High-resolution characterization of 12 classical and non-classical *HLA* loci in Southern Brazilians

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Abstract

The *human leukocyte antigen (HLA*) are the most polymorphic genes in the human genome. Due to their importance for antigen recognition, HLA molecules play a central role in host defense and graft rejection upon transplantation. The aim of this study was to characterize allelic diversity of the classical *HLA* genes *HLA-A, -B, -C, -DRA, -DRB1, -DQA1, -DQB1, -DPA, -DPB1*, and the non-classical class I genes *HLA-E, -F* and *-G* at high-resolution for a population of predominantly European ancestry from Curitiba, Brazil. Genotyping of 108 individuals was performed by next generation sequencing on the MiSeq platform and also by Sanger sequencing. The genotype distributions of all loci were in accordance with Hardy-Weinberg equilibrium ($p > 0.05$) and a total of 202 *HLA* variants at second field resolution were observed for the twelve loci. The strongest linkage disequilibrium ($r^2 = 1.0$, $p < 10^{-5}$) was observed for the following pair of alleles: *HLA-B*42:01:01 ~ HLA-DRB1*03:02:01; HLA-B*14:02:01 ~ HLA-C*08:02:01; B*42:01:01 ~ HLA-C*17:01:01; HLA-DRB1*03:01:01 ~ HLA-DQB1*02:01:01 DRB1*03:01:01 ~ HLA-DQB1*02:01:01; DRB1*13:01:01~ HLA-DQB1*06:03:01 and HLA-DRB1*09:01:02 ~ HLA-DQA1*03:02*. This is the first study to characterize all twelve *HLA* genes at high resolution in a single population. On the basis of the allelic frequencies of worldwide populations and principal component analysis, we confirmed the similarity of the study population to European and other Euro-descendant populations.

Key words: NGS, human leukocyte antigen, population genetics, Curitiba

Introduction

The *human leukocyte antigen* (*HLA)* are the most polymorphic genes in the human genome and are located within the major histocompatibility complex (MHC) on chromosome 6. The HLA molecules are essential for host defense against infection; classical class I and class II molecules present peptide antigens to CD8+ and CD4+ T cells, respectively, and play a pivotal role in self/non-self discrimination. Additionally, HLA class I molecules are involved in natural killer cell regulation.[1] HLA class I and class II molecules differ according to their structure and the region where their genes are located within the MHC complex. HLA class I molecules are formed by an alpha chain that is encoded by an *HLA* gene attached to an invariant beta chain, β2-microglobulin, which is encoded by a non-*HLA* gene. In contrast, HLA class II molecules have an alpha chain attached to a beta chain, which are encoded by two different *HLA* genes.[2]

The classical *HLA* class I genes are *HLA-A, HLA-B* and *HLA-C*, while the non-classical are *HLA-E, HLA-F* and *HLA-G*. The classical *HLA* class II genes are *HLA-DRA, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1 and HLA-DPB1* and the non-classical are *HLA-DMA, HLA-DMB, HLA-DOA and HLA-DOB*, among others.[3] *HLA* allelic variation plays a central role in susceptibility to infection, autoimmune diseases, in graft-versus-host disease after hematopoietic stem cell transplantation and in graft rejection upon transplantation of cells or solid organs.[4]

Most of the classical *HLA* loci have thousands of known alleles, whose frequencies vary remarkably across populations.[5–8] Some alleles occur in most worldwide populations, while others are unique to certain populations. Therefore, characterizing the *HLA* allelic diversity across populations may contribute to the understanding of relationships among different populations.[9] *HLA* may provide valuable information about the evolutionary history of populations, especially if the cis combinations of alleles at different loci (haplotypes) are taken into consideration.[10–12]

The Brazilian population exhibits great diversity due to the long history of migrations and admixture since its multi-ethnic origin. Historically, Brazil underwent two main migration waves after its first colonization by the Portuguese: Africans were brought to work as slaves in sugarcane plantations, mainly in the Northeast region; and Europeans, especially Portuguese, Italian, Spanish and German, came after the abolition of slavery to work in coffee plantations in the South and Southeast regions.[13] Intermarriage between those ethnic groups and with the Native Americans contributed to the ethnic admixture of the contemporary Brazilian population.[14]

Paraná State is located in Southern Brazil and, similar to the other Southern states, its population is of predominantly European ancestry. The State of Paraná had, at the beginning, a greater proportion of immigrants from Italy, but also from Poland in the 1940's and from Japan from 1950 onwards.[15] Curitiba is the capital city of Paraná State and one of the largest Brazilian cities. According to the public data from the Brazilian Institute of Geography and Statistics, 79% of the Curitiba population self-declared themselves as white, 18% as mixed and only 3% as black (https://www.ibge.gov.br). Previous studies from our group showed that the ancestry of the predominantly Euro-descendants individuals from Curitiba was 80.6% European, 12.5% African and 7.0% Amerindian. The predominantly Afro-descendants individuals, on the other hand, exhibited 49.5% African and 41.8% European ancestry.[16,17] This large European component in Afro-descendant individuals has also been observed in other studies[18,19] and evidences the large contribution of the European ancestry in Southern Brazilians.

The *HLA* polymorphism has been deeply studied across populations, however, most studies focused on *HLA-A, HLA-B* and *HLA-DRB1* as their implication for transplantation outcome has been known for longer. The diversity of non-classical *HLA* genes and of genes that encode the alpha chain of the classical class II molecules is poorly known across populations, despite the growing evidence of their impact in disease and transplantation.[20–28] Even scarcer are the studies that describe the classical and non-classical genes in a single population sample.

Here, we characterized the allelic diversity of the classical *HLA* class I *HLA-A, HLA-B* and *HLA-C*, the non-classical class I *HLA-E, HLA-F* and *HLA-G* and the alpha and beta classical *HLA* class II genes *HLA-DRA, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1 and HLA-DPB1* in a sample from Curitiba by the combination of next generation and Sanger sequencing techniques. This is, to the best of our knowledge, the first study describing alleles of all these 12 loci at high-resolution in a single population.

Methods

Population characterization

Peripheral blood samples (8 mL) were collected from 108 unrelated individuals (73.2% female, mean age 29 years) of predominantly European ancestry from Curitiba (25°25′ S, 49°17′ W, Paraná State, Brazil, Figure 1) and its metropolitan region. Individuals who reported known non-European background were not included in this study. We also excluded those who reported acute or chronic health alterations or who were under the use of prescribed medications. All individuals voluntarily agreed to participate and provided written informed consent. The Human Research Ethics Committee of the Federal University of Paraná, in accordance with the Brazilian Federal laws, approved this study under the protocol number CAAE 68282717.0.0000.0102.

DNA extraction

The blood samples were obtained in tubes containing the anticoagulant ethylenediaminetetraacetic acid tripotassium (K3EDTA). The DNA was extracted using the Wizard® Genomic DNA kit (Promega) following the instructions provided by the manufacturer.

HLA class I and class II sequencing

High-resolution genotyping of *HLA-A, -B, -C, -DRB1, -DQB1* and *-DPB1* genes was performed by next generation sequencing, MiSeq platform, with a previously validated protocol.[29]

The class II *HLA-DPA1, -DQA1, -DRA* and the non-classical class I *HLA-E*, *HLA-F*, *HLA-G* genes were genotyped by Sanger sequencing. The exons amplified for each locus were: 2 and 3 for *HLA-DPA1;* 1, 3 and 4 for *HLA-DQA1;* 4 for *HLA-DRA;* 2 and 3 for *HLA-E*; 1, 2, 4 and 5 plus introns 1, 2, 4, and 5 for *HLA-F*; 2, 3, and 4 for *HLA-G*. Primer sequences, annealing temperatures and thermal cycling

conditions are given in the Supplementary Tables 1-4. Sequencing reactions were performed with Big Dye® Terminator Cycle Sequencing Standard v3.1 kits (Life Technologies, Foster City, USA) according to the manufacturer instructions.

Data analysis

Allelic frequencies were obtained by direct counting and haplotypes were estimated by the ELB algorithm using the ARLEQUIN software version 3.5.2.[30] Hardy-Weinberg equilibrium was tested with Genalex v6.502 program (PEAKALL; SMOUSE, 2006). Haplotype frequencies were used to estimate linkage disequilibrium (LD) between pairs of alleles of different *HLA* loci using the exact test with Arlequin v3.5.2 software.

Allelic frequencies were used to perform principal component analysis (PCA) with Minitab 17 Statistics Software. The PCA analysis reduces a large number of variables into a small set that still contains most of the information, called principal components. The components accumulate the maximum variance of the data in a decreasing order, which makes it possible to visualize the variance in dimensions. The first two components comprise most of the variance and populations that have similar *HLA* frequencies will group on the XY graph. For this analysis, we used *HLA-A, HLA-B and HLA-DRB1* allelic frequencies at two-field resolution and included populations from Europe, East Asia and Africa, as well as other Brazilian populations (Table 1). Populations were also compared using the exact test of population differentiation with Arlequin.[31] Frequencies of each haplotype were compared between all populations two by two using the χ^2 test in 2×2 contingency tables.

Results

A total of 202 different *HLA* variants (corresponding to *HLA* alleles in each locus at the resolution level of the second field) were identified: 26 *HLA-A*, 47 *HLA-B*, 22 *HLA-C*, 37 *HLA-DRB1*, 15 *HLA-DQB1*, 23 *HLA-DPB1*, 12 *HLA-DQA1*, 8 *HLA-DPA1*, 2 *HLA-DRA*, 2 *HLA-E*, 3 *HLA-F* and 5 *HLA-G*. Tables 2 and 3 show the allele frequencies at second field resolution for *HLA* class I and class II, respectively. Frequencies of alleles at higher resolution can be found in Supplementary Table 5.

The genotype distributions for all loci were in accordance with Hardy-Weinberg equilibrium ($p > 0.05$).

Frequencies of the most common alleles and haplotypes were compared to those of Maringá, Northern Paraná, the geographically closest population for which high-resolution genotyping for *HLA-DRB1, -DQA1* and *-DQB1* is available.[32] Similar frequencies were observed for *HLA-DRB1*07:01:01* (f = 0.139; p = 0.765), *HLA-DQA1*01:02* (f = 0.178; p = 0.732), *HLA-DQB1*03:01:01* (f = 0.17; p = 0.438) and for the common haplotypes *HLA-DRB1*07:01~DQA1*02:01~DQB1*02:02* (f = 0.103; p = 0.553) and *HLA-DRB1*11:01~DQA1*05:05~DQB1*03:01* haplotypes (f = 0.591; p = 0.385). Since *HLA-A* and *HLA-B* data were not available, this population data was not used in further analyzes.

The *HLA-A~HLA-B~HLA-DRB1, HLA-A~-B~-C~-E~-F~-G and HLA-DRB1~-DQB1~*-DPB1 inferred haplotypes with frequency ≥ 0.015 are shown in Tables 4-6. The list of all inferred haplotypes and their frequencies is available in Supplementary Tables 6-9.

We analyzed the LD between pairs of loci; only haplotypes observed at frequencies ≥ 0.01 and presenting strong LD (D'> 0.8, r^2 >0.6 and $p < 10^{-5}$) are shown (Table 7). Applying these criteria, we observed significant LD between alleles from the following pairs of loci: *HLA-B~HLA-DRB1; HLA-B~HLA-C; HLA-C~HLA-DRB1; HLA-DQB1~HLA-DQA1; HLA-DRB1~HLA-DQB1 and HLA-DRB1~HLA-DQA1.* No significant LD was observed for pairs that included the non-classical loci *HLA-E*, *HLA-F* and *HLA-G*. The pair with the greatest number of alleles in LD was *HLA-DQB1~HLA-DQA1*.

The exact test of population differentiation was based on the *HLA-A*, *HLA-B* and *HLA-DRB1* frequencies (table 8). Frequencies observed in the study population differed significantly from those observed in all the populations, except the Portuguese, Italian and other Brazilian populations. The first two principal components in our analysis represented 39% of the total variance. The grouping was consistent with ancestry and geography (Figure 2). The population from Curitiba grouped with European populations from Germany, Portugal, England and Italy, but

was distant from Asian and African populations. Curitiba also grouped with other Brazilian populations, except Amerindians.

Discussion

The Brazilian population is admixed due to its history of colonization and migrations, and the ethnic background differs between regions of the country.[15,33–36] In our study, we show a great diversity of *HLA* alleles in Curitiba and a large number of inferred haplotypes. The similarity of the allele frequencies found between Curitiba and population of Maringá and other Brazilian populations used for comparisons in our analysis is expected due to the fact that all populations are of predominantly European ancestry.[32,37–39] The novelty of our study is the characterization of 12 classical and non-classical loci at high resolution in a single population. Most studies that describe *HLA* in populations focus on a few loci. Therefore, LD among all those genes as well as haplotypes that include classical and non-classical *HLA* are poorly described.

Brazil was originally occupied by Native American populations and throughout its history received numerous peoples from all over the world, especially from Africa, Europe and Japan. Despite intermarriage being common, the population in the South is of predominantly European ancestry.[13] In fact, the South was colonized after the Northeast and the Southeast regions and did not receive a significant contingent of Africans during the times of slavery; migrants from Eastern Asia were the last to arrive. Accordingly, the most common haplotypes observed in Curitiba are similar to those frequent in Europeans and their frequencies differ significantly from those frequent in Asian or African populations.[6] Despite this, we observed in Curitiba a few *HLA-DRB1~HLA-DQA1~HLA-DQB1* haplotypes that are frequent among Native American populations.[11]

The two most frequent *HLA-A~HLA-B~HLA-DRB1* haplotypes **29:02:01~*44:03:01~*07:01:01* (*f* = 0.025) and **01:01:01~*08:01:01~ *03:01:01* (*f* =

0.02) were also reported as the most common in Italian Americans (*f* = 0.015; *p* = 0.357 and f = 0.049%; *p* = 0.068, respectively) and in Spanish Americans (*f* = 0.023; *p* = 0.922 and *f* = 0.021; *p* = 0.871, respectively).[40] The most frequent classical class I and classical class II haplotypes corresponded to the most common haplotypes in other European populations such as English and Spanish (data not shown).[41,42]

For *HLA* class II, the haplotype *HLA-DRB1*07:01~HLA-DQA1*02:01~HLA-DPA1*01:03~HLA-DPB1*04:01* was observed at high frequency in Spain.[42] Based on our results, this haplotype includes the allele *HLA-DQB1*02:02*, which is the most common class II haplotype in Curitiba (*f* = 0.07) (Supplementary Table 9). Interestingly, *HLA-DQB1*02:02~HLA-DRB1*07:01* was seen in strong LD (D' = 0.953; r^2 = 0.768) as well as the pair *HLA-DQB1*02:02~HLA-DQA1*02:01 (*D' = 0.862; r^2 = 0.680). Our results contribute to the knowledge of previous haplotypes described in European populations.

HLA allelic frequencies were compared across worldwide populations. As expected, Curitiba grouped with Brazilians of predominantly European ancestry as well as with European populations. Because *HLA* frequencies drastically differ among different ethnic groups, Asians and Africans did not group with European and Euro-descendants. It is important to highlight that population grouping in PCA as well as homogeneity of allele and haplotype frequencies do not necessary reflect common ancestry and may be due to stochastic factors, such as gene flow and genetic drift. Therefore, the PCA results should be analyzed carefully as they simply reflect the distribution of *HLA* alleles among these populations, and grouping cannot be interpreted as being necessarily caused by common ancestry.

Polymorphism of *HLA-A*, *-B* and *-DRB1* in Brazil was deeply analyzed based on serologic or low-resolution genotyping volunteer donors enrolled in REDOME (Brazilian Registry of Bone Marrow Donor).[43–46] However, few studies describe the diversity of many *HLA* loci at high resolution in the same population as the present study. Population studies that describe *HLA* frequencies of multiple loci, as well as their LD and haplotype frequencies are important for evolutionary and anthropological studies. Additionally, they impact donor match, as more and more is

shown that *HLA* genes other than the most studied *HLA-A*, *-B*, *-C*, *-DQB1* and *-DRB1* also impact transplantation and graft survival.[20,47] Our results reinforce allelic and haplotypic similarities between the population from Curitiba with European populations, which corroborates other studies that analyzed Euro-descendants from Southern Brazil.[37,48,49]

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Table 1. Populations whose *HLA* frequency data was used for the comparisons.

Population	Reference	Sample size	Ancestry
Brazil Curitiba	Present study	108	Euro-descendant
Brazil Maringá	[50]	641	Euro-descendant
Brazil REDOME Piauí	*	46140	Admixed
Brazil Ribeirão Preto	[39]	108	Admixed
Brazil Terena	[51]	60	Native American
Brazil Rio Grande do Sul	[38]	4428	Euro-descendant
China	[52]	10.000	East Asian
England North west	[53]	161	European
Germany	[54]	8862	European
Italy	[55]	975	European
Japan	[56]	371	East Asian
Kenya	[57]	100	African
Mozambique	[58]	202	African
Portugal	[59]	130	European
South Africa	[60]	159	African

*Data published in Allele Frequency Net Database (http://www.allelefrequencies.net)[5]

Table 2. Allele frequencies of six *HLA* class I genes in 108 individuals from Curitiba, Brazil.

HLA-A		*HLA-B*		*HLA-C*		*HLA-E, -F, -G*	
Alleles	*f*	*Alleles*	*f*	*Alleles*	*f*	*Alleles*	*f*
*A*01:01*	0.075	*B*07:02*	0.082	*C*01:02*	0.024	*E*01:01*	0.538
*A*02:01*	0.226	*B*07:05*	0.005	*C*02:02*	0.052	*E*01:03*	0.462
*A*02:02*	0.009	*B*08:01*	0.034	*C*02:10*	0.010		
*A*02:04*	0.005	*B*13:02*	0.019	*C*03:03*	0.057	*F*01:01*	0.827
*A*02:05*	0.014	*B*14:01*	0.005	*C*03:04*	0.057	*F*01:03*	0.168
*A*02:11*	0.009	*B*14:02*	0.029	*C*03:04*	0.005	*F*01:04*	0.005
*A*02:17*	0.009	*B*15:01*	0.067	*C*04:01*	0.167		
*A*03:01*	0.108	*B*15:03*	0.010	*C*04:29*	0.005	*G*01:01*	0.794
*A*11:01*	0.047	*B*15:09*	0.010	*C*05:01*	0.038	*G*01:03*	0.049
*A*23:01*	0.038	*B*15:10*	0.005	*C*06:02*	0.052	*G*01:04*	0.118
*A*23:05*	0.005	*B*15:15*	0.005	*C*07:01*	0.148	*G*01:05*	0.01
*A*24:02*	0.142	*B*15:17*	0.019	*C*07:02*	0.124	*G*01:06*	0.029
*A*25:01*	0.033	*B*18:01*	0.053	*C*07:04*	0.014		
*A*26:01*	0.033	*B*27:02*	0.005	*C*08:02*	0.029		
*A*29:01*	0.005	*B*27:05*	0.024	*C*12:03*	0.09		
*A*29:02*	0.042	*B*35:01*	0.053	*C*14:02*	0.005		
*A*30:01*	0.028	*B*35:02*	0.024	*C*14:03*	0.005		
*A*30:02*	0.028	*B*35:03*	0.029	*C*15:02*	0.048		
*A*31:01*	0.038	*B*35:08*	0.014	*C*15:05*	0.010		
*A*32:01*	0.024	*B*38:01*	0.038	*C*16:01*	0.043		
*A*33:01*	0.019	*B*39:01*	0.029	*C*16:02*	0.010		
*A*33:03*	0.005	*B*39:02*	0.005	*C*17:01*	0.010		
*A*68:01*	0.028	*B*39:05*	0.010				
*A*68:02*	0.009	*B*39:06*	0.005				
*A*69:01*	0.009	*B*39:06*	0.005				
*A*74:01*	0.009	*B*39:09*	0.010				
		*B*39:13*	0.005				
		*B*40:01*	0.024				
		*B*40:02*	0.014				
		*B*40:04*	0.005				
		*B*41:01*	0.010				
		*B*42:01*	0.010				
		*B*44:02*	0.029				
		*B*44:03*	0.077				
		*B*44:05*	0.014				
		*B*45:01*	0.005				

		*B*47:01*	0.005		
		*B*49:01*	0.034		
		*B*50:01*	0.014		
		*B*51:01*	0.058		
		*B*52:01*	0.010		
		*B*53:01*	0.024		
		*B*55:01*	0.019		
		*B*56:01*	0.005		
		*B*57:01*	0.024		
		*B*58:01*	0.019		
		*B*81:01*	0.005		
HW (*p):*	0.13		0.57	0.77	0.65 0.60 0.70

f = frequency; HW = Hardy-Weinberg; *p* = p-value

Table 3. Allele frequencies of six *HLA* class II genes in a sample of 108 volunteer individuals from Curitiba, Brazil.

HLA-DRB1		*HLA-DQB1*		*HLA-DPB1*		*HLA-DRA, -DQA1, -DPA1*	
Alleles	*f*	*Alleles*	*f*	*Alleles*	*f*	*Alleles*	*f*
*DRB1*01:01*	0.072	*DQB1*02:01*	0.057	*DPB1*01:01*	0.052	*DRA*01:01*	0.656
*DRB1*01:02*	0.034	*DQB1*02:02*	0.118	*DPB1*01:05*	0.009	*DRA*01:02*	0.344
*DRB1*03:01*	0.058	*DQB1*03:01*	0.170	*DPB1*01:24*	0.005		
*DRB1*03:02*	0.010	*DQB1*03:02*	0.104	*DPB1*02:01*	0.132	*DQA1*01:01*	0.091
*DRB1*04:01*	0.019	*DQB1*03:03*	0.033	*DPB1*02:02*	0.014	*DQA1*01:02*	0.178
*DRB1*04:02*	0.029	*DQB1*03:19*	0.019	*DPB1*02:96*	0.005	*DQA1*01:03*	0.063
*DRB1*04:03*	0.010	*DQB1*04:02*	0.104	*DPB1*03:01*	0.047	*DQA1*01:04*	0.038
*DRB1*04:04*	0.019	*DQB1*05:01*	0.132	*DPB1*04:01*	0.316	*DQA1*01:05*	0.029
*DRB1*04:05*	0.014	*DQB1*05:02*	0.033	*DPB1*04:02*	0.151	*DQA1*02:01*	0.130
*DRB1*04:06*	0.005	*DQB1*05:03*	0.033	*DPB1*05:01*	0.028	*DQA1*03:01*	0.106
*DRB1*04:07*	0.01	*DQB1*06:02*	0.066	*DPB1*06:01*	0.024	*DQA1*03:02*	0.010
*DRB1*04:11*	0.019	*DQB1*06:03*	0.066	*DPB1*09:01*	0.009	*DQA1*03:03*	0.034
*DRB1*07:01*	0.139	*DQB1*06:04*	0.052	*DPB1*10:01*	0.038	*DQA1*04:01*	0.101
*DRB1*08:01*	0.048	*DQB1*06:09*	0.014	*DPB1*11:01*	0.028	*DQA1*05:01*	0.063
*DRB1*08:02*	0.010			*DPB1*13:01*	0.019	*DQA1*05:05*	0.159
*DRB1*08:04*	0.014			*DPB1*14:01*	0.033		
*DRB1*08:07*	0.019			*DPB1*15:01*	0.009	*DPA1*01:03*	0.701
*DRB1*09:01*	0.010			*DPB1*16:01*	0.009	*DPA1*01:05*	0.005
*DRB1*10:01*	0.005			*DPB1*17:01*	0.033	*DPA1*02:01*	0.210
*DRB1*11:01*	0.082			*DPB1*18:01*	0.009	*DPA1*02:02*	0.042
*DRB1*11:02*	0.014			*DPB1*19:01*	0.009	*DPA1*02:03*	0.005
*DRB1*11:03*	0.014			*DPB1*23:01*	0.005	*DPA1*02:07*	0.009
*DRB1*11:04*	0.038			*DPB1*27:01*	0.009	*DPA1*03:01*	0.023
*DRB1*12:01*	0.014			*DPB1*45:01*	0.005	*DPA1*04:01*	0.005
*DRB1*12:02*	0.005						
*DRB1*13:01*	0.067						
*DRB1*13:02*	0.077						
*DRB1*13:03*	0.005						
*DRB1*13:05*	0.005						
*DRB1*14:04*	0.005						
*DRB1*14:06*	0.005						
*DRB1*14:54*	0.024						
*DRB1*15:01*	0.043						
*DRB1*15:03*	0.024						
*DRB1*15:11*	0.005						
*DRB1*16:01*	0.029						

HW (*p):*	0.214	0.498	0.240	0.420 0.963 0.125

f = frequency; HW = Hardy-Weinberg; *p* = p-value

Table 4. Most common *HLA-A~HLA-B~HLA-DRB1* haplotypes (frequency ≥ 0.015).

HLA-A~HLA-B~HLA-DRB1	*f*
**29:02:01~*44:03:01~*07:01:01*	0.025
**01:01:01~*08:01:01~*03:01:01*	0.020
**23:01:01~*44:03:01~*07:01:01*	0.015
**02:01:01~*15:01:01~*11:01:01*	0.015

All the haplotypes exhibited significant LD ($p < 0.05$) between all pairs of neighboring loci. A total of 170 haplotypes was inferred by the ELB method (Supplementary table 6). *f* = frequency

Table 5. Most common *HLA* class I haplotypes (frequency ≥ 0.015).

HLA-A~HLA-B~HLA-C~HLA-E~HLA-F~HLA-G	*f*
**01:01:01~*08:01:01~*07:01:01~*01:01~*01:01:01~*01:01:02*	0.024
**02:01:01~*44:02:01~*05:01:01~*01:01~*01:01:01~*01:01:01*	0.024
**29:02:01~*44:03:01~*16:01:01~*01:03~*01:01:01~*01:01:01*	0.024
**03:01:01~*35:01:01~*04:01:01~*01:03~*01:03:01~*01:01:01*	0.018
**11:01:01~*35:01:01~*04:01:01~*01:01~*01:01:02~*01:01:03*	0.018
**02:01:01~*40:01:01~*03:04:01~*01:03~*01:01:01~*01:01:01*	0.018
**03:01:01~*07:02:01~*07:02:01~*01:03~*01:01:01~*01:01:01*	0.018
**02:01:01~*51:01:01~*15:02:01~*01:03~*01:01:01~*01:01:01*	0.018
**02:01:01~*15:01:01~*03:04:01~*01:03~*01:01:01~*01:01:01*	0.018

None of the most frequent inferred haplotypes exhibited significant LD ($p < 0.05$) between all pairs of neighboring loci. A total of 139 haplotypes was inferred by the ELB method (Supplementary table 7). *f* = frequency

Table 6. Most common *HLA-DRB1~HLA-DQB1~HLA-DPB1* haplotypes (frequency ≥ 0.015).

HLA-DRB1~HLA-DQB1~HLA-DPB1	*f*
07:01:01~*02:02:01~*04:01:01	**0.067**
**11:01:01~*03:01:01~*04:02:01*	0.034
01:01:01~*05:01:01~*04:01:01	**0.034**
13:02:01~*06:04:01~*02:01	**0.034**
**15:01:01~*06:02:01~*04:01:01*	0.024
01:02:01~*05:01:01~*04:01:01	**0.024**
**13:01:01~*06:03:01~*04:01:01*	0.019
13:01:01~*06:03:01~*04:02:01	**0.019**
07:01:01~*02:02:01~*11:01:01	**0.019**
**11:04:01~*03:01:01~*04:02:01*	0.019
07:01:01~*02:02:01~*04:02:01	**0.019**
**11:01:01~*03:01:01~*04:01:01*	0.019

In bold, the haplotypes with significant LD ($p < 0.05$) for all pairs of neighboring loci. A total of 112 haplotypes was inferred by the ELB method (Supplementary table 8). *f* = frequency

Table 7. Pairs of alleles at different *HLA* loci that present strong and significant linkage disequilibrium.

Loci	*f*	D'	r^2
HLA-B~HLA-DRB1			
**15:03:01~*08:04:01*	0.01	1.000	0.663
**42:01:01~*03:02:01*	0.01	1.000	1.000
HLA-B~HLA-C			
**07:02:01~*07:02:01*	0.060	1.000	0.623
**14:02:01~*08:02:01*	0.025	1.000	1.000
**42:01:01~*17:01:01*	0.010	1.000	1.000
**44:02:01~*05:01:01*	0.035	1.000	0.743
HLA-DQB1~HLA-DQA1			
**02:01:01~*05:01:01*	0.055	0.903	0.744
**02:02:01~*02:01:01*	0.095	0.862	0.680
**03:01:01~*05:05:01*	0.080	0.849	0.648
**04:02:01~*04:01*	0.055	0.883	0.698
**05:01:01~*01:01*	0.085	0.939	0.594
**05:03:01~*01:04*	0.013	1.000	0.871
**06:03:01~*01:03:01*	0.055	1.000	0.924
HLA-DRB1~HLA-DQB1			
**03:01:01~*02:01:01*	0.055	1.000	1.000
**07:01:01~*02:02:01*	0.120	0.953	0.767
**11:02:01~*03:19:01*	0.015	1.000	0.747
**13:01:01~*06:03:01*	0.060	1.000	1.000
**13:02:01~*06:04:01*	0.050	1.000	0.671
HLA-DRB1~HLA-DQA1			
**03:01:01~*05:01:01*	0.555	0.903	0.744
**07:01:01~*02:01:01*	0.120	0.913	0.769
**09:01:02~*03:02*	0.010	1.000	1.000
**13:01:01~*01:03:01*	0.065	1.000	0.924
**14:54:01~*01:04*	0.015	1.000	0.707

Only alleles with frequencies greater than 0.01. D' > 0.8 and r^2 >0.6 are shown. The significance is $p < 10^{-5}$. *f* = frequency

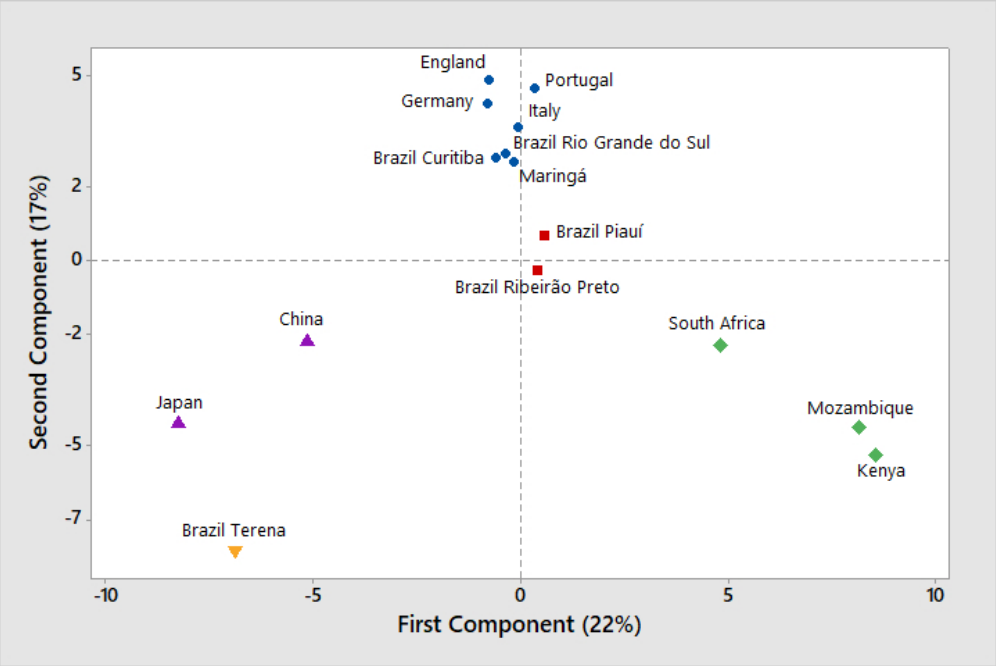
Table 8. Genetic differentiation between Curitiba and other populations based on *HLA-A. HLA-B* and *HLA-DRB1* at second field resolution.

	Curitiba (n = 108) p-value
Brazil Maringá (641)	0.52
Brazil Piauí (46140)	0.83
Brazil Ribeirão Preto (108)	0.95
Brazil Rio Grande do Sul (4428)	0.06
Brazil Terena (60)	$< 10^{-5}$
China (10000)	$< 10^{-5}$
England (161)	$< 10^{-5}$
Germany (8862)	$< 10^{-5}$
Italy (975)	0.09
Japan (371)	$< 10^{-5}$
Kenya (100)	$< 10^{-5}$
Mozambique (202)	$< 10^{-5}$
Portugal (130)	0.99
South Africa (59)	$< 10^{-5}$

Information about the populations and references are in table 1. The *p*-values shown are for test exact of population differentiation.

Figure 1 - Location of the study population. Language spoken: Portuguese; Linguistic family: Indo-European. Geographic location: South America, Brazil, South region; 25°25’ S, 49°17’ W

188x154mm (150 x 150 DPI)



Principal component analysis groups Curitiba with European populations. Each dot represents a population. Circle = European or Euro-descendant; square = Brazilian admixed; diamond = African; triangle = South East Asian or Amerindian. The list of populations and references can be found in Table 1.

110x73mm (165 x 166 DPI)