Yonsei Medical Journal
Vol. 34, No. 4, 1993

Nucleotide Sequence Analysis of HTLV-I Isolate from a Korean Patient with HAM/TSP

**Mineki Saito[1,3], Takashi Moritoyo[3], Jae-Hyeon Park[2], Byung-In Lee[2]
Jin-Soo Kim[2], Jun-ichi Fujisawa[1], Mitsuhiro Osame[3]
and Mitsuaki Yoshida[1]***

Limited nucleotide sequences of human T-cell lymphotropic virus type I (HTLV-1) provirus isolated from the first case of a Korean patient with HTLV-I associated myelopathy and tropical spastic paraparesis (HAM/TSP) were analysed and compared with other isolates from different regions of the world. The sequences of the env, LTR regions (536bp, 690bp respectively) showed 98.7%, 99.3% homologies with the prototype HTLV-I, ATK-1, isolated from a Japanese Adult T-cell leukemia (ATL) patient. A comparison between other isolates from different geographical origins revealed that the Korean HTLV-I isolate is more closely related to Japanese isolates than to those from other geographical origins.

Key Words: HTLV-I, HAM/TSP, polymerase chain reaction, nucleotide sequence analysis

Human T cell leukemia virus type I (HTLV-I) (Poiesz *et al.* 1980, Hinuma *et al.* 1981) is the etiological agent of adult T cell leukemia/lymphoma (Uchiyama *et al.* 1977, Yoshida *et al.* 1982) and HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain *et al.* 1985, Osame *et al.* 1986). Although ATL and HAM/TSP are completely different diseases, HTLV-I isolated from patients with these diseases are very similar and show no specific variations linked to each pathology (Daenke *et al.* 1990, Kinoshita *et al.* 1991).

However, a recent nucleotide sequence analysis of HTLV-I from different geographical areas indicates the presence of area specific variations of HTLV-I, which might be useful in studying the emigration pathways of ancient people carrying this virus (Komurian *et al.* 1991, Gessain *et al.* 1992).

Since we previously reported the first Korean case of HAM/TSP (Park *et al.* 1991), we sequenced the proviral DNA integrated in the peripheral blood lymphocyte of the patient and compared it with three Japanese isolates newly sequenced and with reported HTLV-1 strains from different geographical areas to characterize and determine the geographical subtypes of HTLV-1 infecting this patient.

MATERIALS AND METHODS

A 40-year-old Korean male was diagnosed as HAM/TSP according to the criteria by Osame *et al.* (1987) in 1989. He was born in Korea and had no history of blood transfusion. His antibody titer to HTLV-1 was 8192-

Received August 23, 1993

[1]Department of Cellular and Molecular Biology, Institute of Medical Science, University of Tokyo, Shirokanedai, Minatoku, Tokyo 108, Japan
[2]Department of Neurology, Yonsei University College of Medicine, Seoul, Korea
[3]The Third Department of Internal Medicine, Faculty of Medicine, Kagoshima University, Kagoshima, Japan
All correspondance should be addressed to: Mitsuaki Yoshida, Ph.D. Department of Cellular and Molecular Biology, Institute of Medical Science, University of Tokyo, Shirokanedai, Minati-ku, Tokyo 108

fold in the serum and 512-fold in the CSF by the particle agglutination methods (Fuji Revio Co., Japan). Anti-HTLV-I antibodies of his family members all tested were negative with the exception of his wife. His wife also showed positive antibody titers in the serum and the CSF (256- and 8-fold respectively) without any clinical symptoms.

Peripheral blood lymphocytes (PBL) were separated from the patient, two Japanese HAM/TSP patients and one Japanese asymptomatic carrier by the Ficoll-Hypaque method and cellular DNA was extracted by proteinase K-phenol extraction as previously described. The HTLV-I sequence was amplified by polymerase chain reaction (PCR).

The sequences of the primers were as follows: LTR-I (sense)

5′-ACCATGAGCCCCAAATATCCCCC-3′,

LTR-2 (antisense)

5′-AATTTCTCTCCTGAGAGTGCTATAG-3′, env 13 (sense)

5′-TGTCACCTGTTCCCACCCTA-3′,

and env-14 (antisense)

5′-GCTTCCTTTACAGGGATGAC-3′. The HTLV-I region amplified by these primers mapped at positions 31 to 768 (LTR) and 6078 to 6653 (env) of the Japanese prototype ATK-1 (Seiki *et al.* 1983) DNA sequence.

The PCR was carried out 35 cycles at 94 C

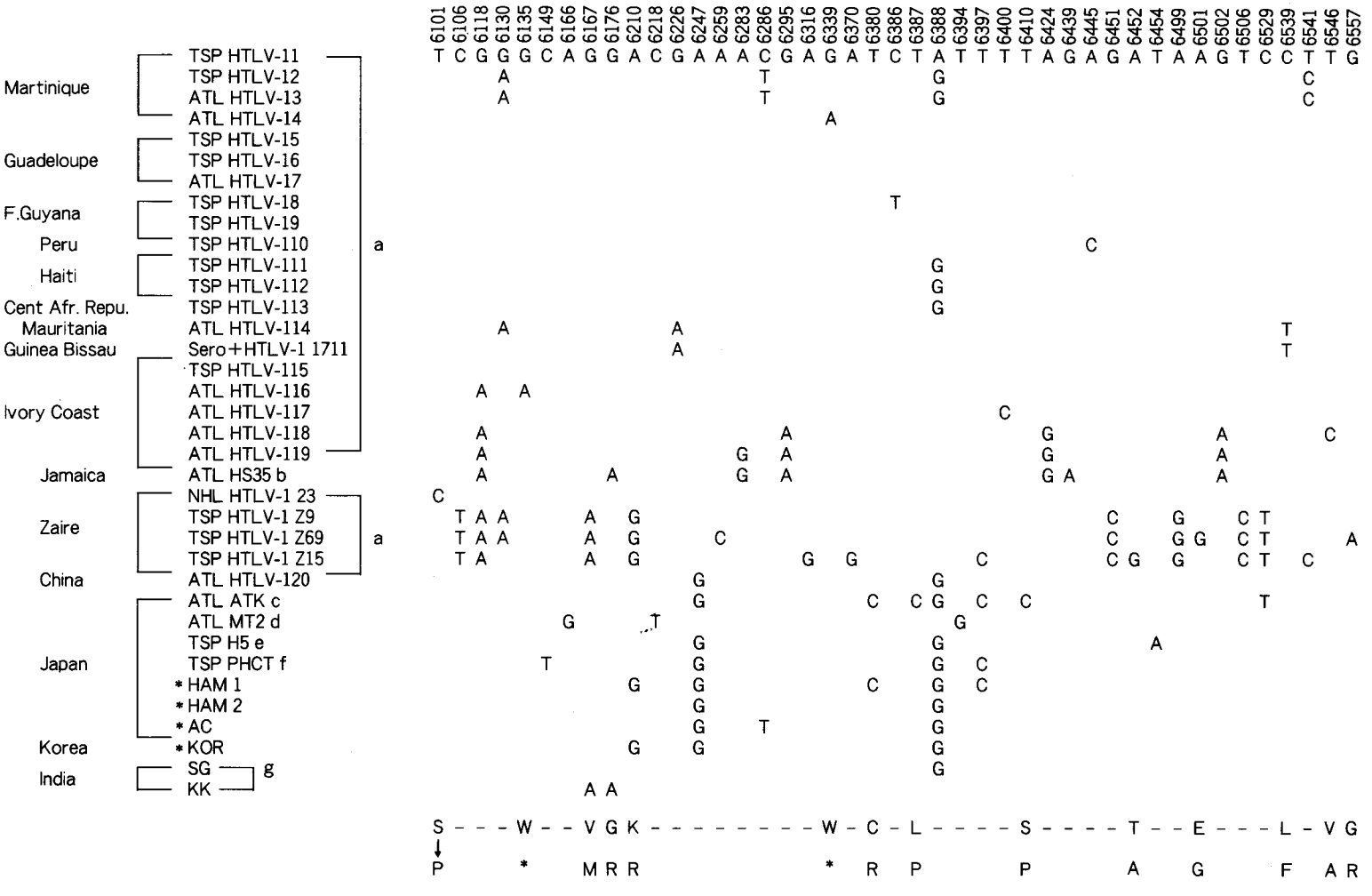
Table 1. Nucleotide variations observed in the LTR region No. 54-743 690bp

Nucleotide No.	ATK-1	HAM1	HAM2	AC	Korean HAM
63	G	*	A	*	*
150	G	A	A	A	A
185	G	*	A	*	*
194	A	*	*	*	G
231	A	G	G	G	*
232	A	G	G	G	G
233	G	X	X	*	*
263	A	*	*	C	*
338	G	A	A	A	A
449	*	*	*	*	T

Nucletoide Variations Observed in the Env region No. 6098-6633 536bp

Nucleotide No.	ATK-1	HAM1	HAM2	AC	Korean HAM
6124§	G	A	*	*	*
6210§	A	G	*	*	G
6246	A	*	G	*	*
6286§	C	*	*	T	*
6343§	G	*	*	C	*
6380§	C	*	T	T	T
6387	C	T	T	T	T
6397§	C	*	T	T	T
6410	C	T	T	T	T
6529§	T	C	C	C	C
6542	C	T	T	*	*
6601	C	*	G	*	*
6622§	T	*	*	C	*
6628	A	C	C	C	C

§: indicating the nucleotide variations causing a change in a deduced amino acids.
X: deletion *indicates nucleotide identity.



*: present study

a. Gessain et al 1992. b. Malik et al 1988. c. Seiki et al 1983. d. Gray et al. 1990.

e. Tsujimoto et al 1988. f. Shirabe et al 1990. g. Hashimoto et al 1993.

At the bottom, nucleotide changes which induce amino acid changes are presented. A dash indicates that no amino acid change, occured has

Fig. 1. *Nuclcotide Changes in HTLV-I Isolates From Different Geographical Areas —env region.*

for 2 min., 55 C for 1 min, 70 C for 2 min, with LTR primers, 35 cycles at 95 C for 1 min, 55 C for 75 sec, 72 C for 90 sec with env primers. The amplified products, 738bp for LTR, 576bp for env were subcloned into SmaI site of Bluescript vector for sequence analysis.

Nucleotide sequences of both DNA strands and at least two clones for each DNA sample were determined by the dideoxynucleotide chain termination method.

RESULTS

We sequenced 738bp of the LTR regions and 576bp of the env regions of the HTLV-I isolated from PBL derived from a Korean patient with HAM/TSP (KOR), two Japanese with HAM/TSP (HAM-1, HAM-2), and one Japanese asymptomatic carrier (AC). We selected these regions for sequence analysis because those were known as variable regions of HTLV-1 and many sequence data on these regions were available. The sequences of these regions without primer sequences were compared with each other and that of prototype ATK-1 sequences (Table 1). Sequences of KOR were highly homologous to that of Japanese isolates ; ATK-1, HAM-1, HAM-2, and AC. Sequence homology between KOR and prototype ATK-1 were 99.3% in the LTR and 98.7% in the env regions. In the env region, base replacements were detected at 7 nucleotides between ATK-1 and KOR that induced 5 amino acid changes out of 178 amino acids. But no deletion or insertion was detected in the env coding sequence, indicating conservation of coding capacities. In the LTR of KOR (690bp), no mutations and deletions were found among three enhancers as the Tax-responsive elements in the U3 region as well as stem structures and the Rex binding site in the R region.

To determine how closely the KOR isolates related to the HTLV-I isolates from various geographical regions of the world, a nucleotide sequence homology analysis was carried out with other HTLV-I isolates from different geographical regions including newly sequenced HAM-1, HAM-2, and AC (Fig. 1).

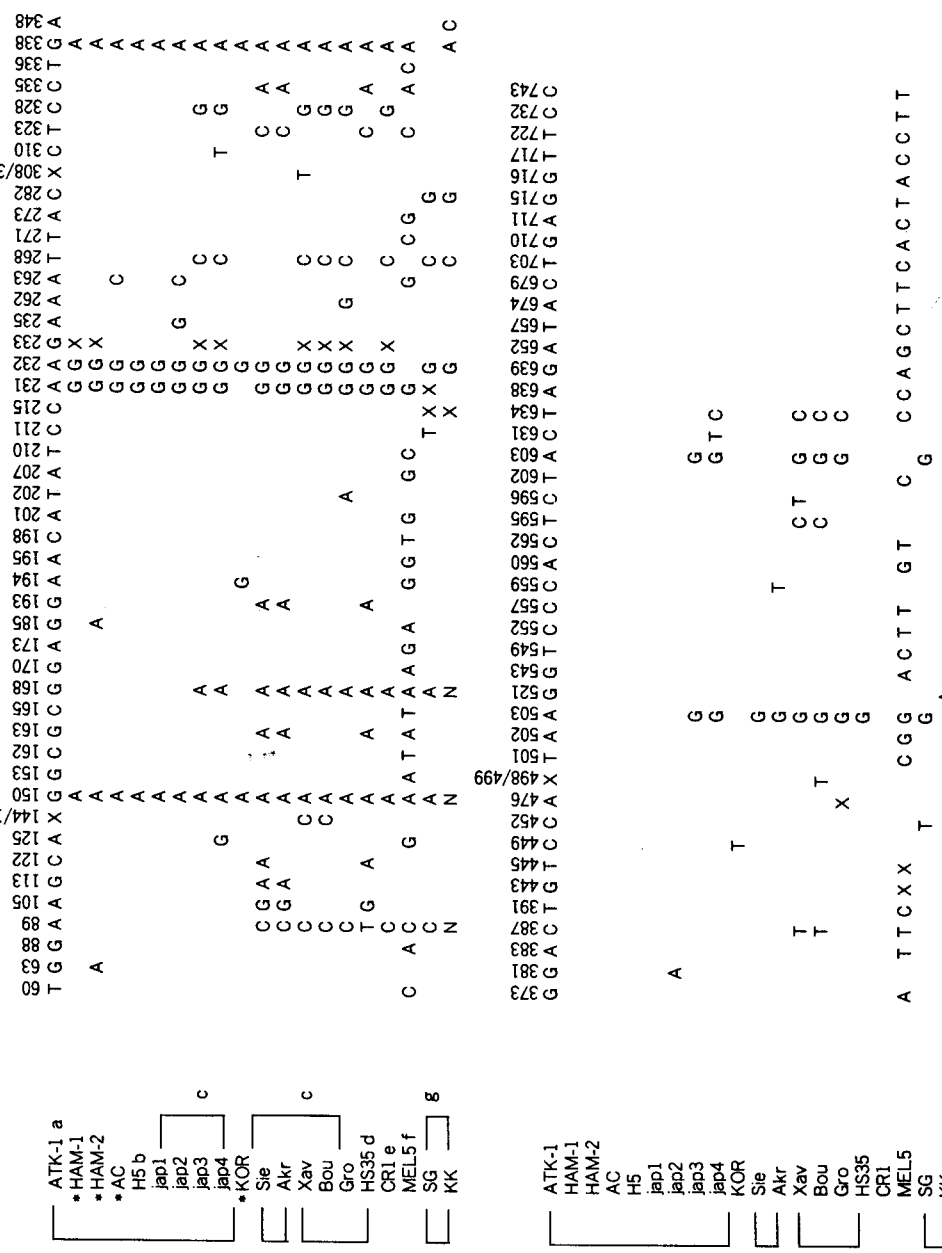
The region of the envelope protein that was sequenced encompassed the cleavage site of the envelope precursor protein and included nearly the entire coding region for the transmembrane glycoprotein gp21. Interestingly, KOR, HAM-1, HAM-2, AC isolates all displayed two nucleotides substitutions (No. 6247 G, No. 6388 G) that were common to all of the Japanese (except MT-2) and Chinese HTLV-I isolates of which sequences for this region have been published(Gessain *et al.* 1992).

The region of LTR was sequenced to be the same region as that reported by Komurian *et al.* who reported the genomic subtypes of HTLV-1 (Komurian *et al.* 1992). According to Komurian's classification, KOR, HAM-2, AC belong to subtype III (Table 2)

Table 2. Restriction site polymorphisms of proviral DNA in the LTR region according to Komurian et al 1992

HTLV-1 classification	Apa I(117) GGGCC↓C	Nde I(320) CA↓TATG	Mae II(603) A↓CGT	Mae III(632) ↓GTNAC	Dra I(501) TTT↓AAA	Sac I(507) GAGCT↓C
Subtype I	−	−	+	+	−	+
Subtype II	+	+	−	−	−	+
Subtype III	+	+	+	+	+	−
*HAM-1	+	+	+	+	+	−
*HAM-2	+	+	+	+	+	−
*AC	+	+	+	+	+	−
*KOR	+	+	+	+	+	−

*Determination of HTLV-1 subtype by sequencing



*present study N: not done X: deletion
a. Seiki et al. 1983 b. Tsujimoto et al. 1988 c. Komurian et al. 1991 d. Malik et al. 1988
e. Josephs et al. 1984 f. Gessain et al. 1993 g. Hashimoto et al. 1993
Nucleotide positions are numbered according to the HTLV-1(ATK-1) sequence in the EMBL data base ATK-1 is used as reference.

Fig. 2. *Nucleotide Changes in HTLV-I Isolates From Different Geographical Areas – LTR region.*

which included 2/3 of the Japanese isolates and some Zairian isolates (Komurian *et al.* 1992).

From this analysis it appears that the KOR strain is more closely related to Japanese and Chinese isolates than those of the other geographical origins.

DISCUSSION

The nucleotide sequences of HTLV-I are highly conserved compared to other retroviruses like HIV and there is no correlation between the specific nucleotide changes and the clinical status of the infected individual (Daenke *et al.* 1990, Kinoshita *et al.* 1991).

On the other hand, it has been demonstrated that nucleotide sequence variations among HTLV-I isolates from different geographical areas existed and it contribute to study of past movements of some human population and origin of the virus (Komurian *et al.* 1991, Gessain *et al.* 1992).

Previous seroepidemiologic studies in the east Asian countries indicated that HTLV-I is endemic in Japan but despite very intimate communication between Korea, China, and Japan, the prevalance of anti-HTLV-I antibodies in Korea(17/6255; 0.27%) and China(1/6884; 0.0%) are extremely low (Lee *et al.* 1986, Zeng *et al.* 1984).

Since the patient is the first case of HAM/TSP in Korea, there is a significant importance to analyse the DNA sequence of the provirus infected in this patient.

Nucleotide sequence of KOR showed close relationship with that of Japanese and Chinese isolates. For example, KOR displayed two nucleotide substitutions in env region (No.6247: G and No.6388: G) that were common to all of the Japanese (except MT-2) and one Chinese HTLV-I isolate as previously reported (Gessain *et al.* 1992). In the LTR region, KOR and other Japanese isolates showed very high homologies with each other and they were all classified into subtype III according to Komurian's classification as the Chinese isolate(1992).

We firstly demonstrated the proviral DNA sequence of HTLV-I from a Korean which showed significant homology with the prototype Japanese isolate ATK-1

Although the homology of HTLV-I isolates from Korean, Chinese and Japanese cases may suggest the possibility of same ancestors of three nations, the rarity of HAM cases in Korea and China may require another explanation.

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