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Association of the HLA-B*52 allele with non-progression to AIDS in Brazilian HIV-1-infected individuals

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Several human leukocyte antigen (HLA) class I alleles are associated with the susceptibility to human immunodeficiency virus-1 (HIV-1) infection and/or AIDS progression. Of these, the HLA-B alleles are considered the strongest genetic determinant of disease outcome. We evaluated the influence of the HLA-B alleles on AIDS progression among HIV-1-positive individuals from Rio de Janeiro, Brazil, who were categorized as rapid progressors (RPs), typical progressors (TPs) or long-term non-progressors (LTNPs). In this study, significant differences in HLA-B allele frequencies were observed among the three progression groups for the B*48, B*49 and B*52 alleles. After controlling for other factors associated with AIDS progression, the presence of the B*52 allele was shown to be a significant protective factor (hazard ratio (HR) 0.49 (95% confidence interval (CI) 0.27–0.90) $P<0.03$). Although no direct association was observed between the presence of the B*27 or B*57 allele and the LTNP profile compared with the TP or RP groups, the adjusted model confirmed that these alleles are protective factors against AIDS progression (HR 0.62 (95% CI 0.38–0.99) $P<0.05$), as previously described. These data corroborate the existence of significant differences in HLA-B allele frequencies among the distinct AIDS progression profiles and further elucidate the role of HLA alleles in the outcome of HIV infections in diverse populations.

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INTRODUCTION

The natural clinical course of human immunodeficiency virus-1 (HIV-1) infection is extremely variable, ranging from the development of AIDS in less than a year to no evidence of progression in >10 years.[1] The majority of HIV-infected individuals (70–80%) present with a typical course of infection that progresses to disease within 10 years. Approximately 10–15% of HIV-1-infected individuals progress to AIDS within 3 years of seroconversion, and these individuals are known as rapid progressors (RPs). A smaller proportion of individuals (5–10%) remains asymptomatic and presents $CD4^+$ T-cell counts $>500\,cells\,mm^{-3}$ for >10 years; these patients are termed long-term non-progressors (LTNPs). To help explain the high variability among AIDS progression profiles, several studies have examined the roles of viral factors,[2–6] the innate and adaptive immune responses[7–19] and host genetic factors[20–26] in disease progression.

Host genetic variation is presently estimated to account for about one-fourth of the observed variation in HIV control.[27] Among the genetic factors involved in the immune response to HIV, the human leukocyte antigens (HLAs) exert the strongest influence on viral control, immune escape and disease progression.[28–31] Several HLA class I alleles are associated with the susceptibility to HIV-1 infection and/or AIDS progression. The HLA-B alleles in particular impose substantially greater selection pressure on HIV-1 and are considered the strongest genetic determinant of disease outcome.[21,32,33] Some associations are well characterized; for example, the impact of HLA-B*27 and B*57 alleles in slower progression to AIDS and long-term control of HIV-1 replication,[24,34–38] whereas the B*35 and B*53 alleles lead to faster progression.[33,39,40]

Based on this background, this study aimed to evaluate the impact of host genetics on AIDS progression based on the distribution of HLA-B class I alleles among HIV-1-infected individuals classified as RPs, typical progressors (TPs) or LTNPs.

RESULTS

Clinical epidemiological characteristics

Of the 3809 HIV-1-seropositive individuals included in this cohort, 496 fulfilled the criteria for the disease progression classification (see Materials and methods section) and were categorized as 182 RPs (36.7%); 289 TPs (58.3%) and 25 LTNPs (5.0%). From this group, a subset of 218 individuals (86 RPs, 39.4%; 115 TPs, 52.8% and 17 LTNPs, 7.8%) had their HLA-B alleles genotyped.

In order to check the representativeness of this subset of 218 individuals, we compared the sociodemographic data of the 3313 unclassified individuals with data from individuals who had their HLA-B alleles genotyped ($n=218$) and those whose alleles could not be determined ($n=278$; Table 1). No difference regarding ethnicity, which could have influence on the HLA-B allele distribution, was observed from these comparisons, indicating that the subgroup of 218 individuals matches the 3313 group in this aspect. Similar results were observed when the sociodemographic data were compared considering the different groups of AIDS progression (Supplementary Table S1, Supplementary Material).

HLA-B alleles

Of the 218 HIV-positive individuals genotyped for their HLA-B alleles, roughly 29 HLA-B allelic groups were identified. The most frequent HLA-B alleles were B*15, B*35, B*44 and B*14 at 11.2%, 11.0%, 9.9% and 7.1% prevalence, respectively. These four common alleles accounted for 39.2% of the overall HLA-B allele

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Table 1. Sociodemographic data from HIV-1-positive individuals from Rio de Janeiro, Brazil, included in the study

	Unclassified individuals	*Classified individuals*		P-*value*[a]	P-*value*[b]
	(n = *3313*) %	*HLA-B not typed* (n = *278*) %	*HLA-B typed* (n = *218*) %		
Gender				0.85	0.25
Male	67.3	64.4	69.7		
Female	32.7	35.6	30.3		
Ethnicity				0.69	0.46
White	54.8	55.4	57.8		
Mulattoes	29.8	28.8	25.7		
Black	15.0	15.1	16.5		
Other[c]	0.1	0.4	0.0		
NR	0.3	0.4	0.0		
Age (years)				0.18	**<0.001**
<30	33.9	47.5	39.4		
30–39	37.8	32.0	38.5		
⩾40	28.2	20.5	22.0		
NR	0.2	0.0	0.0		
Mean age (s.d.)	34.7 (9.2)	32.0 (9.5)	33.4 (8.8)	**<0.001**	**<0.001**
Schooling				0.38	**<0.001**
Fundamental[d]	51.9	40.6	44.5		
Middle	32.3	36.3	38.1		
Graduation	13.6	22.3	17.4		
NR	2.2	0.7	0.0		
Exposure category				0.49	**0.02**
MSM/bisexual	35.2	40.6	49.1		
Heterosexual	45.7	54.6	40.8		
IDU	2.5	3.2	1.4		
Other[e]	3.6	4.1	3.2		
NR	12.9	13.8	5.5		

Abbreviations: HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IDU, injecting drug users; MSM, men who have sex with men; NR, not reported. Bold numbers indicate *P*-value ⩽0.05. [a]Chi-square test, Fisher's exact test or Mann–Whitney test between HLA-B not typed ($n = 278$) and HLA-B typed ($n = 218$). [b]Chi-square test, Fisher's exact test or Mann–Whitney test between unclassified individuals ($n = 3313$) and all classified individuals, including HLA-B typed and not typed. [c]Other = indigenous ($n = 1$), oriental ($n = 3$). [d]Includes illiterate ($n = 94$). [e]Other = blood transfusion ($n = 124$), hemophiliac ($n = 1$), vertical transmission ($n = 4$) and percutaneous exposure ($n = 6$).

frequency. Another 12 alleles presented moderate frequencies ranging from 2 to 7% and accounted for 48.3% of the overall allele frequency. The remaining 12 alleles had frequencies <2% and accounted for 12.5% of the overall allele frequency. Overall, deviation from Hardy–Weinberg equilibrium was not observed in the distribution of HLA-B genotypes in this population ($P > 0.05$).

Given the miscegenation of the Brazilian population, we first analyzed the difference in frequencies of each HLA-B allele between Caucasian and non-Caucasian (Mulattoes and Black) individuals (Supplementary Table S2, Supplementary Material). Except for HLA-B*42 (0.4% vs 3.8%, respectively, $P < 0.02$), no statistically significant differences were evident. Therefore, the following analysis of the HLA-B allele frequencies across the three AIDS progression groups were performed without accounting for the ethnic background of the individuals studied (Table 2). In our study, the frequency of the B*49 allele was higher in the TP group (4.0%) than in the RP group (0.6%, $P < 0.05$, odds ratio (OR) = 0.14). On the other hand, the frequency of the B*48 allele was higher in the LTNP group (5.9%) compared with the RP group (0%, $P < 0.03$, OR = 0.00). Notably, the B*52 allele frequency was higher in the LTNP group (11.8%) than in either the RP group (2.9%, $P < 0.05$, OR = 0.22) or the TP group (2.6%, $P < 0.05$, OR = 0.27; Table 2). The HIV-1-positive individuals in our study had quite diverse HLA-B genotypes, resulting from the combination of the four more common alleles cited above: B*15, B*35, B*44 and B*14. Each one of genotypes B*15:B*44 and B*14:B*15 were found in five individuals; followed by B*35:B*44 and B*14:B*44, with four occurrences each one and B*14:B*35, presented by three individuals. Only 13 individuals (6.0%) presented with a homozygous genotype (data not shown).

The comparison of the three HLA-B alleles significantly associated to disease progression profiles (Table 2) with their respective frequencies reported for the Brazilian general population (REDOME data set) is presented in Table 3. From this analysis, no statistically significant difference was verified in the overall comparison or stratified by ethnicity (white, black and mulattoes) showing that our studied population matched the HLA-B allelic distribution of the Brazilian population.

Survival analysis

In general, the median time between the diagnosis of HIV-1 infection and AIDS development for the 218 evaluated patients was 1816 days (95% confidence interval (CI): 1624–2004 days). The sociodemographic variables identified as having significant differences in the time to AIDS progression were gender ($P < 0.02$), age at date of positive serology ($P < 0.05$) and exposure category ($P < 0.02$). The time until progression to AIDS was significantly different for the clinical variables presence of the B*48 allele ($P < 0.05$, median 4470 vs 1792 days) and presence of the B*52

Table 2. Distribution of HLA-B alleles and statistical comparison of the allele frequencies among the three clinical progression groups from Rio de Janeiro, Brazil ($n = 218$)

Allele	*RPs (2n = 172)*		*TPs (2n = 230)*		*LTNPs (2n = 34)*		*RP* vs *TP*		*RP* vs *LTNP*		*TP* vs *LTNP*	
	n	*af*	n	*af*	n	*af*	P-*value*[a]	*OR*	P-*value*[a]	*OR*	P-*value*[a]	*OR*
B*07	13	0.075	15	0.065	00	0.000	0.69	1.17	0.13	—	0.23	—
B*08	13	0.075	08	0.035	02	0.059	0.07	2.27	1.00	1.31	0.62	0.58
B*13	01	0.006	05	0.022	00	0.000	0.24	0.26	1.00	—	1.00	—
B*14	08	0.046	21	0.091	02	0.059	0.09	0.49	0.67	0.78	0.75	1.61
B*15	19	0.110	24	0.104	06	0.177	0.84	1.07	0.26	0.58	0.24	0.54
B*18	06	0.035	06	0.026	00	0.000	0.61	1.35	0.59	—	1.00	—
B*27	00	0.000	04	0.017	01	0.029	0.14	0.00	0.17	0.00	0.50	0.58
B*35	23	0.134	23	0.100	02	0.059	0.29	1.39	0.39	2.47	0.75	1.78
B*37	01	0.006	02	0.009	00	0.000	1.00	0.67	1.00	—	1.00	—
B*38	02	0.012	04	0.017	01	0.029	1.00	0.66	0.42	0.39	0.50	0.58
B*39	02	0.012	10	0.044	00	0.000	0.06	0.26	1.00	—	0.37	—
B*40	09	0.052	08	0.035	00	0.000	0.39	1.53	0.36	—	0.60	—
B*41	02	0.012	03	0.013	00	0.000	1.00	0.89	1.00	—	1.00	—
B*42	03	0.017	04	0.017	01	0.029	1.00	1.00	0.52	0.59	0.50	0.58
B*44	18	0.105	23	0.100	02	0.059	0.88	1.05	0.54	1.87	0.75	1.78
B*45	05	0.029	07	0.030	01	0.029	0.94	0.95	1.00	0.99	1.00	1.04
B*47	02	0.012	00	0.000	00	0.000	0.18	—	1.00	—	—	0.00
B*48	00	0.000	03	0.013	02	0.059	0.26	0.00	**0.02**	0.00	0.13	0.21
B*49	01	0.006	09	0.040	01	0.029	**0.04**	0.14	0.30	0.19	1.00	1.34
B*50	05	0.029	03	0.013	00	0.000	0.30	2.27	0.59	—	1.00	—
B*51	15	0.087	11	0.048	03	0.089	0.11	1.90	1.00	0.99	0.40	0.52
B*52	05	0.029	06	0.026	04	0.118	1.00	1.12	**0.04**	0.22	**0.04**	0.27
B*53	05	0.029	11	0.048	01	0.029	0.34	0.60	1.00	0.99	1.00	1.66
B*56	00	0.000	01	0.004	01	0.029	1.00	0.00	0.17	0.00	0.24	0.14
B*57	06	0.035	08	0.035	03	0.089	0.99	1.00	0.17	0.37	0.16	0.37
B*58	05	0.029	10	0.044	01	0.029	0.45	0.66	1.00	0.99	1.00	1.50
B*67	00	0.000	01	0.004	00	0.000	1.00	0.00	—	0.00	1.00	—
B*81	02	0.012	00	0.000	00	0.000	0.18	—	1.00	—	—	0.00
B*82	01	0.006	00	0.000	00	0.000	0.43	—	1.00	—	—	0.00

Abbreviations: af, allele frequency; HLA, human leukocyte antigen; LTNP, long-term non-progressor; *n*, observed number of a HLA-B allele; 2n, total number of alleles (allele count); OR, odds ratio; RP, rapid progressor; TP, typical progressor. Bold numbers indicate *P*-value $\leqslant 0.05$. —, undefined *P*-value or OR value.
[a]Chi-square test or Fisher's exact test.

Table 3. Allelic frequencies of the three HLA-B alleles significantly associated with disease progression, comparing HIV-1-positive individuals from Rio de Janeiro, Brazil, and Brazilian general population (data from Brazilian Registry of Bone Marrow Donors-REDOME), stratified according to ethnic background

Allele	*HIV-1*$^+$ *individuals (2n = 436 alleles)*				*Brazilian (REDOME) (2n = 5 706 717 alleles)*				P-*value*[a]			
	Total (2n = 436)	*White (2n = 252)*	*Black (2n = 72)*	*Mulattoes (2n = 112)*	*Total*[b] *(2n = 5706 717)*	*White (2n = 364 014)*	*Black (2n = 345 703)*	*Mulattoes (2n = 352 271)*	*Total*	*White*	*Black*	*Mulattoes*
B*48	0.011	0.012	0.014	0.018	0.007	0.006	0.008	0.009	0.46	0.46	0.48	0.46
B*49	0.025	0.032	0.000	0.027	0.028	0.028	0.027	0.027	0.48	0.48	—	0.50
B*52	0.034	0.036	0.042	0.027	0.020	0.018	0.021	0.022	0.38	0.39	0.43	0.48

Abbreviations: af, allele frequency; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; *n*, observed number of a HLA-B allele; 2n, total number of alleles (allele count); OR, odds ratio. —, undefined *P*-value. [a]Fisher's exact test. [b]The sum of the observed number of HLA-B alleles (*n*) in white, black and mulattoes does not correspond to the total number because REDOME data also includes frequencies of alleles in indigenous, oriental and individuals whose ethnicity was not reported. For purposes of comparison with our data broken down by ethnicity, we included only allele frequencies from white, black and mulattoes' individuals.

allele ($P < 0.02$, median 2618 vs 1775 days), indicating a possible association of these factors with non-progression to AIDS (Figures 1a and b).

In the multivariate analysis, in addition to the variables significantly associated with the risk of progression to AIDS, described above, we also evaluated the variables schooling, presence of the rapid progression allele (B*35) and presence of the slow progression allele (B*27/B*57; Table 4). From this analysis, the variables independently associated with AIDS progression were gender, age at date of positive serology, presence of the slow progression allele (B*27/B*57) and presence of the allele B*52. Although the variable presence of the B*48 allele was not significantly associated with AIDS progression ($P < 0.08$) in the multivariate model, this variable was included in the analysis because it added explanatory power. Controlling for these factors, the risk of developing AIDS was significantly higher among the male patients (hazard ratio (HR) 1.45 (95% CI 1.06–1.97) $P < 0.03$) who were diagnosed with HIV-1 at a later age (HR 1.02 (95% CI

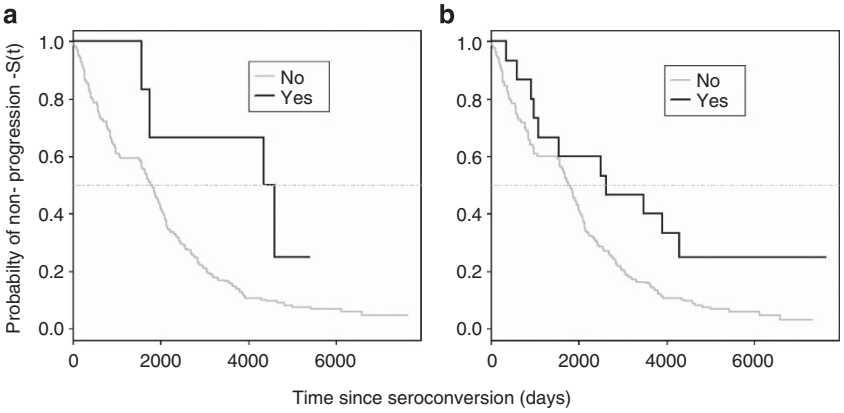

Figure 1. Analysis of disease progression. Kaplan–Meier survival curves for the progression to AIDS from the estimated time of seroconversion stratified by (**a**) the presence of the B*48 allele and (**b**) the presence of the B*52 allele.

Table 4. Bivariate and multivariate analyses of the factors associated with AIDS ($n = 218$)

Factor	*Bivariate*		*Adjusted model*	
	HR	P *Wald*	*HR*	P *Wald*
Sociodemographic characteristics				
Gender				
Female	1 (Ref)		1 (Ref)	
Male	1.47 (1.08; 2.00)	**0.01**	1.45 (1.06; 1.97)	**0.02**
Ethnicity				
White	1 (Ref)			
Black	0.92 (0.62; 1.36)	0.67		
Mulattoes	0.94 (0.68; 1.31)	0.72		
Age (years)	0.99 (0.99; 1.03)	0.06	1.02 (1.00; 1.03)	**0.03**
Schooling				
Graduation	1 (Ref)			
Fundamental	0.74 (0.50; 1.10)	0.14		
Middle	0.98 (0.66; 1.46)	0.93		
Exposure category[a]				
Heterosexual	1 (Ref)			
MSM/bisexual	1.52 (1.13; 2.04)	**0.05**		
Other	1.56 (0.80; 3.04)	0.19		
Clinical characteristics				
Presence of the rapid progression allele (B*35)				
No	1 (Ref)			
Yes	1.22 (0.87; 1.70)	0.26		
Presence of the slow progression allele (B*27/B*57)				
No	1 (Ref)		1 (Ref)	
Yes	0.64 (0.40; 1.03)	0.07	0.62 (0.38; 0.99)	**0.04**
Presence of the B*48 allele				
No	1 (Ref)		1 (Ref)	
Yes	0.37 (0.14; 0.99)	**0.04**	0.41 (0.15; 1.11)	0.08
Presence of the B*49 allele				
No	1 (Ref)			
Yes	0.81 (0.43; 1.54)	0.53		
Presence of the B*52 allele				
No	1 (Ref)		1 (Ref)	
Yes	0.49 (0.27; 0.90)	**0.02**	0.48 (0.26; 0.89)	**0.02**

Abbreviations: HR, hazard ratio; MSM, men who have sex with men; *n*, number of individuals; ref, reference. Bold numbers indicate *P*-value $\leqslant 0.05$. [a]12 missing ($n = 206$).

1.00–1.03) $P < 0.04$). Although no direct association was observed between the B*27 or B*57 alleles and the LTNP profile (Table 2), in the adjusted model these alleles were confirmed as protective factors against AIDS progression (HR 0.62 (95% CI 0.38–0.99) $P < 0.05$). The B*52 allele was found to be a protective factor against AIDS in both the bivariate (HR 0.49 (95% CI 0.27–0.90) $P < 0.03$) and multivariate models (HR 0.48 (95% CI 0.26–0.89) $P < 0.03$).

When selecting the variables to be evaluated in the multivariate model, the variable presence of the rapid progression allele (B*35) was included although the *P*-value (0.26) was > 0.20 in the bivariate analysis. However, no association between this allele and the time to AIDS progression was found using the multivariate model (Table 4). By contrast, the variable exposure category, which was considered significant using the bivariate model, was not evaluated in the adjusted model because it would exclude 12 patients, including 2 from the LTNP group. The final model fit the data properly and was in accordance with the assumptions of Cox model.

DISCUSSION

Host genetic factors have been consistently linked to variations in both susceptibility and resistance to HIV-1 infection and disease progression.[1,41–43] In a previous study, we tried to assess the possible association of CCR5 genotypes and HLA-B alleles with the susceptibility or resistance to HIV-1 infection in injecting drug users from Rio de Janeiro, Brazil, but no association was evident.[44] Here we focused on AIDS progression, evaluating the influence of the HLA-B alleles on disease outcome.

The classification of HIV-1-positive individuals based on their AIDS progression profile is not consistent across the studies, mainly because of the different clinical criteria used to distinguish the RP, TP and LTNP groups. Here we defined these three groups of AIDS progressors based on the time from the estimated date of infection to the occurrence of the first AIDS-defining event. AIDS-defining events included a CD4+ T-lymphocyte count below 350 cells mm^{-3}, AIDS-defining illness, the initiation of antiretroviral therapy or AIDS-related death. The need to apply a standardized and accepted set of clinical definitions for the purpose of disease stratification has already been emphasized.[45] As a result of the strict inclusion criteria adopted in this study to classify the HIV-positive patients across the three AIDS progression profiles, a high proportion of the individuals included in our databank could not be classified. The major obstacle to classification was the late HIV diagnosis, which is common in our country, as in many other regions.[46–49] This factor impeded the classification of a large number of individuals with a potential TP profile. Similarly, the lack of information about the last negative HIV serology limited the classification of possible RPs in our databank.

The HLA class I locus has been characterized as the main factor influencing the outcome of HIV infection.[21,27] This study presents the first evidence of a significant association between HLA-B*52 and slower progression to AIDS. This observation contradicts a previous study in which this allele was not associated with either slower or faster AIDS progression.[50] The association of the B52

antigen with resistance to HIV was first described in 1992 by Fabio *et al*.,[51] but since then there has been no other study corroborating this finding. Recently, HLA-B*52 was linked to a strong effect on viral load during early time points but had no significant effect on the long-term control of HIV-1.[38] This allele has also been described as a protective factor for pulmonary tuberculosis.[52]

We also detected other HLA-B alleles associated with slower progression or non-progression to AIDS that were less striking than that observed for the B*52 allele. In fact, a possible association between the B*48 allele and non-progression to AIDS was initially observed in this study but was not confirmed by the multivariate model results. For the HLA-B*27 and B*57 alleles, we did not find a direct association with non-progression to AIDS, but a correlation was observed based on the higher frequencies of these alleles in the LTNP group (Table 2) and the confirmation of the protective role of these alleles by the multivariate analysis (Table 4). Thus, we may have lacked the statistical power to detect this association because of the low number of individuals with an LTNP profile in our databank. The protective effect of these alleles on the different aspects of HIV-1 infection has been extensively documented in the literature,[21,24,27,36,37,50] including the potent antiviral immunity conferred by the B*27 and B*57 alleles through limiting central memory T-cell infection in LTNP individuals.[53] On the other hand, the previously reported deleterious effect of the HLA-B*35 allele on disease progression[33,40] was not observed in this study despite the higher frequency of this allele in the RP group (13.4% vs 10.0% in the TP group vs 5.9% in the LTNP group). Unlike our findings for the B*27 and B*57 alleles, the multivariate analysis did not confirm the B*35 allele as a risk factor for AIDS in our study group.

Given the high genetic diversity of the Brazilian population, which is characterized by an elevated degree of miscegenation, it is important to compare the allelic frequencies between Caucasians and non-Caucasians. With the exception of the HLA-B*42 allele, which was overrepresented in non-Caucasian individuals ($P<0.02$), all other HLA-B alleles were equally distributed across different ethnicities within the study group. The predominance of HLA-B*42 in non-Caucasians is consistent with the literature[54] and ethnicity information in the REDOME data set and in the Allele Frequency Net Database.[55]

In conclusion, despite the limitations in the classification of the HIV-1-positive individuals because of unavailable data, this study corroborates the importance of the HLA-B alleles in determining the outcome of HIV-1 infection. Here we have described for the first time the protective role of the B*52 allele in disease progression and confirmed the association of the B*27 and B*57 alleles with the LTNP profile. These results reinforce the hypothesis that HLA class I alleles are key determinants of the host immune response against HIV-1 infection and expand our knowledge of the distribution and effects of these alleles in the Brazilian population, where additional studies examining host genetic characterization in the context of infectious diseases are still needed.

MATERIALS AND METHODS

Study group

This retrospective study included HIV-1-seropositive individuals who were undergoing clinical follow-up at Evandro Chagas Clinical Research Institute (IPEC/FIOCRUZ), Rio de Janeiro, Brazil, from 1986 to 2011 ($n=4015$) and were registered in the IPEC database. The inclusion criteria for further classification according to the progression profiles were as follows: (1) the patient had been clinically followed up for at least 60 days, (2) HIV-1 infection was diagnosed before 2010 and (3) the patient was >13 years old. Clinical and laboratory data from a total of 3809 individuals that fulfilled these criteria were considered for the stratification of the HIV-1-infected individuals into the three clinical progression profiles: RP (AIDS progression within 3 years of seroconversion; $\geqslant 1$ CD4 T-cell measurement available; time between the negative serological test and the positive serological test <3 years), TP (AIDS progression >4 years after seroconversion; $\geqslant 2$ CD4 T-cell measurements >350 cells mm^{-3} before AIDS event) and LTNP (asymptomatic HIV-1 infection for >10 year after seroconversion; all CD4 T-cell measurements >500 cells mm^{-3}). Biological material was available in the laboratory biorepository for 218 individuals. These samples were used for genetic characterization and subsequent statistical analyses.

For the RP group, the dates of the last seronegative test and the first seropositive test were used to calculate the estimated date of infection (defined as the midpoint of these two dates with a maximum interval of 36 months). For the other groups, when the date of the last seronegative test was not available, the estimated date of infection was calculated as 6 months before the date of the first seropositive test. Time to disease progression was defined as the time elapsed (in days, after conversion to years) between the estimated date of infection and the first AIDS-defining event. An AIDS-defining event included any one of the following: CD4+ T-lymphocyte count below 350 cells mm^{-3}, AIDS-defining illness (according to the Brazilian Ministry of Health Guidelines),[56] initiation of antiretroviral therapy or AIDS-related death.

Based on the criteria used to estimate the seroconversion date, we were not able to precisely classify individuals who progressed to AIDS within 3 to 4 years of infection because these individuals were in the threshold between the RP and TP groups after applying the estimated seroconversion criteria. The present work was approved by the FIOCRUZ Ethical Research Committee as an anonymous unlinked study (Ethics Committee CAE: 0002.0.009.000-08).

DNA extraction

DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All DNA samples were stored at $-20\,^{\circ}$C until the genomic analyses.

HLA-B typing

The HLA-B typing was performed using a combination of two commercial kits: the INNO LiPA HLA-B Multiplex Plus kit and the INNO LiPA HLA-B Update Plus kit (INNOGENETICS, Ghent, Belgium). The first kit was used to PCR amplify a fragment between the second and fourth exons of the HLA-B locus according to the manufacturer's protocol. After this step, the PCR products were submitted to a hybridization assay using the second kit, which is based on the line probe assay methodology. The identification of the HLA-B alleles was performed using the LiRAS interpretation software, LiPA HLA v6.00 (INNOGENETICS).

Statistical analyses

Sociodemographic characteristics of the subgroups of individuals were tested by the χ^2 test, Fisher's exact test or Mann–Whitney test (the most appropriated in each situation). The HLA-B allele frequencies and genotypes were estimated using the PyPop package.[57] Deviations from Hardy–Weinberg equilibrium were calculated using the method of Guo and Thompson,[58] which was also performed using PyPop. The frequencies of each HLA-B allele were compared (1) among the three clinical progression groups and (2) between HIV-1-positive individuals and the genomic profile from the Brazilian National Registry of Bone Marrow Donors (REDOME) released in March 2013, which represents a reliable and representative sample of the Brazilian population, with almost 3 million registered donors (www.imunogenetica.org). For all comparisons, a χ^2 test or Fisher's exact test was performed using the statistics program Epi Info Version 6.[59]

The Kaplan–Meier method and Cox proportional hazards models were used to evaluate the elapsed time from the first HIV-1-positive serology to the progression to AIDS for individuals who could be categorized into one of the three progression groups and for whom HLA-B data were available. Survival curves were described by the Kaplan–Meier estimator, and the categories of each analyzed variable were compared through a log-rank test. For the evaluation of the factors associated with AIDS progression, variables with a significant effect on the HR and an occurrence of the event $<20\%$ (based on the Wald test) were evaluated in the model. The models were compared using the likelihood ratio rest (analysis of variance). The assumptions of the Cox models and the quality of the adjustment were made, respectively, based on the residuals and coefficient of determination, as well as the likelihood of agreement, to evaluate the discriminatory power and predictive accuracy of the model. All tests were considered

significant if the *P*-value was <0.05. R software version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria) with the Survival library was used to run these analyses.[60]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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