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Divergent Strains of Human T-Lymphotropic Virus Type 1 (HTLV-1) within the Cosmopolitan Subtype in Argentina

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Abstract

HTLV-1 Cosmopolitan subtype Transcontinental subgroup A has been described among aboriginal communities from the northwest endemic area of Argentina. Moreover, Transcontinental subgroup A and the Japanese subgroup B were reported among blood donors from the nonendemic central region of the country. We carried out the first HTLV-1 phylogenetic study in individuals residing in Buenos Aires capital city. Phylogenetic analysis performed on the LTR region showed that all 44 new strains clustered within the Cosmopolitan subtype, with 42 (95.4%) belonging to Transcontinental subgroup A. Of them, 20 (45.5%) strains grouped in the large Latin American cluster and 4 (9.1%) in the small Latin American cluster. The majority of them belonged to individuals of nonblack origin, grouped with Amerindian strains. Three (6.8%) were closely related to South African references and two monophyletic clusters including only HIV/HTLV-1 coinfected individuals were observed. Interestingly, two (4.5%) new sequences (divergent strains) branched off from all five known Cosmopolitan subgroups in a well-supported clade. In summary, these findings show that HTLV-1 Cosmopolitan subtype Transcontinental subgroup A is infecting residents of Buenos Aires, a nonendemic area of Argentina, and confirm the introduction of divergent strains in the country.

Introduction

Human T cell lymphotropic virus type 1 (HTLV-1) and simian T-lymphotropic virus (STLV) belong to the primate T-lymphotropic viruses (PTLVs), classified within four distinct groups: PTLV-1, 2, 3, and 4. While HTLV-1, 2, and 3 have their counterparts in STLVs, only HTLV-4 has recently been identified within the PTLV-4 group.[1] HTLV-1 is phylogenetically divided in four major subtypes: Cosmopolitan (a), Central African (b and d), and Melanesian (c).[2–6] The Cosmopolitan subtype is disseminated worldwide and is composed mainly of five subgroups: Transcontinental (A), Japanese (B), West African (C), North African (D), and Black Peruvian (E).[2,7–9] HTLV-1 is the etiological agent of both adult T cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[10,11] It has also been associated with a number of inflammatory diseases, including uveitis, pediatric infectious dermatitis, polymiositis, and cases of arthritis. [12–15] Viral transmission occurs through sexual contact, blood transfusion, and by sharing injecting equipment as well as from mother to child, mainly by prolonged breastfeeding.[16,17] An estimated 10–20 million people worldwide are infected with HTLV-1. Although the majority of seropositive individuals are considered to be asymptomatic carriers, 5–10% of them develop either ATL or HAM/TSP. The infection is distributed worldwide with local regions of high prevalence including southern Japan, intertropical Africa, the Caribbean, some areas in the Middle East, Melanesia, and South America.[18]

In the Americas, it has been suggested that the HTLV-1 Cosmopolitan subtype was introduced during the multiple pre-Columbian Mongoloid migrations over the Bering Strait, through the post-Columbian migrations during the African slave trade and the Japanese ones. Later, molecular characterization demonstrated that the Transcontinental subgroup A was the most widely distributed. In addition, the Japanese subgroup B was detected in Peru and Brazil (in Japanese immigrants) and the Black Peruvian subgroup E in Peru (from natives of black origin).[8,19]

In Argentina, similar to the other South American countries, an ethnic/geographic restriction for HTLV-1/2 has

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been observed, with the northwest region an endemic area for HTLV-1 and its associated pathologies.[20–22] On the other hand, the infection has also been described among blood donors and different high-risk groups in nonendemic areas of the country.[23–27] Concerning phylogenetic characterization, the Transcontinental subgroup A has been reported in natives and blood donors.[28–30] Recently, the Japanese subgroup B was also detected in two individuals from nonendemic areas.[31]

In recent years, there has been an increasing migration rate from different areas including HTLV-1 endemic regions to Buenos Aires capital city, a nonendemic area of Argentina. Considering this fact, the objective of this study was to carry out a phylogenetic characterization and HTLV-1 strain subtypification of individuals from different at-risk and not at-risk groups residing in Buenos Aires city.

Materials and Methods

Study population

The present study included samples from 44 HTLV-1-positive individuals, some of them referred to the National Reference Center for AIDS for HTLV diagnosis, which were classified as blood donors (BD), pregnant women (PW), HTLV-1-associated myelopathy/tropical spastic paraparesis patients (Neu), and adult T cell leukemia patients (ATL). Other samples were from patients enrolled in previous epidemiological studies.[25–27] After a personal interview with the healthcare staff, patients were invited to sign an informed consent. Enrollment and data collection procedure details have been previously described.[24–27] Participants were classified according to the behavioral information reported during the interview [men who have sex with men (MSM), female sex workers (FSWs), and injecting drug users (IDUs)]. There were a total of 14 BD and 2 PW in the not at-risk group and 3 MSM, 6 FSWs, and 5 IDUs in the at-risk group. There were also 12 Neu and 2 ATL who were clinically diagnosed after a confirmed HTLV-1 infection (Table 1).

Serology

Antibody screening for HTLV-1 was performed by the 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). All samples were also tested for HIV-1 antibodies by particle agglutination assay (SFD HIV $^1/_2$ PA, Bio-Rad, Fujirebio) and by enzyme-linked immunosorbent assay (ELISA) (Enzygnost Anti-HIV $^1/_2$ Plus, Dade Behring). Reactive samples were confirmed by Western blot (New Lav Blot 1, Bio-Rad).

Molecular and phylogenetic analysis

Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Hypaque gradient separation (Pharmacia, Sweden). DNA was extracted using the QIAamp DNA extraction kit (QIAGEN, Hilden, Germany).

Indeterminate and HTLV-1 and -2 coinfected samples were subjected to an "in-house" nested polymerase chain reaction (PCR) to amplify *tax* and *pol* genes. Amplification of the *pol* region was performed with outer primers SK-110-I/SK-111-I and SK-110-II/SK-111-II and inner primers pol 1.1/pol 3.1 and pol 1.2/pol 3.2 for HTLV-1 and HTLV-2, respectively.[32] Amplification of the *Tax* region was carried out with outer primers SK-43-I/SK-44-I and SK-43 II/SK-44 II[32] and inner primers SK-43/SK-44 specific for HTLV-1/2.[33] The sizes of the n-PCR products were 135 bp for *pol* of HTLV-1, 137 bp for *pol* of HTLV-2, and 128 bp for both HTLV-1/2 tax amplification. Restriction enzyme assays for subtyping were performed as described by Tuke *et al*.[33] In order to perform the phylogenetic analysis, the 3′ LTR region was amplified by heminested PCR by using 8200LA/3Vext as outer primers and 8200LA/3Vint as inner primers (528 bp, ATK-1 genome position 8196–8699).[34] 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).[35] BioNeighborjoining (BIONJ) trees were constructed under the HKY+G model with MEGA3.[36] The tree topology obtained was confirmed by a maximum likelihood (ML) analysis performed with PAUP 4.0.[37] The nucleotide evolutionary model was inferred using Modeltest.[38] Model parameters were selected by the model averaging method. The ML tree searches were driven by a batch file containing instructions to perform 10 random addition sequences (RAS) followed by tree bisection reconnection (TBR) holding one tree per replication. Bootstrap values for the ML analysis were obtained with the PHYML program.[39]

Statistical analysis

The similarity percentage data were obtained by comparing the studied and prototype sequences belonging to each subtype (Kruskal-Wallis test). The estimated mean was 95% confidence interval. The Shimodaira–Hasegawua test was performed to statistically compare the likelihood of tree topology obtained by BIONJ and ML methods. In addition, we used the same test to analyze the phylogenetic origin of the divergent strains described here.

Results

Study population

A total of 44 HTLV-1-positive individuals of nonblack origin were studied; 28 (63.6%) were female and 16 (36.4%) were male. Epidemiological and clinical features of the study population are shown in Table 1.

Serological and molecular characterization

Concerning the serological status, 39 (88.6%) samples were HTLV-1 seropositive by Western blot (complete profile), 1 (2.3%) exhibited HTLV-1 and HTLV-2 profiles, and 4 (9.1%) showed indeterminate patterns (according to the stringent criteria issued by the HTLV European Research Network). The indeterminate samples showed different Western blot profiles exhibiting the bands corresponding to p24 (IDU1); p19, p24, and p26 (MSM2); p19 (MSM3); and p19, p26, and p28 (FSW6), respectively. Amplification of *tax* and *pol* genes confirmed all indeterminate cases as HTLV-1 positive and one case as coinfected with HTLV-1 and 2 (IDU6).

Table 1. Epidemiological Features of 44 HTLV-1-Infected Individuals Included in This Study[a]

Group	*Sample ID*	*Age/gender*	*Clinical status*	*Birth place*	*Residence place*	*Risk factors*
Patients with HTLV-1-associated diseases	Neu1	23/F	HAM/TSP	Peru	Buenos Aires	No
	Neu2	25/F	HAM/TSP	Peru	Buenos Aires	No
	Neu3	35/M	HAM/TSP	Argentina	Buenos Aires	HIV^+
	Neu4	67/F	HAM/TSP	Argentina	Chubut/Bs As	No
	Neu5	55/M	HAM/TSP	Argentina	Buenos Aires	HIV^+
	Neu6	25/F	HAM/TSP	Peru	Buenos Aires	No
	Neu7	34/F	HAM/TSP	Argentina	Buenos Aires	No
	Neu8	50/M	HAM/TSP	Argentina	Buenos Aires	No
	Neu10	56/F	HAM/TSP	Argentina	Buenos Aires	No
	Neu11	19/M	HAM/TSP	Peru	Buenos Aires	No
	Neu12	71/F	HAM/TSP	Argentina	Buenos Aires	No
	Neu13	37/F	HAM/TSP	Argentina	Buenos Aires	No
	ATL1	48/F	ATL	Argentina	Buenos Aires	No
	ATL2	67/F	ATL	Argentina	Buenos Aires	No
Pregnant women	PW1	25/F	Healthy carrier	Bolivia	Buenos Aires	No
	PW2	41/F	Healthy carrier	Argentina	Buenos Aires	HIV^+
Blood donors	BD1	45/M	Healthy carrier	Argentina	Buenos Aires	No
	BD2	40/F	Healthy carrier	Argentina	Buenos Aires	HIV^+
	BD3	43/M	Healthy carrier	Paraguay	Buenos Aires	No
	BD4	22/M	Healthy carrier	Argentina	Buenos Aires	No
	BD7	62/F	Healthy carrier	Argentina	Buenos Aires	Japanese descendant
	BD8	57/M	Healthy carrier	Argentina	Buenos Aires	No
	BD10	52/F	Healthy carrier	Argentina	Buenos Aires	No
	BD11	34/M	Healthy carrier	Argentina	Buenos Aires	No
	BD12	48/F	Healthy carrier	Peru	Buenos Aires	No
	BD13	35/F	Healthy carrier	Argentina	Buenos Aires	No
	BD14	46/F	Healthy carrier	Argentina	Buenos Aires	No
	BD15	54/F	HAM/TSP	Argentina	Buenos Aires	HIV^+
	BD16	39/M	Healthy carrier	Peru	Buenos Aires	No
	BD17	51/F	Healthy carrier	Argentina	Buenos Aires	No
Injecting drug users	IDU1	28/M	Healthy carrier	Argentina	Buenos Aires	IDU/HIV^+
	IDU2	29/M	Healthy carrier	Argentina	Buenos Aires	IDU/HIV^+
	IDU3	43/F	Healthy carrier	Argentina	Buenos Aires	IDU/HIV^+
	IDU4	34/M	Healthy carrier	Argentina	Buenos Aires	IDU/HIV^+
	IDU6	28/F	Healthy carrier	Argentina	Buenos Aires	$IDU/HTLV\text{-}2/HIV^+$
Female sex workers	FSW1	45/F	Healthy carrier	Peru	Buenos Aires	FSW
	FWS2	36/F	Healthy carrier	Argentina	Salta/Bs As	FSW
	FWS3	20/F	Healthy carrier	Argentina	Salta/Bs As	FSW
	FWS4	37/F	Healthy carrier	Argentina	Salta/Bs As	FSW
	FWS5	28/F	Healthy carrier	Argentina	Buenos Aires	FSW
	FWS6	47/F	Healthy carrier	Argentina	Buenos Aires	FSW
Men who have sex with men	MSM2	32M	Healthy carrier	Argentina	Buenos Aires	$MSM\ transfusion/HIV^+$
	MSM3	59/M	Healthy carrier	Argentina	Buenos Aires	MSM/HIV^+
	MSM4	61/M	Healthy carrier	Argentina	Buenos Aires	MSM/HIV^+

[a]IDU, injecting drug users; FSWs, female sex workers; MSM, men who have sex with men; TSP/HAM, tropical spastic paraparesis/myelopathy HTLV-1 associated; ATL, adult T leukemia.

Phylogenetic and sequence analyses

To construct a comprehensive phylogenetic dataset, sequences reported here were aligned with 75 HTLV-1 reference strains obtained from the GenBank database, preferentially chosen because of belonging to neighboring countries with high migration rates to Argentina and reference sequences previously reported in South American countries. The Mel 5 reference strain (Melanesian origin, subtype c) was used as an outgroup. Once aligned, the dataset consisted of 492 bp corresponding to the 3′ LTR region. Both BIONJ (data not shown) and ML trees (Fig. 1) showed similar topologies ($p = 0.311$, Shimodaira–Hasegahua test). Cosmopolitan subtype HTLV-1a was separated from HTLV-1 b, c, and d subtypes (bootstrap values: 60% BIONJ, 61% ML). Within the Cosmopolitan subtype, five subgroups could be distinguished as previously described. Although all of them were consistently found by both BIONJ and ML methods, only two subgroups, West African/Caribbean subgroup C (bootstrap values: 85% BIONJ, 79% ML) and North African subgroup D (bootstrap values: 90% BIONJ, 89% ML), were well supported showing bootstraps values higher than 75%.

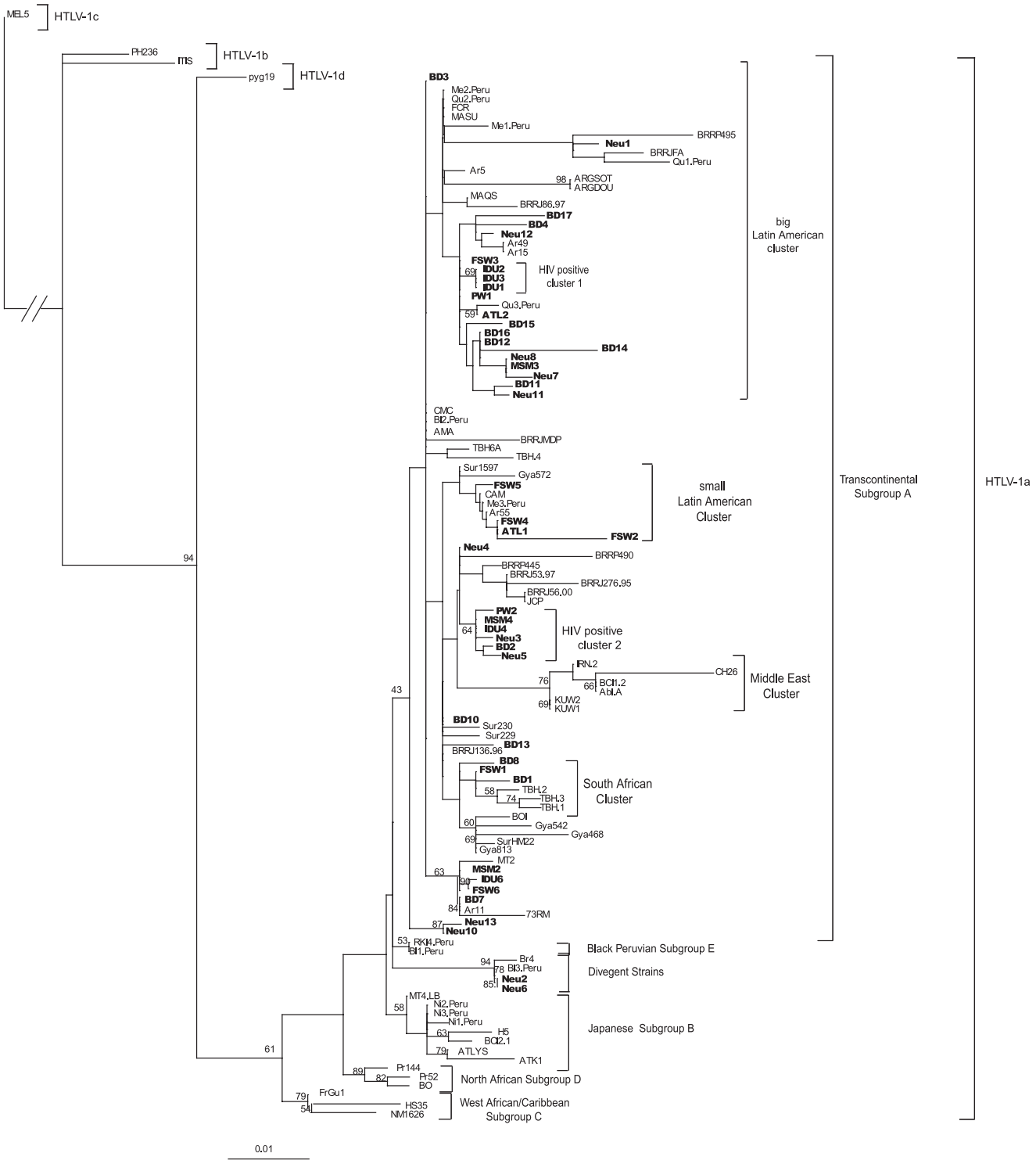

FIG. 1. Rooted maximum likelihood (ML) tree of 119 HTLV-1 strains based upon a 492-bp fragment of the LTR region. ML analysis was performed under the HKY+G nucleotide substitution model. All 44 Argentinian new sequences are shown in bold. The HTLV-1 strain MEL5 was used as the outgroup. Numbers on branches indicate the degree of support for each node. The geographic origin of reference strains included in this analysis are as follows from the bottom to the top: West African/Caribbean subgroup C: NM1626 (French Guiana), HS35 (Caribbean), FrGu1 (French Guiana); North African Subgroup D: BO (Algeria), Pr52 (Morocco), Pr144 (Morocco); Japanese Subgroup B: ATK-1 (Japan), ATLYS (United States, Japan), BCl2.1 (British Colombia), H5 (Japan), Ni1-3.Peru (Peru), MT4.LB (Japan); divergent strains: Bl3.Peru (Peru), Br4 (Brazil); black Peruvian subgroup E: Bl1.Peru (Peru), RKl4.Peru (Peru); Transcontinental subgroup A: 73RM (United States), Ar11 (Argentina), MT2 (Japan), Gya813 (Guyana), SurHM22 (Suriname), Gya468 (Guyana), Gya542 (Guyana), BOI (France), TBH1-3 (South Africa), BRRJ136.96 (Brazil), Sur229-30 (Suriname), KUW1-2 (Kuwait), Abl.A (South Africa), BCl1.2 (British Colombia), CH26 (Chile), IRN2 (Iran), JCP (Brazil), BRRJ56.00 (Brazil), BRRJ276.95 (Brazil), BRRJ53.97 (Brazil), BRRP445 (Brazil), Ar55 (Argentina), Me3.Peru (Peru), CAM (French Guiana), Gya572 (Guyana), Sur1597 (Suriname), TBH4-6 (South Africa), BRRJMDP (Brazil), AMA (Brazil), CMC (Taiwan), Qu3.Peru (Peru), Ar15 (Argentina), Ar49 (Argentina), BRRJ86.97 (Brazil), MAQS (Brazil), ARGDOU (Argentina), ARGSOT (Argentina), Ar5 (Argentina), Qu1.Peru (Peru), BRRJFA (Brazil), BRRP495 (Brazil), Me1.Peru (Peru), MASU (Brazil), FCR (Brazil), Qu2.Peru (Peru), Me2.Peru (Peru), pyg19 (Central Africa Republic), ITIS (Democratic Republic of Congo), PH236 (Gabon), MEL5 (Salomon Islands).

All 44 new strains studied clustered within the Cosmopolitan subtype (bootstrap values: 60% BIONJ, 61% ML), with 42 of them classified as the Transcontinental subgroup (bootstrap values: 46% BIONJ, 43% ML) (Fig. 1). Of them, 20 (45.5%) strains grouped with sequences belonging to a large Latin American cluster and 4 (9.1%) sequences clustered in a small Latin American cluster previously described.[8,19] The majority of them grouped with strains from Amerindians including some from the northwest area of Argentina (Ar5, Ar15, Ar49) while one closely clustered with a strain from a black Brazilian individual (BRRJFA) and four with strains from a Guyanese individual of mixed origin (Gya572) and a Creole of Suriname (Sur1597). Three (6.8%) strains grouped closely to South African references (BIONJ and ML bootstrap values <40%). Two of them (BD1 and BD8) were Argentinean blood donors without risk antecedent for infection and one (FSW1) was a Peruvian female sex worker who reported sexual intercourse with Argentinean, Peruvian, and Bolivian men.

In addition, a total of 12 HTLV-1/HIV coinfected individuals were included in this study. Phylogenetic analysis showed that nine of them (20.5%) grouped together in two subclusters: three (IDU1-3, HIV positive cluster 1) were closely related, clustering in a monophyletic clade within the large Latin American cluster (bootstrap values: 69% BIONJ; 69% ML), and the other six strains (PW2, MSM4, IDU4, Neu3, BD2, and Neu5, HIV positive cluster 2) grouped together, not including any reference sequences (bootstrap values: 58% BIONJ; 64% ML). The remaining three HIV/HTLV-1 coinfected strains seemed to be more distantly related to the other coinfected samples.

Two divergent strains (Neu2 and Neu6) belonging to two HAM/TSP Peruvian sisters with a complete HTLV-1 profile by WB grouped with highly significant support with one Peruvian (Bl3.Peru) and one Brazilian (Br4) reference sequence from individuals of black origin. They branched off from all five Cosmopolitan subgroups in a well-supported clade (bootstrap values: 83% BIONJ, 94% ML: divergent clade) (Fig. 1).[8,29]

In order to elucidate the phylogenetic origin of this divergent group, we compared the likelihood of the best ML tree obtained by a search under constraints that forced the divergent strains to group in a monophyletic clade with either subgroup A or B. We found in both cases an optimal tree where the divergent strains grouped in a monophyletic clade branching off clade A or B (topology A or B, respectively, data not shown). None of them was found to be significantly different (Shimodaira–Hasegawa test, $p = 0.519$ for topology B and $p = 0.238$ for topology A) from the unconstrained ML tree (Fig. 1).

Sequence similarity analysis carried out among studied and prototype sequences revealed that they had the highest similarity to the HTLV-1a prototype ATK-1 (97.5%; 95% CI, 97.4–97.7) and the lowest to HTLV-1d (95.7%; 95% CI, 95.6–95.8), to HTLV-1b (93.2%; 95% CI, 93.0–93.3), and to HTLV-1c (89.4%; 95% CI, 89.2–89.6), consistent with the BIONJ and ML topology. The similarity percentage was significantly different among all groups ($p < 0.05$, Kruskal–Walis test).

Comparison of 492 bp analyzed among divergent strains, Br4 and Bl3.Peru, revealed that Neu2 and Neu6 were identical (100% similitude) to Bl3.Peru, differing from Neu2, Neu6, and Bl3.Peru in one nucleotide position (99.8% similitude) with respect to Br4. When divergent strains were compared among the other 42 studied sequences and the Cosmopolitan prototype, 7 nucleotide differences were observed. Of them, three (G8314A, A8340G, and A8402G) were found only in these divergent strains. On the other hand, one nucleotide position (C8606C) was identical to ATK-1 but different from the other 42 analyzed strains (C8606A, $n = 4$ and C8606G, $n = 38$). The remaining three nucleotide positions were found in the divergent strains and they were also present in some studied samples, differing from ATK-1 (Table 2).

Discussion

HTLV-1 Cosmopolitan subtype Transcontinental subgroup A has been detected in aboriginal communities from the northwest endemic area of Argentina. Moreover, this subgroup and the Japanese subgroup B were recently reported in five individuals including blood donors from the nonendemic central region of the country.[28–31] Although Buenos Aires city is considered nonendemic,[23] a high migration rate from HTLV-1 endemic areas to this capital city has been observed over the past years. Considering these

Table 2. Comparison of Nucleotide Substitutions in the LTR Region of Two Individuals Studied (4971Neu2 and 71419Neu6) with the ATK-1 Prototype and the Other 42 Samples Included in This Study

	Position[a]						
	8314	*8340*	*8402*	*8546*	*8583*	*8606*	*8616*
ATK-1	G	A	A	T	C	C	G
44971Neu2, 71419Neu6 ($n = 2$)	A	G	G	T[b]	T[c]	C	A[d]
Other 42 samples studied ($n = 42$)	G	A	A	A ($n = 40$)	C ($n = 31$)	A ($n = 4$) G ($n = 38$)	G ($n = 3$)

[a]Nucleotides positions were numbered according to the ATK-1 (accession number J02029).
[b]This polymorphism also appeared in two other samples studied (138398Neu10 and 192816Neu13).
[c]This polymorphism also appeared in 11 other samples studied (74215MSM2, 75766FSW6, 78811IDU6, 79253BD3, 100330FSW2, 111606FSW4, 111624FSW5, 127073BD7, 138398Neu10, 192816Neu131, and 95086ATL2).
[d]This polymorphism also appeared in 39 other samples studied (except in 78996IDU, 80151IDU2, and 80830IDU3).

data, we analyzed the phylogenetic and molecular features of HTLV-1 strains from Buenos Aires residents belonging to groups with different at-risk behaviors for HTLV-1/2 transmission.

The analysis of HTLV-1 3′ LTR region demonstrated that all 44 new strains studied belonged to the Cosmopolitan subtype, with the majority classified within Transcontinental subgroup A, positioned in different Transcontinental clusters, suggesting a variable degree of molecular diversity. The majority of the sequences grouped in the large Latin American cluster with strains from Amerindians (some from the northwest area of Argentina: Ar5, Ar15, Ar49) belonged to individuals of nonblack origin. We also identified three strains belonging to the South African cluster that had never been reported in our country before. One of them (FSW1) was obtained from a Peruvian female who reported sexual intercourse with Peruvian, Bolivian, and Argentinean men. These strains clustered with references from a black Brazilian, a Guyanese of mixed origin, and a Creole individual of Suriname.[19,29,40]

These data report the presence of HTLV-1 strains of African origin circulating in Argentina. This result could be geographically explained as a consequence of a high migration rate from South American countries including those with African ethnic background (e.g., Peru) to Buenos Aires city. Although previous studies performed in South America showed that HTLV-1 is found mainly in populations of African ancestry,[40–42] most of the infected individuals in our study were not of black origin, supporting the hypothesis of multiple introductions of HTLV-1 of the Cosmopolitan subtype in the New World.

On the other hand, ML analysis showed the presence of two monophyletic clades (HIV-positive clusters 1 and 2) revealing a close phylogenetic relationship of the retroviruses infecting these individuals. These data could suggest the existence of HTLV-1 strains preferentially spreading among a particular population.

Considering other subgroups of the HTLV-1 Cosmopolitan subtype, the E and B groups have been reported to be infecting Peruvian individuals from Nikkei populations and individuals of black origin, respectively. In addition, subgroup B has also been described in Brazil among Japanese descendants.[8,19] In the present study, even though seven individuals from Peru and one Japanese descendant were included, neither Japanese subgroup B nor Black Peruvian subgroup E strains were identified among HTLV-1-infected Buenos Aires residents.

Regarding the diversity of the HTLV-1 Cosmopolitan subtype, a previous study performed with Latin American strains reported the presence of two sequences from individuals of black origin (Bl3.Peru and Br4) clustered separately from the other known subgroups.[29] We detected two divergent strains (Neu2 and Neu4) belonging to two Peruvian sisters grouping with these sequences and branching off from all five Cosmopolitan subgroups. Moreover, the likelihood-based statistical analysis performed through the Shimodaira–Hasegawa test confirmed this result.

In addition, the sequence analysis of these samples revealed only three different polymorphisms (G8314A, A8340G, and A8402G) between them and ATK-1. Therefore it is not surprising that these positions could be considered the total of the differences that supported them as a divergent clade, especially if we consider the sequence similarity analysis confirmed by the low genomic variability of the studied strains (ranging from 4.3% for ATK-1 to 10.6% for Mel5: $p < 0.05$). Regarding samples from individuals diagnosed with HTLV-1-associated pathologies, the sequence analysis revealed no particular signature pattern of nucleotide substitutions.

In summary, this is the first HTLV-1 phylogenetic study performed on HTLV-1-infected residents of Buenos Aires city, a nonendemic area of Argentina. Our study confirms the only presence of the Cosmopolitan subtype that occurs in other South American countries, suggesting a common origin of these strains. In addition, we observed that the HTLV-1 Transcontinental subgroup was predominant, infecting at-risk and not at-risk individuals. On the other hand, the presence of two divergent strains is described, providing novel data on the genetic variability of HTLV-1. Finally, further analyses including a wider genome region should be performed to determine if these divergent strains could be considered a new subgroup within the HTLV-1 Cosmopolitan subtype.

Sequence Data

The 44 LTR HTLV-1 sequences are available at GenBank with nucleotide accession number EU622582 through EU622625. The accession numbers of the HTLV-1 LTR reference strains used in the phylogenetic analysis are as follow: MEL5 (L02534); ITIS (Z32527); PH236 (L76307); pyg19 (L76310); HS35 (D00294); NM1626 (AF063821); FrGu1 (AY324785); Pr144 (U12807); Bo (U12804); Pr52 (U12806); MT4 (Z31661); Ni1.Peru (Y16484); Ni2.Peru (Y16487); Ni3.Peru (Y16485); ATK-1 (J02029); ATL-YS (U19949); BCI1.2 (U32552); H5 (M37299); Bl3.Peru (Y16483); Br4 (AY324788); Bl1.Peru (Y16481); RK14 (AF054627); AMA (X88871); Bl2.Peru (Y16482); CMC (X88872); BRRJ136-96 (DQ323759); BRRJFA (DQ323757); BRRJ86-97 (DQ323760); ARGDOU (AF007751); ARGSOT (AF007755); Q1.Peru (Y16475); FCR (X88873); MAQS (X88876); MASU (X88877); Me1.Peru (Y16478); Me2.Peru (Y16479); Q2.Peru (Y16476); Q3.Peru (Y16477); Ar15 (AY324778); Ar5 (AY324783); JCP (X88875); BI2.Peru (Y16482); BRRP445 (DQ323755); BRRJ56-00 (DQ323754); BRRJ53-97 (DQ323753); BRRJMDP (DQ323751); BRRP495 (DQ323758); BRRJ276-95 (DQ323750); BRRP490 (DQ323752); BOI (L36905); TBH-1 (L76026); TBH-2 (L76025); TBH-3 (L76034); CAM (AF063819); Me3.Peru (Y16480); Ar55 (AY324782); KUW-1 (L42253); KUW-2 (L42255); IRN-2 (U87261); Abl.A (U87264); BCI2-1 (U32557); CH26 (D23690); TBH-4 (L76028); TBH-6 (L76030); 73RM (M81248); MT2 (L03562); Ar11 (AY324777); Sur229 (AY374468); Sur230 (AY374466), Sur1597 (AY374465); SurHM22 (AY374467); Gya468 (AY374459); Gya542 (AY374460); Gya572 (AY374461); Gya813 (AY374462).

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Disclosure Statement

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