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BRIEF COMMUNICATION

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High *KIR* diversity in Amerindians is maintained using few gene-content haplotypes

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Abstract Interaction between killer cell immunoglobulin-like receptors (KIR) and cognate HLA class I ligands influences the innate and adaptive immune response to infection. The KIR family varies in gene content and allelic polymorphism, thereby, distinguishing individuals and populations. *KIR* gene content was determined for 230 individuals from three Amerindian tribes from Venezuela: the Yucpa, Bari and Warao. Gene-content haplotypes could be assigned to 212 individuals (92%) because only five different haplotypes were present—group *A* and four group *B*. Six different haplotype combinations accounted for >80% of individuals. Each tribe has distinctive genotype frequencies. Despite few haplotypes, all 14 *KIR* genes are at high frequency in the three tribes, with the exception of *2DS3*. Each population has an even frequency of group *A* and *B* haplotypes. Allele-level analysis of *3DL1/S1* distinguished five group *A* haplotypes and six group *B* haplotypes. The high frequency and divergence of the *KIR* haplotypes in the Amerindian tribes provide greater *KIR* diversity than is present in many larger populations. An extreme case being the Yucpa, for whom two gene-content haplotypes account for >90% of the population. These comprise the group *A* haplotype and a group *B* haplotype containing all the *KIR* genes, except *2DS3*, that typify the group *B* haplotypes. Here is clear evidence for balancing selection on the KIR system and the biological importance of both *A* and *B* haplotypes for the survival of human populations.

Keywords *KIR* haplotypes · *KIR* locus genotypes · Amerindians · Balancing selection

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Natural killer (NK) cells are effector cells of innate immunity that help initiate adaptive immunity through interaction with dendritic cells (Moretta and Moretta 2004; Raulet 2004). Killer-cell immunoglobulin receptors (KIR) are expressed on NK cells and some T cells. KIR engage with major histocompatibility complex class I ligands (MHC or HLA in humans) on target cells, producing signals that suppress or activate lymphocyte function (Campbell and Colonna 2001; Lanier 2005). Fourteen KIR are encoded in a region of complex genomic organization that exhibits both gene content and allelic variation (Kelley and Trowsdale 2005; Uhrberg 2005). For HLA class I, which is also highly polymorphic, the study of Amerindians illuminated the role of natural selection in maintaining variability, which in these populations was evident on a background of genetic drift, migration, and admixture (Belich et al. 1992; Salzano 2002; Watkins et al. 1992). Several studies have implicated genetically determined HLA–KIR interactions in immunopathology and resistance to infection (Carrington et al. 2005; Khakoo et al. 2004; Parham 2005). The clinical importance being further exemplified by HLA–KIR combinations that mitigate the complications of tissue transplantation (Cook et al. 2006; Ruggeri et al. 2005). Thus, combinations of HLA and KIR factors are likely to have helped Amerindian populations survive the many infections to which Amerindians have been subject. To identify such combinations, it is first necessary to study the distribution of *KIR* genes in Amerindian populations.

The aim of this study was to analyze *KIR* gene content polymorphism in the Yucpa, Bari, and Warao Amerindian populations of Venezuela. Throughout the centuries of the Indo-Hispanic period, the surviving Indian tribes inhabited the peripheral and less penetrable areas of Venezuela, which represented niches of refuge beyond the steadily advancing frontier of the Creole population. The Yucpa and Bari are neighboring but linguistically distinct tribes; they live in western Venezuela, the region between Lake Maracaibo and the Colombian border. Separating the two tribal territories is the Tucuco River. The Warao live on the Orinoco Delta in eastern Venezuela. For Amerindians, genetic drift due to historical bottleneck and persistently low effective population sizes led to reduced diversity in various polymorphic systems, including HLA. For example, previous analysis showed the Yucpa and Warao have five *HLA-B* alleles and the Bari has nine (Layrisse et al. 2001; Layrisse, unpublished data; Martinez-Arends et al. 1998; Ramos et al. 1995) compared to the global average of ~30 per population. In the Yucpa, >80% of individuals carried *HLA-B*3909*, **3905*, or **52012*, and in the Bari, >80% carried **4002*, **3906*, or **3543*. High frequencies of *HLA-A*02* and *A*24* are characteristic of these tribes, but *A*02* is represented by a different variant in the Bari (**0201*) compared to the Yucpa and Warao (**0204*).

Here, the study panel comprised 230 individuals: 61 Yucpa, 80 Bari, and 89 Warao, and included 10 families with both parents and 27 with one parent. *KIR* genotyping was performed using 15 different pairs of primers to detect the 14 *KIR* genes: *2DL1-5*, *2DS1-5*, *3DL1-3*, and *3DS1* (Uhrberg et al. 1997; Norman et al. 2002). *3DL1/3DS1* alleles were detected using a method to be described elsewhere (Norman et al., in preparation). Frequencies of *KIR* genotypes and haplotypes were estimated by direct counting; Hardy–Weinberg equilibrium was calculated by χ^2 or the Markov Chain method implemented in Arlequin (Schneider et al. 2000). All individuals possessed the framework *KIR* genes *(2DL4*, *3DL2* and *3DL3*). All *KIR* were found in all three populations, apart from *2DS3* that was absent from Bari and Yucpa (Fig. 1). To see if unusual *2DS3* alleles were present, an additional primer pair (Gomez-Lozano and Vilches 2002) was used but with similarly negative result. A total of 21 different *KIR* genotypes were observed, their number and frequencies varying considerably between the three populations. Three genotypes in the Yucpa and five in the Bari and Warao accounted for ~80% of the panel (Fig. 1).

In the absence of family data, it is difficult to estimate the composition of *KIR* haplotypes from heterozygote *KIR* genotypes. Consequently, there is much information on the diversity and distribution of *KIR* genotypes in human populations (Crum et al. 2000; Jiang et al. 2005; Norman et al. 2002; Toneva et al. 2001; Whang et al. 2005; Witt et al. 1999; Yawata et al. 2002), whereas knowledge of *KIR* haplotypes is limited (Hsu et al. 2002; Shilling et al. 2002; Uhrberg et al. 2002; Whang et al. 2005). Because of the relatively few *KIR* haplotypes in the Amerindian tribes, we were able to estimate their composition and frequency from genotype frequencies and some family data, by an iterative process. Five different haplotypes account for at least 218 (92%) of the study panel (Fig. 2).

Almost 90% of Yucpa have one of three *KIR* genotypes, which result from the three possible combinations of haplotypes *h1* and *h2* (Figs. 1 and 2). That the most

	Genotypes	*KIR*														Populations		
#	Assumed Haplotypes	*2DL1*	*2DL2*	*2DL3*	*2DL4*	*3DL1*	*3DL2*	*3DL3*	*2DS1*	*2DS2*	*2DS3*	*2DS4*	*2DS5*	*3DS1*	*2DL5*	Yucpa (n=61) %	Bari (n=80) %	Warao (n=89) %
1	*h1/h1*	■	□	■	■	■	■	■	□	□	□	■	□	□	□	**24.59**	**18.75**	**30.34**
2	*h2/h2*	□	■	□	■	□	■	■	■	■	□	□	■	■	■	**27.87**	1.25	3.37
3a	*h1/h2*	■	■	■	■	■	■	■	■	■	□	■	■	■	■	**36.07**	---	**28.09**
3b	*h3/h4*	■	■	■	■	■	■	■	■	■	□	■	■	■	■	1.64	**12.50**	---
4	*h1/h4*	■	□	■	■	■	■	■	■	□	□	■	■	■	■	4.92	**26.25**	**7.87**
5	*h4/h4*	■	□	■	■	□	■	■	■	□	□	□	■	■	■	---	**10.00**	---
6	*h2/h4*	■	■	■	■	□	■	■	■	■	□	□	■	■	■	1.64	6.25	**10.11**
7	*h1/h3*	■	■	■	■	■	■	■	□	■	□	■	□	□	□	1.64	**11.25**	---
8	*h3/h3*	□	■	□	■	■	■	■	□	■	□	■	□	□	□	---	6.25	---
9	*h1/h5*	■	■	■	■	■	■	■	■	■	■	■	□	□	■	---	---	**8.99**
10	*h2/h3*	□	■	□	■	■	■	■	■	■	□	■	■	■	■	1.64	2.50	---
11	*h2/h5*	□	■	□	■	□	■	■	■	■	■	□	■	■	■	---	---	2.25
12	*h1/?*	■	■	■	■	■	■	■	■	■	□	■	■	□	■	---	2.50	1.12
13	?/?	□	■	□	■	■	■	■	■	□	□	■	■	■	■	---	1.25	---
14	?/?	■	□	■	■	■	■	■	■	■	□	□	■	■	■	---	1.25	---
15	*h1/?*	■	■	■	■	■	■	■	■	□	■	■	■	■	□	---	---	1.12
16	?/?	■	□	■	■	■	■	■	■	□	■	■	□	■	■	---	---	2.25
17	*h1/?*	■	■	■	■	■	■	■	■	□	■	■	■	■	□	---	---	1.12
18	?/?	■	□	■	■	■	■	■	■	□	■	■	■	■	□	---	---	1.12
19	?/?	■	■	■	■	□	■	■	■	■	■	□	■	■	■	---	---	1.12
20	?/?	■	■	■	■	□	■	■	■	■	■	□	□	■	■	---	---	1.12
21	?/?	■	■	■	■	■	■	■	□	■	■	■	□	□	■	---	---	1.12

Fig. 1 Distribution of *KIR* genotypes in Amerindians from Venezuela. *Filled boxes* indicate the presence of *KIR* gene; *open boxes* indicate its absence. For each population the frequencies of the common haplotypes, that together account for ~80% of the panel are shown in bold

Haplotype #	Haplotype name used in previous studies*				*KIR*														Amerindian Populations Haplotype frequency (%)		
	1	2	3	4	*2DL1*	*2DL2*	*2DL3*	*2DL4*	*3DL1*	*3DL2*	*3DL3*	*2DS1*	*2DS2*	*2DS3*	*2DS4*	*2DS5*	*3DS1*	*2DL5*	Yucpa (n=61) %	Bari (n=80) %	Warao (n=89) %
h1	C1-T1	A	2	H29b	■		■	■	■	■	■				■		■	■	45.9	38.8	53.9
h2	C2-T2	B5	14	EM6b		■		■		■	■	■	■			■	■	■	47.5	5.6	23.6
h3	C2-T1	B1	4	H29c		■		■	■	■	■		■		■				2.5	19.4	---
h4	C1-T2	B6	5	H42b	■		■	■		■	■	■				■	■	■	4.1	32.5	9.0
h5						■		■		■	■	■	■	■				■	---	---	5.6

Fig. 2 Distribution of assumed *KIR* haplotypes in Amerindians from Venezuela. *Filled boxes* indicate the presence of *KIR* gene; *open boxes* indicate its absence. **1* Uhrberg 2005, *2* Uhrberg et al. 2002, *3* Hsu et al. 2002, *4* Gomez-Lozano et al. 2002

frequent haplotype (*h1*) is identical to the ubiquitous group *A KIR* haplotype further supports this interpretation (Uhrberg et al. 1997). Here, we consider the *A* haplotype as having its seven characteristic *KIR* (Fig. 2) and *B* haplotypes as having any other combination of *KIR* loci. The *A* haplotype has been found in all populations investigated and is most common in East Asia (Jiang et al. 2005; Norman et al. 2002; Whang et al. 2005; Yawata et al. 2002), the likely origin of Amerindian populations. For the three Amerindian tribes, the range in frequency of the most common genotype (*h1/h1*) and its component haplotype (*A*) (Figs. 1 and 2) is similar to that in most Caucasoid, African, and South Asian populations (Denis et al. 2005; Norman et al. 2002; Witt et al. 1999). Some genotypes present in Amerindians have yet to be detected in other populations. Segregation in families showed that three of these genotypes correspond to homozygosity for three group *B* haplotypes (*h2*, *h3*, and *h4*). The most frequent *B/B* genotype is *h2/h2* in the Yucpa (27.9%), while *h3/h3* (6.2%) and *h4/h4* (10.0%) are common in the Bari (Fig. 1). These haplotypes have been described in other populations (Fig. 2) but rarely at sufficient frequency to observe homozygotes, even though one of them (*h3*) is the second most frequent haplotype in Caucasoids and the fourth most frequent in Koreans (Uhrberg et al. 2002; Whang et al. 2005). The final haplotype (*h5*) is typical of the Warao (Fig. 3, W1 & W2) and the only *2DS3*-containing haplotype found. High-resolution *KIR* subtyping in families confirmed *h5* also lacks *3DL1* and *3DS1*.

Of the three tribes, the Bari presents the highest number of genotypes (after excluding single observations) (Fig. 1). The most frequent *A/B* genotype in Bari comprises haplotypes *h1* and *h4* (26.25%; Fig. 1), as confirmed by family analysis (Fig. 3: B1, 2, B4-6). Ten individuals have genotype *h3/h4*, which is indistinguishable in gene content from genotype *h1/h2*, the most common *A/B* genotype in Yucpa and Warao and also present in Caucasian and Asian populations (Yawata et al. 2002). The distribution of deduced haplotypes within each population was consistent with expectations from Hardy–Weinberg equilibrium, as was also true for the frequencies of group *A* and *B* haplotypes, and *KIR2DL2* and *2DL3*. *KIR2DL2* and *2DL3* segregate as alleles in all populations investigated (Jiang et al. 2005; Norman et al. 2002; Whang et al. 2005; Witt et al. 1999; Yawata et al. 2002).

HLA class I polymorphisms have evolved under balancing selection, likely due to general fitness advantage of heterozygosity. This is interrupted by episodes of positive selection, in which the selected alleles are advantageous in defense against specific disease. Generation and loss of *HLA* alleles is a feature of such evolution, as is the retention of ancient lineages in extant populations (Parham et al. 1997). Whereas new *HLA-B* alleles were generated in Amerindian populations by gametic recombination (Belich et al. 1992; Parham et al. 1997; Watkins et al. 1992), we find no evidence for recombinant *KIR* haplotypes with novel gene content in the Yucpa, Bari, and Warao. Recombination between three centromeric (C1–C3) and three telomeric (T1–T3) can explain much of the *KIR* haplotype diversity in gene content (Uhrberg 2005). Of the nine possible combinations, eight account for ~90% of haplotypes in the Caucasoid population. Four of the nine combinations were found in the Amerindians studied here (Fig. 2). Consistent with the absence of novel gene-content *KIR* haplotypes, the patterns and values of linkage disequilibrium between *KIR* genes in the Amerindians were similar to those observed in other populations (Jiang et al. 2005; Norman et al. 2002; Whang et al. 2005; Yawata et al. 2002) (Fig. S1).

We compared the frequencies with which combinations of KIR and cognate HLA class I ligand occurred in the Yucpa and Bari, the two tribes for which *HLA-C* genotypes

Segregation Pattern	Y1	Y2	B1	B2	B3	B4	B5	B6	W1	W2	W3	W4	W5
Parent 1	*h1/h2*	*h1/h1*	*h1/h4*	*h1/h4*	*h3/h4*	*h1/h1*	*h1/h3*		*h1/h1*	*h2/h5*	*h1/h1*	*h1/h1*	*h1/h2*
Parent 2	*h2/h2*		*h1/h1*	*h4/h4*		*h1/h4*	*h1/h4*						
Siblings	*h2/h2*	*h1/h2*	*h1/h4*	*h4/h4*	*h3/h3*	*h1/h4*	*h1/h4*	*h4/h4* *h1/h4* *h3/h4* *h4/h4*	*h1/h5* *h1/h2*	*h1/h2** *h1/h5*^{V} *h1/h2*	*h1/h2*$^{\square}$	*h1/h2* *h1/h5*	*h1/h1* *h1/h1* *h4/h2*
# of families	2	6	2	1	1	2	1	1	1	1	1	1	1

Fig. 3 Some informative families from the Yucpa (*Y*), Bari (*B*) and Warao (*W*) populations. Each genotype is represented as two assumed haplotypes. Three International Histocompatibility Workshop and Conference (IHWC) cell lines derived from Warao individuals were included in the analysis: *Asterisk* indicates Amala, *inverted triangle* indicates LZL, *rectangle* indicates RML. *KIR* genotypes for AMALA and RML confirm those previously described (Cook et al. 2003). Hsu et al. (2002) suggested the combination of haplotypes *h3* and *h4* for AMALA, but analysis here (family W3) demonstrates the genotype is comprised of *h1* and *h2*

were known (Fig. S2). Of note, the frequency of *HLA-C1* homozygosity combined with *KIR2DL3* homozygosity was higher in the Bari (51%) than the Yucpa (15%). This genotype combination was associated with increased resolution of acute infection with Hepatitis C virus (Khakoo et al. 2004). The effect is attributed to better NK cell activation because of the relative weak inhibitory signal generated by *KIR2D3* interaction with HLA-C1.

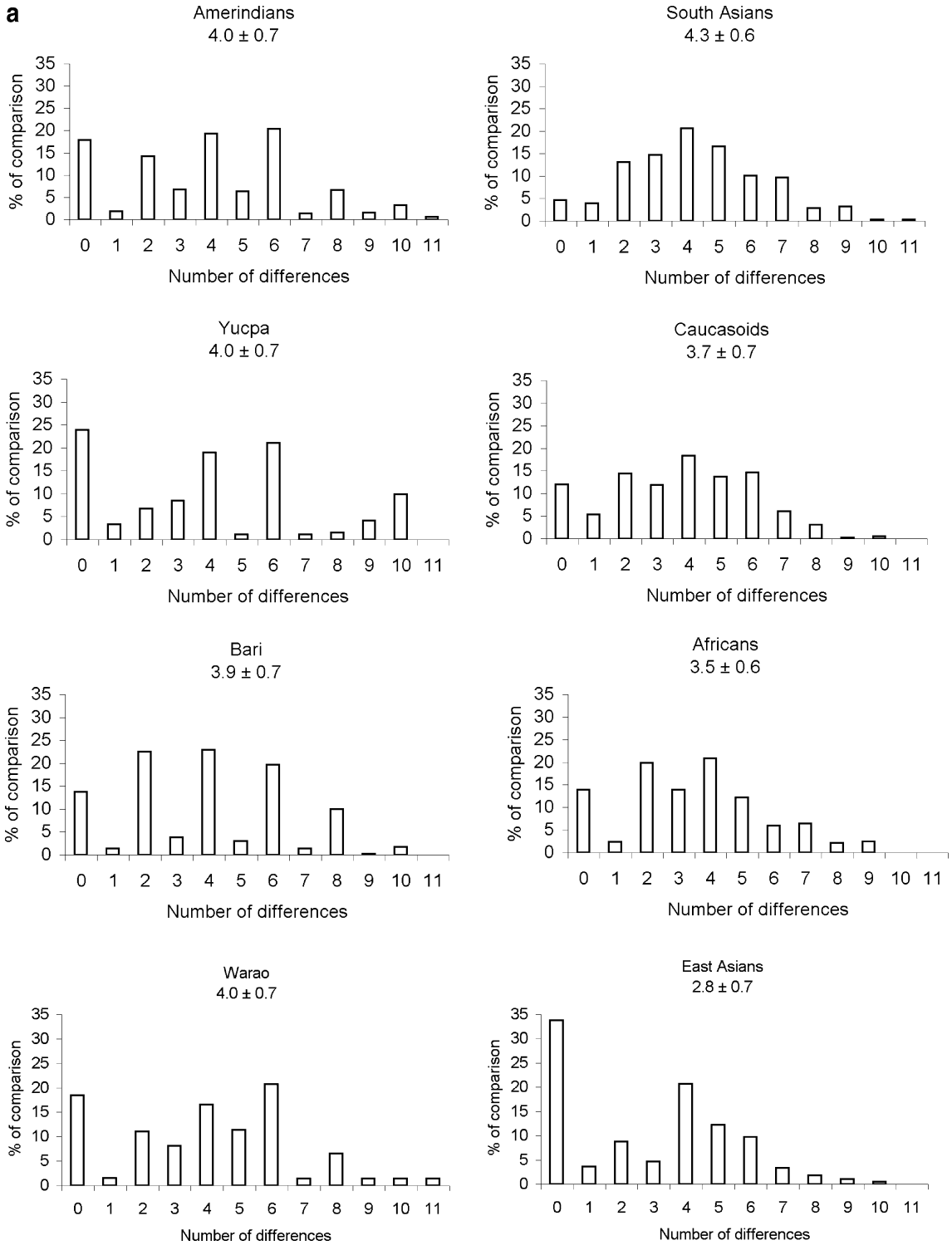
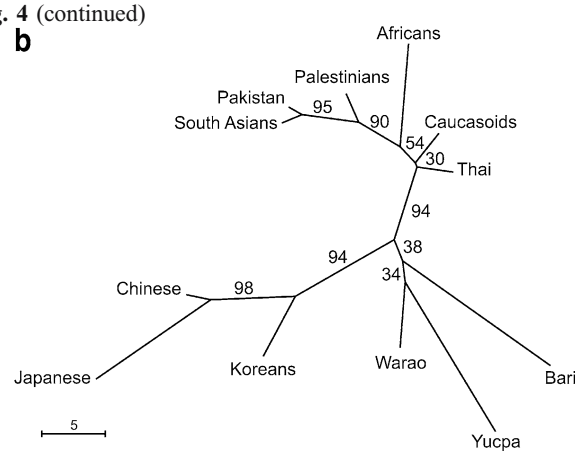


Fig. 4 Differences between *KIR* genotypes within populations. **a** Average pairwise mismatch and mismatch distribution of binary *KIR* genotypes; Mega3.1 (Kumar et al. 2004). Means of differences ± standard error using 10,000 bootstrap replicates. Populations are as described in Jiang et al. (2005); Norman et al. (2002); Whang et al. (2005); Yawata et al. (2002). **b** Neighbor-joining (*NJ*) tree. Differences on the abscissa (in 2% bins) concatenated for each pairwise comparison. Unrooted tree; numbers indicate percentage from 10,000 bootstrap replicates

Fig. 4 (continued)



Another difference is the combination of 2DS2 with HLA-C1 was more frequent in Yucpa (70%) than in Bari (43%). This is a ligand–receptor combination with potential to activate NK cells. The frequency of HLA-Bw4 and its combination with the cognate *KIR3DL1* receptor was similar in the Yucpa and Bari (Fig. S2).

Despite the small numbers of gene-content *KIR* haplotypes, all the *KIR* genes were frequent (>40%) in the Amerindian populations, with the exception of *2DS3*. Furthermore, the frequencies of the group *A* and *B* haplotypes were relatively even in each tribe (Figs. 1 and 2). In Yucpa, the most homogeneous tribe, ten *KIR* distinguish the two very common haplotypes, *h1* (*A*) and *h2* (*B*). To put this striking observation in perspective, we compared the distribution of differences in the number of *KIR* between *KIR* genotypes for Amerindian and other populations (Fig. 4a). This shows that the high incidence of *KIR* genotypes differing by ten *KIR* genes was unique to the Yucpa population. Otherwise, the pattern of within-population distribution in the Venezuelan Indian tribes is similar to that of other populations. Surprisingly, the proportion of similar genotypes (number of *KIR* differences=0) is higher in Japanese, Chinese, and Koreans than in Amerindians, despite more haplotypes being present in these populations (Jiang et al. 2005; Whang et al. 2005; Yawata et al. 2002). This reflects the high frequency of group A haplotypes (73–76%) in East Asians. Using the *Z* test to compare the populations for their intrapopulation variety, the Amerindians were significantly more diverse than East Asians (Chinese, Japanese, Koreans), Africans and Caucasoids and slightly less diverse than South Asian and Palestinian populations ($p<0.001$ for all comparisons) (Fig. 4a). Thus, the Amerindian tribes have maintained a high level of *KIR* diversity despite a restricted number of haplotypes. On phylogenetic comparison, the three Amerindian groups form a cluster, showing the tribes have achieved similar intrapopulation diversity while using different sets of *KIR* haplotypes (Fig. 4b).

Allele-level typing for *3DL1/S1* identified six *3DL1* and two *3DS1* alleles (Fig. 5a). The group A *KIR* gene-content haplotype was split into five allele-level haplotypes by the different *3DL1* alleles. Two group *B KIR* gene-content haplotypes were similarly split: *h2* by *3DS1* variants and *h3* by *3DL1* variants (Fig. 5b). Thus, at the allele level, a comparable number of five group *A* and six group *B* haplotypes are defined, reflecting the even frequencies of the two haplotype groups. That group *A KIR* haplotypes display more allelic variability than group *B KIR* haplotypes was also seen in the Caucasoid and Japanese populations and is likely a general property (Shilling et al. 2002; Yawata et al. 2006). A new variant of *3DS1*, *3DS1*047* (found on 20% of *h2*) is characterized by one non-synonymous nucleotide substitution in the sequence encoding the D1 domain and appears unique to the Warao. The difference in *3DL1/3DS1* heterozygosity between the *A* and *B* haplotypes in total Amerindians was significant ($p<0.01$; 0.55 (A) vs 0.33 (B)). In addition, the *3DL1/S1* sequences displayed unusually high values for Tajima's *D* test statistic (Tajima 1989) (Yucpa, 2.6; Bari, 1.8; and Warao, 3.6; Norman et al., in preparation), evidence for balancing selection.

Fig. 5 *KIR3DL1/S1* allele-level typing of Amerindian populations. **a** Examples of informative families from Yucpa (*Y*), Bari (*B*), and Warao (W). Each genotype is represented as two assumed haplotypes and two correspondent *3DL1/3DS1* alleles. **b** *3DL1/3DS1* alleles split the *h1*, *h2*, and *h3* haplotypes in Amerindians

a

Family	Y3	Y4	B7	W7	W8	W9
Parent 1	*h1/h2 (3DL1*029/ 3DS1*013)*	*h1/h1 (3DL1*01502/ 3DL1*029)*	*h3/h4 (3DL1*01502/ 3DS1*013)*	*h2/h5 (3DS1*013/negative)*	*h1/h1 (3DL1*005/ 3DL1*01502)*	*h1/h1 (3DL1*01502/ 3DL1*005)*
Sibling 1	*h2/h2 (3DS1*013/ 3DS1*013)*	*h1/h1 (3DL1*029/ 3DL1*005)*	*h3/h3 (3DL1*01502/ 3DL1*01502)*	*h1/h2 (3DL1*01502/ 3DS1*013) AMALA*	*h1/h2 (3DL1*01502/ 3DS1*013) RML*	*h1/h1 (3DL1*01502/ 3DL1*005)*
Sibling 2		*h1/h2 (3DL1*029/ 3DS1*013)*		*h1/h5 (3DL1*01502/negative) LZL*		*h1/h2 (3DL1*01502/ 3DS1*047)*
Sibling 3				*h1/h2 (3DL1*01502/ 3DS1*013)*		

b

Haplotypes	*3DL1*	*3DS1*
h1	*01502, 029, 004, 005, 007*	
h2		*013, 047*
h3	*01502, 008*	
h4		*013*

The intrapopulation distribution of *3DL/S1* alleles did not deviate from that expected under Hardy–Weinberg equilibrium. But had we been unable to deduce the presence of *3DL1/S1* negative haplotypes, then it would have seemed perturbed in the Warao ($p<0.05$). Eight Warao individuals with genotype 9 (Fig. 1) appeared homozygous for *3DL1* alleles. Analyzing their family haplotypes revealed that these individuals had one copy of *3DL1* from *h1* and that their second haplotype, *h5*, lacked *3DL1/S1* (Fig. 5a, W7). When absence of the gene was included in the calculations as a distinct allele, Hardy–Weinberg equilibrium was achieved. Haplotypes lacking *3DL1/S1* have been observed at low frequency in Caucasoid and East Asians (Shilling et al. 2002; Hsu et al. 2002; Whang et al. 2005) but can reach frequencies of 10% in South Asians (Norman et al. 2002).

This study shows that the number of *KIR* gene-content haplotypes is much reduced in Amerindians compared to other populations studied. There are also considerable differences between Amerindian populations as is also true for *HLA* class I (Layrisse et al. 2001; Layrisse, unpublished data; Martinez-Arends et al. 1998; Ramos et al. 1995). Such differences indicate how cultural and physical separation, small population size and selection by infectious disease can combine to alter the frequency of *KIR* haplotypes. Despite all this, the Yucpa tribe retains all but the *KIR2DS3* gene at high frequency (>70%), an even balance of *A* and *B* haplotypes and as high a diversity as the majority of other published populations. They do this with only two gene-content *KIR* haplotypes: the group *A* haplotype and a group *B* haplotype that contains all the characteristic group *B* haplotype genes except *KIR2DS3*. Here is good evidence for balancing selection acting on the *KIR* locus, for the biological importance of the group *A* and *B* haplotypes and their vital contribution to human survival.

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