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African Americans Exhibit a Predominant Allele in the Midst of Extensive *KIR2DL1* Allelic Diversity

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Abstract

KIR2DL1 alleles were identified by DNA sequencing of the coding region from amplified genomic DNA from 100 random African Americans. The majority of individuals (97%) carried a *KIR2DL1* locus. Allele *KIR2DL1*00302* was found in 68% of individuals but *KIR2DL1*00401*, **002*, *00303, **006*, and **007* were also frequent. Eleven new alleles were described: *KIR2DL1*00403, *01101,*01102 *012, *013N, *014, *015, *016, *017,*018,* and **019*. Nine of the novel alleles encoded amino acid substitutions located throughout the receptor; one allele carried a stop codon in the exon encoding the first extracellular domain.

Introduction

The natural killer (NK) cell receptor KIR2DL1 is a cell surface inhibitory receptor that recognizes a subset of HLA-C allelic products carrying Lys80 (1,2). Recognition of its ligand results in signals that prevent the NK cell from responding to normal cells. Loss of its HLA ligand on virally infected or malignant cells removes the inhibitory signal and may result in cytotoxicity or in cytokine production (3). The gene encoding KIR2DL1 is located in a cluster of similar genes in the Leukocyte Receptor Complex (4) although some *KIR* haplotypes lack the *KIR2DL1* gene entirely (5). Fifteen alleles encoding ten variant receptors have been identified for *KIR2DL1* (6,7). Studies of a similar receptor, KIR3DL1, have demonstrated that allelic variants may differ in their level of expression at the cell surface and ligand binding affinity (8). The purpose of this study was to evaluate *KIR2DL1* allelic diversity in a population of random African American individuals.

Materials and Methods

Genomic DNA was isolated from 100 unique and unrelated African Americans from the human variation panel obtained 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). Testing for the presence or absence of *KIR2DL1* used polymerase chain reaction (PCR) primers and reaction conditions previously described (9,10,11). A primer pair, described by Gomez-Lozano et al., specific for *KIR2DS1* and *KIR2DL1 *004*, **007*, and **010* was a useful control (12). For sequencing of exons 1 through 9, the strategy based on PCR amplification of genomic DNA followed by DNA sequence analysis has been described (13) with the following exception: The antisense PCR primer used to obtain the amplicon including exon 4 through exon 9 was 2DL1-E89R (TGTGAGGAAGATCGATGCCCTAAG). The latter, described by Murdoch et al (14), was

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useful to identify a 3'untranslated region variation distinguishing *KIR2DL1*0040101* from **0040102*. Allele assignments were obtained by comparison with a *KIR2DL1* cDNA reference library from ImmunoPolymorphismDatabase (IPD)-KIR Release 2.1.0 (6). In this report, the numbering of nucleotides and codons is based on IPD-KIR unless noted. Novel alleles were isolated using allele specific amplification, cloning and/or by using haplotype specific extraction (2DL1-435G; HaploPrep, Qiagen, Valencia, CA). Sequences were submitted to GenBank and novel allele designations were assigned by the *KIR* subcommittee of the World Health Organization Nomenclature Committee for Factors of the HLA System (7). Confirmatory sequences of previously described alleles were submitted to the IPD-KIR database and include: *KIR2DL1*00303* (Cell GM17132; GenBank GQ406045), *KIR2DL1*0040102* (GM17116; GQ844297); *KIR2DL1*006* (GM17177; GQ406047), *KIR2DL1*007* (GM17178; GQ406043), and *KIR2DL1*010* (GM17108, GQ406044). The first four alleles had been previously identified in AfroCaribbean or African American individuals (15,13); the last allele in an individual of European ancestry (14). A phylogenetic tree of *KIR2DL1* was constructed using a maximum likelihood technique with a molecular clock (DNAMLK version 3.66). Since the 5' sequence is unknown for some alleles, the tree was based on the nucleotide sequence from codon -17 through codon 327 (16).

HLA allele identification

To identify the *HLA-A,-B,*-C alleles carried by each individual, PCR primers were used to amplify each locus as previously described (17). Applied Biosystems Big Dye terminator chemistry and an Applied Biosystems Models 3730xl DNA analyzer (PE Applied Biosystems, Foster City, CA) were used to obtain the sequences of both strands of exons 2 and 3. IMGT/HLA database 2.21.0 was used for the interpretation of sequencing results. *DRB1* alleles were amplified and identified using the One Lambda LABType® SSO HD Kit (version RSSOH2B1_002, One Lambda, Canoga Park, California) following manufacturer's protocols. Alleles identical in exons 2 and 3 (class I) or exon 2 (*DRB1*) were not resolved. For those class I samples yielding alternative genotypes, either allele specific sequencing primers, allele specific PCR amplification, or HaploPrep (Qiagen, Valencia, CA) was used to link polymorphisms and to identify the specific allele combination. [In-house primer sequences used for all loci are available at www.dodmarrow.org.] Supplemental Table 1 provides the HLA data.

Results and Discussion

Three individuals (3%) showed a complete absence of the *KIR2DL1*; another study observed 2% of African Americans negative for this locus (18). Forty two individuals carried a single *KIR2DL1* coding sequence and 55 were heterozygous. Of the 13 previously described alleles that differ within the coding region of the gene, eight were observed (Table 1). *KIR2DL1*00302* was the most common allele being found in 68 individuals. Two other frequent alleles included *KIR2DL1*00401* (23 individuals) and *KIR2DL1*002* (13 individuals),. These alleles are the ones most frequently observed in studies of other populations (e.g., Japanese (8), Irish (19)). The G to A transition in the 3' untranslated region of *KIR2DL1*00401* at nucleotide 34 following the stop codon which distinguishes *KIR2DL1*0040101* from **0040102* was observed in this population. We identified the G variant (*KIR2DL1*0040101*) in 14 individuals and the A variant (*KIR2DL1*0040102*) in nine individuals; one individual carried both alleles. The A variant was initially observed in an Afro-Carribean population (15). Other alleles observed in multiple individuals, *KIR2DL1*006* (11 individuals), *KIR2DL1*00303* (8 individuals) and *KIR2DL1*007* (7 individuals), have been previously observed only in populations of African origin (15,19,13).

Eleven novel alleles were characterized. Four of the novel alleles were found in multiple individuals: *KIR2DL1*01102* (2 individuals), *KIR2DL1*012* (5 individuals), *KIR2DL1*014* (3 individuals), and *KIR2DL1*019* (2 individuals). One allele carried a synonymous substitution of the common allele *KIR2DL1*00401* creating *KIR2DL1*00403*. Five novel alleles, *KIR2DL1*01101, KIR2DL1*01102, KIR2DL1*012, KIR2DL1*013N*, and *KIR2DL1*019* (codon 114), carried substitutions that were found in other alleles at the locus. For example, *KIR2DL1*012* carried a substitution altering the first domain at codon 16 altering a proline to an arginine. Twelve of the 15 *KIR2DL1* alleles encode arginine; the other three encode proline at this position. Five novel alleles introduced polymorphism at codons which were previously conserved in all or the majority of alleles altering the amino acid: *KIR2DL1*014, KIR2DL1*015, KIR2DL1*016, KIR2DL1*017, KIR2DL1*018, KIR2DL1*019* (codon 226). Four novel alleles, *KIR2DL1*01101*, *KIR2DL1*01102, KIR2DL1*013N*, and *KIR2DL1*019*, exhibited two substitutions. *KIR2DL1*013N* is interesting because the second substitution in the exon encoding the first extracellular domain creates a termination codon. Other truncated alleles previously described carry termination codons in the exon encoding the second extracellular domain: *KIR2DS3*003N* (substitution), *KIR3DL1*024N* (deletion of nucleotide), and *KIR3DS1*049N* (deletion of nucleotide) (20,21).

The majority of the new alleles described carried nonsynonymous substitutions. The substitutions altered the amino acid sequence of the signal peptide (altered in 2 alleles), domain 1 (1 allele), domain 2 (2 alleles), transmembrane region (2 alleles) and cytoplasmic tail (3 alleles). Only one of the substitutions in the two external domains of KIR introduced a novel amino acid; the other two are observed in other alleles. Based on the crystal structure, none of the extracellular variations appear to impact residues that contact the HLA-C/peptide complex (2). In the cytoplasmic tail, *KIR2DL1*018* (CCA/Pro) has a substitution at codon 282 that was conserved in most alleles (ACA/Thr). This residue falls within the membrane proximal immunoreceptor inhibitory motif (ITIM) motif, adjacent to the tyrosine which is phosphorylated during signaling (22,23). While there is some latitude in amino acids at this position in ITIM motifs, the substitution of a cyclic amino acid (Pro) with properties that affect secondary protein stucture may impact signaling for this form of the receptor.

Figure 1 shows a phylogenetic tree showing the relationships among all of the reported *KIR2DL1* alleles. There are two major clusters of alleles, each containing frequent alleles in the African American population. The novel alleles found in African Americans are found in both of these clusters. The number of *KIR2DL1* alleles observed in this population of 100 individuals is remarkable; 19 alleles with 68% of individuals carrying *KIR2DL1*00302*. In contrast, studies of European American (n=75) or Northern Irish (n=140) populations observed 7 alleles with *KIR2DL1*00302* being found in 63–66% of individuals (13,19).

Identification of HLA-C alleles in the 97 *KIR2DL1* positive African Americans showed that 25 individuals (26%) did not carry an HLA-C allele that would serve as a ligand for KIR2DL1, that is, an HLA-C allelic product with Lys80 (Group 2). In these individuals without the cognate ligand, NK cells expressing, as their only inhibitory KIR receptor, KIR2DL1, would not be “licensed” and are likely to be hyporeactive (24).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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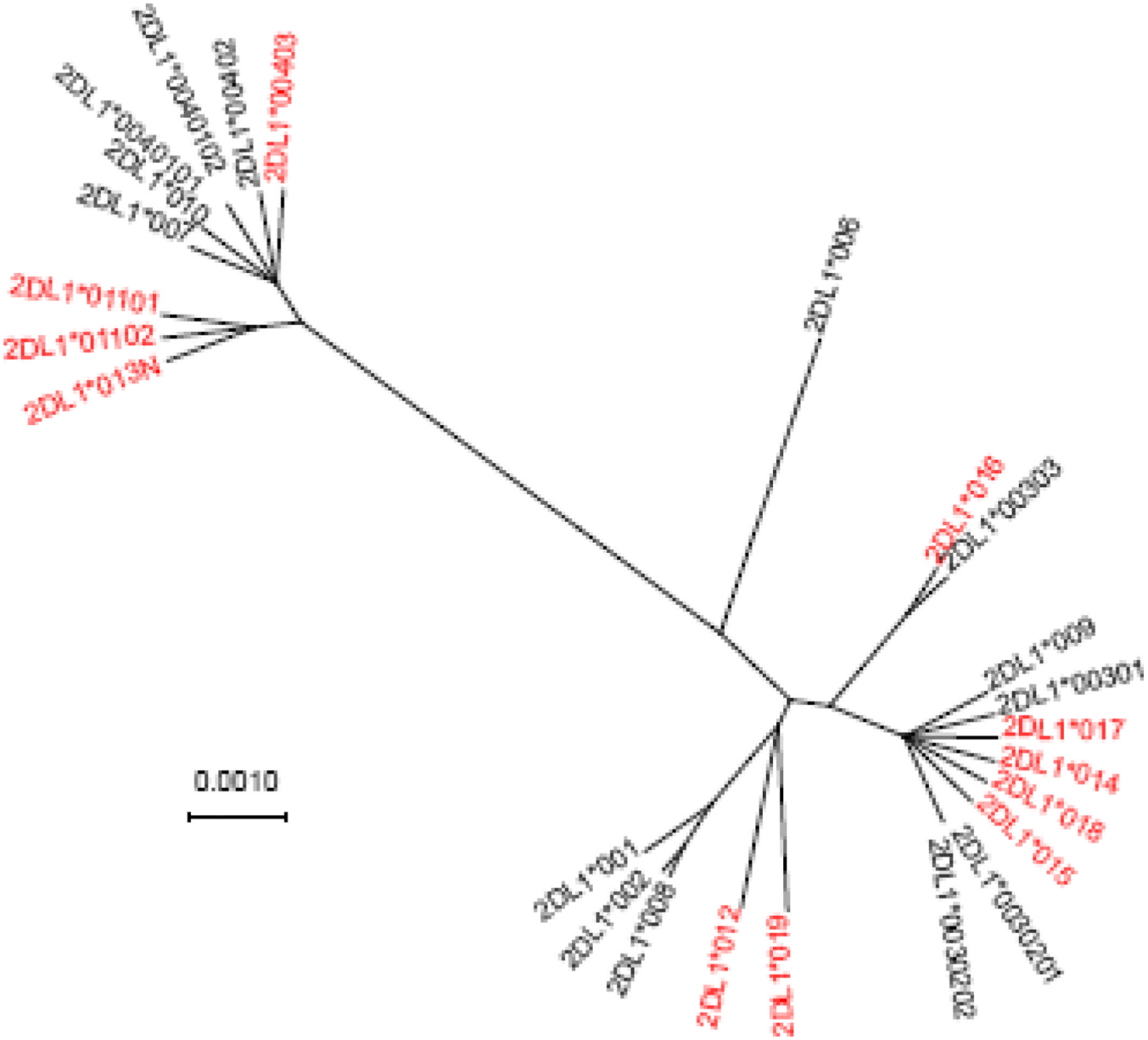

Figure 1.
Phylogenetic tree of *KIR2DL1* alleles. Alleles shown in red were described in this study. Previously described *KIR2DL1*005* was not included because it is missing a portion of the nucleotide sequence encoding the signal peptide.

Table 1

Frequency of *KIR2DL1* Alleles in an African American Panel (n=100)

***KIR2DL1* Allele**	**Number Of Individuals Positive (%)**	***KIR2DL1* Allele**	**Number Of Individuals Positive (%)**
*001	2 (2)	*008	0
*002	13 (13)	*009	0
*00301	0	*010	1 (1)
*00302[a]	68 (68)	*01101[b]	1 (1)
*00303	8 (8)	*01102[b]	2 (2)
*0040101	14 (14)	*012[b]	5 (5)
*0040102	9 (9)	*013N[b]	1 (1)
*00402	0	*014[b]	3 (3)
*00403[b]	1 (1)	*015[b]	1 (1)
*005	0	*016[b]	1 (1)
*006	11 (11)	*017[b]	1 (1)
*007	7 (7)	*018 [b]	1 (1)
		*019[b]	2 (2)

[a] *KIR2DL1*0030201* and *KIR2DL1*0030202* were not individually distinguished in this study.

[b] Novel allele described in this study.

Table 2

Novel *KIR2DL1* alleles observed in African Americans

***KIR2DL1* Novel Allele**	**Most Similar Allele**	**Alteration Nucleotide Codon (Amino Acid)[a]**	**Protein Region Altered**	**GenBank Accession Number**	**Cell Line**
*2DL1*00403*	*2DL1*00401*	254 GCG (A) => GCT (A)	--	EU930369	GM17109
*2DL1*01101*	*2DL1*00401*	-17 GTC (V) => TTC (F), 221 CGA (R) => AGA (R)	Signal peptide	EU930368	GM17159
*2DL1*01102*	*2DL1*00401*	-17 GTC (V) => TTC (F), 186 TAC (Y) => TAT (Y)	Signal peptide	FJ655851	GM17200 [b]
*2DL1*012*	*2DL1*001*	16 CCC (P) => CGC (R)	Domain 1	EU930370	GM17119[b]
*2DL1*013N*	*2DL1*00401*	-17 GTC (V) => TTC (F), 35 GAA (E) => TAA (Stop)	Truncated	EU930371	GM17120
*2DL1*014*	*2DL1*00302*	179 GGC (G) => AGC (S)	Domain 2	EU930372	GM17123 [b]
*2DL1*015*	*2DL1*00302*	275 GAC (D) => GAA (E)	Cytoplasmic	EU930373	GM17124
*2DL1*016*	*2DL1*00303*	296 CGC (R) => TGC (C)	Cytoplasmic	FJ655852	GM17126
*2DL1*017*	*2DL1*00302*	221 CGA (R) => CAA (Q)	Transmembrane	FJ655853	GM17131
*2DL1*018*	*2DL1*00302*	282 ACA (T) => CCA (P)	Cytoplasmic	FJ655854	GM17153
*2DL1*019*	*2DL1*00303*	114 CTG (L) => CCG (P), 226 CTG (L) => GTG (V)	Domain 2 Transmembrane	FJ655855	GM17160 [b]

[a] Most similar known allele => novel allele

[b] This allele was found in more than one individual: *KIR2DL1*01102*, GM17198; *KIR2DL1*012*, GM17141, GM17142, GM17166, GM17185; *KIR2DL1*014*, GM17140, GM17174; *KIR2LD1*019*, GM17102.