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Absence of consistent association between human leukocyte antigen-I and -II alleles and human T-lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis risk in an HTLV-1 French Afro-Caribbean population

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ABSTRACT

Objectives: Human T-cell lymphotropic virus type 1 (HTLV-1) infection leads to the risk of developing HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in less than 5% of cases. The mechanism of disease progression in HAM/TSP remains unknown. A significant role of certain human leukocyte antigen (HLA) genotypes in determining the risk of HAM/TSP has been reported in Japan, where the HLA-A*02 gene has been found to be associated with a lower HTLV-1 provirus load and with protection from HAM/TSP, whereas HLA-DRB1*0101 has been found to be associated with an increased susceptibility to HAM/TSP. The aim of the present case–control study was to investigate the HLA class I and class II allele distribution in HTLV-seropositive French Afro-Caribbean individuals, originating from the French West Indies.
Methods: Associations with HLA class I (A and B) and class II (DRB1 and DQB1) alleles were tested in 123 HAM/TSP patients and 85 asymptomatic HTLV-1 carriers. HLA typing was undertaken on genomic DNA extracted from peripheral blood leukocytes.
Results: In our cohort, no significant effect on either the risk of developing HAM/TSP or HTLV-1 provirus load was found for HLA class I or class II, including HLA-A*02 ($p = 0.43$).
Conclusions: Our findings are in contrast to those in the Japanese population, however the literature on HLA associations in HTLV-1 infections across different populations over the past decade have reported conflicting results and this suggests strong ethnic disparities.

1. Introduction

Tropical spastic paraparesis (TSP) was first reported by Strachan in the late 19th century[1] and was described clinically in a large series of patients from the West Indies by Cruickshank in 1956.[2] Discovered in 1980, human T-cell lymphotropic virus type 1 (HTLV-1) was identified as the etiological agent of adult T-cell leukemia (ATL).[3] HTLV-1-associated myelopathy (HAM) was described by Gessain et al. in 1985,[4] followed by Osame et al.[5] in Japan in 1986. In 1988, the World Health Organization (WHO) recommended "that the disease be known by the acronym HAM/TSP for the time being".[6]

The infection is estimated to affect 10 to 20 million people and HTLV-1 is widespread in the tropics and subtropics.[6–8] There are large endemic foci in the Caribbean, southern Japan, Central and South Africa, and South America, particularly Brazil. HTLV-1 is also present in southern Africa, southern India, northern Iran, the aboriginal populations of northern Australia, and many islands in the tropics. In Europe and North America, the virus is found chiefly in certain immigrant groups and in intravenous drug users. The infection is mainly blood borne. The dominant modes of transmission of HTLV-1 in endemic areas are from mother to child in breast milk and by sexual transmission in adults. Male to female sexual transmission is about four times as frequent as female to male transmission.[9,10] In 1999, the seroprevalence in Martinique, a French island in the Caribbean basin, was estimated to be 2.2%.[11]

HTLV-1 has a preferential tropism for CD4+ T cells and to a lesser extent for CD8+ T cells. After its entry into the cell, the virus is present as a provirus incorporated into the DNA of lymphocytes.[12] Viral replication of HTLV-1 in carriers is very low or even absent and therefore these infected silent cells are undetectable by the immune system for a long period of time. In spite of the

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Table 1
Characteristics of patients with HAM/TSP and of healthy HTLV-1 carriers

	HAM/TSP	Asymptomatic	*p*-Value
n	123	85	
Age, mean ± SD, years	62.27 (1.33)	56.73 (1.75)	0.01
Sex, *n*			
Female	103	59	0.014
Male	20	26	
Gender ratio (M/F)	0.19	0.44	
Follow-up duration, years			
Mean ± SD	13.3 ± 4.38	13.7 ± 4.7	0.517
Median	14	14	
Range	7–26	5–21	
HTLV-1 proviral load, copies/10^6 PBMCs			
n	113	67	
Mean ± SD	83 180.9 (75 658)	23 335.2 (30 902)	<0.0001
Median	67 740	9780	<0.0001

HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HTLV-1, human T-lymphotropic virus type 1; SD, standard deviation; PBMCs, peripheral blood mononuclear cells.

neoplastic, inflammatory, and immunosuppressive effects that can be caused by retroviruses, less than 10% of infected individuals develop HTLV-1-associated diseases in general, including ATL and HAM/TSP, by mechanisms that are incompletely understood.[3,5,13,14]

HAM/TSP is a slowly progressive disabling disorder characterized by spastic paraparesis with bladder and bowel dysfunction and lower back pain.[15,16] The disease course of HAM/TSP appears to be non-homogeneous and a subgroup of patients experiences rapid disease progression, particularly in post-transfusional and post-transplantation cases. In Martinique (French West Indies) the prospective observation of a cohort of 132 HAM/TSP patients over 14 years showed that the median time from disease onset to the assignment of a disability status score (DSS)[17] of 6 (ability to walk with unilateral support ≤100 m without rest) was 6 years, to a score of 6.5 (need of bilateral aid to walk 20 m) was 13 years, and to a score of 8 (essential restriction to a wheelchair) was 21 years. The median time from DSS 6 to 8 was 8 years. DSS 10 (death related to the disease) was reached by a quarter of the patients within 20 years.[18]

It is estimated that among HTLV-1 carriers, the lifetime cumulative risk of developing HAM/TSP ranges from 0.3% to 4%.[14] Several parameters may influence this conversion, including age, route of infection, high provirus load, and host immunological and genetic factors. Twenty years ago, Usuku et al. first suggested that the magnitude of the immune response in HAM/TSP was in part regulated by the immune response genes associated with the host's major histocompatibility complex.[19] The most convincing studies were performed on the Kagoshima population in Japan. The authors demonstrated a clear protective effect of the human leukocyte antigen HLA-A*02 and HLA-Cw*08 alleles against HAM/TSP in HTLV-1-infected individuals, whereas HLA-DRB1*0101 and HLA-B*5401 were found to be associated with an increased susceptibility to HAM/TSP.[20,21] The association of HLA-DRB1*0101 with disease susceptibility was only evident in the absence of the protective effect of HLA-A*02. Moreover, these data were enhanced by the independent associations found between HLA-A*02 genes and a lower HTLV-1 provirus load. Since HTLV-1 contains a dominant A2-restricted epitope, individuals with the HLA-A*02 allele would develop a stronger cytotoxic T-lymphocyte response against HTLV-1, leading to a provirus load decrease. The high prevalence in the human population and the association with proviral load has shown HLA-A*02 to be one of the best candidate genes associated with HAM/TSP conversion among HTLV-1 carriers .

The same authors reported the data from a small sample of 29 HTLV-1 carriers from London, of which 27 were of Caribbean origin, with the same results of a protective effect for A*02 in HAM/TSP: 10 of 14 asymptomatic HTLV-1 carriers were HLA-A*02-positive, compared with four of 15 HAM/TSP patients ($p = 0.02$, Fisher's exact test).[20]

We performed a case–control study to investigate the possible associations of HLA class I (A and B) and class II (DRB1 and DQB1) alleles with HAM/TSP and/or with HTLV-1 provirus load in a large cohort of Afro-Caribbean HTLV-1 carriers followed up in our neurology department.

2. Materials and methods

2.1. Study subjects

The study was carried out in Martinique, an island of the French West Indies situated in the Caribbean Lesser Antilles (14°300N–61°W). One hundred twenty-three consecutive patients who fulfilled the WHO diagnostic criteria of HAM/TSP[22] and 85 consecutive blood asymptomatic HTLV-1 healthy carriers (HCs) followed up in the Department of Neurology, University Hospital of Fort-de-France were recruited. Characteristics of the subjects with HAM/TSP and the HCs are shown in Table 1. As expected, the gender difference between the two groups of HTLV-1 carriers showed a female predominance, mainly in HAM/TSP patients, similar to the results of epidemiological studies.

2.2. HLA typing

Genomic DNA was extracted from peripheral blood leukocytes using the salting out method.[23] HLA class I (A and B) and class II (DRB1 and DQB1) typing was determined by polymerase chain reaction with sequence-specific oligonucleotides (PCR-SSO), using a reverse dot-blot hybridization method (RDB) as previously reported,[24] and according to the manufacturer's recommendations (INNO-LiPA tests from Innogenetics). Briefly, after specific amplification of each locus, the PCR biotinylated product is hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. After hybridization, streptavidin labeled with alkaline phosphatase binds to any biotinylated hybrid, and incubation with the chromogene results in a colored precipitate. The hybridization and coloration steps were performed in an Auto-LiPA apparatus (Innogenetics, Belgium). Positive band patterns were automatically scanned (LiPA Scan) and HLA typing was interpreted using Inno-LiPA interpretation software. For the analysis of HLA class II, 62 probes were used for DRB1 typing, 21 for DQB1 typing, and 21 for DPB1 typing. The same conditions of hybridization and washing temperatures were used whatever the locus considered, allowing the simultaneous