HLA-A, HLA-B, and HLA-DRB1 Allele and Haplotype Frequencies in Renal Transplant Candidates in a Population in Southern Brazil

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Background: Very few studies have examined the diversity of human leukocyte antigens (HLA) in the Brazilian renal transplant candidates. **Methods:** The frequencies of the HLA-A, HLA-B, and HLA-DRB1 alleles, haplotypes and phenotypes were studied in 522 patients with chronic renal failure, renal transplant candidates, registered at the Transplant Centers in north/northwestern Paraná State, southern Brazil. Patients were classified according to the ethnic group (319 whites [Caucasians], 134 mestizos [mixed race descendants of Europeans, Africans, and Amerindians; browns or “pardos”] and 69 blacks). The HLA typing was performed by the polymerase chain reaction sequence-specific oligonucleotide method (PCR-SSO), combined with Luminex technology. **Results:**

Key words: kidney failure, chronic; kidney transplantation; HLA antigens; major histocompatibility complex; polymorphism, genetic

In the analysis of the total samples, 20 HLA-A, 32 HLA-B, and 13 HLA-DRB1 allele groups were identified. The most frequent allele groups for each HLA locus were *HLA-A*02* (25.4%), *HLA-B*44* (10.9%), and *HLA-DRB1*13* (13.9%). The most frequent haplotypes were *HLA-A*01-B*08-DRB1*03* (2.3%), *A*02-B*44-DRB1*07* (1.2%), and *A*03-B*07-DRB1*11* (1.0%). Significant differences ($P < 0.05$) were observed in the *HLA-A*68*, *B*08*, and *B*58* allele frequencies among ethnic groups. **Conclusions:** This study provides the first data on the HLA-A, HLA-B, and HLA-DRB1 allele, phenotype and haplotype frequencies of renal transplant candidates in a population in southern Brazil. J. Clin. Lab. Anal. 30:258–265, 2016. © 2015 Wiley Periodicals, Inc.

INTRODUCTION

Kidney transplantation is considered the best option for therapy and rehabilitation of patients with end-stage renal disease (1). A major limitation to the success of transplantation is the recipient's immunological response to the organ or tissue graft. Recognition of the transplanted cells, as self or nonself, is mainly determined by human leukocyte antigens (HLA) (2). The HLA system is an important marker of the survival of a transplanted organ, and plays a prominent role among the biological systems involved in the rejection process (3–5).

HLA typing tests for both donor and recipient are fundamental in pretransplant evaluation, since the number of

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HLA mismatches may be associated with decreased graft survival (5). Greater HLA compatibility between donor and recipient favors tolerance by the immune system, and increases the graft survival in the case of transplantation from a related living donor, as well as from a deceased donor (5–8).

The Brazilian population includes a mixture of different ethnic groups, with a relatively high proportion of mixed-race individuals or “mestizos” (9). This mixing and ethnic diversity can complicate the search for a donor with the best possible immunological compatibility. Because of the enormous extent of Brazil and the diversity of immigrants who participated in its colonization, the frequencies of the HLA alleles and haplotypes vary in different regions of the country (10–12).

However, in Brazil as a whole and particularly in the southern, very few studies have evaluated HLA diversity (allelic and haplotypic) in the renal transplant candidates. Knowledge of the diversity and distribution of HLA alleles in this population may help in the process of allocation of organs for transplantation and in the understanding of mechanisms associated with susceptibility to different diseases in patients with chronic kidney disease.

This study evaluated the diversity of alleles and haplotypes of HLA-A, HLA-B, and HLA-DRB1 in renal transplant candidates from the northern and northwestern regions of Paraná.

MATERIALS AND METHODS

Samples

The study was conducted with 522 blood samples of patients with chronic renal failure, renal transplant candidates, who were registered at two regional transplant centers: the Central Regional de Transplantes Norte/Londrina (CRTN/Londrina) and the Central Regional de Transplantes Noroeste/Maringá (CRTNO/Maringá), in northern and northeastern Paraná, respectively, in the period from July 2010 to March 2011. Only patients with updated records (active patients and potential transplant recipients) were included. The patients were divided into three ethnic groups: whites (Caucasians, $n = 319$), mestizos (mixed-race descendants of Europeans, Africans, and Amerindians; browns or “pardos”; $n = 134$), and blacks ($n = 69$). The data for ethnic groups were provided by Central Transplants, using self-definition as a criterion for determining the ethnic group, based on the method used by the Brazilian Institute of Geography and Statistics (IBGE) national census survey (the official census of Brazil). This study was approved by the Ethics Committee of the Universidade Estadual de Maringá (protocol no.

333/2011). All procedures followed Resolution 196/1996 of the Brazilian Health Council that rules on research work on humans, and a written informed consent was obtained from all participants.

DNA Extraction and HLA Class I and II Typing

To perform the HLA typing, 5 ml of peripheral blood was collected by venipuncture into vacuum tubes (Vacutainer, Becton and Dickson, Oxford, UK) containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Genomic DNA was extracted with the use of a separation column, using the commercial DNA extraction kit Biopur (Biometrix, Curitiba, Paraná, Brazil), following the manufacturer’s protocol. After the concentration of the DNA obtained was adjusted by optical density, HLA typing was performed by the polymerase chain reaction sequence-specific oligonucleotide method (PCR-SSO) combined with the Luminex technology. In this method, genomic DNA was amplified using a biotinylated sequencing primer locus-specific for HLA class I (HLA-A and HLA-B) and class II (HLA-DRB1) in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA). Subsequently, hybridization was performed with complementary DNA probes conjugated to microspheres (beads) labeled with different fluorochromes to identify complementary sequences of the amplified DNA, using the SSO-LABType commercial kit (One Lambda, Canoga Park, CA) with low-medium resolution, following the manufacturer’s protocol. After hybridization, the results were read using the flow cytometry platform LABScanTM100 (One Lambda), followed by analysis using the program HLA Fusion version 2.0 (One Lambda).

Statistical Analyses

The Arlequin program, version 3.11 (13), was used to calculate the allele and haplotype frequencies and to test for the Hardy–Weinberg equilibrium. The haplotype frequencies were estimated using the expectation maximization algorithm (maximum likelihood method). The Statistica 7.0 and R programs were used to calculate the phenotype frequency and to compare the allele frequencies of the three ethnic groups. Significant differences between the allele frequencies of the ethnic groups were calculated by Fisher’s exact test with Bonferroni correction for multiple comparisons. The P -values less than 0.05 were considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

RESULTS

Among the 522 renal transplant candidates, 314 (60.1%) were males. The predominant ethnic group was white (61.1%), followed by mestizo (25.7%) and black (13.2%).

The HLA-A and HLA-DRB1 loci were in Hardy–Weinberg equilibrium ($P > 0.05$). For the A locus, the observed heterozygosity was 86.4% and the expected heterozygosity was 89.0% ($P = 0.508$). For the DRB1 locus, these values were 87.3 and 89.6%, respectively ($P = 0.309$). A significant difference was observed only for the B locus between the observed and expected heterozygosity ($P = 0.022$), meaning, only the B locus was not in Hardy–Weinberg equilibrium ($P < 0.05$). In the B locus, the observed heterozygosity was 90.6% and the expected heterozygosity was 93.9% ($P = 0.022$).

Table 1 lists the HLA allele and phenotype frequencies in the total samples. In the analysis of the total samples, 20 HLA-A, 32 HLA-B, and 13 HLA-DRB1 allele groups were identified. The *HLA-A*02*, *A*24*, and *A*03* allele groups were the most frequent for the A locus. In the B locus, the most frequent allele groups were *HLA-B*44*, *B*35*, and *B*51*, and in the DRB1 locus the *HLA-DRB1*13*, *DRB1*11*, and *DRB1*04* allele groups were the most frequent.

Table 2 lists the HLA allele frequencies in the total sample and in each ethnic group. There was a wide agreement in the distribution of allele frequencies among the three ethnic groups. Significant differences were observed in the frequency of allele group *HLA-A*68*, between mestizos and blacks (1.9 and 11.6%, respectively), OR: 6.89 (95% CI: 2.47–19.26). For the *HLA-B*08* allele group, there was a significant difference between mestizos and whites (1.9 and 7.2%, respectively), OR: 4.09 (95% CI: 1.61–10.40) and for the *HLA-B*58* allele group, between whites and blacks (1.2 and 6.5%, respectively), OR: 5.5 (95% CI: 2.08–14.51).

The most frequent HLA haplotypes are listed in Table 3. *HLA-A*01-B*08-DRB1*03* (2.3%), *A*02-B*44-DRB1*07* (1.2%), and *A*03-B*07-DRB1*11* (1.0%) were prominent in the HLA haplotype in the total samples. The *HLA-A*01-B*08-DRB1*03* haplotype was the most frequent in the white ethnic group (3.0%). *HLA-A*01-B*08-DRB1*03* (2.9%) and *HLA-A*03-B*07-DRB1*11* (2.9%) were the most frequent in the black ethnic group (2.9%), and *HLA-A*02-B*15-DRB1*13* haplotype in the mestizo ethnic group (2.1%).

DISCUSSION

The Brazilian population is one of the most heterogeneous in the world, composed of a four main ethnic groups including Amerindians, Asians, Africans, and Europeans (9, 10). In the context of organ or tissue transplantation,

this ethnic miscegenation may hinder the search for a donor with the best possible immunological compatibility, especially in the HLA system. Investigation of the diversity of HLA alleles and haplotypes provides important data for studies of the genetic composition and origin of populations (10–12). The frequencies of the HLA alleles and haplotypes may vary according to the predominant ethnic group in a particular region (10–12).

The samples evaluated in the current study were from the state of Paraná in southern Brazil. This state has a strong influence of European settlement, but also has significant numbers of people of African and Amerindian descent (10). These influences can be seen in our results, which show a relatively large contribution of HLA alleles of European origin (14–16), as well as the occurrence of alleles of African origin (17, 18). A similar HLA diversity was also observed in a study of volunteer bone-marrow donors from southern Brazil (12).

Extensive studies of HLA diversity (especially allelic diversity) have been conducted in different regions of Brazil, including in the same region of this study, most of them using apparently healthy individuals (10–12, 19–30). However, to our knowledge, no studies have examined HLA haplotypic and allelic diversity in candidates for renal transplantation in southern Brazil, particularly in Paraná State.

In the current study, a significant difference between the observed and expected heterozygosity was observed only for locus B. The B locus showed a high degree of heterozygosity (90.6%), but the population was not found to be in Hardy–Weinberg equilibrium ($P < 0.05$). The number of heterozygous individuals was lower than expected. This difference can be explained by the number of samples, or according to Chen et al. (31), in different populations of healthy subjects, the deviation from Hardy–Weinberg proportions can be related to natural forces, such as a selective advantage or recent racial admixture, natural selection, or gene flow due to migration; or may reflect difficulties in the identification of HLA. However, because our sample was composed of individuals with chronic kidney disease, that is, nonhealthy individuals, this deviation from Hardy–Weinberg proportions may also be related to the pathological condition itself, whether or not it is related to genetic causes; or because we did not exclude individuals who had some degree of kinship to each other.

Comparing the ethnic groups in our sample, we found differences and similarities in the frequencies of HLA allele groups. The general distribution is very similar to studies with samples from healthy individuals from southern Brazil (states of Paraná and Rio Grande do Sul) (12, 21, 22).

Only some HLA allele groups exhibited significant differences in frequency among the ethnic groups. *HLA-A*68* showed a significant difference in allele frequencies

TABLE 1. HLA Allele and Phenotype Frequencies in the Total Sample ($n = 522$)

HLA locus	F allelic	F phenotypic	HLA locus	F allelic	F phenotypic	HLA locus	F allelic	F phenotypic
<i>A*01</i>	0.0929	0.1858	<i>B*07</i>	0.0699	0.1398	<i>DRB1*01</i>	0.0929	0.1858
<i>A*02</i>	0.2538	0.5077	<i>B*08</i>	0.0536	0.1073	<i>DRB1*03</i>	0.1121	0.2241
<i>A*03</i>	0.0939	0.1877	<i>B*13</i>	0.0172	0.0345	<i>DRB1*04</i>	0.1264	0.2529
<i>A*11</i>	0.0527	0.1054	<i>B*14</i>	0.0316	0.0632	<i>DRB1*07</i>	0.1216	0.2433
<i>A*23</i>	0.0517	0.1034	<i>B*15</i>	0.0843	0.1686	<i>DRB1*08</i>	0.0575	0.1149
<i>A*24</i>	0.1015	0.2031	<i>B*18</i>	0.0498	0.0996	<i>DRB1*09</i>	0.0125	0.0249
<i>A*25</i>	0.0192	0.0383	<i>B*27</i>	0.0259	0.0517	<i>DRB1*10</i>	0.0230	0.0460
<i>A*26</i>	0.0431	0.0862	<i>B*35</i>	0.1073	0.2146	<i>DRB1*11</i>	0.1322	0.2644
<i>A*29</i>	0.0402	0.0805	<i>B*37</i>	0.0125	0.0249	<i>DRB1*12</i>	0.0115	0.0230
<i>A*30</i>	0.0584	0.1169	<i>B*38</i>	0.0268	0.0536	<i>DRB1*13</i>	0.1389	0.2778
<i>A*31</i>	0.0374	0.0747	<i>B*39</i>	0.0364	0.0728	<i>DRB1*14</i>	0.0354	0.0709
<i>A*32</i>	0.0268	0.0536	<i>B*40</i>	0.0489	0.0977	<i>DRB1*15</i>	0.0958	0.1916
<i>A*33</i>	0.0316	0.0632	<i>B*41</i>	0.0201	0.0402	<i>DRB1*16</i>	0.0402	0.0805
<i>A*34</i>	0.0096	0.0192	<i>B*42</i>	0.0239	0.0479			
<i>A*36</i>	0.0048	0.0096	<i>B*44</i>	0.1092	0.2184			
<i>A*66</i>	0.0134	0.0268	<i>B*45</i>	0.0211	0.0421			
<i>A*68</i>	0.0508	0.1015	<i>B*47</i>	0.0019	0.0038			
<i>A*69</i>	0.0010	0.0019	<i>B*48</i>	0.0038	0.0077			
<i>A*74</i>	0.0144	0.0287	<i>B*49</i>	0.0316	0.0632			
<i>A*80</i>	0.0029	0.0057	<i>B*50</i>	0.0268	0.0536			
			<i>B*51</i>	0.0958	0.1916			
			<i>B*52</i>	0.0163	0.0326			
			<i>B*53</i>	0.0211	0.0421			
			<i>B*54</i>	0.0010	0.0019			
			<i>B*55</i>	0.0077	0.0153			
			<i>B*56</i>	0.0019	0.0038			
			<i>B*57</i>	0.0278	0.0556			
			<i>B*58</i>	0.0201	0.0402			
			<i>B*67</i>	0.0019	0.0038			
			<i>B*78</i>	0.0010	0.0019			
			<i>B*81</i>	0.0019	0.0038			
			<i>B*82</i>	0.0010	0.0019			

F, frequencies.

between mestizos and blacks, *HLA-B*08* showed a significant difference between mestizos and whites, and *HLA-B*58* between whites and blacks, demonstrating that these HLA allele groups may be typical of certain ethnic groups in the sample.

However, allele groups that are commonly of European origin, such as *HLA-A*02*, *B*35*, *B*44*, *B*51*, *DRB1*11*, and *DRB1*04*, had high frequencies in whites, mestizos, and blacks, and did not show significant differences among these three ethnic groups. The similarities in allele frequencies found in these ethnic groups reflect the difficulty of defining the ethnicity of an individual based only on physical assessment of color, particularly in populations with a high degree of miscegenation. The Brazilian Institute of Geography and Statistics (IBGE) national census (the official census of Brazil) also used the criteria of self-definition and skin color to determine the ethnicity of the respondents. However, HLA diversity studies can more accurately reveal the ancestry of a population, and are therefore important in populations such as that of Brazil.

Studies of the HLA distribution with samples from populations on five continents demonstrated the existence of differences among the HLA alleles in these populations and the consequent increase of these differences in populations with mixed ethnic groups (32, 33). However, a study of five different samples from Brazilian populations, composed of whites and blacks, showed that the similarities in the frequencies of HLA alleles among these populations are much greater than the differences (29), in concordance with the results of the current study.

In the HLA-A locus, the *HLA-A*02* allele group was the most frequently observed in our sample, as in other studies of HLA frequency conducted in southern Brazil (12, 21). However, the *HLA-A*02* allele group in our sample showed a higher frequency compared to a previous study in Paraná (21), even if compared only between groups of whites. With respect to the study in Rio Grande do Sul (12), the allele group *HLA-A*02* in our sample showed a lower frequency, even compared to studies of groups of Caucasians (whites). In the HLA-B locus, the *HLA-B*44* allele group was the most common, followed

TABLE 2. HLA Allele Frequencies in the Total Sample ($n = 522$) and Comparisons Among Ethnic Groups

HLA locus	Fa total	Fa W	Fa M	Fa B	WXM (<i>P</i> -value)	OR (95% CI)	WXB (<i>P</i> -value)	OR (95% CI)	MXB (<i>P</i> -value)	OR (95% CI)
<i>A*01</i>	0.0929	0.0972	0.0933	0.0725						
<i>A*02</i>	0.2538	0.2602	0.2612	0.2101						
<i>A*03</i>	0.0939	0.0878	0.1194	0.0725						
<i>A*11</i>	0.0527	0.0564	0.0560	0.0290						
<i>A*23</i>	0.0517	0.0549	0.0448	0.0507						
<i>A*24</i>	0.1015	0.1113	0.0821	0.0942						
<i>A*25</i>	0.0192	0.0188	0.0261	0.0072						
<i>A*26</i>	0.0431	0.0408	0.0485	0.0435						
<i>A*29</i>	0.0402	0.0455	0.0299	0.0362						
<i>A*30</i>	0.0584	0.0486	0.0709	0.0797						
<i>A*31</i>	0.0374	0.0455	0.0224	0.0290						
<i>A*32</i>	0.0268	0.0313	0.0224	0.0145						
<i>A*33</i>	0.0316	0.0266	0.0261	0.0652						
<i>A*34</i>	0.0096	0.0047	0.0149	0.0217						
<i>A*36</i>	0.0048	—	0.0112	0.0145						
<i>A*66</i>	0.0134	0.0094	0.0187	0.0217						
<i>A*68</i>	0.0508	0.0502	0.0187	0.1159					0.00114	6.89 (2.47–19.26)
<i>A*69</i>	0.0010	0.0016	—	—						
<i>A*74</i>	0.0144	0.0063	0.0299	0.0217						
<i>A*80</i>	0.0029	0.0031	0.0037	—						
<i>B*07</i>	0.0699	0.0737	0.0448	0.1014						
<i>B*08</i>	0.0536	0.0721	0.0187	0.0362	0.0248	4.09 (1.61–10.40)				
<i>B*13</i>	0.0172	0.0188	0.0187	0.0072						
<i>B*14</i>	0.0316	0.0298	0.0373	0.0290						
<i>B*15</i>	0.0843	0.0721	0.1231	0.0652						
<i>B*18</i>	0.0498	0.0596	0.0373	0.0290						
<i>B*27</i>	0.0259	0.0298	0.0224	0.0145						
<i>B*35</i>	0.1073	0.1034	0.1119	0.1159						
<i>B*37</i>	0.0125	0.0141	0.0112	0.0072						
<i>B*38</i>	0.0268	0.0251	0.0336	0.0217						
<i>B*39</i>	0.0364	0.0345	0.0336	0.0507						
<i>B*40</i>	0.0489	0.0486	0.0410	0.0652						
<i>B*41</i>	0.0201	0.0219	0.0187	0.0145						
<i>B*42</i>	0.0239	0.0157	0.0410	0.0290						
<i>B*44</i>	0.1092	0.1097	0.1082	0.1087						
<i>B*45</i>	0.0211	0.0188	0.0299	0.0145						
<i>B*47</i>	0.0019	0.0016	—	0.0072						
<i>B*48</i>	0.0038	0.0031	0.0037	0.0072						
<i>B*49</i>	0.0316	0.0329	0.0299	0.0290						
<i>B*50</i>	0.0268	0.0235	0.0336	0.0290						
<i>B*51</i>	0.0958	0.1082	0.0933	0.0435						
<i>B*52</i>	0.0163	0.0157	0.0187	0.0145						
<i>B*53</i>	0.0211	0.0172	0.0224	0.0362						
<i>B*54</i>	0.0010	0.0016	—	—						
<i>B*55</i>	0.0077	0.0063	0.0112	0.0072						
<i>B*56</i>	0.0019	0.0031	—	—						
<i>B*57</i>	0.0278	0.0235	0.0336	0.0362						
<i>B*58</i>	0.0201	0.0125	0.0149	0.0652			0.0279	5.5 (2.08–14.51)		
<i>B*67</i>	0.0019	—	0.0075	—						
<i>B*78</i>	0.0010	0.0016	—	—						
<i>B*81</i>	0.0019	0.0016	—	0.0072						
<i>B*82</i>	—	—	—	0.0072						
<i>DRB1*01</i>	0.0929	0.0862	0.0933	0.1232						
<i>DRB1*03</i>	0.1121	0.1144	0.0970	0.1304						
<i>DRB1*04</i>	0.1264	0.1238	0.1343	0.1232						
<i>DRB1*07</i>	0.1216	0.1317	0.1119	0.0942						
<i>DRB1*08</i>	0.0575	0.0596	0.0485	0.0652						
<i>DRB1*09</i>	0.0125	0.0141	0.0075	0.0145						
<i>DRB1*10</i>	0.0230	0.0235	0.0299	0.0072						
<i>DRB1*11</i>	0.1322	0.1285	0.1306	0.1522						
<i>DRB1*12</i>	0.0115	0.0078	0.0187	0.0145						
<i>DRB1*13</i>	0.1389	0.1442	0.1567	0.0797						
<i>DRB1*14</i>	0.0354	0.0408	0.0261	0.0290						
<i>DRB1*15</i>	0.0958	0.0846	0.1119	0.1159						
<i>DRB1*16</i>	0.0402	0.0408	0.0336	0.0507						
2n	1,044	638	268	138						

Fa, allelic frequencies; W, whites; M, mestizos; B, blacks; OR, odds ratio; CI, confidence interval; —, did not occur in this group.

TABLE 3. The 20 Most Common HLA-A-B-DRB1 Haplotype Frequencies in the Total Sample and in the Different Ethnic Groups

HLA haplotype	Total	Whites	Mestizos	Blacks
<i>A*01-B*08-DRB1*03</i>	0.0227	0.0302	—	0.0290
<i>A*02-B*44-DRB1*07</i>	0.0118	0.0157	0.0112	0.0072
<i>A*03-B*07-DRB1*11</i>	0.0104	0.0016	0.0112	0.0290
<i>A*02-B*15-DRB1*04</i>	0.0102	0.0141	0.0048	—
<i>A*02-B*35-DRB1*03</i>	0.0096	0.0031	—	0.0217
<i>A*33-B*14-DRB1*01</i>	0.0096	0.0078	—	0.0217
<i>A*02-B*44-DRB1*13</i>	0.0095	0.0141	0.0037	—
<i>A*30-B*42-DRB1*03</i>	0.0085	0.0094	—	—
<i>A*02-B*39-DRB1*16</i>	0.0085	0.0110	0.0075	—
<i>A*11-B*35-DRB1*01</i>	0.0081	0.0097	0.0037	—
<i>A*02-B*51-DRB1*14</i>	0.0074	0.0047	0.0075	—
<i>A*02-B*18-DRB1*11</i>	0.0067	0.0025	—	—
<i>A*23-B*44-DRB1*07</i>	0.0067	0.0094	0.0037	—
<i>A*24-B*35-DRB1*11</i>	0.0067	0.0117	—	0.0072
<i>A*02-B*07-DRB1*01</i>	0.0066	0.0063	—	—
<i>A*26-B*38-DRB1*13</i>	0.0066	0.0063	0.0037	—
<i>A*02-B*07-DRB1*15</i>	0.0064	0.0094	—	—
<i>A*02-B*15-DRB1*13</i>	0.0064	—	0.0213	—
<i>A*30-B*13-DRB1*07</i>	0.0063	0.0078	—	—
<i>A*02-B*50-DRB1*07</i>	0.0061	0.0078	—	—

—, did not occur in this group.

by *HLA-B*35*. In other studies conducted in southern Brazil, this order is reversed, although the two allele groups were also the most frequently observed in their samples. The allele group *HLA-DRB1*13* was also the most frequent in this locus, in a study in Rio Grande do Sul (12).

Despite the small changes in allele frequencies, the same HLA allele groups in our study are among the most frequent in samples from Paraná (21) and Rio Grande do Sul (12), because these alleles are characteristic of populations of the European origin who actively participated in colonization in these two southern states of Brazil.

Compared to a study of HLA frequency in Pernambuco (30), in northeastern Brazil, where the population is predominantly of African descent, the differences are more pronounced; for example, the *HLA-B*15* allele group (12.3%) was the most frequent for the B locus, whereas in our sample, *HLA-B*44* was the most frequent (10.9%). However, the mestizo group in the current study showed the same frequency as reported for *HLA-B*15*, and this same frequency was also found for this allele group in African-Brazilians in the Paraná study (21).

Similar results were obtained for the most common HLA allele groups among our sample and studies of HLA frequency in other Latin American countries, including Venezuela (34), Colombia (35), Uruguay (36), and Costa Rica (37). This similarity might be explained by the presence of Europeans who participated in the colonization of these countries, including Brazil.

Because of its extensive polymorphism and functions related to the immune system, the HLA system has been increasingly studied as a genetic marker involved in susceptibility to various diseases (38, 39). The frequencies of the *HLA-A*74* and *HLA-DRB1*11* allele group, which, in the current study, were 1.4% and 13.2%, respectively, were positively associated with chronic renal disease, in a study with a small number of the Brazilian patients awaiting renal transplantation (40).

The *HLA-A*01-B*08-DRB1*03* haplotype, typical of whites (caucasians), was the most common in the total sample, and also in the white ethnic group (2.3% and 3.0%), which is in agreement with data reported in other studies in Paraná (2.4% and 2.5%) (21) and Rio Grande do Sul (2.8% and 3.0%) (12). However, variations in haplotype frequencies according to the reported ethnic group were observed in this study. The *HLA-A*01-B*08-DRB1*03* haplotype also was the most frequent in the black ethnic group (2.9%), although less so than among the whites (caucasians, 3.0%); whereas in the mestizo group, this haplotype was not found. Bardi et al. (22) also found, in samples from Paraná, that the *HLA-A*01-B*08-DRB1*03* haplotype was the most frequent in whites (caucasians, 2.7%) and Afro-Brazilians (1.5%). The same haplotype was also reported as the most frequent in North Americans of Italian ancestry (4.9%) and Spanish ancestry (2.1%) (41). Because the majority of the population of north/northwestern Paraná is of the European descent, the data obtained in this study were similar to the HLA genetic profile found in North Americans of the European descent.

The *HLA-A*02-B*15-DRB1*13* haplotype was the most frequent in mestizos in our sample (2.1%). Haplotypes found in this ethnic group may be due to the contribution of HLA alleles of the different European, African, or Amerindian populations who colonized the region.

In this study, the haplotype frequencies were estimated, using as a basis the HLA allele groups found. Knowledge of the genealogy of each individual would be required to identify the inherited haplotypes, and thus to determine the true distribution of haplotypes in the sample. The distribution of HLA haplotypes indicates the ethnicity and ancestry of a population (12).

The genetic similarity of the HLA system, observed through the analysis of HLA allele groups and haplotypes in our sample compared to populations from the same region of the country, can be explained by similar migrations of populations who colonized southern Brazil. This study was not designed to reach conclusions about the effect of evolutionary forces, immigration, or maintaining the genetic diversity of the HLA system in this population. However, our results provide important

information about the diversity and ancestry of this system among the ethnic groups in the region.

In conclusion, the distribution of HLA alleles and haplotypes in renal transplant candidates was very similar to results from other studies of the Brazilian population. Major differences may be observed among the ethnic groups in our sample, and from populations in different regions of the country. The ethnic background influences the distribution of HLA alleles of a population. Southern Brazil has a higher frequency of HLA alleles of the European origin, which was clearly observed in our sample, unlike the Northeast region where the population is more mixed, descended mainly from Africans, and therefore may have a high frequency of HLA alleles and haplotypes that are commonly found in Africa (30).

Data from this study provide knowledge of the frequencies of the HLA-A, HLA-B, and HLA-DRB1 alleles, haplotypes and phenotypes in renal transplant candidates from north/northwestern Paraná. These data reflect the anthropological origin of the population of Paraná, and will be important in understanding the biology of the distribution of these alleles in our population, as well as in studies of susceptibility to different diseases in patients with chronic kidney disease, enabling comparison with other population groups, and contributing in the process of allocation of organs for transplantation in this region and across the country.

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