

Globin Haplotypes of Human T-Cell Lymphotropic Virus Type I–Infected Individuals in Salvador, Bahia, Brazil, Suggest a Post-Columbian African Origin of This Virus

*†Luiz Carlos Alcantara, Jr., ‡Sonia Van Dooren, *Marilda Souza Gonçalves, §Simone Kashima, §Maria Cristina Ramos Costa, *†Fred Luciano Neves Santos, ||Achilea Lisboa Bittencourt, ||Inês Dourado, †||Antonio Andrade Filho, §Dimas Tadeu Covas, ‡Anne-Mieke Vandamme, and *†Bernardo Galvão-Castro

*Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, and †Bahia School of Medicine and Public Health/Foundation for Scientific Development, Salvador, Bahia, Brazil; ‡Rega Institute, Katholieke Universiteit Leuven, Belgium; and §Brazil Blood Center of Ribeirão Preto, São Paulo, and ||Federal University of Bahia, Salvador, Bahia, Brazil

Summary: The city of Salvador, Bahia, Brazil, has sociodemographic characteristics similar to some African cities. Up to now, it has had the highest prevalence of human T-cell lymphotropic virus type I (HTLV-I) infection (1.74%) in the country. To investigate which strains of HTLV-I are circulating in Salvador, we studied isolates from 82 patients infected with HTLV-I: 19 from the general population, 21 from pregnant women, 16 from intravenous drug users, and 26 from patients and their family attending a neurologic clinic. Phylogenetic analysis from part of the LTR fragments showed that most of these isolates belonged to the Transcontinental subgroup of the Cosmopolitan subtype (HTLV-Ia). Only one sample from a pregnant woman was closely related to the Japanese subgroup, suggesting recent introduction of a Japanese HTLV-I lineage into Salvador. β^A -Globin haplotypes were examined in 34 infected individuals and found to be atypical, confirming the racial heterogeneity of this population. A total of 20 chromosomes were characterized as Central African Republic (CAR) haplotype (29.4%), 31 (45.6%) were characterized as Benin (BEN) haplotype, and 17 (25%) were characterized as Senegal (SEN) haplotype. Five patients' genotypes (14.7%) were CAR/CAR; 10 (29.4%), BEN/BEN; 9 (26.5%), CAR/BEN; 2 (5.9%), BEN/SEN; and 7 (20.6%), SEN/SEN. One patient's genotype (2.9%) was CAR/SEN. The β^A -globin haplotype distribution in Salvador is unusual compared with other Brazilian states. Our data support the hypothesis of multiple post-Columbian introductions of African HTLV-Ia strains in Salvador, Bahia, Brazil. **Key Words:** HTLV-1, LTR, β -globin haplotypes, Bantu, Salvador-Brazil, Southern Africa

Human T-cell lymphotropic virus type I (HTLV-I) infection is endemic in Japan, the Caribbean, and some South America and central Africa regions.¹ So far, 6 genetic subtypes have been proposed in the phylogenetic

classification of this virus: Ia, Cosmopolitan (distributed throughout the world)²; Ib, Central African³; Ic, Melanesian (a divergent strain isolated in Papua New Guinea and Australia)⁴; Id, isolated from Central African Republic pygmies and from two patients in Cameroon and Gabon^{5,6}; and Ie and If (recently proposed as new subtypes), identified in samples from 1 Efe pygmy from the Democratic Republic of Congo and 1 individual from Gabon.⁷ The Cosmopolitan subtype is divided into 5 subgroups according to geographic distribution: Transcontinental, Japanese, North African, West African, and Black Peruvian.^{2,8–10}

Address correspondence and reprint requests to Bernardo Galvão-Castro, Advanced Public Health Laboratory, Gonçalo Moniz Research Center, Oswaldo Cruz Foundation Rua Waldemar Falcão 121, Brotas, Salvador, Bahia, Brazil 40295-001. E-mail: bgalvao@cpqgm.fiocruz.br.

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Two hypotheses have been proposed to explain the origin of this retrovirus on the American continent. One postulates that prehistoric migration of infected populations across the Bering Strait brought the virus from northern Asia to the American continent.¹¹ The other suggests that Africans introduced the virus into the American continent during the slave trade.^{12,13}

The 5 geographic regions of Brazil (north, northeast, central west, south, and southeast) have marked social and demographic differences. During the Portuguese colonial period (16th–19th centuries), nearly 4 million Africans were brought to Brazil as part of the slave trade, mainly to the northeast and southeast regions.^{14–16} At the beginning of the 20th century, the south and southeast regions also had large European and Asian migrations. Currently, São Paulo State (southeast region) has the largest Japanese community outside Japan.

In Brazil, HTLV-I was first described in 1986 in the Japanese immigrant population.¹⁷ The prevalence of HTLV-I infection among blood donors in Brazil varies from 0.08% (south and north regions) and 0.33% (southeast region) to 1.35% in Salvador (northeast region).¹⁸ So far, the few isolates studied in this country have all been identified as Cosmopolitan subtype; most of these belong to the Transcontinental subgroup, while a few belong to the Japanese subgroup.¹⁹

The β -globin gene cluster haplotypes have provided an important tool for tracking the origin, evolution, and migration of the human race. Haplotype differences can also be used to detect genetic distance population origin between ethnic groups.^{20,21} The β -globin gene cluster haplotypes have been associated with the presence of hemoglobin S in different ethnic and geographic origins: the Benin (BEN) type originated in midwestern Africa; the Bantu (CAR) type, in south central and eastern Africa; the Senegal (SEN) type, in Atlantic West Africa; the Saudi Arabia–India type, in the Indian subcontinent and the eastern Arabian peninsula; and the Cameroon type, along the west coast of Africa.^{22–25} Nagel and Ranney²⁴ reported the genetic epidemiology of the β -globin gene and about the similarities between the β^A -globin and β^S -globin gene haplotypes. Salvador, Bahia, Brazil, has a high rate of race admixture with a strong African compound and an unusual β^S -globin gene haplotype distribution when compared with those described in other Brazilian states, with the predominance of CAR/BEN heterozygosity and a small frequency of SEN type.^{26–29}

On the basis of those Salvador population aspects and with the probability of describing a possible African origin of HTLV-I in Salvador, we studied β^A -globin gene haplotype characterization to establish the ethnic and geographic origin of the viral host and correlate it with

the viral data. The study included 82 isolates from individuals from Salvador. We established the β^A -globin gene polymorphisms in part of these infected individuals.

METHODS

Study Population

We examined 82 HTLV-I isolates that had been collected between 1996 and 1998 as part of previous studies: 19 isolates from the general population (BA)³⁰; 21 from pregnant women (TP)³¹; 16 from intravenous drug users (IDU)³²; and 26 from patients attending a neurologic clinic and their family members (FNN).

DNA Extraction and PCR Amplification

Genomic DNA was extracted from peripheral blood mononuclear cells by SDS/proteinase K treatment followed by phenol/chloroform extraction or by using a GFX genomic blood DNA purification kit (Amersham Pharmacia Biotech, Piscataway, NJ). To confirm the serologic results, nested PCR analysis was used to amplify the *pol* gene using primers 12P1/SK111 and 12P5/1P1/2P3 as previously described.³³ For amplification of the HTLV-I *tax*LTR gene, all samples were submitted to nested PCR assay using primers LTR1 (5'-CATTCTACTCTCACACGGCCTCATAAGTACTCTT-3') and LTR2 (5'-CGCA GTTCAGGAGGCACCAC-3') to amplify a 574-bp fragment corresponding to nt 8164–8727 in the HTLV-I ATK1 isolate; this was followed by a second round of PCR assay using the primers LTRN1 (5'-TTGAAGAATACACCAACATCCC-3') and LTRN2 (5'-GAACGCG ACTCAACCGCGTGGAT-3') to amplify a 479-bp fragment corresponding to nt 8255–8686 of the HTLV-I ATK1 isolate. The first round used 50- μ L reaction mixtures containing 1.5 μ g of DNA, 1.75 U of Taq DNA polymerase (Perkin–Elmer–Cetus, Norwalk, CT), 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 2.5 mM MgCl₂, 0.25 mM each deoxynucleotide triphosphate, and 30 pmol each primer. Cycling conditions were as follows: 1 cycle at 94°C for 1 minute followed by 25 cycles at 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 45 seconds, and 1 cycle at 72°C for 10 minutes. Five microliters of the initial amplified product was used in the subsequent nested reaction, also performed with 50- μ L reaction mixtures. All amplified products were electrophoresed on 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Nucleotide Sequencing and Phylogenetic Analysis

PCR products amplified from the LTR partial region were purified using the Promega (Madison, WI) Wizard PCR prep system and sequenced in a Perkin–Elmer/ABI Prism 377 DNA Stretch Sequencer using Taq FS Dye terminator cycle sequencing. The same inner PCR primers were used in the sequencing reactions. All reactions were performed twice. A multiple sequence alignment of the examined region and the related sequences in the GenBank/EMBL database was performed with the DAMBE program³⁴ using the Clustal algorithm and further edited with the GeneDoc program.³⁵ Neighbor-joining and maximum-likelihood (ML) phylogenetic analyses were performed with PAUP* version 4.0.2b³⁶ using the HKY85 substitution model including substitution rate heterogeneity. The neighbor-joining tree was constructed with an optimized nucleotide substitution rate matrix and γ shape parameter using empirical base frequencies. The reliability of the

neighbor-joining trees was evaluated by analyzing 1000 bootstrap replicates. For the ML tree reconstruction, a heuristic search with the subtree pruning–grafting–branch swapping algorithm was performed using the neighbor-joining tree as the starting tree including its optimized parameters. A likelihood ratio test was used to calculate the statistical support for the branches (expressed in *P* values). Trees were drawn with the TreeView 1.4 (Glasgow University, Scotland) program.

Accession Numbers

The GenBank accession numbers of the HTLV-I fragments sequenced in our laboratory and included in the phylogenetic study were as follows: BA1239100, BA1978, BA2031, BA2127, BA2326, BA2497, BA2567, BA2746, BA2781, BA2960, BA2993, BA3076200, BA3229, BA3451200, BA3525, BA3565, BA3604, BA4971900, BA2781: AF520453–AF520471; IDU131, IDU150, IDU122, IDU36, IDU5, IDU69, IDU7, IDU71, IDU77, IDU8, TP39, TP41, TP42, TP43, TP44, TP50, TP51, TP52, TP54, TP58, TP59, TP60, TP61, TP64, TP65, TP67, TP70, TP71, TP79, TP81, FNN18, FNN19, FNN22, FNN26, FNN28, FNN29, FNN31, FNN32, FNN35, FNN39, FNN54, FNN57, FNN60, FNN61, FNN64, FNN68, FNN72, FNN73, FNN78, FNN82, FNN83, FNN87, FNN89, FNN9, FNN91, FNN94: AY124592–AY124647; IDU104, IDU108, IDU103, IDU118, IDU122: AF523269–AF523273; IDU126: AF526264; TP62: AY125467.

The other HTLV-I strains were as follows: pyg19, L76310; Afs911, L72212; ITIS, Z32527; MT4LB, Z31661; MEL5, L02534; HS35; D00294; GH78, D23693; CH26, D23690; Bo, U12804; OD, U12805; Pr52, U12806; Pr144, U12807; Ni1.Peru, Y16484; BI1.Peru, Y16481; Me1.Peru, Y16478; B13.Peru, Y16483; B12.Peru, Y16482; Qu1.Peru, Y16475; Me3.Peru, Y16480; Qu3.Peru, Y16477; Me2.Peru, Y16479; Ni2.Peru, Y16487; RK14.Peru, AF054627; ATM, J02030; ATK1, J02029; ATL-YS, U19949; BCI2.1, U32557; BCI1.2, U32552; H5, M37299; 73RM, M81248; MT2, L03562; CR1, K02722; TBH1–TBH4, L76026, L76025, L76034, and L76028; TBH6–TBH7, L76030 and L76029; BOL, L36905; TSP1, M86840; RK12–Rum, AF3012; RK11–Iran, AF3010; AMA, CMC, FCR, and MAQS, X88871–X88873 and X88876; KUW-1, L42253; Abl.A, U87264; CH, M69044; CAM, NAR, and NM1626, AF063819–AF063821; KAPO, YAM, and TOTO, AF076254–AF076256; CR015 and CR01, AF076258 and AF076259; NM1688, NIC, NM2028, and NM2015, AF076261–AF076264; CR08, AF076267.

β^A -Globin PCR Analysis and RFLP

β^A -globin haplotypes were amplified as previously described,³⁷ generating 7 fragments from the β^A -globin gene cluster (5'^G γ , ^G γ , ^A γ , $\Psi\beta$, 3' $\Psi\beta$, 5' β , and 3' β). These fragments were purified by the Promega (Madison, WI) Wizard PCR prep system, and a 5- μ L aliquot was

digested with an appropriated restriction endonuclease (*Xmn*I, *Hind*III, *Hinc*II, *Hinf*I, and *Hpa*I, respectively) used for each site.³⁷ The fragments were analyzed by 3% agarose gel electrophoresis with ethidium bromide under ultraviolet light.

RESULTS

Nucleotide Sequencing and Phylogenetic Analysis

The analysis of the phylogenetic tree showed that all 82 isolates from the infected population cohort belonged to the Cosmopolitan subtype (Fig. 1), 81 of which belonged to the Transcontinental subgroup (*P* < 0.001 for ML). Seventy-four of the 81 isolates, in turn, belonged to the Latin American cluster, and two clades were identified within this cluster (*P* < 0.001 for ML). One strain (Tp58) clustered with the B13.Peru isolate (66% bootstrap replicates; *P* < 0.001 for ML), which had been previously classified as pertaining to the Japanese subgroup when the complete LTR gene was analyzed.¹⁰ This sample was obtained from a 28-year-old woman of African descent who was born in Salvador. She reported that she had had three sexual partners (none of whom with Japanese ethnicity) and denied blood transfusion and intravenous drug use. She also reported being breastfed as a baby. She was seronegative for HIV infection, syphilis, and hepatitis B and C.

Of the 68 β^A -globin chromosomes analyzed, 31 (45.6%) were BEN haplotype, 20 (29.4%) were CAR haplotype, and 17 (25%) were SEN haplotype. Interestingly, none of them were characterized as atypical (Table 1). Ten patients' genotypes were BEN/BEN (29.4%); 9 (26.5%), BEN/CAR; 7 (20.6%), SEN/SEN; 5 (14.7%), CAR/CAR; and 2 (5.9%), BEN/SEN. One patient's (2.9%) genotype was CAR/SEN (Table 1).

Haplotype identification is indicated by plus (+) and minus sign (–) combinations, characterizing the presence or absence of the restriction site (Table 2). Analysis of the 68 chromosomes revealed 24 possible haplotypes. The most common β^A BEN haplotype was XIII (–/–, –/–, –/–, –/–, +/+, +/–), the most common β^A CAR haplotypes were III (+/–, –/–, –/–, +/–, +/+, +/–) and

TABLE 1. Frequency of the atypical haplotypes associated with the β^A -globin gene cluster and frequency of the haplotypes associated with the number of patients studied

Sample	Chromosomes				Patients						
	N	BEN	CAR	SEN	N	BEN/BEN	CAR/CAR	SEN/SEN	BEN/CAR	BEN/SEN	CAR/SEN
FNN	38	18 (47.4%)	14 (36.8%)	6 (15.8%)	19	5 (26.3%)	3 (15.8%)	2 (10.5%)	7 (36.8%)	1 (5.3%)	1 (5.3%)
TP	12	6 (50%)	6 (50%)	—	6	2 (33.4%)	2 (33.3%)	—	2 (33.3%)	—	—
BA	18	7 (38.9%)	—	11 (61.1%)	9	3 (33.3%)	—	5 (55.6%)	—	1 (11.1%)	—
Total	68	31 (45.6%)	20 (29.4%)	17 (25%)	34	10 (29.4%)	5 (14.7%)	7 (20.6%)	9 (26.5%)	2 (5.9%)	1 (2.9%)

BEN, Benin; CAR, Central African Republic; and SEN, Senegal.

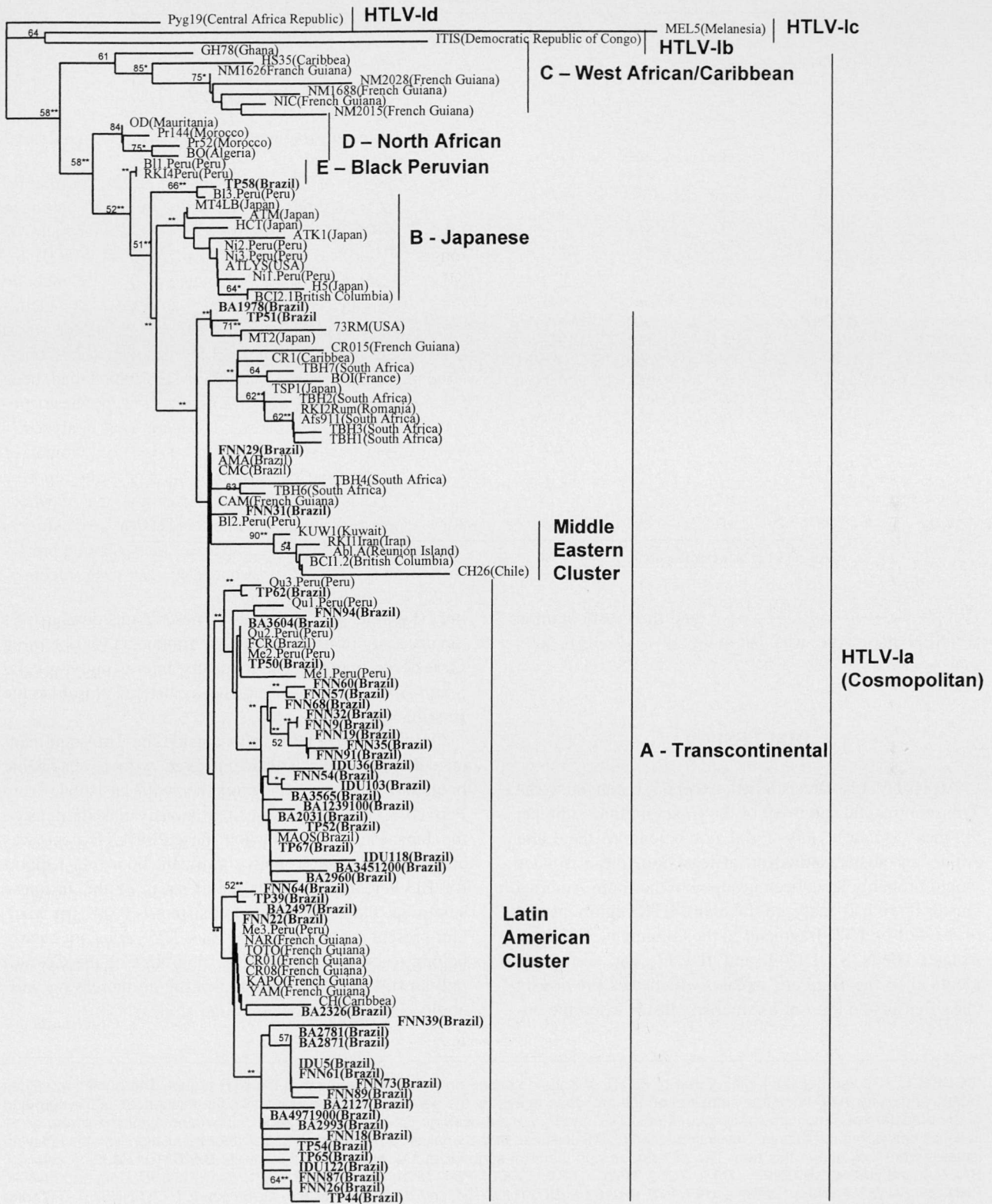


TABLE 2. Frequency of the atypical haplotypes of the β^A -globin gene of HTLV-I from subjects from Salvador, Bahia, Brazil

	XmnI	HindIII	HindIII	HincII	HincII	HinfI	HpaI	N (%)
β^A Benin	-/-	-/-	-/-	-/-	+/+	-/-	-/-	
I	-/-	+/-	-/-	-/-	+/-	+/-	+/-	1 (4.8)
II	-/-	-/-	-/-	-/-	+/-	+/-	+/-	1 (4.8)
III	-/-	+/-	-/-	-/-	+/-	-/-	+/-	1 (4.8)
IV	-/-	+/-	-/-	-/-	+/+	+/-	+/-	1 (4.8)
V	-/-	-/-	-/-	-/-	+/-	+/-	+/-	1 (4.8)
VI	-/-	+/-	+/-	-/-	-/-	+/+	+/-	1 (4.8)
VII	+/-	+/-	-/-	-/-	+/+	+/+	+/-	1 (4.8)
VIII	-/-	+/-	-/-	-/-	+/+	+/+	+/-	1 (4.8)
IX	+/-	+/-	-/-	-/-	+/-	+/+	+/-	1 (4.8)
X	-/-	+/-	+/-	-/-	+/+	+/+	+/-	2 (9.4)
XI	-/-	-/-	-/-	-/-	+/-	+/+	+/-	2 (9.4)
XII	-/-	-/-	-/-	-/-	+/+	+/+	+/-	2 (9.4)
XIII	-/-	-/-	-/-	-/-	-/-	+/-	+/-	6 (28.6)
β^A CAR		+/+	-/-	-/-	-/-	+/+	+/+	
I		+/+	+/-	-/-	+/-	+/+	+/-	1 (16.7)
II		+/+	+/-	-/-	+/-	+/-	+/-	1 (16.7)
III		+/-	-/-	-/-	+/-	+/+	+/-	2 (33.3)
IV		+/-	-/-	-/-	-/-	+/-	+/-	2 (33.3)
β^A Senegal	+/+	+/+	-/-	+/+	+/+	+/+	+/+	
I	+/-	+/+	-/-	-/-	+/+	+/+	+/-	1 (14.3)
II	+/+	+/+	-/-	+/+	+/+	+/+	+/-	1 (14.3)
III	+/+	+/+	-/-	-/-	+/-	+/-	+/-	1 (14.3)
IV	+/-	+/-	-/-	-/-	+/-	+/+	+/-	2 (28.5)
V	+/-	+/-	-/-	+/-	+/-	+/+	+/-	1 (14.3)
VI	+/+	+/+	-/-	+/+	+/-	+/+	+/-	1 (14.3)

CAR, Central African Republic.

IV (+/-, -/-, -/-, -/-, +/-, +/-), and the most common β^A SEN haplotype was IV (+/-, +/-, -/-, -/-, +/-, +/-, +/-).

DISCUSSION

All HTLV-I isolates studied, except 1, belonged to the Transcontinental subgroup of the Cosmopolitan subtype. Of these isolates, only 7 did not belong to the Latin American cluster. However, at least 3 of these isolates would probably have been grouped in the Latin America cluster if we had analyzed the entire LTR region instead of the 420-bp LTR fragment: in the alignment, 3 of these isolates (FNN28, IDU69, and IDU77) had sequences identical to the B12.Peru isolate, which was previously classified inside the Latin America cluster when the en-

tire LTR gene was analyzed.¹⁰ These results demonstrate the necessity of using large gene fragments for obtaining more accurate phylogenetic results. Interestingly, several South African HTLV-I sequences clustered closely to the Brazilian strains.

Only 1 isolate (TP58) was outside the Transcontinental subgroup. This isolate clustered with a reasonable bootstrap support of 66% together with an isolate from Peru (B13.Peru) that had been previously classified inside the Japanese subgroup when the entire LTR gene was analyzed.¹⁰ In spite of no significant bootstrap support for B13.Peru being the ancestral strain of the Japanese subgroup, the *P* value was consistent (<0.001 for ML). Our present analysis indicates that TP58 does not clearly belong to one of the existing subgroups of the Cosmopolitan subtype HTLV-Ia, indicating an increasing variability among the Cosmopolitan strains.

FIGURE 1. Rooted neighbor-joining tree of 82 HTLV-I strains based on a 420-bp fragment of the LTR region. The bootstrap values (>50% and using 1000 bootstrap samples) on the branches represent the percentage of trees for which the sequences at the right end of the branch form a monophyletic group. Mel5, ITIS, and Pyg19 are used as out-groups. Geographic origin and ethnic origin are given in italics between parentheses. Newly sequenced LTRs included in this analysis are the following Salvador population isolates. Only 49 isolates were included in this tree. The other ones with identical sequences are demonstrated in italics: **BA2031** = BA3229, BA3525, BA2746, and BA2567; **BA2497** = BA3076200; **TP61** = TP43, TP59, TP60, TP79, IDU7, IDU22, IDU126, IDU131, IDU150, and FNN78; **FNN72** = FNN82; **FNN9** = FNN83 and FNN70; **IDU103** = IDU71, IDU104, and IDU108; **FNN28** = IDU69 and IDU77; **FNN87** = TP41 and TP42; **FNN72** = FNN82; **IDU5** = IDU8 and TP71; **TP64** = TP81. The isolates FNN28, IDU69, and IDU77 are identical to B12.Peru. **, the ML method was highly significant with *P* < 0.001 or significant with *P* < 0.005.

It has been suggested that the presence of HTLV-I in South America is the result of multiple post-Columbian introductions, mainly from Africa.¹⁰ Approximately 80% of Salvador's population have some African ethnic ancestry. Although most Africans brought to Bahia during the slave trade came from West Africa (Benin, Nigeria, and northern Angola),^{14-16,38-40} there is some evidence that Africans were also brought from other regions of the southern African continent (e.g., Madagascar, Mozambique, and southern Angola) where the CAR (Bantu) β -globin haplotype is prevalent.

The CAR haplotype was identified in 29.4% of the chromosomes, with 26.5% BEN/CAR heterozygous, suggesting a presence of this haplotype in Salvador by immigration of Bantu speakers, possibly from southern Africa. This would be consistent with the close relationship of some HTLV-I Brazilian strains with some South African strains. The other haplotypes (BEN and SEN) suggest importation of blacks from western Africa, although the absence of the West African subgroup among the individuals with HTLV-I infection remains unclear. Further analysis based on the racial admixture and hemoglobin pattern determination will confirm the hypothesis presented here. The presence of SEN and BEN haplotypes in Salvador has been reported previously.²⁸

African people from Bantu-speaking areas were the first slaves exported in large scale to Bahia, depositing strong landmarks of its culture that are still present in the language, religion, folklore, and customs of the people in the region. Therefore, infected South Africans could have introduced the HTLV-I Cosmopolitan subtype of the Transcontinental subgroup in Salvador. Infected individuals who migrated at the gold and diamonds epoch, in turn, could have brought this subgroup to South Africa from Angola or other African countries. Unfortunately, more thorough phylogenetic analysis is not possible, since there are few LTR sequences available from African isolates. The fact that more South African isolates have been characterized, compared with isolates from the rest of Africa, may represent a sampling bias. Therefore, the South African connection should be interpreted with some caution. A possible connection with other Bantu-speaking groups should be investigated, by analyzing more HTLV-I isolates from central Africa.

In conclusion, our results are highly supportive of a post-Columbian introduction of multiple African HTLV-I isolates in Brazil, as has been suggested for other South American countries.^{10,19} Investigation of HTLV-I isolates from different regions and other ethnic groups should be conducted to increase the knowledge of the HTLV-I Brazilian origin.

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