Immunogenetics (2001) 52:195–205
DOI 10.1007/s002510000281

ORIGINAL PAPER

**Paul J. Norman · Henry A.F. Stephens**
**David H. Verity · Dasnayanee Chandanayingyong**
**Robert W. Vaughan**

# Distribution of natural killer cell immunoglobulin-like receptor sequences in three ethnic groups

Received: 21 September 2000 / Revised: 7 November 2000 / Published online: 3 January 2001


**Abstract** Killer cell immunoglobulin-like receptors (KIRs) are members of a group of molecules that specifically recognize HLA class I ligands and are found on subsets of human lymphopoetic cells. The number of *KIR* loci can vary between individuals, resulting in a heterogeneous array of possible *KIR* genes. The range of observed profiles has been explained by the occurrence of two haplotype families termed A and B which can be distinguished on the basis of certain *KIR* sequences. Here we attempted to determine whether the frequencies of putative *KIR* loci and the two haplotype groups vary in three ethnically defined, healthy, and unrelated control populations, namely UK Caucasoid ($n$=136), Palestinian ($n$=105) and Thai ($n$=119). We molecularly typed genomic DNA for the presence of 12 putative *KIR* loci, *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR3DL1*, *KIR3DL2*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, and *KIR3DS1*, using modified PCR sequence-specific primers. The patterns of *KIR* locus frequencies combined with the similar linkage disequilibrium values suggest that there was a distinction in the distribution of the two broad haplotype groups between the populations studied. The A haplotype was always the most prevalent, but the ratio of A to B varied between populations. The frequency of B haplotype was highest in the Palestinians and lowest in the Thais ($P_c$<0.0001).

**Keywords** KIR · PCR-SSP · Population study · *KIR* locus profiles · *KIR* haplotypes

P.J. Norman (✉) · H.A.F. Stephens · R.W. Vaughan
South Thames Tissue Typing, 3rd Floor New Guy's House,
Guy's Hospital, St. Thomas' Street, London, SE1 9RT, UK
E-mail: paul.norman@kcl.ac.uk
Phone: +44-207-9554819
Fax: +44-207-4076370

H.A.F. Stephens
Institute of Urology and Nephrology,
University College Hospital Medical School, London, UK

D.H. Verity
Department of Ophthalmology, St Thomas' Hospital,
London, UK

D. Chandanayingyong
Department of Transfusion Medicine, Faculty of Medicine,
Siriraj Hospital, Bangkok, Thailand

## Introduction

Natural killer (NK) cells have been defined by their ability to spontaneously lyse autologous targets such as certain tumor or virally infected cells and allogeneic transplanted cells (Trinchieri 1989). The interaction of killer cell immunoglobulin-like receptors (KIRs) with HLA class I molecules on target cells contributes to regulation of NK cellular activity (Colonna et al. 1992; D'Andrea et al. 1996; Moretta et al. 1993). KIRs can be expressed on classical NK cells and subpopulations of T cells (Falk et al. 1995; Mingari et al. 1996; Phillips et al. 1995), and have been shown to interact directly with the α helices and bound peptide of their HLA ligands (Boyington et al. 2000). This interaction possibly forms an "immune synapse" during immunosurveillance (Davis et al. 1999; Vales-Gomez et al. 1998a). KIRs have evolved recently in primates (Khakoo et al. 2000; Martin et al. 2000) despite other preexisting NK receptors, such as CD94/NKG2-A and -C which also interact with HLA. CD94/NKG2 is structurally distinct from KIRs but performs an apparently similar function (Braud et al. 1998; Brooks et al. 1997; Lee et al. 1998).

In vitro ligation of different isotypes of KIR by HLA molecules can lead to either inhibition or activation of cytotoxic cell activity (Campbell et al. 1996; Fry et al. 1996; Moretta et al. 1995). Inhibition can override activation, ostensibly as a result of a higher binding affinity of the inhibitory receptors (Biassoni et al. 1997; Vales-Gomez et al. 1998b). These inter-

actions, together with many other regulating events, are presumed to ensure that NK cells are prevented from killing healthy autologous cells while retaining the ability to remove those with abnormal HLA class I expression.

### *KIR diversity*

Within the human population, diversity for KIR is achieved via a combination of variable gene content and allelic polymorphism within these loci (Selvakumar et al. 1997a; Uhrberg et al. 1997). Alternative splicing and differential expression of KIRs within an individual can generate a high somatic diversity of NK cells (Dohring et al. 1996a; Valiante et al. 1997; Wagtmann et al. 1995). Many *KIR* sequences and cDNAs are currently available; although their genetic relationships are not completely characterized, their genomic organization is becoming apparent (Wende et al. 1999; Wilson et al. 1997, 2000). *KIR* genes are positioned on Chromosome 19q13.4 (Baker et al. 1995; Suto et al. 1998). There may be at least 12 distinct loci present in proximity to several genes of similar structure (Borges et al. 1997; Samaridis and Colonna 1997; Wende et al. 2000). These *KIR* sequences have been formed by a combination of duplication events, point mutations, and recombinations (Khakoo et al. 2000; Martin et al. 2000; Shilling et al. 1998).

KIRs have been divided into five families, based on the number of external immunoglobulin (Ig) domains (2D or 3D) and the characteristics of their cytoplasmic domains (see www.ncbi.nlm.nih.gov/PROW). KIRs with three Ig domains have been shown to interact with some HLA-A and HLA-B alleles (Biassoni et al. 1995; Dohring et al. 1996b; Litwin et al. 1994; Pende et al. 1996), and those including two Ig subunits to interact differentially with HLA-Cw or HLA-G (Colonna et al. 1993; Rajagopalan and Long 1999). Irrespective of the number of Ig subunits, the cytoplasmic domain of KIRs can be long and inhibitory because they incorporate immunoreceptor tyrosine-based inhibition motifs (ITIMs), or they can be truncated and activating. Further subclassification is used for products whose genes potentially differ by at least 20 nucleotides from their nearest relatives, which results in 6 inhibitory and 6 activating KIR corresponding to the 12 putative loci (Steffens et al. 1998). However, while this classification system is useful for perceiving the structural and functional differences between KIRs, the sequences corresponding to separate loci and those attributable to allelic variants in the strictest sense have not been entirely confirmed.

### *KIR haplotypes*

The range of gene content observed for the *KIR* region has been explained by the occurrence of several *KIR* haplotypes. According to PCR typing and restriction fragment length polymorphism analysis, two major haplotype groups, A and B, have been proposed to be segregating within the human population (Uhrberg et al. 1997). Additional evidence for the polygenicity and the presence of these haplotypes has been obtained from extensive sequencing and restriction mapping of the region (Wende et al. 2000; Wilson et al. 2000). The two haplotype groups may be defined by the presence of apparently mutually exclusive members of the *KIR2DL* family. Thus a haplotype encompassing *KIR2DL1* and *KIR2DL3* is termed A, while one including *KIR2DL2* is termed B (Uhrberg et al. 1997; Witt et al. 1999). Frequencies of the 12 putative *KIR* loci in Caucasoid populations as well as estimates for linkage disequilibrium between pairwise combinations have been described (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999).

Over 40 different profiles of *KIR* sequences possessed by individuals have been reported so far, in three Caucasoid populations (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999). KIRs are thought to have evolved relatively recently (Wilson et al. 2000) and appear divergent between primate species even when recognizing identical MHC epitopes (Khakoo et al. 2000; Martin et al. 2000). The prevalence of different *KIR* locus haplotypes may therefore be expected to vary between human populations.

In this study we used a PCR sequence-specific priming (SSP) scheme modified after Uhrberg and coworkers (1997) to study the frequencies and profiles of *KIR* present in the genomic DNA of individuals from three ethnically distinct populations, European Caucasoid, Palestinians from the Middle East, and Thais from mainland South-East Asia. We studied only putative *KIR* genes that have an expressed product (Selvakumar et al. 1997b; Uhrberg et al. 1997); thus, the *KIR* locus profiles we have deduced may represent a true KIR phenotype profile. We show that the most frequent *KIR* profile observed in our three populations was the same as that found in the original Caucasoid studies (Crum et al. 2000; Uhrberg et al. 1997; Witt et al. 1999) and we found a number of novel patterns. We also provide evidence that the two broad haplotypes for this region may be present at different frequencies in the three ethnic groups.

## Materials and methods

### *Populations*

The UK Caucasoid population consisted of 136 randomly selected unrelated individuals from south-east England. The unrelated individuals from the Palestinian ($n$=105) and Thai ($n$=119) populations were collected as previously described (Chandanayingyong et al. 1997; Verity et al. 1999).

**Table 1** *KIR* amplification primer combinations (* from Uhrberg et al. 1997; ** after Selvakumar et al. 1997b)

| Target *KIR* | Sense (5′–3′) | Antisense (5′–3′) | Product length (base pairs) |
|---|---|---|---|
| *2DL1** | ACTCACTCCCCCTATCAGG | AGGGCCCAGAGGAAAGTCA | 1790 |
| *2DL2** | CCATGATGGGGTCTCCAAA | GCCCTGCAGAGAACCTACA | 1810 |
| *2DL3** | CCTTCATCGCTGGTGCTG | CAGGAGACAACTTTGGATCA | 800 |
| *2DL4*** | GCCAGTGGGGAAGCCCATGAACT | ACCAACCTGTGACAGAAACAG | 175 |
| *3DL1** | CCATCGGTCCCATGATGCT | AGAGAGAAGGTTTCTCATATG | 1690 |
| *3DL2** | CGGTCCCTTGATGCCTGT | GACCACACGCAGGGCAG | 1950 |
| *2DS1** | TCTCCATCAGTCGCATGAA/G | AGGGCCCAGAGGAAAGTT | 1830 |
| *2DS2** | TGCACAGAGAGGGGAAGTA | CACGCTCTCTCCTGCCAA | 1770 |
| *2DS3* | GACATGTACCATCTATCCAC | GCATCTGTAGGTTCCTCCT | 130 |
| *2DS4** | CTGGCCCTCCCAGGTCA | GGAATGTTCCGTTGATGC | 2000 |
| *2DS5* | AGAGAGGGGACGTTTAACC | TCCGTGGGTGGCAGGGT | 1920 |
| *3DS1** | GGCAGAATATTCCAGGAGG | AGGGGTCCTTAGAGATCCA | 1760 |

*Polymerase chain reaction*

Genomic DNA was extracted from peripheral blood cells by the salting-out method (Miller et al. 1988). PCR-SSP primers for the detection of *KIR* loci in genomic DNA were used as detailed in Table 1. This gives a profile of the *KIR* sequences (or putative loci) possessed by each individual.

The amplification temperatures and conditions were optimized and then used as follows: 10-μl reactions were set up to include 0.1 μg test DNA, buffer IV, 0.2 mM dNTPs, 0.08 mM magnesium chloride, 0.4 units *Taq* DNA polymerase (all Advanced Technologies, UK), and 0.5 μM specific primer mix (except for 3DL1 and 2DS4 which were at a final concentration of 1 μM). Internal controls (forward 5′-CAGTGCCTTCCC AACCATTCCCTTA-3′, reverse 5′-ATCCACTCACGGATTT CTGTTGTGTTTC-3′) specific for a 485-bp human growth hormone gene fragment were included at 0.13 μM in each reaction. All amplifications were performed in a single Perkin Elmer 9600 thermal cycler programmed with a 5-min denaturing step at 94 °C, 10 cycles of 94 °C 10 s, 65 °C 60 s, then 20 cycles of 94 °C 10 s, 61 °C 50 s, 72 °C 30 s. The products were photographed from standard 1% agarose electrophoresis gels containing ethidium bromide. All amplifications were performed in duplicate and any individual who displayed a profile that was unique, or that had not been previously reported was repeated at least once.

PCR-sequence-specific oligonucleotide probe (SSOP) typing for *KIR* sequences was performed exactly as described by Crum and co-workers (2000) on selected individuals. These individuals were also tested for the variant *KIR2DL1v* using the primers 2DL1vf 5′-ACTCACTCCCCCTATCAGG-3′ and 2DL1vr 5′-A GGGCCCAGAGGAAAGTT-3′ (product length ~1750 bp), under the same PCR-SSP reaction conditions as above.

None of the SSP primer combinations for any of the reactions described would theoretically react with the newly characterized *KIR2DL5* (AF204903) or with *KIR3DL3* (AF105233) or any of the known sequences for neighboring pseudogenes according to BLAST searches performed during the study period.

*Statistical analysis*

Observed frequencies of *KIR* occurrence were determined by counting. *KIR* locus frequencies (KLFs) for the putative loci were estimated by the formula KLF=1-$\sqrt{(1-f)}$, where $f$ is the observed frequency of a particular *KIR* sequence in a population. Differences between populations were compared by $\chi^2$, and Yates-corrected *P*-values were subjected to correction for the number of loci investigated ($n$=12).

The linkage disequilibrium parameters ($\Delta$) for two-locus associations were calculated according to Mattiuz and co-workers (1971). This absolute $\Delta$ should not be used comparatively for different pairs of loci; therefore the relative linkage disequilibrium was calculated for each pair using the $\Delta_{max}$ and the observed two-locus haplotype frequency (Baur and Danilovs 1980). The differences between the observed and expected haplotype frequencies were compared by Yates' $\chi^2$ contingency. If a pair of *KIR* is seen with significantly positive linkage disequilibrium and a relatively high two-locus haplotype frequency, then they are likely to be separate genes. Negative linkage disequilibrium values may be indicative of an allelic relationship. Similar methods for estimating the locus frequencies and linkage disequilibrium values had been used previously for *KIR* sequences (Crum et al., 2000; Witt et al. 1999).

## Results

*New SSP pairs*

An alternative primer set for *KIR2DS3* (Table 1) was devised to obviate poor amplification using primers described by Uhrberg and co-workers (1997) under the reaction conditions we describe. Positive reactions correlated precisely with those obtained using the *KIR2DS3* primers from Uhrberg and co-workers (1997) (data not shown) and the resulting gene frequencies for the Caucasoid sample were comparable to other studies on Caucasoid populations (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999). New primers (Table 1.) were designed for *KIR2DS5* because no positive reactions had been observed in previous studies (Uhrberg et al. 1997; Witt et al. 1999); the published primers were suspected to have been designed around a polymorphism atypical for this isotype (confirmed by M. Curran, personal communication and later by Wilson et al. 2000). Furthermore, a selection of positive reactions for *KIR2DS3* and *KIR2DS5* were confirmed by SSOP (data not shown). The frequency of *KIR2DS5* we observed in our Caucasoid population (32% of individuals) was comparable to that reported by Crum and co-workers (35%).

**Table 2** Observed *KIR* frequencies and estimated gene frequencies for Caucasoid, Palestinian, and Thai control populations. $P_c$ is calculated from 2×3 contingency of estimated chromosomes with that locus, corrected for the number of *KIR* investigated ($n$=12) and shown with highest and lowest frequencies compared. No *KIR2DS5* was detected in any of the 360 individuals using the primers of Uhrberg and co-workers (1997)

| | | Inhibitory KIR | | | | | | Activating KIR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *2DL1* | *2DL2* | *2DL3* | *2DL4* | *3DL1* | *3DL2* | *2DS1* | *2DS2* | *2DS3* | *2DS4* | *2DS5* | *3DS1* |
| Caucasoid | % | 91 | 49 | 92 | 100 | 97 | 100 | 45 | 51 | 24 | 96 | 32 | 42 |
| ($n$=136) | Estimated gene frequency | 0.70 | 0.28 | 0.72 | 1.00 | 0.83 | 1.00 | 0.26 | 0.30 | 0.13 | 0.79 | 0.18 | 0.24 |
| Palestinian | % | 83 | 62 | 85 | 100 | 88 | 100 | 44 | 64 | 37 | 88 | 27 | 39 |
| ($n$=105) | Estimated gene frequency | 0.59 | 0.38 | 0.61 | 1.00 | 0.65 | 1.00 | 0.25 | 0.40 | 0.21 | 0.65 | 0.14 | 0.22 |
| Thai ($n$=119) | % | 97 | 42 | 97 | 100 | 93 | 100 | 42 | 44 | 25 | 87 | 23 | 44 |
| | Estimated gene frequency | 0.82 | 0.24 | 0.82 | 1.00 | 0.74 | 1.00 | 0.24 | 0.25 | 0.14 | 0.64 | 0.12 | 0.25 |
| $\chi^2$ (2×3) | | 20.4 | 11.1 | 23.3 | | 20.4 | | | 12.4 | | 17.04 | | |
| $P_c$ | | <0.00001 | <0.04 | <0.0001 | | <0.001 | | | <0.03 | | <0.003 | | |

*Estimated KIR locus frequencies*

The percentage of individuals possessing each particular *KIR* sequence is shown for all three populations in Table 2, together with estimates for the frequencies of the putative *KIR* loci (KLFs). All 12 *KIR* sequences investigated were represented in each of the populations. There was some variation in most KLFs between populations, except for the inhibitory KIR loci *KIR2DL4* and *KIR3DL2*, which were detected in all 360 individuals tested. Two noninhibitory KIR loci, *KIR2DS3* and *KIR2DS5*, were the least frequent loci for all three populations (KLF 0.13–0.21 and 0.12–0.18, respectively).

Significant ($P_c$<0.05) variation in the estimated frequencies occurred for the following loci. *KIR2DL1* and *KIR2DL3* were present at the lowest frequency in the Palestinians (KLF=0.59 and 0.61, respectively) and highest in the Thais (both KLFs 0.82). *KIR3DL1* was at the lowest level in the Palestinians (KLF=0.65) and highest in the Caucasoids (KLF=0.83). *KIR2DL2* and *KIR2DS2* occurred most frequently in the Palestinians (KLF=0.38 and 0.40, respectively) and least in the Thais (KLF=0.24 and 0.25, respectively). *KIR2DS4*, which was the most frequent locus encoding an activating KIR in all populations, was at the highest frequency in the Caucasoid population (KLF=0.79 compared with 0.65 for Palestinians and 0.64 for Thais). *KIR2DS3* was at the highest level in the Palestinians, and *KIR2DS5* was highest in the Caucasoids, although these last two observations did not reach statistical significance.

*Profiles of KIR sequences possessed by individuals*

A total of 49 different *KIR* locus profiles in the genomic DNA from 360 individuals were observed by PCR SSP typing (Fig. 1), 28 of which have not been previously reported. Eleven individuals possessed all 12 tested *KIR* (profile AB4.1) and these included representatives from all three populations. The average number of *KIR* loci per individual ($n$=8), and the modal quantity ($n$=6) did not vary between populations. AA1, which contains 6 *KIR* loci was the most common profile in all three populations.

The addition of primers for *KIR2DS5* into this scheme further resolved six of the previously reported KIR profiles (Fig. 1) (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999). *KIR2DS5* was also seen appended to another of the previously documented profiles, AB7, although it is not possible at this stage to determine whether this was novel.

Both novel and unique profiles were seen in each population; 17 of the novel profiles were unique to one of the three populations, and 10 of the novel profiles were shared between two or more populations (Fig. 1). The novel profiles described occurred at low frequencies; those accounting for more than 2% of the total individuals studied were AA2.1 (4.7%), AB3.1 (3%), AB4.1 (3%), and BB102 (2.2%). All of the profiles that were unique to populations were seen in less than 2% of the respective population, apart from profile C101 which was seen in 2.8% of Palestinians.

In the Thai population, five unique profiles were observed: two were novel and all were of the AA type. Two of the seven profiles seen as unique in our Caucasoid group had been previously documented in Caucasoid populations, whereas only 1 of the 11 profiles unique in this study to the Palestinian population had been previously described (Witt et al. 1999).

Seven individuals were found who tested positive for *KIR2DL2* and negative for either *KIR2DL1* ($n$=5) or *KIR2DL3* ($n$=2) but not both (Fig. 1), and thus may not correspond to either of the two major haplotype groups for at least one chromosome. These individuals were tested for *KIR2DL1* or *KIR2DL3* (respectively) by SSOP and were shown to be positive for the loci in question by this method alone. These individuals may represent novel *KIR* locus profiles.

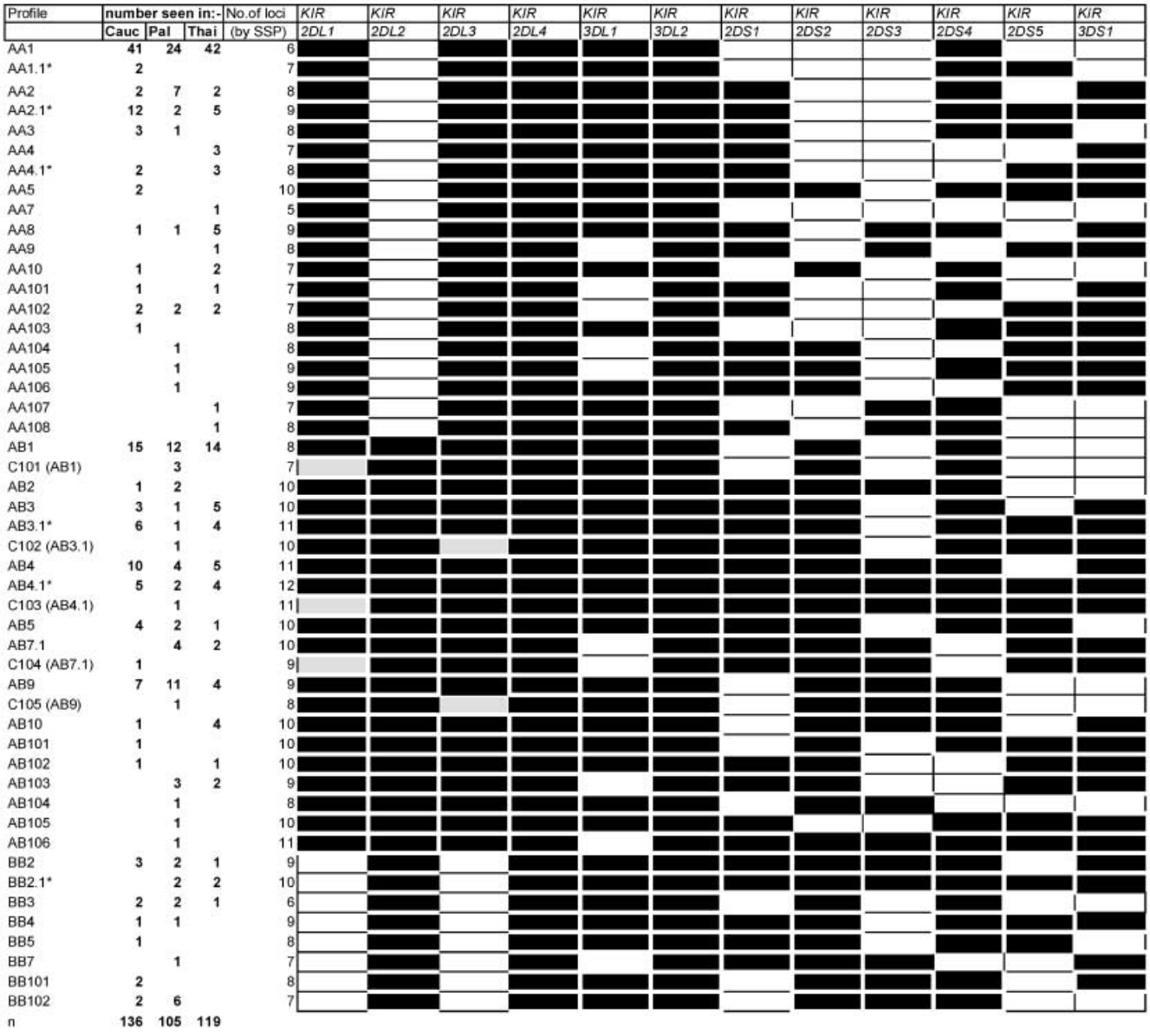


**Fig. 1** *KIR* locus profiles observed and the number of individuals displaying each profile (*black box* presence of KIR sequence, *white box* absence of KIR sequence, *gray box* absent by SSP but detected by SSOP). The nomenclature system from Witt and co-workers (1999) is used for describing these patterns. Profiles including *KIR2DL1* and *KIR2DL3* but not *KIR2DL2* are termed AA, vice versa are BB, and profiles including all three are termed AB; C is used to describe those with one of *KIR2DL1* or *KIR2DL3*; any novel profiles are denoted here by a three-figure suffix (* profiles separated by *KIR2DS5* from those previously documented)

However, if they do possess allelic variants of the loci not detected by SSP, then they would all conform to previously characterized *KIR* locus profiles (shown in parentheses in Fig. 1). To clarify some of the discrepant results between SSP and SSOP for these individuals, we tested the five individuals who were positive for *KIR2DL1* by SSOP only for the variant *KIR2DL1v* (Shilling et al. 1998). Using a PCR-SSP assay with a combination of the forward primer for *KIR2DL1* and reverse for *KIR2DS1* (this primer mixture did not cross-react with any of the known *KIR*) we concluded that four of the five individuals who appeared to lack *KIR2DL1* initially were positive for *KIR2DL1v* (data not shown).

### *Linkage disequilibrium for pairs of KIR loci detected by PCR-SSP*

Estimates for the two-locus haplotype frequencies, linkage disequilibrium parameter $\Delta$, and the relative magnitude of the linkage disequilibrium observed for each pertinent *KIR* locus pair are shown in Table 3. A summary of the most significant associations is shown in Table 4.

**Table 3** Linkage disequilibrium parameters for pairs of *KIR* sequences in Caucasoid (*Cauc*), Palestinian (*Pal*), and Thai populations. [*Δ* linkage disequilibrium parameter, *r* relative linkage disequilibrium (1=threshold), *h* estimated two-locus haplotype frequency (only shown when at least 0.02), *P* indicated where appropriate]

| | | *2DL2* | | | *2DL3* | | | *3DL1* | | | *2DS1* | | | *2DS2* | | | *2DS3* | | | *2DS4* | | | *2DS5* | | | *3DS1* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai | Cauc | Pal | Thai |
| 2DL1 | *Δ* | –0.20 | –0.23 | –0.14 | 0.20 | 0.22 | 0.15 | –0.05 | –0.04 | –0.05 | 0.00 | 0.01 | –0.05 | –0.20 | –0.23 | –0.14 | –0.08 | –0.10 | –0.07 | –0.06 | –0.04 | –0.07 | 0.02 | 0.01 | –0.03 | –0.03 | 0.00 | –0.05 |
| | *r* | 1 | 0.98 | 0.72 | 1 | 0.94 | 1 | 0.08 | 0.09 | 0.08 | 0.01 | 0.10 | 0.25 | 0.94 | 0.92 | 0.67 | 0.82 | 0.81 | 0.61 | 0.11 | 0.09 | 0.12 | 0.46 | 0.27 | 0.32 | 0.15 | 0.02 | 0.23 |
| | *h* | | | 0.06 | 0.72 | 0.60 | 0.82 | 0.54 | 0.37 | 0.56 | 0.18 | 0.17 | 0.15 | | 0.02 | 0.07 | | 0.02 | 0.04 | 0.51 | 0.37 | 0.46 | 0.15 | 0.10 | 0.07 | 0.15 | 0.13 | 0.16 |
| | *P* | <<0.0001 | <<0.0001 | <0.0001 | <<0.0001 | <0.0001 | <0.001 | | | | | | | <<0.0001 | <<0.0001 | <0.0001 | <0.001 | <0.001 | <0.01 | | | | | | | | | |
| 2DL2 | *Δ* | | | | –0.20 | –0.24 | –0.14 | 0.03 | –0.02 | –0.01 | 0.04 | 0.02 | 0.04 | 0.20 | 0.22 | 0.18 | 0.09 | 0.12 | 0.06 | 0.02 | –0.02 | 0.02 | –0.01 | 0.02 | 0.03 | 0.04 | 0.01 | 0.06 |
| | *r* | | | | 1 | 1 | 0.72 | 0.53 | 0.09 | 0.08 | 0.23 | 0.10 | 0.23 | 1 | 0.97 | 1 | 0.94 | 0.94 | 0.56 | 0.35 | 0.09 | 0.23 | 0.17 | 0.27 | 0.31 | 0.24 | 0.04 | 0.32 |
| | *h* | | | | | | 0.06 | 0.26 | 0.23 | 0.16 | 0.12 | 0.11 | 0.10 | 0.28 | 0.37 | 0.24 | 0.12 | 0.20 | 0.09 | 0.24 | 0.23 | 0.17 | 0.04 | 0.08 | 0.06 | 0.11 | 0.09 | 0.12 |
| | *P* | | | | <<0.0001 | <<0.0001 | <0.0001 | | | | | | | <<0.0001 | <<0.0001 | <<0.0001 | <0.001 | <0.001 | 0.02 | | | | | | | | | 0.04 |
| 2DL3 | *Δ* | | | | | | | –0.05 | –0.04 | –0.05 | 0.00 | 0.00 | –0.05 | –0.20 | –0.23 | –0.14 | –0.08 | –0.11 | –0.07 | –0.06 | –0.04 | –0.07 | 0.02 | 0.00 | –0.03 | –0.03 | –0.01 | –0.05 |
| | *r* | | | | | | | 0.08 | 0.10 | 0.08 | 0.01 | 0.00 | 0.25 | 0.94 | 0.97 | 0.67 | 0.82 | 0.91 | 0.61 | 0.11 | 0.10 | 0.12 | 0.46 | 0.07 | 0.32 | 0.15 | 0.09 | 0.23 |
| | *h* | | | | | | | 0.54 | 0.36 | 0.56 | 0.18 | 0.15 | 0.15 | | 0.07 | | 0.02 | 0.01 | 0.04 | 0.51 | 0.36 | 0.46 | 0.15 | 0.09 | 0.07 | 0.15 | 0.12 | 0.16 |
| | *P* | | | | | | | | | | | | | <<0.0001 | <<0.0001 | <0.0001 | <0.001 | <0.0001 | <0.01 | | | | | | | | | |
| 3DL1 | *Δ* | | | | | | | | | | –0.13 | –0.26 | –0.20 | 0.03 | –0.07 | –0.01 | 0.00 | –0.02 | –0.02 | 0.11 | 0.20 | 0.15 | –0.06 | –0.20 | –0.14 | –0.13 | –0.27 | –0.19 |
| | *r* | | | | | | | | | | 0.60 | 1 | 1 | 0.55 | 0.29 | 0.06 | 0.01 | 0.15 | 0.19 | 0.83 | 0.88 | 0.90 | 0.38 | 1 | 1 | 0.66 | 1 | 1 |
| | *h* | | | | | | | | | | 0.09 | | | 0.28 | 0.18 | 0.17 | 0.11 | 0.11 | 0.08 | 0.77 | 0.62 | 0.63 | 0.09 | | | 0.07 | | |
| | *P* | | | | | | | | | | <0.0001 | <<0.0001 | <<0.0001 | | | 0.05 | | | | <0.01 | <0.0001 | <0.01 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <<0.0001 |
| 2DS1 | *Δ* | | | | | | | | | | | | | 0.05 | 0.03 | 0.04 | 0.03 | 0.02 | 0.05 | –0.16 | –0.17 | –0.18 | 0.11 | 0.11 | 0.09 | 0.15 | 0.16 | 0.17 |
| | *r* | | | | | | | | | | | | | 0.26 | 0.18 | 0.19 | 0.36 | 0.15 | 0.50 | 0.77 | 1 | 1 | 0.85 | 1 | 1 | 0.86 | 1 | 0.94 |
| | *h* | | | | | | | | | | | | | 0.12 | 0.13 | 0.10 | 0.07 | 0.07 | 0.08 | 0.05 | | | 0.16 | 0.14 | 0.12 | 0.21 | 0.22 | 0.23 |
| | *P* | | | | | | | | | | | | | | | | | | 0.03 | <<0.0001 | <<0.0001 | <<0.0001 | <0.0001 | <0.001 | <0.001 | <<0.0001 | <0.0001 | <<0.0001 |
| 2DS2 | *Δ* | | | | | | | | | | | | | | | | 0.09 | 0.12 | 0.06 | 0.02 | –0.07 | 0.02 | 0.00 | 0.04 | 0.03 | 0.05 | 0.02 | 0.05 |
| | *r* | | | | | | | | | | | | | | | | 0.94 | 0.94 | 0.54 | 0.04 | 0.29 | 0.26 | 0.04 | 0.43 | 0.29 | 0.28 | 0.14 | 0.28 |
| | *h* | | | | | | | | | | | | | | | | 0.12 | 0.20 | 0.09 | 0.26 | 0.18 | 0.18 | 0.05 | 0.09 | 0.06 | 0.12 | 0.11 | 0.11 |
| | *P* | | | | | | | | | | | | | | | | <0.001 | <0.001 | 0.02 | | | | | | | | | |
| 2DS3 | *Δ* | | | | | | | | | | | | | | | | | | | 0.01 | –0.02 | 0.01 | –0.02 | 0.00 | 0.01 | 0.05 | 0.02 | 0.07 |
| | *r* | | | | | | | | | | | | | | | | | | | 0.33 | 0.15 | 0.22 | 1 | 0.09 | 0.12 | 0.50 | 0.13 | 0.66 |
| | *h* | | | | | | | | | | | | | | | | | | | 0.11 | 0.11 | 0.10 | | 0.03 | 0.03 | 0.08 | 0.07 | 0.10 |
| | *P* | | | | | | | | | | | | | | | | | | | | | | 0.04 | | | 0.015 | | <0.01 |
| 2DS4 | *Δ* | | | | | | | | | | | | | | | | | | | | | | –0.17 | –0.16 | –0.13 | –0.16 | –0.18 | –0.17 |
| | *r* | | | | | | | | | | | | | | | | | | | | | | 1 | 1 | 1 | 0.85 | 1 | 1 |
| | *h* | | | | | | | | | | | | | | | | | | | | | | | | | 0.03 | | |
| | *P* | | | | | | | | | | | | | | | | | | | | | | <<0.0001 | <0.0001 | <0.0001 | <<0.0001 | <<0.0001 | <<0.0001 |
| 2DS5 | *Δ* | | | | | | | | | | | | | | | | | | | | | | | | | 0.09 | 0.09 | 0.08 |
| | *r* | | | | | | | | | | | | | | | | | | | | | | | | | 0.63 | 0.83 | 0.94 |
| | *h* | | | | | | | | | | | | | | | | | | | | | | | | | 0.13 | 0.13 | 0.12 |
| | *P* | | | | | | | | | | | | | | | | | | | | | | | | | <0.001 | <0.001 | <0.001 |

**Table 4** Pairs of *KIR* that displayed significant linkage disequilibrium in UK Caucasoids, Palestinians, and Thai, and compared with previous studies

| Positive linkage disequilibrium | Negative linkage disequilibrium |
|---|---|
| 2DL1–2DL3[a] | 2DL1–2DL2[a,b], 2DL1–2DS2[a], 2DL1–2DS3[a,b] |
| | 2DL2–2DL3[a,b] |
| | 2DL3–2DS2[a], 2DL3–2DS3[a,b] |
| 3DL1–2DS4[a] | 3DL1–2DS1[a,c], 3DL1–2DS5[a,d] |
| | 3DL1–3DS1[a] |
| 2DS1–2DS5[a] | 2DS1–2DS4[c], 2DS4–2DS5[a] |
| 2DS1–3DS1 | 2DS4–3DS1 |
| 2DS5–3DS1[a] | |
| 2DL2–2DS2 | |
| 2DL2–2DS3 | |
| 2DS2–2DS3 | |

[a] Not applied in USA Caucasoid study (Uhrberg et al. 1997)
[b] Not in N. Irish Caucasoid (Crum et al., 2000)
[c] Not in Australian Caucasoid (Witt et al. 1999)
[d] Not in UK Caucasoid population

The linkage disequilibrium values observed were very similar across the populations studied, with a few subtle exceptions. *KIR2DL1* and *KIR2DL3* were strongly associated, consistent with the most common profile and with the observation that less than 2% of all individuals possessed just one of these loci as detected by SSP (Fig. 1). Neither *KIR2DL1* nor *KIR2DL3* were in significant linkage disequilibrium with *KIR3DL1* or *KIR2DS4*, despite relatively high two-locus haplotype frequencies (Table 3) resulting from their appearances together in the most frequent profile (Fig. 1). In contrast, *KIR3DL1* and *KIR2DS4* were associated, and this effect was more evident within the Palestinians (Table 3).

*KIR2DL2* was found in association with *KIR2DS2* and/or *KIR2DS3* and all three loci were negatively associated with *KIR2DL1*/*KIR2DL3*. *KIR2DS1*, *KIR3DS1*, and *KIR2DS5* were all observed in linkage disequilibrium with each other but not with *KIR3DL1* or *KIR2DS4* (Table 4). In the Palestinian and Thai populations, *KIR3DL1* was in negative disequilibrium with *KIR2DS5* (Table 3).

The very obvious negative association of *KIR2DL1/3* with *KIR2DL2* seen in the Caucasoid and Palestinian populations was not so apparent in the Thai population. The negative association for *KIR3DL1* and *KIR2DS5* observed in the Palestinian and Thai populations was not apparent in the Caucasoid population (Table 3).

## Discussion

The frequencies of *KIR* sequences observed overall in our UK Caucasoid population closely resembled those in three recent studies investigating the available KIR repertoire in Caucasoid populations from the USA (52 individuals) (Uhrberg et al. 1997), Australia (147) (Witt et al. 1999), and Northern Ireland (90) (Crum et al., 2000. In these studies, a range of frequencies had been observed for most *KIR* loci. The frequencies of *KIR* sequences that we observed in our UK Caucasoid population all fell within these established ranges. Furthermore, one of the previous studies used a different technique to analyze a similar Caucasoid population (Crum et al., 2000). We compared the results from our Caucasoid population with Palestinian and Thai populations and found small but significant differences in frequencies for several of the *KIR* loci. Although the differences we saw were statistically significant, it is interesting to note the broad similarity between all of the populations that have been studied so far.

Two recently described *KIR* loci were not analyzed using this PCR-SSP detection system. One of them, *KIR3DL3* (Torkar et al. 1998), is likely to be one of the ubiquitous loci (Wilson et al. 2000) and may not be expressed. The sequence for the novel putative inhibitory receptor KIR2DL5 (Vilches et al. 2000) was not detected by this SSP system, nor were any of the (to date) published pseudogene sequences that reside in the *KIR* area.

### *KIR locus profiles*

Over 40 *KIR* sequence profiles had been previously documented (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999) and we saw 49 in 360 individuals. Twenty-eight of these had not been previously reported; thus to date, at least *KIR* 70 profiles have been described by a small number of studies.

One *KIR* locus profile is present at the highest frequency in all of the populations so far investigated. This profile, termed AA1, is composed of six KIR loci: *KIR2DL1*, *KIR2DL3*, *KIR2DL4*, *KIR3DL1*, *KIR3DL2*, and *KIR2DS4*. Profile AA1 probably represents individuals with the fewest number of activating KIRs, although one Thai individual (profile AA7) was observed with no known loci for activating KIRs (Fig. 1). Further investigation of individuals with the AA1 arrangement is required to establish whether this is a result of a selective advantage, and further populations need to be studied to see if this profile remains pervasive.

The variation in the number of *KIR* loci present in all individuals observed so far has been predominantly due to loci for the activating receptors (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999). This was confirmed by our study, in which there were only six possible arrangements of loci for inhibitory KIRs in 98% of individuals. The functional consequences of these findings need to be investigated.

### *Linkage disequilibrium and haplotypes*

Positive linkage disequilibrium values were similar in the three populations we investigated (Table 4) and all of these positive linkage disequilibriums had been previously reported in Caucasoid populations (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999). The association of *KIR2DS1* with *KIR2DS2* was the only previously reported positive linkage disequilibrium that we did not detect. This combination was significant in the Australian population (Witt et al. 1999), and these two putative loci have been linked in preliminary mapping of the area (Wilson et al. 2000). Similar negative linkage disequilibrium values for pairs of *KIR* loci were also reported in the above population studies, where applicable (Table 4).

*KIR2DL1* and *KIR2DL3* were almost always detected together in the three populations we investigated and this pair appeared to be mutually exclusive of *KIR2DL2* by linkage disequilibrium analysis (Fig. 1, Tables 2, 3). These observations suggest that our results are consistent with the segregation of *KIR* region haplotypes into two broad groups distinguished by *KIR2DL1*/*KIR2DL3* (A) and *KIR2DL2* (B). Furthermore, *KIR2DL2* is proposed to have arisen as a recombination event between *KIR2DL1* and *KIR2DL3* (Wilson et al. 2000). In this case, they would appear together as an alternative to *KIR2DL2*, as we observed. A pseudogene (*KIR-Z*) has been implicated in this arrangement that occurs between *KIR2DL1* and *KIR2DL3* (Wilson et al. 2000). Whether *KIR-Z* is absent in individuals who appear to be homozygous for the B haplotype needs to be established.

Certain other *KIR* loci may be associated with either the A or the B haplotype; for example, *KIR2DS2* has been suggested to be characteristic of a group B haplotype (Uhrberg et al. 1997). We observed a strong positive association between *KIR2DS2* and *KIR2DL2* and a negative association between *KIR2DS2* and *KIR2DL1*/*3* in all populations (Table 3.) and we found only one individual with a B haplotype who tested negative for *KIR2DS2* (Fig. 1, profile AB105). However, *KIR2DS2* is not exclusive to B haplotypes, as we also saw this locus in a small number ($n$=8) of individuals who displayed AA profiles. *KIR2DS4* has been suggested as being a characteristic of A haplotypes (Uhrberg et al. 1997), but *KIR2DL1* and *KIR2DL3*, which probably define this haplotype, were not seen to be in association with *KIR2DS4* in any of the populations we studied. Furthermore, *KIR2DS4* was found in the majority of profiles in our study groups (Fig. 1). *KIR2DS4* can therefore be found on most A and most B haplotypes, alongside *KIR3DL1* with which it is associated.

The low frequency of *KIR2DL1* and *KIR2DL3* in the Palestinians and the increased frequency of *KIR2DL2*, *KIR2DS2*, and possibly *KIR2DS3* (Table 2), together with similar linkage disequilibrium values for these loci in all populations (Table 3), would indicate a higher prevalence of the B haplotype in the Palestinian population. In contrast, we found that the B haplotype occurred at the lowest frequency in the Thai population. Indeed only four members of the Thai population were seen without *KIR2DL1* and *KIR2DL3* (Fig. 1). The observation that the relative linkage disequilibrium of *KIR2DL2* with *KIR2DS2* was lower in the Palestinians (Table 3) could suggest an alternative diversification of the B haplotype that is more prevalent in the Palestinian population. Furthermore, six of the seven individuals who did not comply with the simple A or B *KIR* haplotype profile characterization were from the Palestinian population.

### *Distinct loci or alleles?*

Several of the putative loci under investigation may in fact be allelic variants despite a disparity of more than 20 nucleotides. *KIR2DS4* and *KIR2DS1* have been suggested to be allelic variants of the same locus, and *KIR3DL1* and *KIR3DS1* as alleles of another (Gassner et al. 2000; Uhrberg et al. 1997). If a pair of *KIR* loci are allelic, they would have a negative linkage disequilibrium, although this would also occur if they rarely appeared on the same haplotype, regardless of their relative mapped positions and their resolved functions. We detected a strong negative association between *KIR2DS4* and *KIR2DS1* in all populations (Table 3) and only one individual who possessed neither (Fig. 1), which would be consistent with an allelic relationship. However, we observed that *KIR2DS5* and *KIR3DS1* also had a significant negative linkage disequilibrium with *KIR2DS4* (Table 3), which implies that any of these three loci could be allelic variants of *KIR2DS4*. Conversely, *KIR2DS1*, *KIR2DS5*, and *KIR3DS1* were observed in linkage disequilibrium with each other, which suggests they may occur together and on haplotypes not usually containing *KIR2DS4*. This latter situation conforms to the preliminary genetic map of the area and the suggestion that *KIR2DS4* occupies a distinct locus (Wilson et al. 2000).

Wilson and co-workers (2000) also suggest that *KIR3DL1* and *KIR3DS1* occupy the same position but on different haplotypes, and this is further confounded by the proposal that *KIR3DL1* may be represented by more than one polymorphic gene locus

(Vyas et al. 1998). The SSP typing scheme that we employed does not provide any indication of *KIR* gene copy number. Every individual in our study who tested negative for *KIR3DL1* tested positive for *KIR3DS1* ($n$=25). However, individuals have been observed with two alleles of *KIR3DL1* in addition to *KIR3DS1* (Crum et al., 2000; M. Curran, personal communication).

*KIR ligands*

The receptors KIR2DL1 and KIR2DS1 interact with HLA-Cw molecules which display the C2 epitope (e.g., Cw*02,04,05,06,15,1602 and 1701) while KIR2DL2, KIR2DL3, and KIR2DS2 interact with HLA-Cw carrying the alternative C1 epitope (e.g., Cw*03,07,08,12,13,14,1601) (Biassoni et al. 1995; Colonna et al. 1993; Richardson et al. 2000; Winter and Long 1997). KIR3DL1 interacts with HLA class I molecules that contain a Bw4 motif in their $\alpha$ helix and KIR3DL2 interacts with some HLA-A alleles (Dohring et al. 1996b; Pende et al. 1996). No KIR has yet been shown to bind Bw6, which is the alternative HLA-B motif to Bw4, although such a receptor has been proposed to exist (Vyas et al. 1998). KIR2DL4, which is unusual in its extracellular domain organization and in the possession of only one ITIM, probably interacts with HLA-G (Ponte et al. 1999; Rajagopalan and Long 1999) although the functional nature of this interaction has not been confirmed (Lopes-Botet et al. 2000). *KIR2DL4* and *KIR3DL2* form part of the framework for the region of variable gene content (Wilson et al. 2000) and have been found in every individual so far reported (Crum et al., 2000; Uhrberg et al. 1997; Witt et al. 1999) as well as in our study populations. In two of the other population studies mentioned, approximately 10% of individuals did not possess an inhibitory KIR for HLA-C2 (Crum et al., 2000; Witt et al. 1999). In our study, 9% ($n$=32) of the total individuals were in this category; a number of these (16/32) had one HLA-Cw allele, and four individuals had two HLA-Cw alleles for which they had no known inhibitory KIR counterpart (HLA data not shown). We also saw 3% ($n$=4) of Caucasoids, 12% ($n$=13) of Palestinians, and 7% ($n$=8) of Thais who were without a known inhibitory ligand for HLA-Bw4 (Fig. 1), although none of these were actually homozygous for Bw4. Furthermore, Gumperz and co-workers (1996) had previously seen no correlation between KIR3DL1 expression and occurrence of the Bw4 motif. There are several possible explanations for the observations that inhibitory receptors can apparently be expressed for HLA ligands that are not present. There may be a systemic redundancy by which the role of inhibitory KIRs can be fulfilled by the interaction of HLA-E with CD94/NKG2 (Valiante et al. 1997; Young et al. 1998; Zimmer et al. 1998), and other immunoglobulin-related NK inhibitory receptors are being identified (Falco et al. 1999; Ponte et al. 1999). KIR ligation could be more flexible than was first thought, as has been demonstrated by the binding of soluble KIR2DL2 and KIR2DL3 to some C2 as well as C1 epitope-containing HLA-Cw (Winter et al. 1998). KIRs may even be recognizing alternative or additional ligands. There is also the possibility that utilizing a PCR-SSP detection scheme, a novel allele for a polymorphic locus or a functional equivalent may not be detectable. This situation arose with the seven individuals that tested negative for one of *KIR2DL1* or *KIR2DL3*; they all tested positive for the respective *KIR* locus when analyzed by SSOP. We observed that four of the five individuals who appeared to lack *KIR2DL1* were positive for *KIR2DL1v*. This does not imply an allelic relationship between *KIR2DL1* and *KIRD2L1v*, although *KIR2DL1v* is a recombinant of *KIR2DL1* and *KIR2DS1* (Shilling et al. 1998) and may encode an inhibitory receptor that recognizes the same epitope as KIR2DL1.

Hitherto unknown allelic relationships between any pair or any number of the *KIR* sequences would contribute to distortion of the frequencies and the linkage disequilibrium we observed. At present we cannot assume an absolute correlation between these *KIR* sequences and genetic loci. Several allelic variants and subtypes arise by recombination between loci thus, utilizing a PCR-SSP approach which relies on *cis*-encoded polymorphisms should enable unambiguous detection of characterized loci. Conversely, if we were only detecting a certain percentage of alleles at a locus, then the frequency would appear diminished.

We appreciate that the genomic organization of *KIR* is still not completely resolved using this typing system but it is useful in the first instance for studying population differences. The complex *KIR* region is thought to have evolved relatively recently (Wilson et al. 2000) and we demonstrated that *KIR* haplotypes vary in their distribution between ethnically diverse human populations. This observation may be even more apparent with other human populations, and these are under investigation. This work provides further evidence for *KIR* polygenicity and establishes some novel *KIR* profiles. However, detailed studies examining the allelic polymorphism of these loci in individuals and families will be required to fully characterize the available *KIR* haplotypes. Once an individual's potential KIR repertoire is established, it may then be possible to determine the functional implications and even relate these to tansplantation survival, as well as susceptibility to autoimmune, infectious, or malignant diseases.

**Acknowledgements** We are very grateful to everyone involved in the collection of samples from the Palestinian and Thai individuals, and to all the staff from South Thames Tissue Typing, especially Sean Carey and Elli Kondeatis, for help with the Caucasoid control panel assembly. We would like to warmly thank Karen Crum, Susan Logue, Martin Curran, and Derek Middle-

ton from Northern Ireland Tissue Typing for demonstration of SSOP typing and unparalleled hospitality. We would also like to thank Christine Carrington and Andrea Harmer for their helpful discussion. The work reported here was performed in accordance with all appropriate regulations.

## References


Baker E, D'Andrea A, Phillips JH, Sutherland GR, Lanier LL (1995) Natural killer cell receptor for HLA-B allotypes, NKB1: map position 19q13.4. Chromosome Res 3:511

Baur MP, Danilovs JA (1980) Population analysis of HLA-A, B, C, DR and other genetic markers. In: Terasaki PI (ed) Histocompatibilty testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 955–993

Biassoni R, Falco M, Cambiaggi A, Costa P, Verdiani S, Pende D, Conte R, Di Donato C, Parham P, Moretta L (1995) Amino acid substitutions can influence the natural killer (NK) mediated recognition of HLA-C molecules: role of serine-77 and lysine-80 in the target cell protection from lysis mediated by "group 2" or "group 1" NK clones. J Exp Med 182:605–609

Biassoni R, Pessino A, Malaspina A, Cantoni C, Bottino C, Sivori S, Moretta L, Moretta A (1997) Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules. Eur J Immunol 27:3095–3099

Borges L, Hsu ML, Fanger N, Kubin M, Cosman D (1997) A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. J Immunol 159:5192–5196

Boyington JC, Motyka SA, Scuck P, Brooks AG, Sun PD (2000) Crystal structure of an NK cell immunogobulin-like receptor in complex with its class I MHC ligand. Nature 405:537–543

Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, Lanier LL, McMichael AJ (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 391:795–799

Brooks AG, Posch PE, Scorzelli CJ, Borrego F, Coligan JE (1997) NKG2A complexed with CD94 defines a novel inhibitory natural killer cell receptor. J Exp Med 185:795–800

Campbell KS, Dessing M, Lopez-Botet M, Cella M, Colonna M (1996) Tyrosine phosphorylation of a human killer inhibitory receptor recruits protein tyrosine phosphatase 1C. J Exp Med 184:93–100

Chandanayingyong D, Stephens HAF, Klaythong R, Sirikong M, Udee S, Longta P, Chatangpol R, Bejrachandra S, Rungruang E (1997) HLA-A, -B, -DRB1 -DQA1 and -DQB1 polymorphism in Thais. Hum Immunol 53:174–182

Colonna M, Spies T, Strominger JL, Ciccone E, Moretta A, Moretta L, Pende D, Viale O (1992) Alloantigen recognition by two human natural killer cell clones is associated with HLA-C or a closely linked gene. Proc Natl Acad Sci USA 89:7983–85

Colonna M, Borsellino G, Falco M, Ferrara GB, Strominger JL (1993) HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. Proc Natl Acad Sci USA 90:12000–12004

Crum K, Logue S, Curran M, Middleton D (2000) Development of a PCR-SSOP approach capable of defining the natural killer cell inhibitory receptor (KIR) gene sequence repertoires. Tissue Antigens

D'Andrea A, Chang C, Phillips JH, Lanier LL (1996) Regulation of T cell lymphokine production by killer cell inhibitory receptor recognition of self HLA class I alleles. J Exp Med 184:789–94

Davis DM, Chiu I, Fasset M, Cohen GB, Mandelboim O, Strominger J (1999) The human natural killer cell immune synapse. Proc Natl Acad Sci USA 96:15062–15067

Dohring C, Samaridis J, Colonna M (1996a) Alternatively spliced forms of human killer inhibitory receptors. Immunogenetics 44:227–230

Dohring C, Scheidegger D, Samaridis J, Cella M, Colonna M (1996b) A human killer inhibitory receptor specific for HLA-A1,2. J Immunol 156:3098–3101

Falco M, Biassoni R, Bottino C, Vitale M, Sivori S, Augugliaro R, Moretta L, Moretta M (1999) Identification and molecular cloning of p75/AIRM1 a novel member of the sialoadhesion family that functions as an inhibitory receptor in human natural killer cells. J Exp Med 190:793–801

Falk CS, Steinle A, Schendel DJ (1995) Expression of HLA-C molecules confers target cell resistance to some non-major histocompatibility complex-restricted T cells in a manner analogous to allospecific natural killer cells. J Exp Med 182:1005–1018

Fry AM, Lanier LL, Weiss A (1996) Phosphotyrosines in the killer cell inhibitory receptor motif of NKB1 are required for negative signaling and for association with protein tyrosine phosphatase 1C. J Exp Med 184:295–300

Gassner C, Williams L, Vu Q, Suomalainen S, He P, Fortelny S, Geraghty D (2000) Genotyping 3DS1, 3DL1, 2DS1 and 2DS4 of the killer cell inhibitory receptor (KIR) gene locus on chromosome 19. Hum Immunol 61 (suppl 1):S33

Gumperz JE, Valiante NM, Parham P, Lanier LL, Tyan D (1996) Heterogeneous phenotypes of expression of the NKB1 natural killer cell class I receptor among individuals of different human histocompatibility leukocyte antigens types appear genetically regulated, but not linked to major histocompatibililty complex haplotype. J Exp Med 183:1817–1827

Khakoo S, Rajalingam R, Shum, B Weidenbach K, Flodin L, Muir D, Canavez F, Cooper S, Valiante N, Lanier L, Parham P (2000) Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans. Immunity 12:687–698

Lee N, Llano M, Carretero M, Ishitani A, Navarro F, Lopez-Botet M, Geraghty DE (1998) HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci USA 95:5199–5204

Litwin V, Gumperz J, Parham P, Phillips JH, Lanier LL (1994) NKB1: a natural killer cell receptor involved in the recognition of polymorphic HLA-B molecules. J Exp Med 180:537–543

Lopes-Botet M, Bellon T, Llano M, Navarro F, Garcia P, Miguel M de (2000) Paired inhibitory and triggering NK cell receptors for HLA class I molecules. Hum Immunol 61:7–17

Martin AM, Freitas EM, Witt CS, Christiansen FT (2000) The genomic organization and evolution of the natural killer immunoglobulin-like receptor (KIR) gene cluster. Immunogenetics 51:268–280

Mattiuz PL, Ihde D, Piazza A, Ceppellini R, Bodmer WF (1971) New approaches to the population genetic and segregation analysis of the HLA system. In: Terasaki PI (ed) Histocompatibility testing 1970. Munskaard, Copenhagen, pp 193–205

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Mingari MC, Schiavetti F, Ponte M, Vitale C, Maggi E, Romagnani S, Demarest J, Pantaleo G, Fauci AS, Moretta L (1996) Human $CD8^+$ T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. Proc Natl Acad Sci USA 93:12433–12438

Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, Augugliaro R, Barbaresi M, Ciccone E, Moretta L (1993) P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells: anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med 178:597–604

Moretta A, Sivori S, Vitale M, Pende D, Morelli L, Augugliaro R, Bottino C, Moretta L (1995) Existence of both inhibitory (p58) and activatory (p50) receptors for HLA-C molecules in human natural killer cells. J Exp Med 182:875–884

Pende D, Biassoni R, Cantoni C, Verdiani S, Falco M, Donato C di, Accame L, Bottino C, Moretta A, Moretta L (1996) The natural killer cell receptor specific for HLA-A allotypes: a novel member of the p58/p70 family of inhibitory receptors that is characterized by three immunoglobulin-like domains and is expressed as a 140-kD disulphide-linked dimer. J Exp Med 184:505–518

Phillips JH, Gumperz JE, Parham P, Lanier LL (1995) Super-antigen-dependent, cell-mediated cytotoxicity inhibited by MHC class I receptors on T lymphocytes. Science 268:403–405

Ponte M, Cantoni C, Biassoni R, Tradori-Cappai A, Bentivoglio G, Vitale C, Bertone S, Moretta A, Moretta L, Mingari MC (1999) Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor. Proc Natl Acad Sci USA 96:5674–5679

Rajagopalan S, Long EO (1999) A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. J Exp Med 189:1093–1100

Richardson J, Reyburn H, Luque I, Valez-Gomez M, Strominger J (2000) Definition of polymorphic residues on killer IG-like receptor proteins which contribute to the HLA-C binding site. Eur J Immunol 30:1480–1485

Samaridis J, Colonna M (1997) Cloning of novel immunogobulin superfamily receptors expressed on human myeloid and lymphoid cells: structural evidence for new stimulatory and inhibitory pathways. Eur J Immunol 27:660–665

Selvakumar A, Steffens U, Dupont B (1997a) Polymorphism and domain variability of human killer cell inhibitory receptors. Immunol Rev 155:183–196

Selvakumar A, Steffens U, Palanisamy N, Chaganti RS, Dupont B (1997b) Genomic organization and allelic polymorphism of the human killer cell inhibitory receptor gene KIR103. Tissue Antigens 49:564–573

Shilling H, Lienert-Weidenbach K, Valiante N, Uhrberg M, Parham P (1998) Evidence for recombination as a mechanism for KIR diversification. Immunogenetics 48:413–416

Steffens U, Vyas Y, Dupont B, Selvakumar A (1998) Nucleotide and amino acid sequence alignment for human killer cell inhibitory receptors (KIR). Tissue Antigens 51:398–413

Suto Y, Ishikawa Y, Kashara M, Kasai F, Yabe T, Akaza T, Juji T (1998) Gene arrangement of the killer cell inhibitory receptor family on human chromosome 19q13.4 detected by fiber-FISH. Immunogenetics 48:235–241

Torkar M, Norgate Z, Colonna M, Trowsdale J, Wilson MJ (1998) Isotypic variation of novel immunogobuln-like transcript/killer cell inhibitory receptor loci in the leukocyte receptor complex. Eur J Immunol 28:3959–3967

Trinchieri G (1989) Biology of natural killer cells. Adv Immunol 47:187–376

Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, Tyan D, Lanier LL, Parham P (1997) Human diversity in killer cell inhibitory receptor genes. Immunity 7:753–763

Vales-Gomez M, Reyburn HT, Mandelboim M, Strominger JL (1998a) Kinetics of interaction of HLA-C ligands with natural killer cell inhibitory ligands. Immunity 9:337–344

Vales-Gomez M, Reyburn H, Erskine R, Strominger, J (1998b) Differential binding to HLA-C of p50-activating and p58-inhibitory natural killer cell receptors. Proc Natl Acad Sci USA 95:14326–14331

Valiante NM, Uhrberg M, Shilling HG, Lienert-Weidenbach K, Arnett KL, D'Andrea A, Phillips JH, Lanier LL, Parham P (1997) Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. Immunity 7:739–751

Verity DH, Wallace GR, Vaughan RW, Kondeatis E, Madanat W, Zureikat H, Fayyad F, Marr JE, Kanawati CA, Stanford MR (1999) HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behcet's disease. Tissue Antigens 54:264–272

Vilches C, Rajalingam R, Uhrberg M, Gardiner CM, Young NT, Parham P (2000) KIR2DL5, a novel killer-cell receptor with a D0-D2 configuration of Ig-like domains. J Immunol 164:5797–5804

Vyas Y, Selvakumar A, Steffens U, Dupont B (1998) Multiple transcripts of the killer cell immunogobulin-like receptor family, KIR3DL1 (NKB1), are expressed by natural killer cells of a single individual. Tissue Antigens 52:510–519

Wagtmann N, Biassoni R, Cantoni C, Verdiani S, Malnati MS, Vitale M, Bottino C, Moretta L, Moretta A, Long EO (1995) Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. Immunity 2:439–449

Wende H, Colonna M, Ziegler A, Volz A (1999) Organisation of the leukocyte receptor cluster (LRC) on human chromosome 19q13.4 Mamm Genome 10:154–160

Wende H, Volz A, Ziegler A (2000) Extensive gene duplications and large inversion characterize the human leukocyte receptor cluster. Immunogenetics 51:703–713

Wilson MJ, Torkar M, Trowsdale J (1997) Genomic organization of a human killer cell inhibitory receptor gene. Tissue Antigens 49:574–579

Wilson MJ, Torkar M, Haude A, Milne S, Sheer D, Beck S, Trowsdale J (2000) Plasticity in the organization and sequences of human KIR/ILT gene families. Proc Natl Acad Sci USA 97:4778–4783

Winter CC, Long EO (1997) A single amino acid in the p58 killer cell inhibitory receptor controls the ability of natural killer cells to discriminate between the two groups of HLA-C allotypes. J Immunol 158:4026–4028

Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N (1998) Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. J Immunol 161:571–577

Witt CS, Dewing C, Sayer DC, Uhrberg M, Parham P, Christiansen F (1999) Population frequencies and putative haplotypes of the killer cell immunogobulin-like receptor sequences and evidence for recombination. Transplantation 68:227–230

Young NT, Rust NA, Dallman MJ, Cerundolo V, Morris PJ, Welsh KI (1998) Independent contributions of HLA epitopes and killer inhibitory receptor expression to the functional alloreactive specificity of natural killer cells. Hum Immunol 59:700–712

Zimmer J, Donato L, Hanau D, Cazaneve JP, Tongio MM, Moretta A, Salle H de la (1998) Activity and phenotype of natural killer cells in peptide transporter (TAP) deficient patients (type I bare lymphocyte syndrome). J Exp Med 187:117–122