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# The *tax* Gene Sequences Form Two Divergent Monophyletic Lineages Corresponding to Types I and II of Simian and Human T-Cell Leukemia/Lymphotropic Viruses

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Evolutionary associations of human and simian T-cell leukemia/lymphotropic viruses I and II (HTLV-I/II and STLV-I/II) are inferred from phylogenetic analysis of *tax* gene sequences. Samples studied consisted of a geographically diverse assemblage of viral strains obtained from 10 human subjects and 20 individuals representing 12 species of nonhuman primates. Sequence analyses identified distinct substitutions, which distinguished between viral types I and II, irrespective of host species. Phylogenetic reconstruction of nucleotide sequences strongly supported two major evolutionary groups corresponding to viral types I and II. With the type I lineage, clusters were composed of strains from multiple host species. A genetically diverse, monophyletic lineage consisting of eight new viral strains from several species of Asian macaques was identified. The second lineage consisted of a monophyletic assemblage of HTLV-II/STLV-II strains from Africa and the New World, including an isolate from a pygmy chimp (*Pan paniscus*) as an early divergence within the lineage. High levels of genetic variation among strains from Asian STLV-I macaque suggest the virus arose in Asia. Evidence of the origin of the type II virus is less clear, but diversity among HTLV-II variants from a single isolated population of Mbati villagers is suggestive but not proof of an African origin. 

## INTRODUCTION

Since the 1980s numerous strains of human and simian T-cell leukemia/lymphotropic viruses have been identified throughout the world. Serological and genetic analyses have characterized these as oncornaviruses (Gallo, 1986) of at least two major types, HTLV-I/STLV-I and HTLV-II/STLV-II, distinguished by specific areas of endemicity and disease associations. Both HTLV-I and HTLV-II are able to transform T-cells *in vitro* (Miyoshi *et al.,* 1981; Popovic *et al.,* 1983; Markham *et al.,* 1983), but differ in pathogenicity *in vivo.* HTLV-I is the causative agent of adult T-cell leukemia/lymphoma (ATLL) (Poiesz *et al.,* 1980; Hinuma *et al.,* 1981; Miyoshi *et al.,* 1981; Yoshida *et al.,* 1982; Robert-Guroff *et al.,* 1986) and of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain *et al.,* 1985; Rodgers-Johnson *et al.,* 1985; Osame *et al.,* 1986) and is endemic in the Caribbean basin and certain regions within southeast Asia, Africa, and continental America. HTLV-II pathogenicity is less clear, with anecdotal evidence of associations with hairy T-cell leukemia (Kalyanaraman *et al.,* 1982; Conn *et al.,* 1990) and with HAM/TSP (Jacobson *et al.,* 1993), and is endemic within several indigenous populations of North and South America (Lairmore *et al.,* 1990; Biglione *et al.,* 1993; Fujiyama *et al.,* 1993), certain blood donor groups, intravenous drug users (Robert-Guroff *et al.,* 1986; Hjelle *et al.,* 1990; Lee *et al.,* 1989, 1993), and some European countries (Tedder *et al.,* 1984; Lee *et al.,* 1993). In nonhuman primates, multiple strains of STLV-I and STLV-II have been isolated from species throughout the world (Watanabe *et al.,* 1985; Inoue *et al.,* 1986; Saksena *et al.,* 1994; Koralnik *et al.,* 1994; Song *et al.,* 1994; Yanagihara *et al.,* 1994; Vandamme *et al.,* 1996; Van Brussel *et al.,* 1996), with STLV-I reportedly linked with a few cases of an ATLL-like disease (Homma *et al.,* 1984; Blakeslee *et al.,* 1987; McCarthy *et al.,* 1990). Inferences concerning the natural history of HTLV/STLV derived from previous phylogenetic analyses of HTLV-I/

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STLV-I (Koralnik *et al.,* 1994; Ibrahim *et al.,* 1995) suggest interspecies transmission, an event well documented for animals in captivity (Voevodin *et al.,* 1996), and may have contributed significantly to the present global pattern of distribution.

One important characteristic of HTLV/STLV phylogeny is that viral associations exhibit a geographical component (Dekaban *et al.,* 1995). Analyses of HTLV-I/STLV-I has led to the recognition of at least three human subgroups: HTLV-I Cosmopolitan, a recently derived cluster presumed to have been disseminated worldwide by the slave trade (Koralnik *et al.,* 1994); HTLV-I Zaire, identified in central Africa and Italy (Gessain *et al.,* 1992; Boeri *et al.,* 1995); and HTLV-I Melanesia, isolated from patients in Papua New Guinea, Solomon Islands, and Australia (for a recent review see Franchini, 1995). Likewise, several clusters composed of simian strains were identified and had a similar geographic component (Koralnik *et al.,* 1994; Ibrahim *et al.,* 1995). Based on *env* gene sequences, the HTLV-I Melanesia subgroup and a cluster composed of STLV-I from Asian macaques had higher levels of genetic diversity relative to all other simian and human groups identified. Such results implicate Asia as the probable site of origin of HTLV-I/STLV-I. However, the recent discovery of HTLV-II-related viruses in African human and nonhuman primates (Goubou *et al.,* 1992, 1993; Giri *et al.,* 1994; Liu *et al.,* 1994; Gessain *et al.,* 1995) has defined at least three distinct subtypes (Eiraku *et al.,* 1996) and has stimulated further interest in the natural history of this virus.

In the present study, we address the hypothesis of an Asian origin of a common ancestor of type I virus and examine the evolutionary relationships between type I and type II viruses by phylogenetic analysis of a portion of the *tax* gene. Comparative sequence analyses between type I and type II indicate the *tax* gene as most conserved (Cann *et al.,* 1990), thus more apt to resolve distant relationships within the HTLV/STLV phylogeny. We include representatives from recognized human and simian subgroups (Koralnik *et al.,* 1994; Ibrahim *et al.,* 1995; Vandamme *et al.,* 1996) which represent the range of genetic diversity present within type I and type II viruses. In addition, we include an STLV-II isolated from the bonobo chimpanzee (*Pan paniscus*) from Zaire (Giri *et al.,* 1994; Liu *et al.,* 1994; Digilio *et al.,* 1997), 10 previously unreported strains from Asian macaques, HTLV-I from an individual in Zaire, and HTLV-II from three people from an isolated Mbati village in the Central African Republic.

## MATERIALS AND METHODS

### Samples and viral strains

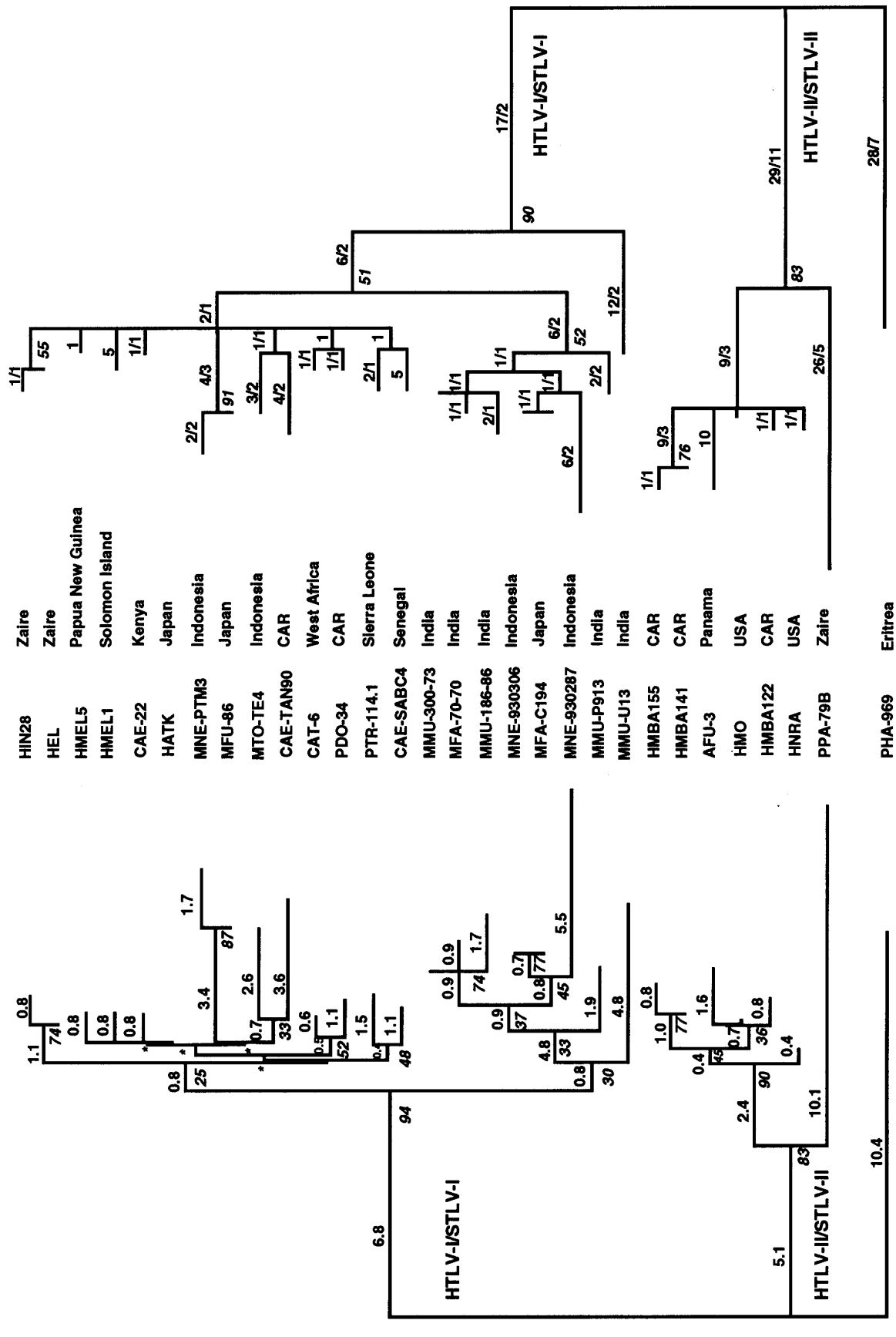
Thirty *tax* sequences were amplified from 10 human (HTLV) and 20 nonhuman (STLV) strains (see Fig. 1). Strains HMBA122, HMBA141, and HMBA155 were obtained from Mbati villagers residing in the Lobaye province, which is located in the eastern part of the Central African Republic. Strain samples HMBA122 and HMBA141 were obtained from adult males, ages 54 and 77, and strain sample HMBA155 was obtained from a 70-year-old female. The HIN28 strain was obtained from a healthy 7-year-old female from the Inongo area in Zaire, and MMU-U13 and MMU-P913 were from macaques housed at the Strasbourg Primatology Center in France. MNE-930287 and MNE-930306 were hosted by pigtailed macaques captured in Sumatra and kept in the Primate Research Center in Bogor, Indonesia. CAT-06 was obtained from a sooty mangabey housed at the Yerkes Regional Primate Research Center in Atlanta, Georgia. MFA-70-70, MMU-186-85, and MMU-300-73 were derived from macaques kept at the New England Regional Primate Research Center in Southborough, Massachusetts (Daniel *et al.,* 1988). Strain MFA-C194 has been previously described (Koralnik *et al.,* 1994). All study animals carrying the simian strains were healthy.

### Polymerase chain reaction (PCR)

Genomic DNA was isolated from peripheral blood mononuclear cells of 13 newly characterized samples included in this study. Viral DNA (40 ng/$\mu$l) was amplified by PCR using the primer sets SK43 (5′-TACGAATTCCGGATACCCGAGTCTACGTGT-3′) and SK44 (5′-TATGAATTCGAGCCGATAACGCGTCCATCG-3′) which contain *Eco*RI restriction sites. These primers amplify a 118-bp fragment of the *tax*/*rex* region of HTLV and STLV. For each reaction, 1 $\mu$g of DNA (25 $\mu$l) was mixed with 75 $\mu$l of PCR mix containing 1$\times$ buffer (100 m*M* Tris–HCl, pH 8.3, 500 m*M* KCl, 15 m*M* $MgCl_2$, and 0.01% w/v gelatin); 0.2 m*M* dATP, dCTP, dGTP, and dTTP; 50 pmol of each primer; and 2.5 U of recombinant *Taq* DNA polymerase (Perkin–Elmer, Branchburg, NJ) for a total volume of 100 $\mu$l. The reaction was performed in a DNA thermal cycler (Perkin–Elmer), and consisted of an initial denaturation step of 5 min at 95°, followed by 35 cycles at 94° for 1 min and 55° for 2 min. For sample MNE-930306, seminested PCR was used to generate a 235-bp *tax* sequence that flanks the SK43/44 sequence. The first round used primer sets PH1F (5′-TTGTCAGCCCACTTCCCAGG-3′) and PH2R (5′-AAGGAGGGGAGTCGAGGGATAAGG-3′), and the second round of amplification used primers PH2F (5′-CCCAGGTTTCGGGCAAAGCCTTCT-3′) and PH2R. PCR conditions and thermal cycling parameters were identical to those described above for primers SK43/44. To prevent contamination, all manipulations were done in a PCR workstation (C.B.S. Scientific Co., Del Mar, CA), reagents were dispensed using aerosol-resistant tips (ART, San Diego, CA), and amplimers and amplicons were handled in separate rooms.

### Cloning and sequencing

SK43/44 amplified fragments were cleaved with the *Eco*RI restriction endonuclease, column purified (Pro-


HIN28 Zaire
HEL Zaire
HMEL5 Papua New Guinea
HMEL1 Solomon Island
CAE-22 Kenya
HATK Japan
MNE-PTM3 Indonesia
MFU-86 Japan
MTO-TE4 Indonesia
CAE-TAN90 CAR
CAT-6 West Africa
PDO-34 CAR
PTR-114.1 Sierra Leone
CAE-SABC4 Senegal
MMU-300-73 India
MFA-70-70 India
MMU-186-86 India
MNE-930306 Indonesia
MFA-C194 Japan
MNE-930287 Indonesia
MMU-P913 India
MMU-U13 India
HMBA155 CAR
HMBA141 CAR
AFU-3 Panama
HMO USA
HMBA122 CAR
HNRA USA
PPA-79B Zaire
PHA-969 Eritrea
HTLV-I/STLV-I
HTLV-II/STLV-II
HTLV-I/STLV-I
HTLV-II/STLV-II

mega, Madison, WI), and ligated into *Eco*RI sites of the Bluescript vector. The resultant plasmid was used to transform HB101 cells (GIBCO, Gaithersburg, MD) by the conventional heat-shock method. The $^{32}$P-labeled SK45 probe (Perkin–Elmer) was used for hybridization; washes were done in 3× SSC, 0.1% SDS at room temperature for 15 min. The nested PCR product (sample MNE-930306) was cloned into the pT7Blue vector (Novagen, Madison, WI). Plasmid DNA for all recombinant clones was extracted, column purified, and sequenced with Sequenase Version 2.0 (United States Biochemical, Cleveland, OH) using the chain-termination method (Sanger *et al.,* 1977) with M13 universal primers.

### Sequence analyses

Viral *tax* sequences were aligned by the method of Needleman and Wunsch (1970) using the GCG computer program (GCG Version 8, 1995) and verified by eye. Sequence site variation was analyzed by MEGA Version 1.01 (Kumar *et al.,* 1993). Phylogenetic reconstruction used both minimum evolution, estimated by the neighbor-joining method (NJ) (Saitou and Nei, 1987), and maximum parsimony analyses (MP) (Swofford, 1993). Although these two methods employed different evolutionary assumptions, concordance between the respective topologies is indicative of the true phylogeny among sequences. Minimum evolution estimated by NJ constructed a phylogeny based on a matrix of Jukes–Cantor (1969) distance among all pairs of sequences. In contrast, character-based MP analyses constructed a tree based on the assumption that minimum-length trees were optimal estimates of the true phylogeny. Bootstrap resampling analyses consisted of 100 iterations and were included to ascertain the reliability of the data to consistently derive the same topology. Strong support for an adjacent node was assumed with bootstrap proportions >70% (Hillis and Bull, 1993).

The computer package PHYLIP Version 3.5 (Felsenstein, 1993) was used for the computation of Jukes–Cantor genetic distances, NJ analyses, and bootstrap resampling with NJ. Maximum parsimony analyses were computed using test version of PAUP* (used with permission by D. Swofford).

## RESULTS

Phylogenetic reconstruction of *tax* gene sequences forms three major evolutionary clusters or clades: HTLV-I/STLV-I, HTLV-II/STLV-II, and a third monotypic lineage composed of a divergent strain of STLV from a wild caught baboon, *Papio hamadryas* (PHA-969) (Fig. 1). Genetic distances among the three groups were approximately equal (Table 1). Within the HTLV-I/STLV-I lineage, the 8 new Asian macaque strains formed a separate cluster in both NJ and MP analyses. The average level of sequence variation within the Asian macaque group (5.4%) was roughly comparable to that estimated among all other HTLV-I/STLV-I groups examined (4.2%) (Table 1). However, the average level of genetic variation between the 8 macaques relative to the other 14 representative strains of HTLV-I/STLV-I nearly doubled (8.0%). The new sequence, HIN28, from a patient from Zaire associates closely with the HTLV-I Zaire strain of HEL. Within the HTLV-II/STLV-II lineage, two of the three sequences from the Mbati villagers cluster together. However, the third sequence, HMBA122, is affiliated with recognized HTLV-II subtypes of HMO (subtype A) and HNRA (subtype B) from the New World. The isolate from a bonobo chimp from Zaire (STLV-79B) was phylogenetically distinct, but strong bootstrap values (83% NJ; MP) support its position as an early divergence within the HTLV-II/STLV-II clade.

Despite a low number of nucleotides examined in this *tax* region, analyses of site variation reveal a small number of changes informative to the phylogenetic placement of 13 newly described sequences. In the HTLV-I/STLV-I lineage, some or all of the 8 Asian macaques share unique substitutions at positions 14 and 102 (Fig. 2). All 8 shared a substitution from A to G at position 102, resulting in an amino acid change from isoleucine to valine (Figure 3). With the exception of MMU-U13, the Asian macaque cluster exhibits a mutation from A to C at position 14. Likewise, in the HTLV-II/STLV-II sequences, the

FIG. 1. Phylogenetic analyses of 30 STLV and HTLV *tax* sequences. Sequences from this study are identified as (a) HTLV-I: HIN28; (b) HTLV-II: HMBA122, HMBA141, HMBA155; (c) STLV-I: CAT-06 (*Cercocebus atys*); MNE-930287, MNE-930306 (*Macaca nemestrina*); MMU-186-85, MMU-300-73, MMU-U13, MMU-P913 (*Macaca mulatta*); and MFA-C194, MFA-70-70 (*Macaca fascicularis*). Previously published sequences are as follows: (a) HTLV-I: HATK (Seiki *et al.,* 1983), HEL (Paine *et al.,* 1991), HMEL 1, HMEL 5 (Gessain *et al.,* 1993); (b) HTLV-II: HMO (Shimotohno *et al.,* 1985), HNRA (Lee *et al.,* 1993); (c) STLV-I: CAE-22 (*Cercopithecus aethiops,* Yanagihara *et al.,* 1993); CAE-TAN90 (*C. aethiops,* Saksena *et al.,* 1993); CAE-SABC4 (*C. aethiops*), PDO-34 (*Papio doghera,* Saksena *et al.,* 1994); MFA-86 (*Macaca fuscata,* Song *et al.,* 1994); MNE-PTM3 (*M. nemestrina,* Watanabe *et al.,* 1994); MTO-TE4 (*Macaca tonkeana,* Ibrahim *et al.,* 1995), PHA-969 (*Papio hamadryas,* Goubou *et al.,* 1994); PTR-114.1 (*Pan troglodytes,* Yanagihara *et al.,* 1993); (d) STLV-II: PPA-79B (*Pan paniscus,* Giri *et al.,* 1994); AFU-3 (*Ateles fusciceps,* Chen *et al.,* 1994). (Left) Tree derived by minimum evolution estimated by neighbor joining using Jukes–Cantor estimates of genetic distance (Table 1). Numbers on branch lengths represent the percentage of sequence divergence. Numbers in italic represent bootstrap proportions in support of adjacent nodes. Asterisks denote nodes not supported in the bootstrap analyses. (Right) Tree derived by maximum parsimony analysis is consensus of 430 equivalent trees obtained by heuristic search employing simple sequence addition (reference taxon, PHA969) and nearest neighbor interchange for branch swapping (length, 202). Numbers on limbs represent number of steps/number of homoplasies. Numbers in italic are bootstrap proportions in support of adjacent nodes. Unlabeled nodes were collapsed to a polytomy in bootstrap analyses. Changes due to transversion were weighted five times relative those due to transitions.

TABLE 1

Genetic Distance (%) Estimated by the Jukes–Cantor (1969) Model of Substitution among All Pairs of *tax* Sequences

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HATK | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HMEL1 | 0.9 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HMEL5 | 0.9 | 1.7 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HEL | 1.7 | 2.6 | 2.6 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HIN28 | 2.6 | 3.5 | 3.5 | 0.9 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CAE-TAN90 | 4.4 | 5.3 | 5.3 | 6.2 | 7.1 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PDO-34 | 1.7 | 2.6 | 2.6 | 3.5 | 4.4 | 6.2 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | |
| CAE-SABC4 | 1.7 | 2.6 | 2.6 | 3.5 | 4.4 | 6.2 | 3.5 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| CAE-22 | 0.9 | 1.7 | 1.7 | 2.6 | 3.5 | 5.3 | 2.6 | 2.6 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | |
| PTR114.1 | 2.6 | 3.5 | 3.5 | 2.6 | 3.5 | 7.1 | 4.4 | 2.6 | 3.5 | 0.0 | | | | | | | | | | | | | | | | | | | | | | |
| CAT-6 | 1.7 | 2.6 | 2.6 | 1.7 | 2.6 | 6.2 | 1.7 | 3.5 | 2.6 | 2.6 | 0.0 | | | | | | | | | | | | | | | | | | | | | |
| PHA-969 | 20.3 | 21.4 | 21.4 | 19.2 | 20.3 | 22.6 | 20.3 | 19.2 | 21.4 | 19.2 | 18.1 | 0.0 | | | | | | | | | | | | | | | | | | | | |
| PPA-79A | 23.7 | 24.9 | 24.9 | 26.1 | 27.3 | 27.3 | 26.1 | 26.1 | 22.6 | 24.9 | 26.1 | 23.7 | 0.0 | | | | | | | | | | | | | | | | | | | |
| MTO-TE4 | 3.5 | 4.4 | 4.4 | 5.3 | 6.2 | 6.2 | 5.3 | 5.3 | 4.4 | 6.2 | 5.3 | 21.4 | 23.7 | 0.0 | | | | | | | | | | | | | | | | | | |
| MFU-86 | 3.5 | 4.4 | 4.4 | 5.3 | 6.2 | 8.0 | 5.3 | 5.3 | 4.4 | 6.2 | 5.3 | 22.6 | 22.6 | 7.1 | 0.0 | | | | | | | | | | | | | | | | | |
| MNE-PTM3 | 5.3 | 6.2 | 6.2 | 7.1 | 6.2 | 8.0 | 7.1 | 7.1 | 6.2 | 8.0 | 7.1 | 24.9 | 24.9 | 9.0 | 1.7 | 0.0 | | | | | | | | | | | | | | | | |
| MFA-C194 | 6.2 | 7.1 | 7.1 | 6.2 | 7.1 | 7.1 | 8.0 | 8.0 | 7.1 | 7.1 | 6.2 | 21.4 | 23.7 | 6.2 | 9.0 | 10.9 | 0.0 | | | | | | | | | | | | | | | |
| MNE-930287 | 10.9 | 11.9 | 11.9 | 10.9 | 11.9 | 13.9 | 12.9 | 12.9 | 11.9 | 11.9 | 10.9 | 28.5 | 26.1 | 12.9 | 11.9 | 13.9 | 6.2 | 0.0 | | | | | | | | | | | | | | |
| MNE-930306 | 6.2 | 7.1 | 7.1 | 6.2 | 7.1 | 7.1 | 8.0 | 8.0 | 7.1 | 7.1 | 6.2 | 21.4 | 23.7 | 6.2 | 9.0 | 10.9 | 0.0 | 6.2 | 0.0 | | | | | | | | | | | | | |
| MFA-70-70 | 6.2 | 7.1 | 7.1 | 6.2 | 7.1 | 9.0 | 8.0 | 8.0 | 7.1 | 7.1 | 6.2 | 19.2 | 26.1 | 8.0 | 9.0 | 10.9 | 3.5 | 8.0 | 3.5 | 0.0 | | | | | | | | | | | | |
| MMY-186-86 | 7.1 | 8.0 | 8.0 | 7.1 | 8.0 | 10.0 | 9.0 | 9.0 | 8.0 | 8.0 | 7.1 | 20.3 | 24.9 | 9.0 | 8.0 | 10.0 | 4.4 | 9.0 | 4.4 | 2.6 | 0.0 | | | | | | | | | | | |
| MMU-300-73 | 5.3 | 6.2 | 6.2 | 5.3 | 6.2 | 8.0 | 7.1 | 7.1 | 6.2 | 6.2 | 5.3 | 20.3 | 24.9 | 7.1 | 8.0 | 10.0 | 2.6 | 7.1 | 2.6 | 0.9 | 1.7 | 0.0 | | | | | | | | | | |
| MMU-U13 | 7.1 | 8.0 | 8.0 | 7.1 | 8.0 | 11.9 | 7.1 | 9.0 | 8.0 | 8.0 | 7.1 | 24.9 | 27.3 | 10.0 | 10.0 | 11.9 | 7.1 | 10.0 | 7.1 | 9.0 | 10.0 | 8.0 | 0.0 | | | | | | | | | |
| MMU-P913 | 5.3 | 6.2 | 6.2 | 5.3 | 6.2 | 8.0 | 7.1 | 7.1 | 6.2 | 6.2 | 5.3 | 23.7 | 28.5 | 7.1 | 6.2 | 8.0 | 4.4 | 9.0 | 4.4 | 4.4 | 5.3 | 3.5 | 8.0 | 0.0 | | | | | | | | |
| HMO | 17.1 | 18.1 | 18.1 | 17.1 | 18.1 | 22.6 | 19.2 | 19.2 | 18.1 | 16.0 | 17.1 | 22.6 | 13.9 | 19.2 | 21.4 | 23.7 | 18.1 | 18.1 | 18.1 | 19.2 | 20.3 | 18.1 | 16.0 | 20.3 | 0.0 | | | | | | | |
| HNRA | 16.0 | 17.1 | 17.1 | 16.0 | 17.1 | 21.4 | 18.1 | 18.1 | 17.1 | 15.0 | 16.0 | 21.4 | 12.9 | 18.1 | 20.3 | 22.6 | 17.1 | 19.2 | 17.1 | 18.1 | 19.2 | 17.1 | 17.1 | 19.2 | 0.9 | 0.0 | | | | | | |
| AFU-3 | 18.3 | 19.4 | 19.4 | 18.3 | 19.4 | 24.0 | 20.5 | 20.5 | 19.4 | 17.2 | 18.3 | 24.0 | 16.2 | 20.5 | 22.8 | 25.2 | 20.5 | 20.5 | 20.5 | 20.5 | 21.7 | 19.4 | 18.3 | 21.7 | 1.7 | 2.6 | 0.0 | | | | | |
| HMBA122 | 18.1 | 19.2 | 19.2 | 18.1 | 19.2 | 23.7 | 20.3 | 20.3 | 19.2 | 17.1 | 18.1 | 21.4 | 12.9 | 20.3 | 20.3 | 22.6 | 19.2 | 19.2 | 19.2 | 20.3 | 19.2 | 19.2 | 17.1 | 21.4 | 0.9 | 1.7 | 2.6 | 0.0 | | | | |
| HMBA141 | 17.1 | 18.1 | 18.1 | 17.1 | 18.1 | 22.6 | 19.2 | 19.2 | 18.1 | 16.0 | 17.1 | 24.9 | 13.9 | 19.2 | 21.4 | 23.7 | 18.1 | 18.1 | 18.1 | 19.2 | 20.3 | 18.1 | 16.0 | 20.3 | 1.7 | 2.6 | 3.5 | 2.6 | 0.0 | | | |
| HMBA155 | 18.1 | 19.2 | 19.2 | 18.1 | 19.2 | 21.4 | 20.3 | 20.3 | 19.2 | 17.1 | 18.1 | 26.1 | 15.0 | 20.3 | 22.6 | 22.6 | 19.2 | 19.2 | 19.2 | 20.3 | 21.4 | 19.2 | 17.1 | 21.4 | 2.6 | 3.5 | 4.4 | 3.5 | 0.9 | 0.0 | | |

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                    20                  40                  60                  80                  100
HATK        TTGGAGACTGTGTACAAGGC GACTGGTGCCCCATCTCTGG GGGACTATGTTCGGCCCGCC TACATCGTCACGCCCTACTG GCCACCTGTCCAGAGCATCA
HMEL1       A.................. .................... .................... .................... ....................
HMEL5       .................. .................... .................... .................... ....................
HEL         ................G... .................... ..................T. .................... ....................
HIN28       .....A.........G... .................... ..................T. .................... ....................
CAE-TAN90   .................. .................... ..............T..... .................... ........C..G........
PDO-34      .................. .................... ....T............... .................... ....................
CAE-SABC4   .................. .................... ................A... .................... ............A.....
CAE-22      ..A............... .................... .................... .................... ....................
PTR-114.1   ................G... .................... ..............A..... .................... ............A.....
CAT-09      ................G... .................... .................... .................... ....................
PHA-969     .C..C..........G.C. ..T...........T..... A..G..T.....C..T..G. ....C..C..T.....G... .....G..C..C..A.....
PPA-79B     ..A.C..T.....T....C. ..T.....T...G.T..A.. T..G..T.....CA...... .G..C..GA.......T..C ........C........C..
MTO-TE4     ...................T .................C.. ............A....... .................... ........C...........
MFU-86      ..............G..... .................... .........C.......... .G..C.............. ....................
MNE-PTM3    .....A........G..... .................... .........C.......... .G..C.............. ....................
MFA-C194    ..............C..G... .................... ............A....... .................... ........C..G.....C..
MNE-930287  ....G..T..C..C..G... .................... ............A....... .G...............T.. ...........G.....C..
MNE-930306  ..............C..G... .................... ............A....... .................... ........C..G.....C..
MFA-70-70   .C............C..G... .................... ...G.........A....... .................... ...........G........
MMU-186-86  ..............C..G... .................... ...G.........A....... ....CT.............. ...........G........
MMU-300-73  ..............C..G... .................... ...G.........A....... .................... ...........G........
MMU-U13     ................G... .................... ....T...CG..C....... .................... ................C..
MMU-P913    ..............C..G..T .................... .........C.......... .................... ...........G........
HMO         ....C..T........G.C. ..T.....T...G....A.. T..T.........CA...... .......A..T.....C... ................C..
HNRA        ....C..T........G.C. ..T.....T...G....A.. T..T.........CA...... .......A..T.....C... ................C..
AFU-3       ....C..T........G.C. ..T.....T...G....A.. T..T.........CA...... .......A..T.....C... ................GA.
HMBA122     ....C..T........G.C. ..T.....T...G....A.. T..T.........CA...... ....C..A..T.....C... ................C..
HMBA141     ....C..T........G.C. ..T.....T...G....A.. T..T.........CA...... .......A........C... ................C..
HMBA155     ....C..T........G.C. ..T.....T...G....A.. T..T.........CA...... .......A........C... ................C..

                    120

HATK        GATCACCTGGGACCCCAT
HMEL1       ..................
HMEL5       ........A.........
HEL         ..................
HIN28       ..................
CAE-TAN90   ............T....C
PDO-34      ...T..............
CAE-SABC4   ..................
CAE-22      ..................
PTR-114.1   ..................
CAT-06      ...T..............
PHA-969     ...T..............
PPA-79B     .C................
MTO-TE4     ..................
MFU-86      ..................
MNE-PTM3    ..................C
MFA-C194    .G................
MNE-930287  AG................
MNE-930306  .G................
MFU-70-70   .G................
MMU-186-86  .G................
MMU-300-73  .G................
MMU-U13     AG................
MMU-P913    .G................
HMO         AC................
HNRA        .C................
AFU-3       AC........*.......
HMBA122     AC................
HMBA141     AC.........T......
HMBA155     AC.........T.....C
```

FIG. 2. Alignment and comparison of nucleotide sequences of the *tax*-encoding region of the *pX* gene (bases 7378 to 7495 for HTLV-I and 7268 to 7385 for HTLV-II) in 13 new strains. For comparison, corresponding sequences are shown for previously described (see legend to Fig. 1) HTLV-I, HTLV-II subgroups, Asian STLV-I, African STLV-I, and PTLV-L. Asterisk represents deletion. Nucleotide sequences for new viral strains have been deposited with GenBank under Accession Nos. U59132 (MFA-C194), U59133 (CAT-06), U59134 (HMBA122), U59135 (HMBA141), U59137 (HIN28), U59138 (MFA-70-70), U59139 (MMU-186-85), U59140 (MMU-300-73), U59141 (MNE-930287), U59142 (MNE-930306), U59143 (MMU-P913), and U59144 (MMU-U13).

```
                          20                  40
HATK          GDCVQGDWCPISGGLCSARL HRHALLATCPEHQITWDPI
HMEL1         .................... ....................
HMEL5         .................... ................U...
HEL           .................... ....................
HIN28         .N.................. ....................
CAE-TAN90     ................V.. ....................
PDO-34        .................... ....................
CAE-SABC4     .................... ....................
CAE-22        .................... ....................
PTR-114.1     ................T.. ....................
CAT-06        .................... ....................
PHA-969       .....A.............. ....................
PPA-79B       S....A....V......T.. ..N.........L.....
MTO-TE4       .................... ....................
MFU-86        .................... ....................
MNE-PTM3      N..................  ..................T
MFA-C194      .................... ............V.....
MNE-930287    .................... ............V.....
MNE-930306    .................... ............V.....
MFA-70-70     .................... ............V.....
MMU-186-86    .................... .C..........V.....
MMU-300-73    .................... ............V.....
MMU-U13       ...............S.... ............V.....
MMU-P913      .................... ............V.....
HMO           .....A....V......T.. ............L.....
HNRA          .....A....V......T.. ............L.....
AFU-3         .....A....V......T.. ..........QKL..T.F
HMBA122       .....A....V......T.. ............L.....
HMBA141       .....A....V......T.. ............L..V..
HMBA155       .....A....V......T.. ............L..V.T
```

FIG. 3. Corresponding amino acid alignment of nucleotide sequences described in Fig. 2. Alignment of the putative amino acid sequences corresponding to the 118-bp *tax*-encoding region of the Cosmopolitan, Zairean, and Melanesian HTLV-I variants, HTLV-II subgroups, STLV-II, PTLV-L, and Asian and African STLV-I. Single-letter amino acid code was used. U represents termination codon.

3 Mbati villager sequences HMBA122 differed from HMBA141 and HMBA155 at 65, 71, and 112. Most likely, the change at position 71 was an ancestral polymorphism as it was found in PHA-969 and the 3 New World sequences.

Further analysis of amino acids reveal clearly defined substitutions that distinguished viral types I and II (Fig. 3). All HTLV-II/STLV-II strains differ with respect to HTLV-I/STLV-I at amino acid positions 6, 11, and 34. In the divergent PHA-969 strain, nucleotide substitutions are synonymous except for alanine rather than a glycine in position 6 (an amino acid characteristic of viral type II). Despite elevated distance estimates relative to all strains examined, the simian strain PPA-79B is most similar to HTLV-II/STLV-II. Of the six substitutions present in the amino acid alignment, three are shared-derived traits with HTLV-II/STLV-II sequences, one is polymorphic among all sequences, and two are unique (autoapomorphic) to PPA-79B.

## DISCUSSION

We report the phylogenetic analysis of HTLV/STLV, including 13 new strains, based on the nucleotide sequence divergence of the evolutionarily conserved *tax* gene. Clearly identified with strong bootstrap support are three major lineages corresponding to HTLV-I/STLV-I, HTLV-II/STLV-II, and a monotypic lineage composed of a single strain from a baboon from Eritrea (PHA-969). The multispecies composition of both HTLV-I/STLV-I and HTLV-II/STLV-II lineages affirm that the distinct evolutionary differences were not related to host phylogeny and corroborate previous evidence for interspecies transmission among primates within the same general geographic region (Koralnik, 1994; Ibrahim, 1995). Additional insights into the natural history of these viruses are inferred from the relative placement of newly described isolates within this study.

Within the HTLV-I/STLV-I lineage, eight new isolates from three different species of Asian macaques further attest to high levels of genetic diversity within Asian STLV-I strains observed in earlier studies (Koralnik *et al.,* 1994; Ibrahim *et al.,* 1995; Vandamme *et al.,* 1996). Within the present study are samples from recognized subgroups based on an *env* gene study (Koralnik *et al.,* 1994) including the following: HATK (*env* Clade 3: Cosmopolitan), HMEL1 and HMEL5 (*env* Clade 1: Melanesia), MNE-PTM3 and MFA-C194 (*env* S1), and PTR-114.1 (*env* S5). Having a slower rate of evolution, *tax* gene sequences do not recapitulate the internal branching order of the *env* phylogeny. However, higher levels of genetic diversity in Asian macaques are inferred by the disparate placement of the two representatives from *env* S1. This consists of splitting MNE-PTM3 apart from MFA-C194, which falls within the monophyletic clade of newly described viruses from Asian macaques.

Average levels of genetic diversity within the Asian macaque cluster were roughly comparable to that among all recognized subgroups included in this analysis. Therefore, assuming that the mutation rate within the *tax* gene is roughly consistent among all strains of HTLV/STLV, the simplest interpretation is that Asian STLV-I strains have a more ancient origin than other type I strains, including those from Africa (HEL, HIN28, CAE-22, CAE-TAN90, CAE-SABC4, and CAT-06). A previous study of the *tax*/*rex* gene (Vandamme *et al.,* 1996) differed in sample composition and included only two STLV-I strains, thus limiting interpretation concerning levels of genetic variation for Asian STLV-I within the *tax* region. In comparison, our results present a slightly stronger argument in favor of an Asian origin for the type I virus.

Phylogenetic analyses of *tax* sequences suggest higher levels of genetic diversity present within HTLV-II/STLV-II than previously described. Research on Amerindian peoples, isolated from one another for thousands of years, revealed variants of HTLV-II that are closely associated with either subtype A or subtype B (Maloney *et al.,* 1992; Biggar *et al.,* 1996). In our analyses of the *tax* gene, HMO and HNRA differed at one site (position 101). In comparison, two of the three Mbati HTLV-II sequences from Africa share two site changes relative to HMO and HNRA. The third sequence, HMBA122, was nearly identical to HMO but also had a unique (autoapomorphic) site change. This level of diversity within an isolated people from the same region implies the virus has resided within the population for some time. In addi-

tion, our analysis unambiguously places the strain, $STLV_{Pan\text{-}p}$, isolated from bonobo chimps from Zaire (Giri *et al.,* 1994; Liu *et al.,* 1994; Digilio *et al.,* 1997), within the type II lineage as an early, ancestral divergence. Consequently, the diversity within the Mbati people combined with the deep divergence of $STLV_{Pan\text{-}p}$ suggests, but does not prove, an African origin for HTLV-II/STLV-II.

Analyses of the conserved *tax* gene illustrate the genetic diversity present within human and simian forms of the T-cell leukemia/lymphotropic virus. Acting as a genetic marker of more ancient events in the evolution of these viruses, *tax* sequences describe considerable variation within Asian macaques and authoctonous human populations in Africa. The results remain inconclusive as to the origin of a common ancestor to all forms of HTLV/STLV, but they indicate that type I and type II strains each arose from a simian host.

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