

Global diversity and evidence for coevolution of *KIR* and *HLA*

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The killer immunoglobulin-like receptor (*KIR*) gene cluster shows extensive genetic diversity, as do the *HLA* class I loci, which encode ligands for *KIR* molecules. We genotyped 1,642 individuals from 30 geographically distinct populations to examine population-level evidence for coevolution of these two functionally related but unlinked gene clusters. We observed strong negative correlations between the presence of activating *KIR* genes and their corresponding *HLA* ligand groups across populations, especially *KIR3DS1* and its putative *HLA-B Bw4-80I* ligands ($r = -0.66$, $P = 0.038$). In contrast, we observed weak positive relationships between the various inhibitory *KIR* genes and their ligands. We observed a negative correlation between distance from East Africa and frequency of activating *KIR* genes and their corresponding ligands, suggesting a balance between selection on *HLA* and *KIR* loci. Most *KIR-HLA* genetic association studies indicate a primary influence of activating *KIR-HLA* genotypes in disease risk^{1,2}; concomitantly, activating receptor-ligand pairs in this study show the strongest signature of coevolution of these two complex genetic systems as compared with inhibitory receptor-ligand pairs.

The *KIR* genes located on chromosome 19q13.4 encode activating and inhibitory receptors that are expressed on natural killer (NK) cells and a subset of T cells. The *KIR* gene cluster shows extensive variability in terms of gene content across haplotypes, probably because of non-allelic homologous recombination occurring between pairs of homologous *KIR* genes³. Over 30 distinct *KIR* haplotypes have been identified based on gene content alone⁴, among which the most salient distinction is the presence or absence of various activating receptor genes. *HLA* class I genes are characterized by extreme allelic polymorphism and encode molecules that bind T cell receptors on cytotoxic T lymphocytes, thereby initiating acquired immune responses. More recently, *HLA* allotypes have been shown to serve as ligands for *KIR*, establishing their importance in both the innate and acquired immune response. The extreme variability at *KIR* and

HLA loci is thought to provide protection against a wide variety of pathogens, with different *KIR-HLA* combinations conferring protection against distinct diseases. Several models for the maintenance of this degree of diversity have been posited, including frequency-dependent selection, heterozygote advantage and selection varying in time and space⁵⁻⁷. Overall, the concept of different *KIR-HLA* combinations protecting against distinct diseases is supported by disease association data^{1,2}. For example, activating *KIR* genotypes tend to be protective against infectious diseases, but they are associated with susceptibility to autoimmune disease, whereas inhibitory genotypes are associated with protection against inflammatory diseases. The receptor-ligand specificity between *KIR* and *HLA* class I represents a classic example of genetic epistasis, where the presence of genes or alleles encoding corresponding receptor-ligand pairs is necessary for functional activity, but the presence of one without the other has no influence on effector cell activity.

Comparisons of *KIR* genes across primate species have demonstrated the rapid evolution of this receptor family—possibly more rapid than the evolution of the genes encoding their major histocompatibility complex (MHC) class I ligands^{4,8,9}. For example, the inhibitory *KIR* specificities for particular amino acids (lysine or asparagine) at position 80 of the MHC-C molecule are preserved between humans and chimpanzees⁸, but the MHC-C^{lys80} specificity is mediated by *KIRs* with differing numbers of extracellular domains in chimpanzees and humans, suggesting particularly rapid evolution of the *KIR* genes. The extensive diversity of the *HLA* and *KIR* gene loci and the central role of their interactions in modulating immune responses are expected to favor the coevolution of genotypic combinations of these two loci in order to maintain appropriate functional interaction. Evidence of *HLA-KIR* coevolution has also been suggested in disease studies^{1,2,10} as well as in the comparative genetic studies across primate species. However, direct evidence from human population studies, which could pinpoint receptor-ligand combinations that are major factors in their coevolution, has been lacking. Significant correlations between frequencies of specific *KIR* genes and *HLA* alleles

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Table 1 Populations, geographic regions, sample sizes and carrier frequencies

No.	Population ^a	Region ^b	<i>n</i>	Carrier frequencies										
				C-group1	C-group2	<i>Bw4</i>	<i>Bw4-80I</i>	<i>KIR2DL1</i>	<i>KIR2DL2</i>	<i>KIR2DL3</i>	<i>KIR3DL1</i>	<i>KIR2DS1</i>	<i>KIR2DS2</i>	<i>KIR3DS1</i>
1	Biaka	AFR	69	69.4	72.6	78.7	49.2	97.1	58.0	80.3	100.0	1.4	56.5	2.9
2	Ethiopian	AFR	31	72.4	75.9	80.0	76.7	90.3	80.6	77.4	96.8	25.8	77.4	22.6
3	Hausa	AFR	37	63.6	84.8	78.8	66.7	100.0	36.7	96.7	100.0	13.8	32.1	3.4
4	Ibo	AFR	48	63.4	82.9	77.5	75.0	95.7	60.9	76.1	100.0	8.3	58.3	6.3
5	Mbuti	AFR	38	67.6	70.3	73.0	45.9	94.4	65.7	61.8	97.2	27.8	65.7	11.1
6	Yoruba	AFR	75	66.7	84.1	65.1	55.6	100.0	38.4	92.0	98.7	14.7	34.7	12.0
7	Adygei	EUR	54	71.7	79.2	77.8	57.4	100.0	50.0	92.5	98.1	35.2	50.0	35.2
8	CEPH_UT	EUR	90	84.4	54.4	62.2	25.6	96.7	56.7	88.9	94.4	40.0	58.9	38.9
9	Danish	EUR	50	82.9	61.0	50.0	20.0	98.0	52.0	96.0	98.0	36.7	52.0	38.0
10	European	EUR	91	81.6	65.5	56.0	26.2	97.8	46.2	92.3	93.3	37.8	46.7	40.0
11	Finns	EUR	35	77.1	62.9	51.4	25.7	100.0	39.4	97.0	90.9	54.5	39.4	53.1
12	Irish	EUR	94	88.2	58.8	65.5	19.5	98.9	52.1	91.5	96.8	28.0	52.1	31.9
13	Russian	EUR	47	84.8	60.9	67.4	26.1	93.5	43.5	86.4	97.9	23.4	43.5	21.3
14	Druze	SWA	116	73.9	80.2	68.5	57.4	96.5	71.9	78.4	96.5	23.7	71.7	30.7
15	Yemenites	SWA	43	83.9	80.6	69.0	58.6	97.7	46.5	95.2	97.7	31.0	50.0	35.7
16	Ami	EAS	40	97.5	32.5	5.0	0.0	92.3	62.5	84.6	100.0	37.5	60.0	32.5
17	Atayal	EAS	42	100.0	9.8	0.0	0.0	100.0	0.0	100.0	100.0	33.3	0.0	34.1
18	Cambodian	EAS	22	88.9	61.1	68.8	12.5	90.9	45.0	85.7	95.2	42.9	42.9	47.6
19	Hakka	EAS	40	100.0	30.0	55.3	31.6	100.0	33.3	97.4	97.5	50.0	33.3	52.5
20	Han_SF	EAS	59	100.0	37.5	68.1	40.4	100.0	13.6	100.0	98.3	37.3	13.6	35.6
21	Han_Taiwan	EAS	48	100.0	38.3	79.1	51.2	97.9	19.1	93.6	97.9	34.8	19.1	37.0
22	Japan	EAS	49	95.5	18.2	59.5	38.1	100.0	8.5	100.0	95.7	47.8	8.5	48.9
23	Micronesia	OCE	36	52.8	88.9	8.6	2.9	97.1	42.9	97.1	97.1	50.0	42.9	51.4
24	Nasioi	OCE	22	95.2	23.8	25.0	0.0	90.9	95.5	59.1	59.1	90.9	90.9	72.7
25	Yakut	NEA	51	91.7	75.0	87.5	45.8	98.0	27.5	92.2	96.1	46.0	37.3	42.0
26	Maya	NAM	50	89.4	38.3	8.7	6.5	94.0	38.0	94.0	94.0	62.0	38.0	56.0
27	Pima	NAM	99	92.6	59.6	23.2	13.7	87.9	63.6	87.6	76.8	76.8	63.6	71.7
28	Karitiana	SAM	55	80.0	52.0	0.0	0.0	85.5	67.3	85.5	81.8	80.0	67.3	80.0
29	Surui	SAM	46	90.5	54.8	7.1	7.1	97.8	26.1	97.8	91.3	47.8	26.1	30.4
30	Ticuna	SAM	65	92.3	53.8	31.3	18.8	93.8	39.1	93.8	93.8	61.9	39.1	46.0
	Total		1642											

^aDetails of the sampling and ethnographic information for these populations can be found at <http://alfred.med.yale.edu/>. ^bAFR = Africa; EUR = Europe; SWA = Southwest Asia; EAS = East Asia; OCE = Oceania; NEA = Northeast Asia; NAM = North America; SAM = South America.

encoding their corresponding ligands would support the hypothesis that these unlinked loci are coevolving.

To assess evidence for coevolution of *KIR* and *HLA* class I at the population level, we genotyped 1,642 unrelated individuals from 30 geographically distinct populations throughout the world (Table 1 and Fig. 1). Each individual was tested for the presence or absence of specific *KIR* genes whose ligands have been defined previously. We grouped *HLA-B* and *HLA-C* alleles according to known ligand specificity for the following inhibitory receptors: (i) *KIR2DL2* and *KIR2DL3*, which segregate as alleles of a single locus, encode molecules that bind *HLA-C* allotypes with Asp80 (C-group1 alleles); (ii) *KIR2DL1* binds *HLA-C* allotypes with Lys80 (C-group2 alleles) and (iii) *KIR3DL1* binds *HLA-B* allotypes that contain the *Bw4* epitope (*Bw4* alleles), which is defined by amino acid variation at positions 77–83 of the *HLA-B* molecules. Data from three studies indicate that *Bw4* molecules with Ile80 (*Bw4-80I* alleles) serve as better ligands for *KIR3DL1* than do *Bw4* molecules with Thr80 (*Bw4-80T* alleles)^{11–13}. Therefore, we considered these two subgroups in analyses involving *Bw4*. The activating *KIR2DS2* and *KIR2DS1* bind to *HLA-C*-group1 allotypes and *HLA-C*-group2 allotypes, respectively, with low affinity. The ligand for *KIR3DS1* is not known definitively, but genetic epidemiological studies^{14,15} point to *Bw4-80I* as its ligand,

which is consistent with the strong similarity between the extracellular domains of *KIR3DS1* and *KIR3DL1*. Similarly, the activating *KIR2DS2* and *KIR2DS1* show 97%–98% sequence similarity in their extracellular domains to the corresponding inhibitory *KIRs* (*KIR2DL2* and *KIR2DL1*, respectively). Frequencies of the presence or absence (that is, phenotypic or carrier frequencies) of the *KIR* genes and the *HLA-B* and *HLA-C* ligand groups are shown in Figure 2.

We investigated population-level evidence for coevolution of the *KIR* and *HLA* loci by determining correlations between the frequencies of functionally relevant receptor–ligand pairs. For each pair, we assessed the significance of these correlations using empirical methods based on genomic comparisons to avoid issues of statistical non-independence among populations and to account for the effects of demographic history¹⁶ (see Methods and Supplementary Note online). The phenotypic frequencies for the activating *KIR3DS1* and *Bw4* ligand group showed a strong negative correlation ($r = -0.63$; $P_{\text{empirical}} = 0.041$; Fig. 3 and Table 2) that was enhanced slightly when we considered the *Bw4-80I* subset of *Bw4* alleles ($r = -0.66$; $P_{\text{empirical}} = 0.038$). In fact, *Bw4-80I* accounts almost completely for the correlation observed between *KIR3DS1* and *Bw4*, as there was only a minimal negative correlation between *KIR3DS1* and the alternative *Bw4-80T* subset ($r = -0.19$; P value not significant). For the activating *KIR2DS1*

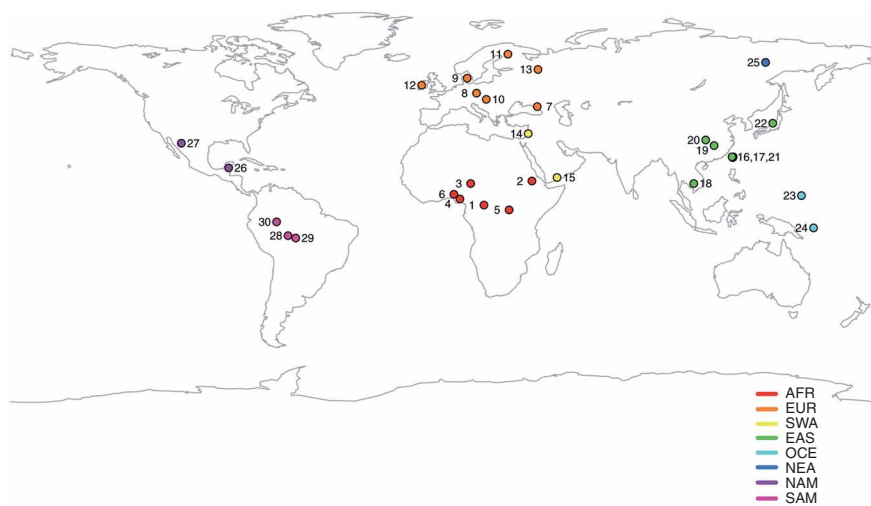


Figure 1 Map of the world showing the location of sampled populations. Numbers correspond to populations in **Table 1**.

and *KIR2DS2* genes, the correlation with C-group2 and C-group1, respectively, was also negative ($r = -0.48$ and -0.37 , respectively; **Supplementary Fig. 1** online) but not significant. *KIR3DL1*, a common inhibitory gene, showed weak, nonsignificant positive correlations with its *Bw4* ligand group and the *Bw4-80I* subset (**Supplementary Fig. 1**). The population-level correlation between *KIR3DL1* and *Bw4-80I* frequencies was even weaker, corresponding with data indicating that *Bw4-80I* molecules serve as better ligands for *KIR3DL1* (refs. 11–13). For the inhibitory *KIR2DL1* and *KIR2DL3* genes, the positive correlation with their ligands was very weak and not significant.

C-group2 alleles and *Bw4* alleles are in significant positive linkage disequilibrium (LD) with one another ($P < 0.001$), as are *KIR3DS1* and *KIR2DS1* ($P < 0.001$). Therefore, it is possible that only one receptor-ligand pair is the dominant factor in shaping the frequency distributions of these two activating *KIR-HLA* ligand pairs and that members of the other pair are simply hitchhiking. Owing to the degree of LD for both sets of genes, it is difficult to assess the individual effects of the two receptor-ligand pairs based on these population-level data. However, the relationship between *KIR3DS1* and *Bw4-80I* frequencies was stronger and more significant than that between any other receptor-ligand pairing, lending credence to the putative receptor-ligand relationship between *KIR3DS1* and *Bw4-80I* molecules, as implicated in previous disease association studies^{14,15,17}.

Although the direction of the *KIR-HLA* associations was predicted *a priori*, we have reported two-sided tests throughout the paper. Even though the negative correlation between *KIR3DS1* and *Bw4* or *Bw4-80I* was the only one to reach statistical significance, the general pattern of negative and positive correlations between activating and inhibitory receptor-ligand pairs, respectively, was very consistent. The single exception is the negative correlation between *KIR2DL2* and C-group1, which is in contrast with the positive correlations between other inhibitory genes tested and their respective ligands. *KIR2DS2* and *KIR2DL2* were virtually in complete LD in all populations tested (except for the Yakut), explaining the identical C-group1 correlations with *KIR2DS2* and *KIR2DL2*. Several lines of evidence suggest that *KIR2DL2* is simply tracking an interaction between *KIR2DS2* and C-group1: (i) the fairly weak correlations between other inhibitory receptors and their ligand groups, (ii) the positive correlations between the other inhibitory receptors and their ligand groups, (iii) the negative

correlation between *KIR2DS2* and its ligand group, consistent with respect to the other activating *KIR* tested and (iv) the fact that *KIR2DL3* recognizes the same ligand group as *KIR2DL2*, yet the correlations with C-group1 are diametrically opposed.

In order to investigate the geographical distribution of gene frequencies, we tested *KIR* and *HLA* ligand group frequencies for correlations with distance from East Africa based on previous results¹⁸ and the latitude and longitude values for the population samples listed in the ALFRED database (**Table 3**). We observed significant positive clines in the frequencies of *KIR3DS1* and *KIR2DS1* with increasing distance from East Africa ($R^2 > 45\%$, $P_{\text{empirical}} = 0.01$); in contrast, we observed more subtle declines in the frequencies of their corresponding ligands, *Bw4-80I* ($R^2 > 35\%$, $P_{\text{empirical}} = 0.004$) and C-group2 ($R^2 > 20\%$; P value not significant), with increasing distance from East

Africa. These data are consistent with the negative correlations that we observed between these activating receptor-ligand pairs (**Fig. 3b** and **Supplementary Fig. 1g**) and indicate an inverse correlation with respect to geography. We have necessarily restricted our analyses to certain *KIR-HLA* pairs with known or suspected receptor-ligand relationships. Although this subset may not represent the entire pattern of coevolution at the *HLA* class I and *KIR* loci, overall, the data suggest the maintenance of a balance between the frequencies of the activating receptors and their corresponding (putative) ligands across populations.

We found it intriguing that some pairs of neighboring populations shared similar *HLA* ligand group frequencies but had highly distinct phenotypic *KIR* gene frequencies (particularly in East Asia and the Americas; **Fig. 2**), as this is consistent with a situation in which the *KIR* genes are evolving at a more rapid rate than the *HLA* class I ligand groupings. A particularly notable example is the two tribal populations from Taiwan: the Atayal from the inland mountain ranges and the Ami from the east coast, which presumably stem from common ancestors settling Taiwan roughly 4,000 to 6,000 years ago¹⁹. The Atayal were completely missing *KIR2DS2* and *KIR2DL2*. In contrast, the Ami sample had some of the highest levels of *KIR2DL2* and *KIR2DS2* worldwide. However, both the Ami and Atayal had very low frequencies of *Bw4*. Additional geographic regions with several neighboring but distinct populations will need to be surveyed for both *KIR* and *HLA* to corroborate and extend these results.

Signatures of balancing selection are evident not only at the *HLA* class I allele level²⁰ but also at the amino acid level²¹, similar to reports on *HLA* class II data²². Notably, recent work has shown that amino acids at residues 77 and 80 in *HLA-C*, which are in strong LD, were among those with the strongest signals for balancing selection relative to other polymorphic *HLA-C* residues (S.J. Mack and H.A. Erlich, personal communication). The importance of position 80 in determining alternative *KIR2D* ligand specificities, along with its involvement in peptide binding, raises the possibility of selection driven by both pathogens and *KIR-HLA* interactions. A very similar situation has been found for position 80 of *HLA-B*, which appears in a cluster of high LD (residues 80–83) showing a signature of balancing selection (S.J. Mack and H.A. Erlich, personal communication) and which determines *KIR3DL1* and *KIR3DS1* specificities. Overall, these data

agree nicely with the balanced relationship between activating receptors and their ligands (or putative ligands) shown in **Figure 3** and give further credibility to the coevolution of the *KIR* and *HLA* loci.

Taken together, the data presented here provide population-level evidence for the evolution of the *KIR* gene cluster owing to selection pressure favoring frequencies of activating *KIR* that suit the locale-specific *HLA* repertoire. Although the only significant correlation between receptor-ligand pairs involved *KIR3DS1* and *Bw4-80I* using our empirical method ($r = -0.66$, $P_{\text{empirical}} = 0.038$), we observed clear negative correlations between each of the other activating *KIR*s and their corresponding ligand groups. The strong correlation

between activating, as opposed to inhibitory, *KIR* with *HLA* ligand makes sense, as NK cell inhibition is essential for preventing harmful responses to self under healthy conditions and is therefore a fixed trait. Thus, it is important that genes encoding inhibitory receptors are common members of *KIR* haplotypes, and indeed there is always at least one known inhibitory *KIR*-*HLA* ligand pairing present across virtually all humans. The activating *KIR* genes, on the other hand, are present on some, but not all, *KIR* haplotypes, and there are a fair number of haplotypes that do not contain any activating *KIR* for which a ligand is known. Activating *KIR*s probably serve as a fine-tuning mechanism: they enhance protection against certain diseases

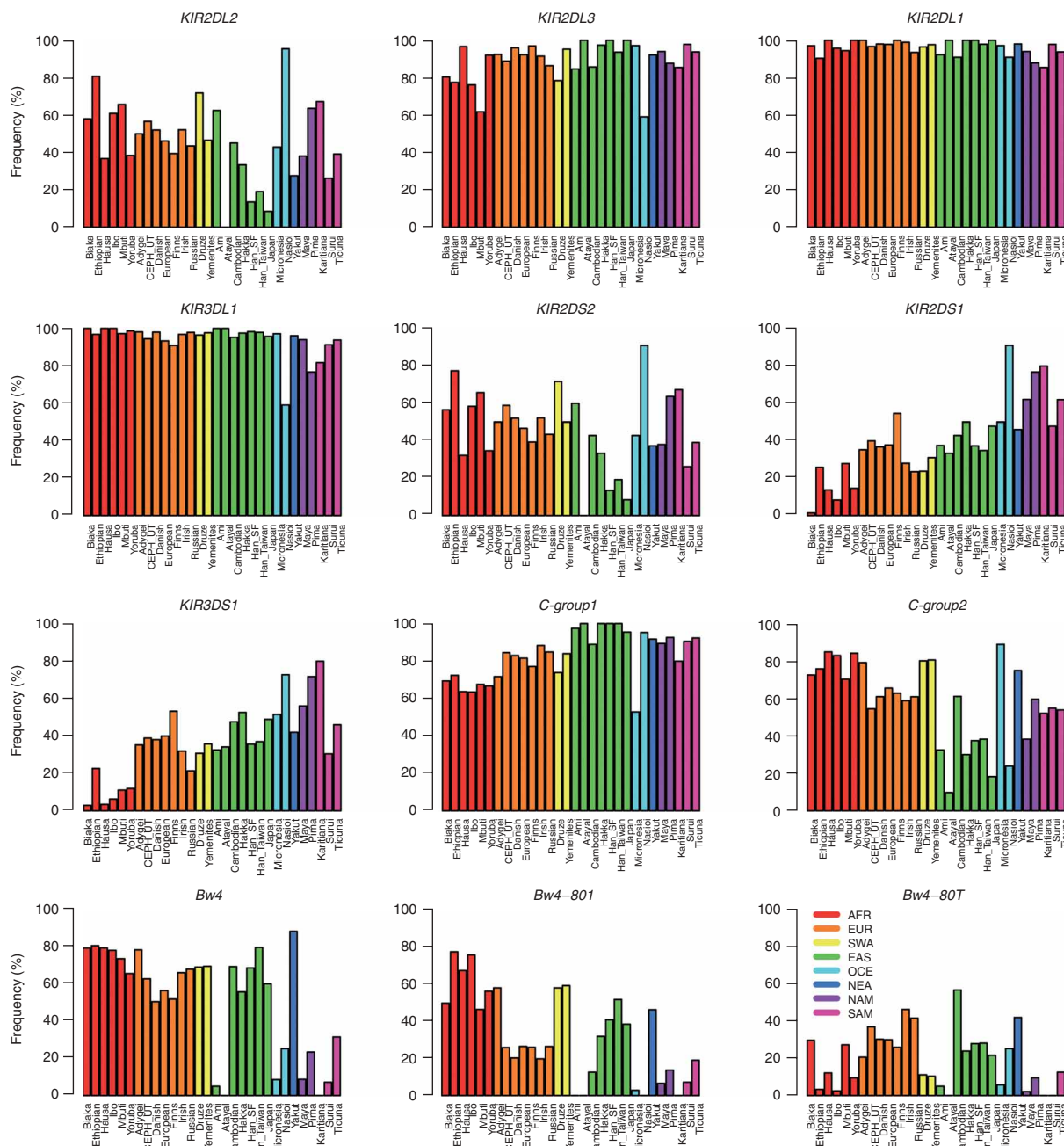


Figure 2 *KIR* and *HLA* ligand carrier frequencies across populations. Populations are grouped by geographic region, and regions are ordered by increasing distance from East Africa. Two activating *KIR* loci (*KIR2DS1* and *KIR3DS1*) show an increasing trend in phenotypic frequency with increasing geographic distance from East Africa. An inverse relationship is seen for the *Bw4* *HLA* ligand group. This trend is stronger for the *Bw4-80I* subset of *Bw4* ligands.

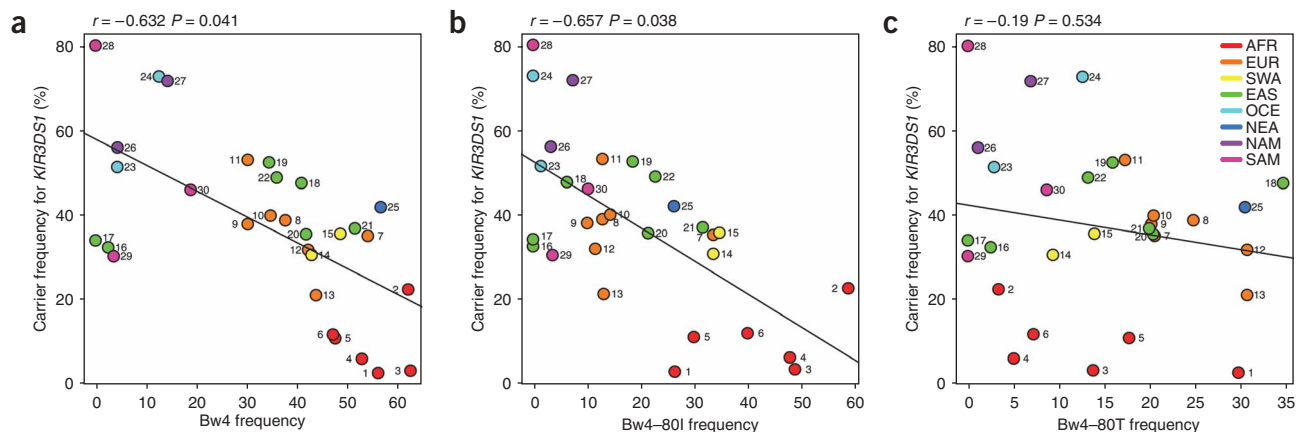


Figure 3 Correlation between *KIR* and *HLA* ligand frequencies. **(a)** There is a strong negative correlation between the carrier frequencies for activating *KIR3DS1* and gene frequencies for its putative *HLA Bw4* ligand group. **(b,c)** The subset of *Bw4-80I* alleles **(b)** accounts for the majority of the relationship of *KIR3DS1* with *Bw4*, as there is little correlation between the frequency of this *KIR* gene and that of *Bw4-80T* alleles **(c)**. There is also a negative, but nonsignificant, correlation for other activating *KIRs* (**Supplementary Figure 1g,h**) and a weaker nonsignificant positive correlation for inhibitory *KIRs* (**Supplementary Figure 1a,b,d,f**). Numbers correspond to populations in **Table 1**, and the solid line represents the estimated regression line.

but may actually confer susceptibility to others. This range in *KIR-*HLA** genotypes, conferring greater activation to greater inhibition, may correlate well with the level of resistance to different types of complex diseases, where the underlying mechanisms of pathogenesis vary as a result of the level of immune responsiveness.

METHODS

Populations. In this study, we genotyped 29 populations from the Allele Frequency Database (ALFRED), a freely accessible collection of allele frequency data in anthropologically defined human populations²³. The populations were classified as belonging to one of the following eight geographic regions: Africa (AFR), Europe (EUR), Southwest Asia (SWA), East Asia (EAS), Oceania (OCE), Northeast Asia (NEA), North America (NAM) or South America (SAM). In addition, 90 Centre d'Etude du Polymorphisme Humain (CEPH) individuals were included in this study. These CEPH individuals from Utah were classified as belonging to the European geographic region based on ancestry. Population names and sample sizes are given in **Table 1**, and the geographic distribution of these populations is given in **Figure 1**. This study was approved by the Protocol Review Office of the National Cancer Institute institutional review board. Informed consent was obtained at the study sites from all participants.

Table 2 Correlation between *KIR* and *HLA* ligand frequencies

Locus pair	Correlation (r^a)	Empirical P value ^b
<i>KIR3DL1</i> , <i>Bw4</i>	0.426	0.218
<i>KIR3DL1</i> , <i>Bw480i</i>	0.416	0.191
<i>KIR3DL1</i> , <i>Bw480t</i>	0.171	0.758
<i>KIR3DS1</i> , <i>Bw4</i>	-0.632	0.041
<i>KIR3DS1</i> , <i>Bw480i</i>	-0.657	0.038
<i>KIR3DS1</i> , <i>Bw480t</i>	-0.190	0.534
<i>KIR2DL1</i> , C-group2	0.046	0.924
<i>KIR2DL2</i> , C-group1	-0.366	0.542
<i>KIR2DL3</i> , C-group1	0.184	0.328
<i>KIR2DS1</i> , C-group2	-0.478	0.149
<i>KIR2DS2</i> , C-group1	-0.371	0.479

^aPearson product-moment correlation between *KIR* carrier frequency (CF) and *HLA* ligand gene frequency (GF). ^bThe empirical P value is the proportion of times that a larger absolute correlation than the true correlation between *KIR* GF and *HLA* GF was observed in the empirical distribution, generated by computing correlations between GFs for 10,000 pairs of unlinked sites typed in the same populations as the present study from the ALFRED database (see Methods).

***KIR* genotyping.** Genomic DNA was genotyped for presence or absence of the following *KIR* genes: *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3*, *KIR3DS1*, *KIR2DP1* and *KIR3DP1*. Genotyping was performed using PCR amplification with two pairs of primers specific for each locus (PCR-SSP) as previously described^{24,25}. The *KIR* genes are named based on their structure, including the number of extracellular domains they possess (for example, 2D or 3D) and the length of their intracytoplasmic tail (L for long, or S for short). *KIR* genes with a long intracytoplasmic tail send inhibitory signals, and those with a short intracytoplasmic tail send activating signals.

***HLA* class I genotyping.** Genomic DNA was amplified using locus-specific primers flanking exons 2 and 3. The PCR products were blotted on nylon membranes and hybridized with a panel of sequence-specific oligonucleotide (SSO) probes. Alleles were assigned by the reaction patterns of the SSO probes. Ambiguous SSOP typing results were resolved by sequencing analysis.

Table 3 Relationship between distance from East Africa and *KIR/HLA* frequency

Locus	Distance from East Africa			
	Slope ^a	R^2 ^b	P value ^c (regression)	P value ^d (empirical)
<i>KIR2DL1</i>	-0.000164	5.1%	0.247	0.098
<i>KIR2DL2</i>	-0.000018	0.5%	0.731	0.747
<i>KIR2DL3</i>	0.000072	1.8%	0.497	0.327
<i>KIR3DL1</i>	-0.000248	17.7%	0.026	0.019
<i>KIR2DS1</i>	0.000127	47.7%	<0.001	0.010
<i>KIR2DS2</i>	-0.000020	0.6%	0.691	0.745
<i>KIR3DS1</i>	0.000118	45.6%	<0.001	0.010
C-group2	-0.000052	20.3%	0.016	0.286
<i>Bw4</i>	-0.000197	32.4%	0.002	0.022
<i>Bw4-80I</i>	-0.000314	35.3%	0.001	0.004
<i>Bw4-80T</i>	-0.000163	18.7%	0.022	0.083

^aSlope of the relationship between $GF^* = \log(GF/(1 - GF))$ on the distance from East Africa, where GF is the gene frequency. ^bProportion of variation explained (R^2) values for the regression of GF^* on the distance from East Africa. ^cThe ordinary regression-based P value. ^dThe empirical P value is the proportion of times that a stronger relationship between GF^* and distance from East Africa was observed in the empirical distribution, generated by computing the above regression for 538 sites from the ALFRED database typed in the same populations as the present study (see Methods).

Statistical methods. For *HLA-B* and *HLA-C*, analyses of allele frequencies (gene frequencies) were carried out using the PyPop software package²⁶. The methods implemented in PyPop are given in ref. 27. *KIR* carrier frequencies (CF) were determined for each of the 30 population samples. Gene frequencies (GF), assuming HWP, were computed via Bernstein's formula²⁸ as $GF_i = 1 - \sqrt{(1 - CF_i)}$.

The significance of the correlation between *KIR* and *HLA* frequencies was assessed using both empirical and permutation-based approaches to avoid issues of non-independence among populations and to account for the effects of demographic history. In each case, this was accomplished by comparing the observed statistic to a null distribution of values in the same set of populations to account for any correlation induced by shared history. The ordinary *P* value for the Pearson product moment correlation does not account for this non-independence. Although the direction of the *KIR-HLA* associations was predicted *a priori*, we have reported more conservative two-sided tests throughout the paper based on the methods described below.

In the empirical approach, we used gene frequencies for 538 sites in 202 genes, typed in the same set of populations as the present study and provided in the ALFRED database, to generate an empirical distribution of correlations between gene frequencies for pairs of unlinked sites. The empirical *P* value ($P_{\text{empirical}}$) represents the proportion of times that the correlation between two randomly chosen sites on different chromosomes was larger in absolute value than the correlation between *KIR* gene frequencies and *HLA* gene frequencies. Each of the sites that we used had a heterozygosity of at least 0.25.

The empirical distribution used to assess the strength of the relationship between the distance from East Africa and the *KIR* gene frequency (or *HLA* gene frequency) was based on the results relating distance from East Africa and each of the 538 sites from ALFRED. The dependent variable used in the regressions was $GF^* = \log(GF / (1 - GF))$, where *GF* is the gene frequency. The empirical *P* value represents the proportion of times that transformed gene frequencies (GF^*) in the empirical distribution showed a stronger relationship with distance from East Africa than did *KIR* (or the *HLA* ligand grouping). A weight of $1/k$ was used when there were *k* sites in the same gene.

Fisher's exact test was used to test for nonrandom association between the presence of pairs of *KIR* genes. The strength of the association was measured using Cramer's *V* statistic²⁹. This measure of association, sometimes referred to as W_n , is equivalent to the correlation coefficient ($r = \sqrt{D_{11}^2 / (p_1 p_2 q_1 q_2)}$) between two biallelic loci but has a range from -1 to +1. Fisher's method for combining *P* values³⁰ was used to test for significant LD across a set of populations for a locus pair.

URLs. Details of the sampling and ethnographic information for the studied populations can be found at <http://alfred.med.yale.edu/>. SSO protocols are available at <http://www.ihwg.org/protocols/protocol.htm>. PyPop is available at <http://www.pypop.org/>.

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

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AUTHOR CONTRIBUTIONS

M.C. designed and supervised the project and prepared the manuscript; R.M.S. contributed to the design of the project, developed the statistical methods and prepared the manuscript; M.P.M. performed *KIR* genotyping and prepared the manuscript; X.G. performed HLA genotyping; D.M. and M.Y. provided

intellectual input; J.R.K. and K.K.K. provided samples and intellectual input; all authors discussed the results and commented on the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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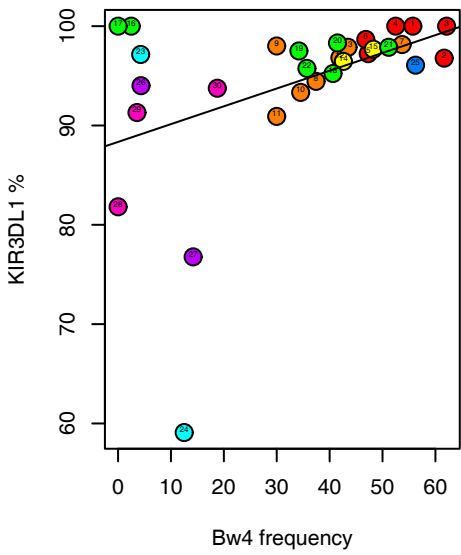
Global diversity and evidence for co-evolution of *KIR* and *HLA* genes

Richard M. Single^{1,6}, Maureen P. Martin^{2,6}, Xiaojiang Gao², Diogo Meyer³, Meredith Yeager⁴, Judith R. Kidd⁵, Kenneth K. Kidd⁵, and Mary Carrington²

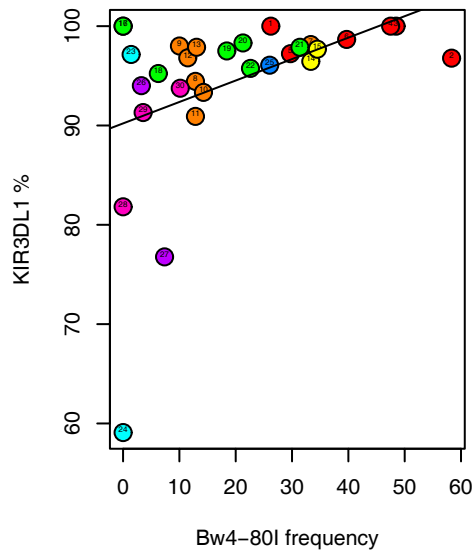
Supplementary Figure 1. Correlation between *KIR* and *HLA* ligand frequencies.

While the only significant receptor-ligand correlation was between *KIR3DS1* and *Bw4/Bw4-80I* (Figure 1), the pattern of negative and positive correlations between activating and inhibitory receptor-ligand pairs, respectively, was very consistent. The activating *KIR2DS1* showed a negative correlation with its *C-group2* ligands (g), mirroring that of *KIR3DS1* and *Bw4-80I* (Figure 3b), but not significantly so. Likewise, the activating *KIR2DS2* gene had a negative, but weaker correlation with its *C-group1* ligands (h). There was a weaker positive correlation for all but one inhibitory *KIR* (a, b, d, f). Although the inhibitory *KIR2DL2* showed a negative correlation with its *C-group1* ligand (e), several lines of evidence point to its tracking the *KIR2DS2-C-group1* relationship (see text for details). The subset of *Bw4-80I* alleles (b) has a much stronger correlation with *KIR3DL1* than do the *Bw4-80T* alleles, a pattern consistent with correlations between *KIR3DS1* and these subsets of the *Bw4* ligand group. Population numbers correspond to those in Table 1 and the solid line represents the estimated regression line.

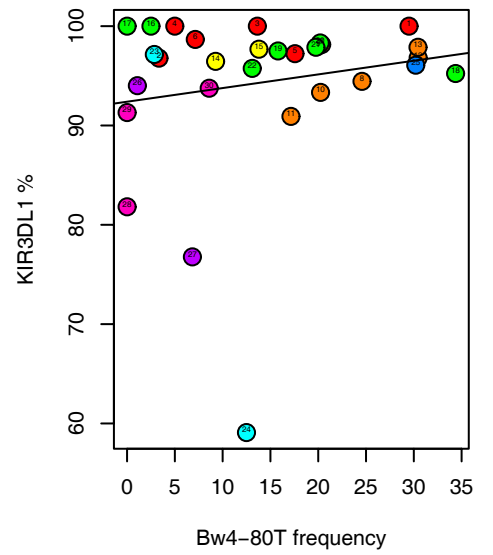
(a) $r = 0.426$ $p = 0.218$



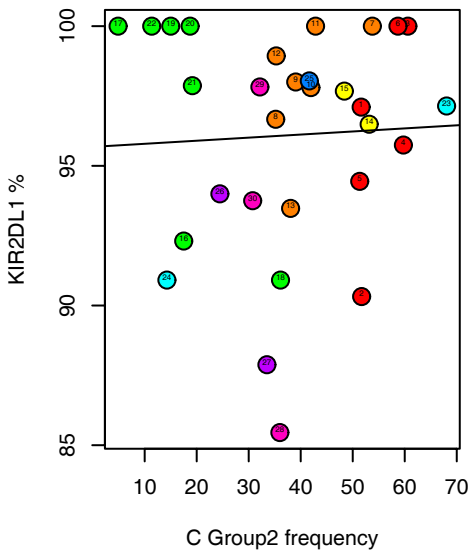
(b) $r = 0.416$ $p = 0.191$



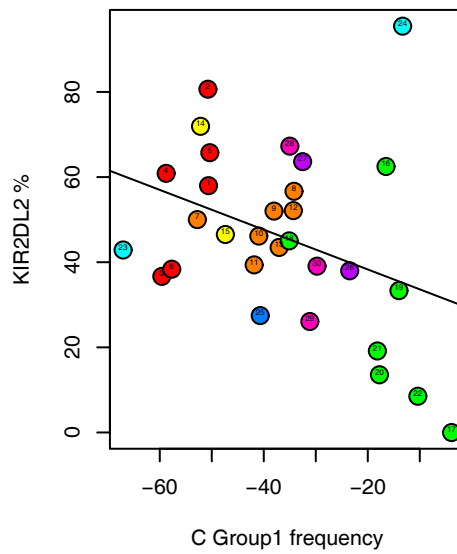
(c) $r = 0.171$ $p = 0.758$



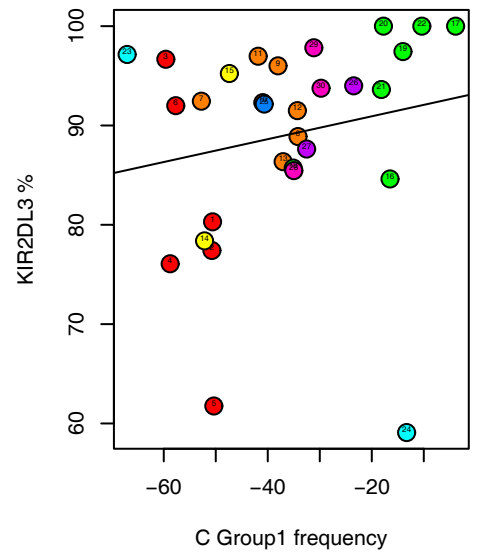
(d) $r = 0.046$ $p = 0.924$



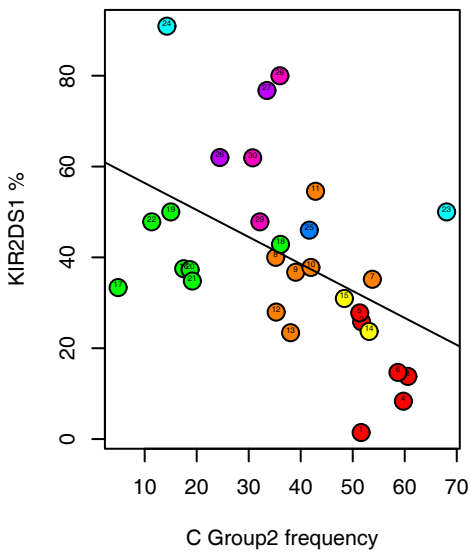
(e) $r = -0.366$ $p = 0.542$



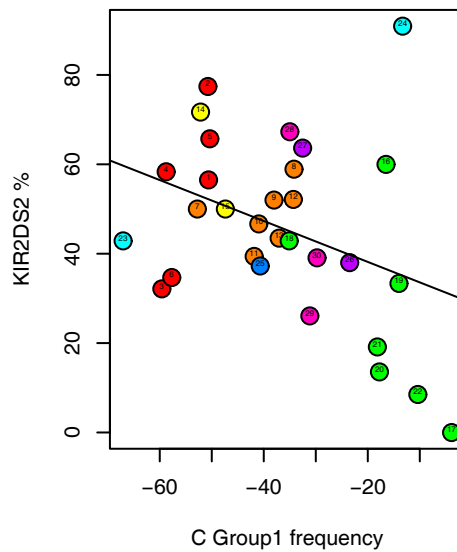
(f) $r = 0.184$ $p = 0.328$



(g) $r = -0.478$ $p = 0.149$



(h) $r = -0.371$ $p = 0.479$



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Supplementary Note

We assessed/validated our conclusions about the significance of correlations between frequencies for *KIR* and their corresponding *HLA* ligand groups by constructing a permutation test. As with the empirical approach based on genomic comparisons, this permutation approach avoids issues of non-independence among populations due to shared demographic history. In the permutation-based approach, labels for the *C group1/2* (or *Bw4/6*) status of each *HLA-C* (or *HLA-B*) allele were randomly assigned before computing “reassigned *HLA*” gene frequencies at each iteration. The correlation between *KIR* carrier frequencies and “reassigned *HLA*” gene frequencies was computed 10,000 times in order to generate a permutation distribution of correlation coefficients under the null hypothesis. The permutation p-value represents the proportion of times that a larger absolute correlation than the true *KIR-HLA* correlation was observed in the permutation distribution. Both the empirical and permutation methods generated consistent results for the significance of correlations between functionally-relevant *KIR/HLA* pairs.