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Sequence Note

Sequence of the *env* Gene of Some KwaZulu-Natal, South African Strains of HTLV Type I

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ABSTRACT

Phylogenetic analysis of HTLV-I suggests three main subtypes, namely, cosmopolitan, Central African, and Australo-Melanesian. HTLV-I is endemic in KwaZulu-Natal, South Africa. However, sequence data on the local strains are limited to the LTR region. The *env* gene of the local strain was amplified and sequenced from the peripheral blood of five seropositive individuals. Four had HTLV-I-associated myelopathy and one had infective eczema. The sequence analysis of the *env* gene showed a greater then 99% homology of the local strains. They were closely related to the North American strains (99.3%), followed by the Japanese strains (98.3–98.9%). Phylogenetic studies linked the local strains to the cosmopolitan subtype. This study provides new sequence data on the *env* gene of the local HTLV-I strain.

THE HUMAN T cell lymphotropic virus type I (HTLV-I) is causally linked to HTLV-I-associated myelopathy (HAM/TSP) and adult T cell leukemia/lymphoma (ATL). HTLV-I is endemic in the indigenous population of KwaZulu-Natal in South Africa, with a seroprevalence in the community of 2.6%.[1]

Phylogenetic analysis of the local KwaZulu-Natal strain has been confined to an analysis of the long terminal repeat (LTR) region.[2,3] The molecular study by Mahieux *et al.*[4] included analysis of a fragment of the *env* gene of strains from another area (The Free State) of this country. We report on the sequence analysis of the *env* region of HTLV-I from five seropositive individuals in KwaZulu-Natal.

One of the main objectives was to type and determine the most predominant local isolate. A local isolate was defined as that isolate that could be regarded as the predominant virus in KwaZulu-Natal. It was statistically calculated that isolates from five different patients would be adequate for this purpose.

Venous blood was drawn from five HTLV-I-seropositive individuals. Four had HAM/TSP and one had infective eczema. DNA was extracted from the peripheral blood by the scaled-down protocol of the Genomix kit (Talent, Srl, Italy). Primers were designed to target a 1.5-kb product of the *env* gene from nucleotides 5146 to 6681 (forward primer 5146–5186, 5′ CAT GCC CAA GAC CCG TCG GAG 3′; reverse primer 6661–6681, 5′ AGG GGC TGA GAA GGC CAA AGA 3′).

An internal primer pair was also designed for a nested polymerase chain reaction (PCR) from nucleotide 5166 to 6658 (forward primer 5166–5186, 5′ GGC CCC GCC CAT CCC AAA GAA 3′; reverse primer 6638–6658, 5′ ACT CAG GTT TTA TAA GAG AGT 3′).

A reaction mix was prepared as follows: 10 μl of 10× buffer (Boehringer Mannheim, Indianapolis, IN), dNTPs (250 μM each; Pharmacia, Piscataway, NJ), 2 U of *Taq* polymerase (Boehringer Mannheim), 2 mM Mg^{2+}, and 20 pmol of each primer in a 100-μl total volume. One microgram of DNA was added to each mix.

The cycling conditions were as follows: 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 60°C for 30 sec, and 72°C for 30 sec. A final cycle of 72°C for 7 min concluded the PCR,

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TABLE 1. LIST OF STRAINS USED FOR ANALYSIS OF *env* GENE

Strain	*Geographical origin*	*Accession No.*	*Ref.*
CH	N. America	M67490	Paine *et al.* (1991)[5]
EL	Zaire	M67514	Paine *et al.* (1991)[5]
GP68 (MT-2)	Japan	M23823	Takeuchi *et al.* (1991)[6]
H5	Japan	M37299	Tsujimoto *et al.* (1991)[7]
HA (HUT-102)	United States	M61048	De *et al.* (1991)[8]
HB (JA-1)	Japan	M61049	De *et al.* (1991)[8]
HC (JA-2)	Japan	M61050	De *et al.* (1991)[8]
HE (AF-3)	W. Africa	M61052	De *et al.* (1991)[8]
HG (AF-3)	W. Africa	M61054	De *et al.* (1991)[8]
HJ (AF-6)	W. Africa	M61057	De *et al.* (1991)[8]
HK (MT-2)	Japan	M61058	De *et al.* (1991)[8]
HL (ATK)	Japan	M61059	De *et al.* (1991)[8]
HL12091	Martinique	L76069	Baylot *et al.* (1991)[9]
HL1RHK30	Japan	L03561	Zhao *et al.* (1991)[10]
HL1RHK34	Japan	L03562	Zhao *et al.* (1991)[10]
HL2HENV	United States	L20734	Lee *et al.* (1991)[11]
HM (US-1)	United States	M64263	De *et al.* (1991)[8]
HTLVIENV(MT-2)	Japan	X56949	Astier-Gin *et al.* (1991)[12]
HTVPRCAR	Caribbean	D13784	Malik *et al.* (1991)[13]
HTVPROP(ATK-1)	Japan	J02029	Seiki *et al.* (1991)[14]
HL12055	French Guiana	L76056	Baylot *et al.* (1991)[9]
MEL1	Papua New Guinea	L02533	Gessain *et al.* (1991)[15]
SP	N. America	M67514	Paine *et al.* (1991)[5]
ST	Chile	L13774	Dekaban *et al.* (1991)[16]
STLENVI (PtM3)	Asia	M11373	Watanabe *et al.* (1991)[17]

which was then held at 4°C. Three microliters of the PCR product of the first step was used in the second step of the reaction.

The PCR product was purified to allow size selection and removal of DNA polymerase. Briefly, equal volumes of the PCR product and a 24:1 chloroform–isoamyl alcohol mix were vortexed for 1 min, then centrifuged for 1 min. The upper aqueous layer was used. Cycle sequencing was not performed on the PCR-amplified segments as the sequencing process did not work well with the primers that we designed. To overcome this problem all of the isolates were cloned and then sequenced. Only one clone per patient was sequenced. The product was then cloned using the pMOS blue T-vector cloning kit (Amersham, Life Science, Buckinghamshire, UK). This system exploits the template-independent activity of *Taq* polymerase, which preferentially adds a single adenine to the 3′ end of double-stranded DNA. The PCR products were inserted into compatible thymidine-tailed vectors, for which the pMOS blue T-vector has been specifically constructed.

Clones were screened by PCR and alkaline lysis of plasmid minipreps. The two restriction enzymes used were *Bam*HI and *Hin*dIII.

For sequencing of the insert, DNA was extracted, using a Qiagen (Chatsworth, CA) plasmid kit. Sequencing was performed with the automated laser fluorescent (ALF) express (Pharmacia-Biotech) DNA sequencer, according to the manufacturer instructions.

For the phylogenetic analysis, the sequences were aligned by DNASIS v7.00 (Hitachi Software Engineering, San Bruno, CA, 1991) with minimal manual editing. Analysis was performed using the PHYLIP (phylogenetic) inference package, version 3.2) software package. Trees were constructed for the entire *env* gene (1.5 kb) and partial *env* gene ($\sim$ 500 bp). The latter allowed comparison with a large number of published sequences. All strains included in the analysis are listed in the Table 1.[5–17] The tree-building methods used were the neighbor-joining (NJ) method and the maximum likelihood (ML) method.

The sequence analysis of the 1.5-kb *env* gene showed a 0.1 to 0.9% diversity among the local strains. They were closely related to the North American strains CH and SP (99.3%), followed by the Japanese strains (HTLV-I/ENV, 98.9%; H5, 98.6%; and HTVPROP, 98.3%), the Caribbean strain (HTVPRCAR, 97.9%), and the central African strain (EL, 97.4%). The Melanesian strain differed from the local strains by 7.5%.

When compared with the North American strain (CH) at the amino acid level, all of the local isolates showed an alanine-to-glycine change at codon 72 (Fig. 1). Two isolates showed a threonine-to-alanine change at codon 187. Other single isolate changes were isoleucine to threonine at codon 9, lysine to arginine at codon 352 and threonine to alanine at codon 493. The local isolates did not share the amino acid change of isoleucine to methionine at codon 326 and methionine to isoleucine at codon 412 that occurred in the CH strain.

Phylogenetic analysis of the 1.5-kb segment by the NJ method showed that the local strains clustered with the cosmopolitan subtype (Fig. 2), especially the North American strains CH and SP. Statistical analysis (1000 bootstrap replications) showed the branching pattern among the local strains and North American strains to be unstable, with a bootstrap confidence level (BCL) of 14%. The Central African and Melanesian strains were clearly distinct and separate from the cosmopolitan strain.

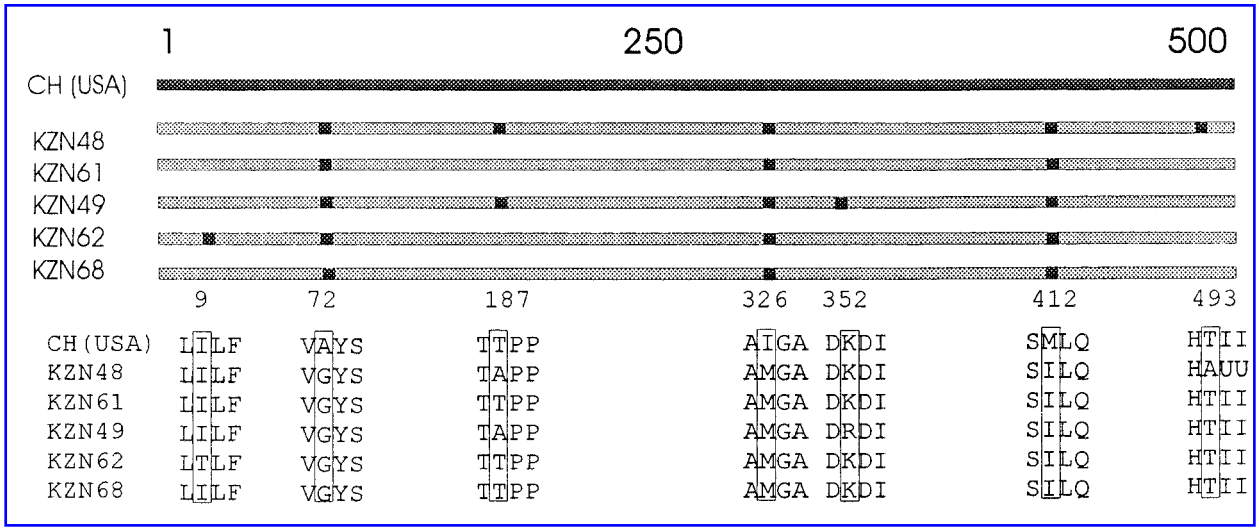

FIG. 1. List of strains used for analysis of *env* gene: Alignment of variable positions in amino acid sequences is presented. The top (solid) line represents amino acids 1–500 of the HTLV-I *env* gene of the CH (USA) isolate. The five gray lines represent equivalent amino acid sequences for the KZN isolates 48–68. The solid squares identify amino acid positions that differ from the CH (USA) strain. The actual amino acids are listed at the bottom.

The partial *env* gene analysis allowed the inclusion of more cosmopolitan strains (Fig. 3). The results were the same as with the 1.5-kb *env* analysis. The local strains still clustered with the cosmopolitan subtype. The Martinique strain was also included in this cluster.

Phylogenetic studies of HTLV-I isolates from endemic areas in the world suggest three main subtypes.[16,18–21] These subtypes are the cosmopolitan, Central African, and Melanesian. The most widespread type is the cosmopolitan variety, which includes isolates from Japan, Africa, South America, and the Caribbean. Miura *et al.*[19] further subdivided the cosmopolitan group into subgroups A, B, and C, where the A subgroup has Caribbean, Japanese, South American, and Iranian strains, the B subgroup has mainly Japanese strains, and the C subgroup has mainly West African and Caribbean strains. The Central African strain is distinct from other isolates from Africa, ap-

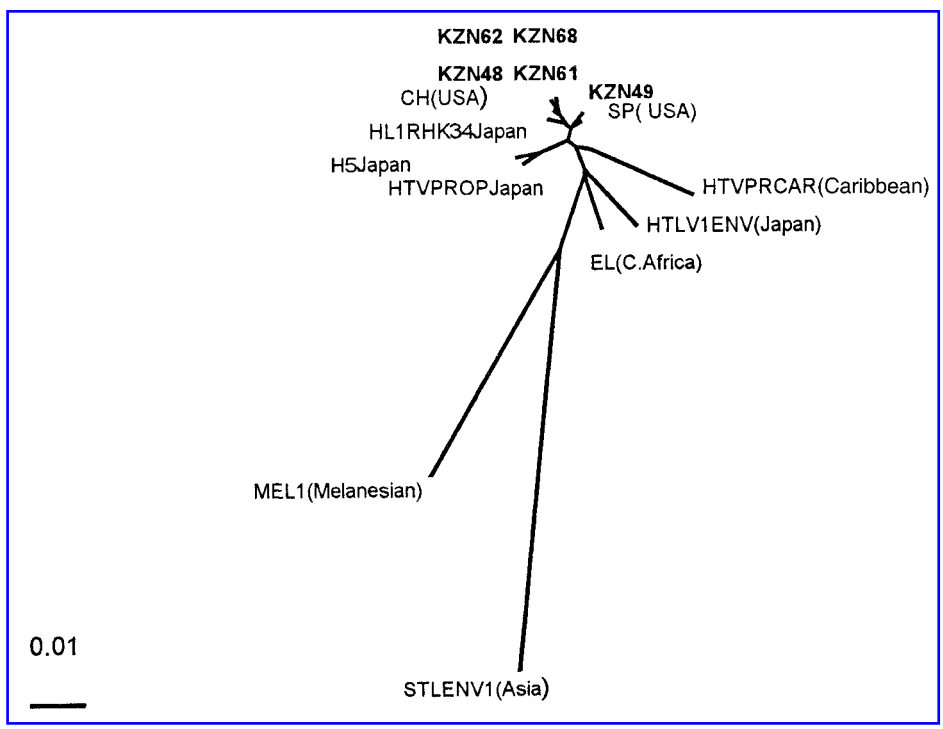

FIG. 2. HTLV-I phylogenetic tree (1.5-kb *env* nucleotide analysis), using the maximum likelihood (ML) and neighbor-joining (NJ) methods. Phylogenetic analysis was performed with the PHYLIP software package. Local strains are presented in boldface.

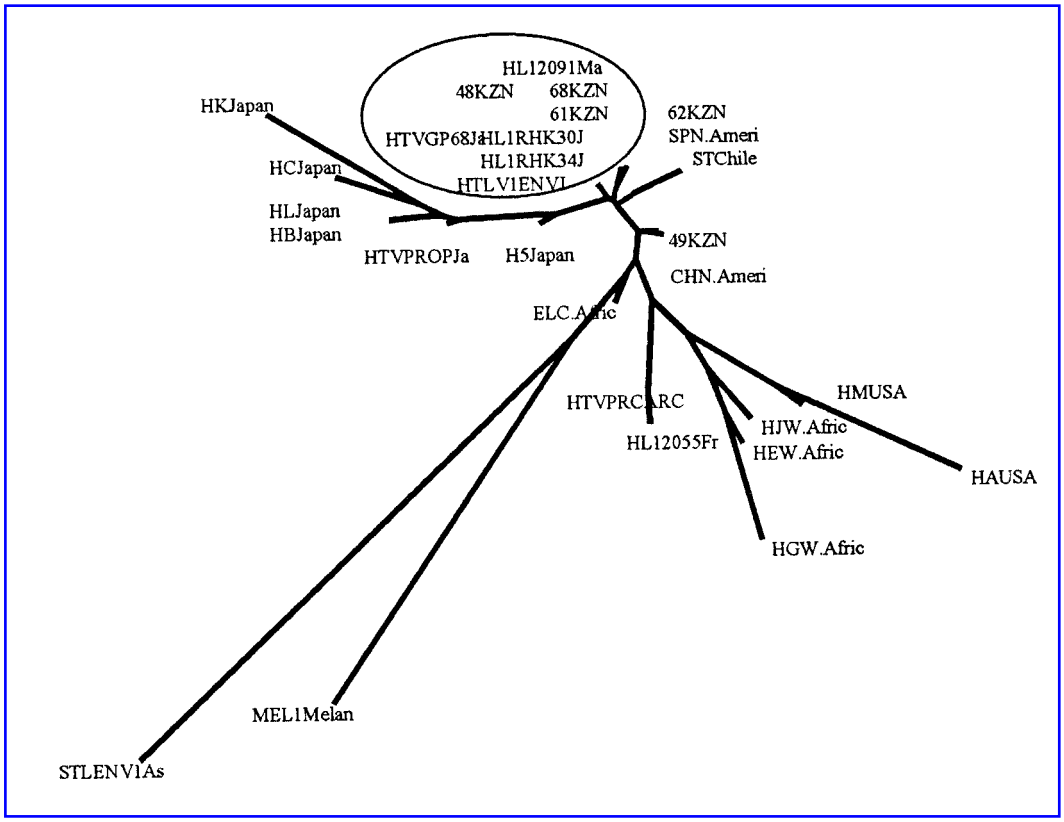

FIG. 3. HTLV-I phylogenetic tree (581-bp region of the *env* gene). The tree was constructed by the ML and NJ methods. Phylogenetic analysis was performed with the PHYLIP software package.

pears to have a fairly localized distribution, and is more closely related to a simian T cell lymphotropic virus type I (STLV-I) isolated from a Sierra Leone chimpanzee. This observation suggests recent interspecies transmission. Mahieux *et al.*[4] proposed a new, distinct African cluster that they position between the Central African and Austro-Melanesian subtype.

The Austro-Melanesian subtype is genetically distinct and was found in populations with no prior contact with either the Africans or Japanese. Further, this variant has occurred in areas devoid of subhuman primates. These observations suggest that this subtype evolved independently somewhere in the Southeast Asian land mass and also suggest an ancient association between Melanesians and Australian aboriginals.[20]

In our study we randomly chose one clone per isolate. As the variation and mutation rates of HTLV are not as high as for other retroviruses we felt that one clone would be representative of the isolate. The high sequence similarity between the isolates verified the approach that was taken.

Our study provides new sequence data on the *env* gene of the local KwaZulu-Natal HTLV-I strain. The data links these strains to the cosmopolitan subtype and complements LTR sequence data obtained by others.[2,3] An unexpected observation arising from our *env* data is the close linking of the local strains to the North American strains, yet the local strains are distinct and phylogenetically somewhat removed from the Caribbean and West African strains. The reasons for these findings are not clear. However, the findings do suggest that the explanation that the virus was spread to the New World by the slave trade is simplistic or incomplete.

Yamashita *et al.*[2] are incorrect in their speculation that the local strain was imported from India, as HAM/TSP is not seen in South Africans of Indian descent. To date we have seen approximately 200 cases of HAM/TSP (Refs. 1 and 22, and data on file). All of the patients were black. Furthermore, blood donor screening has not demonstrated any seropositivity in those of Indian descent.[23]

There are no serological or sequence data for HTLV-I in other southern African countries such as Mozambique, Zimbabwe, Zambia, and Angola. It is important to obtain these data before evaluating the presence and spread of the different subtypes in southern Africa.

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