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Predominance of Human Lymphotropic T-cell Virus type 2 (HTLV-2) subtype b in urban populations of Argentina.

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ABSTRACT

HTLV-2b infection has been described among aborigines from Northern Argentina, while HTLV-2a has been described in an injecting drug user (IDU) from a Central region, similar to the situation in Spain, USA and Brazil. In this study, 22 of the 26 strains analyzed from blood donors and HIV-1+ individuals were HTLV-2b (84.6%) clustering with Amerindian references, while 4 HIV-1+ (15.4%) were HTLV-2a. HTLV-2a sequences were closely related to Brazilian references in contrast to the previous Argentinean IDU strain which clustered with Africans and Amerindians from North America. In summary, these findings show that HTLV-2b is the major strain circulating in an urban population of Argentina. HTLV-2a/b could have been introduced from endemic South American countries such as Brazil and due to the contact with other populations such as IDUs from Europe despite its introduction due to the increasing internal migration of aborigines to big urban centers. Considering this results and recent data about the dissemination of HTLV-1 in residents of Buenos Aires city, new studies among non-at-risk groups for HTLV-1/2 infection should be performed.

Keywords: HTLV-2, phylogeny, HIV-1, blood donors, Argentina

INTRODUCTION

Human T-Lymphotropic Viruses (HTLVs) and their analogues Simian T-Lymphotropic Viruses (STLVs) are collectively called Primate T-Lymphotropic Viruses (PTLVs) with PTLV-1, PTLV-2, and PTLV-3 being composed of both viruses (HTLV/STLV), respectively. The PTLV-4 group currently has only one member, HTLV-4, since a simian counterpart has yet to be identified [1]. Human T- Lymphotropic virus type 1 and 2 were the first human retroviruses to be discovered in 1980 and 1982, respectively [2, 3]. While HTLV-1 infection is mainly associated with adult T-cell leukaemia and with a chronic neurological disorder, known as tropical spastic paraparesis/ HTLV-1 associated myelopathy (HAM/TSP) [3, 4]; HTLV-2 is not associated with a specific disease, but there is accumulating evidence that the infection may be related to neurological disorders similar to HAM/TSP and increased rates of infectious diseases [5-8]. Both retroviruses can be transmitted through sexual contact, blood transfusion and by sharing injecting equipment as well as from mother to child, mainly by prolonged breast-feeding [9-12].

These retroviruses are estimated to be infecting 15-20 million people in the world and is known to be endemic among aborigine groups throughout the Americas, certain Pygmy groups in Africa and injecting drug users (IDUs) worldwide [13-22]. In the Americas, an ethnic/geographic restriction of the infection has been observed being HTLV-1 detected in natives of the highlands from Venezuela, Colombia, Peru, Bolivia, and Chile and HTLV-2 among Amerindians from the lowlands of Venezuela, Colombia, Paraguay, and Brazil [23].

Phylogenetically, HTLV-2 is divided in three subtypes: HTLV-2a, HTLV-2b and HTLV-2d [10, 24-26]. Subtype HTLV-2a is present mainly in IDUs of Brazil, USA, Northern Europe, South East Asia (Vietnam) and some original communities such as Navajo and Pueblo in the USA and Kayapo in Brazil [18, 27-32]. HTLV-2b is present in Bakola Pygmies of Cameroon, inhabitants of Zaire and Gabon and is also predominantly distributed among Amerindian populations such as the Navajo, Pueblo and Seminole in the USA, the Guaymi

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in Panama, the Guahibo in Venezuela, the Wayu and the Tunebo in Colombia and the Tiriyos in Brazil [18, 20, 32-36]. This subtype was also reported in IDUs in Southern Europe (Italy and Spain) and urban populations in Brazil [27, 30, 37, 38]. In Brazil, a unique HTLV-2 subtype was detected among IDUs, HIV-1 infected individuals and Amerindians, formerly called HTLV-2c [10, 24, 39, 40]. HTLV-2c was characterized by its separate clustering within subtype HTLV-2a [24]. This subtype possessed a long transactivating protein Tax similar to HTLV-2b and the *env*, and LTR genomic regions similar to HTLV-2a, albeit complete nucleotide sequences of several genomes had unequivocally demonstrated that the Brazilian HTLV-2c strains were molecular variants of the HTLV-2a subtype [35, 39, 41, 42]. Subtype d has been found in an Efe Pygmy population in Congo, Africa [26].

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In Argentina, similarly to other South American countries, an ethnic/geographic restriction for HTLV-1/2 has been observed. There is a known endemic region for HTLV-1 infection in the Northwest among Aymara populations; and an HTLV-2 endemic region in the Northern Gran Chaco among Tobas, Wichis and Pilagas [43, 44]. On the other hand, the infection has also been described among blood donors and different high-risk groups of the country [45, 46]. Concerning the phylogenetic characterization, while only subtype HTLV-2b has been reported as endemic in Amerindians from Gran Chaco region, only one case of HTLV-2a was described in a non-aboriginal HIV positive IDU from Rosario, Santa Fe, Central area of Argentina [9, 47]. The objective of this study was to carry out the phylogenetic characterization and the subtypification of HTLV-2 positive individuals in an urban population of Argentina.

MATERIALS AND METHODS

Population studied

The present study included samples from 26 HTLV-2 positive individuals, seven of them referred to the National Reference Center for AIDS in Buenos Aires, Argentina for HTLV-1/2 diagnosis and 19 were HIV-1 seropositive individuals enrolled in a previous epidemiological study [48, 49]. After a personal interview with healthcare staff, patients were invited to sign an informed consent. Institutional review board and the scientific ethical committee at the University of Buenos Aires approved the study protocols. Enrollment and data collection procedure details have been previously described elsewhere [48, 49].

Serology and molecular diagnosis for HTLV-1/2

Antibody screening for HTLV-1/2 was performed by particle agglutination technique (SERODIA-HTLV-I, FUJIREBIO, Tokyo, Japan) and reactive samples were subjected to Western Blot confirmation (HTLV blot 2.4, Genelabs Diagnostics, Science Park, Singapore). According to the manufacturer criteria, HTLV-2 positive samples showed reactivity to p19 or p24, GD21 and rgp46-II. Indeterminate and HTLV samples were subjected to an "in-house" nested-PCR to amplify *tax* and *pol* genes. Amplification of *pol* region was performed with outer primers SK-110-I/SK-111-I, SK-110-II/SK-111-II and inner primers pol 1.1/pol 3.1, pol 1.2/pol 3.2 for HTLV-1 and HTLV-2 respectively [50]. Amplification of *tax* region was carried out with outer primers SK-43-I/SK-44-I, SK-43 II/SK-44 II and inner primers SK-43/SK-44 specific for HTLV-1/2 [50, 51]. The size of the n-PCR products were 135bp for *pol* of HTLV-1 and 137bp for *pol* of HTLV-2 and 128bp for both HTLV-1/2 *tax* amplification [51].

Molecular and Phylogenetic analysis

Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradient separation (Pharmacia, Sweden). DNA was extracted using QIAamp DNA extraction kit (QIAGEN, Hilden, Germany). In order to perform the phylogenetic analysis,

the LTR was amplified by nested-PCR by using BSQF6/BSDR3 as outer primers, and BSQF2/BSDR4 as inner primers, respectively (LTR 665bp, Mo genome reference, position 8253-8918) [32, 52]. Direct sequencing reactions were done by using an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Version 3.0 mixture (Applied Biosystems). Sequences were generated on an ABI Prism® 3100 Genetic Analyzer according to the manufacturers' instructions. Sequence alignment was carried out by using Clustal W (BioEdit 7.0.4.1 sequence alignment editor) and hand optimization was performed. [53, 54]. The phylogenetic analysis was performed by Neighbour Joining (NJ) by using MEGA 4.0 [55] and the topology obtained was confirmed by Maximum Likelihood (ML) with the PHYML programme [55]. The substitution model was chosen using MODELTEST 3.0. The tree topology was visualized with TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

Statistical analysis

The similarity percentage data was obtained by comparing the studied and prototype sequences belonging to each subtype (Kruskal-Wallis test). The estimated mean was 95% Confidence Interval.

RESULTS

Epidemiological features

A total of 26 HTLV-2 positive individuals were studied of which 9 (34.6%) were females and 17 (65.4%) males. Out of the total, seven were blood donors (BD) and 19 were HIV-1 positive individuals of which 17 had a history of injecting drug use and the remaining two had an HIV-1 positive partner. Considering the residing place one was a resident of Formosa city in the Northern Province of Formosa (BDAR1), one was residing in Ushuaia city, the Southernmost province of Tierra del Fuego (BDAR4) and the remaining 24 individuals were residents of Buenos Aires city. Epidemiological and clinical features of the study population are given in Table 1.

Serological and molecular characterization

Concerning the serological status, 22 (84,6%) samples were HTLV-2 seropositive by Western Blot (complete profile), two (7.7%) (BDAR6 and BDAR7) were HTLV and two (7,7%) exhibited an indeterminate pattern (according to the stringent criteria issued by the HTLV European Research Network). The indeterminate samples (IDU2 and IDU9), both belonging to the IDU population, showed different Western Blot profiles exhibiting the bands corresponding to GD21, p24, p28, p32 and p53 (IDU2); and GD21 and p24 (IDU9), respectively. Amplification of *tax* and *pol* genes confirmed HTLV-2 infection in both HTLV and seroindeterminate cases.

Phylogenetic and Sequence analyses

To construct a comprehensive phylogenetic dataset, the sequences reported here were aligned with 76 HTLV-2 reference strains obtained from the GenBank database, preferentially those belonging to neighbouring countries with high migration rates to Argentina and reference sequences previously reported worldwide. An STLV-2 reference strain (PP1664) was used as outgroup. Once aligned, the dataset consisted of 596bp corresponding to the 3'LTR region. Subtype HTLV-2d was consistently separated from subtypes HTLV-2a and HTLV-2b while the former two subtypes showed a bootstrap value of 84% supporting these two clusters.

Out of the 26 samples, four samples belonging to HIV-1 positive individuals (three of whom were also IDUs and a partner of an HIV-1 seropositive individual) clustered within subtype a, closer to Brazilian isolates ~~previously described as HTLV-2c~~. The remaining 22 samples were HTLV-2 subtype b being 15 of them HIV-1 positive (14 IDUs and a partner of an HIV-1 seropositive individual) while the other seven were blood donors including one from Formosa province, HTLV-2 endemic area and one from Tierra del Fuego province, a non-endemic area (Figure 1).

The four new HTLV-2a sequences from HIV individuals (HIVAR1, HIVAR2, HIVAR14, HIVAR16) clustered with Brazilian references (BRPOA6, BRPOA8, BH223, BH339, KAY73, BAIDU148, BAIDU25, Belem10, Kayapo78, KAyapo79) ~~belonging to the formerly called HTLV-2c subgroup~~ with a high bootstrap value (69%) and form a clear distinct subgroup within subtype HTLV-2a (figure 1). The 22 remaining studied strains including all the blood donors clustered within HTLV-2b subtype (bootstrap value: 84%), being 20 of them closely related to previous isolations from Amerindians of Argentina, Colombia, United States and African individuals including a healthy male from Gabon and a Pygmy from Cameroon (Figure 1). Two HTLV-2b sequences belonging to two sisters who were blood donors (BDAR6 and BDAR7) inhabiting Buenos Aires but having parents from a province of the North of the country (Chaco) where HTLV-2 is present, clustered with a bootstrap value of 75% with strains from Amerindians of Colombia (WYU1) and Venezuela (G2) and also closely related to North American (SFIDU, SFIDU4) and European (SPAN129) IDUs strains. Especially in the four cases in which the individuals had a strong relation to endemic areas, there is a higher probability of having an Indian origin.

The sequence analysis for HTLV-2a samples compared to MO as a reference, revealed a similarity ranging from 96.1% (95% CI, 94.5-97.7) to 98.2% (95% CI, 96.9-99.3). Regarding HTLV-2b, sequence similarity to NRA ranged from 98.5% (95% CI, 97.4-99.6) to 99.3% (95% CI, 98.3-99.8). Among the new HTLV-2a sequences, HIVAR1 and HIVAR2 were identical (100% similitude); HIVAR15 showed differences in two nucleotide positions (T8502G and G8686C) respect to HIVAR1 and HIVAR2 with a 99.7% (95% CI, 98.8-99.9) similarity; and HIVAR16 resulted the most different sequence compared to HIVAR1, HIVAR2 and HIVAR15 with a similarity of 97.7% (95% CI, 96.4-99.9) and 14 nucleotide changes (Table 2).

When analyzing HTLV-2b, 13 sequences (BDAR1, HIVAR3, HIVAR4, HIVAR6, HIVAR7, HIVAR8, HIVAR10, HIVAR11, HIVAR12, HIVAR14, HIVAR17, HIVAR18, HIVAR19) showed a 100% similarity. When comparing these 13 sequences with BDAR2 and BDAR4, they showed a similarity of 99.5% (95% CI, 98.5-99.9) with three nucleotide changes (C8288T, A8321C and C8340T); BDAR5 revealed a similarity of 99.7% (95% CI, 98.8-99.9) with two nucleotide changes (G8347A and C8747G); HIVAR5, HIVAR9 and HIVAR13 showed a similarity of 99.8% (95% CI, 99.1-99.7) with one nucleotide change (G8597A, T8502G and A8366T, respectively) while for BDAR6 and BDAR7 were 99.2% (95% CI, 98.1- 99.7) with five nucleotide changes (A8485T, C8503G, T8504G, G8531A and T8596C).

The sequence analysis of these samples revealed 25 different polymorphisms within subtype HTLV-2a and 20 in HTLV-2b (Table 2) compared to MO and NRA, respectively. As shown in table 2, BDAR6 and BDAR7 were the most divergent sequences among HTLV-2b, closer to strains from Colombian and Venezuelan Amerindians.

DISCUSSION

This study describes for the first time the phylogeny of HTLV-2 strains circulating among urban populations in Argentina. Out of the 26 studied sequences, the majority of them clustered within HTLV-2b as previously described in other South American countries with an important Amerindian ethnic component. Moreover, this molecular genotype has been described in previous studies conducted in New York City, Vietnam and Southern Europe where HTLV-2b was more frequently found among IDUs populations. In contrast, HTLV-2a is found as predominant among IDUs from other regions of USA, Northern Europe (UK, Ireland and Sweden), Brazil and South East Asia (Vietnam) and also in Amerindians such as Navajo and Pueblo in the USA and Kayapo in Brazil [18, 27-32]. The only study conducted in Argentina in 1996 in an IDU from Rosario (IVDUros) reported the

presence of one HTLV-2a sequence [47]. In contrast, these results demonstrated that only four among 26 of the new HTLV-2 analyzed strains (HIVAR1, HIVAR2, HIVAR15, HIVAR16) clustered together with high bootstrap values within subtype a being subtype b the major circulating strain of HTLV-2 among IDUs of Buenos Aires city. These sequences were closely related to a group of HTLV-2a, subvariant c. While IVDUros was closer to African and North American aborigine isolates, the new HTLV-2a sequences were found to be closely related to Brazilian aborigine isolates suggesting the introduction of these strains from Brazil.

On the other hand, our data shows that 84.6% (22/26) of the HTLV-2 strains circulating in this studied population of Argentina belongs to subtype b. Out of the 22 HTLV-2b sequences, 14 belonged to HIV-1 positive-IDU individuals Furthermore, all 7 blood donors clustered within this subtype, suggesting that b is also circulating in blood donors in non-endemic areas such as Buenos Aires and the Southernmost region of the country, Ushuaia. These individuals did not report any risk factor except for BDAR3 who received a blood transfusion. Within subtype b, the two blood donors who were sisters residing in Buenos Aires positioned in a different cluster among Colombian, Venezuelan and Panamean strains from aborigines (WYU1, Y5 and G12) suggesting some degree of molecular diversity, which was also confirmed in the analysis of nucleotide similarity.

HTLV-2 subtype b was previously described as endemic in Amerindians, including Pilagas and Wichis of Gran Chaco Region in Argentina. The presence of this subtype among urban inhabitants of the country supports the theory that there had been significant interaction between these groups and aborigine populations. The antecedents associated to HTLV-2 infection were having received a blood transfusion and having relation to endemic areas as four blood donors lived and/or had relatives coming from these areas. Moreover, this data correlates to migrations of aborigines to big cities and the introduction of drugs in this population. Although Buenos Aires city is considered non-endemic, a high migration

rate from HTLV-2 endemic areas, to the capital city has been observed over the last years. There has also been a high rate of migration and tourism to and for Argentina, especially from Southern Europe, USA and Brazil which could have contributed to the spread of HTLV-2b in our country.

Regarding the sequence analysis of these samples, they revealed 25 different polymorphisms within subtype HTLV-2a and 20 in HTLV-2b compared to MO and NRA reference strains, respectively. It is not surprising that these positions in HTLV-2a could be considered the total of differences supporting them in a divergent clade (subvariant c) from MO, especially if we consider the sequence similarity analysis confirmed by the low genomic variability of the studied strains (ranging from 1.9% to 3.9% for MO). Regarding HTLV-2b, most of the samples showed the same substitutions and the sequence similarity ranged from 0.7% to 1.5% compared to NRA. BDAR6 and BDAR7 were the most divergent sequences among HTLV-2b, with nucleotide substitutions similar to strains from Amerindians from Venezuela and Panama (Y5 and G12, respectively).

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In summary, this is the first HTLV-2 phylogenetic study performed in urban populations of Argentina, including individuals coming from the Northern and Southernmost provinces of the country. Our study confirms the presence of HTLV-2a and HTLV-2b as it occurs in other South American countries such as Brazil, suggesting a common origin of these strains. It is highly probable that HTLV-2 originated in Africa and was brought to the Americas with human migration across the Bering Strait 11,000 to 13,000 years ago[40, 56, 57]. On the basis of archeological, anthropological and genetic evidence, migration to South America may have occurred via two independent routes, one leading directly towards the Amazon region and another along the Pacific Coast, paralleling the Andes. These different migratory pathways taken by the Amerindian ancestors could have resulted in the introduction of HTLV-2a in Argentina from Brazil and of HTLV-2b being actively transmitted from aboriginal populations to, and subsequently within, urban

areas. Moreover, HTLV-2a could have also been introduced in urban areas of Argentina due to the contact with other populations such as IDUs from Europe.

Besides, this study confirms the presence of HTLV-2a among HIV-1 positive individuals of Argentina although HTLV-2b was the predominant subtype infecting this population and blood donors suggesting its introduction by the increasing interaction with aborigine populations.

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SEQUENCE DATA

The 26 LTR sequences presented here are available at GenBank with nucleotide accession numbers: GenBank ID: JN222942 (BDAR1), GenBank ID: JN222943 (BDAR2), GenBank ID: JN222944 (BDAR3), GenBank ID: JN222945 (BDAR4), GenBank ID: JN222946 (BDAR5), GenBank ID: JN222947 (BDAR6), GenBank ID: JN222948 (BDAR7), GenBank ID: JN222949 (HIVAR1), GenBank ID: JN222950 (HIVAR2), GenBank ID: JN222951 (HIVAR3), GenBank ID: JN222952 (HIVAR4), GenBank ID: JN222953 (HIVAR5), GenBank ID: JN222954 (HIVAR6), GenBank ID: JN222955 (HIVAR7), GenBank ID: JN222956 (HIVAR8), GenBank ID: JN222957 (HIVAR9), GenBank ID: JN222958 (HIVAR10), GenBank ID: JN222959 (HIVAR11), GenBank ID: JN222960 (HIVAR12), GenBank ID: JN222961 (HIVAR13),

GenBank ID: JN222962 (HIVAR14), GenBank ID: JN222963 (HIVAR15), GenBank ID: JN222964 (HIVAR16), GenBank ID: JN222965 (HIVAR17), GenBank ID: JN222966 (HIVAR18) and GenBank ID: JN222967 (HIVAR19). The accession numbers of the HTLV-2 LTR reference strains used in the phylogenetic analysis are as follow: PP1664 (GenBank ID: Y14570), Efe2 (GenBank ID: Y14365), GuyII (GenBank ID: AF262408), BH223 (GenBank ID: AY509597), BH339 (GenBank ID: AY509599), BAIDU86 (GenBank ID: AF401492), Kay73 (GenBank ID: L42509), Tyrio80 (GenBank ID: AF139391), SPWV (GenBank ID: AF139382), Kay139 (GenBank ID: L42508), BRAZA21 (GenBank ID: U10253), Kayapo83 (GenBank ID: AF139390), Oklnd15-8 (GenBank ID: U73015), IVDUros (GenBank ID: AF054272), PH230 (GenBank ID: Z46838), Dub991(GenBank ID: AF032993), NOR2N (GenBank ID: U10258), MO (GenBank ID: M10060), CH610 (GenBank ID: U46557), Pilaga (GenBank ID: AF054271), SPAN129 (GenBank ID: U10265), ITA50A (GenBank ID: U10255), Gu (GenBank ID: X89270), ny185 (GenBank ID: U10259), ITA47A (GenBank ID: U10254), PUERBAG (GenBank ID: U10261), WYU1 (GenBank ID: U12792), G12 (GenBank ID: L11456), SFIDU6-4 (GenBank ID: U73018), Y5 (GenBank ID: AF005395), PENN7A (GenBank ID: U10260), SEM1051 (GenBank ID: U10264), NRA (GenBank ID: L20734), FOR6 (GenBank ID: AF054273), Oklnd14-17 (GenBank ID: U73009), PYGCAM1 (GenBank ID: Z46888), Gab (GenBank ID: Y13051), SEM1050 (GenBank ID: U10263) and WYU2 (GenBank ID: U12794).

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TABLE 1. Epidemiological features of HTLV-2 seropositive blood donors and HIV-1 positive individuals of Argentina.

Group	Sample ID	Age/ gender	Residence place	Risk factors	HTLV-2 subtype
Blood donors	BDAR1	NI/F	Formosa	Born in an endemic area (Formosa)	HTLV-2b
	BDAR2	55/M	Buenos Aires	NOT KNOWN	HTLV-2b
	BDAR3	47/M	Buenos Aires	Born in Tucumán, blood transfusion	HTLV-2b
	BDAR4	45/M	Ushuaia	Born in an endemic area (Formosa)	HTLV-2b
	BDAR5	28/M	Buenos Aires	NOT KNOWN	HTLV-2b
	BDAR6	56/F	Buenos Aires	Parents from endemic area (Chaco)	HTLV-2b
	BDAR7	54/F	Buenos Aires	Parents from endemic area (Chaco)	HTLV-2b
HIV-1+	HIVAR1	23/F	Buenos Aires	SEX	**HTLV-2a**
	HIVAR2	31/M	Buenos Aires	IDU	**HTLV-2a**
	HIVAR3	39/M	Buenos Aires	IDU	HTLV-2b
	HIVAR4	26/M	Buenos Aires	IDU	HTLV-2b
	HIVAR5	35/M	Buenos Aires	IDU	HTLV-2b
	HIVAR6	43/F	Buenos Aires	SEX	HTLV-2b
	HIVAR7	28/M	Buenos Aires	IDU	HTLV-2b
	HIVAR8	32/M	Buenos Aires	IDU	HTLV-2b
	HIVAR9	28/F	Buenos Aires	IDU	HTLV-2b
	HIVAR10	21/F	Buenos Aires	IDU	HTLV-2b
	HIVAR11	24/M	Buenos Aires	IDU	HTLV-2b
	HIVAR12	28/M	Buenos Aires	IDU	HTLV-2b
	HIVAR13	45/F	Buenos Aires	IDU	HTLV-2b
	HIVAR14	30/M	Buenos Aires	IDU	HTLV-2b
	HIVAR15	29/M	Buenos Aires	IDU	**HTLV-2a**
	HIVAR16	31/M	Buenos Aires	IDU	**HTLV-2a**
	HIVAR17	28/M	Buenos Aires	IDU	HTLV-2b
	HIVAR18	29/M	Buenos Aires	IDU	HTLV-2b
	HIVAR19	24/F	Buenos Aires	IDU	HTLV-2b

Note: IDU, Intravenous Drug Users; NI: no information ; SEX: sexual transmission

FIGURES

Figure 1: Rooted Neighbour joining (NJ) tree of 103 HTLV-2 strains based upon a 596-bp fragment of the 3'LTR region. All 26 Argentinean new sequences are marked with squares (HIV positive) (■) and dots (BD) (●). The STLV strain PP1664 was used as outgroup. Numbers on branches indicate the support for each node. The geographic origin of reference strains included in this analysis are as follows from the bottom to the top: PP1664 (Simian), Efe2 (Congo), GuyII (French Guyana), BH223 (Brasil), BH339 (Brasil), BAIDU86 (Brasil), Kay73 (Brasil), Tyrio80 (Brasil), SPWV (Brasil), Kay139 (Brasil), BRAZA21 (Brasil), Kayapo83 (Brasil), Oklnd15-8 (USA), IVDUros (Argentina), PH230 (Cameroon), Dub991(Ireland), NOR2N (Europe), MO (USA), CH610 (Paraguay/Argentina), Pilaga (Argentina), SPAN129 (Spain), ITA50A (Italy), Gu (Europe), ny185 (USA), ITA47A (Italy), PUERBAG (USA), WYU1 (Colombia), G12 (Panama), SFIDU6-4 (USA), Y5 (Venezuela), PENN7A (USA), SEM1051 (USA), NRA (USA), FOR6 (Argentina), Oklnd14-17 (USA), PYGCAM (Cameroon), Gab (Gabon), SEM1050 (USA) and WYU2 (Colombia).