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KIR haplotypes defined by segregation analysis in 59 Centre d'Etude Polymorphisme Humain (CEPH) families

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Abstract

The killer cell immunoglobulin-like receptor (KIR) gene cluster exhibits extensive allelic and haplotypic diversity. Variation at the locus is associated with an increasing number of human diseases, reminiscent of the *HLA* loci. Characterization of diversity at the *KIR* locus has progressed over the past several years, particularly since the sequence of entire *KIR* haplotypes have become available. To determine the extent of *KIR* haplotypic variability among individuals of northern European descent, we genotyped 59 CEPH families for presence/absence of all *KIR* genes and performed limited allelic subtyping at several *KIR* loci. A total of 20 unique haplotypes differing in gene content were identified, the most common of which was the previously defined A haplotype ($f=0.52$). Several unusual haplotypes that probably arose as a consequence of unequal crossing over events were also identified. Linkage disequilibrium (LD) analysis indicated strong negative and positive LD between several pairs of genes, values that may be useful in determining haplotypic structure when family data are not available. These data provide a resource to aid in the interpretation of disease association data involving individuals of European descent.

Keywords

KIR haplotypes; CEPH; Linkage disequilibrium

Natural killer (NK) cells are central to the innate immune system and participate in early responses against infected or transformed cells through production of cytokines and direct cytotoxicity (Bancroft 1993; Biron et al. 1999; Trinchieri 1989). They also interact with components of the adaptive immune system including T cells and dendritic cells (Raulet

2004). NK cell function is regulated by a network of inhibitory and activating receptors (Colucci et al. 2002; Lanier 2005), including the highly polymorphic killer cell immunoglobulin-like receptors (KIRs). *KIR* haplotypes vary in the type and number of genes present (Uhrberg et al. 1997), and some of the *KIR* genes show extensive allelic polymorphism to the extent that it is unlikely for any two randomly selected individuals to have identical *KIR* genotypes (Shilling et al. 2002a). *KIR* gene expression patterns vary clonally (Valiante et al. 1997), adding yet another layer of complexity to the system. HLA class I allotypes are ligands for KIR, and variability at *HLA-A*, *HLA-B*, and *HLA-C* is thought to be due in part to selection pressure through disease morbidity and mortality (Hill et al. 1991; Klein et al. 1993), processes that may result in part from KIR-HLA interactions. Comparisons of *KIR* genes across primates indicate rapid evolution of this receptor family (Khakoo and Carrington 2006; Khakoo et al. 2000; Vilches and Parham 2002), and recent data suggest that the *KIR* and *HLA* loci are indeed coevolving (Norman et al. 2007; Single et al. 2007).

The *KIR* locus maps to chromosome 19q13.4 within the leukocyte receptor complex (*LRC*), where the genes are tandemly arrayed over a segment of about 100–200 kb (Wilson et al. 2000). The *KIR* genes show high sequence similarity that may facilitate the occurrence of non-allelic homologous recombination (Carrington and Cullen 2004; Lupski 1998) and explain to some extent the expansion and contraction of *KIR* haplotypes (Martin et al. 2000; Wilson et al. 2000). The elasticity at the *KIR* locus has resulted in the generation of *KIR* haplotypes that may have two (or more) copies of the same gene on a single haplotype, a rearranged gene order, or novel hybrid genes (Gomez-Lozano et al. 2005; Martin et al. 2003; Williams et al. 2003).

Based on gene content, *KIR* haplotypes have been divided into two groups, termed A and B, which were originally differentiated by the presence of a 24-kb *HindIII* fragment on Southern blot analysis (Uhrberg et al. 1997). Haplotype A is uniform in terms of gene content (*3DL3*, *2DL3*, *2DL1*, *2DP1*, *3DP1*, *2DL4*, *3DL1*, *2DS4*, and *3DL2*), whereas the B haplotypes contain variable numbers and combinations of *KIR* genes. Human populations can vary remarkably in the frequencies of *KIR* genes and haplotypes, possibly a reflection of selection pressures conferred by population-specific diseases (Cook et al. 2003; Crum et al. 2000; Denis et al. 2005; Frassati et al. 2006; Gendzekhadze et al. 2006; Jiang et al. 2005; Kulkarni et al. 2008a; Middleton et al. 2007; Norman et al. 2001; Rajalingam et al. 2002; Toneva et al. 2001; Velickovic et al. 2006; Whang et al. 2005; Witt et al. 1999; Yawata et al. 2002). The frequency distribution of haplotypes A and B are roughly equal in Caucasian populations, but this balance is not maintained in some other populations, such as in Australian Aborigines where the A haplotype has a frequency of only about 13% (Toneva et al. 2001). Currently, more than 40 different B haplotypes have been described based on segregation analysis (summarized in Khakoo and Carrington 2006), and this number is likely to increase as more populations are genotyped. Given the role of *KIR* in both arms of the immune response, their specificity for HLA class I allotypes, and their extensive diversity, it is not surprising that variation at the *KIR* locus has been shown to influence resistance and susceptibility to a number of human diseases (reviewed in Kulkarni et al. 2008b). However, the diversity of the *KIR* genes/haplotypes and strong linkage disequilibrium (LD) between pairs of *KIR* loci imparts a challenge when attempting to determine the gene(s) or allelic variants that directly contribute to disease phenotypes.

In order to more fully characterize the *KIR* locus in Caucasians, we typed for the presence/absence status of 16 *KIR* genes (Martin and Carrington 2007) in members of 57 CEPH families of European descent and determined haplotypes based on segregation analysis (Fig. 1). These families included one Amish family, ten French families, and 46 families from Utah. All genotyping data will be uploaded to the Centre d'Etude Polymorphisme Humain

(CEPH) database (<http://www.cephb.fr/en/cephdb/>), which is freely accessible to the scientific community. The framework genes *KIR3DL3*, *KIR3DP1*, *KIR2DL4*, and *KIR3DL2* were present in all individuals, as were *KIR2DL2/KIR2DL3* (alleles of the same locus) and *KIR3DL1/KIR3DS1* (also alleles of the same locus). Overall, carrier frequencies were similar to those found for other Caucasian populations (Cook et al. 2003; Hsu et al. 2002; Norman et al. 2001; Uhrberg et al. 1997, 2002; Witt et al. 1999), as were gene frequencies (Table 1). We also performed allelic subtyping (Table 2) for *KIR2DL4* and limited subtyping for *KIR2DL5* in order to distinguish *2DL5A* and *2DL5B*, for *KIR2DS4* to determine presence/absence of the deletion variant ($\Delta 2DS4$), and for *KIR3DP1* to identify individuals with the variant allele (*3DP1v*) that actually contains an exon 2.

Figure 1 lists the unique haplotypes identified by segregation analysis based on presence/absence of *KIR* genes along with the haplotypes defined by allelic subtyping. Some of these haplotypes are not necessarily definitive, since our typing method does not determine gene copy number; that is, if both parents have a particular gene and all offspring also carry that gene, it is impossible to know by our typing method whether both parents have two copies of the gene (one on each chromosome 19) or one parent has two copies and the other parent has only one copy, as in both cases, all offspring will type positive for the gene. Therefore, to generate these haplotypes, several assumptions were made based on gene frequencies, patterns of linkage disequilibrium, and consistent observations across other studies: (1) *3DL3* and *3DL2* are present on all haplotypes; (2) if *2DS4* is present, *2DS1* is absent and vice versa; (3) if *2DL1* is present, *2DP1* is present; (4) if *3DP1v* is present, *2DL1* and *2DP1* are absent; (5) *2DL5B* is always assumed to be in the centromeric position, and *2DL5A* is always assumed to be in the telomeric position; and (6) *2DS3* and *2DS5* were arbitrarily placed in the telomeric position, even though they can map to either half of the locus. Deduced haplotypes were further supported by the estimated haplotypes produced by the HAPLO-IHP (haplotype inference using identified haplotypes patterns) software (Yoo et al. 2007; see review by Single et al. in this issue). A total of 20 unique haplotypes based on presence/absence of *KIR* genes alone were identified with frequencies ranging from 51.7% to <1%. The most common haplotype was the A haplotype (51.7%), which is consistent with previous studies of Caucasians. The most common B haplotype was detected at a frequency of 17.4% overall (Fig. 1, haplotype 8) and 36% among the set of B haplotypes. The other B haplotypes were relatively rare with overall frequencies ranging from 7.3% to <1%, and of the 19 B haplotypes, 13 of them were observed only once or twice.

A few of the haplotypes deduced from the analysis were unusual in that they did not follow the general characteristics of *KIR* haplotypes. For example, haplotype 17 appears to contain only three genes (*3DL3*, *2DS1*, and *3DL2*), and it is missing the framework genes *3DP1* and *2DL4*, as well as *2DL2/3* and *3DL1/S1*. Full characterization and sequence of this haplotype will be published elsewhere (Traherne et al., in preparation). Haplotype 20 on the other hand is an extended haplotype containing two copies of both *2DL4* and *3DL1/S1* as well as a *2DL5A/3DP1* hybrid, which we described previously (Martin et al. 2003). The hybrid *2DL5A/3DP1* gene has now been officially designated *3DP1*004* (Gomez-Lozano et al. 2005). Haplotype 18 is missing *2DL2/3*, and haplotype 19 is missing both *2DL4* and *3DL1/S1*. These unique haplotypes probably arose as a consequence of unequal crossing over.

Allelic variation of *KIRs* creates further diversity such that it is extremely unlikely that any two individuals will be 100% identical (Shilling et al. 2002b). Thus far, the number of alleles per *KIR* locus ranges from 5 to 55 (<http://www.ebi.ac.uk/ipd/kir/database>). Variation tends to occur throughout all regions of the gene, but many of the amino acid residues that vary among *KIR* allotypes are found in the extracellular domains, which might sometimes result from selection of polymorphisms that alter their interaction with ligands (Fan et al. 2001; Gardiner et al. 2001; Snyder et al. 1999) and/or expression levels (Gardiner et al.

2001; Thomas et al. 2008; Yawata et al. 2006). Subtyping of *KIR2DL4*, *KIR2DL5* (A vs B), *KIR2DS4* (Δ vs full length), and *3DP1* (*3DP1v* vs *3DP1 Δ ex2*) extended the number of unique haplotypes to 31 (Fig. 1). The most frequent haplotype was an A haplotype containing *2DL4*008* and *2DS4* (25.8% overall; 50% of the A subset of haplotypes). Interestingly, *KIR2DL4*008* is not expressed on the cell surface due to a deletion of an adenine in the transmembrane domain (exon 6) that results in a frameshift leading to a premature stop codon four codons into exon 7, which along with exon 8 encodes the cytoplasmic domain of the molecule (Witt et al. 2000). Likewise, *2DS4* is also not expressed as a consequence of a 22-bp deletion in the D2 domain. Thus, the most common *KIR* haplotype contains no activating *KIR* that is expressed on the cell surface. *2DL4*005* was always found on a haplotype containing *KIR3DS1* and is therefore not present on the A haplotype in this population. However, of the 14 haplotypes containing *KIR3DS1*, two did not contain *2DL4*005*, but rather contained alleles very similar to *2DL4*005*, both of which were probably derived from *2DL4*005*. For the most part, the majority of unexpressed *2DL4* alleles (**008*, **011*) were found on haplotypes with *2DS4*, while the expressed alleles (**001*, **002*) tended to be found with the expressed *2DS4* allotypes. *2DL4*006*, which encodes an allotype that is expressed, was observed only once and was found on a *2DS4* haplotype.

Pairwise LD analysis was performed for the *KIR* loci using Cramer's V statistic (Hedrick 1987) excluding the framework genes *3DL3*, *3DP1*, *2DL4*, and *3DL2* (Table 3). The significance of overall LD between two loci was tested using Fisher's exact test. In general, the LD values were similar to previously published LD values. As expected, there was a significant negative LD between *2DS2* and genes present on the A haplotype, including *2DL3* and *2DL1*, and to a lesser extent *3DL1* and *2DS4*. *2DL3* and *2DL1*, on the other hand, were in negative LD with several activating receptors found on the B haplotypes, including *2DS3*, *2DS1*, and *2DL5B*. Overall, LD (negative or positive) was stronger between genes located on the same half of the locus as delineated by *2DL4*; that is, genes found centromeric to *2DL4* were in stronger negative or positive LD with each other than with those telomeric to *2DL4* and vice versa. One exception was *2DS3*, which can be found at either end of the locus. It is possible that the framework genes might in fact impose structural constraints on recombination between the centromeric and telomeric genes, explaining at least in part the LD values observed. Table 4 shows LD values at the allelic level, which were obtained using Lewontin's normalized measure ($D'_{ij} = D_{ij}/D_{\max}$), which quantifies the deviation from random association for alleles at two loci (Lewontin 1964). Complete negative or positive LD between certain pairs of alleles raises the possibility that allelic subtyping might be a useful tool for predicting haplotypes when segregation analysis is not an option. As mentioned above, for example, *2DL4*005* is almost always found on a haplotype with *KIR3DS1*, and consequently, it is not seen on haplotypes with *KIR3DL1*.

Concluding remarks

We have shown that there is extensive variability at the *KIR* locus in families of Northern European descent, which likely contributes to differences across individuals in both their innate and adaptive immune responses to various pathogens. Functional consequences of *KIR* haplotype variation in infectious diseases, cancer, and autoimmune diseases are quite plausible, and an in depth understanding of the molecular genetic properties of the locus is essential for accurate interpretation of such disease association data.

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Fig. 1.

KIR haplotypes in CEPH based on gene content and allelic variation determined by segregation analysis. The gene order is based on published sequences of *KIR* haplotypes that were sequenced in their entirety. One exception is the extended haplotype 20 where the duplicated genes are not in their proper order, but for simplicity, they have been placed in their usual location (see Martin et al. 2003 for the exact order). Genes depicted as colored boxes have been subtyped, at least to some extent. The *2DS4* alleles containing the 22bp deletion are designated by "Δ". The variant *3DP1* alleles that contain exon 2 are designated by "v". *KIR2DS3* and *2DS5* can be found on the centromeric end between *2DL5* and *2DP1* and on the telomeric end between *2DL5* and *2DS1*, but since it was not possible to

determine the exact location of these genes, they were arbitrarily placed on the telomeric end in this figure

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Table 1

KIR gene frequencies in unrelated CEPH individuals

Locus	Gene frequency (%)	Carrier frequency (%)
3DL3	100.00	100.00
2DS2	35.39	60.67
2DL2	34.27	58.43
2DL3	64.04	88.76
2DL5B	14.04	24.72
2DP1	78.09	96.63
2DL1	78.09	96.63
3DP1	99.44	100.00
2DL4	98.88	100.00
3DL1	76.97	94.38
3DS1	22.47	39.33
2DL5A	21.35	37.08
2DS3	17.42	29.21
2DS5	16.29	30.34
2DS1	22.47	39.33
2DS4	77.53	94.38
3DL2	100.00	100.00

Table 2*KIR* allele frequencies in unrelated CEPH individuals

Locus	Allele	Frequency (%)	Count
2DL2/3	Absent	1.69	3
	<i>2DL2</i>	34.27	61
	<i>2DL3</i>	64.04	114
3DP1	Absent	0.56	1
	$\Delta ex2$	77.53	138
	$\Delta ex2+004^a$	0.56	1
	<i>3DP1v</i>	21.35	38
	Absent	1.12	2
2DL4	<i>001</i>	21.91	39
	<i>005</i>	20.22	36
	<i>005-like</i>	0.56	1
	<i>006</i>	1.12	2
	<i>008</i>	39.89	71
	<i>008+005^a</i>	0.56	1
	<i>011</i>	14.61	26
	Absent	1.12	2
3DL1/S1	<i>3DL1</i>	76.40	136
	<i>3DL1+3DS1^a</i>	0.56	1
	<i>3DS1</i>	21.91	39
	Absent	22.47	40
2DS4	Δ	57.30	102
	<i>FL</i>	20.22	36
	Absent	22.47	40

For *3DP1*, $\Delta ex2$ =alleles missing exon 2; v=alleles containing exon 2. For *2DS4*, Δ =deletion alleles; *FL*=full length alleles

^aTwo copies present on the same haplotype

Table 3

Linkage disequilibrium analysis of *KIR* genes in CEPH

	W_n											
	2DL2	2DL3	2DL5B	2DP1	2DL1	3DL1	3DS1	2DL5A	2DS3	2DS5	2DS1	2DS4
2DS2	0.976	-0.988	0.512	-0.687	-0.687	-0.181	0.193	0.130	0.373	0.151	0.164	-0.164
	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	0.014	NS	<0.001	NS	0.038	0.038
2DL2		-0.964	0.526	-0.705	-0.705	-0.139	0.150	0.086	0.386	0.098	0.122	-0.122
		<0.001	<0.001	<0.001	<0.001	NS	NS	NS	<0.001	NS	NS	NS
2DL3			-0.506	0.707	0.707	0.202	-0.186	-0.124	-0.366	-0.145	-0.186	0.186
			<0.001	<0.001	<0.001	0.009	0.016	NS	<0.001	NS	0.016	0.016
2DL5B				0.136	0.136	-0.316	0.325	0.223	0.838	0.172	0.286	-0.286
				NS	NS	<0.001	<0.001	0.007	<0.001	0.037	<0.001	<0.001
2DP1					1.000	-0.064	0.090	0.110	0.172	0.050	0.090	-0.090
					<0.001	NS	NS	NS	0.029	NS	NS	NS
2DL1						-0.064	0.090	0.110	0.172	0.050	0.090	-0.090
						NS	NS	NS	0.029	NS	NS	NS
3DL1							-0.952	-0.887	-0.382	-0.806	-0.984	0.984
							<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3DS1								0.902	0.427	0.783	0.936	-0.936
								<0.001	<0.001	<0.001	<0.001	<0.001
2DL5A									0.411	0.810	0.902	-0.902
									<0.001	<0.001	<0.001	<0.001
2DS3										0.118	0.356	-0.356
										NS	<0.001	<0.001
2DS5											0.819	-0.819
											<0.001	<0.001
2DS1											-1.000	<0.001
												<0.001

LD values with $p < 0.001$ in bold. *2DL4*, *3DL2*, *3DL3*, and *3DP1* have been omitted from the table, as all three have a frequency of 100%

NS not significant

Table 4

Linkage disequilibrium analysis at the allelic level

	2DL2/3			3DP1		2DL4					3DL1/S1			2DS4		
	0	2	3	$\Delta ex2$	v	001	005	006	008	011	0	L1	S1	0	Δ	FL
2DL2/3	0			-0.140	-1.000	-1.000	0.582	-1.000	-1.000	-1.000	0.491	-1.000	0.573	1.000	-1.000	-1.000
	2			-0.962	1.000	0.103	0.155	0.239	-0.096	-0.650	0.239	-0.203	0.181	0.163	-0.228	0.155
	3			1.000	-1.000	-0.079	-0.219	-0.219	0.138	0.666	-1.000	0.294	-0.239	-0.258	0.264	-0.133
3DP1	$\Delta ex2$	-0.140	-0.962	1.000												
	v	-1.000	1.000	-1.000												
2DL4	001	-1.000	0.103	-0.079	-0.107	0.124	-0.349	-1.000	0.124	-0.625	-1.000	-1.000	-0.883	-1.000	-0.821	0.964
	005	0.582	0.155	-0.219	0.382	0.124	-0.349	-1.000	0.124	-0.625	-1.000	-1.000	-0.883	-1.000	-0.821	0.964
	006	-1.000	0.239	-0.219	1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000
	008	-1.000	-0.096	0.138	-0.085	0.124	-0.085	0.124	-0.085	0.644	-0.355	-0.152	0.315	0.333	0.040	-0.140
	011	-1.000	-0.650	0.666	0.644	-0.625	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000
	0	0.491	0.239	-1.000	-0.355	-1.000	-1.000	-1.000	-1.000	-1.000	1.000	-1.000	-1.000	1.000	-1.000	-1.000
3DL1/S1	0	0.491	0.239	-1.000	-0.355	-1.000	-1.000	-1.000	-1.000	-1.000	1.000	-1.000	-1.000	1.000	-1.000	-1.000
	L1	-1.000	-0.203	0.294	-0.152	0.331	-0.891	1.000	1.000	0.830	-0.817	-0.822	0.813	-0.822	0.813	-0.802
	S1	0.573	0.181	-0.239	0.315	-0.279	-0.883	1.000	-1.000	-0.817	-1.000	-1.000	0.967	-1.000	0.958	0.882
2DS4	0	1.000	0.163	-0.258	0.333	-0.414	-1.000	-1.000	-1.000	-0.822	1.000	-1.000	0.967	0.967	-1.000	-0.873
	Δ	-1.000	-0.228	0.264	0.040	-0.036	-0.821	-1.000	1.000	0.813	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000
	FL	-1.000	0.155	-0.133	-0.140	0.152	0.964	-1.000	-1.000	-0.802	-1.000	-1.000	-0.873	-1.000	-0.873	-1.000

0=absence of gene. For 3DP1, $\Delta ex2$ =the alleles missing exon 2; v = the alleles containing exon 2. For 2DS4, FL=full length alleles; Δ =deletion alleles