HTLV-2 Horizontal and Vertical Transmission in a Family from a Brazilian Urban Area: Seroepidemiological, Clinical and Molecular Study

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ABSTRACT
Human T cell lymphotropic virus type 2 (HTLV-2) has been shown to be endemic in Brazilian Indians and among intravenous drug users in urban areas, but transmission of this infection seems to be infrequent in the general population living in urban areas in Brazil. Six persons in three generations of a Brazilian family were evaluated to assess HTLV-2 transmission and its molecular features in the positive cases. The index was detected during screening (HTLV EIA) of donated blood in Fundação Hemominas, Belo Horizonte, Brazil. Confirmatory serological test and viral typing were performed by Western blotting and polymerase chain reaction. The family consisted of husband, wife (index case), three daughters, and the mother of the index case. The husband and one daughter were found positive, thus pointing to horizontal and vertical transmission. The husband was a truck driver, who reported casual sex during frequent traveling. The positive daughter was breast-fed for 3 months, as opposed to the remaining two (seronegative), who breast-fed for 1 month. The index case’s mother was negative. To identify HTLV-2 subtype(s), phylogenetic analysis of the noncoding long terminal repeat region and part of the env and tax coding regions was performed. These new isolates from Belo Horizonte are related to subtype IIa but present a molecular variant with extended tax, previously reported in subtype IIc. Analyzing both LTR and env regions, the family’s sequences clustered with isolates of Brazilian intravenous drug users and transfusion transmitted virus.

INTRODUCTION
Human T cell lymphotropic virus type 2 (HTLV-2) is endemic in many Indian communities and intravenous drug users (IVDUs) in South America, especially in Brazil. HTLV-2 is transmitted through contaminated blood transfusion, including needle sharing of IVDU, sexual intercourse, and from mother to child by breast-feeding. HTLV-2 may have been introduced into the American continent through migration of an HTLV-2-infected Asian population around 15,000–35,000 years ago, over the Bering land bridge. In the past few decades the virus seems to be transmitted from American Indians to IVDUs and spread due to the practice of needle sharing. Although HTLV-2 was not initially associated with a clear-cut disease, there is accumulating reports that the infection may be related to neurological disorders and increased rates of infectious diseases. High rates for HTLV-2 infection are found among IVDUs from urban areas, but it is uncommon among positive HTLV-I/II blood donors from Belo Horizonte, Brazil, with less than 3% of the total of HTLV-I/II infected individuals.
In this report, we describe HTLV-2 transmission, clinical and laboratory features of the infection, and phylogenetic aspects of the virus in three generations of a Brazilian family, identified from a positive blood donor candidate (index case) in Belo Horizonte, Minas Gerais State.

**MATeRIALS AND METHODS**

**Study population and HTLV-2 serology**

Belo Horizonte is the capital city of Minas Gerais State and its metropolitan area has close to four million inhabitants—the fourth largest urban area in Brazil. We studied a family from Sete Lagoas City, located 116 km far from Belo Horizonte.

Informed consent was obtained from those willing to participate in the GIPH cohort study, approved by the Committee on Human Subjects of the Hemominas Foundation. In addition, blood samples were collected and the subjects were examined by physicians. The GIPH cohort study protocol included medical appointments and laboratory examinations for 2 years.

**HTLV-2 serologic diagnosis**

The Brazilian family studied was composed of a wife (index case), husband, three daughters, and the index case’s mother. The couple was married for 12 years. Antibodies to HTLV-1/2 in serum were detected with a commercial kit (EIA, Ortho, USA). Repeatedly reactive samples were submitted to Western blot (Genelabs Diagnostics), and were interpreted according to the manufacturer’s instructions.

**Polymerase chain reaction (PCR), HTLV-2 cloning and sequencing**

Mononuclear cells from peripheral blood (PBMC) were obtained by Ficoll-Hypaque gradient separation (Pharmacia, Sweden). Genomic DNA from PBMC was extracted using a DNAzol kit (GIBCO-BRL). The HTLV-2 nested polymerase chain reaction (PCR) of part of the long terminal repeat (LTR), env and tax regions, was performed as described previously.2,12 All amplified products were analyzed in a 2% agarose gel electrophoresis followed by ethidium bromide staining. The HTLV-2 tax amplified products were purified using the Promega Wizard PCR Prep system and sequenced directly in a Perkin-Elmer/ABI Prism 377 DNA Stretch Sequencer by Taq FS Dye terminator cycle sequencing. The same PCR inner primers were used in the sequencing reactions.

The amplicons from both LTR (661 bp) and env (631 bp) were cloned in a pGEM-T easy vector (PROMEGA), according to the manufacturer. Clones from each gene region fragment and patient were selected after testing by PCR. Plasmidial DNA was purified by the Wizard plus SV minipreps system (Promega). At least two clones of each region were sequenced (ABI PRISM, Applied Biosystems INC.). Sequences were obtained from both strands, repeated at least once, and analyzed using the software packages BLASTN, BLASTX, and BLASTX available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and deposited at GenBank (NCBI, Bethesda, MD).

**Phylogenetic analysis**

Multiple sequence alignments for the HTLV-2 gpl20-env, LTR, and tax region of the studied samples together with related sequences in the GenBank/EMBL database were done with the Dastme program13 using the Clustal-W algorithm and further edited in the GeneDoc program,14 resulting in alignments with 586, 657, and 433 bp, respectively. Neighbor-joining (NJ) and maximum-likelihood (ML) trees were performed by PAUP* program version 4.02b5 using the HKY85 substitution model including substitution rate heterogeneity for env (transition/transversion rate = 3.32 and a parameter = 0.485483) and LTR (transition/transversion rate = 1.89 and a parameter = 0.603905), and the GTR substitution model including substitution rate heterogeneity for tax (transition/transversion rate = 3.57 and a parameter = 0.36662). The reliability of the NJ trees was evaluated by analyzing 1000 bootstrap replicates. The trees were drawn with the TreeView 1.4 program (Glasgow University, Scotland).

**RESULTS**

Among six family members tested, there were HTLV-2 seropositive (EIA and WB) the index case, the husband and a daughter (BH223, BH339, and BH115 HTLV-2 isolates, respectively). All of them were HTLV-I negative. The reactive samples were tested by PCR, which was positive for HTLV-2 only. The infected child was 7 years old and had been breast-fed by her mother for 3 months, as opposed to her two sisters, who breast-fed for a month. No member of this family received a blood transfusion.

The index case, a 39-year-old female was first seen on July 1998, after being diagnosed as positive for HTLV-2. Other screening tests for blood donors were negative (HBV, HCV, HBV, Chagas’ disease, syphilis). She had no risk factors that could account for her HTLV-2-positive status other than sexual contact with her HTLV-2-positive husband. Although she was breast-fed, her mother was HTLV-2 negative. At her first appointment she complained of slight dizziness, leg weakness and pain, and lower back pain. During physical examination, no hepatomegaly, splenomegaly, or axillomgaly was detected. No neurological changes were noted. Laboratory analyses showed a leukocyte count of 11 x 10^3 cells/mm^3, with 1.0 x 10^3 lymphocytes/mm^3. Eight atypical lymphocytes were observed. Phenotypic evaluation of peripheral blood leukocytes was normal. HTLV-1 in cerebrospinal fluid was negative. The stool examination for parasites was negative (Hoffman–Pons–Jenner and Baerman–Moraes techniques). Angiologic examination of the lower limbs showed no alterations that could account for her leg pain.

Ophthalmological examination revealed the presence of keratoconjunctivitis sicca, which was treated, and the eye examination became normal. Dermatological examination was normal. In 2003, besides the initial complaints, she was under treatment for epicondylitis on her right elbow and had initiated hormonotherapy after early menopause. Clinical and neurological examinations continued to be normal. She presented at the time atypical lymphocytes in peripheral blood (1.0 x 10^3/m^3). Her husband was under treatment for bowel cancer, being currently...
in remission, with no complaints. He was 44 years old and had a positive family history of bowel cancer (father). The daughter was asymptomatic in her first visit (7 years old) and had normal laboratory tests and physical examination at the time. In July 2002 she had bilateral halux nail injury with extensive infectious complications, which demanded surgical drainage. Peripher al blood cell examination revealed giant platelets, but was otherwise normal.

**HTLV-2 phylogenetic analysis**

The phylogenetic analysis of the fragments of 657 and 586 bp, part of the LTR (Fig. 1) and gp21-env (Fig. 2) regions, respectively, demonstrated that all isolates belonged to subtype 2a, supported by bootstrap values of 76% (LTR) and 88% (env).

In the LTR analysis, the isolates from Belo Horizonte formed a separate cluster within subtype IIa with 76% of the bootstrap support and grouped with Brazilian samples from IDU and transfused people, with 82% bootstrap.

A fragment of 523 bp comprising part of the tax gene, and corresponding to the 7752–8274 bp position in the HTLV-2a Mo prototype, was studied in all three samples from Belo Horizonte (Fig. 3). All isolates had the same sequence in the final alignment. Phylogenetic analysis of these isolates has grouped them with other Brazilian prototypes isolated from Kayapo Indians (K96 and RP329) with a bootstrap value of 53% and they belonged to the Brazilian HTLV-2a molecular variant, previously classified as subtype IIc.\(^{16,17}\)

**FIG. 1.** Rooted NJ tree of three new HTLV-2 strains along with a representative set of HTLV-2 strains based upon a 586-bp fragment of the *env* region. The bootstrap values (above 50% and using 1000 bootstrap samples) on the branches represent the percentage of trees for which the sequences at one end of the branch form a monophyletic group. Efe2 and PP1664 strains are used as outgroups. The geographical origin and ethnic origin are given in parentheses. Newly sequenced *env* included in this analysis are in bold.
DISCUSSION
We have described the occurrence of horizontal and vertical transmission of HTLV-2 in the same family. The husband, a truck driver, reported casual sex in his trips to northeastern Brazil. Considering that the wife’s (index case) mother was negative, sexual intercourse was the probable source of infection in the family. The infected child was breast-fed longer (3 months) than her negative, older sisters, who were breast-fed for 1 month. Being younger, she could also have been exposed to a higher proviral load, possibly due to progression of the infection in the mother.18

The index case had persistent neurological symptoms, which, although they do not fulfill the criteria of HAM/TSP, deserve appropriate follow-up, with physical and image examinations to elicit neural damage.

Of note is the persistent infection in the halux of the infected child, which could also point out a deficiency of the immune system, as previously reported in association with HTLV-2.11

The index case had persistent neurological symptoms, which, although they do not fulfill the criteria of HAM/TSP, deserve appropriate follow-up, with physical and image examinations to elicit neural damage.

The presence of giant platelets does not seem to be a consequence of HTLV-2 infection, since they can also be seen in the negative controls of the GIPH cohort. Phylogenetic analysis of BH223, BH315, and BH339 using env and LTR sequences, which demonstrated that these isolates belonged to subtype IIa,

FIG. 2. Rooted NJ tree of three HTLV-2 strains based upon a 626-bp fragment of the LTR region. The bootstrap values (above 50% and using 1000 bootstrap samples) on the branches represent the percentage of trees for which the sequences at one end of the branch form a monophyletic group. Efe2 and PP1664 strains are used as outgroups. The geographical origin and ethnic origin are given in parentheses. Newly sequenced LTR included in this analysis are in bold.

FIG. 3. Rooted NJ tree of three HTLV-2 strains based upon a 530-bp fragment of the tax region. The bootstrap values (above 50% and using 1000 bootstrap samples) on the branches represent the percentage of trees for which the sequences at one end of the branch form a monophyletic group. Efe2 and PP1664 strains are used as outgroups. The geographical origin and ethnic origin are given in parentheses. New tax sequences included in this analysis are in bold (BH226, BH315, and BH339).
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clustering with isolates from Brazilian IDUs and blood trans-
mitt... at position 8203. These results demonstrate the usefulness of family studies coupled with phylogenetic analysis to infer possible transmission routes of HTLV-2 and to genetically characterize strains to determine the circulating viruses in urban and rural areas in Brazil.

ACKNOWLEDGMENTS

The authors would like to thank Fapemig, Fundaes Hemo-

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