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Thirty allele-level haplotypes centered around *KIR2DL5* define the diversity in an African American population

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Abstract

KIR2DL5 alleles were physically linked to alleles at adjacent *KIR* loci to define this region of *KIR* haplotypes in 55 gene positive random African Americans. The majority carried *KIR2DL5B*. Three *KIR2DL5A* and six *KIR2DL5B* alleles that have been previously described and 11 novel *KIR2DL5* alleles were identified by DNA sequencing. Novel alleles included variation that may impact promoter activity; two alleles carried nonsynonymous coding region variation. Based on linkage with *KIR2DS1, KIR2DS3, KIR2DS5, KIR2DL2, KIR2DL3*, and *KIR3DS1* alleles, 7 haplotypes of *KIR2DL5A* and 23 haplotypes of *KIR2DL5B* were observed. The phylogenetic relationships among the *KIR2DL5* alleles predicted their association with either *KIR2DS3* (6 alleles) or *KIR2DS5* (7 alleles). All of the *KIR2DL5A* alleles were linked either to *KIR3DS1*01301* or *KIR3DS1*049N*. The majority of the *KIR2DL5B* alleles were linked to seven *KIR2DL2* alleles; two were linked to a novel allele of *KIR2DL3*. These findings underscore the diversity of KIR haplotypes present in this population.

Keywords

natural killer cell; cell surface receptor; killer cell immunoglobulin-like receptor; haplotypes; polymorphism; population study

Introduction

The human natural killer cell immunoglobulin-like receptor (KIR) *KIR2DL5* gene arises from an ancient lineage with orthologs identified in nonhuman primates (Hershberger et al. 2005;Khakoo et al. 2000;Rajalingam et al. 2004). KIR2DL5 is classified as an inhibitory receptor but its ligand is not yet known (Estefania et al. 2007;Yusa et al. 2004). Its D0-D2 domain structure (specified by exons 3 and 5 in the absence of exon 4) distinguish it and KIR2DL4 from the other two domain KIR that are characterized by a D1-D2 structure (specified by exons 4 and 5 with the inactivation of exon 3) (Vilches et al. 2000b).

The gene encoding KIR2DL5 is duplicated in some *KIR* haplotypes (Gomez-Lozano et al. 2002). *KIR2DL5B* lies in the centromeric region of the *KIR* gene complex, flanked by *KIR2DL2* (or *KIR2DL3)* at its 5' end and by *KIR2DS3* (or *KIR2DS5)* at its 3' end (Du et al. 2008;Ordonez et al. 2008). *KIR2DL5A* lies in the telomeric region flanked by *KIR3DS1*

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(5') and *KIR2DS3* (or *KIR2DS5*) and *KIR2DS1* (3'). The *KIR2DL5* gene is usually found in a subset of *KIR* haplotypes designated as B which are marked by an increased activating *KIR* gene content (Hsu et al. 2002;Marsh et al. 2003a).

Both *KIR2DL5A* and *KIR2DL5B* are polymorphic, encoding eight and thirteen alleles respectively (IPD-KIR database release 2.1.0) (Robinson et al. 2010). Many of the *KIR2DL5B* alleles are not expressed due to variation in transcription factor binding sites, including runt-related transcription factor-3 (RUNX3) (also known as AML2) (Vilches et al. 2000a;Vilches et al. 2000b), Yin Yang 1 (YY1) and specificity protein 1 (Sp1)(Davies et al. 2007;Li et al. 2008). Variation in the balance between forward and reverse transcripts impacted by allelic polymorphism alters expression levels. Reduced promoter activity may lead to epigenetic silencing mediated by methylation (Gomez-Lozano et al. 2007).

The purpose of this study was to evaluate *2DL5* allelic diversity in an African American population and to identify alleles at flanking loci to define this region of *KIR* haplotypes.

Materials and Methods

Human studies were approved by the Georgetown University Institutional Review Board and conform to standards laid down in the 1964 Declaration of Helsinki. Genomic DNA was isolated from Epstein Barr Virus-transformed B-cell lines from 100 unique and unrelated African Americans from the National Institute of General Medical Sciences (NIGMS) Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/nigms/) using a QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA). Characterization of the *KIR2DS1, KIR2DS3, KIR2DS5*, and *KIR3DS1* alleles carried in this population has already been reported (Hou et al. 2009;Jiang et al. 2010). The strategy for *KIR2DL5 a*llele characterization has been described previously; two overlapping amplicons cover the 5' upstream region from -274 through the 3'UTR (Mulrooney et al. 2008). *KIR2DL2/2DL3* alleles were identified by the DNA sequencing of overlapping amplicons using PCR primers listed in supplemental Table 1. An exception was *KIR2DL3* amplicons B and C where one of the two PCR primers yielding each amplicon annealed in intron 5 but did not produce overlapping fragments. In some cases, other closely related *KIR* loci coamplified with some of the amplicons and required additional strategies. KIR2DL2-999T and KIR2DL3-1316T HaploPrep™ separation kits (Qiagen, Valencia, CA) were used to isolate specific *KIR2DL2/2DL3* alleles from those cell lines shown to carry *KIR2DL1, KIR2DS2* or both *KIR2DL2* and *KIR2DL3*. Probe 2DL2-999T targets nucleotide position 708 in exon 6 shared by all known *KIR2DL2* alleles except *KIR2DL2*004* and absent from *KIR2DL3*. The probe 2DL3-1316T targets nucleotide 1024 in exon 9 which is found in all alleles of *KIR2DL3* but not in *KIR2DL2*. Cleavage of *KIR2DP1* with the restriction enzyme *BclI* eliminated its coamplification with amplicon A of *KIR2DL3* and reduced the background observed in sequencing amplicon B. Genomic DNA (2ug) was digested using 3 ul *Bc1I* (15 Units/ul; New England BioLabs, Ipswich, MA) in 1X NE buffer 3 in a total volume of 200 ul at 50°C for 1hr. DNA was isolated using phenol: chloroform and ethanol precipitation and PCR amplification performed as described.

KIR2DL5 and *KIR2DL2/KIR2DL3* amplicons were purified using AmPure magnetic beads (Agencourt Bioscience, Beverly MA). Sequencing was performed using Applied Biosystems' BigDye Terminator Ready Reaction mix (Applied Biosystems, Foster City, CA). Sequencing primers were positioned to obtain the sequence of both strands of each amplicon (supplemental Table 2 for *KIR2DL2/2DL3*) (Mulrooney et al. 2008). Some of the primers for *KIR2DL2/2DL3* were specific for polymorphisms that distinguished alleles and/or reduced the background from contaminating loci. The reactions were purified using CleanSEQ (Agencourt Bioscience). Sequencing products were detected using an Applied

Biosystems 3730XL DNA analyzer. Sample files were analyzed using Sequencher (Genecodes Corp., Ann Arbor, MI) and Assign SBT 3.2.7 (Conexio Genomics, Applecross, Western Australia) software. Sequences were compared to known *KIR* sequences obtained from the IPD-KIR database version 2.1.0 to determine allelic assignments. Proximal promoter sequences were compared to known *KIR* sequences from sources previously described (Mulrooney et al. 2008). In this report, the numbering of nucleotides and codons are based on IPD-KIR unless noted.

In heterozygotes, novel alleles were isolated using allele specific amplification, HaploPrep kits (Qiagen, Valencia, CA) or by cloning and characterized by DNA sequencing. Allele designations for novel alleles were assigned by the WHO Nomenclature Committee for Factors of the HLA System (Marsh et al. 2003b).

Adjacent genes, *KIR2DS3, KIR2DS5*, and *KIR2DS1*, were identified in all *KIR2DL5* positive cells by extracting DNA fragments with probes 2DS3-867A, 2DS5-493C or 2DS1-563A (HaploPrep) followed by detection of *KIR2DL5* on the fragment by sequencing. Long-range PCR was used to link *KIR3DS1* or *KIR2DL2/2DL3* to *KIR2DL5* alleles in cases of a novel allele or a novel allele combination defining a haplotype using a sense primer annealing in the 5' gene (3DS1-SSPF- GGCAGAATATTCCAGGAGG, 2DL2-E6F-708T- TCACCCACTGAACCAAGCTCT, and 2DL3-SSPF-CCTTCATCGCTGGTGCTG) and an antisense primer annealing in *KIR2DL5* (GGGGTCACAGGGCCCATGAGGAT). Haplotype specific extraction was also used to link *KIR2DL2/2DL3* or *KIR3DL1/KIR3DS1 to KIR2DL5* using probes 2DL2-999T, 2DL3-1316T, 3DL1-1156C, 3DS1-556T, and 2DL5-784A (HaploPrep).

The nucleotide sequences of *KIR2DL5* were aligned by MUSCLE (Edgar 2004) and a maximum likelihood tree was built using PHYML (version 3.0, BIONJ distance-based tree as the starting tree, GTR as the substitution model)(Guindon and Gascuel 2003).

Results

KIR2DL5B predominates in a population with high *KIR2DL5* allelic diversity

Fifty five of 100 random African Americans carried *KIR2DL5*; 31% carried *KIR2DL5A* and 82% carried *KIR2DL5B*. This is consistent with the distribution previously observed in a population of 32 African Americans (Du et al. 2008). In contrast, in European Americans, the majority of *KIR2DL5* positive individuals carry *KIR2DL5A* (83%) compared to *KIR2DL5B* (49%) (Mulrooney et al. 2008). Sequencing of 254 base pairs of the 5' upstream region and the entire coding region of *KIR2DL5* identified three known *KIR2DL5A* alleles, six known *KIR2DL5B* alleles, and 11 novel alleles (Table 1). A comparison with a similar study in European Americans (n=74) (Mulrooney et al. 2008) shows three alleles present in >10% of gene positive individuals in African Americans and four in European Americans; two of these frequent alleles (*KIR2DL5A*0010101* found in 22% of African Americans and 63% of European Americans; *KIR2DL5B*0020101* found in 33% and 29%, respectively) are common in both populations. Frequent *KIR2DL5B*00601* (20%) in African Americans was not observed in European Americans; frequent *KIR2DL5B*0020102* (14%) in European Americans was not observed in African Americans. Seventeen alleles are found at frequencies <10%--each of these alleles was found in from one to three African American individuals. In contrast, in European Americans, three alleles at frequencies <10% were found in single individuals. Thus, African Americans appear to carry a broader spectrum of *KIR2DL5* alleles compared to an European American population.

The identity of the 11 novel alleles as *KIR2DL5A* versus *KIR2DL5B* was determined by sequence homology to known alleles and by physically linking the new allele to its 5'

neighboring gene, either to *KIR3DS1* for *KIR2DL5A* or to *KIR2DL2* (or *KIR2DL3)* for *KIR2DL5B* (Hsu et al. 2002). Novel alleles differing in their coding sequences included *KIR2DL5A*01201* with a substitution in the signal peptide and *KIR2DL5B*01301* with a substitution in the second extracellular domain (Table 2). Six alleles had synonymous substitutions including variants of the two novel alleles described above.

Six of the novel alleles exhibited variation in their 5' upstream regions from their most similar allele. Four of these alleles, all *KIR2DL5B* alleles, join *KIR2DL5B*003* in carrying the expressed version of the RUNX3 site: *KIR2DL5B*0020106, *00202, *0070102*, and **01303*. Furthermore, two of these alleles, *KIR2DL5B*0020106* and *KIR2DL5B*0070102*, exhibit identical nucleotide changes which alter the binding sites for transcription factors key in the regulation of *KIR* expression (Davies et al. 2007;Li et al. 2008). Substitution at nucleotide -176 restores the sequence of the Ying Yang 1 (YY1) site; disruption of this site in most *KIR2DL5* alleles is thought to increase the strength of the antisense promoter reducing gene expression of *KIR2DL5*. Variation at -27 alters an Sp1 site, creating a better consensus sequence for specificity protein 1 (Sp1) binding. This latter variation, also found in *KIR2DL5B*003* and *KIR2DL5B*0070101*, has been shown to decrease forward promoter activity reducing expression compared to *KIR2DL5A*001* (Li et al. 2008). It is not known how these two nucleotide substitutions will impact the balance between sense and antisense transcripts but expression may vary from the other *KIR2DL5B* alleles.

Eleven novel *KIR2DL2/2DL3* alleles alter the amino acid sequence of the gene located 5' of *KIR2DL5B*—Alleles present at the *KIR2DL2/2DL3* locus were identified by DNA sequencing in preparation for defining their association with *KIR2DL5B* alleles. In addition to six previously reported alleles, four *KIR2DL2* and seven *KIR2DL3* novel alleles were detected in 100 African Americans (Table 3). Two of the four novel *KIR2DL2* alleles carried single nonsynonymous substitutions altering D1 or transmembrane regions compared to their most similar alleles. Five of seven novel *KIR2DL3* alleles carried one or two nonsynonymous substitutions altering D1, D2 or cytoplasmic tail regions.

Linkage defines 30 allelic haplotypes centered around *KIR2DL5*

Table 4 shows the linkage of *KIR2DL5* alleles with alleles at nearby loci defining these centromeric and telomeric regions of the *KIR* haplotypes. The association with *KIR2DS3* or *KIR2DS5* was observed to parallel the nucleotide sequence homology as identified by a phylogenetic tree of *KIR2DL5* coding sequences (Figure 1). Alleles clustered with K*IR2DL5B*0020101* are associated with *KIR2DS3*; alleles clustered with *KIR2DL5A*0010101* are associated with *KIR2DS5*. Both *KIR2DL5A* and *KIR2DL5B* alleles and both expressed and non-expressed alleles are found in each cluster.

Linkage of telomeric *KIR2DL5A* to specific alleles of *KIR2DS3* or *KIR2DS5* noted by other investigators were observed [*KIR2DL5A*0010101* with *KIR2DS5*00201* (11 individuals); *KIR2DL5A*0050101* with *KIR2DS3*002* (2 individuals)] (Middleton et al. 2007;Ordonez et al. 2008). Three additional haplotypes of *KIR2DL5A* with *KIR2DS5* were identified: one silent variation of *KIR2DL5A*0010101 (KIR2DL5A*00102)* remains associated with *KIR2DS5*00201* and two new haplotypes include novel alleles, *KIR2DL5A*01201* and *KIR2DL5A*01202*. Five of the six *KIR2DL5A* haplotypes include *KIR3DS1*01301*; one is linked to *KIR3DS1*049N*. Although also found in the absence of *KIR2DL5A, KIR2DS1* was found linked to *KIR2DL5A* in 15 of 16 individuals. The majority of haplotypes contain the predominant *KIR2DS1*00201* (Middleton et al. 2007); one included *KIR2DS1*006*.

In the centromeric *KIR2DL5B* complex, the previously noted association of *KIR2DL5B*0020101/03* with *KIR2DS3*00103 (*15 out of 55 *KIR2DL5* positive

individuals) was the most common haplotype in African Americans (Ordonez et al. 2008)). If other flanking loci were considered, 23 haplotypes of *KIR2DL5B* were observed: nine with *KIR2DS3* and 14 with *KIR2DS5*. Alleles closely related to *KIR2DL5B*0020101/03 (*e.g. *KIR2DL5B*0020104* or *KIR2DL5B*010)* (Figure 1) remain associated with *KIR2DS3*00103* or its silent variants. Other alleles of *KIR2DL5B* are associated with a number of *KIR2DS5* alleles.

The centromeric *KIR2DL5B* alleles are primarily associated with *KIR2DL2* alleles. Seven *KIR2DL2* alleles were identified in linkage with *KIR2DL5B*; the same alleles were found in association with haplotypes carrying either *KIR2DS3* or *KIR2DS5*. Two haplotypes carried K*IR2DL3*014* in association with two different *KIR2DL5B* alleles, one in association with *KIR2DS3* and one with *KIR2DS5*.

Discussion

Few studies have explored KIR haplotype diversity at allele-level resolution. This study of 55 KIR2DL5 positive random African Americans has detected extensive diversity at both the allelic and the haplotypic levels for KIR. Twenty *KIR2DL5* alleles are embedded within 30 multi-gene complexes defining either centromeric and telomeric regions of the KIR gene cluster. These gene complexes are further differentiated by the linked alleles at neighboring loci. Specific *KIR2DL5* alleles were associated either with *KIR2DS3*, an apparently non-expressed locus (VandenBussche et al. 2008), or with *KIR2DS5*, a diverse locus in African Americans (Hou et al. 2009). Other sources of haplotype variability included allelic variation at *KIR2DS3* (6 alleles) and *KIR2DS*5 (7 alleles) as well as at *KIR2DS1* (2 alleles), *KIR3DS1* (2 alleles), and *KIR2DL2/KIR2DL3* (8 alleles). This diversity is reminiscent of the allelic diversity of the HLA haplotypes although the extent of allelic variation at a *KIR* locus is more subtle. *KIR* allelic products differ only modestly from one another while HLA allelic variation can be more extensive resulting in many amino acid differences.

The impact of genetic variation on the expressed KIR repertoire and the functional activity of NK cells is yet to be fully understood. The presence of specific *KIR* genes has been associated with susceptibility or resistance to infectious and autoimmune diseases and to malignancy (Bashirova et al. 2006) (Khakoo and Carrington 2006). In human immunodeficiency virus (HIV) infected individuals, the presence of *KIR3DS1* is associated with a delayed progression to AIDS (Martin et al. 2002a) and KIR3DS1 expressing cells inhibit viral replication (Alter et al. 2007). In autoimmune subjects with psoriasis, those carrying activating *KIR2DS2* and *KIR2DS1* are more likely to develop psoriatic arthritis than subjects without these genes (Martin et al. 2002b). In hematopoietic progenitor cell transplantation for acute myelogenous leukemia, a decreased frequency of relapse has been noted in transplants with donors carrying haplotypes with increased numbers of activating *KIR* genes (i.e., KIR B haplotypes) (Cooley et al. 2009). The mechanisms by which individual KIR impact disease susceptibility, progression and outcome is not yet clear. While resistance in diseases associated with the presence of inhibitory KIR receptors with known HLA ligands may result from a decreased activation threshold for NK cells (Khakoo et al. 2004), the lack of information on the ligands of other KIR including KIR2DL5 hinders an understanding of mechanisms for those KIR.

Less is known about the impact of KIR allelic polymorphism on the immune response. Allelic variation alters the level of protein expression and the affinity of ligand binding as demonstrated for KIR3DL1 (Sharma et al. 2009;Yawata et al. 2008) and KIR2DL2/KIR2DL3 (Moesta et al. 2008). In HIV infection, allotypic variation of KIR3DL1 influences disease progression and levels of the pathogen in plasma (Martin et al. 2007). In hepatitis C infections, the presence of the KIR2DL3 subset of the KIR2DL2/KIR2DL3 alleles is

associated with the resolution of infection in those carrying the appropriate HLA-C ligand (Khakoo et al. 2004). Thus, the unique *KIR* haplotypes observed in the African American population, coupled with diversity in their HLA ligands, likely result in unique immune response profiles for an individual.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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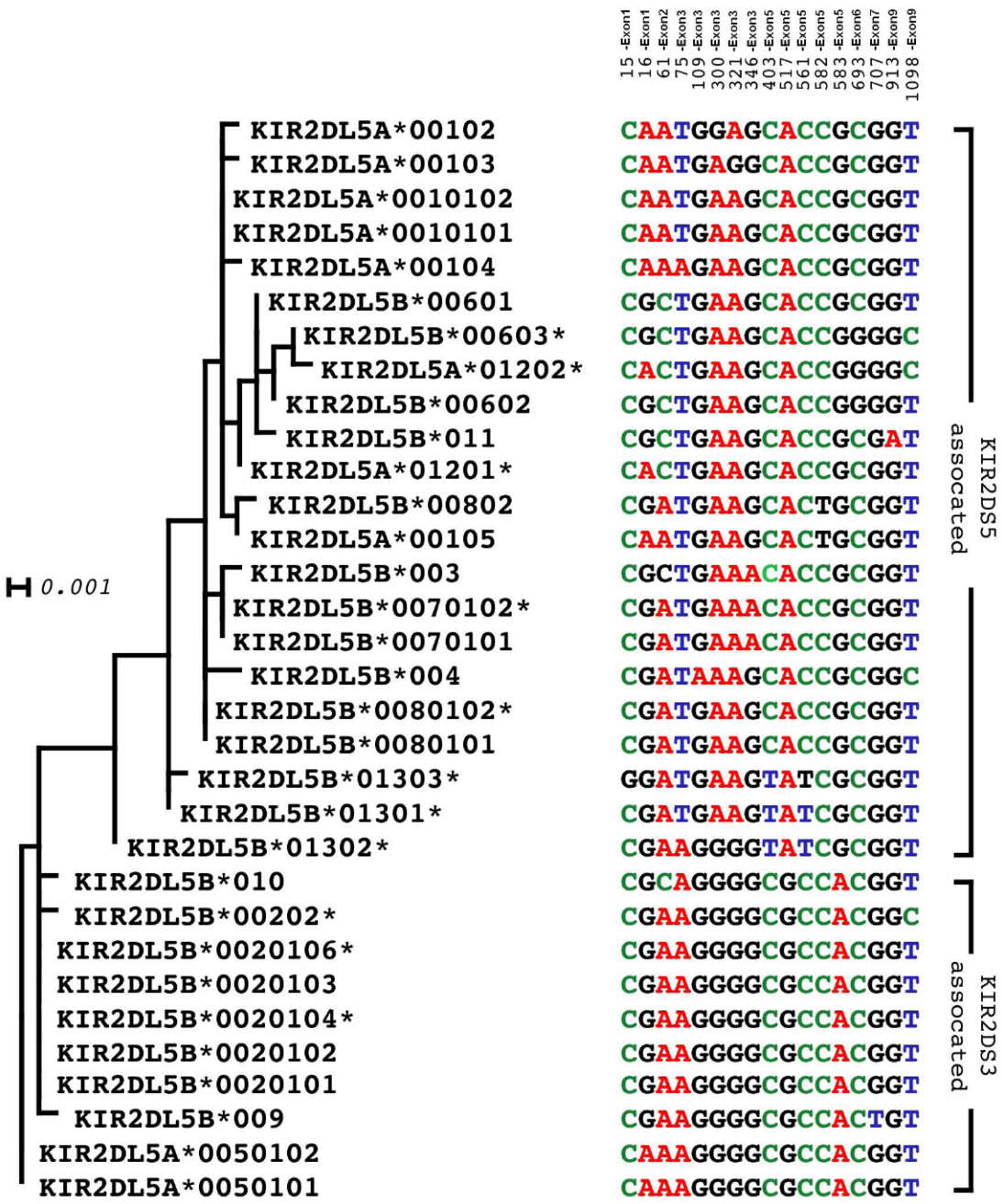

Figure 1.

Phylogenetic tree of *KIR2DL5* alleles. Alleles cluster into two major groups which correspond with the *KIR2DS3* or *KIR2DS5* associations observed in our study. The nucleotides that differ among the alleles are shown with their position in the coding sequence. Asterisks following the allele names identify alleles identified in this study.

Table 1

Number of individuals carrying *KIR2DL5* alleles in a population of 100 random African Americans[a].

KIR2DL5A*	**Number individuals**	***KIR2DL5B****	**Number individuals**
0010101	12	0020101 (or 0020103)[c]	18
00102	1	0020104[b]	1
0050101	2	0020106[b]	2
01201[b]	1	00202[b]	1
01202[b]	1	003	1
		004	3
		00601	11
		00603[b]	3
		0070102[b]	1
		0080101	3
		0080102[b]	1
		010	1
		01301[b]	1
		01302[b]	1
		01303[b]	1

[a]Seven individuals carried *KIR2DL5A* and *KIR2DL5B* and five carried two *KIR2DL5B* alleles. One individual apparently carrying three *KIR2DL5* alleles was not included in this table.

[b]Novel, described in this study

[c]*KIR2DL5B*0020101* and *KIR2DL5B*0020103* differ in an intron and were not distinguished in this study.

Table 2

Novel alleles

Novel allele	Most similar allele	Promoter variation[a]	Codon (amino acid) substitution[a]	Gene 5'	Cell	GenBank accession number
2DL5A*01201	2DL5A*0010101		-1 ACA (T) => CCA (P)	KIR3DS1	GM17114	FJ824674
2DL5A*01202	2DL5A*01201		210 TCC (S) => TCG (S), 345 GCT (A) => GCC (A)	KIR3DS1	GM17102	FJ804063
2DL5B*0020104	2DL5B*0020101	-23 T => C		KIR2DL2	GM17173	FJ804070
2DL5B*0020106	2DL5B*0020101	-239G => T, -215T => C, -208C => T, -206A => G, -176G => A, -159A => G, -154T => C, -120C => T, -97A => G, -84G => A, -27C => T, -23T => C, -10C => T		KIR2DL3	GM17129 GM17178	FJ804065
2DL5B*00202	2DL5B*0020101	-215T => C, -208C => T, -206A => G, -154T => C, -97A => G, -84G => A, -23T => C	345 GCT (A) => GCC (A)	KIR2DL2	GM17187	GU121962
2DL5B*00603	2DL5B*00601		210 TCC (S) => TCG (S), 345 GCT (A) => GCC (A)	KIR2DL2	GM17141 GM17160 GM17166	FJ804069
2DL5B*0070102	2DL5B*0070101	-206A => G, -176G => A, -120C => T		KIR2DL3	GM17190	FJ804067
2DL5B*0080102	2DL5B*0080101	-23T => C		KIR2DL2	GM17177	FJ804071
2DL5B*01301	2DL5B*0080101		114 CGC (R) => TGC (C), 166 CAC (H) => CAT (H)	KIR2DL2	GM17113	FJ804064
2DL5B*01302	2DL5B*01301		4 GGT (G) => GGA (G), 79 CCA (P) => CCG (P), 86 TCA (S) => TCG (S)	KIR2DL2	GM17128	FJ804066
2DL5B*01303	2DL5B*01301	-215T => C, 154T => C, -97A => G-84G => A,-23T => C	-17 GTC (V) => GTG (V)	KIR2DL2	GM17183	FJ824675

[a]Most similar allele => novel allele

Table 3

Novel *KIR2DL2/KIR2DL3* alleles

Sample ID	Locus	Most Similar Allele	WHO name[a]	Codons (Amino Acid) Altered	GenBank Accession No.
GM17109	2DL2	003	2DL2*00601	16 CGC (R) => CCC (P)	EU933932
GM17115	2DL2	003	2DL2*00602	16 CGC (R) => CCC (P), 31 GAT (D) => GAC (D)	EU933933
GM17134	2DL2	001	2DL2*007	232 GTC (V) => GCC (A)	EU933935
GM17140	2DL2	001	2DL2*00102	80 TAC (Y) => TAT (Y)	EU933931
GM17113	2DL3	001	2DL3*00102	246 CGC (R) => CGG (R)	GU138980
GM17159	2DL3	006	2DL3*012	255 GTT (V) => GCT (A), 299 TCT (S) => TTT (F)	GU138983
GM17160	2DL3	001	2DL3*00103	59G TC (V) => GTT (V)	GU138984
GM17172	2DL3	001	2DL3*013	131 CGG (R) => CAG (Q)	GU138986
GM17127	2DL3	001	2DL3*011	114 CCG (P) => CTG (L)	GU138982
GM17190	2DL3	005	2DL3*014	11 CGG (R) => CTG (L), 142 GAG (E) => GAA (E), 297 CAC (H) => CGC (R)	GU138979
GM17199	2DL3	001	2DL3*016	123 AGC (S) => AAT (N), 282 TAT (Y) => TAC (Y)	GU573764

[a]The names are alleles are assigned by the World Health Organization Nomenclature Committee for Factors of the HLA System (Marsh et al. 2003b).

Table 4

Centromeric and telomeric haplotypes[a] associated with *KIR2DL5*

Haplo	No. Individuals	2DL2 2DL3	2DL5B	2DS3 2DS5	Haplo	No. Individuals	3DS1	2DL5A	2DS3 2DS5	2DS1
C1	14	2*00101	*0020101/03	3*00103	T1	9	*01301	*0010101	5*00201	*00201
C2	1	2*00101	*0020101/03	3*00105	T2	1	*01301	*0010101	5*00201	*006
C3	1	2*00101	*0020101/03	3*004	T3	1	*049N	*0010101	5*00201	*00201
C4	1	2*00102	*0020101/03	3*00103	T4	1	*01301	*00102	5*00201	*00201
C5	1	2*00101	*0020104	3*00103	T5	2	*01301	*0050101	3*002	*00201
C6	1	2*00101	*0020106	3*00103	T6	1	*01301	*01201	5*009	*00201
C7	1	3*014	*0020106	3*00103	T7	1	*01301	*01202	5*006	-
C8[b]	1	2*00301	*00202	3*00106						
C9	1	2*004	*003	5*003						
C10	3	2*00101	*004	5*006						
C11	7	2*00602	*00601	5*007						
C12	2	2*00301	*00601	5*009						
C13	1	2*00301	*00601	5*00201						
C14	1	2*00601	*00601	5*009						
C15	3	2*00602	*00603	5*006						
C16	1	3*014	*0070102	5*003						
C17	1	2*00101	*0080101	5*00201						
C18	1	2*00101	*0080101	5*007						
C19	1[a]	2*007	*0080101	5*007						
C20	1	2*00301	*0080102	5*009						
C21	1	2*00601	*010	3*00104						
C22	1	2*00101	*01301	5*005						
C23	1	2*00101	*01302	5*00801						

[a]Each haplotype was identified by the physical linkage of adjacent loci in all individuals carrying the haplotype. The exceptions were *KIR2DS1* which was not physically linked to adjacent loci in all individuals carrying a particular haplotype and haplotypes C20 (linkage of *KIR2DL2*) and haplotype C8 which were predicted based on common associations. It is not known whether the gene segments carried in the centromeric gene complex are located on the same chromosome as the telomeric gene complex in individuals who carry both. The number of individuals carrying the haplotype are listed in the table; the table does not include two KIR2DL5 positive individuals in whom haplotypes could not be determined by physical linkage or be predicted. This included the individual carrying novel allele *KIR2DL5*01303*.