2011

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Immunogenetics
DOI 10.1007/s00251-011-0565-1

BRIEF COMMUNICATION

Diversity of the *KIR* gene cluster in an urban Brazilian population

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Received: 26 October 2009 / Accepted: 30 July 2011

Abstract The activity of natural killer cells depends on the balance between activating and inhibitory signals coming from their receptors. Among these are the killer cell immunoglobulin-like receptors (KIR) that recognize specific HLA class I allotypes. Here we characterized *KIR* genetic diversity and their HLA ligands in the population of Curitiba, Paraná State (n=164), and compared it with other worldwide populations. The distribution of *2DL4* alleles was also analyzed. The Curitiba population did not differ significantly from European and Euro-descendant populations, but as an admixed population showed higher genetic diversity. We found 27 *KIR* profiles, many of them uncommon in European populations, in agreement with the elevated historically recent gene flow in the study population. The frequencies of *KIR* genes and their respective HLA ligands were distributed independently and none of the analyzed individuals lacked functional KIR–HLA ligand combinations. *KIR* gene frequencies of 33 worldwide populations were consistent with geographic and ethnic distribution, in agreement with demography being the major factor shaping the observed gene content diversity of the *KIR* locus.

Keywords *KIR* genes · *KIR* diversity · Brazilian population · HLA ligands · *2DL4* allele frequencies

Natural killer (NK) cells are crucial in innate immunity and influence the outcome of antigen-specific immune responses (Biron et al. 1999). They also contribute to placental development and the outcome of pregnancy (Trowsdale and Moffett 2008). NK cell function depends on complex cellular interactions mediated by membrane-bound and soluble gene products. Of prime importance are the inhibitory and activating killer cell immunoglobulin-like receptors (KIR) that bind major histocompatibility complex class I molecules. *KIR* are encoded by a cluster of homologous genes located on human chromosome 19q13.4 (Wende et al. 1999; Liu et al. 2000). *KIR* diversity is observed as presence/absence of genes, resulting in expansion and contraction of *KIR* haplotypes (Wende et al. 2000; Martin et al. 2003) and further diversity is provided by allelic variation of individual *KIR* genes. Almost 400 *KIR* profiles (i.e., genotypes defined by *KIR* gene content) have been identified among 190 populations catalogued online (www.allelefrequencies.net—Gonzalez-Galarza et al. 2011). The presence or absence of certain *KIR* genes as well as some KIR–HLA receptor–ligand combinations have been

Electronic supplementary material The online version of this article (doi:10.1007/s00251-011-0565-1) contains supplementary material, which is available to authorized users.

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Published online: 18 August 2011

shown to associate with various diseases (Khakoo and Carrington 2006; Jiao et al. 2008; Levinson et al. 2008). *KIR* genes may also be genetic landmarks of populations (Rayes et al. 2008) and there are suggestions that *KIR* gene frequencies may be influenced by host/pathogen interactions (Kulkarni et al. 2008). There is also strong evidence that *HLA* and *KIR* are co-evolving (Single et al. 2007; Norman et al. 2007)

Much interest has been shown in the analysis of *KIR* gene frequencies in human populations as a means to understand the evolutionary causes and functional consequences of its polymorphism. However, the data thus far has been limited to a few geographic regions around the world and few Brazilian populations are represented (Middleton et al. 2008; Rudnick et al. 2008). Regarding allelic diversity, even less is known. In this study, we aimed to characterize *KIR* gene content polymorphism and *2DL4* allelic diversity in an urban Brazilian population and compare this with other populations. We also tested for correlations between *KIR* and *HLA* ligand frequencies that might support the hypothesis of co-evolution of these two unlinked loci.

The population from Curitiba (the Capital city of Paraná State, Brazil, located at 25°25′ S and 49°17′ W) is of mixed, predominantly European ancestry. A detailed description can be found in Probst et al. (2000) and Braun-Prado et al. (2000). The individuals tested (n=164) were unrelated, healthy volunteers. All subjects were informed about the aims of the study and formally agreed to participate. The study has been performed according to Brazilian laws and was approved by the ethics committee of the Federal University of Paraná. A subset of individuals (n=99) was sequenced for *2DL4*. The number of individuals in some analyses may vary because of quantitative and qualitative limitations of DNA. We tested for presence/absence of 15 *KIR* genes by PCR-SSP with two pairs of specific primers for each locus (Martin and Carrington 2008; Kulkarni et al. 2010). Unusual profiles were carefully analyzed and were retyped two to three times to validate the results. *HLA-A*, *HLA-B*, and *HLA-C* were previously genotyped by the PCR-SSOP method (Braun-Prado et al. 2000). The Alellefrequencies.net database (Gonzalez-Galarza et al. 2011) was used as the reference to identify *KIR* profiles and to obtain data for comparisons between populations. Allelic subtyping of *2DL4* was carried out by amplification of exons 3, 4, 6, and 8 using gene-specific primers and the products were sequenced using the Big Dye terminator kit (Applied Biosystems). The primer sequences are available on request.

The carrier frequency (F) of each *KIR* gene (presence/absence) was obtained by direct counting. *KIR* gene frequencies were estimated using Bernstein's formula $f_G = 1-\sqrt{(1-F)}$, where F corresponds to the frequency of carriers. Frequencies of the two pairs of "allelic genes", *2DL2–2DL3* and *3DL1–3DS1*, were estimated by two approaches: (1) like the other *KIR* genes as just described, considering them as independent from each other, and (2) considering that they segregate as alleles of one locus with three alleles, two of which are codominant (the "genes") and the third one being recessive (absence of the gene). Haplotype A and B frequencies were also calculated by Bernstein's formula. The linkage disequilibrium (LD) parameter D corresponds to the difference between the estimated two-loci haplotype frequencies and the haplotype frequencies expected at equilibrium and was calculated as described by Mattiuz et al. (1970). D′, the LD coefficient standardized by the maximum value it can take given the allele frequencies (Lewontin 1964), was estimated with the Arlequin software package version 3.16 (Excoffier et al. 2005).

Thirty-two previously described worldwide populations were compared with the study population by the exact test of population differentiation included in the Arlequin software package v. 3.16. The non-informative pseudogenes and framework genes were excluded from the analysis. Genetic distances were estimated by Nei's method (Nei 1972) using PHYLIP—version 3.6 (Felsenstein 2004). A dendrogram was drawn by the neighbor-joining method (Saitou and Nei 1987) and visualized with the TreeView software (Page 1996). The expected frequency of co-occurrence of *KIR* and their *HLA* class I ligands was estimated and compared to the observed frequency using the chi-square test. The receptor/ligand pairs tested were *KIR3DL1/S1+HLA Bw4*, *KIR 2DL2/3+HLA-C* group 1, and *KIR2DL1+HLA-C* group 2. In addition, we counted in each individual the number of functional *KIR–HLA* combinations to estimate their average number and to determine the proportion of individuals in the population lacking functional receptor/ligand combinations.

The carrier frequencies of the *KIR* genes in the study population varied from 31.9% (*2DS5*) to 100% (*2DL4*, *3DL2*, and *3DL3*). With the exception of *2DS4* (93.6%), no other activating *KIR* gene reached a carrier frequency of 60%. Overall, the frequencies of the inhibitory *KIR* genes were higher and more homogeneous among worldwide populations than for the activating genes (Table 1) and *KIR* gene frequencies in the study population are in agreement with the predominantly European ancestry of this population. Among all the *KIR* genes, only *2DL2* and *2DL3* differed significantly in frequencies between the Curitiba population and other populations with European ancestry (Table 1). The frequency of *2DL2* differed significantly between Curitiba and France and *2DL3* differed between Curitiba and some European and Euro-descendant populations, including the population from Belo Horizonte. The reason for this heterogeneity is not clear but it may reflect the ethnic admixture of some of these populations. It may also be related to the partially overlapping functions of

Table 1 *KIR* gene frequencies in the population of Curitiba and in worldwide populations

n	Curitiba 153			Czech 125	BHorizonte 90	Argentina 102	France 108	Ireland 200	Palestine 105	India 145	USAfricans 58	Senegal 118	Camoros 54	Singapore 47	Hong Kong 100	Japan 132	China 106	S. Korea 154	Amazon 40
	F (%)	f_G	f_A	*F* (%)															
2DL1	97.4	0.84	–	95.0	96.7	96.1	97.0	96.0	83.0***	99.3	98.3	100.0	100.0	100.0	99.0	100.0	99.0	99.4	93.0
2DL2	59.4	0.36	0.36	59.0	52.2	62.5	50.0***	51.0	62.0	62.8	56.9	55.0	41.0*	28.2***	28.0***	11.4***	17.3***	14.3***	65.0
2DL3	84.6	0.61	0.55	86.0	94.4*	86.5	91.0***	93.0*	85.0	81.9	82.0	90.0	93.0	100.0**	98.0***	100.0***	99.0***	99.4***	80.0
2DL4	100.0	1.00	–	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2DL5	51.9	0.31	–	52.0	58.9	55.7	47.0	44.0	63.0	71.0***	53.4	52.0	62.7	39.1	45.0	35.6**	68.7*	38.3**	85.0***
2DP1	94.7	0.77	–	94.0	96.7	96.1	97.0	97.0	NT	99.3	98.3	100.0	100.0	100.0	99.0	100.0	99.0	NT	NT
2DS1	42.1	0.24	–	43.0	37.8	45.4	36.0	34.0	44.0	62.8***	22.4	13.0***	17.0***	28.3	40.0	33.3	34.3	37.7	88.0***
2DS2	59.2	0.36	–	57.0	53.3	54.5	51.0	52.0	64.0	62.8	46.6	42.0**	30.0***	28.3***	28.0***	11.4***	17.3***	16.9***	58.0
2DS3	32.5	0.18	–	36.0	38.9	28.6	31.0	28.0	37.0	53.8***	27.6	24.0	20.0	17.4*	25.0	13.6***	12.5***	16.2***	10.0**
2DS4	93.6	0.75	–	92.0	95.6	95.0	96.0	95.0	88.0	86.1***	100.0	100.0**	96.0	97.8	94.0	99.2	94.2	94.2	78.0**
2DS5	31.9	0.17	–	26.0	32.2	35.4	27.0	26.0	27.0	51.0***	37.9	30.0	37.0	21.7	26.0	22.0	23.1	26.6	90.0***
3DL1	92.7	0.73	0.74	94.0	95.6	95.0	96.0	95.0	88.0	87.4	100.0*	99.0*	98.0	97.8	94.0	99.2**	94.2	94.2	65.0***
3DS1	37.3	0.21	0.26	38.0	41.1	42.0	44.0	35.0	39.0	62.2***	13.8***	4.0***	15.0**	30.4	39.0	31.9	32.7	36.4	70.0***
3DL2	100.0	1.00	–	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
3DL3	100.0	1.00	–	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The asterisks indicate *p* values for the populations that differed significantly from Curitiba: *$0.01 \leq p < 0.05$; **$0.001 \leq p < 0.01$, and ***$p < 0.001$. References are listed in Table 3. The populations are named as in the dendrogram

n number of individuals in the sample, *F* carrier frequency, f_G gene frequency obtained by Bernstein's method, f_A the gene frequency, considering the allelic status of *2DL2* and *2DL3*, and of *3DL1*, *3DS1*, *NT* not tested

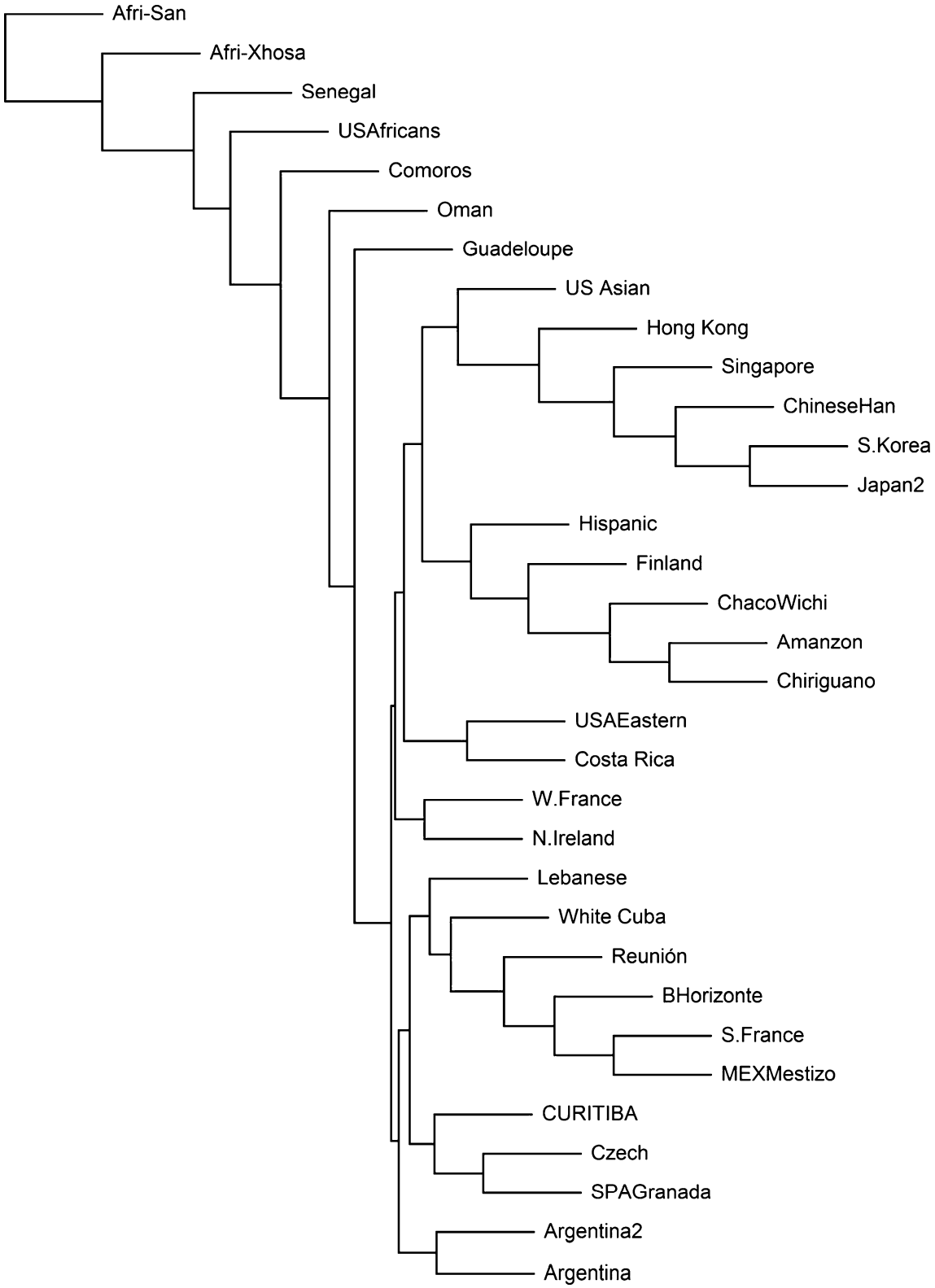


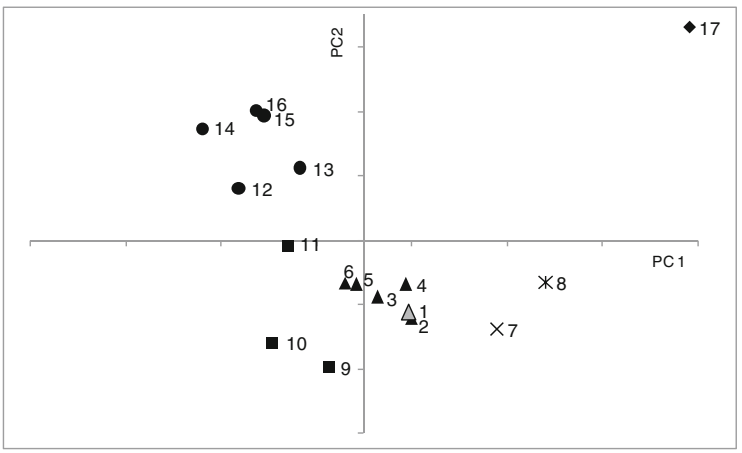
Fig. 1 Nei's genetic distances of 33 worldwide populations visualized in a neighbor-joining dendrogram. Population references are given in Table 3

these genes. *2DL2* and *2DL3* segregate as alleles of one locus and both bind HLA-C group 1 molecules although ligand binding is stronger for 2DL2 (Moesta et al. 2008). Thus, *2DL2* and *2DL3* may be exposed to less severe functional constraints in comparison to other inhibitory *KIR* genes. Relaxation of selection may also permit greater fluctuation of frequencies as a consequence of demographic factors. The two-locus LD pattern was similar to other population studies (see Supplementary Figure 1).

We characterized genetic distances based on *KIR* gene carrier frequencies of 33 populations, and the dendrogram generated is consistent with geography and ancestry (Fig. 1). In general, the European populations tended to cluster together including the Curitiba population, but Finland was somewhat separated from other Europeans. The most divergent are the Africans and they did not cluster in a single clade. It should however be stressed that internal nodes of the dendrogram do not represent a common ancestral population and that grouping of populations may result not only from common ancestry but also from gene flow (admixture), natural selection, or demographic factors resulting in random genetic drift, bottleneck, and founder effects. The principal component analysis (Fig. 2) also revealed that common ancestry is the major factor resulting in similarity of *KIR* gene frequencies, followed by gene flow and possibly random demographic factors.

The profiles observed in the study population are listed in Fig. 3. For comparison, the profiles found in Brazilians from Belo Horizonte (Middleton et al. 2008) are also shown. We identified 27 profiles, eight of which accounted for 77.3% of all observed profiles. The profile representing homozygosity for haplotype A (profile 1 in Fig. 3) was the most frequent (F=28.8%), as reported for other European populations. The frequency of A and B haplotypes were estimated to be 0.54 and 0.46, respectively. The less frequent profiles that are uncommon in Europeans or Euro-descendants reflect the heterogeneous ancestry of this population. Apart from Europeans (Portuguese, Italian, Polish, German, Ukrainian, and others), Africans, as well as Amerindians who had lived in South America before the colonization, contributed to the present gene pool of our study population (Probst et al. 2000). Previous results from our group showed that the ancestry of this population is approximately 87% European, 8% African, and 5% Amerindian (Braun-Prado et al. 2000; Probst et al. 2000 and unpublished results). Therefore, the occurrence of profiles characteristic of non-Europeans and a higher diversity than that seen in other European populations is not unexpected. *KIR* haplotypes could be more informative as ancestry markers, but the recessive absence of *KIR* genes makes it difficult to accurately infer haplotypes in samples of unrelated individuals and segregation analysis would require analysis of a large sample of extended families. The *KIR* complex also has the potential to be a good ancestry marker after more studies describing its allele diversity become available.

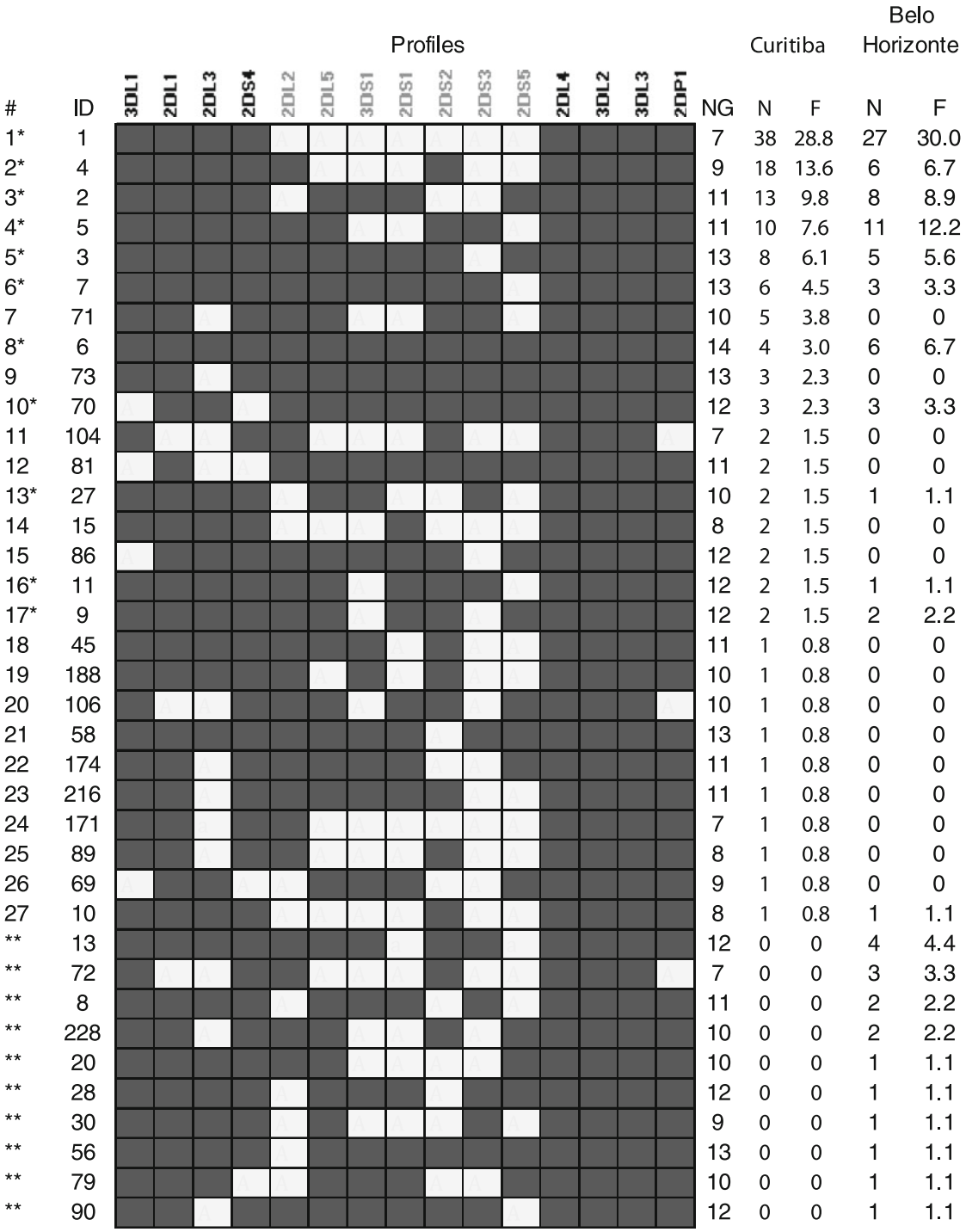
We next compared *2DL4* allele frequencies between Curitiba and Belo Horizonte, another urban Brazilian



▲ = Europeans or Euro-descendants; ■ = Africans or African-descendants; ● = Asians; ◆ = Amerindians; ✕ = Palestinians; ✳ = Asian Indians.

1 = Curitiba; 2 = Czech Republic; 3 = Belo Horizonte; 4 = Argentina; 5 = West France; 6 = Northern Ireland; 7 = Palestine; 8 = Asian Indians; 9 = US Africans; 10 = Senegal; 11 = Comoros; 12 = Singapore 13 = Hong Kong; 14 = Japan; 15 = Han Chinese; 16 = South Korea; 17 = Amazonian Indians

Fig. 2 Principal components analysis of 17 worldwide populations, including the population of Curitiba



#	ID	NG	Curitiba N	Curitiba F	Belo Horizonte N	Belo Horizonte F
1*	1	7	38	28.8	27	30.0
2*	4	9	18	13.6	6	6.7
3*	2	11	13	9.8	8	8.9
4*	5	11	10	7.6	11	12.2
5*	3	13	8	6.1	5	5.6
6*	7	13	6	4.5	3	3.3
7	71	10	5	3.8	0	0
8*	6	14	4	3.0	6	6.7
9	73	13	3	2.3	0	0
10*	70	12	3	2.3	3	3.3
11	104	7	2	1.5	0	0
12	81	11	2	1.5	0	0
13*	27	10	2	1.5	1	1.1
14	15	8	2	1.5	0	0
15	86	12	2	1.5	0	0
16*	11	12	2	1.5	1	1.1
17*	9	12	2	1.5	2	2.2
18	45	11	1	0.8	0	0
19	188	10	1	0.8	0	0
20	106	10	1	0.8	0	0
21	58	13	1	0.8	0	0
22	174	11	1	0.8	0	0
23	216	11	1	0.8	0	0
24	171	7	1	0.8	0	0
25	89	8	1	0.8	0	0
26	69	9	1	0.8	0	0
27	10	8	1	0.8	1	1.1
**	13	12	0	0	4	4.4
**	72	7	0	0	3	3.3
**	8	11	0	0	2	2.2
**	228	10	0	0	2	2.2
**	20	10	0	0	1	1.1
**	28	12	0	0	1	1.1
**	30	9	0	0	1	1.1
**	56	13	0	0	1	1.1
**	79	10	0	0	1	1.1
**	90	12	0	0	1	1.1

Fig. 3 KIR profiles observed in the population from Curitiba and Belo Horizonte. Profiles found in Curitiba (present study) and Belo Horizonte, another urban Brazilian population (Middleton et al. 2008). * Profiles found in both populations. ** Profiles not found in Curitiba. ID is the identification number in Allefrequencies.net (Gonzalez-Galarza et al. 2011); *NG* is the number of genes per profile (excluding pseudogenes), *N* number of individuals with a given profile, *F* relative frequency (%). Genes shown in *gray* are found exclusively on B haplotypes. *Filled boxes* indicate presence of a *KIR* gene and *open boxes* indicate absence of the gene

population (Table 2, top) and found that there were significant differences in allele frequencies (See Table 3 for references of Belo Horizonte and other populations used for comparisons). However, this difference can be explained by the low typing resolution used in the Belo Horizonte study, where only exons 3 and 4 that encode the extracellular domains were sequenced (Williams et al. 2004; Middleton et al. 2008). The allele pairs *00202* and

Table 2 *KIR2DL4* allele frequencies in Curitiba and in Belo Horizonte

Allele	Curitiba (n=99)		Belo Horizonte (n=90)
	f_A	F (%)	F (%)
*2DL4*00102/5*	0.28	44.4	50.0
*2DL4*00501*	0.21	35.4	53.3
*2DL4*00802*	0.15	28.3	0.0
*2DL4*00801*	0.13	23.2	0.0
*2DL4*011*	0.11	15.2	0.0
*2DL4*00103*	0.06	10.1	0.0
*2DL4*006*	0.04	5.1	9.0
*2DL4*010*	0.01	2.0	0.0
*2DL4*00104*	0.01	1.0	0.0
*2DL4*00202*	0.00	0.0	27.9
*2DL4*00201*	0.00	0.0	28.7
Allele groups[a]			
*2DL4*00102/5+00103*	0.34	54.5	50.0
*2DL4*00501+011*	0.32	50.5	53.3
*2DL4*00802+00201*	0.15	28.3	28.7
*2DL4*00801+00202*	0.13	23.2	27.9

Data for Belo Horizonte from Middleton et al. (2008)

n number of individuals, f_A allele frequency, *F(%)* carrier frequency

[a] Alleles were grouped considering the ambiguities of the low resolution typing employed for the analysis of Belo Horizonte's population (Middleton et al. 2008)

00801, and *00201* and *00802*, differ only in an adenine deletion in exon 6, which encodes the transmembrane domain. Differences in exons 6 and 8 also discriminate between *00501* and *011*, and between *00102* and *00103*, respectively. When the ambiguous allele pairs were grouped (Table 2, bottom), their frequencies did not differ significantly (p=0.67). This is noteworthy because of the differential contribution of Europeans to these two populations: while the European ancestry component of Belo Horizonte is essentially Iberian, in Curitiba, apart from the Portuguese, other Mediterranean and central and eastern Europeans account for a high proportion of the immigrants (Probst et al. 2000). Further, the French (Buhler et al. 2009) and the USA populations (Gedil et al. 2005) typed at the same high resolution as in our study, show *2DL4* allele frequencies similar to those seen in Curitiba (p>0.05; data not shown). Thus, the similarity between Curitiba and Belo Horizonte provides additional evidence of low differentiation across Europeans for *2DL4*. Based on the adenine deletion in exon 6, nucleotide position 811, two groups of *2DL4* alleles have been defined, referred to as 9A (the deleted) and 10A (Witt et al. 2000). The two groups differ markedly with respect to *2DL4* expression and function. Deletion alleles cannot be expressed as membrane-bound receptors due to excision of the transmembrane domain as a consequence of the frame shift caused by the adenine deletion. (Goodridge et al. 2003; Goodridge et al, 2007). In our study population, 57.6% of the individuals have a deleted 9A allele in at least one chromosome and 18.2% are homozygous for 9A and therefore lack cell surface 2DL4 receptors. It has been suggested that these two allele groups are maintained at intermediate frequency by balancing natural selection (Witt et al. 2000; Goodridge et al. 2003). This hypothesis is supported by the high frequency of both the deleted and the undeleted allele groups. *2DS4* also exhibits deletion and nondeletion allelic variants. A group of *2DS4* alleles have a deletion of 22 bp in exon 5, encompassing codons 131 to 137 and the first nucleotide of codon 138 within the D2 extracellular domain (Maxwell et al. 2002; Hsu et al. 2002). This results in a frame shift yielding a truncated protein with no transmembrane or cytoplasmic domains and is therefore not expressed on the cell surface. The truncated *2DS4* carrier frequency in Curitiba was 75%. In France this allele group is also very common (carrier frequency 84%), while in Senegal the frequency is lower (carrier frequency 48%) (Denis et al. 2005). *2DS4* is the only classical activating receptor found on the A haplotype, and interestingly we estimated that in Curitiba 33% of individuals homozygous for haplotype A (corresponding to 13.6% of the detected profiles) are homozygous for the deletion, and therefore lack a functional classical activating KIR.

Because *KIR* and *HLA* are both highly polymorphic and because these two gene clusters segregate independently, an individual may have receptor and no ligand or vice versa. Previous studies have also highlighted the relevance of *KIR* with their *HLA* ligands in disease pathogenesis and resistance to viral infections, thus we evaluated the *KIR–HLA* class I ligand combinations in our population. With respect to 2DL1, 2DL2, 2DL3, and 3DL1, the present population sample exhibited an average of 2.9 functional receptor–ligand pairs per individual and each individual carried at least two functional receptor–ligand pairs. The inhibitory 2DL1 binds HLA-C group 2 allotypes with lysine at position 80, and 2DL2/3 receptors bind HLA-C group 1 allotypes, with asparagine at the same position. 3DL1 recognizes HLA-A and HLA-B allotypes with the Bw4 motif. The Bw4 positive HLA-A molecules (A*23, A*24, A*25, and A*32) have isoleucine at position 80 (Bw4-80I) that is also present in a subset of the HLA-B Bw4 molecules. HLA-B Bw4-80I molecules exhibit stronger interactions with their cognate KIR receptors than Bw4-80T (threonine) bearing HLA-B molecules (Cella et al. 1994; Carr et al. 2005). In the present study, we observed a similar frequency of Bw4-80I from both the *HLA-A* and *HLA-B* loci (0.20 and 0.24, respectively). Of the individuals lacking HLA-B Bw4, 47% had a Bw4 HLA-A allele. The high frequency of HLA-A molecules

Table 3 References for the populations used in the estimation of genetic distances and principal components analysis

Name as in the dendrogram	Population name and sample size	Reference
Amazon	Amazon Amerindians 40	Ewerton et al. 2007
Argentina	Argentina 102	Unpublished data *
Argentina 2	Argentina Buenos Aires 365	Flores et al. 2007
ChacoWichi	Argentina Chaco Wichis 82	Flores et al. 2007
Chiriguano	Argentina Chiriguanos 54	Flores et al. 2007
Czech	Czech Population	Pavlova et al. 2008
BHorizonte	Brazil Belo Horizonte 90	Middleton et al. 2008
ChineseHan	China Zhejiang Han 104	Jiang et al. 2005
Comoros	Comoros 54	Frassati et al. 2006
Costa Rica	Costa Rica Guanacaste 117	Carrington et al. 2005
White Cuba	Cuban White 70	Middleton et al. 2008
Curitiba	Euro-descendants 164	Present study
Finland	Finland Helsinki 101	Denis et al. 2005
S. France	France Southeast pop2 38	Frassati et al. 2006
W. France	France West 108	Denis et al. 2005
Guadeloupe	Guadeloupe 118	Denis et al. 2005
Hong Kong	Hong Kong Chinese 100	Middleton et al. 2008
N. Ireland	Ireland Northern pop2 154	Middleton et al. 2008
Japan2	Japanese 132	Yawata et al. 2006
Lebanese	Lebanon 120	Mahfouz et al. 2006
MEX Mestizo	Mexico Veracruz Mestizos 51	Contreras et al. 2007
Oman	Oman 99	Middleton et al. 2008
Palestine	Palestine Jordan 105	Norman et al. 2001
Reunion	Reunion 101	Denis et al. 2005
Senegal	Senegal 90	Denis et al. 2005
Singapore	Singapore Chinese 47	Middleton et al. 2008
Afri-San	South African San 91	Middleton et al. 2008
Afri-Xhosa	South African Xhosa 50	Middleton et al. 2008
S. Korea	South Korea 154	Whang et al. 2005
SPAGranada	Spain Granada 100 WS	Middleton et al. 2007
US Africans	US California African Americans 58	Du et al. 2007
US Asian	US California Asian Americans 150	Du et al. 2007
USAEastern	Euro-descendants from USA 213	Carrington et al. 2005
Hispanic	US California Hispanics 128	Du et al. 2007
India	India 145	Kulkarni et al. 2008

*Population data available on Allelefrequencies.net database (Gonzalez-Galarza et al. 2011)

with the Bw4 epitope suggest that these HLA-A and HLA-B allotypes may be equally important for NK cell function.

The frequencies of the HLA ligands are shown in Supplementary Table 1. All ligands and their respective receptors were distributed independently (p=0.44, data not shown). This might indicate that natural selection acting on specific receptor/ligand combinations is not a major factor determining their population frequencies. Alternatively, the KIR–HLA frequencies might be at equilibrium in this population. However, this result should be interpreted with caution, because for a statistically significant association to be observed in such a population sample, the selection pressure would need to be unusually strong.

The heterogeneous colonization and the continued migrations during the five centuries of Brazil's history as well as the high frequency of interethnic unions, has resulted in an admixed population whose ancestry differs among geographic regions. This is the first study describing *KIR2DL4* allele frequencies in a Southern Brazilian population of predominately European background and only a few South American populations have thus far been analyzed with respect to *KIR* gene content variation and

KIR gene allelic frequencies. The European origins of the population of Curitiba are more heterogeneous than that of most other Brazilian populations. Nevertheless, its *KIR* gene content and *2DL4* allelic diversity are generally similar to those of most European populations. More effort should be dedicated to analyze other admixed and unique isolated populations of various ancestries existing in Brazil and all over the world in order to provide more informative data for evolutionary and functional analyses of *KIR* polymorphism.

Acknowledgments We thank the staff of the Laboratory of Human Molecular Genetics at the Department of Genetics of the Federal University of Paraná for assistance and support. Special thanks to Liana Alves de Oliveira for her expertise and valuable comments. We also thank Fuh-Mei Duh and Colm O'huigin for helpful advice and Maria Dias da Silva for reading this manuscript. This project received financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), PRONEX, Institutos do Milênio, Fundação Araucária, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This project was funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. This Research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

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Supplementary Table 1. Frequencies of HLA ligands for KIR3DL1/S1, KIR2DL1 and KIR2DL2/3 in the population of Curitiba.

Ligands	Allele Frequencies	Carrier frequencies (%)
C1 (HLA-C)	0.66	83.6
C2 (HLA-C)	0.34	50.9
HLA-B Bw4	0.39	62.7
Bw4-80I (HLA-B)	0.24	45.2
Bw4-80I (HLA-A)	0.20	33.8

Supplementary Figure 1. Linkage disequilibrium between pairs of *KIR* genes in the population of Curitiba. The colors indicate D' values following the color scale on the extreme right. The asterisks indicate significant *p* values: ***** $0.01 \leq p < 0.05$; ****** $0.001 \leq p < 0.01$ and ******* $p < 0.001$. Strong negative LD was observed for the allelic genes *KIR2DL2/2DL3*, *KIR3DL1/3DS1* and the gene pairs *KIR2DS4*/*KIR2DS5*, *KIR2DS4*/*KIR3DS1*, *KIR2DS5*/*KIR3DL1* and *KIR2DP1*/*KIR2DL2*. Besides *KIR2DL1*/*KIR2DP1*, strong positive LD occurred for the pairs *KIR2DS1*/*KIR2DS5*, *KIR2DS5*/*KIR2DL5*, *KIR2DL1*/*KIR2DL2*, *KIR2DL5*/*KIR2DS1*, *KIR2DL5*/*KIR3DS1*, *KIR2DL3*/*KIR2DP1*, *KIR2DL2*/*KIR2DS2* and *KIR2DS1*/*KIR3DS1*.

	2DL2	2DL3	2DL5	2DS1	2DS2	2DS3	2DS4	2DS5	3DL1	3DS1	2DP1
2DL1	1.00	1.00 ***	0.03	0.07	1.00	0.96	0.91	0.15	0.97	1.00	1.00 ***
2DL2		0.75 ***	0.47 ***	0.33 *	0.88 ***	0.75 ***	0.21	0.23	0.16	0.24	1.00 *
2DL3			0.21	0.16	0.79 ***	0.63 ***	0.33	0.16	0.09	0.01	0.90 ***
2DL5				0.91 ***	0.41 ***	0.78 ***	0.43 *	0.98 ***	0.54 **	0.91 ***	0.30
2DS1					0.27 ***	0.29 **	0.55 **	1.00 ***	0.12 **	0.88 ***	0.07
2DS2						0.79 ***	0.16	0.10	0.82	1.00	1.00 *
2DS3							0.37	0.01	0.26	0.25	0.69
2DS4								1.00 ***	0.18 ***	1.00 ***	0.17
2DS5									1.00 ***	0.86 ***	0.07
3DL1										1.00 ***	0.86
3DS1											0.49