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Human T Cell Lymphotropic Virus Type 2a Strains Among HIV Type 1-Coinfected Patients from Brazil Have Originated Mostly from Brazilian Amerindians

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Abstract

The human T cell lymphotropic virus type 2 (HTLV-2) is found mainly in Amerindians and in intravenous drug users (IDUs) from urban areas of the United States, Europe, and Latin America. Worldwide, HTLV-2a and HTLV-2b subtypes are the most prevalent. Phylogenetic analysis of HTLV-2 isolates from Brazil showed the HTLV-2a subtype, variant -2c, which spread from Indians to the general population and IDUs. The present study searched for the types of HTLV-2 that predominate among HIV-1-coinfected patients from southern and southeastern Brazil. Molecular characterization of the LTR, *env*, and *tax* regions of 38 isolates confirmed the HTLV-2c variant in 37 patients, and one HTLV-2b in a patient from Paraguay. Phylogenetic analysis of sequences showed different clades of HTLV-2 associated with risk factors and geographic region. These clades could represent different routes of virus transmission and/or little diverse evolutionary rates of virus. Taking into account the results obtained in the present study and the lack of the prototypic North American HTLV-2a strain and HTLV-2b subtypes commonly detected among HIV-coinfected individuals worldwide, we could speculate on the introduction of Brazilian HTLV-2 strains in such populations before the introduction of HIV.

Introduction

The human T cell lymphotropic virus type 2 (HTLV-2) is found mainly in American Indians and in intravenous drug users (IDUs) in urban areas of the United States, Europe, and Latin America.[1–3] In Brazil, considered the country with the largest number of people infected by HTLVs,[4] HTLV-2 is considered endemic among indigenous populations of the Amazon region and among Indians of the south region, and is present in IDUs of urban areas and in patients with HIV/AIDS.[5–10]

The HTLV-1/2 are low replicating viruses, thus have little genetic sequence variation.[11,12] However, the e*nv* structural gene and the long terminal repeat (LTR) region containing the viral promoter are the most variable regions, thus are useful for virus subtyping.[3] The LTR represents a noncoding region and consequently is not subject to the same evolutionary constraints as coding regions. The regulatory gene *tax*, which is engaged in viral transcription, diverges among isolates by nucleotide differences and size of the encoded Tax regulatory protein; thus this region is also valuable for molecular and epidemiological analysis.[7,13,14] Still, the *tax* region allows us to discriminate the molecular variant of Brazilian strains named HTLV-2c, which encodes a long transactivating protein Tax because of the loss of the stop codon in the *tax* gene position of 2735 and the gain of 25 amino acids (Fig. 1).[13] Interestingly, the Brazilian HTLV-2 strain has the *tax* region similar to the HTLV-2b subtype and the *env* and LTR genomic regions similar to the HTLV-2a subtype.[7,13,15] The occurrence of the HTLV-2c variant in Brazil is intriguing and its origin is not completely clarified. It is thought that HTLV-2 reached the New World approximately 15,000 years ago by the human (Paleo Indians) migratory wave that crossed the Bering land bridge. Two different migratory routes paralleling the Andean Cordillera along the Pacific Coast and toward the Amazon region could have independently resulted in the emergence of the HTLV-2c strain,

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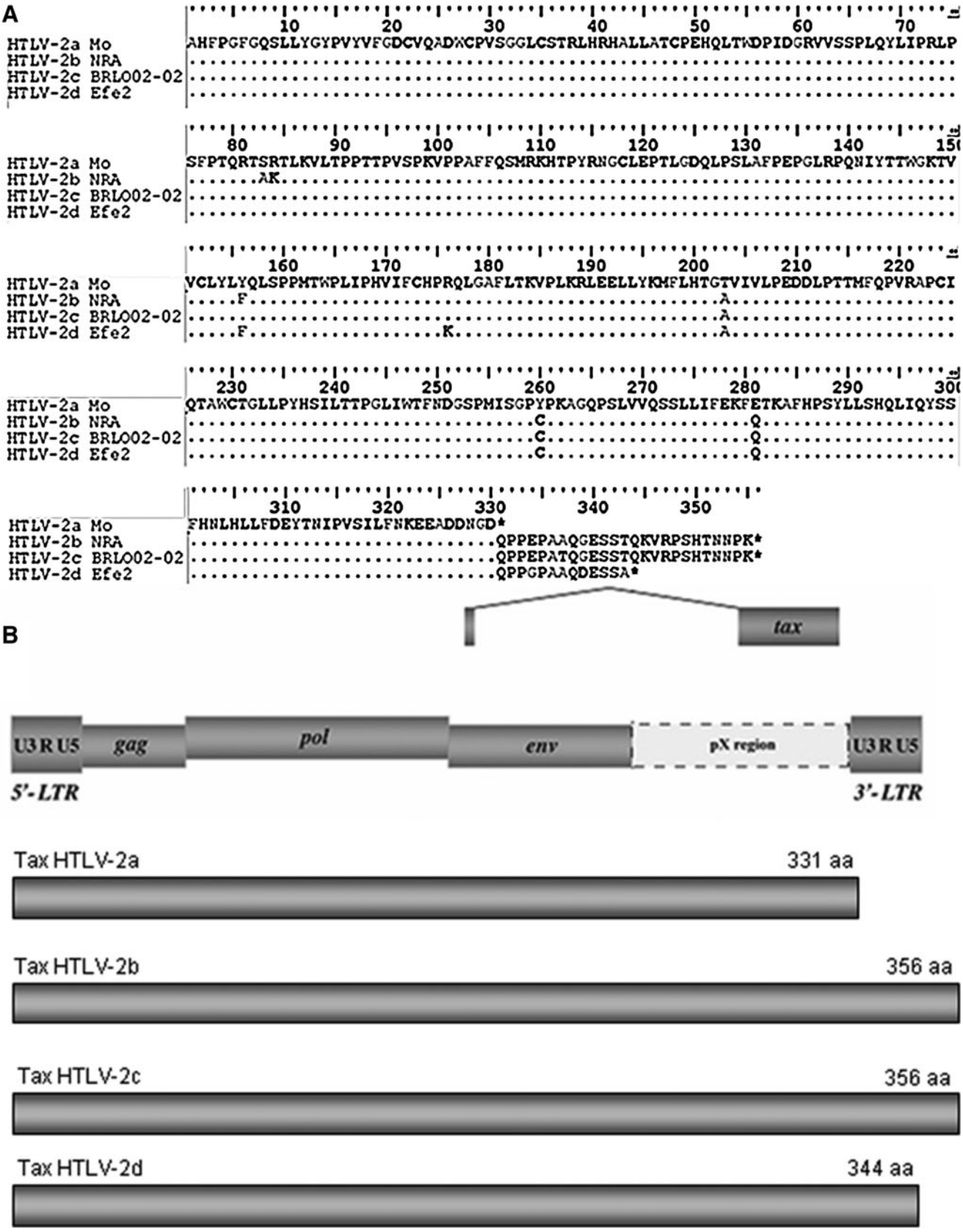

FIG. 1. Amino acid alignment of the *tax* region **(A)** and schematic Tax protein structure **(B)** among human T cell lymphotropic virus (HTLV)-2a (Mo prototype), HTLV-2b (NRA prototype), HTLV-2c (BRLO02-02), and HTLV-2d (Efe2) subtypes, highlighting the stop codon position in each sequence that results in an additional 25 amino acids at the C-terminal end of the Tax protein of HTLV-2b and HTLV-2c and 13 amino acids in HTLV-2d subtypes in relation to the HTLV-2a subtype. Note that the first amino acids are encoded in the *env* region not displayed in these sequence alignments.

perhaps from a prototype 2a, or in the introduction of 2c exclusively into the Amazon region.[7]

Therefore, HTLV-2 molecular characterization worldwide disclosed four HTLV subtypes: HTLV-2a and HTLV-2b, the most prevalent among IDUs from urban areas of the Americas and Europe and in the indigenous population of the Americas, and with sporadic distribution in Asia and Africa; the HTLV-2c variant detected in the indigenous population of the Brazilian Amazon and in IDUs from urban populations in Brazil; and the HTLV-2d detected in pygmy tribes in Africa.[13,16–21]

In 2010, we started the characterization of HTLV-2 isolates that circulate in HIV-coinfected individuals from southern Brazil using nucleotide sequence and restriction fragment length polymorphism (RFLP) analysis of the LTR region, and confirmed that all HTLV-2 strains belonging to the 2a subtype. Moreover, we observed an association of molecular variants and sexual risk factor and injecting drug use.[22] Now, in order to expand these findings, confirm the HTLV-2 subtype prevalent in HIV-coinfected individuals from the south and southeast regions, and provide information regarding the characterization and the origin of HTLV-2 in such populations, the present study was conducted.

Materials and Methods

The samples analyzed consisted of blood samples obtained from 38 patients with HIV/AIDS attending the AIDS Reference Centers who were coinfected with HTLV-2: 28 from Londrina city and vicinities in Paraná (PR) state (BRLO, southern Brazil) of whom we have complete epidemiological data,[9,22] and 10 from São Paulo and Jundiaí cities in São Paulo (SP) state (BRSP, southeastern Brazil) of whom we have only data concerning gender and age. The available data on such patients are described in Table 1 and the map showing the

Table 1. Epidemiological Data and Human T Cell Lymphotropic Virus Type 2 Sequences Accession Numbers Obtained from 38 HIV/Human T Cell Lymphotropic Virus Type 2-Coinfected Patients Attending AIDS Reference Centers in Paraná (South) and São Paulo (Southeast) States of Brazil

Patient code	*Gender/age (years)*	*Locality (city–state)*	*Risk factor*	*GenBank AN LTR*	*GenBank AN env*	*GenBank AN tax*
BRLO02-02	F/26	Londrina–PR	IDU		HM770414	JN887712
BRLO03-02	F/39	Londrina–PR	Sexual		HM770390	JN887713
BRLO05-02	M/56	Londrina–PR	IDU		HM770391	
BRLO07-02	M/41	Londrina–PR	IDU	GU573730[a]	HM770392	
BRLO09-02	M/30	Londrina–PR	IDU	GU573731[a]	HM770393	JN887714
BRLO10-02	M/45	Londrina–PR	Sexual		HM770394	
BRLO11-02	M/35	Londrina–PR	IDU		HM770395	
BRLO12-02	F/33	Londrina–PR	IDU	GU573732[a]	HM770396	JN887715
BRLO18-02	F/29	Londrina–PR	Sexual	GU573733[a]	HM770397	
BRLO19-02	M/34	Londrina–PR	IDU	GU573734[a]	HM770398	JN887716
BRLO21-02	F/30	Londrina–PR	Sexual	GU573735[a]	HM770399	JN887717
BRLO22-02	M/46	Londrina–PR	Sexual	GU573736[a]	HM770400	JN887718
BRLO23-02	F/39	Londrina–PR	Sexual	GU573737[a]	HM770401	
BRLO24-02	M/37	Londrina–PR	Sexual	GU573738[a]	HM770402	
BRLO25-02	F/33	Londrina–PR	IDU		HM770415	
BRLO26-02	M/37	Londrina–PR	IDU	GU573739[a]	HM770403	JN887719
BRLO27-02	F/31	Londrina–PR	Sexual	GU573740[a]	HM770404	JN887720
BRLO28-02	M/42	Londrina–PR	Sex + IDU	GU573741[a]	HM770405	JN887721
BRLO29-02	F/34	Londrina–PR	Blood	GU573742[a]	HM770406	
BRLO31-02	M/29	Londrina–PR	IDU	GU573743[a]		JN887722
BRLO32-02	M/34	Londrina–PR	IDU		HM770407	JN887723
BRLO35-02	M/34	Londrina–PR	IDU		HM770408	JN887724
BRLO37-02	M/46	Londrina–PR	Sex + IDU	JQ435902	JQ435911	JN887725
BRLO38-02	F/27	Londrina–PR	Sexual		HM770409	JN887726
BRLO39-02	M/32	Londrina–PR	IDU		HM770410	JN887727
BRLO43-02	M/34	Londrina–PR	IDU		HM770411	JN887728
BRLO45-02	M/45	Londrina–PR	Sexual		HM770412	JN887729
BRLO49-02	M/35	Londrina–PR	Sex + IDU		HM770413	JN887730
BRSP91-08	M/47	São Paulo–SP	unknown	JQ435903	HM770416	
BRSP111-08	M/42	São Paulo–SP	unknown		HM770417	
BRSP160-08	M/46	São Paulo–SP	unknown	JQ435904	HM770418	
BRSP171-08	F/33	Jundiaí–SP	unknown	JQ435905	HM770423	JN887731
BRSP172-08	M/46	Jundiaí–SP	unknown		HM770424	JN887732
BRSP239-08	M/52	São Paulo–SP	unknown	JQ435906	HM770419	JN887733
BRSP319-08	F/47	São Paulo–SP	unknown	JQ435907	HM770420	JN887734
BRSP348-08	M/35	Jundiaí–SP	unknown	JQ435908	HM770425	JN887735
BRSP84-09	M/36	Jundiaí–SP	unknown	JQ435909	HM770421	
BRSP130-09	M/29	Jundiaí–SP	unknown	JQ435910	HM770422	JN887736

[a]Sequences published by Magri *et al*., 2010.[22]

M, male; F, female; PR, Paraná state; SP, São Paulo state; IDU, intravenous drug users; AN, accession numbers; LTR, long terminal repeat.

location and distances among the cities is presented in Fig. 2. Signed informed consent was obtained from patients, and the study was approved by the Ethics Committee of all participant institutions.

DNA samples were extracted from peripheral blood leukocytes. The established protocol for amplification and sequencing the LTR region was previously published,[22] and for the *env* and *tax* regions were based on protocols previously described,[13,23,24] in which one primer has undergone minor nucleotide modification and thermal cycling conditions were adjusted (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/aid). Still, in both protocols the polymerase chain reaction (PCR) and nested PCR were improved in stability using the Go*Taq* Colorless Master Mix (Promega Corporation, Madison, WI). In addition, to recover a few cases of HTLV-2-positive *tax* nested PCR that generated bad sequences, the sequencing was repeated with the primers described in Supplementary Table S2 and using three additional primers: Px107 *Forward* 5′ACC CCA TGT CAT ATT CTG CCA3′ (nt. 7713–7733), Px108 *Reverse* 5′AGC CTT TAC TTG GGA TTG TTT3′ (nt. 8265–8285), and Px104 *Reverse* 5′AAG TTC TTC TAA TCG TTT TAG3′ (nt. 7771–7791).[13]

Sequencing was performed using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster, CA). All of the sequencing chromatograms were assembled and edited with Sequencher 4.7 software. Multiple alignments were performed using the Clustal W multiple-sequence alignment tool from BioEdit Sequence Alignment Editor, version 7.0.5.3, software with a reference set available in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank), in which the nucleotide and amino acid substitutions were searched. HTLV-2 Mo and HTLV-2 NRA were used as prototypic examples of HTLV-2a and HTLV-2b subtypes, respectively; the first was identified in a T cell line derived from an atypical variant of hairy cell leukemia from patient Mo and the second from a patient with $CD8^+$ T cell lymphoproliferative disorder, both in the United States.[25] HTLV-2 subtyping was screened with the NCBI Genotyping (www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi) tool website. Neighbor-joining (NJ) and maximum likelihood (ML) phylogenetic trees were constructed based on appropriate nucleotide substitution models determined by Modeltest v3.7 using PAUP v4b10 software (HKY + i + G model for the LTR and *env* regions and TrN + G model for the *tax* region), and showed similar topologies. Bootstrapping was performed with the stepwise addition algorithm for 1,000 replicates. The Efe2 (HTLV-2d) sequence was used as the outgroup. HTLV-1 was not used as the outgroup because it was previously indicated that the analysis could decrease the signal-to-noise value, resulting in serious topological errors.[26] MEGA4 software was used to estimate nucleotide distances.

Results

Although all efforts were made to improve the amplification and sequencing of all HTLV-2 isolates, we were not able to sequence some genomic segments (Table 1). The protocol established for the LTR allowed us to obtain sequences of 458 bp from 23 patients (15 from the southern region, 14 previously published,[22] and 8 from southeastern Brazil), sequences of 1,065 bp for *env* from 37 patients (27 from the southern region and 10 from southeastern Brazil), and sequences of 1,068 bp for *tax* from 25 patients (19 from the south and 6 from the southeast regions of Brazil). All GenBank accession numbers are presented in Table 1 and in the section Sequence Data.

The sequences were analyzed by the NCBI Genotyping tool and by phylogeny and all belong to the HTLV-2a subtype (Figs. 3, 4, and 5), except one (BRLO38-02) that belongs to the HTLV-2b subtype (Figs. 4 and 5). The variant -2c was confirmed in all HTLV-2a isolates by *tax* sequence analysis using the Clustal W multiple-sequence alignment tool, which

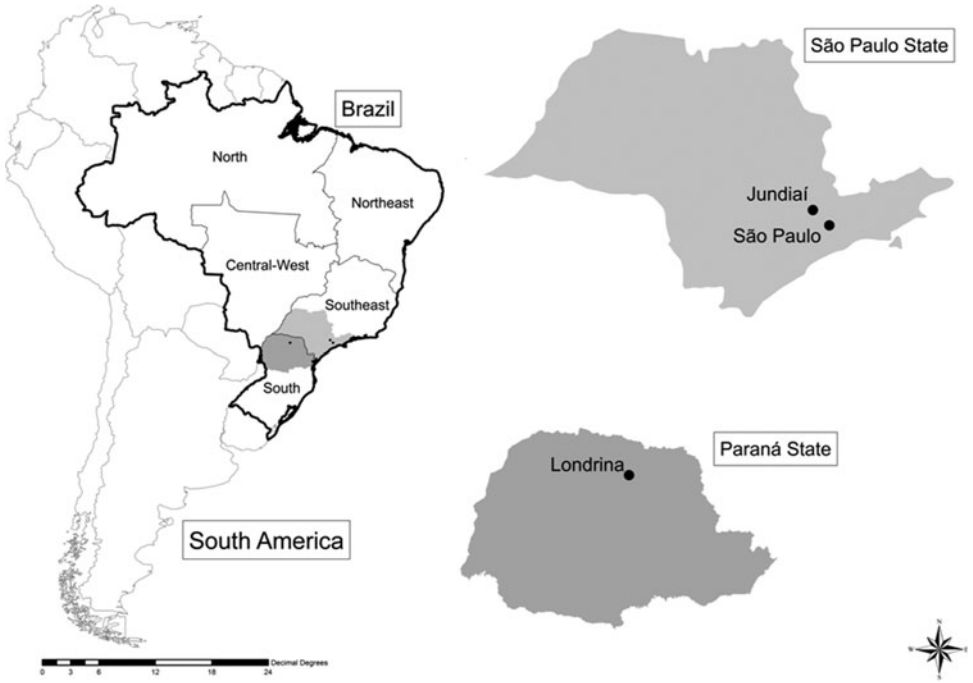

FIG. 2. Map of Brazil showing the location of the São Paulo state (highlighting São Paulo and Jundiaí cities, distance 37 miles) and the Paraná state (highlighting Londrina city, 330 miles from São Paulo city).

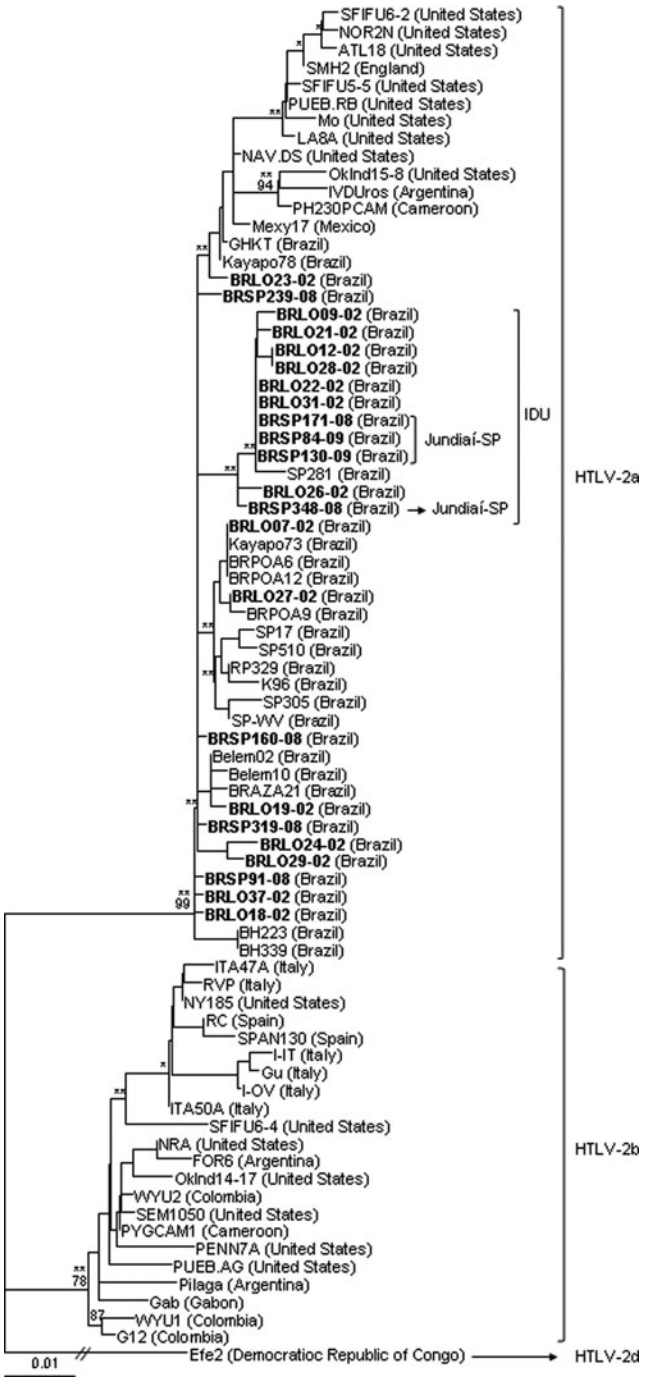

FIG. 3. Dendrogram showing the phylogenetic relationship between 458 bp of the long terminal repeat (LTR) (nt. 181–638 in relation to the Mo prototype—AN M10060) region of the HTLV-2 strains, including sequences from the south and southeast regions of Brazil (GenBank AN GU573730–GU573743, and JQ435902–JQ435910) in bold. Bootstrap values above 65% and zero length using the likelihood ratio test with $p < 0.001$ (**) and $p \leq 0.05$ (*) in key branches are depicted. The HTLV-2d Efe2 isolate was used as the outgroup.

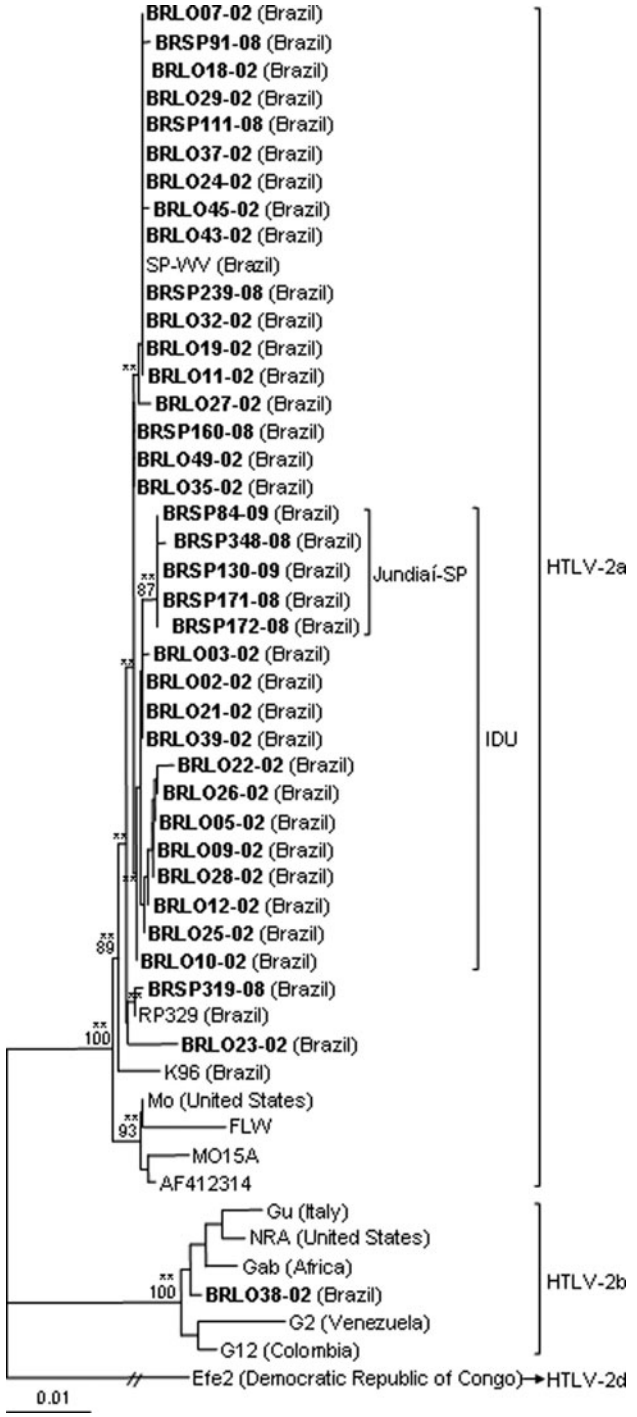

FIG. 4. Dendrogram showing the phylogenetic relationship between 1,065 bp of the *env* (nt. 5573–6637 in relation to the Mo prototype—AN M10060) region of the HTLV-2 strains, including sequences from the south and southeast regions of Brazil (GenBank AN HM770390–HM770425 and JQ435911) in bold. Bootstrap values above 65% and zero length using the likelihood ratio test with $p < 0.001$ (**) and $p \leq 0.05$ (*) in key branches are depicted. The HTLV-2d Efe2 isolate was used as the outgroup.

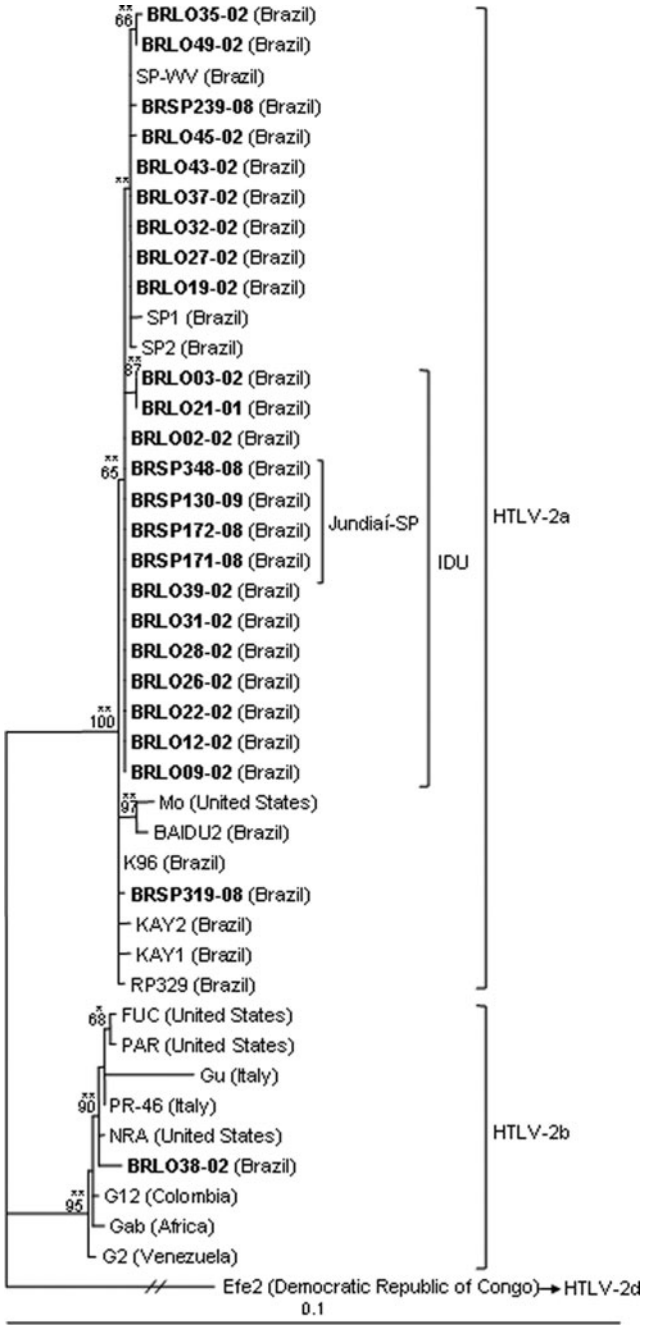

FIG. 5. Dendrogram showing the phylogenetic relationship between 1,068 bp of the *tax* (nt. 7213–8280 in relation to the Mo prototype—AN M10060) region of the HTLV-2 strains, including sequences from the south and southeast regions of Brazil (GenBank AN JN887712–JN887736) in bold. Bootstrap values above 65% and zero length using the likelihood ratio test with $p < 0.001$ (**) and $p \leq 0.05$ (*) in key branches are depicted. The HTLV-2d Efe2 isolate was used as the outgroup.

disclosed the loss of the stop codon in the *tax* gene position of 2735 and an additional 25 amino acids at the C-terminal end of the Tax protein (Fig. 1).

Phylogenetic sequence analysis of the *env* tree in relation to demographic and epidemiological data of patients disclosed an association between viral variants and type of exposure to the virus (IDU, $p < 0.001$) and the place of origin of the patient (isolates from Jundiaí city in São Paulo State are present in a single clade, bootstrap = 87%, $p < 0.001$) (Fig. 4). But when the sequences were analyzed by the LTR and *tax* phylogenetic trees, it was observed that the sequences from Jundiaí clustered together on the IDU branch, not separately (LTR and *tax*: $p < 0.001$) (Figs. 3 and 5, respectively).

Molecular analysis of the LTR, *env*, and *tax* sequences of HTLV-2, using MEGA4, showed high nucleotide similarity between the Brazilian sequences (99.3%, 99.6%, and 99.9%, respectively) and in relation to the HTLV-2a Mo prototype (97.8%, 99.2%, and 99.3%, respectively), in contrast to the low similarities detected in relation to the HTLV2-b NRA prototype (similarity of 95.2%, 95.7%, and 96.7%, respectively). When the sequences were stratified into two groups, one from Londrina and another from São Paulo, no differences were found. The similarities between the BRLO38-02 (HTLV-2b subtype) sequences and the NRA (HTLV-2b prototype) were 99.3% for the *env* and 99.5% for the *tax* region; unfortunately, the LTR region amplification did not function as required.

Regarding the presence of nucleotide substitutions in the LTR region of HTLV-2 in relation to the Mo prototype, in 100% of sequences G214A, T265C, C401T, and C551G substitutions were detected, while G316C, G317T, A448G, and T630C nucleotide substitutions were detected at frequencies of 96.3%, 96.3%, 92.6%, and 85.2%, respectively. The T315G, C320G, and G522A nucleotide substitutions were detected in 44.4% of sequences.

Concerning the presence of mutations in the *env* region, six nucleotide changes were very conserved among the isolates at positions T5726C, A5794G, T6109C, C6226T, C6379T, and A6580G with frequencies of 100%, 97.2%, 91.6%, 100%, 100%, and 100% relative to the Mo prototype, respectively. Among these nucleotide substitutions the only one that was a nonsynonymous mutation and thus generating an amino acid change was the T5726C. When the sequences were converted into amino acids the change S1909P was observed.

The sequences of the *tax* region of HTLV-2 were also analyzed for the presence of mutations in relation to the Mo prototype. The main nucleotide substitutions observed were A7819G, A7991G, A7798G, G8053C, and T8203A, with a frequency of 100%. Nucleotide substitutions C7611T, T7686C, and A7825G were detected at frequencies of 96%, 92%, and 36%, respectively. Amino acid changes of T2607A, Y2664C, and E2685Q and the loss of the stop codon 2735Q and the gain of 25 amino acids were detected in 100% of the sequences (Fig. 1). The *tax* sequence of the isolated BRLO38-02 was also aligned with the NRA prototype (HTLV-2b) and few nucleotide changes were detected (T7554C, T7611C, T7902C, T8031C, and G8142A), without amino acid change.

Discussion and Conclusions

The selected and optimized protocols in the present study proved useful to generate long fragments of *env* and *tax* regions of the HTLV-2 proviral genome and enabled robust phylogenetic analysis, although they still were not be able to amplify all the study samples. The sequencing of the *tax* region was nearly full: only lacking were the three initial nucleotides located at the beginning of the viral *env* gene that characterize the start codon of Tax (amino acid methionine), and by explicing encodes a protein of 356 amino acids in

length (Fig. 1). The function of this long transactivator protein was poorly analyzed. There is only one study that compared the function of the Tax proteins of HTLV-1 and HTLV-2 subtypes a, b, c, and d, in CREB and NF-κB-mediated transactivation. Using the full-length HTLV-1 LTR and a 21-bp repeat reporter in 293T cells, no significant difference in Tax transactivation was detected, except for some HTLV-2a Tax isolates, including the Mo prototype. It was suggested that all HTLV-2 subtypes except the HTLV-2a subtype have a pathogenic potential equivalent to that of HTLV-1.[27] Unfortunately this study was not designed to address this issue, but the Brazilian HTLV-2 isolates of the present study could help us to add information concerning this matter in the future.

The molecular characterization of the LTR, *env*, and *tax* region of HTLV-2 isolates from southern and southeastern Brazil corroborated the results obtained in a previous study of the LTR region[22] and with data from the literature, in which Brazilian isolates clustered in the clade of the HTLV-2a subtype.[13,15,21] The phylogenetic tree of the *env* region showed the presence of a group almost exclusively of IDUs, also found in the LTR and *tax* trees. These clades could represent a different route of virus transmission among IDUs or a little diverse evolutionary virus rate, as described in European IDUs.[22,28] Furthermore, the clade in the *env* phylogenetic tree, which contains isolates from a specific geographic region (Jundiaí city 37 miles from São Paulo city), and the clades of the same sequences in the LTR and *tax* phylogenetic trees allow us to suggest that the risk factor for acquiring retroviruses in patients from Jundiaí was the use of injecting drugs. In addition, a single local monophyletic subcluster closed to IDUs suggests *in situ* dissemination of a local clade.

The HTLV-2b subtype found in one sample (BRLO38-02) of the present study (the *env* and *tax* regions) was associated with ethnic background; the patient was from Paraguay, a country endemic for HTLV-2b. Consistent with this finding, the HTLV-2b subtype has been found in the state of Rio Grande do Sul in Brazil,[20] bordering countries where the HTLV-2b subtype prevails. We could speculate that with the commercial agreement of Mercosul, the increasing population flux across borders warrants monitoring this viral subtype in the country.

When the present sequences were compared with the HTLV-2 Mo prototype, a high nucleotide similarity of 99.2% in the *env* region was observed, and this result is in line with the results found in IDUs living in Salvador (99%), northeastern Brazil.[19] Still, the amino acid change S1909P in 100% of the *env* sequences was also previously reported in Brazilian strains, and could represent a molecular signature of HTLV-2a (variant 2c) strains from Brazil.[21,29]

In relation to the *tax* sequences, the present results corroborated the previous reports that characterize the Brazilian HTLV-2c variant (often considered a molecular variant of -2a), highlighting the long Tax protein.[7,13,15] In addition, high similarity in *tax* was observed among isolates from the present study (99.9%) and also in relation to the Mo prototype (99.3%). Therefore, it is necessary to emphasize that HTLV replication, unlike other retroviruses, is primarily through the clonal expansion of cells that are infected via mitosis and not so much by the use of the reverse transcriptase.[30] During mitosis the cellular DNA polymerases are used and the new cells contain high fidelity copies of the original provirus.[11] This fact could help to explain why HTLV has high genetic similarity, as opposed to HIV.[31]

Concerning the evolutionary rate of HTLV-2, Salemi and collaborators calibrated a molecular clock of the LTR region of HTLV-2 and estimated a fixation rate between 1.08×10^{-4} and 2.7×10^{-5} nucleotide substitutions per site per year for the 2a and 2b European IDU strains, and concluded that this rate is very low among RNA viruses.[26] For example, HIV-1 (the *env* region) was measured up to 1.6×10^{-2} substitutions per site per year.[32]

High prevalences of HTLV-2 have been described in geographically isolated groups that should have different viral dynamics and evolution.[11,26,33] Interestingly, phylogenetic analysis of LTR sequences from the Kayapo Indians of Brazil clustered the sequences in a phylogroup named A-II, in which sequences from one Mexican prostitute and two prostitutes from Ghana and Cameroon clustered together.[34] This finding could suggest that HTLV-2a may have evolved from a common ancestor long before the HTLV-2a-infected ancestors of the Kayapo introduced this subtype into the Americas. Corroborating this hypothesis, Mauclère and collaborators proposed that HTLV-2 appeared in Africa, and then some of the strains left this continent during the period of human migration and became the ancestors of HTLV-2a/c or HTLV-2b. Additionally they also speculated that the HTLV-2b-bearing populations may have migrated through Asia and then separated into subgroups: some went to America and others returned to Africa. Lastly, the remaining virus became the HTLV-2d subtype.[35] Also other studies agreed that HTLV-2 was originally brought from Asia into the Americas during the migration of the Asian populations over the Bering land bridge.[33,36,37] These data and Mauclère's data accommodate the hypothesis of the entrance of HTLV-2 into the Americas by the Bering land bridge a long time ago.

In Europe it is believed that the -2a subtype was introduced to IDUs in Eastern Europe and the -2b in Western Europe in at least two separate periods.[37] In Spain and Italy, for example, HTLV-2b remains the most prevalent subtype, although today new cases of this infection are decreasing.[38,39] In Brazil, all data point to the HTLV-2c molecular variant as formerly present in Indians native tribes, with posterior dissemination to the urban population during its formation, through interethnic contact during the intense event of miscegenation, by sexual intercourse, and is maintained in Indians mostly by breast feeding.[7,13,19,33,34]

In conclusion, since we demonstrated the absence of the HTLV-2b and prototype North American HTLV-2a subtypes in the present study population, we suggest that these individuals had little interaction with individuals or blood products from other geographic areas, and also with individuals coinfected with HIV/HTLV-2 outside Brazil. In addition, we could speculate that there were different routes and origins of HIV and HTLV-2 in Brazil, probably through prior infection by HTLV-2 among IDUs and later on HIV. Because of the development and recent increase in the population movement in South American migration in the past years, surveillance of HTLV-2 infection is required and opportune in Brazil.

Sequence Data

The GenBank accession numbers of the 71 HTLV-2 new sequences obtained in our laboratory and included in the phylogenetic analysis are as follows: **LTR**: BRLO37-02

(JQ435902), BRSP91-08 (JQ435903), BRSP160-08 (JQ435904), BRSP171-08 (JQ435905), BRSP239-08 (JQ435906), BRSP319-08 (JQ435907), BRSP348-08 (JQ435908), BRSP84-09 (JQ435909), BRSP130-09 (JQ435910); ***env***: BRLO02-02 (HM770414), BRLO03-02 (HM770390), BRLO05-02 (HM770391), BRLO07-02 (HM770392), BRLO09-02 (HM770393), BRLO10-02 (HM770394), BRLO11-02 (HM770395), BRLO12-02 (HM770396), BRLO18-02 (HM770397), BRLO19-02 (HM770398), BRLO21-02 (HM770399), BRLO22-02 (HM770400), BRLO23-02 (HM770401), BRLO24-02 (HM770402), BRLO25-02 (HM770415), BRLO26-02 (HM770403), BRLO27-02 (HM770404), BRLO28-02 (HM770405), BRLO29-02 (HM770406), BRLO32-02 (HM770407), BRLO35-02 (HM770408), BRLO37-02 (JQ435911), BRLO38-02 (HM770409), BRLO39-02 (HM770410), BRLO43-02 (HM770411), BRLO45-02 (HM770412), BRLO49-02 (HM770413), BRSP91-08 (HM770416), BRSP111-08 (HM770417), BRSP160-08 (HM770418), BRSP171-08 (HM770423), BRSP172-08 (HM770423), BRSP239-08 (HM770419), BRSP319-08 (HM770420), BRSP348-08 (HM770425), BRSP84-09 (HM770421), BRSP130-09 (HM770422); ***tax***: BRLO02-02 (JN887712), BRLO03-02 (JN887713), BRLO09-02 (JN887714), BRLO12-02 (JN887715), BRLO19-02 (JN887716), BRLO21-02 (JN887717), BRLO22-02 (JN887718), BRLO26-02 (JN887719), BRLO27-02 (JN887720), BRLO28-02 (JN887721), BRLO31-02 (JN887722), BRLO32-02 (JN887723), BRLO35-02 (JN887724), BRLO37-02 (JN887725), BRLO38-02 (JN887726), BRLO39-02 (JN887727), BRLO43-02 (JN887728), BRLO45-02 (JN887729), BRLO49-02 (JN887730), BRSP171-08 (JN887731), BRSP172-08 (JN887732), BRSP239-08 (JN887733), BRSP319-08 (JN887734), BRSP348-08 (JN887735), BRSP130-09 (JN887736).

The GenBank accession numbers of the HTLV-2 reference sequences included in the phylogenetic study are as follows: **LTR:** BRLO7-02 (GU573730), BRLO9-02 (GU573731), BRLO12-02 (GU573732), BRLO18-02 (GU573733), BRLO19-02 (GU573734), BRLO21-02 (GU573735), BRLO22-02 (GU573736), BRLO23-02 (GU573737), BRLO24-02 (GU573738), BRLO26-02 (GU573739), BRLO27-02 (GU573740), BRLO28-02 (GU573741), BRLO29-02 (GU573742), BRLO31-02 (GU573743), SFIFU6 2 (U73022), NOR2N (U10258), ATL18 (U10252), SMH2 (Y09148), SFIFU5 5 (U73010), PUEBRB (U10262), Mo (M10060), LA8A (U10256), NAV.DS (U10257), Oklnd15 8 (U73015), IVDUros (AF054272), PH230PCAM (Z46838), Mexy17 (L42510), GHKT (L42507), RP329 (AF326583), K96 (AF326584), Kayapo78 (AF139388), SP-WV (AF139382), Kayapo73 (L42509), BRPOA6 (DQ028606), BRPOA12 (DQ028613), BRPOA9 (DQ028608), Belem10 (AF139393), Belem02 (AF139392), BH223 (AY509600), BH339 (AY509602), RC (L77235), SPAN130 (U10266), PortVs (AY622979), PortNn (AY622978), I-IT (Y09151), Gu (X89270), I-OV (Y09155), ITA47A (U10254), NY185 (U10259), RVP (L77244), ITA50A (U10255), SFIFU6-4 (U73018), BRPOA10 (DQ028611), FOR6 (AF054273), NRA (L20734), Oklnd14-17 (U73009), WYU2 (U12794), SEM1050 (U10263), PYGCAM1 (Z46888), PENN7A (U10260), PUEB AG (U10261), Pilaga (AF054271), Gab (Y13051), WYU1 (U12792), G12 (L11456), BRPOA11 (DQ028612), Efe2 (Y14365); ***env***: Mo (M10060), MO15A (K02024), RP329 (AF326583), Gab (Y13051), Gu (X89270), FLW (S67545), AF412314 (AF412314), SP-WV (AF139382), G2 (AF074965), G12 (L11456), NRA (L20734), k96 (AF326584), Efe2 (Y14365); ***tax***: Mo (M10060), K96 (AF326584), NRA (L20734), G12 (L11456), RP329 (AF326583), BAIDU2 (AF401496), SP-WV (AF139382), SP2 (U32872), SP1 (U32873), FUC (U32882), PAR (U32880), KAY1 (U32875), KAY2 (U32874), G2 (AF074965), Gab (Y13051), Gu (X89270), PR-46 (DQ022075), Efe2 (Y14365).

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Author Disclosure Statement

No competing financial interests exist.

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