B*48, B*50, B*5401, B*55, B*56, B*67, B*7801, B*8101, B*8201</i>	
	HLA-B	Gly	Asn	Leu	Arg	Gly	<i>B*7301</i>	

<sup>a</sup>Only alleles observed in the individuals who were typed are listed (see URL in Methods).

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analysis from 59 families from the Centre d'Etude du Polymorphisme Humain (M.C., unpublished data) and sequence analyses of *KIR* haplotypes<sup>6</sup> indicate that *KIR3DL1* and *KIR3DS1*, which were originally thought to be separate genes, segregate as alleles. Several *KIR3DL1* alleles that differ by 1–21 bp have been identified<sup>8</sup>. We refer to this locus as *KIR3DL1/3DS1*.

*KIR3DL1* encodes molecules with long cytoplasmic tails that inhibit NK-cell activity on ligand binding<sup>9</sup>. *KIR3DS1* encodes molecules with short cytoplasmic tails and a charged lysine residue in the transmembrane region—the hallmarks of an activating *KIR*<sup>10,11</sup>. Other activating *KIR*s, such as *KIR2DS2*, mediate their activating signal through an associated transmembrane adapter called DAP12 after interaction with an appropriate ligand<sup>10</sup>. This is probably also the case for *KIR3DS1*, although it has not been demonstrated. Inspection of the nucleotide sequence of the allele *KIR3DS1* suggests that it may have been derived from an unequal crossing-over event between exons encoding the extracellular domains of an ancestral *KIR3DL1* and exons encoding the transmembrane and cytoplasmic domains of an ancestral activating *KIR* gene.

All HLA-B molecules express one of two mutually exclusive serological epitopes, Bw4 and Bw6, that are specified by five variable amino acids spanning positions 77–83 (Table 1)<sup>12</sup>. Bw6 is present in about two-thirds of all HLA-B molecules and is found exclusively on HLA-B molecules, whereas Bw4 is present in the remaining third of HLA-B and in several HLA-A molecules (see URL in Methods). Receptor-ligand binding and lysis inhibition assays have shown that Bw4-containing HLA-B molecules are ligands for the NK-cell receptor *KIR3DL1*, and thereby inhibit the lysis of target cells expressing Bw4 (ref. 13). But some Bw4-positive HLA-B types seem to be more effective than others in these assays<sup>7,14</sup>, and Bw4-positive HLA-A molecules are not lig-

ands for *KIR3DL1* (ref. 15), which indicates that there are additional requirements for *KIR3DL1* recognition. The ligand for *KIR3DS1* has not been identified, but *KIR3DL1* and *KIR3DS1* share about 97% amino-acid sequence similarity in their extracellular domains and they may share a similar set of ligands, as do other inhibitory and activating *KIR*s that have high sequence similarity in their extracellular domains<sup>11</sup>.

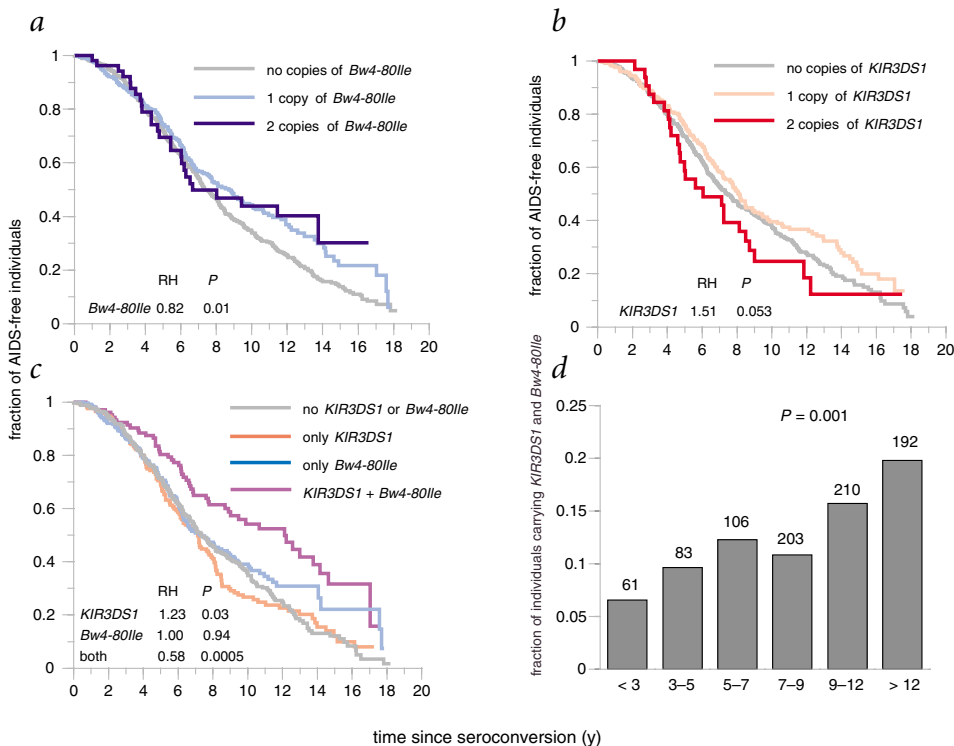
Functional studies have shown the importance of CTLs in controlling HIV-1, and genetic epidemiological data have supported such conclusions by indicating the strong influence of HLA class I loci on progression to AIDS<sup>16–19</sup>. It has been suggested that homozygosity with respect to Bw4-positive *HLA-B* alleles (*HLA-B Bw4*) is associated with a slower diminution of CD4<sup>+</sup> T-cell count<sup>20</sup>, although alternative epidemiological and mechanistic interpretations for these findings are plausible<sup>21</sup>.

As several data indicate that the *HLA-B* locus may have a primary role in regulating outcome after HIV-1 infection, we have examined whether *KIR3DL1/3DS1* acts in concert with the *HLA-B* locus to control HIV-1 in an innate response early after infection. Initially, we tested the potential effect of *HLA-B Bw4* on AIDS progression using a model that considered all other established *HLA* genetic effects, as well as those involving *CCR5* and *CCR2* genotypes that have been previously shown to affect AIDS progression, as covariates. These included *HLA* class I zygosity, *B\*35-Px*, *B\*2705*, *B\*5701* and *CCR5Δ32* heterozygosity and dominant *CCR2-64I* (ref. 17).

The protective influence of *HLA-B Bw4* on the rate of progression to AIDS was confirmed, albeit weakly, in a survival analysis of 1,039 study participants (Table 2). Bw4 molecules can be divided into two groups on the basis of whether isoleucine or threonine is present at position 80 (Bw4-80Ile and Bw4-80Thr, respectively; Table 1). Receptor-binding and lysis-inhibition data suggest that

**Fig. 1** Effect of *HLA-B Bw4-80Ile* and *KIR3DS1* on AIDS progression.

**a–c**, Kaplan–Meier survival analyses comparing the effects of *HLA-B Bw4-80Ile* and *KIR3DS1* separately (**a,b**) and combined (**c**) on progression to AIDS (1993 definition). The individuals were seroconverters of all racial groups in combined cohorts. Relative hazard (RH) and significance (*P*) are given for Cox model analyses with the covariates described in Table 2 (**a,b**). RH and *P* for the combined factors (**c**) are calculated as described in Table 2. **a**, Effect of *HLA-B Bw4-80Ile* heterozygosity (light blue) or homozygosity (blue). **b**, Effect of *KIR3DS1* heterozygosity (pink) or homozygosity (red). **c**, Effect of the combined presence of *KIR3DS1* and *HLA-B Bw4-80Ile* (purple) compared with the effects of the presence of only *KIR3DS1* (red) or only *HLA-B Bw4-80Ile* (blue). In **a–c**, gray represents individuals who lacked the factors in question. **d**, Disease-category analyses comparing the frequency of *KIR3DS1* + *HLA-B Bw4-80Ile* (one or more copies of each) genotypes in rapid and slow progressors to AIDS 1993. The individuals were European American seroconverters and seroprevalents in combined cohorts. Only seroconverters who progressed to the defined end points were considered in the first three (rapid) groups. For the slow groups, all seroconverters and seroprevalents who progressed to the defined end points or had their last known follow up or censorship in the specified time were included. The number of individuals in each category is shown above the bars.



HLA-B molecules containing Bw4-80Ile may be more effective ligands for KIR3DL1 than are those containing Bw4-80Thr<sup>7,14</sup>, whereas neither HLA-A Bw4 nor HLA-B Bw6 molecules bind to KIR3DL1 (refs 13,15). In addition, residue 80 of HLA-C is crucial in determining binding specificity for receptors containing two immunoglobulin domains, as shown by cytotoxicity assays<sup>22</sup> and X-ray crystallography<sup>23</sup>. We therefore grouped *HLA-B Bw4* alleles dichotomously according to amino-acid variation at position 80 and determined the effect of these two groups on AIDS progression.

A comparison of study participants with *HLA-B Bw4-80Ile* against those without these alleles indicated that the protective effect of *HLA-B Bw4* against AIDS progression could be attributed to the *Bw4-80Ile* genotype (Fig. 1a and Table 2). No influence of the *Bw4-80Thr* allele was observed (Table 2).

*HLA-A Bw4* alleles, all of which encode isoleucine at position 80, had no detectable effect on AIDS progression (data not shown).

We examined the association of *HLA-B Bw4-80Ile* with protection against AIDS progression in the context of the *KIR3DL1* and *KIR3DS1* subtypes. First, we compared the effect of the *KIR3DL1* and *KIR3DS1* alleles on progression to AIDS in all individuals without considering the influence of *HLA-B Bw4-80Ile*. In survival analyses of combined cohorts, a modest recessive influence of *KIR3DS1* leading to more rapid AIDS progression was observed consistently (relative hazard (RH) = 1.31–1.86 for different AIDS end points; Fig. 1b and Table 2). This accelerating influence was apparent in Cox model analyses in which the other known AIDS restriction genotypes (*HLA* zygosity, *B\*27*, *B\*57*, *B\*35-Px*, *CCR5-Δ32* and *CCR2-64I*) were considered as covariates; these analyses indicated an independent recessive effect of the *KIR3DS1* gene.

To explore the possible interaction between the observed effects of *HLA-B Bw4-80Ile* and those of *KIR3DS1*, we carried out survival analyses to determine the effects of *HLA-B Bw4-80Ile* in the absence of *KIR3DS1*, *KIR3DS1* in the absence of *HLA-B Bw4-80Ile*, and the two factors combined. We used the three genotypes as covariates, along with the five confounding covariates described above, in the multivariate Cox model regression. The combination of *KIR3DS1* and *HLA-B Bw4-80Ile* was highly protective with respect to several AIDS end points, suggesting that there is an

**Table 2 • Independent effect of *HLA-B Bw4* subtypes and *KIR3DS1* on progression to AIDS-related end points**

Genetic variable	Frequency <sup>b</sup>		AIDS outcome	No. of individuals	RH <sup>a</sup>	P value
	EA	AA				
<i>HLA-B Bw4</i>	0.40	0.43	CD4 < 200	1,001	0.91	0.18
			AIDS 1993	1,005	0.89	0.07
			AIDS 1987	1,016	0.86	0.05
			death	1,015	0.88	0.15
<i>HLA-B Bw4-80Ile</i>	0.19	0.35	CD4 < 200	998	0.87	0.11
			AIDS 1993	1,002	0.82	0.01
			AIDS 1987	1,013	0.83	0.05
			death	1,012	0.79	0.04
<i>HLA-B Bw4-80Thr</i>	0.22	0.08	CD4 < 200	998	1.00	0.98
			AIDS 1993	1,002	1.04	0.64
			AIDS 1987	1,013	0.95	0.59
			death	1,012	1.03	0.78
<i>KIR3DS1</i>	0.22	0.05	CD4 < 200	912	1.50	0.06
			AIDS 1993	915	1.51	0.05
			AIDS 1987	925	1.31	0.32
			death	924	1.86	0.02

<sup>a</sup>Cox model survival analysis of the effect of *HLA-Bw4* and *KIR3DS1* on progression to four AIDS outcomes. The individuals were seroconverters of all racial groups in combined cohorts. The effect of the presence of *HLA-Bw4* or *Bw4* subtypes was tested with a codominant model using as the Cox model explanatory variable either (i) the individual's number of *HLA-B Bw4* alleles or (ii) the number of alleles of the *HLA-B Bw4-80Ile* or *HLA-B Bw4-80Thr* subtypes. A recessive model was used for *KIR3DS1*. The following genetic factors that affect AIDS progression were considered as confounding covariates in each analysis: protective genotypes of the chemokine receptors *CCR2* or *CCR5*, the HLA alleles *B\*27*, *B\*57* and alleles of the *B\*35Px* group, and overall homozygosity at *HLA-A*, *HLA-B* and *HLA-C* loci. Because the *Bw4-80Ile* and *Bw4-80Thr* subtypes together comprise all of the *HLA-B Bw4* allele group, the absence of an association ( $P > 0.5$ ) between the *HLA-B Bw4-80Thr* subtype and progression to any AIDS outcome indicates that the *HLA-B Bw4-80Ile* subtype is responsible for the observed (and previously reported<sup>20</sup>) association between the Bw4 epitope and AIDS progression. This is consistent with the significant association ( $P \leq 0.05$ ) observed between *HLA-B Bw4-80Ile* and progression to three of four AIDS outcomes.<sup>9</sup> The allele frequency of each variable is also shown for European Americans (EA) and African Americans (AA).

epistatic or synergistic interaction of the two unlinked alleles on the kinetics of AIDS progression (Fig. 1c,d and Table 3). This synergistic interaction is notable because the observed *HLA-B Bw4-80Ile* protection (Fig. 1a and Table 2) is eliminated when the influence of the combined *KIR3DS1* + *HLA-B Bw4-80Ile* genotype is included as a covariate (Table 3).

We interpret this discordance in the *HLA-B Bw4-80Ile* effect to indicate that the *Bw4-80Ile* protection is derived completely from *KIR3DS1* + *HLA-B Bw4-80Ile* epistasis. In support of this interpretation, the effect of *HLA-B Bw4-80Ile* (determined using the model described in Table 2) in European Americans is significant ( $P = 0.03$ – $0.0008$ ), whereas it is not significant in African Americans for any outcome ( $P = 0.18$ – $0.68$ ). This corresponds to a low frequency of *KIR3DS1* in African Americans ( $f = 0.05$ ) relative to that in European Americans ( $f = 0.22$ ). In addition, the influence of *KIR3DS1* in

**Table 3 • Synergistic effect of *KIR3DS1* and *HLA-B Bw4-80Ile* on progression to AIDS-related end points**

Genetic variable	AIDS outcome	No. of individuals	RH <sup>a</sup>	P value
<i>KIR3DS1</i>	CD4 < 200	904	1.26	0.02
	AIDS 1993	907	1.23	0.03
	AIDS 1987	917	1.14	0.26
	death	916	1.36	0.01
<i>Bw4-80Ile</i>	CD4 < 200	904	1.05	0.66
	AIDS 1993	907	1.00	0.94
	AIDS 1987	917	0.92	0.50
	death	916	0.95	0.70
<i>KIR3DS1</i> + <i>Bw4-80Ile</i>	CD4 < 200	904	0.58	0.001
	AIDS 1993	907	0.58	0.0005
	AIDS 1987	917	0.74	0.10
	death	916	0.65	0.04

<sup>a</sup>Results of a single Cox model survival analysis with three codominant explanatory variables: (i) the individual's number of *KIR3DS1* alleles, (ii) the number of *HLA-B Bw4-80Ile* alleles and (iii) the combination of *KIR3DS1* and *HLA-B Bw4-80Ile*, calculated as the product of the first two variables. Other factors relating to *HLA* and *CCR* alleles were considered as confounding covariates as described in Table 2. Alternative analyses were also carried out considering the effects of *KIR3DS1* and *HLA-B Bw4-80Ile* as dominant, both separately and in combination (see Web Table B online).

**Table 4 • Synergistic effect of *KIR3DS1* and *HLA-B Bw4-80Ile* on progression to AIDS-related end points stratified by ethnicity**

Genetic variable	AIDS outcome	European Americans			African Americans		
		No. of individuals	RH	<i>P</i> value	No. of individuals	RH	<i>P</i> value
<i>KIR3DS1</i>	CD4 < 200	631	1.21	0.07	234	2.66	0.02
	AIDS 1993	633	1.21	0.06	235	2.23	0.05
	AIDS 1987	640	1.12	0.33	238	0.50	0.46
	death	639	1.34	0.02	238	0.69	0.74
<i>Bw4-80Ile</i>	CD4 < 200	631	1.02	0.87	234	1.20	0.40
	AIDS 1993	633	0.93	0.55	235	1.33	0.12
	AIDS 1987	640	0.80	0.16	238	1.37	0.20
	death	639	0.84	0.29	238	1.43	0.27
<i>KIR3DS1</i> + <i>Bw4-80Ile</i>	CD4 < 200	631	0.60	0.006	234	0.18	0.01
	AIDS 1993	633	0.59	0.003	235	0.25	0.03
	AIDS 1987	640	0.78	0.23	238	1.76	0.59
	death	639	0.70	0.11	238	1.52	0.74

accelerating progression to AIDS is enhanced when the epistatic interaction between *KIR3DS1* + *HLA-B Bw4-80Ile* is considered as a covariate (Table 3), which confirms the observed effect of *KIR3DS1* in analyses not adjusted for combinatorial effects (Fig. 1c and Table 2). That *KIR3DS1* exerts a modest accelerating influence makes the synergistic influence of *KIR3DS1* + *HLA-B Bw4-80Ile* in slowing AIDS progression particularly notable.

Protection against AIDS conferred by the epistatic *KIR3DS1* + *HLA-B Bw4-80Ile* genotype was apparent in Caucasian and African American cohorts (Table 4) and in combined ethnic groups (Table 3). Consistent RHs, albeit not always statistically significant, were observed in survival analyses of separate risk groups and cohorts (see Web Table A online). A categorical analysis of rapid versus slow progressors, which included several seroprevalent study participants in addition to seroconverters, also showed consistently elevated frequencies of the protective dual genotype (*KIR3DS1* + *HLA-B Bw4-80Ile*) in slow progressors to AIDS (Fig. 1d and Web Fig. A online). Thus, replication in groups stratified by ethnicity, risk (parenteral or mucosal infection) and cohort consistently support epistatic protection by the *KIR3DS1* + *HLA-B Bw4-80Ile* genotype against rapid AIDS progression.

The synergistic effect of *KIR3DS1* and *HLA-B Bw4-80Ile* was strongest in outcomes that took into account CD4<sup>+</sup> T-cell counts of less than 200 per mm<sup>3</sup> (CD4 < 200 and AIDS 1993 definition; Table 3). No combinatorial effect of *HLA-A Bw4-80Ile* alleles with *KIR3DS1* was observed (*P* > 0.19), which emphasizes the specificity of the observed effect for *HLA-B* as opposed to other class I loci. Four subtypes of the Bw4 epitope, three of which are found in *HLA-B* molecules, were observed among the samples typed in this study (Table 1). Among *HLA-B* Bw4 molecules, positions 77, 80 and 81 vary by two or three amino acids at each site and are conserved at the remaining four positions of the epitope. Cox model analysis of each variant at positions 77 and 81 indicated that they are not associated with the protective effect observed between *KIR3DS1* and *HLA-B Bw4-80Ile*. Together, the data suggest that *HLA-B Bw4-80Ile* molecules behave as ligands for *KIR3DS1* and that HIV-1-infected cells expressing *HLA-B Bw4-80Ile* may be prone to NK-cell activity that is regulated in part by ligand binding to *KIR3DS1* expressed on the NK-cell surface.

Some KIRs are expressed on a subset of T cells with a memory phenotype<sup>24</sup>, which suggests that they may regulate T-cell as well as NK-cell activity. Indeed, masking of inhibitory NK receptors on CTLs from HIV-infected individuals by monoclonal antibody has been shown to increase HIV-specific CTL activity<sup>25</sup>. It will therefore be important to determine whether our observations reflect a functional interaction of *KIR3DS1* and *HLA-B Bw4-80Ile* on T cells, on NK cells, or on both.

The *KIR3DS1* allele seems to influence two opposing mechanisms of viral control: one for AIDS susceptibility (*KIR3DS1* homozygosity in the absence of *HLA-B Bw4-80Ile*) and the other for protection (*KIR3DS1* + *HLA-B Bw4-80Ile*). The protective effect of *KIR3DS1* + *HLA-B Bw4-80Ile* seems to be dominant over susceptibility conferred by *KIR3DS1* homozygosity, because the ten study participants who were homozygous for *KIR3DS1* and positive for *HLA-B Bw4-80Ile* progressed slowly to AIDS (1993 definition; approaching

statistical significance, RH = 0.43, *P* = 0.09). The protective effect of heterozygous *KIR3DS1/3DL1* + *HLA-B Bw4-80Ile* cannot be readily attributed to *KIR3DL1*, because the presence of two copies of *KIR3DL1* along with *HLA-B Bw4-80Ile* did not confer a protective effect. It is possible, however, that *KIR3DS1* is not directly responsible for the observed protection, but is only a marker of a haplotype that contains the actual disease locus. If so, then this operative locus would still have to interact with *HLA-B Bw4-80Ile* or with a locus in linkage disequilibrium with *HLA-B Bw4-80Ile*. This remains a possibility, because both *KIR3DS1* and *HLA-B* are located in genomic segments that contain other related genes involved in the immune response.

Data indicating a primary role for the *HLA-B* locus in AIDS progression and the receptor–ligand relationship of *HLA-B* molecules with *KIR3DL1* lend credence, however, to a model in which *KIR3DS1* binds *HLA-B* molecules containing Bw4-80Ile, leading to activation of NK cells, T cells, or both and elimination of HIV-1-infected cells. Such events may occur before the generation of HIV-specific CTLs and continue in concert with CTLs once an acquired immune response has been initiated. A swift nonspecific response to viral infection mediated through KIR molecules would correlate well with data showing that rapid immune responses after viral exposure are beneficial in controlling the virus<sup>26</sup>. Theoretically, this same genetic combination should be protective against other viral infections, as there is no reason to suppose that the response is specific to HIV.

The recessive susceptibility effect of *KIR3DS1* (in the absence of *HLA-B Bw4-80Ile*) may be due to other genes in linkage disequilibrium with *KIR3DS1* or, alternatively, to a relatively weak dominant-protective effect of *KIR3DL1* that appears neutral in comparison to the strong protective effect of *KIR3DS1* + *HLA-B Bw4-80Ile*. Differential binding of a specific antibody against *KIR3DL1* to NK cells carrying various *KIR3DL1* alleles<sup>8</sup> raises the possibility that allelic variation at this locus might affect expression of the molecule; *KIR3DL1* subtyping may provide some clues to potential effects of this group of alleles.

Survival analyses of *KIR3DS1* with individual *HLA-B Bw4-80Ile* alleles suggested that no single allele or minor subset of *HLA-B Bw4-80Ile* alleles could account for the observed protective effect of *KIR3DS1* + *HLA-B Bw4-80Ile* as a whole, although the power to detect such effects was diminished. But protection by the *Bw4-80Ile*-positive *HLA-B\*5701* allele was evident in the presence (CD4 < 200, RH = 0.37, *P* = 0.05) or absence (CD4 < 200, RH = 0.52, *P* = 0.008) of *KIR3DS1*, which suggests that the strong protection conferred by this allele is not completely dependent on *KIR3DS1*. Similarly, *HLA-B\*27* protection is probably independent of a *KIR3DS1* interaction because the most

common  $B^*27$  allele in our cohorts,  $B^*2705$  (83% of all  $B^*27$  alleles), contains  $Bw4-80Thr$ —the  $Bw4$  motif that does not confer protection in the presence of  $KIR3DS1$ . Although we cannot rule out the possibility of  $KIR$  involvement in  $B^*27$  protection, the ability of  $B^*27$  molecules to present a highly conserved immunodominant HIV-1 epitope—one that may require two mutations to escape CTL recognition and still maintain viral fitness<sup>27</sup>—may be at least partly responsible for its observed protective effect.

Because of the extensive polymorphism at the  $HLA$  class I loci, strategies for grouping alleles according to similarity in specificity for peptide motifs can enhance the power and information content of  $HLA$ –disease association studies<sup>19,28</sup>. Peptides differing at positions 7 and 8, both of which are non-anchor residues, can sometimes affect recognition of  $HLA$  class I ligands by specific  $KIR$  molecules<sup>29</sup>. But it is unlikely that the epistatic effect of  $KIR3DS1 + HLA-B\ Bw4-80Ile$  can be explained by peptide preference of the various  $HLA-B\ Bw4-80Ile$  molecules, because members of this group differ markedly in their preference for peptides.

The interaction between common alleles at polymorphic loci in disease resistance, as shown here for  $KIR3DS1$  and  $HLA-B\ Bw4-80Ile$  in HIV-1 infection, represents a form of genetic epistasis that is distinct from cases where mutations in two or more different genes result in a disease phenotype<sup>30</sup>. Although inherently difficult to detect, the synergistic interaction between alleles at unlinked loci may be common in complex disorders such as infectious diseases. Identification of such interactions may provide new approaches to therapeutic and vaccine development.

## Methods

**Subjects.** Individuals infected with HIV-1 for whom dates of seroconversion were known were derived from five cohorts: the Multicenter AIDS Cohort study (MACS), the Multicenter Hemophilia Cohort Study (MHCS), the Hemophilia Growth and Development Study (HGDS), the San Francisco City Clinic Cohort (SFCCC) and the AIDS Linked to Intra-venous Experience (ALIVE) Study. Seroconverters from the MACS and ALIVE cohorts were representative of all HIV-infected individuals, whereas individuals from the SFCCC and MHCS cohorts showed a moderate survival bias because biological samples were unavailable for individuals with the most rapid rates of progression to AIDS. The study was approved by the Protocol Review Office of the NCI institutional review board. Informed consent was obtained at the study sites from all individuals.

**HLA class I typing.** We amplified genomic DNA using locus-specific primers that flanked exons 2 and 3. The PCR products were blotted on nylon membranes and hybridized with sequence-specific oligonucleotide (SSO) probes. We assigned alleles by the reaction patterns of the SSO probes. Ambiguous SSO probe typing results were resolved by sequencing analysis as described<sup>19</sup>.

**KIR genotyping.** Genomic DNA was typed for presence or absence of  $KIR3DL1$  and  $KIR3DS1$  by PCR with sequence-specific priming (PCR–SSP). We carried out PCR amplification with two pairs of specific primers for each locus. Internal control primers for a fragment of 796 bp of the third intron of  $DRB1$  were also included in each PCR. We amplified 20–50 ng DNA in a volume of 20  $\mu$ l containing 200  $\mu$ M dNTP, 100–500 nM of specific primer, 100 nM of internal control primer, 2 mM  $MgCl_2$ , 67 mM Tris, 16.6 mM  $(NH_4)_2SO_4$ , and 0.5 U of *Taq* polymerase. Cycling was carried out in a GeneAmp PCR system 9700 thermal cycler (PE Applied Biosystems) as follows: 1 min at 96 °C; 5 cycles of 96 °C for 25 s, 65 °C for 45 s, 72 °C for 30 s; 21 cycles of 96 °C for 25 s, 60 °C for 45 s, 72 °C for 30 s; 5 cycles of 96 °C for 25 s, 55 °C for 1 min, 72 °C for 2 min; and a final extension step of 10 min at 72 °C. We separated PCR products in 1.5% agarose gels containing ethidium bromide and visualized the products under ultraviolet light. Primer sequences are available from the authors on request.

**Statistical analysis.** Survival analyses were carried out on seroconverters from all of the cohorts combined and included all individuals without

regard to racial group. Four AIDS-related outcomes were considered as end points of survival analysis: a  $CD4^+$  T-lymphocyte count of <200 per  $mm^3$ , progression to AIDS according to the 1993 definition of the US Centers for Disease Control (CDC), progression to AIDS according to the more stringent 1987 CDC definition, and death. We carried out Kaplan–Meier and Cox model analyses using the LIFETEST and PHREG procedures of the SAS system (SAS Institute). Genetic factors with a confirmed effect on progression to AIDS (protective genotypes of the chemokine receptors  $CCR2$  and  $CCR5$ ,  $HLA$  alleles  $B^*27$ ,  $B^*57$  and alleles of the  $B^*35Px$  group, and overall homozygosity at the  $HLA-A$ ,  $HLA-B$  and  $HLA-C$  loci) were included as covariates in Cox model analyses. Subjects in the Cox model analyses were stratified by race and age at seroconversion.

**URL.**  $HLA$  class I and II sequence alignments, <http://www.anthonynolan.org.uk/HIG/data.html>.

*Note: Supplementary information is available on the Nature Genetics website.*

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## Competing interests

The authors declare that they have no competing financial interests.

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