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Molecular Study of HBZ and gp21 Human T Cell Leukemia Virus Type 1 Proteins Isolated from Different Clinical Profile Infected Individuals

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Abstract

Human T cell leukemia virus type 1 (HTLV-1) is associated with a neurological syndrome named tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM) and the disease progression involves viral factors. The gp21 glycoprotein is involved in envelope trafficking and membrane targeting while the bZIP protein is indispensable for cell growth and proliferation. This study aimed to assess the molecular diversity of gp21 and HBZ proteins in TSP/HAM and healthy carriers. DNA samples from HTLV-1-infected individuals were submitted to PCR and sequencing, and the molecular analyses were performed using bioinformatics tools. From eight gp21-analyzed sequences one amino acid change (Y477H) was associated with the switch of a helix to coil structure at secondary structure prediction. From 10 HBZ analyzed sequences, two amino acid changes were identified (S9P and T95I) at the activation domain. One mutation (R112C) located at the nuclear localization signal was present in 66.7% and 25% of healthy carriers (HC) and TSP/HAM groups, respectively. This is the first report of mutations in the HBZ region. These polymorphisms might be important for viral fitness.

Human T cell leukemia virus type 1 (HTLV-1), initially isolated from a patient with cutaneous T cell lymphoma in the early 1980s, was the first identified human retrovirus.[1] Apart from inflammatory disorders more recently associated with HTLV-1 infection, such as arthritis, uveitis, dermatitis, lymphadenitis, and Sjögren's syndrome,[2] HTLV-1 is classically the etiological agent of tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM).[3] The oldest epidemiological data estimated that around 0.25–3.8% of the infected population develops TSP/HAM.[3,4] The evaluation of risk progression to TSP/HAM includes factors such as proviral load and genetic factors such as human leukocyte antigen (HLA).

HTLV-1 envelope glycoproteins are synthesized as precursors and are processed in the Golgi apparatus to yield a mature functional SU-TM protein complex. The TM (gp21) protein structure is long rods comprising an N-terminal central triple-stranded coiled coil, a disulfide bonded loop associated with a chain reversal at the base of the rod, and a structurally diverse C-terminal ectodomain segment that packs against the exterior of the central coiled coil in an antiparallel manner.[5] Through characterization of neutralizing antibodies, peptides, and *env* mutants, it was possible to show that the gp21 harbors motifs that direct Env trafficking, membrane targeting, and virion incorporation.[6]

More recently, the HTLV-1 bZIP factor gene (HBZ), which is encoded in the minus strand of the HTLV-1 genome, has been shown to be consistently expressed in adult T cell leukemia (ATL) cells and to be indispensable for the growth, survival, and proliferation of these cells.[7] As HBZ mRNA expression in TSP/HAM patients is well correlated with disease severity, it can play a critical role in disease development.[8] Despite this evidence, there is no study that has focused on the molecular diversity of HBZ in individuals with different clinical status. Therefore, considering the influence of genetic variability in determining viral fitness, the present study aimed to assess, briefly, the molecular diversity of gp21 (structural) and HBZ (regulatory) proteins in TSP/HAM individuals and in health carriers.

During 2010, blood samples were collected from 10 individuals frequently followed at the HTLV Reference Center of Bahia School of Medicine and Public Health, in Salvador, located in northeastern Brazil. All patients were included in the study through inclusion criteria consisting of neurological evaluation, provided they had similar proviral load and age.

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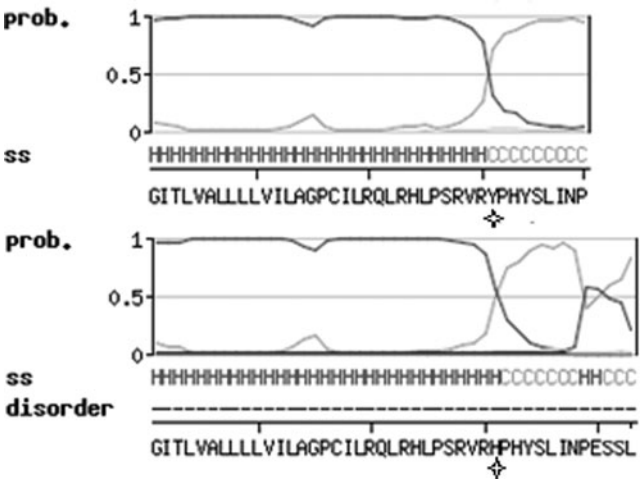


FIG. 1. Results of human T cell leukemia virus type 1 (HTLV-1) gp21 secondary structure prediction. The polymorphism Y477H is identified as ✧.

This study was approved by the Ethnic Committee of the Bahia School of Medicine and Public Health. Informed consent was obtained from all patients.

DNA was extracted using the spin column DNA extraction system (Qiagen, Hilden, Germany) and HTLV-1 proviral load was quantified using a real-time TaqMan polymerase chain reaction (PCR) method, as described previously.[9]

The viruses gp21 and HBZ coding sequences isolated from peripheral blood mononuclear cells (PBMCs) of HTLV-1-infected patients were amplified by PCR. Briefly, gp21F/gp21R were designed and used to amplify a 525-pb fragment, corresponding to the entire gp21 coding region (position 6118 to 6643 relative to the ATK1 genome). For HBZ, S1/AS4 were used to amplify a 517-pb fragment (position 7289 to 7806 relative to the AB219938 genome), corresponding to the unspliced HBZ fragment, as previously described.[10]

The PCR products were purified using a Qiaquick Gel Extraction kit (Qiagen, QIAamp DNA miniKit, Qiagen, Hilden, Düsseldorf, Germany) and sequenced in an ABI Prism 3100 DNA Sequencer (Applied Biosystems Inc., Foster City, CA) using Taq FS Dye terminator (Applied Biosystems) cycle sequencing. The same PCR primers were used in the sequencing reactions.

The mutation/polymorphism identification was performed, manually, through visualization of the alignment using Bioedit software[11] and GenBank sequences as reference strains. The SWISS-MODEL online tool (http://swissmodel.expasy.org/)[12] was used as a fully automated protein structure homology-modeling server, to infer the possible influence of the amino acid changes at protein secondary structure. The protein domain analysis was also performed using GeneDoc software[13] and the Prosite tool, as previously described.[14]

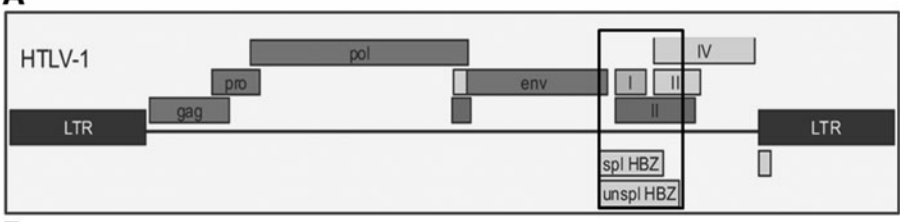
The gp21 and HBZ nucleotide sequences previously deposited in GenBank and used in this study as references are listed below with their corresponding accession number: AB219938 (HBZ), J02029 (gp21), and U81865–U81869 (gp21). The new gp21 and HBZ nucleotide sequences that were generated in this study were deposited in GenBank, and their corresponding accession number are KF053886–KF053893 (gp21) and KF053876–KF053885 (HBZ).

Our cohort was composed of 10 HTLV-1-infected individuals including six health carriers (HC) and four TSP/HAM patients. The medians of HTLV-1 proviral load of HC and TSP/HAM patients were similar (PV log = 5.0). Since the progression to TSP/HAM is multifactorial and dependent not only on viral factors, but also on the host genetic background, we also considered the age of the individuals and both groups had a quite similar median of age: 53 (38–72) and 58 (41–73) years for HC and TSP/HAM patients, respectively.

Eight gp21 sequences (461nt) were successfully generated and submitted to molecular characterization: four from HC and four from TSP/HAM individuals. Regarding the amino acid changes, it was possible to identify one (Y477H) change with 87.5% ($N = 7$) of frequency. This mutation can be a protein signature of these individuals, because it is not frequent (20%) in other available and analyzed (GenBank) gp21 sequences generated from HTLV-1-infected individuals in Brazil. It is sited at the cytoplasmatic domain of the protein and the prediction of the secondary structure shows that there are structural differences at the gp21 C-terminal region between the wild and mutated alleles (Fig. 1), especially because the Y477H polymorphism was associated with the switch of the helix to coil structure.

Due to the reduced number of sequences in this report, it was not possible to infer an association between gp21 mutations and clinical status, but it is important to study the gp21 variability because this protein is associated with Env

A



B

HBZ encoding region		pX gene	
NT site	AA site	NT site p12	AA site p12
C336T	R112C	C101T	P34L

FIG. 2. Schematic figure of ORF I (pX gene) and HBZ encoding region overlapping **(A)**. Summarized results of HBZ characterization in comparison to pX gene changes **(B)**. NT, nucleotide; AA, amino acid.

trafficking and consequently with the expression of HTLV-1 glycoproteins at the infected cells surface.

Ten HBZ sequences (517nt) were generated and submitted to molecular characterization: six from HC individuals and four from TSP/HAM patients. Two amino acid changes (S9P and T95I) were identified in 100% of the analyzed sequences. The third (R112C) most frequent mutation was found in 66.7% and 25% of HC and TSP/HAM groups, respectively (Fisher test, $p = 0.5238$). It is important to note that the mutations S9P and T95I are located within the activation HBZ domain,[15] as the R112C is located within the HBZ nuclear localization signal.[15] It is important to note that the three mutations identified in the HBZ protein resulted in changes in amino acids with different biochemistry characteristics, which can be related to changes in the HBZ structure and, consequently, in protein functioning.

The analysis of posttranslation modification sites shows that the same potential protein domains were found between the HC and TSP/HAM sequence groups. The functional motifs identified in all HBZ sequences were two N-myristylation sites (1 and 138 amino acid positions), three casein kinase II phosphorylation sites (25, 45, and 69 amino acid positions), and one protein kinase C phosphorylation site (146 amino acid position).

Considering that the HBZ protein is encoded by the negative strand in a region homologous to the positive strand that encodes the pX gene, the HBZ mutations could be responsible for changes in the proteins encoded by the pX gene. Comparing the HBZ sequences to a pX reference sequence (ATK1-J02029), we found that the mutation R112C is located at the same site of the P34L mutation in the p12 protein (ORF-I of pX) (Fig. 2). The p12 I L34 position was already identified as prevalent among Brazilian strains and was not found in the Argentinean sequences.[16]

In this study, we identified a genomic mutation in the sequences from both HC and TSP/HAM patients that was related to structural modification of the gp21 and that may interfere with Env trafficking, membrane targeting, and virion incorporation processes. Moreover, we found three mutations located at the HBZ region whose expression has been correlated with disease development. Despite the reduced number of HBZ sequences, this is the first report involving the identification of mutations in this genomic region. Therefore we believe that these amino acid changes should be more closely investigated.

Author Disclosure Statement

No competing financial interests exist.

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