Gene Structure and Promoter Variation of Expressed and Nonexpressed Variants of the KIR2DL5 Gene

Carlos Vilches, Clair M. Gardiner and Peter Parham

*J Immunol* 2000; 165:6416-6421; doi: 10.4049/jimmunol.165.11.6416

http://www.jimmunol.org/content/165/11/6416

References

This article cites 27 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/165/11/6416.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Gene Structure and Promoter Variation of Expressed and Nonexpressed Variants of the KIR2DL5 Gene

Carlos Vilches, Clair M. Gardiner, and Peter Parham

Two variants of the novel KIR2DL5 gene (KIR2DL5.1 and .2) were identified in genomic DNA of a single donor. However, only the KIR2DL5.1 variant was transcribed in PBMC. In this study, analysis of seven additional donors reveals two new variants of the KIR2DL5 gene and indicates that transcription, or its lack, are consistently associated with particular variants of this gene. Comparison of the complete nucleotide sequences of the exons and introns of KIR2DL5.1 and KIR2DL5.2 reveals no structural abnormalities, but similar open reading frames for both variants. In contrast, the promoter region of KIR2DL5 shows a high degree of sequence polymorphism that is likely relevant for expression. Substitution within a putative binding site for the transcription factor acute myeloid leukemia gene 1 could determine the lack of expression for some KIR2DL5 variants.


Killer cell Ig-like receptors (KIR) control the response of human NK cells by delivering inhibitory or activating signals upon recognition of MHC class I ligands on the surface of potential target cells. KIR comprise a family of proteins with related but distinct structures, whose genes are located in chromosome 19. We and others have recently described KIR2DL5, a new member of this family whose ligand has yet to be identified. KIR2DL5 shares some structural features with KIR2DL4, a receptor for HLA-G (5, 6), that distinguish them from other KIR: a D0-D2 configuration of extracellular Ig-like domains and a particularly long cytoplasmic tail. The primary structures of KIR2DL4 and KIR2DL5 are 79% identical and differ in the number of immunoreceptor tyrosine inhibitory motifs: one in KIR2DL4, two in KIR2DL5, as well as in the presence (KIR2DL4) or absence (KIR2DL5) of a positively charged residue in the transmembrane region. Also, their distribution in the population and patterns of expression are different: whereas KIR2DL4 is present in all individuals and transcribed in all NK cells (5, 7, 8), KIR2DL5 is a common feature of B haplo-

types, found in ~50% of the population, and is clonally expressed by NK cells and T lymphocytes (3).

Whereas two KIR2DL5 variants (KIR2DL5.1 and .2) were found in the genomic DNA of donor NV, mRNA could only be detected for the KIR2DL5.1 gene and not for KIR2DL5.2 (3). Exons 3 and 4 of KIR2DL5.2, which encode the extracellular Ig-like domains, differ from those of KIR2DL5.1 by only five nucleotide changes, of which three are synonymous and two determine amino acid substitutions in the D2 domain. Introns 2 and 3 of both variants were found to differ by a single nucleotide replacement (our unpublished observation), all their splicing sites conforming to the AG/GT rule. Since the sequenced regions of KIR2DL5.2 harbored no obvious structural defects, we could not ascertain whether it represents a null version of the KIR2DL5 gene or whether its lack of expression in the donor studied was due to some form of negative regulation.

To establish whether expression or its lack correlate with particular structural variants of the KIR2DL5 gene and to assess further its polymorphism, we have studied the expression and nucleotide sequence of this gene in different donors. We have also investigated the organization of the KIR2DL5 gene and verified the structural integrity of the exons and introns of KIR2DL5.2 by determination of the complete nucleotide sequence. Finally, we have characterized the promoter regions of expressed and nonexpressed KIR2DL5 variants in different donors to identify the elements that regulate transcription of this gene.

Materials and Methods

Analysis of sequence polymorphism in the coding region of KIR2DL5 in seven donors

Genomic DNA from seven donors was analyzed by PCR with sequence-specific primers (PCR-SSP), using primers that recognize single nucleotide differences between variants. .1 and .2 of the KIR2DL5 gene. Of the seven donors, four (WCS, WCJ, CC, and VH) were of African-American origin, two (RR and SK) were Asian Indians, and one (BS) was Chinese. Donors WCS and CC were the parents of WCJ; VH was the mother of donor CC. PCR-SSP typing of KIR2DL5.1 and .2 was performed using primer pairs F153/Rc939 and Fa139/Ra964, respectively, as previously described (3). F153/Rc939 recognize, in addition to KIR2DL5.1, the new variants described in this article, KIR2DL5.3 and .4.

RNA isolated from PBMC of all seven donors was submitted to RT-PCR using primers FLcon63 and LRg1769, which amplify the entire codon of KIR2DL5. We and others (3, 4) have recently described KIR2DL5, a new member of this family whose ligand has yet to be identified. KIR2DL5 shares some structural features with KIR2DL4, a receptor for HLA-G (5, 6), that distinguish them from other KIR: a D0-D2 configuration of extracellular Ig-like domains and a particularly long cytoplasmic tail. The primary structures of KIR2DL4 and KIR2DL5 are 79% identical and differ in the number of immunoreceptor tyrosine inhibitory motifs: one in KIR2DL4, two in KIR2DL5, as well as in the presence (KIR2DL4) or absence (KIR2DL5) of a positively charged residue in the transmembrane region. Also, their distribution in the population and patterns of expression are different: whereas KIR2DL4 is present in all individuals and transcribed in all NK cells (5, 7, 8), KIR2DL5 is a common feature of B haplo-

Copyright © 2000 by The American Association of Immunologists 0022-1767/00/$02.00

Received for publication June 23, 2000. Accepted for publication September 8, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grant AI17892 from U.S. Public Health Service (to P.P.); C.V. was supported by a fellowship from the Instituto de Salud Carlos III (Madrid, Spain; FIS BAE 98/5105); C.M.G. was supported by a grant from the leukemia Research Foundation.

2 The sequences reported in this article have been submitted to GenBank under accession numbers AF217485 (KIR2DL5.1 gene and promoter), AF217486 (KIR2DL5.2 gene and promoter), AF217487 (KIR2DL5.3 cDNA), AF260137 (KIR2DL5.3 promoter region), and AF260138–AF260141 (KIR2DL5.4 promoter region and exons 1–4).

3 Current address: Servicio de Immunologia, H.U. Clinica Puerta de Hierro, San Martin de Porres 4, 28035 Madrid, Spain.

4 Address correspondence and reprint requests to Dr. Peter Parham, Departments of Structural Biology and Microbiology and Immunology, Stanford University School of Medicine, Sherman Fairchild Building, Stanford, CA 94305. E-mail address: peparha@leland.stanford.edu.

5 Abbreviations used in this paper: KIR, killer-cell Ig-like receptor; UTR, untranslated region; SSP, sequence-specific primer; AML1, acute myeloid leukemia gene 1; Ets-1, erythroblastosis virus oncogene homologue 1.
(BS, SK, WCS, WJ, and VH), and their nucleotide sequences were determined after molecular cloning. No amplification products were obtained from cDNA of donors RR and CC. Genomic DNA samples of donors RR2 and CC were amplified from genomic DNA of donors CC and VH by PCR with primers Fcon113 and Ra964 (3). The PCR products were cloned and the nucleotide sequences of exons 2, 3, and 4 were determined.

In all cloning experiments, PCR products were inserted into the pCR2.1-TOPO or pc4-TOPO plasmid vectors (Invitrogen, San Diego, CA). Plasmids were isolated from individual bacterial clones using the Qiagen Spin mininprep kit (Qiagen, Chatsworth, CA). Nucleotide sequences were determined using dye-labeled deoxy-terminators and either 373A or 377 automated DNA sequencers (PE Applied Biosystems, Foster City, CA).

**Genomic DNA cloning of KIR2DL5.1 and KIR2DL5.2**

To amplify the complete KIR2DL5.1 and KIR2DL5.2 genes of donor NV, long-range PCR was performed on his genomic DNA using three different primer combinations. First, we used the Fcon63 and LRg1769 primers, which recognize the 5’ and 3’ untranslated regions (UTR) of KIR2DL5. Clones corresponding to both KIR2DL5.1 and 2 were obtained from this PCR product, as assessed by partial nucleotide sequencing, which also revealed that these variants have a single nucleotide polymorphism in exon 1. Based on this polymorphism, we designed new forward primers recognizing exon 1 of KIR2DL5.1 and KIR2DL5.2: LFa80 (5’-GACGACCATGTACACATGCATGAC-3’), and LFc80 (5’-GACGACCATGTACACATGCATGACCTG-3’), respectively. LFa80 and LFc80 were combined with LRg1769 to amplify separately the two variants of KIR2DL5.2.

In all PCR experiments, 200 ng of DNA were amplified in 25 μl of high-salt PCR buffer containing 0.5 μl of TaqPlus Long-DNA polymerase (Stratagene, La Jolla, CA) and 10 pmol of each primer. The PCR profile was 5 min of denaturation at 95°C and 30 cycles of 40 s at 94°C, 50 s at 68°C, and 20 min at 72°C, followed by a 5-min extension period at 72°C. PCR products with lengths of 9 kb were obtained and they were cloned as above. One clone for each KIR2DL5 variant was selected among those derived from the LFc63/LRg1769 amplification, and two additional clones for each of the variants were selected from the variant-specific amplifications. Complete nucleotide sequences were determined for both DNA strands of all six clones.

**Molecular cloning of the promoters of four KIR2DL5 variants**

Two 2.5-μg samples of genomic DNA of donor NV were digested separately with the restriction endonucleases DraI and SacI according to the recommendations of the manufacturer (Genome Walker kit; Clontech, Palo Alto, CA). Digests (Dral-DNA and SacI-DNA, hereafter) were purified by extraction with phenol and chloroform, precipitated with ethanol and 3 M sodium acetate (pH 4.96), and resuspended in 20 μl of TE buffer. One-fifth of each of the fractions ligated to the Genome Walker adaptron during 16 h at 16°C. The ligation reaction was stopped by a 5-min incubation at 72°C and diluted with 9 vol of TE buffer. One-fifth of each sample was amplified with 5 pmol of each LF1450 and LRcon364 (LRcon364: 5’-TGCTGACCACTCAATGGGGAGCCTG-3’). One hundred nanograms of DNA was amplified with 5 pmol of each LFI1450 and LRcon364 in the following PCR conditions: 2 min denaturation at 95°C; 10 cycles of 2 s at 94°C, 30 s at 94°C, and 20 min at 70°C. Similarly, the promoter region of KIR2DL5 was isolated from genomic DNA of donors VH, BS, SK, RR, WJS, and CC using LFI1450 and LR302 primers in the same conditions.

**Sequence analysis**

Nucleotide sequences were aligned and compared using AutoAssembler (PE Applied Biosystems) and the Wisconsin Package Version 10.0 (Genetics Computer Group, Madison, WI). Analysis of potential regulatory elements in the promoter region of KIR2DL5 variants was performed using free text analyses of MatInspector Professional (Genomatix, http://genomatix.gsf.de/free services/) (9) and the TRANSFAC database (10).

**Results**

**Definition of four KIR2DL5 variants:** two that are expressed and two that are not

Previously, we identified two variants of KIR2DL5 in the genomic DNA of one individual, donor NV (3). Of the two variants, KIR2DL5.1 was transcribed in NK cells and T lymphocytes, whereas KIR2DL5.2 was not. To investigate variation in KIR2DL5 further, we analyzed genomic DNA of an additional seven donors using PCR-SSP and oligonucleotide primers that discriminate sequence polymorphisms in exons 3 and 4 of KIR2DL5.1 and KIR2DL5.2. Of the seven donors analyzed (Table I), one typed for both variants, one typed only for KIR2DL5.2, and five donors typed for just KIR2DL5.1. Subsequent analysis (see below) showed that three of the latter five donors actually have novel variants of KIR2DL5.

To investigate the expression of KIR2DL5 variants, RNA was made from the PBMC of each donor and subjected to RT-PCR using primers that amplify the entire coding region of KIR2DL5. Complementary DNA from five donors gave PCR products, and two (RR and CC) did not. The PCR products were cloned and their nucleotide sequences were determined. Three donors (VH, BS, and SK) gave cDNA clones identical in sequence to KIR2DL5.1. Clones corresponding to a novel form of KIR2DL5 were obtained from two related donors (WCS and WCI). This KIR2DL5.3 variant differs from KIR2DL5.1 by three nonsynonymous substitutions: one each in exons 1, 2, and 3 (Table II). At all three positions, KIR2DL5.3 shows identity with other KIR genes, whereas KIR2DL5.1 has a unique substitution. Although genomic DNA from donor VH typed positively for both KIR2DL5.1 and 2, only cDNA clones for KIR2DL5.1 were obtained.

| Table 1. Distribution and expression of four KIR2DL5 variants in eight donors |
|---|---|---|---|---|
| Donor | KIR2DL5.1 | KIR2DL5.2 | KIR2DL5.3 | KIR2DL5.4 |
| NV | + (+) | - | - | - |
| VH | + (+) | + (+) | - | - |
| RS | + (+) | - | - | - |
| WCI | - | - | + (+) | - |
| WCS | - | - | - | - |
| SK | - | - | - | - |
| SS | - | - | - | - |

* Symbols indicate the presence or absence of each KIR2DL5 variant in genomic DNA and mRNA (in parentheses) of each donor.

Since donor VH is the mother of donor CC, lack of inheritance of either VH’s variants KIR2DL5.1 or KIR2DL5.2 by donor CC suggests that these two genes are located on the same KIR haplotype. This possibility is further supported by the observation that KIR2DL5.1 and KIR2DL5.2 are placed downstream of different KIR genes (this article) and by genotype analysis of KIR2DL5 variants in two additional families (C. Vilches, unpublished observation).

Downloaded from http://www.jimmunol.org/ by guest on April 16, 2017
By performing PCR on VH genomic DNA, clones corresponding to a fragment containing exons 2-4 of KIR2DL5 were obtained and these were sequenced. Two types of clones were obtained with genomic fragment of (Table II). When the oligonucleotide primers that amplified the gene, the nucleotide sequences for all of the exons and introns of the KIR2DL5.1 and KIR2DL5.2 genes were determined and compared. Clones containing all of the exons were obtained from genomic DNA by a long-range PCR using primers that recognized the 5' and 3' UTR of KIR2DL5. For each variant, complete sequences were determined for both strands of three clones derived from two different PCR amplifications, a strategy designed to eliminate the effect of polymerase errors during PCR.

The KIR2DL5 gene contains eight exons that are organized similar to those of the KIR2DL4 gene (11). The KIR2DL5 exons extend over ~9.3 kb of DNA, which is about 1.5 kb shorter than for KIR2DL4, due mainly to differences in the lengths of introns 1, 4, and 5 (Table III). The overall organization of the KIR2DL5.1 and 2 genes is the same (Table III). In both variants, the exons make an open reading frame of 1128 nt and they are flanked by splicing sites that conform to the consensus motifs. Within the exons, there is a total of seven nucleotide substitutions, none of which are predicted to have an effect on gene expression (Tables II and III). Within the introns, KIR2DL5.1 and 2 differ by 32 nt substitutions.

Table II. Diversity in the sequence and expression of the KIR2DL5 gene

<table>
<thead>
<tr>
<th>KIR2DL5 Variant</th>
<th>mRNA Expression</th>
<th>Leader</th>
<th>D0 Domain</th>
<th>D2 Domain</th>
<th>3' UTR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GTC</td>
<td>ACA</td>
<td>GGT</td>
<td>ATA</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>Val</td>
<td>Thr</td>
<td>Gly</td>
<td>Ser</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td></td>
<td>GGA</td>
<td>CCG</td>
<td>GAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>syn</td>
<td>syn</td>
<td>TCG</td>
<td>syn</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>CCA</td>
<td>Pro</td>
<td>ACT</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
</tbody>
</table>

*The nucleotide sequence of KIR2DL5 variants in polymorphic positions of exons is shown in comparison to a KIR2DL5 consensus. Codons are numbered from the start codon; nucleotides in the 3' UTR are numbered from the last nucleotide after the stop codon. ND, not determined. Transcription or its lack is shown for each variant.

By contrast, a similar analysis of genomic clones from donor CC revealed a novel variant, KIR2DL5.4, which differs from KIR2DL5.1 by one nucleotide change in exon 1 and one in exon 3 (Table II). When the oligonucleotide primers that amplified the genomic fragment of KIR2DL5.4 were tested against cDNA from donor CC, no product was obtained. This result confirmed that KIR2DL5.4 is not expressed and ruled out the possibility that lack of amplification in the first RT-PCR experiments was due to poly-A.

Table III. Comparison of the structures of KIR2DL5.1, KIR2DL5.2, and KIR2DL4 genes

<table>
<thead>
<tr>
<th>Changes in KIR2DL5.2 Compared to KIR2DL5.1</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substitutions</td>
<td>Insertions/deletions</td>
</tr>
<tr>
<td>Exon 1</td>
<td>1</td>
</tr>
<tr>
<td>Intron 1</td>
<td>7</td>
</tr>
<tr>
<td>Exon 2</td>
<td>0</td>
</tr>
<tr>
<td>Intron 2</td>
<td>0</td>
</tr>
<tr>
<td>Exon 3</td>
<td>3</td>
</tr>
<tr>
<td>Intron 3</td>
<td>1</td>
</tr>
<tr>
<td>Exon 4</td>
<td>2</td>
</tr>
<tr>
<td>Intron 4</td>
<td>4</td>
</tr>
<tr>
<td>Exon 5</td>
<td>0</td>
</tr>
<tr>
<td>Intron 5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>4-bp del</td>
</tr>
<tr>
<td>Exon 6</td>
<td>0</td>
</tr>
<tr>
<td>Intron 6</td>
<td>0</td>
</tr>
<tr>
<td>Exon 7</td>
<td>0</td>
</tr>
<tr>
<td>Intron 7</td>
<td>0</td>
</tr>
<tr>
<td>Exon 8</td>
<td>1</td>
</tr>
<tr>
<td>Sum</td>
<td>39 (0.42%)</td>
</tr>
</tbody>
</table>

*The lengths of KIR2DL4 exons and introns were deduced from GenBank sequences with accession numbers AL133414 and AC011501; differences between the latter sequences are indicated.

* The lengths indicated for the first and last exons of KIR2DL4 and KIR2DL5 correspond to the DNA segments amplified by the PCR primers FLcon63 and LRg1769.

* ins Insertion; del, deletion.
Promoters of expressed KIR2DL5 variants contain an acute myeloid leukemia gene 1 (AML1) site that is mutated in the nontranscribed variants

As the exons and introns revealed no feature that could account for the nonexpression of KIR2DL5.2, we turned to compare the promoter regions of KIR2DL5 variants. Genomic DNA from donor NV, who has both KIR2DL5.1 and .2, was digested with either DraI or Scal restriction enzymes that cut neither KIR2DL5 variant in the DNA segment encompassing exons 1–4. An artificial adaptor sequence was ligated to the blunt-ended DNA fragments produced by the digest. Nested PCR was then performed using antisense primers specific for exon 3 of KIR2DL5 and primers targeted at the adaptor sequence. PCR products of ~2 and ~4 kb were obtained from the DraI- and Scal-digested DNA, respectively.

These fragments were cloned and sequenced. Clones corresponding to KIR2DL5.1 and .2 were obtained from the DraI digest. They contained ~500 bp of DNA that was derived from the region upstream of the putative start codon. In contrast, only clones corresponding to KIR2DL5.2 were obtained from the Scal digest. These clones contained 2793 bp of DNA from the region upstream of the putative start codon, of which the 5’-most 763 bp were identified as the 3’-most exon of the KIR2DL2 gene. Thus, the end of the KIR2DL2 gene and the start codon of KIR2DL5.2 are separated by only 2030 bp.

By taking advantage of the close juxtaposition of KIR genes (Ref. 4 and this article), we were able to isolate clones that encompassed the entire noncoding upstream region of the KIR2DL5.1 gene. PCR was performed using an antisense primer specific for exon 3 of KIR2DL5.1 in combination with a sense primer recognizing the last exon of all KIR. The product of this PCR contained exon 9 of KIR3DS1 at its 5’ end and exons 1–3 of KIR2DL5 at its 3’ end. Determination of the nucleotide sequence of this product showed that the region 5’ of the start codon is ~1.2 kb upstream of the KIR3DS1 promoter. The nucleotide sequence of a 1244–1299-bp segment upstream of the initiation codon was determined. This allowed the promoters of all four KIR2DL5 variants to be compared, as well as several independently isolated KIR2DL5.1 and .2 variants. In all isolates of KIR2DL5.1, we found KIR3DS1 to be the gene upstream of KIR2DL5.1, and in all isolates of KIR2DL5.2 and .4, we found the upstream gene to be KIR2DL2. The KIR gene sequence found upstream of KIR2DL5.3 was not a complete match with any of the known KIR genes, being closest to the 3’-most exon of KIR2DL2 (data not shown).

The promoter sequences were identical in the KIR2DL5.1 genes obtained from four donors (NV, VH, BS, and SK). Similarly, the KIR2DL5.2 promoter sequences from three donors (NV, RR, and VH) were identical and they were also identical to the KIR2DL5.4 promoter. A promoter of a different sequence was found in KIR2DL5.3, which differs from those of KIR2DL5.1 and .2 by 28 and 32 substitutions, respectively. It contains some unique substitutions, in addition to elements in common with each of the other two promoters (Fig. 1).

Comparison of the promoter sequences of the expressed and nonexpressed KIR2DL5 variants was made (Fig. 1). The 58-bp insertion, which was first found in KIR2DL5.2, is unlikely to be responsible for the lack of expression because this insertion is also present in the expressed variant KIR2DL5.3. More likely candidates are 11 changes that are specific to the nonexpressed variants.

A search for regulatory elements of transcription was performed in the promoters of KIR2DL5 variants, and several transcription factor binding sites that are present in the expressed KIR2DL5 variants are absent in the nonexpressed variants were found (Fig. 1). Of these, only AML1 is known to function in hematopoietic cells (12). In the T cell lineage, AML1 cooperates with the transcription factor erythroblastosis virus oncogene homologue 1 (Ets-1) in the activation of the TCR-β chain enhancer (13). Indeed, two potential binding sites for Ets-1 were found flanking the AML1-binding motif (14) present in positions ~95 to ~100 of KIR2DL5.1 and .3 and which is mutated from TGTGTT to TGATGT in the nontranscribed variants KIR2DL5.2 and .4 (Fig. 1).

FIGURE 1. Sequence variability in the KIR2DL5 gene promoter. The nucleotide sequence of a 1244–1299-bp segment upstream of the start codon (of which ~400 bp are shown) was determined for all four variants in seven donors. Within this ~1.2-kb region, KIR2DL5.1 and KIR2DL5.2/4 differ by 20 substitutions (1.6%); KIR2DL5.1 and KIR2DL5.3 by 28 substitutions (2.26%); and KIR2DL5.2/4 and KIR2DL5.3 by 32 substitutions (2.46%). Full stops indicate identity with KIR2DL5.1 and dashes, absence of nucleotides. Potential binding sites for Ets-1 and human transcription factors that are unique of expressed variants of KIR2DL5 (.1 and .3) are underlined and boldfaced. Sox-5, SRY-related HMG-box gene 5 (25); CDP-CR3, CCAAT displacement protein CR3 (26); WHN, winged-helix nuda (27); and AML1 (14). The first nucleotide of the longest cDNA obtained during 5’ rapid amplification of cDNA ends cloning of KIR2DL5.1 (3) is pointed out by an arrow.

KIR2DL5.1 caataactcgtcggaaacattaaataacatcatttagttcattacaccggagaaaggttgatgattotcagatagaaacAA      -119
KIR2DL5.2 & .4    aaaaaaaaaaacaatcgtaggacttttcgatctctgtaag -342
KIR2DL5.3      aaaaaaaaaaacaatcgtaggacttttctgtaagctctgtaag -342

(continued) -300

KIR2DL5.1     aaaaaaaaaaacaatcgtaggacttttcgatctctgtaag -222
KIR2DL5.2 & .4    aaaaaaaaaaacaatcgtaggactttttctgtaagctctgtaag -222
KIR2DL5.3      aaaaaaaaaaacaatcgtaggacttttctgtaagctctgtaag -222

WHN           -200   Ets-1

KIR2DL5.2  agagcaagctgtctctctgcatggaaacacactggcttcagtttaagacgtgtgattotcagatagaaacAA      -122
KIR2DL5.3      agagcaagctgtctctctgcatggaaacacactggcttcagtttaagacgtgtgattotcagatagaaacAA      -122

AML1         -10   Met

KIR2DL5.1  agagcaagctgtctctctgcatggaaacacactggcttcagtttaagacgtgtgattotcagatagaaacAA      19
KIR2DL5.2 & .4    agagcaagctgtctctctgcatggaaacacactggcttcagtttaagacgtgtgattotcagatagaaacAA      19
KIR2DL5.3      agagcaagctgtctctctgcatggaaacacactggcttcagtttaagacgtgtgattotcagatagaaacAA      19

FIGURE 1. Sequence variability in the KIR2DL5 gene promoter. The nucleotide sequence of a 1244–1299-bp segment upstream of the start codon (of which ~400 bp are shown) was determined for all four variants in seven donors. Within this ~1.2-kb region, KIR2DL5.1 and KIR2DL5.2/4 differ by 20 substitutions (1.6%); KIR2DL5.1 and KIR2DL5.3 by 28 substitutions (2.26%); and KIR2DL5.2/4 and KIR2DL5.3 by 32 substitutions (2.46%). Full stops indicate identity with KIR2DL5.1 and dashes, absence of nucleotides. Potential binding sites for Ets-1 and human transcription factors that are unique of expressed variants of KIR2DL5 (.1 and .3) are underlined and boldfaced. Sox-5, SRY-related HMG-box gene 5 (25); CDP-CR3, CCAAT displacement protein CR3 (26); WHN, winged-helix nuda (27); and AML1 (14). The first nucleotide of the longest cDNA obtained during 5’ rapid amplification of cDNA ends cloning of KIR2DL5.1 (3) is pointed out by an arrow.
Common to all four KIR2DL5 variants are binding sites for transcription factors that are expressed ubiquitously in all cells or in hematopoietic lineage cells (15–17): AP-1, Sp1, Oct-1, GATA, NFAT-1, Ikaros, STATs, Ets-1, and AML1 (data not shown). No TATA box was found in the vicinity of the transcription start site, as assessed by rapid amplification of cDNA ends in KIR2DL5.1 (3). Absence of a TATA box is associated with variability in the site in which transcription is initiated, as has been recently shown for the murine NK cell receptor 2B4 (18).

Discussion

Previous identification of two variants of a novel KIR gene (KIR2DL5.1 and .2) in genomic DNA of a single individual was followed by the unexpected finding that only the first variant (KIR2DL5.1) was transcribed (3). Absence of mRNA from the KIR2DL5.2 variant could be due to unknown structural defects or to specific silencing of the gene in this donor. Regarding the latter possibility, it was of interest that paternally imprinted mononucleic expression had been described for PIR-A and –B, murine genes that are structurally related to KIR and located in a syntenic chromosomal region (19). To distinguish between lack of expression due to structural defect or to gene regulation, we studied the structure, variation, and expression of KIR2DL5 genes in a panel of eight donors who represent the major ethnic groups.

The expression of each KIR2DL5 variant, or its lack, are consistently reproduced in different individuals (Table II). This argues against the possibility of KIR2DL5.2 nonexpression being determined by some sort of regulation that could vary in different donors. In particular, lack of KIR2DL5.2 mRNA in one donor (RR) in whom this variant was found in the absence of KIR2DL5.1 excluded mononucleic expression as the cause of KIR2DL5.2 being a silent gene. Two additional variants of the gene, one expressed (KIR2DL5.3) and one nonexpressed (KIR2DL5.4), were found in this study. Altogether, the results of this survey are consistent with expression or its lack being constitutive features of certain variants of the KIR2DL5 gene.

Genomic DNA cloning of KIR2DL5.1 and KIR2DL5.2 after long-range PCR provided the complete exon and intron sequences of both KIR gene variants. However, we found no structural abnormalities in KIR2DL5.2 that might explain its lack of expression or suggest that KIR2DL5.2 is a pseudogene, all its differences from KIR2DL5.1 being minor in nature. In contrast, analysis of the KIR2DL5 promoter revealed a high level of sequence polymorphism that is likely relevant for expression. Whereas the promoters of each of the expressed variants have distinctive sequences, the two nontranscribed versions of the gene have the identical promoter. The identity of KIR2DL5.2 and .4 promoter sequences contrasts with the coding regions of these variants being among the most divergent within the KIR2DL5 group (Table II) and supports their lack of expression being determined by shared features in their promoters.

One of the changes that distinguish the promoters of expressed and nonexpressed KIR2DL5 variants takes place in a putative binding site for the transcription factor AML1, also known as CBP or PEBP2 (14). AML1 controls the expression of several genes that are essential for the development and function of hematopoietic cells, including those encoding Ag receptors of T and B lymphocytes and granzyme B (12, 20, 21). This protease is present in the lytic granules of NK cells (22), an indication of AML1 activity in these cells. Also, cooperation of AML1 with Ets-1 (13, 23) and the presence of potential binding sites for the latter transcription factor in the proximal promoter of KIR2DL5 make it likely that AML1 and Ets-1 participate in the regulation of KIR2DL5 expression. The observed mutation of an AML1 binding site in the promoters of KIR2DL5.2 and KIR2DL5.4 might thus be responsible for their lack of transcription, an issue that will be illuminated by functional analysis of KIR2DL5 promoters. However, we cannot exclude that expression of KIR2DL5 could be affected by unknown polymorphisms at more distant regulatory elements.

Comparing the sequences of KIR2DL5 variants also reveals aspects that are intriguing from the evolutionary aspect. Because KIR2DL5.2 is not expressed, non-sense mutations and other aberrations could have accumulated in this gene due to the lack of selective pressure. However, no such abnormalities were found, which indicates a recent inactivation of KIR2DL5.2. In addition, the similarity in the coding regions and introns of KIR2DL5 variants (0.42% differences, Table III) is consistent with their recent separation from a common ancestor. In contrast, the promoters of expressed and nonexpressed variants differ by 1.61–2.46% (Fig. 1). This 3.8- to 5.8-fold higher number of differences could mean that the promoters of the different variants have accumulated mutations at a higher rate than structural parts of the gene. Alternatively, stretches of new sequence motifs could have been introduced in their promoters by recombination with other KIR genes. In the case of KIR2DL5.1, its location downstream of a KIR gene different from other KIR2DL5 variants indicates that recombination indeed affected its upstream region at some moment of evolution.

Before the description of KIR2DL5 and KIRC1/KIR44 (3, 24), all KIR genes had been shown to be expressed in every individual in whom they are present (8). Variability in the expression of KIR2DL5 is an exception to this rule and adds to the variation in the number and combination of KIR genes present in the genome of different humans (8). Also, nonexpression of structurally normal KIR2DL5 variants parallels the lack of transcription of KIRC1/KIR44, a gene that also contains an open reading frame similar to that of other KIR (24). Since mRNA of KIRC1/KIR44 has only been sought in seven individuals (3, 24), the variation observed in transcription of KIR2DL5 prompts further analysis of the expression of KIRC1/KIR44 in a wider population sample.

KIR2DL5 is the first KIR gene whose promoter has been structurally characterized. Comparison of its sequence with those of other KIR genes should shed light on the three types of KIR gene expression observed in NK cells: clonal distribution of certain KIR2DL5 variants and most KIR genes (3, 7), lack of transcription of other KIR2DL5 variants and KIRC1/KIR44 (3, 24), and constitutive transcription of KIR2DL4 (5, 7). In addition, our study opens a way to the functional analysis of the KIR2DL5 promoter, which should illustrate how the expression of its variants is controlled. Since KIR can modulate the function of NK cells and subsets of T lymphocytes, the issue of how KIR gene expression is regulated is relevant for better understanding of both natural and adaptive immunity and tolerance.

Acknowledgments

We thank Dr. Yumiko Watanabe and Benny Shum for helpful suggestions.

References


