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In Contrast to Other Stimulatory Natural Killer Cell Immunoglobulin-Like Receptor Loci, Several KIR2DS5 Alleles Predominate in African Americans

LiHua Hou, **Minghua Chen**, **Bo Jiang**, **Kanthi Kariyawasam**, **Jennifer Ng**, and **Carolyn Katovich Hurley**
Departments of Pediatrics and Oncology, CW Bill Young Marrow Donor Recruitment and Research Program, Georgetown University Medical Center, Washington, DC

Abstract

The five two domain stimulatory KIR genes carried by 100 random African Americans were characterized by DNA sequencing of genomic DNA covering the majority of coding exons. The frequency of individual loci was similar to that found in European Americans with the exception of a reduced frequency for *KIR2DS1* in African Americans. New alleles were identified at the *KIR2DS1* (**008*), *KIR2DS2* (**006*), *KIR2DS3* (**00104, *00105, *00106, *004*), *KIR2DS4* (**00103, *00104, *009, *011, *012, *013*), and *KIR2DS5* (**006, *007, *00801, *00802, *009*) loci. The distribution of alleles at each locus was similar to that found in a European American population except for *KIR2DS5*. *KIR2DS5* exhibits a single allele in European Americans; the same allele is found at reduced frequency (41% of gene positive individuals) accompanied by *KIR2DS5*006* (18%), *KIR2DS5*007* (26%), and six other alleles (25%) in African Americans.

Introduction

A balance of inhibitory and stimulatory signals determines the activation state of natural killer (NK) cells. These cells express a number of cell surface stimulatory receptors which detect ligands associated with abnormal cells: NKG2D detecting stress proteins; natural cytotoxicity receptor NKp46, viral components; and CD16 Fc receptor, antigen antibody complexes [1]. Also expressed by human NK cells are the stimulatory killer cell immunoglobulin-like receptors (sKIR). The six sKIR (KIR2DS1-KIR2DS5 and KIR3DS1) belong to a larger family of homologous receptors that also includes inhibitory receptors KIR2DL1-KIR2DL5 and KIR3DL1-KIR3DL3 [2]. The similarity of sKIR extracellular domains to those of inhibitory KIR (iKIR) suggests that sKIR recognize the same or similar ligands, i.e., major histocompatibility complex (MHC) class I molecules. The identification of ligands for KIR2DS1[3] [4] and KIR2DS4 [5] supports this although the binding to MHC molecules is weak suggesting that specific MHC-antigenic peptide complexes might be required or that other pathogen-associated MHC-like ligands might exist [6].

Like several other stimulatory receptors, sKIR have a short cytoplasmic domain and signal through an associated immunotyrosine activation motif (ITAM)-bearing adaptor protein, DAP12 for the sKIR. When antibodies directed to sKIR are used to trigger these receptors on NK cells, cytotoxicity is increased and cytokines (interferon gamma, tumor necrosis factor) are produced [7-9].

Corresponding Author: Carolyn Katovich Hurley, Ph.D., E404 Research Building, Georgetown University Medical Center, 3970 Reservoir Road, NW, Washington DC 20057 USA; Fax: 202-687-6440; Phone: 202-687-2157; hurleyc@georgetown.edu.

The genes encoding all *KIR* are clustered on chromosome 19 [10] and many haplotypes encoding variable numbers of *KIR* genes segregate in the population [11]. Haplotypes designated as "A" carry only *KIR2DS4* as their sKIR in combination with iKIR *KIR2DL1*, *KIR2DL3*, *KIR3DL1*, and *KIR3DL2*. Haplotypes with other sKIR loci are designated as "B"; *KIR2DS4* is found on some of the B haplotypes. Some haplotypes may encode no surface expressed *KIR2DS* loci [12]; others encode several. The allelic products of one sKIR locus, *KIR2DS3*, do not appear to be surface expressed and the locus may be nonfunctional [13]. In addition to the diversity in receptor gene repertoire, the *KIR* loci themselves are polymorphic [14]. For the majority of sKIR, most populations studied have one or two predominant alleles at a locus with small numbers of individuals carrying other alleles [15-17]. The exception is *KIR2DS4* which is more polymorphic. The *KIR* allelic variation may impact the level of cell surface expression and/or the affinity of interaction with ligand [16,18]. Another level of diversity is the expression of specific KIR on cell surfaces of individual NK clones. KIR are expressed by the CD56 dim subset of NK cells and individual cells in this subset express receptors encoded by subsets of their *KIR* genes. Some cells may express a single KIR while others might express two or more. The specific receptors expressed depends, in part, on the HLA ligands expressed [16] with the overall repertoire appearing to balance the reactivity to missing self ligands [19].

The goal of this study was to examine the allelic diversity in the two domain *sKIR* genes carried by a random population of African Americans and to contrast their diversity with that observed in other populations.

Materials and Methods

Population studied

This study used Epstein Barr Virus-transformed B-cell lines from the National Institute of General Medical Sciences (NIGMS) Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/nigms/). Genomic DNA was isolated from 100 unique and unrelated African Americans from the human variation panel using a QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA) following manufacturer's instructions.

KIR polymerase chain reaction amplification

Testing for the presence or absence of specific stimulatory 2D *KIR* used polymerase chain reaction (PCR) primers and reaction conditions previously described [15,20,21]. Amplified DNA products were visualized by agarose gel electrophoresis.

To identify alleles at each locus, *sKIR* genes were amplified from genomic DNA by PCR with two pairs of primers designed to yield overlapping amplicons covering most of the exons as previously described [15]. One exception was that the antisense exon 9 primer for *KIR2DS4* was replaced to accommodate a novel polymorphism that impacted primer annealing. The new primer, 5′TGAAATGGAGAATTGTGGGCTAAG [22], anneals in the 3′ untranslated region. Amplicons were purified using AmPure magnetic beads (Agencourt Bioscience, Beverly MA) according to the manufacturer's protocol.

Nested PCR was used to isolate *KIR2DS4* alleles with and without a known exon 5 deletion in heterozygotes as previously described [15]. When required, PCR products were cloned using the TOPO TA cloning kit (Invitrogen). Qiagen HaploPrep reagents 488A (*KIR2DS2*) and 744d22 (*KIR2DS4*) (Valencia, CA) were used to isolate alleles [23] in some cases following manufacturer's instructions.

KIR sequencing and analysis

Sequencing primers were positioned to obtain the sequence of both strands of each amplicon [15]. Sequencing was performed using Applied Biosystems' BigDye Terminator Ready Reaction mix according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). The reactions were purified using CleanSEQ (Agencourt Bioscience) according to the manufacturer's protocol. 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. The sequences were compared to locus specific databases (ImmunoPolymorphismDatabase (IPD)-KIR Release 2.0.0) of known *KIR* sequences [24] created using Library Builder software (Conexio Genomics) to identify alleles. In this report, the numbering of nucleotides and codons is based on IPD-KIR unless noted. 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 [25]. Confirmatory sequences of previously described alleles were submitted to the IPD-KIR database and include: *KIR2DS1*004* (GenBank FJ374884), *KIR2DS1*006* (EU915286), *KIR2DS2*00102* (FJ374880), *KIR2DS5*003* (FJ374879), *KIR2DS5*004* (FJ374881), *KIR2DS5*005* (FJ374882).

Results

The percent of African Americans carrying specific two domain stimulatory genes is similar to other populations

The presence or absence of specific two domain stimulatory *KIR* genes in a population of 100 unrelated African Americans is shown in Table 1. The percentages are similar to that previously reported for 58 random African Americans from a Los Angeles blood donor population [26]. Since many African Americans trace their ancestry to slaves originating from the west coast of Africa [27], the percentages were compared also to percentages found in two west African populations (n=62 [28], n=90 [29]). The results are comparable with the exception of *KIR2DS1* which was lower in a population from Senegal (27% vs 13%) and *KIR2DS5* which was lower in both populations from west Africa (39% vs 24% (or 30%)). The lower percentage for *KIR2DS5* is not explained by a failure in the earlier studies of primers to anneal to the novel alleles we are describing in this study. In a comparison with a European American population (n=77), percentages are similar except for *KIR2DS1* where 27% of African Americans carried the gene in comparison with 42% of European Americans [15]. **KIR2DS1.** Like most populations studied [15-17], the predominant allele in African Americans is *KIR2DS1*00201* found in 81% of gene positive individuals (Table 1). The previously described *KIR2DS1*004*, previously reported in a single Black individual [30], was identified in two individuals and the sequence was extended to include all of the leader sequence (GenBankFJ374884). *KIR2DS1*006*, previously found in an European American [15], was observed for a second time. One new allele, *KIR2DS1*008*, was detected in three individuals (Tables 1, 2). It differs by three nucleotides from *KIR2DS1*00201*, sharing codon 90 with *KIR2DS1*004* (GTG/val; other alleles have TTG/leu) but has a unique substitution of two nucleotides in codon 123 altering asn (AAT) to ser (AGC). Codon 123 is located in the second extracellular domain and the serine substitution alters a potential N-linked glycosylation site. **KIR2DS2**. Like European Americans [15], the predominant allele is *KIR2DS2*00101* although *KIR2DS2*00102* was observed in five of 44 gene-positive individuals (Table 1). The latter confirms an allele previously identified in an individual of mixed race [31]. A new allele, *KIR2DS2*006*, differs from *KIR2DS2*00102* by a single nucleotide substitution at codon 250 altering AAC (asn) to GAC (asp) in the transmembrane region; the same codon is found in *KIR2DS2*005*. At the protein level, the African American population is more homogeneous than the European

American population, expressing two KIR2DS2 receptors compared to three [15], but more diverse than the Japanese population with one expressed receptor [16]. **KIR2DS3**. The predominant allele is *KIR2DS3*00103* (Table 1). *KIR2DS3*002* was observed twice, being previously found in Caucasians. Four new alleles were detected (Table 2). Silent variants of the predominant allele were designated *KIR2DS3*00104*, *KIR2DS3*00105*, and *KIR2DS3*00106*. *KIR2DS3*004* differs at codon 131 from the other alleles at the locus (TGG/trp was altered to CGG/arg) and at codon 262 (a silent substitution). The amino acid substitution alters the second extracellular domain. Like the other alleles at this locus, *KIR2DS3*004* has greatly reduced surface expression [13].

KIR2DS4

Alleles encoding all five known *KIR2DS4*-encoded polypeptides were found in African Americans (Table 1, Figure 1). *KIR2DS4*00101* predominates as it does in a Japanese population and European Americans [15,16]. Other alleles are frequent; the second most common allele in European Americans, *KIR2DL4*003* [17], is found in 25% of the gene positive African American panel. Six novel alleles were identified. Two novel alleles, *KIR2DS4*00103* and *KIR2DS*00104*, differ at the nucleotide level from *KIR2DS4*00101*; their substitutions are unique. *KIR2DS4*00104* was first identified by an inconsistency between the sequence-specific priming (SSP) results indicating the presence of at least two *KIR2DS4* alleles differing for the known deletion and the initial DNA sequencing results yielding a single allele. The novel allele, *KIR2DS4*00104*, differs in the annealing site of our antisense primer for the B amplicon and was identified in five individuals. *KIR2DS4*011*, observed twice, differs from *KIR2DS4*00101* at codon 250 in the transmembrane region altering the conserved GAC (asp) to AAC (asn).

Three other novel alleles carried the deletion observed in *KIR2DS4*003* [12]. *KIR2DS4*009* differs from *KIR2DS4*003* by three nucleotide changes at previously conserved positions causing two amino acid substitutions at 158 (leu replaced by phe) and 171 (ser replaced by leu) in the portion of the unique polypeptide sequence introduced by the deletion. *KIR2DS4*012* differs from *KIR2DS4*006* by a single nucleotide substitution altering codon 74 from the conserved ala to glu in the first extracellular domain. The third novel deletion allele, *KIR2DS4*013*, differs from *KIR2DS4*006* by five nucleotide changes. Four alter the nucleotide sequence following the deletion but do not impact the amino acid sequence. One at codon 4 (GTC (val) to GCC (ala)) alters a conserved amino acid in the first domain sequence. **KIR2DS5**. In contrast with studies in other populations [15-17] [32], *KIR2DS5* is more diverse in African Americans. While *KIR2DS5*00201* predominates, it is carried only by 41% of individuals carrying *KIR2DS5* alleles (Table 1, Figure 1). In contrast, *KIR2DS5*00201* is the only allele found in individuals of European [15,17] and Japanese ancestry [16]. Alleles *KIR2DS5*003*, *KIR2DS5*004* and *KIR2DS5*005*, previously observed in African Blacks [31,32] and reported in single individuals, were found in African Americans. Five novel alleles were identified. An inconsistency between gene presence [33] or absence [20] using different locus specific primers signaled the presence of a novel allele, *KIR2DS5*009*, observed in 3 individuals. A substitution at codon 176 altered a conserved primer annealing site and introduced a new amino acid (thr instead of arg). Novel alleles *KIR2DS5*006* and *KIR2DS5*007* were found in multiple individuals including 18% and 26%, respectively, of the gene positive population. Both nonsynonymous substitutions alter previously conserved codons in the extracellular portion of the receptor. Amino acid substitutions in *KIR2DS5*00801* and *KIR2DS5*00802* alter the amino terminus of the mature receptor from his to arg. None of the novel *KIR2DS5* alleles carry the substitutions known to result in the loss of surface expression observed for *KIR2DS5*001* [34].

Discussion

The two domain sKIR carried by African Americans resemble that of European Americans [15]and other populations [16,17,28,29] at the level of gene presence or absence. One exception is *KIR2DS1* which appears at a lower frequency in African Americans (this study and [26]) (27% in African Americans compared to 42% in European Americans) and this decrease is mirrored in African populations [28,29,35]. A second exception is *KIR2DS5* being found in a smaller percent of individuals in west African populations compared to African Americans. Other loci are present in similar numbers of individuals (less than +/-10%) in African Americans and European Americans.

With the exception of *KIR2DS5*, the distribution of *sKIR* alleles at each locus is similar to European Americans. *KIR2DS1, KIR2DS2,* and *KIR2DS3* all show the same predominant allele at each locus. *KIR2DS4* is more polymorphic but, again, both population groups exhibit the same predominant alleles. The finding of a single predominant allele also applies to the single three domain sKIR. A single allele, *KIR3DS1*013*, accounts for 97% of the alleles identified in a study of 28 world-wide populations [36]. The exception is *KIR2DS5* where all gene positive European Americans carry *KIR2DS5*00201* but three alleles predominate in African Americans: *KIR2DS5*00201* (41%), *KIR2DS5*007* (26%), and *KIR2DS5*006* (18%). These alleles encode receptors that differ from one another at three polymorphic positions in the extracellular portion of the receptor, two in the second domain and one just adjacent to the cell membrane. The substitutions are nonconservative (P154T, R157G, and E216K) and may impact the properties of the receptor.

Most individuals (>90%) who carry a locus exhibit a single allele although it is not possible to determine if they carry one or more copies without an analysis of haplotypes. For example, out of 27 individuals positive for *KIR2DS1*, 26 exhibit a single allele; single alleles are observed in all 44 *KIR2DS2* gene positive individuals and 21 out of 23 *KIR2DS3* gene positive individuals. This likely reflects the predominance of a single allele at these loci but even *KIR2DS5* which is more polymorphic with three relatively frequent alleles exhibits 36 individuals with single alleles out of 39 individuals carrying the locus. In contrast, 46% (45 out of 97) of *KIR2DS4* positive individuals are heterozygotes.

The 17 novel *KIR2DS* alleles described here impact 24 codons. The vast majority occur at previously conserved positions; 14 codon changes are nonsynonymous. The predominance of amino acid changes suggests a positive selection for protein variation. The majority of the nonsynonymous substitutions (12/14) impact the extracellular domains. Positive selection for amino acid substitutions in the extracellular domains has been reported previously for KIR3DL1/KIR3DS1 using tests that consider codon variation in the ratio of nonsynonymous to synonymous (dN/dS) substitutions [36].

The total number of sKIR loci present varies among individuals. Overall, only four out of 100 African Americans carry all five two domain sKIR. Multiple individuals carry four (15 individuals), three (25), and two (19) sKIR loci. Thirty seven individuals (37%) carry only one locus; the single locus is always *KIR2DS4* suggesting that these individuals carry two haplotypes designated as “A”. Previous studies observed AA haplotypes in 35% of Africans [28], 30% of Caucasoids [37] and 59% of Japanese [16]. There are no individuals who fail to carry at least one sKIR gene but there are seven individuals who carry, as their only 2D sKIR, *KIR2DS4* with an allele which lacks cell surface expression [12]. One further individual lacks a surface expressed *KIR2DS4* allele and carries only the apparently nonexpressed *KIR2DS3* locus [13]. Thus individuals run the gamut from carrying all five sKIR loci to those who carry a single locus encoding an apparently defective receptor.

While the sKIR profile in populations is still limited in the heterogeneity, the number of sKIR alleles that are discovered is increasing. Once the role of these receptors in the NK cell response is known, we can begin to evaluate the impact of this allelic diversity in health and in disease states.

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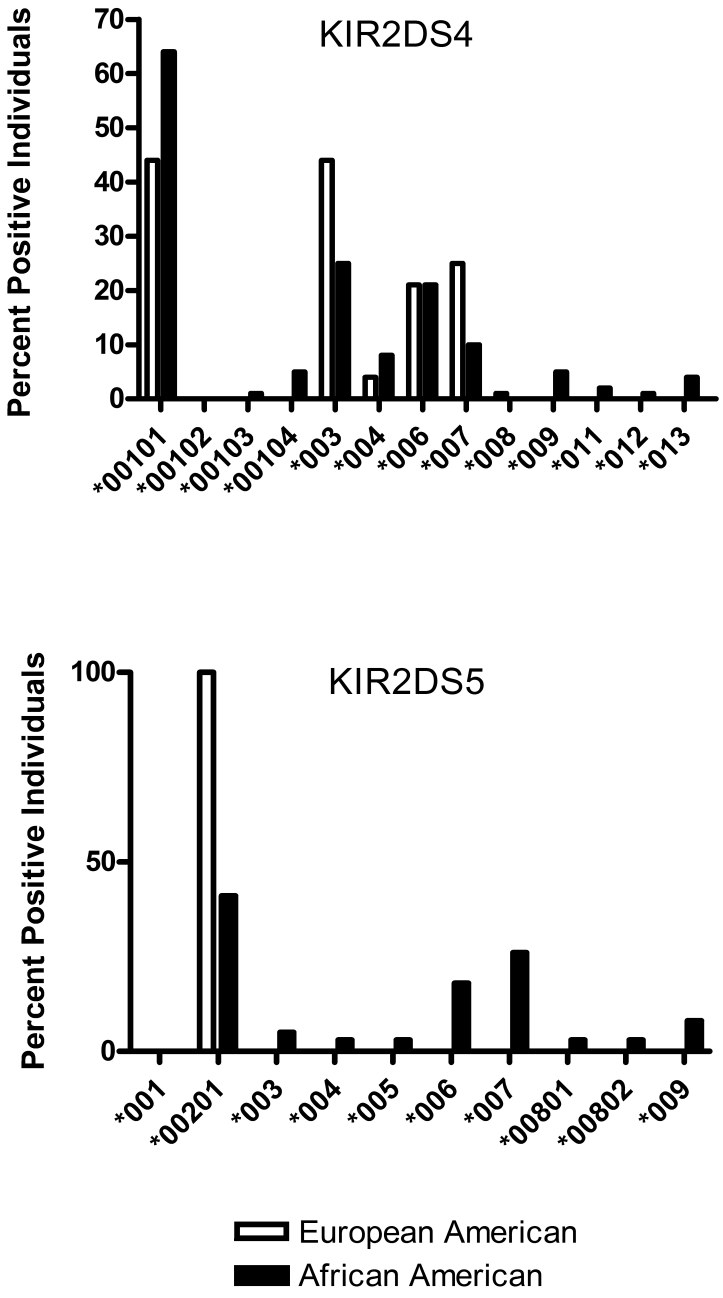

Figure 1. Percentage of gene positive individuals with *KIR2DS4* and *KIR2DS5* alleles in two populations

DNA sequencing was used to identify stimulatory KIR alleles in a panel of random African Americans (n=100) as compared to a panel of European Americans (n=77) [15].

Table 1

Percent of individuals positive for 2DS alleles

Locus / Allele	Percent of total individuals	Locus / Allele	Percent of total individuals
2DS1*	27 (27/100)	**2DS4***	97 (97/100)
00201	22 (22/100)	00101[a]	62 (62/100)
004	2	00103[b]	1
006	1	00104[b]	5
008[b]	3	003	24
Other	0	004	8
2DS2*	44 (44/100)	006[b]	20
00101	36 (36/100)	007[b]	10
00102	5	009[b]	5
006[b]	3	011[b]	2
Other	0	012[b]	1
2DS3*	23 (23/100)	013[b]	4
00103	19 (19/100)	Other	0
00104[b]	1	**2DS5***	39 (39/100)
00105[b]	1	00201	16 (16/100)
00106[b]	1	003	2
002	2	004	1
004[b]	1	005	1
Other	0	006[b]	7
		007[b]	10
		00801[b]	1
		00802[b]	1
		009[b]	3
		Other	0

[a] Alleles differing outside of coding regions were not resolved.

[b] Novel allele described in this study.

Table 2

Novel alleles

Novel Allele[a]	Similar Allele	Codon (Amino Acid) Altered[b]	GenBank Accession No.	Sample ID
2DS1				
2DS1*008	KIR2DS1*00201	90 TTG (L) > GTG (V), 123 AAT (N) > AGC (S)	EU277008	GM17113
2DS2				
2DS2*006	KIR2DS2*00102	250 AAC (N) > GAC (D)	FJ457922	GM17148
2DS3				
2DS3*00104	KIR2DS3*00103	157 AAC (N) > AAT (N)	EU277007	GM17109
2DS3*00105	KIR2DS3*00103	178 TTC (F) > TTT (F)	EU915289	GM17200
2DS3*00106	KIR2DS3*00103	262 GCG (A) > GCA (A)	EU915290	GM17187
2DS3*004	KIR2DS3*00103	131 TGG (W) > CGG (R), 262 GCG (A) > GCA (A)	EU277009	GM17114
2DS4-full length				
2DS4*00103	KIR2DS4*00101	162 GCC (A) > GCT (A)	EU277010	GM17114
2DS4*00104	KIR2DS4*00101	280 GTG (V) > GTA (V)	EU933934	GM17127
2DS4*011	KIR2DS4*00101	250 GAC (D) > AAC (N)	EU915288	GM17165
2DS4 deletion				
2DS4*009	KIR2DS4*003	112 GCC (A) > GCA (A), 158[c] CTC (L) > TTC (F), 171[c] TCG (S) > TTG (L)	EU277011	GM17123
2DS4*012	KIR2DS4*006	74 GCA (A) > GAA (E)	EU915291	GM17199
2DS4*013	KIR2DS4*006	4 GTC (V) > GCC (A), 149[c] TCA (S) > TCC (S), 179[c] ACG (T) > ACA (T), nucleotides 787-789 CCG > GCA 3′ after termination codon	FJ374883	GM17141
2DS5				
2DS5*006	KIR2DS5*005	154 CCC (P) > ACC (T)	EU277005	GM17102
2DS5*007	KIR2DS5*005	216 GAA (E) > AAA (K)	EU277006	GM17103
2DS5*00801	KIR2DS5*005	1 CAT (H) > CGT (R), 224 CAC (H) > CAT (H)	EU277004	GM17128
2DS5*00802	KIR2DS5*005	1 CAT (H) > CGT (R)	FJ374885	GM17183
2DS5*009	KIR2DS5*00201	176 AGA (R) > ACA (T)	EU915287	GM17148

[a] Designation assigned by the WHO Committee for Factors of the HLA System [25].

[b] Numbering based on the IPD-KIR database [24]; most similar known allele listed first.

[c] 2DS4*009, *012, *013 carry the deletion found in 2DS4*003 and the numbering of codons and amino acids is based on alignment with 2DS4*003 following codon 130. Alleles with the deletion have a frameshift which dramatically alters the amino acid sequence beginning at codon 131 through a termination codon at 219.