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A deep look at KIR–HLA in Amerindians: Comprehensive meta-analysis reveals limited diversity of *KIR* haplotypes

Danillo G. Augusto [a,1,2], Jill A. Hollenbach [b], Maria Luiza Petzl-Erler [a,*]

[a] Departamento de Genética, Universidade Federal do Paraná, Curitiba, Brazil
[b] Department of Neurology, University of California, San Francisco School of Medicine, United States

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ABSTRACT

Killer-cell immunoglobulin-like receptors (KIR) are highly polymorphic and have been associated to several diseases. Their ligands are specific human leukocyte antigens (HLA) molecules, expressed on the majority of cells. Only few genetically isolated populations have been characterized for the frequency of KIR–HLA combinations. The aim of this work was summarize and reanalyze the data described in recent publications regarding *KIR* and *HLA* in Amerindians. In total, 1258 individuals from 23 Amerindian populations were analyzed. All population samples were previously genotyped for *KIR* presence/absence polymorphism; *KIR* allelic content was poorly described. Only 9 of the 23 populations were genotyped for *HLA* class I. Based on the *KIR* gene-content profiles, we estimated the most common Amerindian *KIR* gene-content haplotypes, information never reported before for many of these populations. When the *HLA* genes started to be analyzed in many of these groups, *KIR* genes were still not well characterized. Therefore, they have never been analyzed in a joint context. We thoroughly examined the *HLA* haplotypes of these populations; for the first time, we are showing the frequencies of the known HLA ligands of most of these populations, which had been separately studied for both *KIR* and *HLA*. Amerindians exhibits a low diversity of *KIR* gene-content haplotypes when compared to most worldwide population. We compared the *KIR–HLA* diversity within and between Amerindian groups trying to understand the natural causes of variation. This study corroborates the hypothesis that demographic factors such as founder effect played a major role in shaping *KIR* diversity in Amerindians and may contribute to understand the importance of KIR–HLA for human health and disease.

1. Introduction

Killer-cell immunoglobulin-like receptors (KIR) are expressed on the surface of natural killer (NK) cells. This family of receptors is responsible for activating and inhibitory signals that modulate the NK cell response against infected and altered cells [1,2], differentiate self from non-self [3,4] and influence placentation [5]. Because of these critical roles in innate immunity and reproduction, KIR variation likely is under selective pressure.

The *KIR* genes are located in the leukocyte receptor complex (LCR) at 19q13.4 [6,7], and have shown extensive polymorphism at both the gene-content and allelic levels. The *KIR* have evolved rapidly, facilitated by unequal crossing over between the several paralogous *KIR* genes [6,8]. Although many studies have described *KIR* diversity in worldwide populations, only a few genetically isolated populations have been studied, and even fewer have analyzed KIR in the context of their well-established ligands, the human leukocyte antigen (HLA) class I molecules.

KIR gene-content polymorphism has been associated with several infectious [9–11] and other complex diseases [12–15]. Specific combinations of KIR with HLA have also been associated with protection or susceptibility to numerous diseases [16–19]. We recently described *KIR* diversity in two of the largest Brazilian Amerindians groups, Kaingang and Guarani, demonstrating that demographic factors are the most important forces shaping *KIR* frequencies in these populations [20]. However, it is clear that natural selection is also driving functional KIR and HLA combinations in populations [21–23]. Examination of genetically isolated populations permits analysis of the evolutionary basis for *KIR* variation, allowing insight into the role of these receptors in health and

* Corresponding author at: Departamento de Genética, Universidade Federal do Paraná, Caixa Postal 19071, 81531-980 Curitiba, Brazil. Fax: +55 (41) 33611793.
E-mail address: perler@ufpr.br (M.L. Petzl-Erler).
[1] Current address: Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, United States.
[2] CNPq, Science Without Borders.

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disease. Here, we perform a meta-analysis of *KIR* and *HLA* polymorphism in all Amerindian population studied to date, providing new results that expand current understanding of *KIR* diversity in human populations.

2. Materials and methods

2.1. Populations

Many Amerindian populations remain genetically isolated. Distinct cultures and languages from different linguistic stocks form a barrier to admixture. Based on the available data on Allelefrequencies.net, the literature and unpublished data from our research group, *KIR* diversity has been examined in 23 Amerindian groups, but *HLA* data is available for only nine (Table 1). The total size of these isolated populations is generally less than 10,000, but the Mexicans Purepechas and Tarahumara have population sizes of around 100,000 and 400,000, respectively. Only a few populations were represented by samples of less than 50 individuals. The Amerindian populations examined are native to Brazil, Argentina, Mexico and Venezuela. The sample 'Amazon' is a mixture of five isolated Brazilian populations (Fig. 1), therefore does not represent a population.

2.2. KIR genotyping

Population samples were genotyped in former studies by PCR-SSP (sequence specific primers) [24–26] or PCR-SSOP (specific sequence oligonucleotide probes) [27]. The methods used for the populations are summarized in Table 1.

2.3. Statistical analyzes

Genetic distances were estimated based on the frequencies of *KIR* gene-content genotypes. The estimation was performed according to Nei's method [28] using PHYLIP—version 3.6 [29]. A dendrogram was drawn by the neighbor-joining method [30] and visualized with TreeView software [31]. Populations were compared with the study population by the exact test of population differentiation in the Arlequin software package v. 3.5 [32].

Principal component analyses (PCA) were performed in Minitab® statistics software using *KIR* gene frequencies. A set of observations of probably correlated variables is converted into a set of values of linearly uncorrelated variables called principal components. The first two principal components have the largest possible variance, accounting for as much of the variability in the data as possible. Grouping in the XY graph shows similarity, being method a powerful tool to compare populations.

We analyzed gene-content haplotype diversity for the 12 populations for which the full data set of *KIR* genes was available. In the absence of family data, it is necessary to estimate population-level haplotype frequencies using the Expectation–Maximization (EM) algorithm. Haplotype estimation for the *KIR* region poses particular obstacles. Because some *KIR* genes are present only on certain haplotypes, the list of possible *KIR* haplotypes excludes some loci combinations that might be possible based only on the observed genotypic data. We performed haplotype estimation for the Amerindian populations using a modified EM algorithm [33] that utilizes an *a priori* list of known/possible haplotypes as a constraint. With this method, estimates are produced only where the observed genotype can be generated from at least one pair of haplotypes in the list. Genotypes that cannot be explained by haplotypes from this list are left unresolved. As a result, haplotype frequency estimates may sum to less than one in some populations.

To calculate the frequencies of HLA ligands we examined the literature for the frequencies of the *HLA* haplotypes in each population. With the exception of Tarahumara, there were no Amerindian haplotypes that carried the Bw4 epitope on both *HLA-A* and *HLA-B* loci. Correlations between *KIR* genes and HLA ligands were calculated by permutation approach as described by Single et al. [22].

3. Results

Amerindians show low diversity of *KIR* gene-content (Fig. 2). Most of these populations have more than 70% of the diversity explained by three or four gene-content profiles that are shared

Table 1
Amerindian populations of this meta-analysis study.

ID	Population	Geographic coordinates	Country	Population size	Language	Sample size	*KIR* and *HLA*	All *KIR*	Method	References
1	Wichis (Chaco)	24°56′S; 61°31′W	Argentina	36,000	wichi	82	Yes	Yes	SSOP	[66]
2	Chiriguanos	23°08′S; 64°30′W	Argentina	42,000	guarani	54	Yes	Yes	SSOP	[66]
3	Wichis (Salta)	23°8′S; 64°2′W	Argentina	36,000	wichi	19	No	Yes	SSOP	[66]
4	Kayapo*	7°28′S; 52°44′W and 3°46′S; 51°35′W	Brazil	8638	jê	27	No	Yes	SSP	[34]
5	Waiampi*	1°23′S; 52°47′W	Brazil	905	waiampi	3	No	Yes	SSP	[34]
6	Awa-Guaja*	3°45′S; 46°08′W	Brazil	355	guaja	2	No	Yes	SSP	[34]
7	Parakana*	5°30′S; 52°40′W	Brazil	1266	akwawa	4	No	Yes	SSP	[34]
8	Arara Laranjal*	3°30′S; 53°21′W	Brazil	453	karib	4	No	Yes	SSP	[34]
9	Karitiana	9°0′S; 65°0′W	Brazil	320	karitiana	55	No	No	SSP	[22]
10	Surui	9°0′S; 62°0′W	Brazil	1172	surui	46	No	No	SSP	[22]
11	Ticuna	3°15′S; 68°35′W	Brazil	8000	ticuna	65	No	No	SSP	[22]
12	Guarani Kaiowá	23°06′S,55°12′W and 23°12′S,55°06′W	Brazil	31,000	guarani	96	Yes	Yes	SSP	[20]
13	Guarani Ñandeva	23°48′S,54°30′W	Brazil	13,000	guarani	50	Yes	Yes	SSP	[20]
14	Guarani M'byá	25°18′S; 52°32′W	Brazil	7000	guarani	81	Yes	Yes	SSP	[20]
15	Kaingang	25°18′S; 52°32′W	Brazil	1750	kaingang	100	Yes	Yes	SSP	[20]
16	Huicholes	21°44′N; 105°13′W	Mexico	10,000	wixárika	73	No	Yes	SSP	[47]
17	Purepechas	19°46′N; 101°11′W	Mexico	120,000	purépecha	53	No	Yes	SSP	[47]
18	Sierra Madre Pima	31°19′N; 112°55′W	Mexico	2000	uto-aztecan	99	No	No	SSP	[22]
19	Tarahumara	27°30′N; 107°45′W	Mexico	106,000	tarahumara	65	No	Yes	SSP	[47]
20	Yucatan Maya	20°58′N; 89°37′W	Mexico	400,000	yucatec maya	50	No	No	SSP	[22]
21	Bari	10°0′N; 72°57′W	Venezuela	1520	bari	80	Yes	Yes	SSP	[46]
22	Warao	9°00′N; 61°50′W	Venezuela	24,005	warao	89	Yes	Yes	SSP	[46]
23	Yucpa	10°17′N; 72°67′W	Venezuela	4 174	yucpa	61	Yes	Yes	SSP	[46]

* Populations that were grouped and named Amazon. "*KIR* and *HLA*" means the populations that have all *KIR* and *HLA* class I described. "All *KIR*" means populations that have all *KIR* genes genotyped.