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Conserved KIR Allele-Level Haplotypes Are Altered by Microvariation in Individuals with European Ancestry

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Abstract

Natural killer cell immunoglobulin-like receptor (KIR) haplotype-specific DNA fragments were sequenced to identify centromeric and telomeric allele level haplotype structures and their frequencies from 76 unrelated individuals with European ancestry. Analysis was simplified by redefining the 5' boundary of the centromeric KIR gene cluster to include only exons 7-9 of KIR3DL3. Three consensus allele level haplotypes were identified for a centromeric gene presence/absence structure designated as Cen-A1. KIR3DL3*00201 (ex 7-9)—KIR2DL3*001—KIR2DL1*00302 was the most frequent (37.5%) centromeric structure. Single consensus haplotypes were observed for haplotype structures Cen-B1 and Cen-B2. Six Tel-A1 and two Tel-B1 consensus haplotypes were observed; the most prevalent (23.0%) was KIR2DL4*00102—KIR3DL1*002—KIR2DS4*00101—KIR3DL2*002. A small number of nucleotide substitutions (3) in the coding regions of the functional KIR genes created microvariants of the consensus haplotypes. Eight less common haplotype structures were also detected. Four carried hybrid genes formed during gene deletion events, two carried an insertion with a 2DL5/3DP1 fusion gene, and two included a very large insertion. These data show that the KIR gene complex is composed of a limited number of conserved allele level centromeric and telomeric haplotypes that have diversified by mutation, recombination within a locus, and unequal crossing over.

Keywords

Natural killer cell; killer immunoglobulin-like receptor; haplotype; population study

Introduction

The recognition of malignant or infected cells by natural killer (NK) cells is controlled by both inhibitory and stimulatory receptors (1;2). Of the 14 members of the killer cell immunoglobulin-like receptor (KIR) family, many (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1, KIR3DL2, and KIR3DL3) are inhibitory receptors that prevent NK destruction of normal cells. Some of these receptors recognize subsets of human leukocyte antigens (HLA) as their ligands (3-6). Stimulatory receptors that may initiate NK destruction of damaged cells include KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, and KIR3DS1; some of these receptors recognize HLA ligands but the ligands of others are not yet known (7;8) (9). KIR2DL4 has the capacity to be both stimulatory and inhibitory (10); it recognizes HLA-G as its ligand (11). Together, KIR and other NK receptors, like the NKG2 and the natural cytotoxicity receptors, control NK reactivity.

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Conflict of interest

The authors have no competing financial interests related to this work.

The genes encoding KIR are found in two adjacent clusters within the human leukocyte receptor complex on chromosome 19 (12). Within each cluster, KIR genes, lying in a head to tail orientation, are separated by about 2 Kb. Studies of KIR gene presence or absence in various individuals have noted that not all 14 KIR genes are found in a single haplotype (13-15). KIR2DL2 and KIR2DL3 appear to be encoded as allotypes; individual copies of chromosome 19 carry one or the other but not both genes. This is also true for KIR3DL1 and KIR3DS1. Three KIR genes (KIR2DL5, KIR2DS3, KIR2DS5) appear to be duplicated and may appear in the centromeric and/or the telomeric cluster (16-18). The complex also includes two pseudogenes, KIR2DP1 and KIR3DP1. Framework genes, found in most KIR haplotypes, flank each cluster: KIR3DL3 and KIR3DP1 flank the centromeric cluster while KIR2DL4 and KIR3DL2 flank the telomeric cluster.

DNA sequencing of single copies of chromosome 19 (18;19) and family segregation (15;20) (21) have defined three common centromeric (Cen-A1, Cen-B1, Cen-B2) and two common telomeric (Tel-A1, Tel-B1) haplotype structures based on the presence and absence of specific KIR genes. As examples, Cen-A1 is comprised of KIR3DL3—KIR2DL3—KIR2DP1—KIR2DL1—KIR3DP1 in this gene order while Tel-A1 includes KIR2DL4—KIR3DL1—KIR2DS4—KIR3DL2 (Table 1). Recombination within the region separating the two KIR gene clusters generates various centromeric--telomeric combinations. Less common KIR haplotypes appear to have been generated by unequal crossing over in this multigene family. Some haplotypes carry duplicated KIR genes (22); others carry large deletions (23). Some are marked by the presence of chimeric genes generated by the splicing of two KIR genes to form, for example, an expressed form of the KIR3DP1 pseudogene (24) or a hybrid KIR3DL1/KIR3DL2 gene (25). Overall, the KIR gene complex is a model for genetic complexity.

In addition to variation in the presence or absence of genes, the KIR genes are also polymorphic (26). This structural variation can impact levels of surface expression, ligand binding and NK cell activity (27-30). Family segregation, linkage disequilibrium, and haplotype isolation have defined allelic associations for segments of some KIR haplotypes (16;18;20;21;31;32). This study extends those observations to define the frequency and allelic content of KIR haplotypes in individuals of European ancestry using strategies to isolate haplotypes for DNA sequencing.

Results

KIR haplotype structures were examined in a panel of 76 unrelated individuals with European ancestry. Previously, gene amplification assays had been used to identify the presence or absence of KIR genes in these individuals and comprehensive DNA sequencing of essentially all exons had been applied to identify alleles at the 14 expressed KIR loci (32-39). Sixty eight individuals appeared to carry only the centromeric (A1, B1, B2) and telomeric (A1, B1) haplotype structures previously defined (18) (Tables 1 and 2). Eight individuals carried “other” haplotype organizations. Isolation of haplotype-specific DNA fragments and limited DNA sequencing were used to define the allelic linkages in these 76 individuals.

The frequencies at which centromeric or telomeric common haplotypes were found in the 76 individuals studied are similar to that observed by Pyo and colleagues in a random Caucasian population (Figure 1). The frequencies of Cen-A1, Cen-B1, and Cen-B2 were 67.1%, 8.6%, and 17.8% in our study compared to 71.9%, 10.4% and 16.7% in the earlier study. The frequencies of Tel-A1 and Tel-B1 were 76.3% and 19.1% in our study versus 82.3% and 16.7% in the Pyo et al. study (18). The frequency of “other” haplotypes was higher in our study (eight of 76 individuals) compared to one in 48 individuals in the Pyo et

al. study. This difference may be the result of the inability of the screening assays used in the earlier study to detect less common gene organizations. The distribution of haplotype structures in individuals carrying only common structures is given in Table 2.

Although this study was not able to link specific centromeric and telomeric haplotypes, the combinations of centromeric and telomeric structures found were consistent with their frequencies in the population. The majority of individuals (31.8%, n=21 of 66 with known haplotypes) carried Cen-A1/Cen-A1 and Tel-A1/Tel-A1 haplotypes. Also frequent were Cen-A1/Cen-A1 with Tel-A1/Tel-B1 (16.7%) and Cen-A1/Cen-B2 with Tel-A1/Tel-A1 (21.2%). The associations between centromeric and telomeric KIR haplotype structures appear to be random ($p=0.47$).

Redefining the centromeric framework gene, KIR3DL3

Extensive diversity is found in the KIR3DL3 alleles associated with the centromeric haplotypes. Cen-A1 haplotypes alone were found with 26 different KIR3DL3 alleles. However, if the KIR3DL3 gene is divided into two segments based on a hotspot for recombination separating exons encoding the extracellular domains (exons 1-5) from exons encoding the intracellular segment (exons 7-9) (40), the extensive nucleotide variation is localized to exons 1-5. The nucleotide sequences of exons 7-9 of KIR3DL3 are highly conserved (Table 3). Only five consensus sequences for this 3' region are found among all the known alleles of KIR3DL3 (n=75). These conserved sequences have specific associations with centromeric haplotype structures, Cen-A1 and Cen-B (18) and with specific consensus KIR haplotypes within those haplotype structures as described below.

Exons 1-5 of KIR3DL3 found associated with the centromeric haplotype structures exhibit a similar level diversity as found when all known KIR3DL3 alleles are compared. Figure 2 shows a mismatch comparison of pairs of KIR3DL3 alleles based on the nucleotide sequences of exons 1-5. The plot compares the frequency distribution of pairwise comparisons of all KIR3DL3 alleles (n=75) to the 16 alleles found within a related cluster (consensus haplotype I as described below) of Cen-A1 haplotypes. Both comparisons exhibit a bimodal distribution suggesting that they are composed of recently related lineages with a small number of nucleotide variations and more distantly related lineages with greater sequence differences. So, the KIR3DL3 alleles associated with a consensus haplotype of Cen-A1 are as different from one another as is found if all the KIR alleles are examined without regard to shared haplotypes. This diversity in KIR lineages associated with a conserved haplotype has been previously noted (18).

Cen-A1

Thirty two different haplotypes were observed from 1 to 17 times in the 102 haplotypes designated as Cen-A1 (Table 4). KIR3DL3*00901--KIR2DL3*001--KIR2DL1*00302 (17 copies) and KIR3DL3*00101--KIR2DL3*002--KIR2DL1*002 (15copies) were the two most common Cen-A1 haplotypes. The 32 Cen-A1 haplotypes can be clustered into three consensus haplotypes where haplotypes within each cluster share essentially all alleles at the included loci if exons 1-5 of the most centromeric locus, KIR3DL3, are excluded. The consensus haplotype is the most frequent haplotype within the consensus cluster. While haplotypes differ from the consensus sequence by 3 nucleotides in the coding regions of expressed alleles, the three consensus haplotypes differ from one another by seven to 11 nucleotides (Figure 3). The frequencies of the three centromeric consensus haplotype clusters are shown in Figure 4.

Eighteen distinct haplotypes share a consensus haplotype (designated Cen-A1-I for Cen-A1, consensus haplotype I) comprised of KIR3DL3*00201 (exons 7-9)--KIR2DL3*001—

KIR2DL1*00302 (Table 4). The majority (37.5%) of the centromeric haplotypes carry this consensus (Figure 4). Minor differences from the Cen-A1-I consensus include a haplotype carrying KIR2DL3*003, an allele differing by two nucleotides from KIR2DL3*001, and a haplotype carrying KIR2DL1*009, an allele differing by two nucleotides from KIR2DL1*00302. Four haplotypes differ by a single nucleotide substitution in exons 7-9 creating KIR3DL3*00101. A single nucleotide substitution in the exon 7-9 sequence is also observed for the haplotypes associated with KIR3DL3*00902 (Table 3). Thus, a conserved haplotype defines this group with less common variant haplotypes differing only subtly from the conserved coding sequences of the KIR genes present.

The ten Cen-A1 consensus II (Cen-A1-II) haplotypes share KIR3DL3*00101 (exons 7-9)--KIR2DL3*002--KIR2DL1*002. This consensus appears at a frequency of 26.3% in individuals with European ancestry. Microvariation generates haplotypes carrying KIR2DL1*008 or KIR3DL3*00102 (exons 7-9); both alleles differ by a single nucleotide from the consensus allele at the locus. KIR3DL3*025 (exons 7-9)--KIR2DL3*005--KIR2DL1*001 form the third consensus haplotype (Cen-A1-III) with a frequency of 3.3%. Four haplotypes were identified that share these alleles but differ for the 5' regions of KIR3DL3; one differs by a single nucleotide substitution within exons 7-9 of the consensus KIR3DL3 sequence (Table 3).

Cen-B

Six Cen-B1 haplotypes were observed with KIR3DL3*00301--KIR2DS2*00101--KIR2DL2*001--KIR2DL5B*0020101 (or*0020103)--KIR2DS3*00103—KIR2DL1*00401 as the most common, found in six of the 13 Cen-B1 copies (Table 5)(Figure 4). Of the seven allele divergent Cen-B2 haplotypes observed, there were 15 copies of KIR3DL3*00301--KIR2DS2*00101--KIR2DL2*003 out of 27 total Cen-B2 copies (Table 6). Based on the alleles shared at KIR3DL3 exons 7-9, KIR2DS2, and KIR2DL2, it appears that Cen-B2 was generated from Cen-B1 by a gene deletion event (or Cen-B1 generated by a gene insertion event). Allelic variation in the three functional loci shared by the two haplotypes is restricted to a single nucleotide difference at KIR2DL2*001 versus KIR2DL2*003. The Cen-B1-I and Cen-B2-I consensus haplotypes are found at frequencies of 8.6% and 17.8%, respectively. Microvariation similar to that observed for Cen-A1 within a consensus was observed.

Tel-A1

Twenty three allele content haplotypes were identified for Tel-A1 (Table 7). The most common were KIR2DL4*00102--KIR3DL1*002--KIR2DS4*00101--KIR3DL2*002 (20 copies) and KIR2DL4*0080101 (or*0080103)--KIR3DL1*00101--KIR2DS4*003--KIR3DL2*00101 (19 copies). The haplotypes fall into six consensus haplotypes (I-VI), distinguished by the lineage of the KIR3DL1 alleles (41) and by the extent of nucleotide differences between the combined coding regions. Consensus haplotypes I-III carry the KIR3DL1*015 lineage alleles. Clusters IV and V include the lineage KIR3DL1*005 alleles. Tel-A1-VI carries the interlineage recombinant KIR3DL1*00101. The consensus haplotypes differ from one another by from six (consensus IV versus VI) to 30 nucleotides (consensus I versus V) with an average of 18.2 nucleotides. For this comparison, the 22 bp deletion found in some of the KIR2DS4 alleles was counted as a single alteration. The relationships among the Tel-A1 consensus haplotypes are shown in a Neighbor-Joining tree (Figure 5). The frequencies of the telomeric A consensus haplotypes are shown in Figure 4. They vary from 23.0% for Tel-A1-I to 2.0% for Tel-A1-III.

Within a consensus haplotype cluster, there is a frequent consensus haplotype with microvariation creating the others. For example, the consensus haplotype for Tel-A1-I is KIR2DL4*00102—KIR3DL1*002—KIR2DS4*00101—KIR3DL2*002 found 20 times

within the 35 haplotypes in this group. The other three haplotypes in this group differ by one to two nucleotides from the consensus at individual loci forming microvariants KIR2DL4*00103, KIR3DL1*01502, KIR3DL1*01702, and KIR3DL2*021. The second most prevalent haplotype is KIR2DL4*00102—KIR3DL1*01502—KIR2DS4*00101—KIR3DL2*002 found 11 times. The other two haplotypes were observed one and three times in this population.

An exception to the microvariation is found within the consensus VI haplotype. The KIR3DL1*00101 interlineage recombinant fusing exons 1-3 of KIR3DL1*005 and exons 4-9 of KIR3DL1*015, is replaced by KIR3DL1*009. This allele is a second interlineage recombinant joining exons 1-3 of KIR3DS1*013 and exons 4-9 of KIR3DL1*00101 (41). Thus recombination may also cause variation within a consensus haplotype.

Tel-B1

Six Tel-B1 haplotypes were identified with KIR2DL4*00501--KIR3DS1*01301--KIR2DL5A*0010101--KIR2DS5*00201--KIR2DS1*00201--KIR3DL2*00701 the most frequent at 21 copies (Table 8). Again, microvariation is noted. Furthermore, a single gene conversion event with a Cen-B1 haplotype may have created the second consensus haplotypes carrying KIR2DL5A*0050101—KIR2DS3*002. It has been noted previously that both KIR2DL5A*0050101 and KIR2DS3 are fusion genes (42) (18;43). KIR2DL5*0050101 shares the 5' regulatory region and exon 1 with the consensus allele in the major Tel-B1 haplotype, KIR2DL5A*0010101 and the remainder of its sequence with KIR2DL5B*0020101 while KIR2DS3*002 shares its 5' sequence with KIR2DS3*00103 and its 3' sequence with KIR2DS5. Since the surrounding genes in Tel-B1 are shared, this suggests that a single gene conversion-like event created this haplotype from the consensus. The frequencies of the consensus haplotypes I and II are 15.8% and 3.3%, respectively.

Gene extended/contracted haplotypes

Eight of the 76 individuals carried allele combinations that did not fit the expected common haplotype structures. Comparison to previously reported expanded or contracted haplotypes and to the centromeric and telomeric haplotype structures, and physical linkage of adjacent alleles were used to assign genes and alleles to haplotypes in each cell. The number of KIR2DL4 genes in each of the eight individuals was confirmed by a quantitative polymerase chain reaction.

Four of the eight individuals carried haplotypes with deletions of KIR genes resulting in hybrid genes fusing the 5' region of a centromeric gene with the 3' region of a telomeric gene at the recombination site. For example, RDP71 carried a haplotype with a large deletion and only four genes: KIR3DL3*00602, KIR2DS1*00502, KIR3DL2*017, and a KIR2DL3/2DP1 fusion gene (Table 9). This haplotype had been previously observed in a CEPH family (designated haplotype "j")(23). KIR2DS1*00502 is a second hybrid allele in this haplotype linking the 5' sequence of KIR2DL1 and the 3' sequence of KIR2DS1. The alleles carried by the three loci of the CEPH haplotype are shared with cell RDP71 with the exception of a single nucleotide substitution in the KIR3DL2 allele (*007 in the CEPH family vs *017 in this study). Partial sequences of KIR2DL3/2DP1 covering exons 1-4 of KIR2DL3 and exons 6-9 of KIR2DP1 showed identity to the previously reported fusion gene sequence (GenBank accession numbers CU041340 and CU633846).

In the deletion haplotype found in RDP69, KIR2DL5B*008 is a fusion between KIR2DL5B*0020101 and KIR2DL5A*0010101 in which the recombination can be localized between nucleotide 16 in exon 1 and nucleotide 75 in exon 3. Likewise, in a third contracted haplotype in RDP76, KIR2DS2*005 is a fusion between KIR2DS2*0010101 and

KIR2DS3*00103 with the recombination occurring between exon 5 and exon 7. The haplotypes observed in RDP69 and RDP76 were observed, at the gene presence/absence level, by Gourraud and colleagues (21). A final contracted haplotype links the centromeric KIR2DL5B*0020102 to telomeric KIR2DS3*002 in RDP27. KIR2DS3*002 has previously been noted to be a hybrid gene of KIR2DS3*00103 and KIR2DS5 (43). A haplotype apparently carrying the same deletion as RDP27 on the basis of genes present or absent was observed previously in a Spanish family (C43)(16).

Two (RDP10, RDP64) of the 76 individuals studied (2.6%) carried a haplotype with an insertion of several KIR genes, previously found in European populations at a frequency of 0.8-4.5% (22;24;44) (Table 9). The haplotype is marked by the presence of a chimeric gene carrying the 5' regulatory and exon 1 sequence of KIR2DL5A fused to the 3' sequence of KIR3DP1. Because the chimeric gene was identified by sequence-specific amplification, it is not known if its sequence is identical to the original report identifying the gene as KIR3DP1*004 (24). Comparison of the haplotypes in RDP10 and RDP64 suggests that they originated independently. RDP10 arose from a Tel-A1-I consensus haplotype based on the four telomeric genes while RDP64 arose from Tel-A1-VI (Table 7). Other allele level haplotypes carrying the KIR2DL5A/3DP1 fusion gene identified in families (22;24;25) carry other alleles in the telomeric segment suggesting that this insertion has arisen independently several times.

Two individuals (RDP81, RDP14) (2.6%) carried a haplotype with an extensive insertion, previously observed in a Northern Irish family (12/04) (16). The two individuals carried the same alleles at each locus with the exception that one (RDP14) carried KIR3DS1*058 at one of the two KIR3DS1 loci instead of KIR3DS1*01301. The two alleles differ by a single nucleotide substitution. The previously reported haplotype carries the same alleles at the ten loci that were typed as the cells identified here suggesting that these haplotype are ancestrally related. The haplotype carries 14 functional genes and is comprised of segments of known Cen-A1-I (genes 1-3 starting from the centromere), Tel-B1-II (genes 4- 6), Cen-B1-I (genes 7-8), Tel-B1-I (genes 9-14) allelic haplotypes. Partnering haplotypes in the eight individuals with extended or contracted haplotypes comprised the more common centromeric and telomeric structures.

Discussion

Using a panel of 76 European Americans with alleles previously identified for all functional KIR loci, we isolated haplotype-specific DNA fragments to identify linked alleles. These data complement and extend haplotype information obtained by the comprehensive DNA sequencing of overlapping genomic clones obtained from 27 KIR haplotypes derived from multiple ethnic groups (18;19;45). Our in-depth analysis of the structure and frequency of KIR allele level haplotypes in individuals of European ancestry suggests that conserved haplotypes predominant in the population and shows the extent to which microvariation has impacted those haplotypes.

The five conserved gene presence/absence haplotype structures (Cen-A1, Cen-B1, Cen-B2, Tel- A1, Tel-B1) were the only structures found in the majority of individuals (89.5%). Conserved structures were also found in single haplotypes from the eight individuals who also carried an extended or a contracted haplotype. The haplotypes characterized in this study using physical linkage carry essentially all of the two locus allele combinations previously noted in a linkage study of families from Northern Ireland (21). For example, the earlier study noted the association of KIR3DL1*01502 with KIR3DL2*002 with a 100% positive predictive value. This association was observed in our cohort as a variant of the Tel-A1 consensus I haplotype (Table 7). There were two exceptions from the many associations

observed in the family linkage study which are explained by the lower resolution typing of the earlier study. The association of KIR3DL1*005 (and KIR2DL4*011) with KIR2DS4*003 was not observed in our study. KIR2DS4*003 differs by a single nucleotide from KIR2DS4*007, the allele we observed in association with KIR3DL1*005 (Table 7, Tel-A1-IV) and an allele not yet identified when the family samples were typed (20). Each of the consensus haplotypes described here has been previously observed (18) with a few exceptions. While the consensus haplotypes for Tel-A1-I and Tel-A1-V were not observed, one of the microvariant haplotypes in those clusters were characterized in the earlier study. Two consensus haplotypes, Cen-A1-III and Tel-A1-IV, were not found in the earlier study but this may be due to the limited number of cells investigated by fosmid sequencing. Six of the allele level haplotypes observed in our study were identified as extended haplotypes in a previous study of linkage disequilibrium (21). Thus, our independent dataset and analysis supports and extends the allele-level haplotype data previously reported in world populations.

The majority of the centromeric and telomeric consensus haplotypes observed in this cohort with European ancestry are found at frequencies between 10-20%. Three exceptions are haplotypes that appear at frequencies less than 5% (Cen-A1-III, Tel-A1-III, Tel-B1-II). These haplotypes may have originated more recently. Two other exceptions are the Cen-A1-I and Cen-A1-II haplotypes that are found at frequencies of 37.5% and 26.3%, respectively. These two haplotypes exhibit the most variation with 17 and 9 microvariant haplotypes, respectively. Many of the microvariant haplotypes are found in multiple individuals suggesting that these two consensus haplotypes may be more ancient or may be favored by selection.

At first glance, the diversity of KIR haplotypes appears extensive. Forty five different haplotypes were observed within the centromeric region with 32 classified as Cen-A1. However, the diversity within Cen-A1 was reduced to three consensus haplotypes by eliminating the KIR3DL3 sequences 5' of a hotspot of recombination and by identifying variation at other loci as limited to 3 nucleotide substitutions. Likewise, the 29 telomeric haplotypes fell into 8 consensus haplotypes. The telomeric haplotypes are more diverse than the centromeric haplotypes as previously noted (18;21).

It has been noted that hot spots of recombination, as found in the intron dividing extracellular from intracellular coding segments of KIR3DL3 and in the region separating the centromeric and telomeric KIR haplotypes, are often flanked by regions with reduced crossing over called haplotype blocks (46;47). This is consistent with the conserved allele level KIR haplotype structures observed. This is especially true for the exon 7-9 region of human KIR3DL3 which is thought to be very ancient (40). The conserved haplotypes noted here with modest variation within the alleles carried suggests that these extended KIR haplotypes comprise optimal allele combinations that have been preserved over evolutionary time.

But how do the diverse 5' sequences of KIR3DL3 fit into the picture? The 5' exon 1-5 sequences of KIR3DL3 appear to form a region distinct from the conserved centromeric and telomeric segments. There appears to have been extensive exchange among the various KIR haplotypes so that there is no association of this region with exons 7-9 nor with specific centromeric haplotype structures. The impact of a hotspot of recombination on the KIR3DL3 protein itself is not clear. KIR3DL3 lacks exon 6 which encodes the stem region of KIR (48) and cell surface expression has not been observed (49) suggesting that this locus might not be functional. However, comparison of the sequence of the extracellular region with that of higher primates shows extensive conservation and the impact of purifying

selection (40). Solution to this conundrum may come when the function of KIR3DL3, if there is any, is understood.

Gene expanded or contracted haplotype appeared in 10.5% of individuals with European ancestry studied. The creation of these haplotypes appears to be facilitated by the unique features of the KIR gene complex in terms of the dense clustering of repeat elements in the KIR introns (23). Such domain shuffling as a mechanism for generating new receptors has been noted in a comparison among higher primates (50). These less common haplotypes are sometimes difficult to detect in routine testing but are often marked by unique fusion genes providing a marker for these “other” haplotypes. By comparison with previous reports of similar structures, it is clear that some of these allele level haplotypes, such as the three gene-content contracted haplotype first described by Traherne et al. (23) will be found multiple times in the population. In contrast, other haplotypes such as those bearing the KIR2DL5A/3DP1 fusion gene (22;24;25), vary in their allele content suggesting that the same gene organization has arisen independently. Regardless, it is possible to identify the allele level consensus centromeric and telomeric haplotypes that gave rise to these expanded and contracted haplotypes.

KIR alleles are inherited on conserved haplotypes and it is this association of alleles on a haplotype that generates an immune response profile (Table 10). For example, the Cen-A1-I haplotype carries two inhibitory KIR with HLA-C ligands. KIR2DL3*001, a receptor with lower ligand binding affinity for HLA-C groups 1 and 2 compared to KIR2DL2 (28) is paired with KIR2DL1*00302 which strongly binds HLA-C group 2 (51). Likewise, Tel-A1 includes haplotypes with strong (KIR3DL1*00501, KIR3DL1*00101, consensus IV, VI), intermediate (KIR3DL1*01502, KIR3DL1*020, consensus I, II), and weak (KIR3DL1*007, consensus III) HLA-Bw4 binding. The presence of Tel-B1-I with Cen-B2 in an individual who expresses only HLA-C group 2 may weaken the inhibitory signal of KIR2DL2 by providing a stimulatory signal through the KIR2DS1 receptor with its similar ligand specificity (52;53). Some haplotypes carry apparently nonfunctional alleles such as Cen-B1 with KIR2DL5B and KIR2DS3 (42;54) or Tel-A1-V with two secreted receptors (KIR2DL4*00802, KIR2DS4*006)(55;56) and one predominantly misfolded receptor (KIR3DL1*00401)(57). The impacts of haplotypes encoding KIR receptors with unknown ligands (eg KIR2DL5) or where our understanding of any functional differences among allelic products is rudimentary (e.g., alleles of KIR3DL2) are yet to be determined. Furthermore, licensing induced by HLA allelic products in NK cells expressing these haplotypes will also impact immune activity (58). However, since KIR haplotypes are conserved, it will be possible to define immune profiles for these consensus haplotypes and apply this knowledge to our understanding of the role of KIR in health and disease.

Materials and Methods

KIR alleles carried by 76 unrelated individuals with European ancestry were identified by DNA sequencing of the coding regions of all functional KIR loci and are described in previous publications (32- 39). These individuals were either donors or recipients of hematopoietic stem cells. The characteristics of this cohort have been previously described (59); KIR genotyping was performed retrospectively. The cells were obtained from the National Marrow Donor Program Research Repository.

PyPop (Python for Population genetics, version 0.7.0 <http://www.pypop.org>) was used to carry out Hardy-Weinberg testing for three KIR loci found in all haplotypes, one in the centromeric gene cluster and two in the telomeric cluster (60;61). Allele frequencies were determined by gene counting and assumed that each individual carried two copies of each locus. Individuals (8) carrying gene expanded/contracted haplotypes were not included in

the analysis. Allele frequencies at each locus were evaluated for deviations from Hardy-Weinberg equilibrium proportions using the exact test of Guo and Thompson (62). Chi-square tests were investigated for a pooled set of all heterozygotes and a pooled set of all homozygotes (63). The tests measured the goodness of fit of KIR2DL2/2DL3, KIR3DL1/3DS1, and KIR3DL2 genotypes to Hardy-Weinberg proportions. This test measures the degree to which observed genotype frequencies differ from those expected based on the allele frequencies for that population, assuming that the population is suitably large and experiences random mating. No overall deviation from Hardy-Weinberg proportions were noted (p-values=0.9165, 0.4587, 0.7527, respectively).

Centromeric and telomeric haplotype structures were predicted based on the structures of Cen- A1, Cen-B1, Cen-B2, Tel-A1 and Tel-B1 (18). Cells were selected for further haplotype characterization based on these predictions. Haplotype specific extraction (HSE) (Qiagen, Valencia, CA) was used to isolate a DNA fragment carrying a single allele at a locus. Supplemental Table 1 lists the HSE and amplification reagents used. Once isolated, the alleles carried by the DNA fragment were identified by DNA sequencing for expressed genes and sequence-specific amplification for the pseudogenes and some fusion genes. For example, haplotypes predicted as Cen-A1 were confirmed by physically linking KIR3DL3 to KIR2DL3 using haplotype specific extraction with KIR3DL3 group specific probes (3DL3-1018A or 3DL3-161A) followed by DNA sequencing of segments of KIR3DL3 and KIR2DL3 coding regions found on that fragment of DNA. A similar strategy was used for cells carrying unexpected haplotype structures. Exceptions included the following: An extended polymerase chain reaction (PCR) (32) was performed to link KIR2DL2 to KIR2DL5 in haplotypes carrying Cen-B1 with DNA sequencing to identify the presence of the alleles at the loci. An extended PCR and sequencing were also used to link KIR3DS1 and KIR2DL5 in Tel-B1 haplotypes. Alleles in the second KIR haplotype in a cell were defined as those alleles not assigned by physical linkage to the first haplotype. In some cells, KIR2DL3-KIR2DL1 Cen-A1 haplotypes were assumed based on common associations. Amplification reactions were used to identify the presence of KIR2DP1, KIR3DP1, a KIR2DL5A-3DP1 fusion gene (24;64-66) and a 2DL3/2DP1 fusion gene (23)(Supplemental Table 1).

TaqMan copy number assays were used to measure KIR2DL4 copy number variation (Applied Biosystems, Inc., Foster City, CA). The 20ul reaction volume contained 4 ul (5 ng/ul) DNA, 10 ul TaqMan 2X PCR Master Mix, 1 ul 2DL4 CNV assay solution (Sense Primer- TCAGGACAAGCCCTTCTG, Antisense Primer- ACCCCATCTTTCTTGTACAGTG, and KIR2DL4-probe- CTGTGGTGCCTCAAGGAGG-labeled with FAM dye as previously described (22)), 1 ul reference assay RNaseP solution, and 4ul water. The quantitative PCR was performed using a StepOnePlus Real-time PCR cycler (Applied Biosystems, Inc.) with the following cycling conditions: 95°C for 10 min hold and 40 cycles of 95°C for 15 sec and 60°C for 60 sec. The results were analyzed using CopyCaller v1.0 (relative quantitation) (Applied Biosystems).

A mismatch distribution for exons 1-5 of the KIR3DL3 alleles using a constant population size was calculated using DnaSP v5 (67-69). SNAP (<http://www.hiv.lanl.gov>) was used to identify the number of nucleotide differences among the pairs. A Neighbor-Joining method (70) was used to infer the evolutionary history of haplotypes. The bootstrap consensus tree was inferred from 1000 replicates (71) using MEGA4 (72) with complete deletion to adjust for gaps and missing data and using a nucleotide--maximum composite likelihood model with uniform rates among sites. Independence of the centromeric and telomeric haplotype structures was tested using the exact option for the chi-square test in StatXact (Cytel Software Corp., Cambridge, MA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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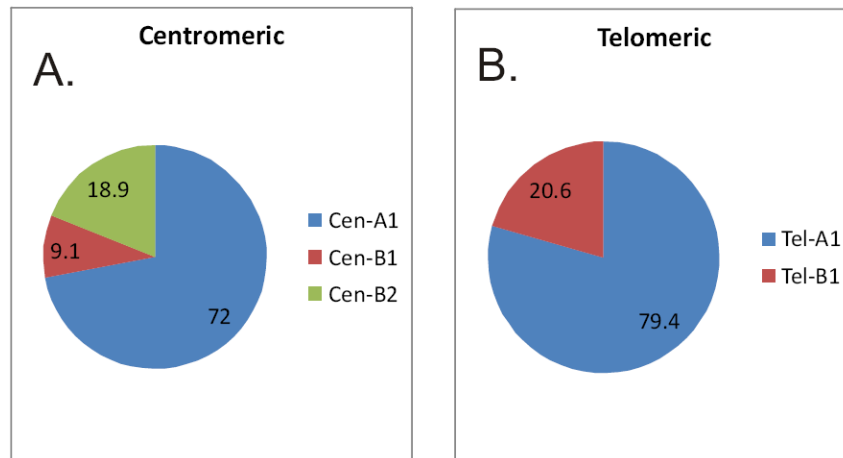


Figure 1. Frequencies of (A.) Cen-A1, Cen-B1, Cen-B2 and (B.) Tel-A1 and Tel-B1 haplotype structures in 76 unrelated individuals of European ancestry. The centromeric structures in two individuals could not be defined (ND). In addition to common haplotype structures, eight individuals carried extended or contracted haplotypes (labeled as “other”).

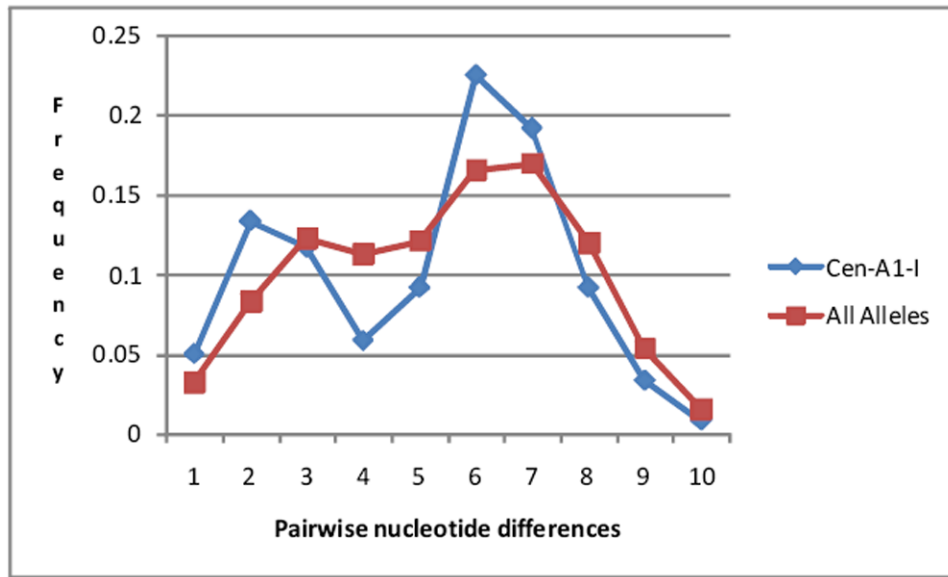


Figure 2. Mismatch distribution of pairwise comparisons of exon 1-5 nucleotide sequences among alleles at the KIR3DL3 locus. Blue is the distribution observed in a comparison of 16 KIR3DL3 alleles that were identified within the Cen-A1-I consensus group. Red is the distribution observed in a comparison of 75 KIR3DL3 alleles.

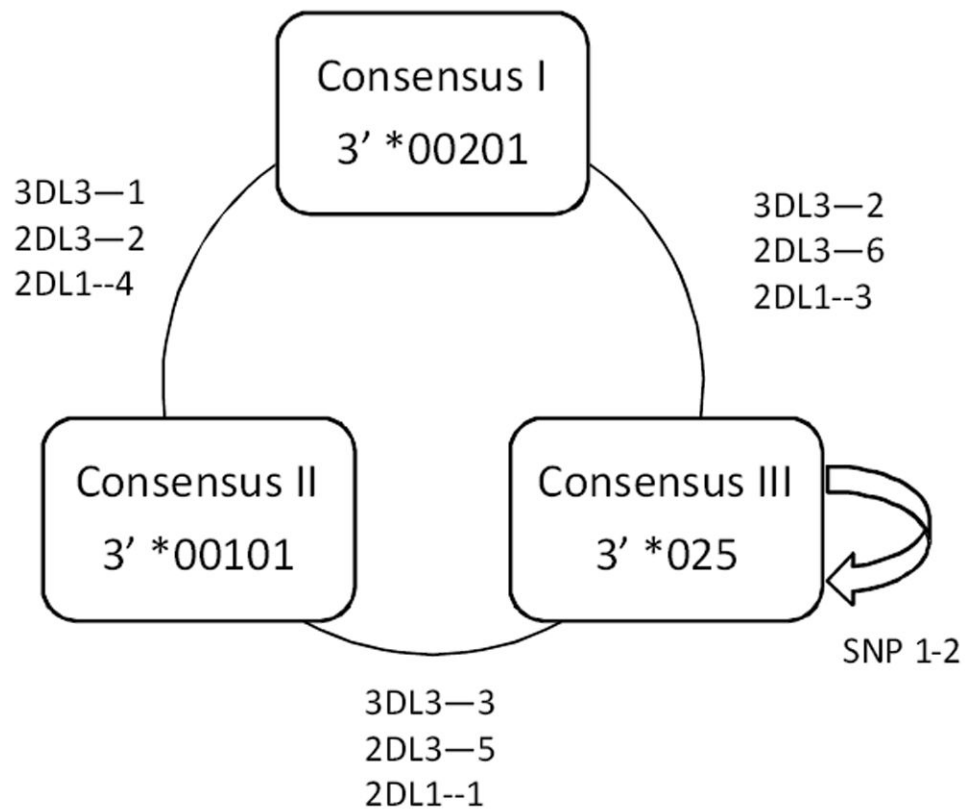


Figure 3.

The 32 Cen-A1 haplotypes described in Table 4 were reduced to ten haplotypes based on the nucleotide sequences of KIR3DL3 exons7-9, KIR2DL3, and KIR2DL1. The evolutionary history among these ten haplotypes was then inferred using the Neighbor-Joining method. The alleles carried by each haplotype are indicated in the figure; for example, CenA2 00101-002-002 refers to the Cen-A1 haplotype in consensus group II that carries KIR3DL3*00101 (exons7-9)-KIR2DL3*002-KIR2DL1*002. Brackets indicate the haplotypes within the three consensus groups; arrows indicate the three consensus haplotypes defining those groups. Haplotypes within each consensus group differ from the consensus by 3 single nucleotide polymorphisms. The consensus haplotypes differ from one another by from seven to 11 single nucleotide polymorphisms. The nucleotide sequences of the alleles at each locus in each Cen-A1 haplotype were concatenated for this analysis. The bootstrap consensus tree was inferred from 1000 replicates. The percentage of replicate trees in which the associated haplotypes clustered together in the bootstrap test is shown next to the branches.

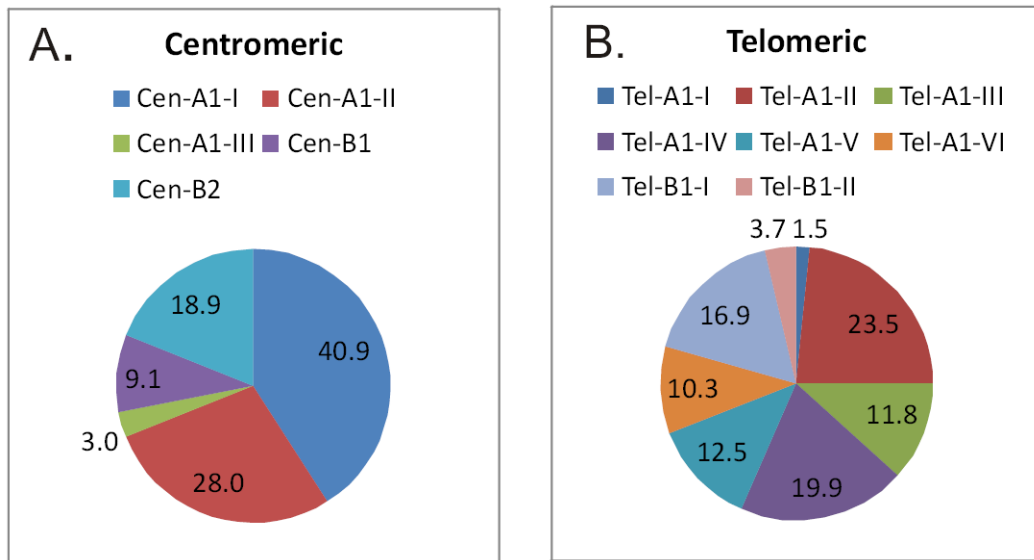


Figure 4. Frequencies of (A.) centromeric and (B.) telomeric consensus haplotypes in 76 individuals of European ancestry. The centromeric structures in two individuals were unclear (labeled “ND”). In addition to common haplotype structures, eight individuals carried extended or contracted haplotypes (labeled as “other”).

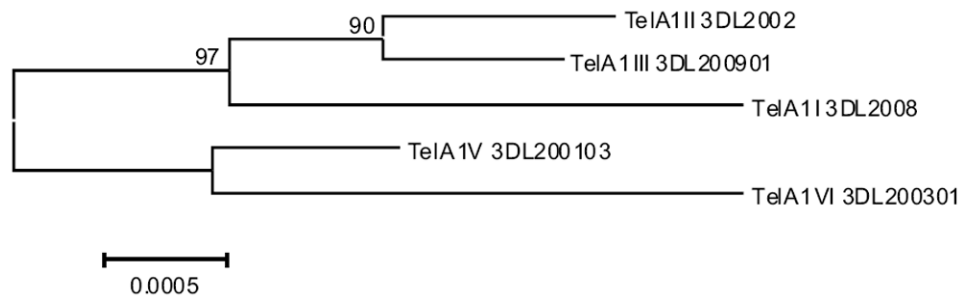


Figure 5.

The evolutionary history among five consensus Tel-A1 haplotypes (I-V) was inferred using the Neighbor-Joining method. The nucleotide sequences of the alleles at each locus in each Tel-A1 consensus haplotype were concatenated for this analysis. The bootstrap consensus tree was inferred from 1000 replicates. The percentage of replicate trees in which the associated haplotypes clustered together in the bootstrap test is shown next to the branches. The sixth consensus haplotype IV was not included because of the presence of interlineage recombinants of KIR3DL1.

Table 1

Genes carried by centromeric and telomeric KIR haplotypes.

Haplotype Structure ¹	Genes Included (centromere to telomere)
Cen-A1	KIR3DL3-KIR2DL3-KIR2DP1-KIR2DL1-KIR3DP1
Cen-B1	KIR3DL3-KIR2DS2-KIR2DL2-KIR2DL5B-KIR2DS3 (or KIR2DS5)-KIR2DP1-KIR2DL1-KIR3DP1
Cen-B2	KIR3DL3-KIR2DS2-KIR2DL2-KIR3DP1
Tel-A1	KIR2DL4-KIR3DL1-KIR2DS4-KIR3DL2
Tel-B1	KIR2DL4-KIR3DS1-KIR2DL5A-KIR2DS3 (or KIR2DS5)-KIR2DS1-KIR3DL2

¹Based on previous studies (15; 18-20) (21) (18).

Table 2

Haplotype structures in 68 individuals with European ancestry¹

Centromeric Haplotype ²	Percent (No. Individuals)	Telomeric Haplotype	Percent (No. Individuals)
Cen-A1/Cen-A1	48.5 (33)	Tel-A1/Tel-A1	61.8 (42)
Cen-A1/Cen-B1	16.2 (11)	Tel-A1/Tel-B1	35.3 (24)
Cen-A1/Cen-B2	26.5 (18)	Tel-B1/Tel-B1	2.9 (2)
Cen-B1/Cen-B1	0		
Cen-B1/Cen-B2	1.5 (1)		
Cen-B2/Cen-B2	4.4 (3)		

¹Eight of the 76 individuals had at least one haplotype that did not conform to the five centromeric and telomeric organizations and are not included in these totals.

²Two of 68 individuals carried either Cen-B1/Cen-B1 or Cen-B1/Cen-B2 and are not included in the centromeric tally. Both cells carried the same centromeric assignments: KIR3DL3*00301,*01402, KIR2DS2*00101, KIR2DL2*001, KIR2DL5B*0020101 (or *0020103), KIR2DS3*00103, KIR2DL1*00401.

Table 3

Nucleotide sequence differences in exons 7-9 among KIR3DL3 alleles

Exons 7-9 ¹	Position of Nucleotide Differences							Observed ² KIR3DL3 Alleles Included
	961	969	971	1042	1074	1119	1119	
00101	A	C	T	G	A	A	A	*00101, *00103, *00602, *01302, *01303, *01306, *026
00102	-	-	-	-	G	-	-	*00102, *00601, *01305, *01307, *017
00201	C	-	-	-	-	-	-	*00201, *00202, *00206, *00207, *005, *00801, *00901, *010, *01102, *015, *030
00301	T	-	C	-	-	-	-	*00301, *007, *01402, *01404, *01405, *016
00902 ³	C	T	-	-	-	-	-	*00902
025	C	-	-	C	-	T	T	*025, *027, *031
029 ³	C	-	A	C	-	T	T	*029

¹The numerical "allele" designation of exons 7-9 is based on the lowest numbered allele with the consensus sequence in exons 7-9.

²Only alleles observed in this study are included in the table.

³KIR3DL3*00902 and KIR3DL3*029 are microvariants of KIR3DL3*00201 and KIR3DL3*025 exon 7-9 sequences, respectively.

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Table 4

Cen-A1 haplotypes¹

Haplotype	3DL3 Exons			No. Observed
	3DL3* 7-9 ²	2DL3*	2DL1*	
I--Consensus	00201	001	00302	57
00901	00201	001	00302	17 ³
00901	00201	001	009	1
00902	00201v ⁴	001	00302	3
00902	00201v	003	00302	1
00202	00201	001	00302	6
00206	00201	001	00302	3 ³
030	00201	001	00302	2
010	00201	001	00302	2 ³
00201	00201	001	00302	2
00207	00201	001	00302	4
00801	00201	001	00302	2
005	00201	001	00302	1
01102	00201	001	00302	1
015	00201	001	00302	2
00101	00101	001	00302	6
01302	00101	001	00302	1
01306	00101	001	00302	2
01303	00101	001	00302	1
II--Consensus	00101	002	002	40
00101	00101	002	002	15 ³
00101	00101	002	008	1
00103	00101	002	002	1
026	00101	002	002	1
01302	00101	002	002	3
00102	00102	002	002	6
00601	00102	002	002	3

Haplotype	3DL3 Exons		2DL3*	2DL1*	No. Observed
	3DL3*	7-9 ²			
01305	00102	002	002	002	3
01307	00102	002	002	002	1
017	00102	002	002	002	6
III-Consensus	025	005	001	001	5
025	025	005	001	001	2
031	025	005	001	001	1
029	025v	005	001	001	1
027	025	005	001	001	1

¹Based on 74 individuals where the centromeric haplotypes could be defined. Two other individuals carrying expected haplotype structures were not included because it was not possible to determine whether they carried Cen-B1/-B1 or Cen-B1/-B2.

²The designation is based on the lowest numbered allele with the consensus sequence in exons 7-9 (Table 3).

³Haplotype previously reported (18).

⁴Blue shading marks alleles that differ by one or two nucleotide changes from the consensus allele. Only the exon 7-9 segment of KIR3DL3 is included in this comparison. The 3' nucleotide sequences of KIR3DL3*00902 (labeled 00201v) and KIR3DL3*029 (labeled 025v) are one nucleotide different from their consensus sequences (Table 3).

Table 5

Cen-B1 haplotypes¹

Haplotype	3DL3		2DS2*	2DL2*	2DL5B*	2DS3*	2DL1*	No. Observed	
	3DL3*	Exons 7-9 ²							
I--Consensus	00301	00301	00101	001	0020101 ³	00103	00401	13	
00301	00301	00101	00101	001	0020101	00103	00401	6 ⁴	
00301	00301	002 ⁵	001	0020102 ⁶	00103	00401	00401	3	
00301	00301	002	001	0020102	00103	00402	00401	1	
00301	00301	002	001	009	00103	00401	00401	1	
01402	00301	00101	005	0020101	00103	00401	00401	1	
007	00301	00101	001	0020101	00103	00401	00401	1	

¹Based on 74 individuals where the centromeric haplotypes could be defined. Two other individuals carrying expected haplotype structures were not included because it was not possible to determine whether they carried Cen-B1/-B1 or Cen-B1/-B2.

²The designation is based on the lowest numbered allele with the consensus sequence in exons 7-9 (Table 3).

³Not distinguished from KIR2DL5B*0020103 in this study.

⁴Haplotype previously observed (18).

⁵Blue shading marks alleles that differ by a single nucleotide change from the consensus allele. Only the exon 7-9 segment of KIR3DL3 is included in this comparison.

⁶KIR2DL5B*0020102 has the same nucleotide coding sequence as the consensus allele.

Table 6

Cen-B2 haplotypes¹

Haplotype	3DL3* Exons 7-9 ²	3DL3 Exons 7-9 ²	2DS2* 00101	2DL2* 003	2DL1 ³ neg	No. Observed
I--Consensus		00301	00101	003	neg	27
00301	00301	00301	00101	003	neg	15 ⁴
01405	00301	00301	00101	003	neg	2
00301	00301	00301	00101	001 ⁵	neg	2
01404	00301	00301	00101	003	neg	1
01402	00301	00301	00101	001	neg	1 ⁴
007	00301	00301	00101	003	neg	5
016	00301	00301	00101	001	neg	1

¹Based on 74 individuals where the centromeric haplotypes could be defined. Two other individuals carrying expected haplotype structures were not included because it was not possible to determine whether they carried Cen-B1/-B1 or Cen-B1/-B2.

²The designation is based on the lowest numbered allele with the consensus sequence in exons 7-9 (Table 3).

³Neg. negative for the gene. Some of these haplotypes were tested for the presence of KIR2DP1 and found to be negative.

⁴Haplotype observed previously (18).

⁵Blue shading marks an allele that differs by two nucleotide changes from the consensus allele. Only the exon 7-9 segment of KIR3DL3 is included in this comparison.

Table 7

Tel-A1 haplotypes⁷

Haplotype	2DL4*	3DL1*	2DS4*	3DL2*	No. Observed
I-Consensus	00102	002	00101	002	35
	00102	002	00101	002	20
	00102	01502 ³	00101	002	11 ²
	00102	01702	00101	021	1
	00103	002	00101	002	3
II-Consensus	00103	008	003	00901	16
	00103	008	003	00901	11 ²
	00103	008	003	019	3
	00103	020	00101 ⁴	00902	2
III-Consensus	00602	007	004	008	3
	00602	007	004	008	3 ²
IV--Consensus	011	00501	007	00103	19
	011	00501	007	00103	14
	011	00501	007	010	5
V--Consensus	00802	00401	006	00301	16
	00802	00401	006	00301	4
	00802	00402	006	00301	1
	00802	00401	006	005	5 ²
	00802	019	006	005	2
	00201	00401	006	005	1
	00802	00401	006	011	2
	00802	00401	006	020	1
VI--Consensus	0080101 ⁵	00101	003	00101	27
	0080101	00101	003	00101	19 ²
	0080104 ⁶	00101	003	00101	1
	0080101	00101	008	00101	1
	0080101	00101	003	011	4

Haplotype	2DL4*	3DL1*	2DS4*	3DL2*	No. Observed
	0080101	0097	003	011	1
	0080101	00101	003	00901	1

¹Based on 76 individuals where the telomeric haplotypes could be defined.

²Allele level haplotype previously observed (18). Five of the haplotypes on this table (without KIR2DS4 typing) were deduced by Norman et al. in a European population as part of a haplotype with a duplication (25).

³Blue shading marks alleles that differ by one or two nucleotide changes from the consensus allele.

⁴KIR2DS4*003 differs from KIR2DS4*00101 by a 22 base pair deletion.

⁵KIR2DL4*0080103 not excluded.

⁶KIR2DL4*0080104 has the same coding sequence as the consensus allele.

⁷KIR3DL1*009 is an interlineage recombinant, sharing the sequences of exons 1-3 with KIR3DS1 and exons 4-9 with KIR3DL1*00101 (41).

Table 8

Tel-B1 haplotypes¹

Haplotype	2DL4*	3DS1*	2DL5A*	2DS3*	2DS5*	2DS1*	3DL2*	No. Observed
I-Consensus	00501	01301	0010101	00201	00201	00201	00701	24
	00501	01301	0010101	00201	00201	00201	00701	21 ²
	00501	01301	0010101	00201	00201	006 ³	00701	1
	00501	01301	0010101	00201	00201	00501	00701	1
	00501	01301	0010101	00201	00201	00201	018	1 ²
II-Consensus	00501	01301	0050101	002	00201	00201	00701	5
	00501	01301	0050101	002	00201	00201	00701	4 ²
	00501	01301	0050102 ⁴	002	00201	00201	00701	1

¹ Based on 76 individuals where the telomeric haplotypes could be defined.

² Allele level haplotype previously reported (18).

³ Blue shading marks alleles that differ by one to three nucleotide changes from the common allele in the cluster.

⁴ KIR2DL5A*0050102 has the same coding region sequence as the consensus allele.

Table 9

Gene contracted or expanded KIR haplotypes

Sample ID	Haplotype ¹	Reference	Fusion Gene(s)
Contracted			
RDP27	3DL3*00301--2DS2*002--2DL2*001--2DL5B*0020102--2DS3*002--2DS1*00201--3DL2*00701 ²	(16)	KIR2DS3*002 (2DS3/2DS5)
RDP69	3DL3*00301--2DS2*00101--2DL2*001--2DL5B*008--2DS5*00201--2DS1*00201--3DL2*00701 ²	(21)	KIR2DL5B*008 (2DL5B/2DL5A)
RDP71	3DL3*00602--2DL3/2DP1--2DS1*00502--3DL2*017 ²	(23)	KIR2DL3/KIR2DP1 KIR2DS1*00502 ³ (2DL1/2DS1)
RDP76	3DL3*00301--2DS2*005--2DP1--2DL1*00401--3DP1--known telomeric region	(21)	KIR2DS2*005 (2DS2/2DS3)
Expanded			
RDP10	Known centromeric region--2DL4*00501--3DS1*01301--2DL5A/3DP1--2DL4*00102--3DL1*002--2DS4*00101--3DL2*002	(22;24;44)	KIR2DL5A/3DP1
RDP64	Known centromeric region--2DL4*00501--3DS1*01301--2DL5A/3DP1--2DL4*0080101/0080103--3DL1*00101--2DS4*003--3DL2*009variant ⁴		
RDP81	3DL3*015--2DL3*001--[2DP1 ⁵ --2DL1*00302--[3DP1]--2DL4*00501--3DS1*01301--2DL5A*0050101--2DS3*00103--[2DP1]--2DL1*00401--[3DP1]--2DL4*00501--3DS1*01301--2DL5A*0010101--2DS5*002--2DS1*00201--3DL2*00701	(16)	
RDP14	3DL3*015--2DL3*001--[2DP1]--2DL1*00302--[3DP1]--2DL4*00501--3DS1*01301 (or *058)--2DL5A*0050101--2DS3*00103--[2DP1]--2DL1*00401--[3DP1]--2DL4*00501--3DS1*058 (or *01301)--2DL5A*0010101--2DS5*002--2DS1*00201--3DL2*00701		

¹The order of the genes based on physical linkage of alleles, by known allele-level haplotype observed in this study, and/or on previous literature.

²These haplotypes do not appear to carry intact KIR2DP1 and KIR3DP1 genes.

³KIR2DS1*00502 had previously been named as KIR2DS1*007.

⁴Allele designation requested, not yet assigned.

⁵Positions of KIR2DP1 and KIR3DP1 based on Ordonez et al. (16)

Table 10

Immune response profile¹ of KIR Haplotypes

Centromeric									
Gene	3DL3	2DS2	2DL2	2DL3	2DL1	2DL5B	2DS3		
Ligand	?	?	HLA-Cg1/HLA-Cg2 (affinity for Cg2 is weak)	HLA-Cg1/HLA-Cg2 (ligand affinity lower than 2DL2)	HLA-Cg2	?	?		?
Cen-A1-I	++ ²	Absent	Absent	Weak (001) ³	Strong (00302)	Absent	Absent		Absent
Cen-A1-II	++	Absent	Absent	++ (002)	Strong (002)	Absent	Absent		Absent
Cen-A1-III	++	Absent	Absent	++ (005)	Strong (001)	Absent	Absent		Absent
Cen-B1	++	++	Strong (001)	Absent	Weak (00401)	Not expressed (0020101)	Misfolded (00103)		
Cen-B2	++	++	Strong (003)	Absent	Absent	Absent	Absent		Absent

Telomeric												
Gene	2DL4	3DS1	3DL1	2DS4	2DS1	3DL2	2DL5A	2DS3	2DS5			
Ligand	HLA-G	?	HLA-Bw4	HLA-A*1102 C subset	HLA-Cg2	HLA-A3,11, B27d; CpG	?	?	?			
Tel-A1-I	++ (00102)	Absent	Intermediate (01502)	++ (00101)	Absent	++	Absent	Absent	Absent			
Tel-A1-II	++ (00103)	Absent	Intermediate (020)	Secreted (003)	Absent	++	Absent	Absent	Absent			
Tel-A1-III	++ (00602)	Absent	Weak (007) ^a	Secreted (004)	Absent	++	Absent	Absent	Absent			
Tel-A1-IV	Secreted (011)	Absent	Strong (00501)	Secreted (007)	Absent	++	Absent	Absent	Absent			
Tel-A1-V	Secreted (00802)	Absent	Misfolded (00401)	Secreted (006)	Absent	++	Absent	Absent	Absent			
Tel-A1-VI	Secreted (0080101)	Absent	Strong (00101)	Secreted (003)	Absent	++	Absent	Absent	Absent			
Tel-B1-I	++ (00501)	++	Absent	Absent	Weak	++	++ (0010101)	Absent	++			
Tel-B1-II	++ (00501)	++	Absent	Absent	Weak	++	++ (0050101)	Misfolded (002)	Absent			

¹The characteristics of KIR allelic products and their ligands have been described: KIR2DL1 (51;73), KIR2DL2/KIR2DL3 (28), KIR2DL4 (11;55), KIR2DL5 (42), KIR2DS1 (52), KIR2DS3 (54), KIR2DS4 (9;56;74), KIR3DL1 (29;57), KIR3DL2 (6;75;76).

²++ indicates gene is present for loci in which the functional impact of allelic variation has not been characterized.

³Alleles are given in parentheses.