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BRIEF COMMUNICATION

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Evidence for recombination as a mechanism for KIR diversification

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Killer cell inhibitory receptors (KIR) are a family of immunoglobulin (Ig)-like cell-surface glycoproteins which are expressed on human natural killer (NK) and some T cells. Certain KIR recognize polymorphic determinants of HLA class I molecules (Gumperz et al. 1995; Litwin et al. 1994; Mandelboim et al. 1997; Moretta et al. 1993), the ligands for other KIR have yet to be defined. Over forty putative receptors have been identified, and they can be distinguished according to the number of their extracellular Ig domains and the lengths of their cytoplasmic tails (Biassoni et al. 1996; Colonnna and Samaridis 1995; D'Andrea et al. 1995; Döhring et al. 1996; Wagtmann et al. 1995a). Long cytoplasmic tails contain immune-receptor tyrosine-based inhibitory motifs (ITIMs), which can confer inhibitory activity on KIR (reviewed in Valiante et al. 1997a). KIR with short cytoplasmic tails lack ITIMs, and are believed to act as noninhibitory, or stimulatory, receptors (Biassoni et al. 1996; Moretta et al. 1995).

Comparison of KIR sequences reveals a "patchwork" pattern of variability in which most substitutions are found in more than one sequence (Salter et al. 1997; Selvakumar et al. 1997; Valiante et al. 1997a). This pattern, which is analogous to that seen for highly polymorphic major histocompatibility complex (MHC) class I and class II loci, provides evidence for the role of genetic recombination in the generation of KIR diversity (Parham et al. 1988, 1995). However, in contrast to the study of MHC genes, analysis of KIR sequences has yet to identify triplets of related sequences which define potential events of recombination. Here we describe such a relationship, in which a newly discovered KIR is homologous to KIR2DL1 family members (e.g., NKAT1) in the 5' and 3' regions, but resembles KIR2DS1 family members (e.g., Eb6Act1) in an internal segment.

In the course of an NK-cell receptor repertoire study (Valiante et al. 1997b), KIR cDNA were cloned and sequenced from two blood donors (PP and NV). Multiple copies of each cDNA were fully sequenced to eliminate artifacts generated by polymerase chain reaction (PCR) amplification prior to cloning. A total of twenty-two different KIR were characterized and of these, nine have previously undiscovered sequences. Nucleotide differences that distinguish the new KIR from closely related, previously characterized KIR sequences are scattered throughout the coding region, although there are no nonsynonymous changes in the exons encoding the cytoplasmic domain. Although KIR2DL1v, KIR2DL3v, KIR2DL4v3, and KIR3DL1v each contain a unique polymorphism, most of the substitutions are shared with other KIR sequences. (see Table 1) Eight of the new KIR differ from known KIR sequences by only one, two, or three nucleotide changes. In contrast, the ninth sequence, provisionally named KIR2DL1v, differs by eight or more nucleotide substitutions from every other KIR (Valiante et al. 1997b). The KIR most closely related to KIR2DL1v are those of the KIR2DL1 family, which differ from KIR2DL1v by eight to ten nucleotide substitutions. KIR2DL1v differs from NKAT1 by a cluster of six nucleotide substitutions within positions 523–709 and by additional substitutions at positions 110 and 799. Other KIR2DL1 differ from KIR2DL1v by the cluster of six nucleotide substitutions, but have different additional sites of nucleotide substitution. Nucleotides 523–709 of KIR2DL1v are identical in sequence to the homologous region of KIR2DS1 receptors (Fig. 1). This pattern of sequence similarity suggests that either a gene conversion or double recombination event between KIR2DL1 and KIR2DS1 occurred in the evolution of these KIR. Additional substitutions have also contributed to their evolution and the substitution at position 799 in KIR2DL1v is unique among the KIR so far defined. It

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Table 1 New KIR variants

New KIR sequence (donor)	Closest known KIR sequence	Substitutions:[a] Nucleotide[b]	Amino acid[c]
KIR2DL1v (NV)[d]	NKAT1	C to G (110)	P to R (16)
		C to A (523)	P to T (154)
		T to C (549)	–
		G to A (550)	D to N (163)
		C to T (576)	–
		A to G (608)	H to R (182)
		A to G (709)	K to E (216)
		C to ***T*** (799)	R to ***C*** (246)
KIR 2DL3v (PP)	023GB	A to G (581)	–
		G to ***T*** (724)	R to ***I*** (221)
KIR2DL4v1 (PP and NV)	cl12.11c	G to A (1024)	–
KIR2DL4v2 (PP)	cl15.212	A to G (479)	–
KIR2DL4v3 (NV)	cl15.212	A to ***G*** (158)	Y to ***C*** (30)
		A to G (479)	–
		C to T (761)	–
KIR2DS1v (NV)	Eb6Act1	G to A (271)	R to K (70)
KIR2DS3v (NV)	NKAT7	C to T (882)	–
KIR3DL1v (NV)	NKB1/cl2	C to G (775)	R to G (238)
		C to ***G*** (998)	S to ***C*** (312)
KIR3DS1v (NV)	123FM	C to T (5)	S to L (LP2)[e]
		A to G (497)	H to R (145)

[a] Unique substitutions are in bold italics. Multiple copies of each KIR were sequenced to eliminate artifacts generated by PCR amplification prior to cloning
[b] Numbering begins at first nucleotide of the translation initiation codon
[c] Numbering begins at first residue of the mature protein. Dashes indicate synonymous substitutions.
[d] It is unlikely that KIR2DL1v is a chimeric product generated during PCR amplification, as donor NV does not express KIR sequences which could serve as templates. Furthermore, KIR2DL1v has also been identified in the donor's mother (unpublished data)
[e] (LP2) denotes position 2 of the leader peptide
Genbank accession numbers are KIR2DL1v (AF022045), KIR2DL3v (AF022048), KIR2DL4v1 (AF034771), KIR2DL4v2 (AF034772), KIR2DL4v3 (AF034773), KIR2DS1v (AF0022046), KIR2DS3v (AF022047), KIR3DL1v (AF022049), KIR3DS1v (AF022044), NKAT1 (L41267), 023GB (U73395), cl12.11c (X999479), cl15.212 (X97229), Eb6Actl (X89892), NKAT7 (L76670), NKB1 (U31415), and 123FM (U73396)

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KIR2DL1 and KIR2DS1 receptors recognize HLA-C allotypes, such as HLA-Cw*0401 and HLA-Cw*0202, which possess asparagine 77 and lysine 80 in the heavy chain sequence (Colonna et al. 1993; Kim et al. 1997; Mandelboim et al. 1997; Moretta et al. 1993; Wagtmann et al. 1995b). Cytoxicity assays with NK clones expressing KIR2DL1v have shown that this receptor binds to HLA-Cw*0202 and HLA-Cw*0401 and functions as a KIR2DL1 receptor (Valiante et al. 1997b and unpublished observations). The KIR2DL1v protein differs from other KIR2DL1 at five amino acid residues, including three in the membrane proximal Ig domain, called D2 (residues 154, 163, and 182), one in the stem (216), and one in the transmembrane region (246) (Fig. 2A).

A recently determined crystal structure of the extracellular portion of a KIR2DL1 (cl42) shows that this molecule resembles human growth hormone receptor (hGHR) and prolactin receptor (hPRLR) (Fan et al. 1997). The KIR2DL1 crystal structure allows placement of the Ig domain differences between KIR2DL1v and other KIR2DL1. Substitutions of threonine (T) at position 154 and asparagine (N) at 163 are restricted to KIR2DS1 family members and KIR2DL1v. Residue 154 lies on a loop at the membrane-distal end of D2, within what appears to be a cluster of variability between residues 148–156 (Selvakumar et al. 1997a). Crystal structure determinations of hGHR and hPRLR, co-crystallized with growth hormone, implicate loops near the interdomain elbow in ligand binding (De Vos et al. 1992; Somers et al. 1994). By analogy, loops at the elbow between the two KIR Ig domains are expected to be involved in HLA-C binding, but not the loop containing residue 154 (Fan et al. 1997). In addition, exchanging proline for threonine at 154 in soluble KIR2DS1 has no affect on HLA-Cw*0401 binding, nor does the reciprocal substitution of threonine 154 for proline in KIR2DL1. (Biassoni et al. 1997) Position 163, on the exposed face of the β-sheet opposite

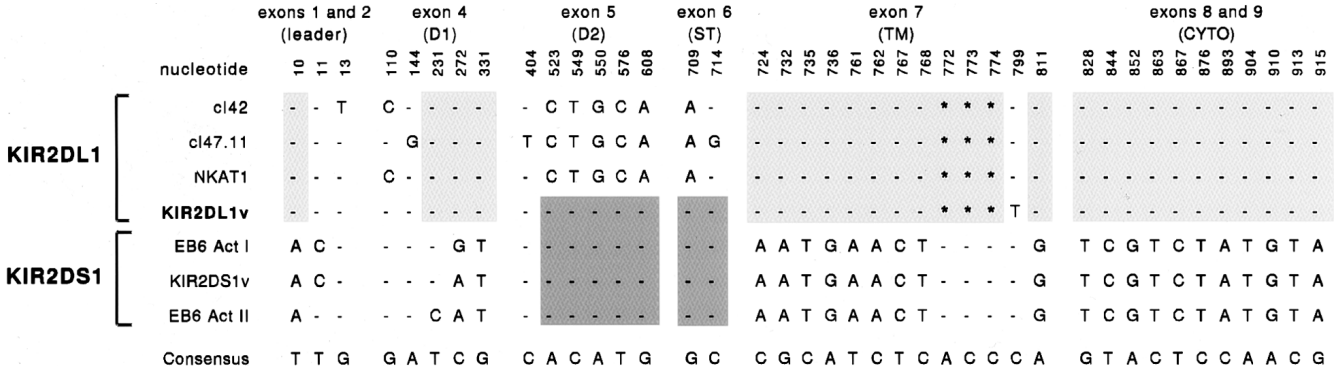
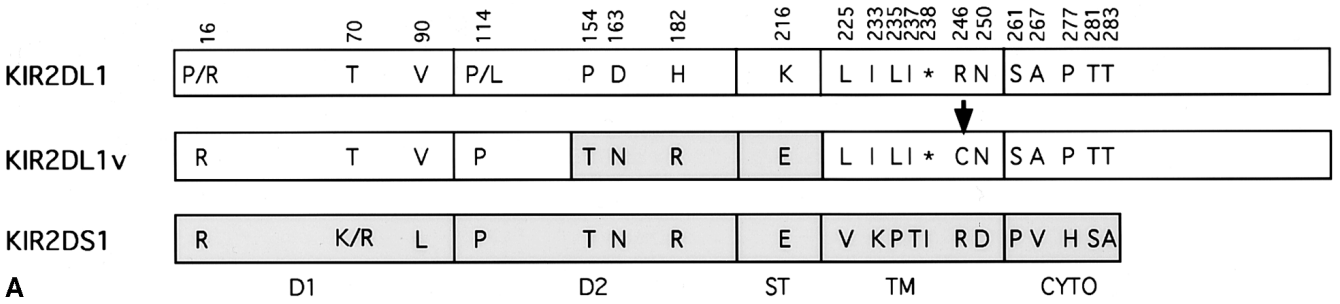


Fig. 1 Nucleotide differences between KIR2DL1 and KIR2DS1 sequences. Positions at which KIR2DL1v is identical to all other KIR2DL1 sequences are *boxed* in *light grey* and positions identical KIR2DS1 are *boxed* in *dark grey*. Exon 3 is a pseudo-exon which does not appear in mature RNA transcripts (Wilson et al. 1997). *Numbering* starts at the first nucleotide of the translation initiation codon. Consensus represents KIR2DL1 and KIR2DS1 families only. Identities with the consensus are indicated by a *dash*. *Asterisks* indicate deletions. *ST* stem; *TM* transmembrane domain; *CYTO* cytoplasmic domain. GenBank accession numbers are cl42(U24076), cl47.11(U24078), NKAT1 (L41267), KIR2DL1v (AF022045), EB6ActI (X89892), KIR2DS1v (AF022046), and Eb6ActII (X98858)

the interdomain interface, is removed from the putative binding site, but could be involved in receptor dimerization. In contrast, residue 182, changed from histidine (H) to arginine (R) in KIR2DL1v, sits on a loop at the tip of the interdomain elbow, opposite residue 44, which controls KIR2D specificity (Winter and Long 1997). All known KIR have either histidine or arginine at position 182, suggesting that a basic residue may be required at this site for ligand binding (Fig. 2B). Similarly, other sites in elbow loops, notably residues 45 and 70, have been shown to influence HLA-C binding (Biassoni et al. 1997; E.O. Long, personal communication).

In the stem region, KIR2DL1v and KIR2DS1 receptors share a glutamate (E) at position 216 with KIR2DL2, KIR2DL3, and KIR2DS5 receptors. All other known KIR express lysine (K) at this position, indicating that a charged residue may be needed here, possibly for receptor stabilization or dimerization (Fan et al. 1997). The unique substitution at nucleotide position 799 gives rise to a cysteine (C) at position 246 in the transmembrane domain of KIR2DL1v (Fig. 2A). Functional studies show that KIR2DL1v binds at least



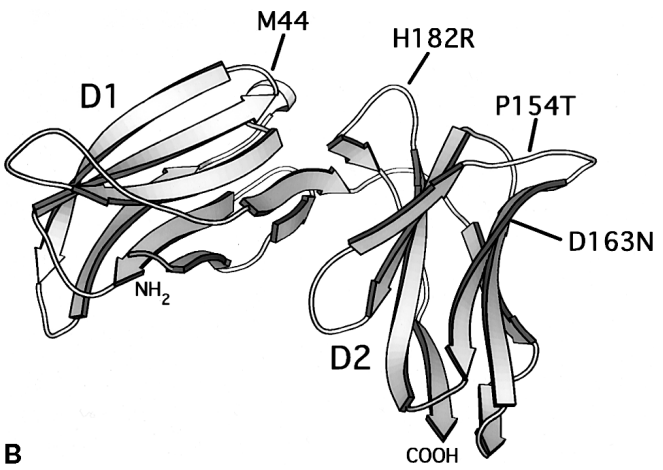


Fig. 2 **A** Schematic diagram showing amino acid differences between mature KIR2DL1, KIR2DL1v and KIR2DS1 receptors. Shaded regions of KIR2DL1v are identical to KIR2DS1. The unique cysteine in the KIR2DL1v transmembrane domain is marked by an *arrow*. An *asterisk* indicates a deletion. *Numbering* starts at the first residue of the mature KIR proteins. *D1* membrane-distal Ig domain; *D2* membrane-proximal Ig domain; *ST* stem; *TM* transmembrane domain; *CYTO* cytoplasmic domain. **B** Amino acid substitutions in the extracellular domains of the KIR2DL1v receptor. KIR2DL1v differs from other KIR2DL1 proteins by a proline to threonine substitution at residue 154 (P154T), an aspartate to asparagine substitution at 163 (D163N), and a histidine to arginine change at 182 (H182R). The putative class I binding site is at the tip of the interdomain interface. A methimine at position 44 (M44) confers specificity for the N77K80 class I motif. *D1* membrane-distal Ig domain; *D2* membrane-proximal Ig domain. Crystal structure coordinates were supplied by Q.R. Fan (Fan et al. 1997) and the ribbon diagram was prepared using MolScript (Kraulis 1991)

two KIR2DL1 ligands, HLA-Cw*0202 and HLA-Cw*0401. However, a thorough analysis of KIR2DL1v specificity, which may reveal differences between KIR2DL1v and other KIR2DL1 receptors, has yet to be performed.

In this study, characterization of the KIR from two individuals yielded nine new KIR cDNA sequences. The number of new KIR found in only two donors suggests that many additional KIR sequences await discovery. A population study, designed to produce a database of KIR alleles comparable to those available for MHC class I and class II, will be needed to understand fully the nature of KIR sequence diversity. The relationship between the KIR2DL1 and KIR2DS1 families and KIR2DL1v is the first evidence clearly implicating either gene conversion or double recombination as a mechanism for KIR diversification. This observation is consistent with the more general patchwork pattern of variation seen in the known KIR (Salter et al. 1997; Selvakumar et al. 1997; Valiante et al. 1997a), and suggests that the diversity in KIR and their MHC class I ligands was generated by similar mechanisms.

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