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# *KIR* genes polymorphism in Argentinean Caucasoid and Amerindian populations

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## Abstract

In natural killer cells, killer immunoglobulin-like receptors (KIRs) loci code for either inhibitory or activating receptors, and according to the number of genes present in each individual, it is possible to identify a high rate of polymorphism in the populations. We performed KIR typing by polymerase chain reaction–sequence-specific oligonucleotide probing in 402 Argentinean Caucasoid and in two Amerindian populations (101 Wichis and 54 Chiriguanos) from the North of Argentina. *KIR2DL4*, *KIR3DL2*, *KIR3DL3* and *KIR3DP1* were always present, whereas the frequencies of *KIR2DL1*, *KIR2DL3*, *KIR2DS4*, *KIR3DL1* and *KIR2DP1* ranged between 84% and 96%. The frequencies of *KIR2DS2*, *KIR2DL2*, *KIR2DL5*, *KIR2DS5*, *KIR2DS1* and *KIR3DS1* ranged between 41% and 62%. The *KIR2DS3* with a frequency of 29% in Argentinean Caucasoid population was present at a very low frequency in Amerindian populations. Haplotype segregation studies performed in 10 Wichi families showed the presence of only three haplotypes: A, B5 and B1. The Amerindian populations showed several similarities to Asian but not to Caucasoid populations with regard to the frequency of *KIR2DS3*, full-length *KIR2DS4* gene and *KIR2DL4* alleles.

## Introduction

Natural killer (NK) cells are known to be a very important component of the innate immune system, providing a first line of defense against infectious agents (1). NK cells lyse infected and tumor cells without the need for prior sensitization or major histocompatibility complex restriction. New insights into the NK cell biology have suggested major roles for these cells in health and disease by acting not only as direct cytotoxic killers but also as a source of cytokines. NK cell function is tightly regulated by a balance between positive and negative signals emanating from activating and inhibitory receptors (2). The NK cell receptors (NKRs) belong to three main families: the killer immunoglobulin-like receptors (KIRs), the C-type lectin (CD94/NKGs) and the immunoglobulin-like transcripts (ILTs or LIRs) (3–5). All NKR families have both activating and inhibitory members, and most of them engage with human leukocyte antigen (HLA) class I ligands on target cells. However, specific signaling pathways remain to be fully elucidated (6).

The inhibition or activation function of KIRs is determined by the protein structure. KIRs are grouped according to whether they have two domains (2D) or three domains (3D) and whether they possess a short (S) or long (L) cytoplasmic tail. Those with long cytoplasmic tails containing immunoreceptor tyrosine-based inhibition motifs (ITIMs)

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have an inhibitory function (7), whereas those with short cytoplasmic tails have a potentially activating function mediated by immunoreceptor tyrosine-based activation motifs (8). Two ITIMs are present on the long cytoplasmic tails with exception of *KIR2DL4*. The inhibitory function of this gene has been questioned because its expressed protein possesses only one ITIM and a charged residue motif in the transmembrane region, which are typical of activating receptors (9, 10).

The ligands for some KIR have shown to be subsets of HLA class I molecules, HLA-Cw, HLA-Bw4, HLA-A (HLA-A3 and HLA-A11 are ligands for KIR3DL2) and HLA-G, which is a ligand for KIR2DL4. The HLA allotypes can be divided into two major groups on the basis of a dimorphism at position 80 of the D1 domain, which affects the KIR–HLA interaction: C1 and C2 for HLA-Cw (Asn/Lys) and Bw4 and Bw6 for HLA-B (Thr-Ile/Asn). Group C1 are ligands for inhibitory *KIR2DL2*/*KIR2DL3* and activating *KIR2DS2*, group C2 are ligands for inhibitory *KIR2DL1* and activating *KIR2DS1* and group Bw4 are ligands of *KIR3DL1* (11, 12). Additionally, the inhibitory receptors have more affinity than the activating receptors.

KIR are encoded by a family of genes on chromosome 19, exhibiting extensive haplotypic variation in gene number and content, as well as allelic polymorphism for individual genes. KIR multigene families consist of 14 genes and two pseudogenes. On the basis of gene content, two groups of KIR haplotype have been defined 'A' and 'B'. Common to both groups of haplotypes are the 'framework genes': *KIR3DL2*, *KIR3DL3* and *KIR2DL4* (13). The simpler A haplotype containing seven genes and two pseudogenes is characterized by the dominance of genes coding for inhibitory receptors (*KIR3DL3*, *KIR2DL3*, *KIR2DP1*, *KIR2DL1*, *KIR3DP1*, *KIR2DL4*, *KIR3DL1* and *KIR3DL2*) and has only one activating gene (*KIR2DS4*). In contrast, the group B haplotypes are more diverse and have several genes coding activating receptors. Analysis of KIR genotype has shown that the relative frequencies of A and B haplotypes vary between populations (13–15). Recent studies on KIR interaction with HLA ligands have suggested its role in immunopathology (16, 17). Thus, it has become important to characterize the KIR–HLA combinations in different ethnic populations.

Here, we report the frequency of each of the 16 KIRs, their respective ligands HLA-C and the corresponding *KIR* gene profiles in the Argentinean Caucasoid population and in two closed Amerindian communities from Argentina.

## Materials and methods

### Subjects

The study included 402 unrelated individuals from Buenos Aires (latitude 34°28′ longitude 58°28′) who were defined as Argentinean Caucasoid and 155 unrelated individuals from two Amerindian populations from Argentina: 54 Chiriguanos from Salta province and 101 Wichis from Chaco and Salta province.

Argentinean Caucasoids were recruited from the Greater Buenos Aires (GBA) area, which includes Buenos Aires city and surroundings. GBA has a population of more than 12,000,000 inhabitants, which represent almost a third of the total Argentinean population. According to the official Census, the GBA has had an unusual demographic behavior. Up to 1914, more than 50% of its population was composed of European immigrants, mainly Spaniards and Italians. Between 1914 and 1947, associated with the industrial expansion, the internal migration from the provinces started representing the so-called 'criollos', an admixture of the native populations with the Spaniards that colonized the country. In 1980, the percentage of foreigners was only 13% (10% Europeans). The Amerindian communities belong to the linguistic family Mataco-Mataguayo. Those from Salta (north west of Argentina) are located in the city of Oran at latitude 23°8′ longitude 64°20′. Those from Chaco province (north east of Argentina) are located in a reserve near Nueva Pompeya at latitude 24°56′ longitude 61°31′.

### KIR and HLA-C typing by polymerase chain reaction-sequence-specific oligonucleotide probing

Genomic DNA was obtained by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. All conditions to identify the presence or absence of each *KIR* gene (*KIR2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *2DP1*, *3DL1*, *3DL2*, *3DL3*, *3DS1* and *3DP1*) have been previously documented (18). Briefly, two polymerase chain reaction (PCR) amplifications were performed, the PCR-1 for domains D1 and D2 combined and the PCR-2 for the transmembrane/cytoplasmic region. Nineteen 5′-digoxigenin-labeled probes were used in a sequence-specific oligonucleotide probing (SSOP) approach, 13 for PCR-1 and 6 for PCR-2. The *KIR* gene content in each individual was inferred after compound analysis of all probes.

Nine *KIR2DL4* alleles (*KIR2DL4*00101*, **00102*, **00201*, **00202*, **003*, **004*, **005*, **006*, **007*) were identified with a PCR amplification of the D0 and D2 domain and hybridized with 14 probes (5′-digoxigenin label) as described elsewhere (19).

HLA-Cw genotyping was performed within exons 2 and 3 of the *HLA-C* gene. Primers and conditions for PCR amplification were those described by Cereb et al. (20). Medium resolution SSOP typing used 37 probes (5′-digoxigenin label), and the 15 allelic groups of HLA-Cw were identified (HLA-Cw*01, *02, *03, *04, *05, *06, *07, *08, *12, *13, *14, *15, *16, *17, *18) (21). One of these

probes (TGACCGAGTGAACCTGC) is specific for the *HLA-C* alleles that belong to the C1 group (Asn 80) and another (sequence ACCGAGTGAGCCTGCG) anneals with the *HLA-C* alleles that belong to the C2 group (Lys 80).

The *KIR2DS4* gene was amplified by PCR with primers previously described (22) to analyze the full-length and the deleted version of the *KIR2DS4* gene. In the electrophoresis gel, two PCR products were identified, *KIR2DS4* PCR deletion, *KIR2DS4* PCR full length and the samples carrying both PCR products.

### Analysis of *KIR* gene family diversity

Two approaches have been used to define the *KIR* gene content of individuals. The first approach was determined by presence or absence of *KIR* genes in unrelated individuals. A second approach was the analysis of the haplotype segregation. This study was performed in 10 different Wichi families (only the parents of these families were included in the study of unrelated individuals).

### Statistical analysis

For HLA-C, Hardy–Weinberg equilibrium was tested using the ARLEQUIN v2.0 package for all populations studied. The genotype frequencies were compared between each Amerindian population and Caucasoid population with two-sided Fisher's exact test for $2 \times 2$ contingency tables. $P$ values ($P$c) $<0.05$ were considered statistically significant, after correction by multiplying by the number of comparisons made.

## Results

### Ethnic differences of *KIR* gene frequencies

The study of *KIR* genes performed in the Argentinean population included Caucasoid ($n = 402$) and two Amerindian populations (54 Chiriguanos and 101 Wichis). The three 'framework' genes *KIR2DL4*, *KIR3DL2* and *KIR3DL3* and the pseudogene *KIR3DP1* were present in all individuals and not included in Table 1. The frequencies of *KIR2DL2*, *KIR2DL3*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS5*, *KIR2DS4*, *KIR3DL1* and *KIR3DS1* were similar in all three populations. Although the Wichi population differs from the Caucasoid in that the Wichis have lower frequencies of *KIR2DL1* and *KIR2DP1* ($P$c $< 0.005$). In addition, *KIR2DS3* represents the activating gene present at the lowest frequency in our Argentinean Caucasoid population (29%), but it is present at a very low frequency in the Amerindian populations (3% in Wichis and 6% in Chiriguanos; $P$c $<$ 0.005). The distribution of KIR genotypes previously reported in the Amerindian populations from Venezuela

**Table 1** The distribution of *KIR* gene frequency in Argentinean Caucasoid and Argentinean Amerindian populations in comparison with other populations[a]

| | *KIR* gene | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population[b] | 2DS2 | 2DL2 | 2DL3 | 2DS3 | 2DP1 | 2DL1 | 3DL1 | 3DS1 | 2DL5 | 2DS5 | 2DS1 | 2DS4 |
| Caucasoids, $n = 402$ | 55[c] | 56 | 87 | 29 | 96 | 96 | 95 | 42 | 56 | 36 | 46 | 95 |
| Wichis, $n = 101$ | 61 | 62 | 84 | 3 | 84 | 84 | 89 | 54 | 53 | 52 | 53 | 89 |
| Chiriguanos, $n = 54$ | 41 | 44 | 87 | 6 | 91 | 91 | 87 | 57 | 59 | 56 | 57 | 87 |
| Japanese (27) | 17 | 16 | 100 | 17 | 100 | 100 | 97 | 33 | 39 | 28 | 34 | 96 |
| Chinese Han (26) | 17 | 17 | 99 | 12 | 99 | 99 | 94 | 33 | 35 | 23 | 34 | 94 |
| Australian aborigines (25) | 85 | 79 | 67 | 81 | NT | 72 | 55 | 78 | NT | NT | 82 | 51 |
| African (29) | 45 | 52 | 85 | 19 | NT | 79 | 98 | 13 | 52 | 24 | 23 | 97 |
| Trinidad South Asian (29) | 69 | 64 | 83 | 27 | NT | 82 | 88 | 44 | 74 | 37 | 55 | 81 |
| Karachi South Asian (29) | 69 | 67 | 91 | 45 | NT | 90 | 81 | 56 | 78 | 48 | 60 | 72 |
| Mexico Mestizo (24) | 44 | 43 | 100 | 17 | NT | 100 | 99 | 42 | 49 | 40 | 42 | 98 |
| Huichol (24) | 34 | 34 | 100 | 16 | NT | 100 | 97 | 56 | 56 | 48 | 56 | 97 |
| Purepecha (24) | 34 | 34 | 100 | 4 | NT | 100 | 98 | 62 | 62 | 62 | 62 | 98 |
| Tarahumara (24) | 34 | 34 | 100 | 0 | NT | 100 | 98 | 66 | 66 | 66 | 66 | 98 |
| Vietnamese (25) | 41 | 45 | 66 | 34 | NT | 98 | 88 | 41 | NT | NT | 37 | 88 |
| Thai (14) | 44 | 42 | 97 | 25 | NT | 97 | 93 | 44 | NT | 23 | 42 | 87 |
| Palestinian (14) | 64 | 62 | 85 | 37 | NT | 83 | 88 | 39 | NT | 27 | 44 | 88 |
| Greek (28) | 54 | 50 | 88 | 37 | NT | 89 | 90 | 46 | NT | 21 | 43 | 88 |

NT, not tested.

[a] KIR population frequency data are held at www.allelefrequencies.net.

[b] Numbers in parentheses correspond to the references of previously reported data.

[c] Figures represent percentage of individuals positive within the studied population.

indicated that all *KIR* genes were found, excepting 2DS3 that was absent in Yucpa and Bari populations (23). Similar findings appeared in two ethnic groups from Mexico (24). Interestingly, KIR2DS3 was found with high frequency in Australian aborigines (25). Table 1 also includes for comparison of *KIR* gene frequency distribution in Chinese (26), Japanese (27), Vietnamese (25), Thai, Palestinian (14), Greek (28), African and South Asian (29) populations.

### Restricted *KIR* gene profiles in Amerindian populations

Variations in the number and type of *KIR* genes generate a diversity of *KIR* gene profile in human populations. Gene profiles, based on the presence or absence of *KIR* genes, showed the presence of 47 different KIR genotypes in the Argentinean Caucasoid population (Figure 1). The Amerindian populations showed a much more restricted number of *KIR* gene profiles (12 in Wichis and 18 in Chiriguanos).

The organization of genotype 1 with seven genes and two pseudogenes (characterized by genes coding for inhibitory receptors and only one activating gene *KIR2DS4*) corresponded to the group A haplotype. We assumed that individuals with this genotype are homozygous for the group A KIR haplotype. When compared with Caucasoid, the Wichi population showed a highly significant increased frequency of the profiles 2 and 3 ($Pc < 0.005$).

### Wichis have a reduced number of KIR haplotypes

To determine the presence of haplotypes, we performed an analysis of 10 Wichi families. Segregated haplotypes were established by including 70 members from these 10 families. Three of these families are shown in Figure 2. The distribution of assumed KIR haplotypes in the Wichi families showed the presence of only three different haplotypes: 1 – the A haplotype or *h1* (present in all 10 families) represents the most frequent haplotype observed in Caucasoid and Amerindian populations; 2 – B5 or *h2* haplotype (present in 9 families) and 3 – B1 or *h3* haplotype (present in 6 of these families). These three haplotypes are characterized by the absence of the *KIR2DS3* gene. B1 and B5 haplotypes have been described in other populations (23, 30, 31) but rarely at sufficient frequency to observe homozygotes although B1 is the second most frequent haplotype in Caucasoid and the fourth most frequent in Korean populations (31). Because of the relatively few haplotypes detected in Amerindian populations, we were able to estimate their composition and frequency from the genotype frequencies and these family data by an iterative process. For example, the assumed haplotypes depicted in Figure 1, would be *h1*/*h1*, *h2*/*h2* and *h1*/*h2* for the first three genotypes. In Argentinean Caucasoid population, these three genotypes account for 29% of the population *vs* 70.3 in Wichis and 48.2 in Chiriguanos.

### Typing of *KIR2DS4* gene variants

KIR2DS4 is the only activating receptor present on the A haplotype. The most common allele of KIR2DS4 has a deletion so individuals with two A haplotypes may have none or very few activating receptors. The typing in all three populations, for the full-length or the deleted form of the *KIR2DS4* gene, showed that 78% of Caucasoid population showed the presence of the deleted version. In contrast, only 26% of Wichis and 42% of Chiriguanos showed the deleted version (Table 2).

### Characterization of individuals homozygous for haplotype AA

We also analyzed in each population the frequency of AA genotype with the observed presence of their HLA-C ligands. It was found that *HLA-C* alleles were in Hardy–Weinberg equilibrium. The AA genotype was found slightly increased in both Amerindian populations.

When considering HLA-C molecules as ligands for KIR on NK cells, Wichi individuals carrying AA genotype showed an HLA-C2 frequency of 33.3%, which contrasted with the frequency observed in Caucasoid (65.4%) and Chiriguano (66.7%) populations ($P < 0.01$ Wichis *vs* Caucasian; Figure 3).

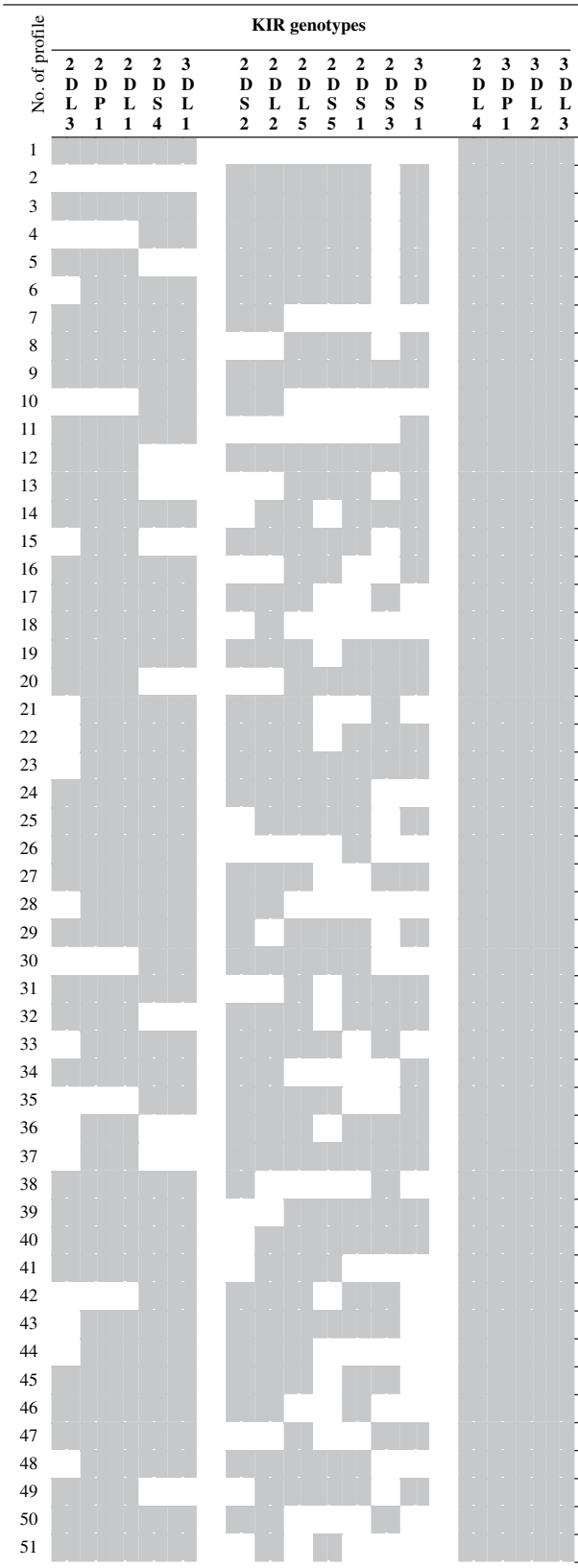
### *KIR2DL4* alleles

We also typed the allelic frequencies of the nine alleles of *KIR2DL4* documented in the Nomenclature Report (*KIR2DL4*00101*, **00102*, **00201*, **00202*, **003*, **004*, **005*, **006*, **007*) (32) by PCR-SSOP, although there are now 20 recognized alleles listed on the IPD–KIR Sequence Database (www.ebi.ac.uk/ipd/kir). The most frequent allele in Amerindian populations was represented by *KIR2DL4*00102*, with a gene frequency that ranged between 0.52 and 0.53 *vs* 0.31 in Argentinean Caucasoid population ($Pc < 0.005$) (Table 3). In Chiriguano and Wichi population, the *KIR2DL4*00201* was either absent or present at very low frequencies in both Amerindian populations ($Pc < 0.005$). We identified the presence of three *KIR2DL4* alleles (*KIR2DL4*00102*, **00201* and **005*) in a single Argentinean Caucasoid individual. This result could be in-line with a previous report, which suggested that during synapsis, misalignment of *KIR* genes on the two parental homologous chromosomes and an unequal crossover event might result in a progeny haplotype containing two copies of KIR2DL4 (33).

## Discussion

This study was designed to compare the KIR frequency and KIR genetic profiles between Argentinean populations, which include three different groups: a population defined as Argentinean Caucasoid and two close Amerindian



| No. of profile | Caucasian (*n*=402) | Wichis (*n*=101) | Chiriguanos (*n*=54) |
|---|---|---|---|
| 1 | 24.0 | 30.7 | 31.5 |
| 2 | — | 8.9[b] | 3.7 |
| 3 | 5.0 | 30.7[b] | 13 |
| 4 | 1.0 | 4.0 | 3.7 |
| 5 | 0.7 | 1.0 | 3.7 |
| 6 | — | — | 1.9 |
| 7 | 14.0 | 12.9 | 5.6 |
| 8 | 14.0 | 5.0 | 16.7 |
| 9 | 3.7 | 1.0 | — |
| 10 | 1.5 | 3.0 | 1.9 |
| 11 | 0.2 | — | 1.9 |
| 12 | 2.0 | — | 1.9 |
| 13 | 1.0 | — | 1.9 |
| 14 | 0.2 | — | 1.9 |
| 15 | — | — | 1.9 |
| 16 | — | — | 1.9 |
| 17 | 5.7 | — | — |
| 18 | 0.2 | — | — |
| 19 | 4.0 | — | — |
| 20 | 0.2 | 1.0 | — |
| 21 | 3.0 | — | — |
| 22 | 1.7 | — | — |
| 23 | 2.0 | — | — |
| 24 | 2.5 | — | 3.7 |
| 25 | 0.5 | 1.0 | 1.9 |
| 26 | 2.0 | — | — |
| 27 | 2.0 | — | — |
| 28 | 1.0 | — | — |
| 29 | 0.5 | — | — |
| 30 | 0.7 | — | — |
| 31 | 1.2 | 1.2 | 1.9 |
| 32 | 0.2 | — | — |
| 33 | 0.2 | — | — |
| 34 | 0.2 | — | — |
| 35 | 0.2 | — | — |
| 36 | 0.2 | — | — |
| 37 | 0.2 | — | — |
| 38 | 0.2 | — | — |
| 39 | 0.2 | — | — |
| 40 | 0.2 | — | — |
| 41 | 0.2 | — | — |
| 42 | 0.2 | — | — |
| 43 | 0.2 | — | — |
| 44 | 0.2 | — | — |
| 45 | 0.2 | — | — |
| 46 | 0.2 | — | — |
| 47 | 0.2 | — | — |
| 48 | 0.2 | — | — |
| 49 | 0.2 | — | — |
| 50 | 0.2 | — | — |
| 51 | 0.2 | — | — |

**Figure 1** *KIR* gene profiles observed in Caucasian and Amerindian populations. Black boxes indicate presence of *KIR* gene and white boxes, absence of *KIR* gene. Fifty-one *KIR* gene profiles are represented. Only 12 profiles were present in Wichis and 18 were present in Chiriguanos. The profile 1 corresponds to the AA genotype. Other genotypes included individuals who are either heterozygous for A or B haplotypes (3) or B homozygous (2). Genotypes 1, 3, 4, 5, 7, 8, 10, 25 and 31 were detected in all three populations studied. When compared with Caucasoids, both Amerindian populations showed a higher frequency of the profile 3. The profile 2 was present only in the Amerindian populations. KIR, killer immunoglobulin-like receptor.[a] Represents frequencies of unrelated individuals carrying each *KIR* gene profile.[b] Significant after correction for the number of comparisons made ($P_c < 0.005$).

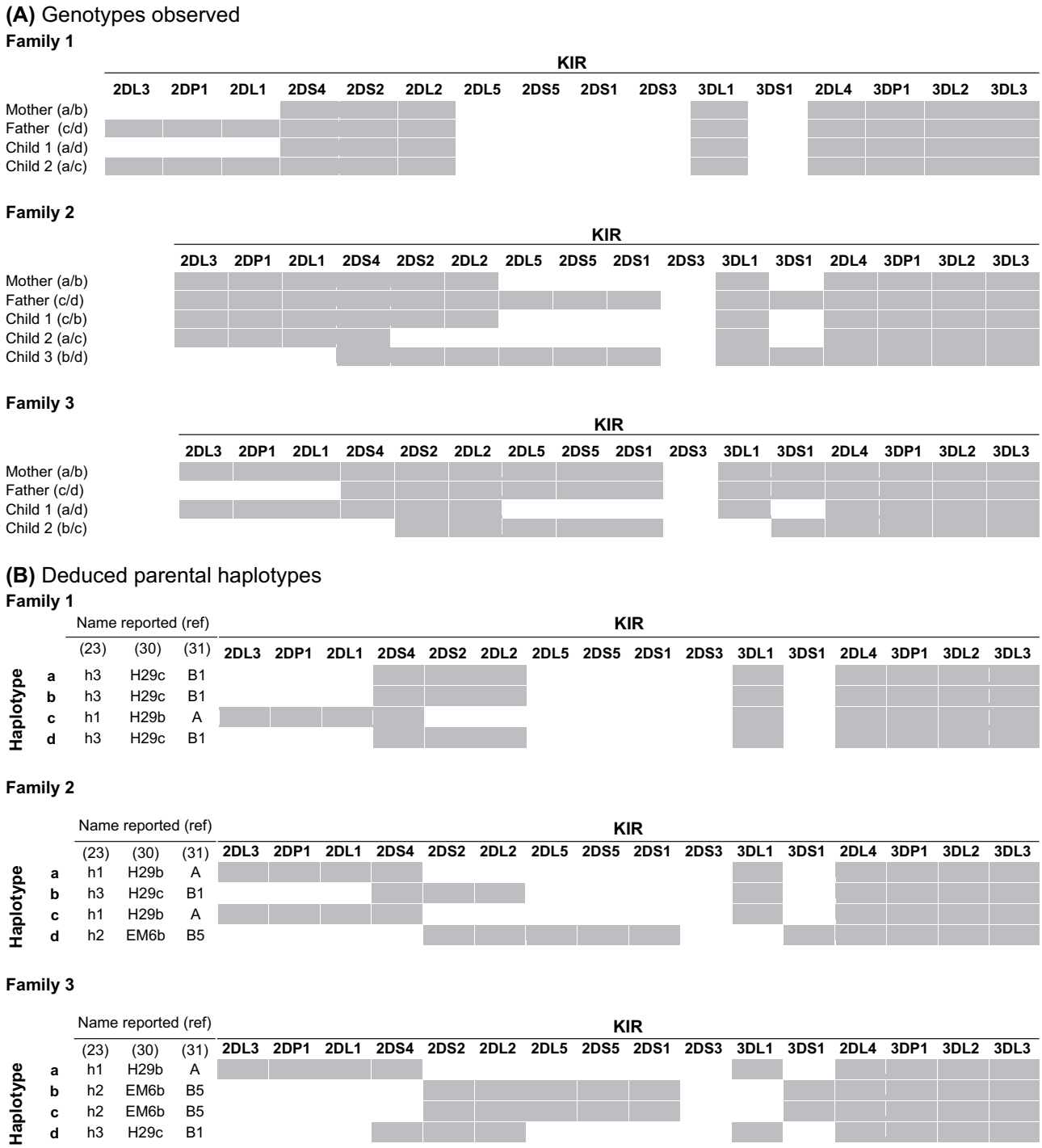



**Figure 2** KIR haplotypes detected in Wichi families. Confirmed presence (black boxes) or absence (white boxes) of *KIR* gene. (A) KIR genotypes of members of three informative families are depicted. (B) Deduced parental *KIR* gene haplotypes are indicated. The nomenclature described by Uhrberg (31) is used for the assignment of the haplotype. KIR, killer immunoglobulin-like receptor.

communities from the North of Argentina. With the exception of *KIR2DS3*, all *KIR* genes were present at similar frequencies in all populations studied. *KIR2DS3* represents the activating gene present at the lowest frequency in our Argentinean Caucasoid population, but it is present at a very low frequency in Wichi and Chiriguano populations. The absence or very low frequency of *KIR2DS3* has also been reported in three Amerindian

**Table 2** Percentage of individuals having full-length and deleted form of *KIR2DS4* gene in Caucasoid and Amerindian populations

| | Frequency (%) | | |
|---|---|---|---|
| *KIR2DS4* | Caucasoid ($n = 138$) | Wichis ($n = 92$) | Chiriguanos ($n = 38$) |
| Full length | 50.7 | 91.3[a] | 86.8[a] |
| Deleted form | 78.3 | 26.1[a] | 42.1[a] |

[a] Represent those comparisons between the Amerindian and the Caucasoid populations, which are significant after correction for the number of comparisons made ($Pc < 0.005$).

communities from Venezuela (23) and in two ethnic groups from Mexico (24). The comparison of the frequency of *KIR2DS3* with other populations, which included Vietnamese, Thai, African, Caucasoid, Palestinian, Trinidad Asian, South Asian, North Indian, Australian Aborigine and Japanese shows that Amerindians followed by Japanese individuals have the lowest frequency of this gene. Although *KIR2DS3* was reported present at low frequencies in Asian populations (Japanese and Chinese), this gene was found in most Australian aborigines (25). A likely hypothesis previously suggested by Uhrberg (34) indicates that the ratio of A and B haplotypes in Japanese and Australian aborigines diverged subsequent to the separation of these populations, probably because of a differential selection pressures induced by locally acting epidemiological challenges. Thus, migration of the Asian ancestors, the likely origin of Amerindian populations, to the American continent might have initiated after they diverged from Australian aborigines.

A second major finding was the restricted *KIR* gene diversity observed in Amerindian populations. Only 20 different KIR genotypes were observed in Amerindian populations. In contrast, 47 different genotypes were identified in Caucasoid population. The lower number of

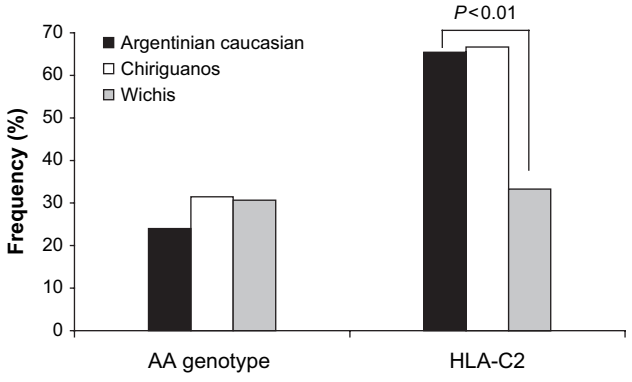



**Figure 3** HLA-C2 presence in the individuals carrying the AA KIR genotype. Analysis of the frequency of HLA-C2 within the individuals carrying the AA KIR genotype. Wichis show the lower frequency of HLA-C2 within the AA individuals. KIR, killer immunoglobulin-like receptor.

**Table 3** Genotype frequencies of *KIR2DL4* alleles in the Caucasoid and Amerindian populations

| | Genotype frequency | | |
|---|---|---|---|
| *KIR2DL4* | Caucasoid ($n = 122$) | Chiriguanos ($n = 51$) | Wichis ($n = 45$) |
| **00102* | 0.306 | 0.529[a] | 0.522[a] |
| **00201* | 0.155 | 0.020[a] | 0.000[a] |
| **00202* | 0.159 | 0.039 | 0.022 |
| **005* | 0.335 | 0.456 | 0.412 |
| **006* | 0.045 | 0.000 | 0.000 |

[a] Represent those comparisons between the Amerindian and the Caucasoid populations, which are significant after correction for the number of comparisons made ($Pc < 0.005$).

Amerindians in this study and inbreeding in Amerindian populations compared with large Caucasoid populations living in a large city could partially explain the restricted KIR genetic repertoire found in Amerindian populations. However, the genetic repertoire observed in Amerindian populations showed an increased frequency of haplotypes with little representation in Caucasoid population. Although the sample size of our Amerindian population is relatively small, the restricted gene diversity observed in our Amerindian populations is in-line with the studies performed on ethnical groups from Mexico and Venezuela (23, 24).

Although group A haplotypes do not vary in gene content, they show extensive variability on the allelic level (35). An interesting point to ascertain is whether there is a difference in the frequency of alleles of these genes. Some of these alleles will not be expressed, while others will have different levels of expression. This could have important ramifications when selecting these alleles in populations that are more likely to be prone to infectious diseases, somewhat akin to the many new *HLA* alleles, which have been found in Amerindian populations. In the three populations examined for *KIR2DL4* alleles, we only found five of the nine alleles. However, because our genotyping method could only distinguish 9 alleles from the 20 *KIR2DL4* alleles currently recognized, we cannot exclude that some of the newer alleles are present in the Amerindian populations. The frequency of the *KIR2DL4* alleles in our Caucasoid population was remarkably similar to that in Northern Ireland (18). In contrast, Amerindian populations showed the predominance of the *KIR2DL4*00102* allele, which is higher than in Caucasoid and more similar in frequency to that reported in Chinese from Hong Kong and that reported in the Xhosa population from South Africa (19). These similarities include the absence or low frequency of *KIR2DL4*00201* and *KIR2DL4*00202*. Another similarity between Amerindian and Japanese populations is that, in comparison with the Caucasoid population, they have similar lower frequencies of the nonfunctional *KIR2DS4* gene (36).

This study reveals that Wichi individuals carrying AA genotype showed a diminished frequency of HLA-C2. A previous study (37) described that a combination of maternal KIR AA genotype with fetal HLA-C2 is associated with an increased risk of developing preeclampsia during pregnancy. Selection by preeclampsia might explain the inverse correlation between C2 and the A haplotype in human populations, as exemplified by the low C2 and high A frequencies in the Japanese (36). However, it is important to note that the correlations reported in this study do not address the fitness of allogeneic combinations of maternal and fetal factors, but the *KIR* and *HLA* genes brought together in the child's genotype.

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