The influence of human leukocyte antigen class I loci -A, -B, -C, and class II HLA-DRB1 alleles in a Brazilian HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) population and association with disease outcome and proviral load

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Abstract

Background

Around ten million people are infected with HTLV-1 worldwide, and 1–4% develop HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), characterized by an important degeneration of the spinal cord, that can lead to death. Distinct HLA alleles have been associated with either HAM/TSP susceptibility or protection. However, these HLA alleles set may change according to the population studied. Brazil is the second country in the number of HTLV-1 infected people and there are few reports addressing the HLA influence on HTLV-1 infection as well as on disease outcome.

Results

The objective of this study was to evaluate the influence of HLA alleles as a risk factor for HAM/TSP and the proviral load (PVL) levels, clinical progression, and death outcomes in an admixed Brazilian population. The HLA-A, -B, -C, and -DRB1 were genotyped in 375 HTLV-1-infected individuals divided into asymptomatic carriers (AC) (n = 165) and HAM/TSP (n = 210) in a longitudinal cohort from eight to 22 years of follow-up. The alleles HLA-A*68 and -C*07 were related to HAM/TSP risk in multivariate analysis. The alleles HLA-A*33, and -A*36 were associated with protection against disease progression in HAM/TSP patients, while HLA-B*37, -C*12, -C*14, and -DRB1*08 were associated with increased risk of death. In the AC group, the presence of HLA-B*45, -B*47, -B*58, -C*06 and -DRB1*15 alleles influenced an increased PVL, in an adjusted linear regression model, while -A*30, -A*34, -B*40, -C*06, -C*17 and -DRB1*09 alleles were associated with increased PVL in HAM/TSP group compared to HAM/TSP individuals not carrying these alleles. All these alleles were also related to increased PVL associated with clinical progression outcome. Increased PVL associated with the death outcome was linked to the presence of HLA-A*30.

Conclusions

PVL has been associated with HLA, and several alleles were related in AC and HAM/TSP patients with or without interacting with clinical progression outcomes. Understanding the prognostic value of HLA in HAM/TSP pathogenesis can provide important biomarkers tools to improve clinical management and contribute to the discovery of new therapeutic interventions.

Background

The human T cell lymphotropic virus (HTLV-1) is a retrovirus associated with two primary diseases: the adult T cell leukemia/lymphoma (ATLL) [1] and the HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [2, 3]. Approximately 5 to 10 million people are infected with HTLV-1 worldwide [4]. Japan and Brazil are countries with the highest estimated total numbers of HTLV1infected people [5].
The HTLV-1 prevalence in Brazil ranges from 146.3 to 390.2/100,000 inhabitants [4]. Although most infected individuals remain asymptomatic, around 0.25–3.8% develop HAM/TSP over time [6]. This neurological disease is characterized as a slowly progressing spinal cord disease. The leg muscles gradually become weak. The limbs are stiff, the movements are slow, and walking becomes more difficult. Muscle spasms in the legs are common. Other HTLV-1-associated diseases, such as neurogenic bladder [7] and ocular manifestations [8], can also be present among HTLV-1-positive individuals. These clinical outcomes are associated with increased HTLV-1 proviral loads (PVL) [9]; however, it is still unclear what determines the high PVL in these patients. The feature that only a small percentage of HTLV-1 carriers will develop some symptoms has not yet been fully elucidated.

The neurological injury in HAM/TSP may be a consequence of an inflammatory reaction triggered by the recognition of infected cells by cytotoxic T lymphocytes, followed by the release of cytokines and damage to the central nervous system. Factors related to the HTLV-1/host interaction may be involved in the risk of developing HAM/TSP [9].

In the search for a biomarker that can identify who is more prone to develop the disease, depending on the population's geographic distribution, some studies have shown that HLA alleles can influence the course of the infection.

The HLA alleles A*31, B*07, B*54, C*07, DRB1*01 and DQB1*05 were described to be related to a higher risk of developing HAM/TSP in Japan [10–14]. DRB1*01, in the absence of A*02 and C*08, was related to a higher risk of developing HAM/TSP in the Iranian population [15, 16]. In addition, B*07 and DRB1*01 were related to an increased risk of developing HAM/TSP in Spain, where the majority of the individuals studied were from Latin America [17]. In South America, the allele B*35 was associated with a higher risk of developing HAM/TSP in Argentina [18]. On the other hand, the HLA-A*02, -C*08, -B*40, -DRB1*15, and -DQB1*06 were related to protection in Japan [11–13]. However, in Iran, Caribbean, Peruvian, and Spain populations, the same HLA alleles were unrelated to HAM/TSP susceptibility or protection [15–17, 19–22]. Few studies were conducted in Brazil, enrolling few HAM/TSP patients, without correlation with clinical progression and evaluating specific HLA alleles [23, 24].

Our objective was to describe the genetic polymorphism in the HLA-A, -B, -C, and -DRB1 loci in a subset of the Brazilian population infected with HTLV-1 in a longitudinal follow-up cohort at a Reference Center for Infectious Diseases in Rio de Janeiro, Brazil.

**Results**

**Patients and clinical data.**

Table 1 contains demographic and clinical data. Three hundred and seventy-five individuals infected with HTLV-1 were randomly selected from the HTLV-1 cohort, corresponding to 41.7% of all HTLV-1 infected individuals followed as outpatients at the INI/Fiocruz. The enrolled participants lived predominantly in the metropolitan area of Rio de Janeiro City. They were divided into HAM/TSP (n=210) and AC (n=165)
subgroups. The mean age of the HAM/TSP group (60.9±11.8 years) was slightly greater than the AC group (57.4±15.9 years, \(p=0.01\)). The mean clinical follow-up time was 13.1±6.31 and 15.4±7.40 years for HAM/TSP and AC, respectively. The distribution of gender and ethnicity (a self-identified skin color, described as white or non-white (black and mixed)) was similar in both groups. Also, the HAM/TSP group presented a higher frequency of other clinical manifestations such as the neurogenic bladder (79.5%) and ophthalmic alterations (10.5% of the patients) compared to the AC group, \(p<0.01\). PVL was higher in the HAM/TSP group compared to the AC group (p>0.01).
Table 1. Demographic and Clinical data in HAM/TSP and AC Brazilian patients.

<table>
<thead>
<tr>
<th></th>
<th>HAM-TSP (n=210)</th>
<th>AC (n=165)</th>
<th>OR (95%CI)/mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>132 (62.9)</td>
<td>90 (54.5)</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>78 (37.1)</td>
<td>75 (45.5)</td>
<td>0.71 (0.47-1.07)</td>
</tr>
<tr>
<td><strong>Age (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (±sd)</td>
<td>60.9 (11.8)</td>
<td>57.4 (15.9)</td>
<td>1.02 (1-1.03)</td>
</tr>
<tr>
<td><strong>Ethnicity (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>103 (49.0)</td>
<td>77 (46.7)</td>
<td>-</td>
</tr>
<tr>
<td>Non-white</td>
<td>106 (50.5)</td>
<td>88 (53.3)</td>
<td>0.9 (0.6-1.36)</td>
</tr>
<tr>
<td>N/D</td>
<td>1 (0.5)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical follow-up (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (sd)</td>
<td>13.1 (6.31)</td>
<td>15.4 (7.40)</td>
<td>0.95 (0.92-0.98)</td>
</tr>
<tr>
<td><strong>Clinical progression (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34 (16.2)</td>
<td>152 (92.2)</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>148 (70.5)</td>
<td>07 (4.2)</td>
<td>60.15 (29.38-123.15)</td>
</tr>
<tr>
<td>N/D</td>
<td>28 (13.3)</td>
<td>6 (3.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical INI grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (±sd)</td>
<td>19.7 (9.48)</td>
<td>0.906 (2.80)</td>
<td>1.67 (1.49-1.87)</td>
</tr>
<tr>
<td><strong>Neurogenic bladder (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41 (19.5)</td>
<td>154 (93.3)</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>167 (79.5)</td>
<td>11 (6.7)</td>
<td>57.02 (28.3-114.9)</td>
</tr>
<tr>
<td>N/D</td>
<td>2 (1.0)</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
### Ophthalmic alterations (n, %)

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>8.98 (3.48-23.17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>188 (89.5)</td>
<td>22 (10.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 (97)</td>
<td>5 (3)</td>
<td></td>
</tr>
</tbody>
</table>

### Proviral load

<table>
<thead>
<tr>
<th></th>
<th>mean (±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>8.36 (6.80)</td>
</tr>
<tr>
<td>HAM</td>
<td>4.17 (5.64)</td>
</tr>
<tr>
<td>N/D</td>
<td>1.15 (1.09-1.22)</td>
</tr>
</tbody>
</table>

### Death outcome (n, %)

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>47.81 (2.88-793.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>164 (78.1)</td>
<td>24(11.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 (97.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>N/D</td>
<td>22 (10.5)</td>
<td>5 (3.0)</td>
<td></td>
</tr>
</tbody>
</table>

AC= asymptomatic control, HAM-TSP= Human T cell lymphotropic virus-associated myelopathy/tropical spastic paraparesis, N/D=not determined, OR=odds ratio and 95% confidence interval. $^\$missing data (N/D) were not considered for OR calculation and statistical analysis.

During the clinical follow-up, 24 (11.4%) patients died from secondary causes related to HTLV-1 infection, such as recurrent urinary infections, bedsores, and sepsis. All of these individuals were HAM/TSP patients. The clinical progression was investigated during the follow-up, and 148 (70.5%) HAM/TSP and only seven (4.2%) AC patients, showed a deteriorating outcome ($p\!<\!0.01$). The clinical follow-up in the AC progressors (v.g., from AC to HAM/TSP) subgroup ranged from three to 25 years (17.86±8.51 years).

Figure 1 shows the clinical progression throughout two to 27 years (14,20+/-6,84 years) of follow-up in the HAM/TSP progressors subgroup. The clinical progression occurred more rapidly according to the severity of the patient’s condition. Patients with the worse disability, such as those with bilateral walking difficulties (HAM/TSP Bilateral-Progressors), progressed more quickly than those with unilateral walking difficulties (HAM/TSP Unilateral-Progressors) or those able to walk without support (HAM/TSP walking).

A Log-Rank Mantel-Cox test showed a statistically significant difference between subgroups ($p\!<\!0.0001$). The difference in clinical evolution between those individuals with HAM/TSP with unilateral support and bilateral support was significant ($p\!=\!0.0462$); however, there was no difference in clinical development between individuals who needed unilateral support and those with an independent gait.

### HLA genotyping

The frequencies of the HLA -A, -B, -C, and -DRB1 alleles in the studied population were compatible with those found at the Brazilian Bone Marrow Volunteer Donor Registry (REDOME), except for the HLA-A*68 allele, with a frequency of 10.37% in the HTLV-1 infected patients versus 6.14% in the REDOME [29].
difference was mainly in the HAM/TSP patients (13.29%). Supplemental Table 1 described all allelic frequencies found in our studied population. The locus B is in linkage disequilibrium for both HAM/TSP and AC groups, \( p < 0.0000 \) and \( p < 0.0003 \), respectively. In contrast, all the other loci are in Hardy-Weinberg equilibrium for both groups. After univariate and multivariate analysis, alleles HLA-A*68 and -C*07 showed a higher frequency in HAM/TSP group than in the AC group, even after being adjusted by age, gender, ethnicity, and time of clinical follow-up (Table 2, OR 2.03 (1.19-3.46, 95%CI), \( p = 0.01 \), and OR 1.61 (1.04-2.48, 95%CI), \( p = 0.03 \), respectively), and were associated to HAM/TSP risk. As HLA-C*07 in the absence of -A*02 has been previously described as a risk factor for HAM/TSP [24], we tested its association in the absence of the -A*02 allele. The contingency table analysis showed that the presence or absence of the -A*02 did not influence the -C*07 allele distribution in both groups.

Due to the small number of affected patients with neurogenic bladder and ophthalmological manifestations was not possible to correlate these data with HLA genotyping.

Table 2. HLA allele's frequency in HAM/TSP and AC Brazilian patients.

<table>
<thead>
<tr>
<th>HLA Alleles (%)</th>
<th>HAM-TSP (n=210)</th>
<th>AC (n=165)</th>
<th>OR (95%CI)</th>
<th>P-value</th>
<th>OR-Adjusted (95%CI)*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>-A*68</td>
<td>55 (13.3)</td>
<td>23 (7.3)</td>
<td>1.96 (1.17-3.26)</td>
<td>0.01</td>
<td>2.03 (1.19-3.46)</td>
<td>0.01</td>
</tr>
<tr>
<td>-C*07</td>
<td>63 (22.8)</td>
<td>47 (16.1)</td>
<td>1.54 (1.01-2.35)</td>
<td>0.04</td>
<td>1.61 (1.04-2.48)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

AC= asymptomatic control, HAM/TSP= Human T cell lymphotropic virus-associated myelopathy/tropical spastic paraparesis, OR= odds ratio 95% confidence interval, * \( p \) values adjusted by age, sex, ethnicity and time of clinical follow-up.

HLA genotypes and clinical outcomes

Logistic regression was performed to analyze whether HLA-A, -B, -C, and -DRB1 alleles were associated with different clinical progression outcomes in HAM/TSP patients. Only seven AC patients progressed to HAM/TSP during the clinical follow-up (17.86±8.51 years), while 148 patients in HAM/TSP group showed clinical progression. Therefore, we only analyzed the association between HLA and disease progression in the HAM/TSP group (Table 3). The alleles -A*33 (OR 0.28 (0.09-0.91 95%CI), \( p = 0.03 \)) and -A*36 (OR 0.12 (0.02-0.9 95%CI), \( p = 0.04 \)) were associated with protection against disease progression in the adjusted model. Regardless of whether -B*50 was associated with disease progression in the HAM/TSP group (\( p = 0.04 \)), there was no association when data were adjusted for age, sex, ethnicity, and length of clinical follow-up (Supplemental Table 2). Logistic regression was also performed to verify HLA alleles' association with the outcome of death in the HAM/TSP group. The alleles -B*37 (OR 7.12 (1.48-34.22, CI95%), \( p = 0.01 \)), -C*12 (OR 6.25 (1.71-22.8, CI95%), \( p = 0.01 \)), and -C*14 (OR 8.85 (1.68-46.67, CI95%), \( p = 0.01 \)) were associated with death in HAM/TSP patients in the adjusted model (Table 4).
Table 3. HLA allele’s frequency in HAM/TSP associated with disease progression.

<table>
<thead>
<tr>
<th>Progression</th>
<th>No (n=34)</th>
<th>Yes (n=148)</th>
<th>OR (95%CI)</th>
<th>P-value</th>
<th>OR-Adjusted (95%CI)*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alelles n</td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-A*33</td>
<td>6 (9)</td>
<td>7 (2.4)</td>
<td>0.25 (0.08-0.78)</td>
<td>0.02</td>
<td>0.28 (0.09-0.91)</td>
<td>0.03</td>
</tr>
<tr>
<td>-A*36</td>
<td>2 (3)</td>
<td>2 (0.7)</td>
<td>0.23 (0.03-1.64)</td>
<td>0.14</td>
<td>0.12 (0.02-0.9)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

AC= asymptomatic control, HAM/TSP= Human T cell lymphotropic virus-associated myelopathy/tropical spastic paraparesis, OR= odds ratio 95% confidence interval, * p values adjusted by age, sex, ethnicity and time of clinical follow-up.

Table 4. HLA allele’s frequency in HAM/TSP associated with death.

<table>
<thead>
<tr>
<th>Death</th>
<th>No (n=164)</th>
<th>Yes (n=24)</th>
<th>OR (95%CI)</th>
<th>P-value</th>
<th>OR-Adjusted (95%CI)*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alelles n</td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-B*37</td>
<td>7 (2.8)</td>
<td>3 (9.7)</td>
<td>3.75 (0.92-15.33)</td>
<td>0.07</td>
<td>7.12 (1.48-34.32)</td>
<td>0.01</td>
</tr>
<tr>
<td>-C*12</td>
<td>11 (4.5)</td>
<td>5 (15.6)</td>
<td>3.92 (1.27-12.14)</td>
<td>0.02</td>
<td>6.25 (1.71-22.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>-C*14</td>
<td>4 (1.6)</td>
<td>4 (12.5)</td>
<td>8.57 (2.03-36.18)</td>
<td>&lt;0.01</td>
<td>8.85 (1.68-46.67)</td>
<td>0.01</td>
</tr>
<tr>
<td>-DRB1*08</td>
<td>9 (3.6)</td>
<td>3 (16.7)</td>
<td>5.36 (1.31-21.87)</td>
<td>0.02</td>
<td>9.1 (1.72-48.04)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

AC= asymptomatic control, HAM/TSP= Human T cell lymphotropic virus-associated myelopathy/tropical spastic paraparesis, OR= odds ratio 95% confidence interval, * p values adjusted by age, sex, ethnicity and time of clinical follow-up.

**HLA genotypes, PVL, and its association with clinical progression outcome**

Several HLA alleles have been associated with PVL in Japan [11,30] but not in other populations. We analyzed whether HLA alleles in our population alter the PVL. We performed an independent case and
control analysis because PVL was higher in the HAM/TSP group compared to the AC group (Table 1). Table 5 describes, through linear regression analysis, the role of the HLA alleles in the PVL in AC patients. Individuals in this group, who carry the alleles -B*45 ($\beta$ 12.808 (1.573;24.044, 95%CI) $p$= 0.026), -B*47 ($\beta$ 16.955 (5.794;28.116, 95%CI), $p$=0.003), -B*58 ($\beta$ 3.943 (0.564;7.322, 95%CI), $p$=0.022), -C*06 ($\beta$ 3.904 (1.016;6.792, 95%CI), $p$=0.008) and -DRB1*15 ($\beta$ 3.381 (0.741;6.021, 95%CI), $p$=0.012) had an increased PVL compared to those who do not carry those alleles ($\beta$, 95%CI as showed in Table 5), after adjustment. In the univariate analysis, carriers of the alleles -A*30 and -A*66 also presented higher PVL (Table 5). However, after adjusting the multivariate analysis, there was no difference between carriers and non-carriers in the PVL related to these alleles.
Table 5. Linear regression analysis of proviral load and HLA-A, -B, -C and -DRB1 loci in AC Brazilian patients.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Status</th>
<th>( \beta ) (95%CI)</th>
<th>P-value</th>
<th>( \beta ) (95%CI), adjusted*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>-A*30</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Allele present</td>
<td>3.128 (0.247;6.009)</td>
<td>0.033</td>
<td>2.56 (-0.323;5.443)</td>
<td>0.081</td>
</tr>
<tr>
<td>-A*66</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Allele present</td>
<td>4.772 (0.167;9.378)</td>
<td>0.042</td>
<td>3.826 (-0.774;8.426)</td>
<td>0.103</td>
</tr>
<tr>
<td>-B*45</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Allele present</td>
<td>13.32 (2.03;24.611)</td>
<td>0.021</td>
<td>12.808 (1.573;24.044)</td>
<td>0.026</td>
</tr>
<tr>
<td>-B*47</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Allele present</td>
<td>17.378 (6.183;28.573)</td>
<td>0.002</td>
<td>16.955 (5.794;28.116)</td>
<td>0.003</td>
</tr>
<tr>
<td>-B*58</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Allele present</td>
<td>4.2 (0.864;7.536)</td>
<td>0.014</td>
<td>3.943 (0.564;7.322)</td>
<td>0.022</td>
</tr>
<tr>
<td>-C*06</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Allele present</td>
<td>3.912 (1.019;6.805)</td>
<td>0.008</td>
<td>3.904 (1.016;6.792)</td>
<td>0.008</td>
</tr>
<tr>
<td>-DRB1*15</td>
<td>Allele absent</td>
<td>-</td>
<td>-</td>
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<td></td>
<td>Allele present</td>
<td>3.407 (0.773;6.041)</td>
<td>0.011</td>
<td>3.381 (0.741;6.021)</td>
<td>0.012</td>
</tr>
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</table>
AC= asymptomatic control, HAM/TSP= Human T cell lymphotropic virus-associated myelopathy/tropical spastic paraparesis, *p values adjusted by age, sex, ethnicity and time of clinical follow-up clinical follow-up, 95% CI= confidence interval.

In the HAM/TSP group, individuals who carry the A*30 (β 3.033 (0.252;5.8130, 95%CI), p=0.033), -A*34 (β 9.172 (2.755;15.588, 95%CI), p=0.005), B*40 (β 5.13 (0.831;9.429, 95%CI), p=0.02), -C*06 (β 4.016 (0.883;7.15, 95%CI), p=0.012), -C*17 (β 7.048 (0.696;13.399, 95%CI), p=0.03) and -DRB1*09 (β 6.874 (2.341;11.408, 95%CI), p=0.003) alleles had an increased PVL compared to those who do not carry those alleles (β, 95%IC as showed in Table 6). Indeed, these alleles were also related to an increased PVL and a worse clinical progression (Table 6). On the other hand, only the allele -A*30 (β 24.634 (11.282;37.985, 95%CI) was associated with a higher PVL and death (Table 6). Due to the low frequency of -DR*09 carries, it was not possible to calculate the β value in the multivariate model. None of the alleles -A*34, -B*40, -C*06, -C*17, and –DRB1*09 were associated with both death outcome and the PVL levels in HAM/TSP patients.

Discussion

Brazil is a country with continental proportions and endemic to HTLV-1. Its population is composed of miscegenation among individuals of Indigenous, African, Asia, and European origin. Some studies have associated HLA genetic polymorphism with protection or susceptibility to HAM/TSP development. This study evaluated the influence of HLA class I and II gene polymorphism in HTLV-1 infected individuals in a longitudinal follow-up and its association with the PVL levels, clinical progression, and death outcomes. AC and HAM/TSP patients were accompanied for two to a maximum of 27 years, and clinical progression was related to HAM/TSP patients. The lifetime risk of HAM/TSP is different among ethnic groups [31]. Some studies report that it can take up to 21 years from the onset of infection to using a wheelchair in HAM/TSP [32]. Our study could not specify the median time patients became wheelchair users because the walking condition was only assessed in the first and the last clinical consultation. However, HAM/TSP patients that presented a clinical progression were in the cohort for at least eight years. Regarding mortality in HAM/TSP patients, it was pointed out that it would be a consequence of complications of the disease itself and co-infections with HCV/HIV [33].

In our cohort, PVL and neurogenic bladder were significantly higher in HAM/TSP than in the AC group, reinforcing what has already been published [28, 34]. The close relationship between HTLV-1 infection and uveitis has been identified concerning the ophthalmologic alterations, indicating that HTLV-1 is also highly associated with intraocular inflammatory disorder [35, 36]. Some HLA alleles have been related to uveitis. The HLA-A*29 is strongly associated with Birdshot uveitis in individuals of Western-European ancestry, while HLA-B*27 is associated with uveitis, particularly in ankylosing spondylitis [37, 38]. In our cohort, ophthalmic manifestations were more frequent in the HAM/TSP group, but it was not possible to correlate it with any HLA due to the small number of affected patients.

Only two studies with small sample sizes were carried out in Brazil to investigate the HLA association with HTLV-1 infection [23, 24]. Borducchi et al. [23] analyzed a small number of HTLV-1 infected patients.
(n = 71), including AC, ATLL, and HAM/TSP. No statistical difference was determined among these groups because of the small sample size. This study showed a tendency to a lower HLA-A2 frequency in HAM/TSP white patients and an association of HLA-DR11 with HAM/TSP only in the Mestizo patients, in disagreement with our study even after adjustment by ethnicity. Unfortunately, that report did not mention the number of HAM/TSP patients and did not compare their results using multivariable analysis. In another study \[^{24}\] , only alleles previously associated with protection and disease risk were tested in a small cohort of 84 AC and 9 HAM/TSP patients. They identified that the alleles HLA-C*07 in the absence of -A*02 was associated with an increased risk of disease, and alleles C*08 and B*07 had no association with the development of HAM/TSP. In our studied population, the presence or absence of A*02 did not influence the C*07 allele, and B*07 and C*08 also showed no association with the disease.

In our study, the most frequent allele observed at locus A was A*02 (19.02%, Supplementary Table 1). Still, unlike in the Japanese studies \[^{11, 12}\] , neither A*02 nor the HLA-C*08 alleles were associated with the protection or increased risk for HAM/TSP.

Our results showed that the alleles -A*68, -C*07, and -DRB1*10 had a higher frequency among HAM/TSP patients than in the AC individuals, leading to an increased risk of HAM/TSP. To our knowledge, no previous studies indicated the association of -A*68 and -DRB1*10 with HAM/TSP. However, we should take into account that the -A*68 allele had a higher frequency in our study cohort when compared to the frequency of this allele in the Brazilian general population \[^{29}\] . Indeed, in an HIV-positive African population, the allele -A*68 was associated with the rapid transmission of HIV in serum discordant heterosexual partners \[^{39}\] . The -C*07 allele was cited in the Japanese population as a risk factor for HAM/TSP \[^{13}\] , as well as in our study. Interestingly, in an Argentine population, -C*07 was associated with an increased risk of ATLL but not with HAM/TSP \[^{18}\] . This allele was underrepresented in AIDS Brazilian patients conferring protection against cytomegalovirus retinitis \[^{40}\] .

This is the first study that analyzed whether HLA alleles could influence clinical progression in a longitudinal cohort. Patients were clinically followed for 20 years, and their walking clinical progression condition was determined. In our cohort -B*58, -C*07, and -DRB1*07 were associated with clinical progression. The -B*58 and -DRB1*07 alleles influenced risk and protection to clinical progression, respectively, but lost significance when adjusted for demographic factors. HLA-C*07 increased the risk of developing HAM/TSP and influenced the clinical progression condition. The role of this allele needed to be clarified in other populations and concerning the physiopathological properties.

There is a clear association between HLA alleles and the PVL levels \[^{17–19}\] . We identified new alleles that were associated with increased PVL in the AC group, carriers of the alleles -B*45, -B*58, -C*06, and -DRB1*15, and in the HAM/TSP group, carriers of the alleles -A*30, -A*34, -B*40, -C*06, -C*17 and -DRB1*09. Although the presence of these alleles influenced an increased PVL, among these alleles, only B*40 was cited in a Japanese population study \[^{13}\] , where its distribution was associated with a protective effect.
Further, we analyzed if the presence of the HLA alleles were responsible for the increase in PVL regarding the clinical progression outcome.

In the AC group, carriers of the alleles -B*47, -B*58, -C*06, and -DRB1*15 had an increased PVL and a higher risk of clinical progression. In the HAM/TSP group, the alleles -A*30, -A*34, -B*40, -C*06, -C*17, and -DRB1*09 also influenced PVL and risk for clinical progression. Intriguingly, the alleles that influence PVL and clinical progression in the AC group are not the same as in the HAM/TSP group. Among these alleles, the only correlation with other studies we found in the -DRB1*15 allele, which in the Japanese study had a protective effect [13] and is cited with a tendency to a higher frequency in HAM/TSP patients than in HCs in a French Afro-Caribbean population [22], but without association with PVL. It is the first time these associations are described in the Brazilian population.

Moreover, we can highlight that the presence of the HLA-C*06 allele is associated with PVL, clinical progression, and death outcomes for the first time in our population. Studies carried out in ethnically different populations have not demonstrated equanimity in their results regarding risk or protection for the development of HAM/TSP. The host's genetic background must have an important role in this issue.

Regardless of the originality of this work with associations of HLA and HTLV-1 infection and clinical progression in a longitudinal cohort follow-up, it should point to some study limitations. The majority of the individuals included in this cohort were natural from Rio de Janeiro and could not reflect the genetic diversity of the Brazilian population. In addition, despite our study having recruited a higher number of participants than others made in the Brazilian population, the inclusion of more individuals could increase the strength of the comparisons between some HLAs regarding their role in HTLV-1 infection.

The HLA system is vital in response to infections. In the case of HTLV-1 infection, where a minority of individuals carrying the virus will develop the neurological disease, the genetic determinants could be seen as an interplay between different immunological events. However, more studies should be conducted to monitor the HLA profile in AC patients at risk of developing the disease in longitudinal follow-up.

Conclusions

In this study, we investigated the influence of HLA polymorphisms on the susceptibility for the development of HAM/TSP and clinical progression in a longitudinal cohort of an admixed Brazilian population. We found that HLA-A*68 and -C*07 carriers presented augmented risk for HAM/TSP development in HTLV-1 infected individuals. In addition, HLA-B*37, -C*12, -C*14, and -DRB1*08 were associated with an augmented risk of death in HAM/TSP individuals. In contrast, the alleles HLA-A*33, and -A*36 were related to protection against disease progression in these individuals. We also showed that asymptomatic HLA-B*45, -B*47, -B*58, -C*06 and -DRB1*15 carriers have increased proviral load, while -A*30, -A*34, -B*40, -C*06, -C*17 and -DRB1*09 alleles were associated with this characteristic on HAM/TSP patients. We also demonstrated that patients with high PVL and carrying these alleles presented disease clinical progression, while the presence of HLA-A*30 in these patients was associated
with death outcome. This is the first study to evaluate the influence of HLA alleles on the risk for HAM/TSP development as well as in clinical progression and death, in an admixed Brazilian population. Brazil has one of the highest rates of HTLV-1 infection, and studies addressing genetic factors associated with HAM/TSP risk could prevent disease worsening through earlier medical care.

**Methods**

**Patients and samples**

HTLV-1 positive patients were enrolled from the longitudinal cohort of the Laboratory for Clinical Research in Neuroinfections at the Evandro Chagas National Institute of Infectious Diseases, FIOCRUZ (INI/Fiocruz), Rio de Janeiro, RJ, Brazil. The Research Ethics Committee of the INI/FIOCRUZ (CAAE-0012.0.009.000-08) approved this study, and all subjects provided written informed consent. Volunteers were consecutively followed from 1999 to 2020 in a longitudinal cohort from eight to 22 years. A case-control study was designed to identify HLA's influence on the disease disability status, PVL, disease progression, and death outcomes. A comprehensive medical history relating to neurological illnesses was taken at the time of inclusion in the cohort and annually for the AC patients and biannually for the HAM/TSP patients.

Clinical classification inclusion criteria followed the World Health Organization guidelines for HAM/TSP [25]. Patients’ inclusion criteria were defined by positive serology and divided into two groups: individuals with HAM/TSP and HTLV-1 asymptomatic carriers (AC). The HAM/TSP participants were classified according to their disability status using the INI clinical disability scale as described elsewhere [26].

Clinical progression outcome was defined by loss in patients’ ability to walk during the follow-up. Clinical progression was determined by comparing patients’ performance at the first clinical visit, when patients were included in the cohort, with the last clinical visit. A survival curve was generated to analyze the longitudinal clinical progression in HAM/TSP patients, using each of their clinical classifications regarding the ability to walk (walk without assistance, with unilateral or bilateral support, and the need for a wheelchair).

**HLA genotyping**

Peripheral blood (5 mL) was collected in EDTA tubes from all individuals, and DNA was extracted using the Puregene commercial kit (Gentra Systems Inc., Minneapolis, MN, USA), following the manufacturer's instructions.

First, the HTLV-1 cohort was HLA genotyped through an in-house PCR-SSP (polymerase chain reaction-sequence-specific primer) from 1999 to 2007 (n=273 and throughout PCR-SSO (polymerase chain reaction with specific oligonucleotide probes) technique (n=102).
The HLA class I loci (-A, -B, and -C) genotyping was performed in an in-house PCR-SSP according to the methodology established by the Organ Transplant Laboratory from Oxford University [27]. Primers were donated by the Department of Immunology at the St Mary’s School of Medicine, Imperial College, London, UK; and ii) PCR-SSO technique, using DNA LABType® trading system One Lambda Inc., Canoga Park, CA, USA was performed, according to manufacturer’s instructions, that provides SSO probes for the sequence-specific oligonucleotide linked to fluorescent microspheres for the identification of HLA alleles in genomic DNA samples.

**Proviral load quantification**

The HTLV-1 PVL DNA was measured by real-time PCR assay (SmartCycle II; Cepheid) using the TaqMan system (Applied Biosystems) to amplify a 159bp fragment of tax gene. A standard curve was generated using the β-globin gene as a reference. DNA from the TARL-2 cell line, which contains a single copy of the provirus HTLV-1, was used to establish the standard curve for tax gene quantification. The proviral load (PVL) in \(10^4\) cells was calculated by the following equation: \([(\text{copy number of tax gene})/ (\text{copy number of β-globin gene}/2)] \times 100\)[28].

**Statistical Analysis**

Descriptive analyses of clinical and demographic characteristics were performed. Outcomes (clinical status – HAM/TSP or AC, disease progression, and death) and alleles (HLA-A, -B, -C, and -DR) were associated using absolute and relative frequencies or mean and standard deviation. Hardy-Weinberg equilibrium was calculated by Exact test using a Markov chain to define the equilibrium in the allelic distribution in HLA-A, -B, -C, and -DR locus between HAM/TSP and AC patients. Prism 7 was used to determine a survival curve to analyze the walk ability’s clinical progression. The Mann-Whitney U test evaluated quantitative variables, whereas the univariate Odds Ratio (OR) was used for categorical variables. We used a multivariate generalized linear Binomial model to estimate OR and Ci=Is. Linear models were tested to estimate the average difference in PVL among alleles and outcomes. A \(p\)-value < 0.05 was considered significant. All analysis was performed using R software version 3.6.3.

**Declarations**

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the INI/FIOCRUZ (CAAE-0012.0.009.000-08) and all participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

DS, LCP and APV performed the HLA typing experiments; AQCA recruited the patients and collected the clinical data; DS, LCP, MGBA and EHR designed the study; JCA performed the statistical analysis; DS, LCP, EHR and MGBA analyzed the data and interpreted the results; DS, LCP, EHR, JCA, AQCA and MGBA contributed to the writing of the manuscript. All authors contributed critically to the manuscript conception. All authors read and approved the final manuscript.

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Not applicable

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Tables

Table 6 is available in the Supplementary Files section.

Figures
Longitudinal clinical progression in HAM/TSP patients related to walking condition ability. A log-Rank Mantel-Cox test evaluated the statistical difference between HAM/TSP Progressors subgroups. The difference between the HAM/TSP Unilateral-Progressor versus Bilateral-Progressor groups was statistically significant ($p=0.0462$). The comparison of clinical progression between the HAM/TSP walking and the bilateral walking condition group was also statistically significant ($p<0.0001$). No difference was observed when comparing HAM/TSP walking versus HAM/TSP Unilateral-Progressors patients.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table6Schoretal.xlsx
- SupplementaryTablesSchoretal.docx