Published in final edited form as:
*Tissue Antigens*. 2010 July ; 76(1): 64–66. doi:10.1111/j.1399-0039.2010.01468.x.

# The profile of KIR3DL1 and KIR3DS1 alleles in an African American population resembles that found in African populations

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## Abstract

KIR3DL1 and KIR3DS1 allele frequencies were determined by DNA sequencing of the complete coding regions from 100 random unrelated African Americans. Alleles 3DL1 *01501 (29 individuals) and 3DL1*01502 (23 individuals) were most frequently observed in addition to 21 other known alleles and seven new alleles: KIR3DL1*01503, *03102, *064, *065, *066, *067, and *068. Two of the new alleles (KIR3DL1*064, *065) created additional variants of the chimeric KIR3DL1/KIR3DL2 gene. KIR3DS1*01301 (94% of the gene positive individuals) and KIR3DS1*049N (11%) were identified in the 18 individuals carrying this gene. Three individuals appeared to carry a KIR haplotype with a KIR3DL1/S1 duplication. The profile of alleles resembles that found in African populations but also shows signs of admixture.

Killer cell immunoglobulin-like receptors (KIRs) are expressed mainly on natural killer (NK) cells (1). Signaling through these and other receptors on NK cells determine the cell's activation state. Fifty two alleles have been identified at the inhibitory KIR3DL1 locus (2). Also considered alleles at this locus are the fourteen alleles of KIR3DS1, a chimeric gene resulting from a fusion between KIR3DL1 and a stimulatory KIR locus (3). Still other KIR3DL1 alleles have arisen by fusion of KIR3DL1 with KIR3DL2 (4). Allelic variation has been shown to alter the level of KIR3DL1 surface expression and the affinity of KIR3DL1 for its ligand, HLA-Bw4 (5). The goal of this study was to investigate KIR3DL1 and KIR3DS1 allelic diversity in African Americans by DNA sequencing of the entire coding regions of these genes.

The majority (81%) of 100 random African Americans were negative for KIR3DS1 and positive for KIR3DL1. Eighteen individuals (18%) were positive for both KIR3DS1 and KIR3DL1; one individual (1%) was negative for both. No individuals carried KIR3DS1 alone. The frequency of KIR3DS1 positive individuals (18%) is similar to the frequency observed in other African American populations (8-16%) and reflects the low frequency observed in Sub-Saharan Africa (3,6). KIR3DL1 is found at a similar high frequency in African American (99%) and other populations.

Twenty three alleles out of 52 known KIR3DL1 alleles were observed (Table 1). KIR3DL1*01501 and KIR3DL1*01502 were the most frequent alleles being observed in 29 and 23 individuals, respectively. These and other common alleles, KIR3DL1*00101, *00401, *00501, *007, *01701, *020, *022, and *031 were also frequently found in African populations (3). Several of the less frequent alleles, KIR3DL1*002, *00402, *008, *019,

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*029 have been found in other African American but not African populations (3) suggesting that they might have been contributed by admixture with European or Native American populations.

The KIR3DL1/KIR3DL2 hybrid is found in 5% of this African American population. Norman et al. observed this hybrid in sub-Saharan Africans and related populations at frequencies of up to 6.5%. Three alleles have been described for KIR3DL1/KIR3DL2 but only KIR3DL1*059 was observed in our study being identified in three individuals. Two individuals carried new variants of KIR3DL1*059. GM17119 carried an allele, KIR3DL1*065, which altered codon 54 in the amino-terminal extracellular domain D0 from ile to leu. Novel allele, KIR3DL1*064 from GM17177 carried a substitution at codon 150, altering ile to asn in the second extracellular domain D1.

Five other new alleles of KIR3DL1 were also identified; all altered frequent alleles found in this population. Two individuals (GM17128, GM17197) carried KIR3DL1*067 in which codon 350 in the cytoplasmic region was altered from ala to val in KIR3DL1*00101. Ser was altered to thr at codon 62 in the first extracellular domain D0 of KIR3DL1*01701 forming KIR3DL1*066 in two individuals (GM17124, GM17191). KIR3DL1*068 (GM17166) has one silent (codon 377) and two nonsynonymous codon changes from KIR3DL1*007. The substitution at codon 378 (ala to thr) falls within the second ITIM (7) but does not alter the consensus motif of (I/V)xYxx(L/V). Codon 381 is altered from asp to asn. Two alleles had silent substitutions: variation in codons 171 and 86, respectively, created alleles KIR3DL1*01503 (GM17109) and KIR3DL1*03102 (GM17170).

Two KIR3DS1 alleles out of the 14 known alleles were detected in African Americans, KIR3DS1*01301 (17 individuals) and KIR3DS1*049N (2 individuals). The high frequency of KIR3DS1*01301 among the KIR3DS1 alleles has been observed in other populations (3). Luo et al. first described 3DL1*049N in European Americans with a frequency of 2% (8) and it has not been previously observed in populations of African origin (3). This again may reflect admixture.

GM17102 and GM17130 carried two KIR3DL1 alleles (KIR3DL1*00101,*007 and KIR3DL1*01501,*01502, respectively) and one 3DS1 allele (KIR3DS1*01301) suggesting that they carried a previously described haplotype carrying both KIR3DL1 and KIR3DS1 (9,4). Cell GM17151 carried two KIR3DS1 alleles (KIR3DS1*01301, KIR3DS1*049N) and one KIR3DL1 allele (KIR3DL1*00101). Based on an analysis of these and other loci in the samples, it is likely that these are the two frequent haplotypes found in Hispanic and European populations as described by Norman et al. (4), again suggesting the contribution from admixture.

In the random African Americans in this study, HLA-B molecules carrying a Bw4 epitope, a KIR3DL1 ligand (10), were found in 75% of individuals (Hou et al., submitted). Fifty three percent of these individuals carried, as their only HLA-Bw4, alleles encoding ile at codon 80; 30% carried only thr at codon 80; and 8% carried both variants. In African populations, the frequencies of HLA-Bw4 (65-80%) and Bw4-80Ile (46-77%) (6) are similar to that observed for this study. Of the 99 individuals carrying KIR3DL1 alleles, 74 also expressed HLA-Bw4. Of the remaining 25 KIR3DL1 positive individuals without HLA-Bw4, six carried an HLA-A allele encoding a molecule bearing a Bw4 epitope thought to potentially bind KIR3DL1 (11,12).

A comparison of the allele profile from a random panel of 100 African Americans showed extensive similarity to KIR3DL1 alleles identified in African populations. Less frequent KIR3DL1 alleles in our population were observed in other studies of African Americans and in European and Native American populations suggesting that they were introduced by

admixture. Alleles resulting from apparent unequal crossing over events were also observed for KIR3DL1 including new variants of the KIR3DL1/KIR3DL2 fusion gene previously found in populations of African origin. KIR3DS1 was more homogeneous with the common KIR3DS1*01301 allele and, again, potential admixture suggested by the presence of a null allele previously observed in an individual with European ancestry and by the presence of haplotypes carrying both KIR3DL1 and KIR3DS1 previously found in European populations. Further diversity in the form of several new KIR3DL1 alleles encoding amino acid substitutions increase the diversity of this locus. The diversity of KIR3DL1 in this population and the presence of its ligand, HLA-Bw4, coupled with an understanding of the impact of allelic diversity on KIR function will aid in the understanding differences in immune responsiveness among individuals.

## Acknowledgments

This research is supported by funding from the Office of Naval Research N00014-07-41-1108. The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, the Department of Defense, or the U.S. government.

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## Table 1

Frequency on KIR3DL1 and KIR3DS1 alleles[a] in unrelated African American individuals (n=100)

| KIR3DL1 | No. of individuals (= % of individuals) | KIR3DL1 | No. of individuals (= % of individuals) | KIR3DS1 | No. of individuals (= % of individuals) |
|---|---|---|---|---|---|
| 3DL1*00101 | 17 | 3DL1*022 | 6 | 3DS1*01301 | 17 |
| 3DL1*002 | 3 | 3DL1*023 | 1 | 3DS1*049N | 2 |
| 3DL1*00401 | 14 | 3DL1*025 | 2 | 3DS1*058 | 0 |
| 3DL1*00402 | 3 | 3DL1*028 | 1 | Absent | 82 |
| 3DL1*00501 | 11 | 3DL1*029 | 1 | | |
| 3DL1*007 | 8 | 3DL1*031 | 10 | | |
| 3DL1*008 | 3 | 3DL1*033 | 2 | | |
| 3DL1*01501 | 29 | 3DL1*039 | 1 | | |
| 3DL1*01502 | 23 | 3DL1*041 | 2 | | |
| 3DL1*016 | 1 | 3DL1*059 | 3 | | |
| 3DL1*01701 | 10 | New alleles[b] | 9 | | |
| 3DL1*019 | 2 | Absent | 1 | | |
| 3DL1*020 | 5 | | | | |

[a]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). Sequence specific priming followed by gel electrophoresis was used to test for the presence or absence of KIR3DL1 and KIR3DS1 using polymerase chain reaction (PCR) primers previously described (13). Five sets of PCR primers were used to generate overlapping amplicons containing all alleles of the KIR3DL1 locus except the KIR3DL1/KIR3DL2 alleles (KIR3DL1*059,*060,*061). DNA were amplified as previously reported (14) with the following exceptions. Exons 8 and 9 were amplified using primers described by Sun et al. (15). An amplicon including exon 4 through exon 9 of the KIR3DL1/KIR3DL2 alleles was amplified by primers 3DL1 SSPF (16) and 3DL2 Exon9R (GGCTGTTGTCTCCCTAGAAA)(17). Together the KIR3DL1 amplicons included exon 1 through the 3'untranslated region (UTR) covering a region of approximately 14,700 base pairs. Two primers pairs were used to amplify KIR3DS1 including exon 1 to the 3'UTR as previously described (14). For some amplicons, the Roche Expand High Fidelity PCR System (Roche Applied Science, Indianapolis, IN) was used to improve PCR product yield and amplicon fidelity according to the manufacturer's protocol. Amplifications were carried out in a 2720 model thermal cycler (Applied Biosystems, Foster City, CA). The initial DNA denaturation was performed with one cycle of 92°C for two minutes. The PCR amplification included 10 cycles of 92°C for 10 sec; 60°C for 30 sec; 68°C for 11 min followed by 45 cycles of 92°C for 15 sec; 57°C for 30 sec; 68°C for 11 min (increasing the 11 min extension time by 20 sec each cycle). A final extension was carried out for 7 min at 68°C. DNA sequencing used primers and methods already described (14) with the addition of three KIR3DL1 primers TGGAGCACCCTAGTCTCACC (annealing in intron 2, antisense) ATTCAGGAGGTGGGACAGTG (intron 3, antisense), and ACCCTCACTCATTCCAGGTG (intron 4, sense). Sequences were compared to the IPD-KIR version 2.1.0 database (February 2009) to determine allelic assignments.

[b]This includes seven novel alleles, five identified in single individuals and two identified in two individuals each. Novel alleles were isolated using allele specific amplification, cloning (TOPO-XL, Invitrogen, Carlsbad, CA) and/or by using haplotype specific extraction (3DL1-199G, 3DL1-208A,3DL1-229T; HaploPrep, Qiagen, Valencia, CA). KIR allele designations for novel alleles were assigned by the WHO Nomenclature Committee for Factors of the HLA System (18).