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Natural killer cell immunoglobulin-like receptor (KIR) locus profiles in African and South Asian populations

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Natural killer (NK) and some T cells express killer cell immunoglobulin-like receptors (KIRs), which interact with HLA class I expressed by target cells and consequently regulate cytolytic activity. The number of KIR loci can vary and so a range of genetic profiles is observed. We have determined the KIR genetic profiles from one African (n = 62) and two South Asian (n = 108, n = 78) populations. Several of the KIRs are present at significantly different frequencies between the two major ethnic groups (eg KIR2DS4 gene frequency 0.82 African, 0.47 S Asian. $P_c < 1 \times 10^{-6}$) and this is due to uneven distribution of two KIR haplotype families 'A' and 'B'. All three populations described here displayed a greater degree of diversity of KIR genetic profiles than other populations investigated, which indicates further complexity of underlying haplotypes; in this respect we describe two individuals who appear homozygous for a large deletion including the previously ubiquitous 2DL4. We have also reanalysed three populations that we studied previously, for the presence of a KIR which is now known to be an indicator of the 'B' haplotype. South Asians had the highest overall frequencies of all KIR loci characteristic of 'B' haplotypes ($P_c < 0.0001$ to < 0.004). Furthermore, gene frequency independent deviances in the linkage disequilibrium were apparent between populations.
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Introduction

Killer cell immunoglobulin-like receptors (KIRs) are members of a group of regulatory molecules found on subsets of lymphocytes. In humans, ligation of KIR isotypes by HLA Class I proteins can lead to either inhibition or activation of cytotoxic cell activity (reviewed by Lanier[1]). Although there are many such interactions influencing natural cytotoxic cells (see Raulet *et al*[2] and Moretta *et al*[3]) KIRs could potentially play a significant role in the control of the immune response. Consequently KIRs have been implicated in several diseases including natural killer (NK) and T cell lymphomas,[4,5] coeliac disease[6] and rheumatoid arthritis,[7,8] in the potentially beneficial graft *vs* leukaemia responses[9] and also in the transition to memory phenotype for $CD8^+$ T cells.[10]

Variable gene content is observed for the *KIR* region at chromosome 19q13.4[11] and the variety of genetic profiles thus observed has been explained by two broad haplotype groups (termed A and B) which are distinguishable by the presence or absence of up to 15 loci. Over 70 of these *KIR* profiles have been reported so far in approximately 650 individuals.[11–14]

Aside from subtypes of particular *KIR* (eg, *2DL5* and *3DL1*), all of these putative *KIR* loci have an expressed product,[11,15–17] thus the *KIR* genetic profile may be a reflection of the repertoire of KIR available to an individual. We have previously reported the distribution of NK cell receptor repertoires in three distinct populations, Caucasoid, Palestinian and Thai, and observed statistically significant differences in gene frequencies for several of the *KIR* loci.[14] Here we examine two more distinct ethnic groups from three population samples. We have studied the genetic profiles of individuals of African and South Asian descent from Trinidad and a further South Asian population from Karachi (Pakistan). This African population originated primarily from West Africa, and the Trinidad South Asians originated from Bangladesh, India and Pakistan.[18] The Karachi South Asian population consisted of northern Indian, Punjabi, Memon and Ismaili.[19] We have also reassessed the initial three popu-

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lations in the context of *2DL5*, a recently characterized KIR locus that defines the 'B' haplotype group[16,20] and compare all six groups with respect to their distributions of *KIR* loci and the linkage disequilibrium observed between them.

Results

KIR locus frequencies

The percentages of individuals possessing each particular *KIR* sequence are shown for all three populations in Table 1, alongside estimates for the gene frequencies of the KIR loci. All of the 14 *KIR* were present in all populations and there was some variation in most, principally when the African is compared with the two South Asian populations. The two South Asian populations had very similar frequencies for most loci with no significant difference between them. *KIR*s *3DL2* and *3DL3* were detected in every individual. *KIR2DL4* was detected in every individual excluding one Trinidad South Asian and one Karachi South Asian (Table 1 and profile X101, Figure 1).

The African population had significantly higher frequencies of *3DL1* and *2DS4* and lower frequencies of *2DL5*, *2DS1* and *3DS1* than both the South Asian populations (Table 1). *KIR3DS1* was only detected in eight individuals (13%) from this African population sample; this is considerably less frequent than any of the other populations we have studied (approximately 30–40%). We also note the comparatively low frequency of *2DS2* in this African population sample (Table 1).

KIR locus profiles

Overall, 25 profiles were observed from the 62 Africans, 41 from 108 Trinidad South Asians and 30 from 78 Karachi South Asians (Figure 1). We also observed 28 profiles of the initial 12 *KIR* which were not apparent in the first three populations we studied.[14] The African and South Asians exhibited a wider range of profiles than any of the Caucasoid, Palestinian or Thai populations we reported previously (ratio of profiles: individuals = 0.39 for African and Trinidad South Asian, 0.38 for Karachi South Asian, 0.29 for Palestinian, 0.23 for Thai and 0.22 for UK Caucasoid). Many profiles were population specific, although most new profiles described here were shared by only a small number of individuals. Population unique profiles that were more frequently occurring included AB8, which was observed in five (8.1%) Africans, AB7.1 in seven (9%) Karachi South Asians and AA5.1 and AB5.1, which were each seen in four (3.7%) Trinidad South Asians.

Profile AA1, which has been the most predominant profile reported overall[11–14] was also prevalent in these populations and accounted for 22 (35.5%) Africans, 17 (15.7%) Trinidad South Asians and nine (11.5%) Karachi South Asians. After AA1, profiles AB1/1.1 (9.7%), AB8/8.1 (9.7%), AB9 (8.1%) and AA2.1 (6.4%) were the most common in the African group and AB1/1.1 (9.3%), AB5/5.1 (9.3%), AB9 (8.3%) and AA2/2.1 (8.3%) in the Trinidad South Asian group. In the Karachi South Asians, profiles AA1 and AB9 were equally common at 11.5%; other frequently occurring profiles in this group were AB7.1 at 9%, AA2.1 at 7.7% and AB1 at 6.4%.

KIR2DL1v and *2DL5* in six populations

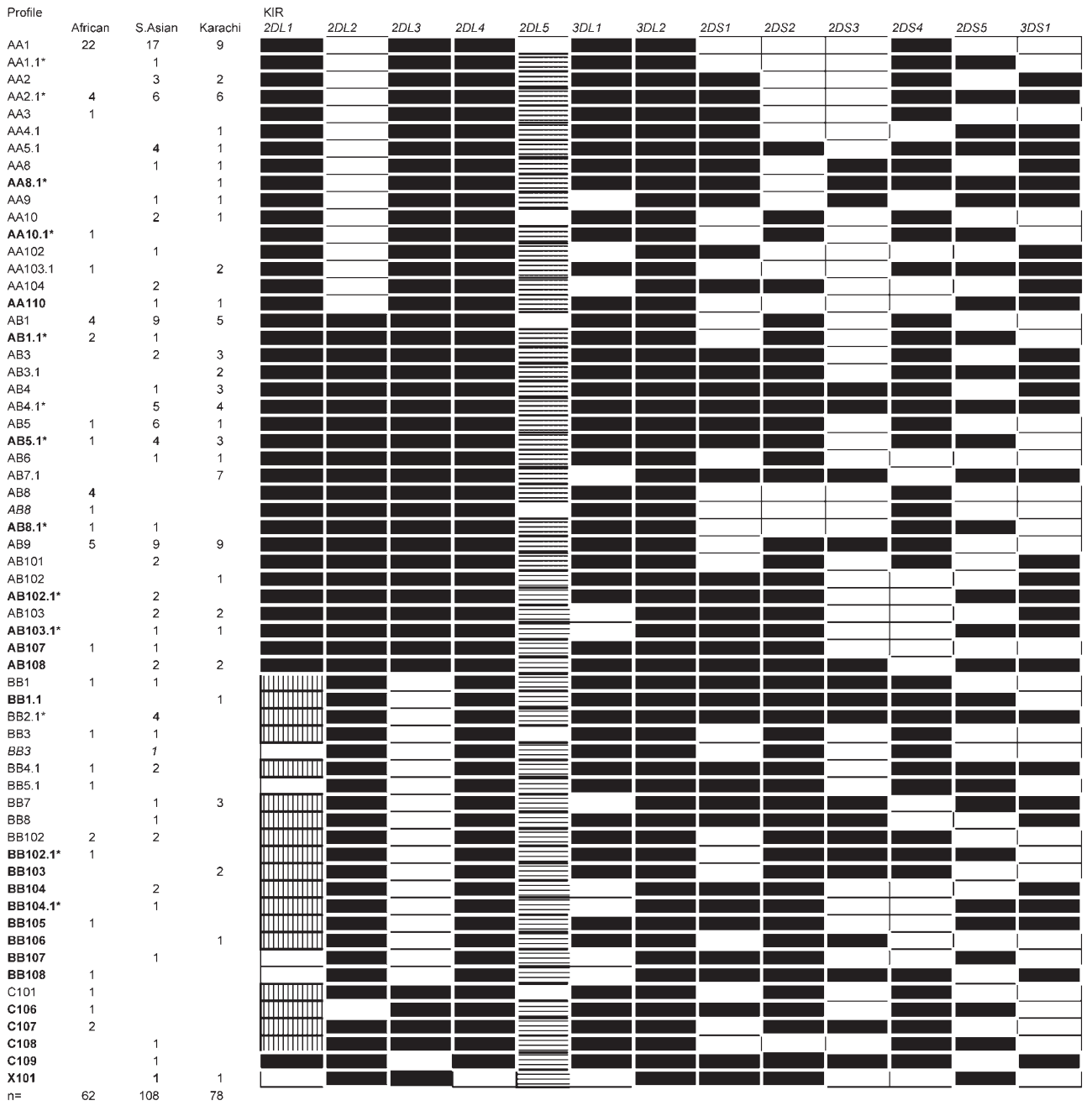
KIR2DL5 had not been characterised when we studied the first three population samples[14]; we therefore tested all of the original individuals for the presence of this locus. The percentage of individuals with *2DL5* in our Caucasoid panel (55%, Table 2) was comparable with the previously reported 52% from a panel of 108 Caucasoid donors.[16] Estimated gene frequencies for *2DL5* were highest in the two South Asian and lowest in the African and Thai populations (Tables 1 and 3). Screening for *2DL5* did not affect the number of KIR locus profiles in the first three populations (Table 3) and resulted in only two additional profiles from these latest populations (AB8 and BB3; Figure 1). *KIR2DL5* was absent from just five genetic profiles including the two most frequently observed in total (AA1 and AB1). *KIR2DL5* was observed in linkage disequilibrium positively with *2DL2* and negatively with *2DL1*, *2DL3*, *3DL1* and *2DS4* in all six populations (Table 4).

There were 13 individuals from the 608 we have now investigated in which neither *2DL1* nor *2DL1v* (*2DL1*004*) were detected (Figure 1 and Table 3). Estimated gene fre-

Table 1 Observed KIR frequencies and estimated KIR locus gene frequencies

		Inhibitory KIR-							*Non-inhibitory KIR-*					
		2DL1	*2DL2*	*2DL3*	*2DL4*	*2DL5*	*3DL1*	*3DL2*	*2DS1*	*2DS2*	*2DS3*	*2DS4*	*2DS5*	*3DS1*
African	%	79	52	85	100	52	98	100	23	45	19	97	24	13
(n = 62)	gf	**0.54**	**0.30**	**0.62**	**1.00**	**0.30**	**0.87**	**1.00**	**0.12**	**0.26**	**0.10**	**0.82**	**0.13**	**0.07**
Trinidad S. Asian	%	82	64	83	99	74	88	100	55	69	27	81	37	44
(n = 108)	gf	**0.58**	**0.40**	**0.59**	**0.90**	**0.49**	**0.65**	**1.00**	**0.33**	**0.45**	**0.14**	**0.56**	**0.21**	**0.25**
Karachi S. Asian	%	90	67	91	99	78	81	100	60	69	45	72	48	56
(n = 78)	gf	**0.68**	**0.42**	**0.70**	**0.89**	**0.53**	**0.56**	**1.00**	**0.37**	**0.45**	**0.26**	**0.47**	**0.28**	**0.34**
African to	P_c* <					0.01	2×10^{-4}		5×10^{-4}			2×10^{-5}		1×10^{-3}
Trinidad South Asian	χ^2					11.2	18.7		16.9			22.9		15.3
African to	P_c* <					1×10^{-3}	1×10^{-7}		5×10^{-5}		0.01	1×10^{-7}		3×10^{-6}
Karachi South Asian	χ^2					14.5	30.6		21.3		11.3	35.6		27.1

% = percentage of individuals positive; gf = gene frequency.
*P_c is calculated from 2 × 2 contingency of estimated chromosomes with that locus, corrected for the number of loci (n = 13).



Profile	African	S.Asian	Karachi
AA1	22	17	9
AA1.1*		1	
AA2		3	2
AA2.1*	4	6	6
AA3	1		
AA4.1			1
AA5.1		4	1
AA8		1	1
AA8.1*			1
AA9		1	1
AA10		2	1
AA10.1*	1		
AA102		1	
AA103.1	1		2
AA104		2	
AA110		1	1
AB1	4	9	5
AB1.1*	2	1	
AB3		2	3
AB3.1			2
AB4		1	3
AB4.1*		5	4
AB5	1	6	1
AB5.1*	1	4	3
AB6		1	1
AB7.1			7
AB8	4		
AB8	1		
AB8.1*	1	1	
AB9	5	9	9
AB101		2	
AB102			1
AB102.1*		2	
AB103		2	2
AB103.1*		1	1
AB107	1	1	
AB108		2	2
BB1	1	1	
BB1.1			1
BB2.1*		4	
BB3	1	1	
BB3		1	
BB4.1	1	2	
BB5.1	1		
BB7		1	3
BB8		1	
BB102	2	2	
BB102.1*	1		
BB103			2
BB104		2	
BB104.1*		1	
BB105	1		
BB106			1
BB107		1	
BB108	1		
C101	1		
C106	1		
C107	2		
C108		1	
C109		1	
X101		1	1
n=	62	108	78

Figure 1 *KIR* locus profiles observed in African and South Asian population samples. (black box) presence of KIR sequence; (white box) indicates absence; (vertical stripes) *KIR2DL1* absent but *KIR2DL1v* detected; (horizontal stripes) *KIR2DL5*. Previous nomenclature system[12] is adapted for describing these patterns; any novel profiles are shown in bold. *Profiles further resolved using *KIR2DS5* (to aid comparison with those previously documented). IHWC cell line DNA with common profiles: TAB089 and BM16 = AA1, OLGA = AA2.1, JVM = AB1, MANIKA = AB4.1, DBB = AB9.

quencies for *2DL1*004* were similar for the African, both South Asian, and Palestinian groups (Table 2). Gene frequencies for *2DL1* are now estimated to be higher than previous assessments would have been for all six populations. For clarity and ease of comparison with previous works, *2DL1*004* results were not incorporated into the initial linkage disequilibrium analysis.

Linkage disequilibrium

Many pairs of loci were calculated to be in highly significant positive or negative linkage disequilibrium for all populations (Table 4). The overall patterns of linkage disequilibrium were similar for all populations, however we observed that the magnitude of many of the values obtained differed between the ethnic groups. Fitting of a log-linear model indicated which of these variations were not wholly attributable to differences in respective gene frequencies (Table 5). For example, strong negative linkage disequilibrium between *2DS5* and *2DS4* was only seen in Karachi South Asians (Table 4). This observation may be attributed to the highest overall frequency of *2DS5* in the Karachi South Asians (Table 1), as our log-

Table 2 *KIR2DL1*004* and *2DL5* gene frequencies in six population samples

		African	*Trinidad S. Asian*	*Karachi S. Asian*	*Caucasoid*	*Palestinian*	*Thai*
previous *2DL1* estimate	gf	***0.54***	***0.58***	***0.68***	***0.70***	***0.59***	***0.82***
*2DL1*004*	%	35	39	38	28	38	13
	gf	**0.20**	**0.22**	**0.22**	**0.15**	**0.21**	**0.07**
*total *2DL1*	%	97	97	99	98	97	99
	gf	**0.82**	**0.83**	**0.89**	**0.85**	**0.83**	**0.91**
2DL5	%				55	63	50
	gf				**0.33**	**0.39**	**0.29**

*From Figure 1 and Table 3.
% = percentage of individuals positive; gf = gene frequency.

Table 3 KIR locus profiles from Caucasoid, Palestinian and Thai populations reanalysed with *2DL1*004*, *2DL5* and *3DL3*

Profile designation[a]	*2DL1*004*[b] +/–	*2DL5*[c] +/–	*No. of individuals*
AA1		–	116
AA10		–	3
AB1		–	44
BB2	+	+	6
BB2.1	+	+	5
BB3	–	–	5
BB4	+	+	2
BB5	–	+	1
BB7	–	+	1
BB101	+	+	2
BB102	+	+	8

[a]Profiles designated after Witt and co-workers.[12]
[b]*2DL1*004*(*2DL1v*) is shown for those individuals for which no *2DL1* was detected in the initial study.
[c]*2DL5* was detected in all individuals with profiles not shown.
3DL3 was detected in every individual.

linear model did not detect any significant differences between the populations (Table 5). In a second example, the statistical interaction between *2DL5* and *3DS1* described in terms of Δ and two-locus haplotype frequency (*h*) was seen to be similar for all populations except the Africans, whilst relative disequilibrium, (*r*) varied throughout all the groups (Table 4). We detected a significantly lower frequency of *3DS1* in the Africans compared with the other populations (Table 1). However, this did not account for all of the deviation in *r*, as the log-linear analysis indicated significant linkage disequilibrium differences between several populations for this particular locus pair (Table 5, viii). There were nine pairs of *KIR* loci where linkage disequilibrium varied in a frequency independent manner between populations (Table 5). The greatest number of linkage disequilibrium differences noted were between the Thai and African and between Thai and Trinidad South Asian populations (Table 5, x); the least number of differences being noted between the African and Trinidad South Asian populations.

Profile X101

Individuals with this profile appeared to be negative for *2DL4*. These were the first from over 900 individuals that have now been analysed (Figure 1)[12–14,21] PCR-sequence specific oligonucleotide probe (PCR-SSOP) typing confirmed that this is more likely to represent a missing KIR than a novel allele as the SSOP method uses several probes for *2DL4* (Table 6). The X101 profile also appeared to lack several other loci (*2DL1* (and *2DL1*004*), *2DS3*, *2DS4*, *3DL1* and *3DS1*) which, if one examines the characterised *KIR* haplotypes,[22] may indicate a novel haplotype presenting a large deletion spanning *2DL4*. PCR-SSOP typing corroborated these observations and revealed that profile X101 lacks *KIR-X* (*KIR 3Dp1*), which is a putative pseudogene positioned between *2DL1* and *2DL4*.[22] PCR-SSOP also confirmed that these individuals possess all the *KIR* indicated in Figure 1, with the addition of *KIR-Z* (*KIR2Dp1*; another possible pseudogene).

Discussion

Previous population studies examining the polygenicity of the KIR region have focused on Caucasoid population samples. In the first, 52 USA Caucasoids were analysed for 10 of the putative loci (*2DL1–3*, *3DL1–2*, *2DS1–4* and *3DS1*).[11] Another study tested 147 Australian Caucasoids for 11 loci (*2DL4* added).[12] The third population to be reported, namely Northern Ireland Caucasoid ($n = 90$), was analysed for these 11 plus *2DS5* using PCR-SSOP.[13] A further Australian Caucasoid ($n = 32$), as well as Vietnamese ($n = 59$) and Australian Aborigine ($n = 67$) populations have also been described recently for the original 10 loci.[21] We have now studied six distinct populations, UK Caucasoid, Palestinian, Thai, South Asians from Karachi and African and South Asian descended populations from Trinidad, for all 12 KIR loci plus the more recently described *2DL5* and *3DL3*. We have also tested our study groups for a *KIR* variant that was not detected by the original primer combinations. There is close correlation for the *KIR* gene frequencies between the UK and the other Caucasoid populations, but we have observed many differences between the distinct ethnic populations. The profiles of most of the highly significant two-locus associations would appear to be similar for all of the populations now reported.[11–14,21] However, we have noted some differences in the magnitude of linkage disequilibrium for pairs of loci between populations that are not directly attributable to differences in locus frequencies.

Locus profiles

We have observed a total of 79 different genetic profiles of 13 *KIR* loci in 608 individuals, encompassing five dif-

Table 4 Linkage disequilibrium values for pairs of KIR loci

	2DL2			*2DL3*			*3DL1*			*2DS1*			*2DS2*			*2DS3*			*2DS4*			*2DS5*			*3DS1*			*2DL5*			*Cauc*	*Pal*	*Thai*
	African	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*	*African*	*TSA*	*KSA*			
2DL1																																	
Δ	−0.19	−0.25	−0.18	0.21	0.23	0.20	0.07	0.09	0.09	−0.07	−0.07	−0.01	−0.34	−0.14	−0.18	−0.10	−0.06	−0.12	0.04	0.07	0.08	−0.04	−0.05	−0.04	−0.03	−0.04	00.04	−0.14	−0.12	−0.15	−0.10	−0.05	−0.04
r	1	1	0.64	0.73	0.91	0.91	0.17	0.33	0.48	1	0.36	0.02	1	0.52	0.59	1	0.76	0.71	0.12	0.30	0.55	0.56	0.40	0.24	0.71	0.27	0.38	0.84	0.43	0.41	0.42	0.22	0.17
h			0.10	0.54	0.57	0.68	0.54	0.47	0.47		0.12	0.25		0.12	0.13			0.05	0.49	0.39	0.40	0.03	0.07	0.13		0.11	0.27	0.03	0.16	0.21	0.14	0.18	0.20
P	<0.0001	≪0.0001	<0.0001	<0.01	<0.0001	<0.001							<0.0001	<0.001	<0.001		<0.01	<0.001										<0.001	<0.01	<0.001	<0.001		
2DL2																																	
Δ				−0.27	−0.25	−0.17	−0.09	−0.02	−0.14	0.01	0.04	0.04	0.16	0.20	0.23	0.07	0.06	0.11	−0.12	−0.05	−0.11	0.00	0.01	0.02	−0.01	−0.02	−0.02	0.13	0.12	0.11	0.09	0.10	0.09
r				1	1	0.58	0.33	0.06	0.59	0.12	0.18	0.21	0.87	0.76	0.91	1	0.81	0.77	0.50	0.22	0.56	0.04	0.09	0.16	0.70	0.17	0.16	0.59	0.41	0.34	0.37	0.41	0.40
h						0.12	0.18	0.24	0.10	0.05	0.17	0.20	0.24	0.38	0.42	0.10	0.12	0.22	0.12	0.17	0.09	0.04	0.09	0.13		0.08	0.12	0.22	0.31	0.33	0.19	0.25	0.16
P				≪0.0001	≪0.0001	<0.001			<0.001				<0.01	<0.0001	<0.0001														<0.01		<0.01	<0.01	<0.01
2DL3																																	
Δ							0.08	0.07	0.06	−0.08	−0.08	0.01	−0.28	−0.23	−0.17	−0.09	−0.08	−0.22	0.06	0.06	0.07	−0.05	−0.02	−0.03	−0.04	−0.07	0.03	−0.14	−0.12	−0.14	−0.09	−0.10	−0.04
r							0.24	0.28	0.38	1	0.43	0.07	1	0.85	0.53	1	1	1	0.19	0.24	0.48	0.60	0.19	0.15	1	0.46	0.28	0.73	0.40	0.37	0.39	0.43	0.17
h							0.62	0.46	0.46		0.11	0.27		0.04	0.15				0.57	0.39	0.40	0.03	0.10	0.15		0.08	0.27	0.05	0.17	0.23	0.15	0.13	0.20
P										<0.01			<0.0001	<0.0001	<0.001		<0.001	≪0.0001										<0.01	<0.01	<0.01	<0.01		
3DL1																																	
Δ										−0.11	−0.23	−0.28	−0.09	−0.06	−0.13	−0.11	0.02	−0.10	−0.02	0.19	0.21	0.02	−0.02	−0.20	−0.12	−0.12	−0.18	−0.09	−0.18	−0.20	−0.10	−0.20	−0.16
r										1	1	1	0.42	0.19	0.52	1	0.40	0.69	0.03	1	1	1	0.17	1	1	0.74	0.92	0.33	0.57	0.68	0.37	0.77	0.73
h													0.13	0.24	0.12		0.11	0.05	0.69	0.56	0.47	0.13	0.11			0.04		0.18	0.13	0.09	0.18	0.06	0.06
P										<0.001	<0.0001	≪0.0001			<0.01	<0.01		<0.01		<0.0001	<0.001			≪0.0001		<0.0001	≪0.0001		<0.0001	<0.001	<0.01	≪0.0001	≪0.0001
2DS1																																	
Δ													0.03	0.07	0.04	−0.01	0.03	0.03	−0.16	−0.16	−0.14	0.06	0.10	0.15	0.05	0.15	0.18	0.07	0.16	0.16	0.17	0.15	0.16
r													0.14	0.23	0.15	0.59	0.28	0.16	1	0.88	0.80	0.50	0.74	0.82	0.84	0.88	0.85	0.27	0.49	0.47	0.71	0.50	0.74
h													0.06	0.21	0.21		0.07	0.12			0.03	0.07	0.17	0.25	0.06	0.23	0.31	0.11	0.32	0.36	0.26	0.25	0.23
P																			<0.01	≪0.0001	<0.0001		<0.01	<0.01		<0.0001	<0.001		<0.001	<0.01	≪0.0001	<0.001	≪0.0001
2DS2																																	
Δ																0.08	0.06	0.11	−0.13	−0.08	0.10	0.03	0.01	0.01	−0.01	0.02	−0.03	0.11	0.12	0.10	0.09	0.11	0.08
r																1	0.77	0.75	0.62	0.31	0.47	0.28	0.09	0.08	0.42	0.13	0.18	0.47	0.46	0.34	0.41	0.46	0.38
h																0.10	0.12	0.22	0.08	0.17	0.11	0.06	0.10	0.13		0.13	0.12	0.19	0.34	0.34	0.20	0.26	0.16
P																			<0.01										<0.01		<0.01		
2DS3																																	
Δ																			0.02	0.00	−0.07	−0.02	0.02	0.01	−0.01	0.03	0.03	0.07	0.07	0.11	0.09	0.13	0.10
r																			0.02	0.01	0.61	1	0.12	0.08	0.78	0.16	0.11	0.26	0.17	0.27	0.33	0.40	0.38
h																			0.10	0.08	0.05		0.05	0.08		0.07	0.12	0.10	0.13	0.24	0.14	0.21	0.14
P																															<0.001	<0.001	<0.001
2DS4																																	
Δ																						−0.03	−0.05	−0.15	−0.04	−0.14	−0.15	−0.12	−0.13	−0.13	−0.16	−0.20	−0.24
r																						0.28	0.39	1	0.74	0.96	0.97	0.50	0.49	0.54	0.61	0.77	1
h																						0.08	0.07					0.12	0.14	0.12	0.10	0.06	
P																								≪0.001		≪0.0001	<0.0001	<0.01	<0.01	<0.01	≪0.0001	≪0.0001	<0.0001
2DS5																																	
Δ																									0.05	0.08	0.14	0.08	0.09	0.11	0.12	0.09	0.09
r																									0.84	0.42	0.56	0.30	0.22	0.27	0.44	0.25	0.35
h																									0.06	0.14	0.23	0.12	0.18	0.24	0.18	0.14	0.13
P																										<0.01	<0.01				≪0.0001	<0.01	<0.001
3DS1																																	
Δ																												0.03	0.11	0.13	0.15	0.13	0.18
r																												0.12	0.32	0.37	0.59	0.42	0.81
h																												0.06	0.24	0.31	0.23	0.21	0.25
P																													<0.01	<0.01	≪0.0001	<0.001	≪0.0001

TSA = Trinidad South Asian; KSA = Karachi South Asian; Cauc = UK Caucasoid; Pal = Palestinian. Δ = Δ linkage disequilibrium parameter. *r* = relative linkage disequilibrium (1 = threshold). *h* = estimated two-locus haplotype frequency (only shown when >0.02). *P* (Yates corrected) indicated where appropriate.

Table 5 Log-linear model for linkage disequilibrium applied to six populations

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	0.005	.	0.005	.		
TSA	0.02	.	0.02	.	.	
KSA	.	.	.	0.02	.	.

i. 2DL2 vs 2DS2

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	.	0.03	.	.		
TSA	.	.	0.006	.	.	
KSA	.	.	0.01	.	.	.

ii. 2DL2 vs 2DS1

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	.	0.04	0.02	.		
TSA	.	0.001	0.002	.	.	
KSA	.	.	.	.	.	.

iii. 3DL1 vs 2DS5

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	.	0.02	0.02	.		
TSA	.	0.009	0.008	.	.	
KSA	.	.	.	.	.	.

iv. 2DS1 vs 2DS5

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	0.02	.				
Thai	0.03	.	.			
African	.	0.05	0.03	.		
TSA	.	0.01	.	.	.	
KSA	.	0.03	.	.	.	.

v. 2DS1 vs 3DS1

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	0.04	.				
Thai	.	0.007	.			
African	.	.	0.01	.		
TSA	.	.	.	.	.	
KSA	.	.	0.02	.	.	.

vi. 2DS3 vs 3DS1

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	.	.	.	.		
TSA	0.03	0.02	0.02	.	.	
KSA	.	.	.	.	.	.

vii. 2DS4 vs 2DS5

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	0.02	.	0.008	.		
TSA	.	.	.	.	.	
KSA	0.02	.	0.003	.	.	.

viii. 3DS1 vs 2DL5

	Cauc	Pal	Thai	Afri	TSA	KSA
Cauc	.					
Pal	.	.				
Thai	.	.	.			
African	0.02	0.005	.	.		
TSA	0.02	0.004	.	.	.	
KSA	.	.	.	.	.	.

*ix. 2DL1 *004 vs 2DS2*

	Cauc	**Pal**	**Thai**	**Afri**	**TSA**	**KSA**
Cauc	.					
Pal	**2**	.				
Thai	**1**	**2**	.			
African	**4**	**4**	**6**	.		
TSA	**3**	**5**	**6**	**0**	.	
KSA	**1**	**1**	**3**	**1**	**1**	.

x. Total

Pairs of loci with gene frequency independent deviations in linkage disequilibrium between populations ($P < 0.05$) (i–ix) further analysed to determine which populations were significantly different ($P <$ value shown). (x) Total number of instances where two populations had significant differences in linkage disequilibrium. TSA = Trinidad South Asian, KSA = Karachi South Asian.

ferent ethnic groups and six populations. This variety is predominantly due to loci for the non-inhibitory receptors, as has been the case for all populations where these profiles have been illustrated (Figure 1).[11–14] There appears to be a greater variety of profiles in the African and South Asian populations than the others we have investigated, which may be due to further complexity of the *KIR* haplotypes segregating in these populations. We have identified 25 novel profiles in these African and South Asian populations. Several of these profiles appear infrequently in certain population groups, such as AB8 which was detected in six Africans (9.7%, Figure 1.) and had only been observed in one Australian Caucasoid.[12]

The most frequently occurring profile so far reported has been AA1, which comprises of seven *KIR* (now *3DL3* is included) and includes the fewest non-inhibitory KIR loci (see Figure 1). AA1 was the most prevalent in all Caucasoid populations as well as the Palestinian and Thai[11–14] and remained the most common in these three groups reported here, although to a lesser degree in the South Asians. The reduced prevalence of AA1 in the South Asians is reflected in the modal number of loci pos-

Table 6 Extra PCR primers for *KIR*

(i) SSP combinations

KIR	*'forward' sequence (5′–3′)*	*3′ position*	*'reverse' sequence (5′–3′)*	*3′ position*	*Product length (base pairs)*
2DL1v[a]	ACTCACTCCCCCTATCAGG	331 exon 4	AGGGCCCAGAGGAAAGTT	550 exon 5	1800
2DL5[b]	TCGGGGTTCACACCCRCG	302 exon 3	CCGGCTGGGCTGAGAGT	375 exon 5	1000
3DL3	TTCTTCTTGCTGGAGGGGC	(54 exon 2)	CTTCAGACACCACAGTGCC	(105 exon 3)	850

(ii) SSOP probes (5′–3′)

KIR	*probe sequence*	*position*	*probe sequence*	*position*
2DL4	CATCTTCACGCTGTAC	190 exon 3	GCCTGCGGGACACAGAAC	840 exon 8
2DL5	GGGTTTACCATCTTC	175 exon 3	CATTGCTGCTGCTCCA	795 exon 8
3DL3	CTGAAGGACAACATGTG	(135 exon 3)	CACGATGCGGGTTCCCAG	(530 exon 4)
KIR-X (3DP)	GAAACACCGTTTTCATAG	(*exon 3*)	GAAGTTTAATGACACTTTG	(*exon 4*)
KIR-Z (2DP)	CAGGGACGTACAGAT	(*exon 3*))	CCATGATGGAAGACCTG	(*exon 4*)

Nucleotide positions according to cDNA from start of leader sequence. Exon numbers corresponding to alignment with *3DL/S*. If no cDNA available, then alignments in brackets. Locus nomenclature according to HUGO (see http://www.gene.ucl.ac.uk/nomenclature/genefamily/kir.html); allele nomenclature.[17]
[a]*2DL1v* represents *2DL1*004* (AF022045); *2DL1*001* (L41267 (NKAT1)), *2DL1*002* (U24076, NM14218), *2DL1*00301* (U24078), *2DL1*00302* (AF285431) and *2DL1*005* (AF285432) are all detected by original 2DL1 primers.
[b]Specific for all known subtypes of *2DL5* (*2DL5.1*:AF204903 and *2DL5.2*:AF204905, *2DL5.3*:AF217487 and *2DL5.4*:AF260138[16,20] also three new alleles AF272157, AF271607 and AF271608.

sessed for each population: seven for Africans (also for UK Caucasoid, Palestinian and Thai,[14] and 11 for both South Asian groups (Figure 1). It is also interesting to note that the AA1 profile was rarely observed in the Australian Aborigine population.[21] It still remains to be determined whether AA1 profile offers a protective advantage, or is merely a consequence of unrelated environmentally induced population bottlenecks; which may have been severe in some cases.[23]

Profile X101 and 2DL4 deletion haplotypes

Loci for the inhibitory 2DL4 and 3DL2 have been detected in every individual investigated to date.[12–14,21] However, we were unable to detect *2DL4* in one individual from each of our South Asian groups (profile X101, Figure 1). Furthermore, neither *3DL1*, *3DS1* nor *KIR-X* were detected in these individuals. If one examines the nucleotide sequence organization of the area[22,24,25] it appears most likely that these individuals are homozygous for a *KIR* haplotype with a large deletion spanning these loci (45–65 kb deletion; complete *KIR* region spans 100–160 kb). The observation that profile X101 incorporates *2DL2*, *2DL3* and *KIR-Z* suggests that these haplotypes contain an alternative arrangement of *2DL2/3/-Z* in addition to the deletion, as all three loci would not be predicted to be on the same haplotype. Nevertheless, *2DL4* deletion haplotypes must be present in approximately 10% individuals in both South Asian populations in order to comply with Hardy-Weinberg equilibrium. The finding of healthy individuals completely lacking *2DL4* suggests redundancy of function for this KIR, which has been implicated in foetal survival due to interaction with HLA-G[26,27] and was thought to be essential for several species.[28]

***KIR2DL5* and haplotype characterisation**

Uhrberg and coworkers originally defined *KIR* 'B' haplotypes by the presence of a 24 kb *Hind*III fragment on Southern blot analysis and these haplotypes exhibited a greater number of loci for the non-inhibitory KIR.[11] Subsequently, Witt and coworkers proposed that the haplotype families could be distinguished by the presence of certain loci.[12] 'A' haplotypes were defined by the presence of *2DL1* and *2DL3*, and 'B' haplotypes by the presence of *2DL2*. *KIRs 2DL1* and *2DL3* were observed in Hardy-Weinberg equilibrium with *2DL2* for this Australian Caucasoid population and also for all six populations that we have investigated. Furthermore, *2DL2* has been observed in linkage disequilibrium with certain loci for non-inhibitory KIR in all populations.[11–14,21] However, the model is somewhat confounded by *2DL1v* (*2DL1*004*) which is a recombinant of *2DL1* and *2DS1* and may encode an inhibitory receptor that recognizes the same epitope as KIR2DL1.[29] We can now see that only 13 individuals from the 608 we have investigated did not possess *2DL1* (Figure 1 and Table 3) and so *2DL1 per se* is therefore not a marker for the 'A' haplotype. Another confounding locus is *2DL5*, which characterises the 'B' haplotype.[16] Our results clearly indicate that no individual from the previously designated AB1 profile was seen to possess *2DL5* and there were many other discrepancies (Figure 1 and Table 3). We now believe that the original model appeared consistent for most individuals assigned as BB homozygous because *2DL1*004* is in linkage disequilibrium with *2DL2* and also one of the known *2DL5* subtypes (own unpublished observations). One further corollary is the finding that *2DL5* may be duplicated on the same gametic haplotype in some instances.[20]

A nomenclature system needs to be devised that

accommodates the complexity of KIR polygenicity and polymorphism. Such a system should be designed to describe adequately the genetic profiles and with a view to the disclosure of their underlying haplotypes.

Linkage disequilibrium and comparison of all six populations

There appears to be uneven distributions of most of the loci between the populations described which are consistent with the combinations of loci that are in linkage disequilibrium and probably form the two major haplotypes. Both of our South Asian populations had elevated frequencies of *2DL2* and *2DS1–3*, *2DS5* and *2DL5* ('B' haplotype) and lower frequencies of *2DL1*, *2DL3*, *3DL1* and *2DS4* ('A' haplotype) (Table 1), and similar patterns were evident in the Australian Aborigine population.[21] The converse profile was apparent for the Thai and African populations, where the highest frequencies of *2DL1*, *2DL3*, *3DL1* and *2DS4* and the lowest frequencies of *2DL5*, *2DS1–3*, *2DS5* and *3DS1* were observed overall (Table 1).[14]

We previously suggested that of the three populations we studied, the Palestinians had the highest frequency of 'B' haplotypes and Thais the highest 'A'. This observation was based on the estimated gene frequencies of *KIR* and their linkage disequilibrium profiles.[14] Re-examination of these populations, in the context of *2DL5*, has confirmed our original deductions and even higher frequencies of 'B' haplotypes are now evident in South Asian populations (Table 1).

We have used a log-linear model to ascertain which inter-population deviations in linkage disequilibrium are not influenced by the inter-population differences in gene frequencies of the loci in question. Overall we can see that the Thai and South Asian groups would appear to be the most disparate in terms of deviations in linkage disequilibrium as well as gene frequencies, whereas the African and South Asian groups have diverse gene frequencies but similar linkage disequilibrium (Table 5). These observations all provide evidence for differences in the structure and/or frequencies of the KIR gene content haplotypes that are segregating in the various populations we have studied. We anticipate that the information gained from these populations will be of value towards the characterization of these *KIR* haplotypes.

At present we cannot assume an absolute correlation between the *KIR* sequences and genetic loci, as we may only be detecting a certain percentage of alleles at a locus, or recombination events may have occurred between certain loci that lead to an apparent increase in their frequency. The observed correlation between the SSP and SSOP reduces these possibilities,[14] although it would be difficult to speculate how many loci we could be underestimating. Allelic variants of several *KIR*s have been described recently[30] and the typing systems used here remain consistent with these. It should also be noted that many sequences publicly available are derived from mRNA and remain unconfirmed by DNA sequencing; such a cDNA sequence appears to be a fusion of *2DL1* and *3DL1* (4M1#6) and if this were produced from a single gene then it would appear positive for *2DL3* but negative for *3DL1* using PCR-SSP. Thus the PCR determined profiles described may not be an entirely accurate reflection of the gene content for the *KIR* region for all individuals, although they highlight that there are considerable differences between populations.

The calculations for linkage disequilibrium that we have used may be restricted by the lack of true genotype information in some cases; for example, *2DS4* and *3DL1* have exhibited strong positive linkage disequilibrium in all populations that have been investigated in a similar manner.[12–14] However, linkage disequilibrium was not detected between these loci in the Africans. It has been stated that the effective distance for linkage disequilibrium may be shorter throughout the genome for African populations,[23] although in this case the reduced association was probably due to high gene frequencies of *2DS4* and *3DL1*, which occurred together in all but three of the Africans (Figure 1, profiles AB107, BB105 and BB108).

In summary, we have shown that *KIR* locus frequencies can be significantly different between ethnic populations, we have established yet further *KIR* genetic profiles and provided evidence for inter-population diversity of the *KIR* haplotypes that can determine an individual's KIR repertoire. More populations need to be investigated if we are to develop a comprehensive understanding of the biological implications of these variations, especially in the context of the highly polymorphic nature and ethnic distribution of their ligands. We feel that KIR repertoires should be considered, perhaps even on an individual basis, in many functional and/or disease studies concerning HLA and the immune response.

Materials and methods

Populations

Sixty-two unrelated healthy individuals of African and 108 of South Asian descent from the island of Trinidad were studied. Documentation of the family history of each individual for three generations allowed confirmation of ethnicity. Full details of sample collection are discussed elsewhere.[18] Seventy-eight healthy unrelated South Asian individuals from Karachi were also analysed; Hameed and coworkers[19] describe full details of ethnicity and sample collection. We also re-analysed three previous populations[14] using further PCR-SSP primer combinations which are described below.

PCR-SSP

We tested genomic DNA from the three new populations using precisely the same PCR sequence specific priming (PCR-SSP) combinations as detailed previously.[14] All six populations were also tested for the recently identified *2DL5*,[16] *3DL3* (eg from AC0666) and *2DL1v* (*2DL1*004*)[29] (Table 6).

Reactions of 10 μl were set up to include 0.1 μg test DNA, Buffer IV, 0.2 mM dNTP, 1.08 mM magnesium chloride, 0.3U *Taq* DNA polymerase (all Advanced Technologies, UK), and 0.5 μM specific primer mix (except for *3DL1*, *2DS4* which were at a final concentration of 1 μM). Internal controls (f 5′-CAGTGCCTTCCCAACCATTCCC TTA-3′, r 5′-ATCCACTCACGGATTTCTGTTGTGTTTC-3′; Oswel DNA, Southampton, UK.) specific for a 485 base pair human growth hormone fragment were included at 0.067 μM in each reaction. All amplifications were performed in duplicate under thermal cycling conditions previously described,[14] and any individual sample which displayed a profile that was unique, or had not

been previously reported, was repeated at least once more. Six widely available cell line samples with the most common *KIR* genetic profiles are illustrated in Figure 1.

PCR-SSOP

Where appropriate, PCR-SSOP typing for *KIR* sequences was performed exactly as described[13] and with additional probes for *2DL5* and *3DL3*, pseudogenes *KIR-Z* (*2Dp1*) and *KIR-X* (*3Dp1*) and two further probes for *2DL4* (Table 6).

Statistical analysis

Percentages of each *KIR* in each population were determined by direct counting. Gene frequencies (gf) and linkage disequilirium Δ values, haplotype frequencies (*h*) and relative disequilibrium (*r*) for two locus associations were calculated exactly as previously described.[14] These parameters give an estimate of linkage disequilibrium between two loci in a population that is generally not dependant on the respective frequencies of the loci. However, when comparing populations then deviances in linkage disequilibrium are not necessarily precluded by a difference in gf between populations. We therefore fitted a log-linear model to adjust for any differences in *KIR* frequencies before comparing linkage disequilibrium between the population groups.

The analysis was performed in pairs of genes and consisted of two stages. In the first stage the log-linear model was fitted to data from all six populations to test whether the linkage disequilibrium between pairs of genes differed between populations independently of gene frequencies. If a statistically significant difference in the linkage disequilibrium between at least two of the populations was identified, then that gene pair entered the second stage, where the log-linear model was fitted to all possible pairs of populations in order to determine where any differences lay. Analysis was performed using SPlus (version 6. Insightful, Seattle, WA, USA) and all significant testing was carried out at the 5% level. Details on fitting log-linear models to contingency tables can be found in Everitt (1994).[31]

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