Human T-cell Lymphotropic virus type I (HTLV-I) $p12^I$ is dispensable for HTLV-I transmission and maintenance of infection in vivo

Running title; Sequence variations in HTLV-I p12

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Abstract

The function of the $p12^I$ protein of Human T-cell Lymphotropic virus type-I (HTLV-I) has been under debate. p12K (Lysine) and p12R (Arginine) variants of this protein at amino acid 88, and a shorter life of p12K had been reported by other group. Because HTLV-I-associated myelopathy / tropical spastic paraparesis (HAM/TSP) patients usually have a higher provirus load than asymptomatic HTLV-I carriers (ACs), and $p12^I$ had been suggested to confer a proliferative effect on HTLV-I infected cells in-vitro, it is possible that the relatively unstable p12K is less frequent in HAM/TSP patients than in ACs. To elucidate whether p12K and other alterations in the *p12* gene were related with the outcome of HTLV-I infection, we sequenced the *p12* gene in 144 HAM/TSP patients, 41 Adult T-cell leukemia (ATL) patients and in 46 ACs. p12K was observed only in two HAM/TSP patients, but was not present either in ATL patients or in ACs. Interestingly, a premature termination codon in *p12* was observed in 5.6% of HAM/TSP patients and in 4.9% of ATL patients but none was found in ACs. The *p12* initiation codon was destroyed in one HAM/TSP patient. These HTLV-I variants with truncated p12 protein or with a destroyed initiation codon in *p12* gene appeared to have been transmitted in the subjects' families. These findings suggest that *p12* is dispensable for the transmission and maintenance of HTLV-I infection, although it is premature to conclude that sequence varitation in the *p12* gene is associated with differences in the outcome of HTLV-I infection.

Key Words,

HTLV-I, p12, HAM/TSP, ATL, asymptomatic carrier, infectivity

Introduction

Human T-cell Lymphotropic virus type I (HTLV-I) was first isolated from a cutaneous T-lymphoma in 1980,[1] and was determined to be the etiologic agent of adult T-cell leukemia (ATL).[2] In 1985 an association was reported between tropical spastic paraparesis, which had been considered to be a degenerative disorder, and HTLV-I.[3] In 1986 HTLV-I was reported to be associated with a similar syndrome which was called HTLV-I-associated myelopathy (HAM);[4] the condition is now called HAM/TSP. One of the intriguing questions with HTLV-I infection is why the same HTLV-I causes two distinct diseases. Another question with HTLV-I infection is why only a small proportion of infected people, approximately 2-3% of infected individuals, develop ATL,[5] and a further 0.25% develops HAM/TSP in Japan,[6] while the majority (about 97%) of HTLV-I-infected individuals develop no associated disease. To date, HTLV-I provirus isolated from ATL and HAM/TSP patients were reported to be indistinguishable in the LTR and env regions.[7] However, we recently reported the existence of subgroups in the *tax* gene and different risks of HAM/TSP among different HTLV-I subgroups.[8] Tax is a multifunctional protein encoded by the open reading frame IV of the pX region of HTLV-I which can trans-activate HTLV-I transcription through LTR,[9] Tax can also transactivate many cytokine genes[10,11] and proto-oncogenes.[12] These observations raise the interesting possibility that differences in Tax may influence the outcome of HTLV-I infection. $p12^{I}$ is another protein encoded by the open reading frame I of the pX region, the function of which is not well elucidated.[13] The $p12^{I}$ protein can bind to the 16-kDa subunit of the H+-ATPase proton pump[14] and to the β and γc chains of the interleukin 2 (IL-2) receptor.[15] Although $p12^{I}$ does not influence the infectivity of HTLV-I in in vitro culture, it has

been suggested that $p12^I$ is necessary for persistent HTLV-I infection in the rabbit model.[16] When $p12^I$ was expressed under experimental conditions, $p12^I$ was reported to reside in the endoplasmic reticulum and Golgi,[17] and associated with several proteins such as calcium binding protein,[18] and free major histocompatibility complex class I heavy chain (MHC-I-Hc).[19] Association of $p12^I$ with calreticulin modulates activation of nuclear factor of activated T cells, and enhances STAT5 activation.[20] Binding of the $p12^I$ with MHC-I-HC was shown to result in a significant decrease in the surface level of MHC-I on human T cells.[19] These previously reported findings suggested that $p12^I$ might confer a proliferative advantage on HTLV-I infected cell and also may affect the result of HTLV-I infection.

The $p12^I$ protein with lysine in the C-terminal region (position 88: p12K) was shown to be a substrate for ubiquitylation and degradation by the proteasome. Also, the p12K variant has a half-life significantly shorter than that of $p12^I$ with arginine in the same position (p12R).[21] There have been conflicting results on the prevalence of these p12K and p12R alleles in HAM/TSP patients and asymptomatic carriers (ACs). p12K was reported to be more frequent in HAM/TSP patients than in asymptomatic carriers in one study.[21] Another study reported that p12K was observed in a small minority of both HAM/TSP patients and ACs.[22] Although it had been suggested that $p12^I$ confers a proliferative advantage of HTLV-I infected cells in vitro, the effect of $p12^I$ in vivo, if it is indeed expressed, may be different. If p12K, which is more unstable than p12R, is more frequently observed in HAM/TSP patients (who usually have higher HTLV-I provirus load, than in AC), one possibility is that a shorter life of p12K is associated with greater proliferation of HTLV-I infected cells in vivo. We therefore set out to examine whether p12K variant is present at a significantly higher

frequency in HAM/TSP patients than in ACs in Japan, and whether the p12R/K allele was associated with the HTLV-I tax viral genotype, that we previously reported to influence the risk of HAM/TSP development in this Japanese population. We then extended our study to examine other variations in the *p12* gene, to test whether $p12^I$ is dispensable for HTLV-I infection in the human, and whether variations in the p12 gene were associated with different risks of HTLV-I associated diseases. We discuss the role of $p12^I$ in the establishment of stable infection in the human, and the association between sequence variations in the p12 gene of HTLV-I and HTLV-I related diseases.

Materials and methods

Study population. One hundred and forty four cases of HAM/TSP were compared with 41 ATL patients (30 acute type, 3 lymphoma type, 6 chronic type and 2 smoldering type ATL) and 46 randomly selected HTLV-I seropositive asymptomatic blood donors (ACs). All cases and controls were of Japanese ethnic origin and resided in Kagoshima prefecture, Japan. The diagnosis of HAM/TSP was made according to the World Health Organization diagnostic criteria.[23] The diagnosis and clinical subtype of ATL was made according to Shimoyama's criteria.[24] Asymptomatic carriers were randomly selected from 111 individuals who were notified by the Red Cross following blood donation that they were infected with HTLV-I and subsequently attended our clinic.[25]

Sequencing of HTLV-I p12 gene. All DNA samples extracted from PBMCs were sequenced from position 6801 to 7199 (numbered as in the reference strain ATK),[26] which included the entire HTLV-I p12 gene (nucleotides 6834 to 7130). PCR was done on the extracted DNA to amplify proviral DNA, and nucleotide sequences were

determined in both directions. One hundred nanogram of DNA was amplified by 35 cycles of PCR using Expand high fidelity PCR system (Boehringer Mannheim, Japan) and 1μM primers (ORFI03+: 5'- CGTCAGATACCCCCATTACTC-3' (6598--6619) and ORFI02-: 5'- AGCCGATAACGCGTCCATCGAT -3' (7472—7493)). Each PCR cycle consisted of denaturation at 94°C for 60 s, annealing at 58°C for 75 s, extension at 72°C for 90 s and extension of the final cycle at 72°C for 10 min. Amplified DNA products were purified using QIA quick purification kit (Qiagen, Japan) and 0.1μg of PCR products were sequenced using dye terminator DNA sequencing kit (Applied Biosystems, Japan) with 3.2pmol of each primers (ORFI03+, ORFI02-) in an automatic sequencer (377 DNA Sequencer, Applied Biosystems).

Restriction fragment length polymorphism analysis of HTLV-I tax

Subgroup analysis of the Japanese HTLV-I tax genes was performed as described elsewhere.[8] Substitution at nucleotide position 8344 of the *tax* gene creates an AccII restriction site. One μl of the 1st PCR product of the *tax* gene amplified with primers PXO1+ and PXO2- was subjected to a further 20 cycles of PCR with primers PXI3+ and PXI3-. Two μl of the nested PCR product was digested with 5 U of AccII (Takara, Japan) in a 10μl reaction volume at 37°C for 1 hour and the product was electrophoresed on a 1 % agarose gel. When the PCR product was cut by ACCII, the sample was identified as tax A, and when uncut, the sample was identified as tax B.

Proviral load measurement. The HTLV-I provirus load in peripheral blood mononuclear cells (PBMC) was measured in HAM/TSP patients and ACs as described.[27] A quantitative PCR reaction was performed using the ABI PRISM 7700 sequence detector (Perkin-Elmer Applied Biosystems). The amount of HTLV-I proviral DNA was calculated as follows: copy number of HTLV-1 (*tax*) per 10^4 PBMC =

[copy number of *tax* / (copy number of β-actin/2)] x 10^4. The lower limit of detection was 1 copy per 10^4 PBMC.

Results

Existence of p12K variant at low frequency in HAM/TSP patients

We analyzed 231 samples from 144 HAM/TSP patients, 41 ATL patients and 46 ACs, all of them residing in Kagoshima, southern Japan. The grand consensus sequence of these 231 samples differed from the reference ATK strain at nucleotide position 6906 (A to G; leading to the amino acid change from Serine in ATK to Glycine in the grand consensus sequence), 6984 and 6985 (G to A in both positions; leading to the amino acid change from Glycine in ATK to Asparagine in the grand consensus sequence). There were HTLV-I subgroup-specific nucleotides at nucleotide position 6900 and 6905. The nucleotide at position 6900 was T and the corresponding amino acid was Serine in all of the HTLV-I sequences with *tax* A (31 cases in this study); this nucleotide was C and the amino acid was Proline in all of the HTLV-I with *tax* B (200 cases in this study). The nucleotide at position 6905 was G in all HTLV-I sequences with *tax* A (31 cases in this study); in tax B this nucleotide was T but this difference does not correspond with an amino acid alteration. In Figure 1, we summarize the observed nucleotide alterations and amino acid changes that were not specific to an HTLV-I subgroup. In table 1, we summarize amino acid changes that may influence the function of $p12^I$. The p12K variant was observed only in HAM/TSP patients; however, it was present at a low frequency (2 out of 144; 1.39%). All other samples, including HAM/TSP, ATL and asymptomatic carriers had the p12R variant. One of the p12K subjects was a 65 year old female, who had had HAM/TSP for 8 years, the other

was a 72 year old female, who had had HAM/TSP for 22 years. In each of these subjects, the Tax subgroup was Tax B. Serum anti-HTLV-I antibody titer and HTLV-I provirus load are summarized in Table 2. The HTLV-I provirus load tended to be lower in these two subjects, although a meaningful statistical analysis was not applicable because of the small number.

Premature stop codon preceding the p12R/K allele, alteration of initiation codon in p12 and other alteration

Interestingly, in 7 out of 144 HAM/TSP patients, a nucleotide substitution from G to A at nt. positions 7087 (one patient) or 7088 (six patients), created a premature stop codon just upstream of the p12R/K allele (amino acid at position 87 of the $p12^{I}$ protein was changed from W to Stop). In another HAM/TSP patient, a nucleotide substitution from G to A at nt. position 7078 also created a premature stop codon five amino acids upstream of the p12R/K allele (amino acid at position 82 of the p12 protein was changed from W to Stop). In one other HAM/TSP patient, nucleotide substitutions from T to C at nt. 7086 and from G to A at nt. 7088 were observed simultaneously, resulting in an amino acid substitution from W to R, one amino acid upstream of the p12R/K allele. In one other HAM/TSP patient, a nucleotide substitution from G to A at nt. position 6836 destroyed the initiation codon of p12 (M to I). In ATL cases, there were 2 patients with a premature stop codon just upstream of the p12R/K allele out of 41 patients. However, in asymptomatic carriers, there was no subject with a premature stop codon in the p12 gene. Ages and other laboratory findings are summarized in Table 2. There was an ATL specific nucleotide alteration at nt position 6909 in 3 out of 41 ATL patients (Fig. 1B) which was the most frequently observed alteration. The nucleotide alteration from G to A at this position changed the

amino acid from Aspartate to Asparagine.

HTLV-I subgroup-specific nucleotide alteration, p12R/K allele and the premature stop codon in p12 are stable over time (Table3)

To examine if subgroup specific nucleotide alterations, the p12R/K allele and the premature stop codon were stable over time, we sequenced the HTLV-I p12 gene at different time points in cases with these nucleotide alterations. The nucleotide at position 6900 was T in HTLV-I sequences with *tax* A and this residue was C in HTLV-I with *tax* B. The nucleotide at position 6905 was G in HTLV-I sequences with *tax* A and this residue was T in HTLV-I with *tax* B. These nucleotide alterations existed stably for 7 years in #HAM70 case (Table 3). The p12R allele existed stably for 7 years in #HAM57 case. Also, the p12K allele existed stably for 9 years in #HAM79 case (Table 3). The premature stop codon in p12 gene was also stably maintained in #HAM105 and in #HAM181 for 9 years in both cases (Table 3). No case was found in which these sequence variants changed over time in one individual.

HTLV-I with premature stop codon in p12 gene is transmissible (Table 4)

To examine whether HTLV-I with a premature stop codon in the p12 gene is transmissible, we sequenced the p12 gene in asymptomatic carriers who are family members of cases with a premature stop codon in the p12 gene. HTLV-I carriers who are family members of three HAM/TSP patients with a p12 premature stop codon were examined. Each family member had a sequence of the p12 gene that was identical with that of the HAM/TSP patients in their respective family. For example, the husband of #HAM 181 had an identical p12 sequence with substitution at 7078, which created a stop codon that was observed only in this family. The wife of #HAM201 had

an identical p12 gene sequence with substitutions at 6840, 7094 (creating a stop codon), 7134, in addition to the HTLV-I subgroup-specific substitutions at 6900 and 6905. A sister and the mother of #HAM790 had an identical p12 gene sequence with the stop codon at nucleotide 7094.

HTLV-I with destroyed initiation codon in the p12 gene was also transmitted (Table 4)

To examine whether HTLV-I with a destroyed initiation codon in the p12 gene was also transmitted, we checked family members of patient #HAM271 who carried an HTLV-I provirus with a destroyed initiation codon. Samples from two sisters of this patient were available, one of whom was previously diagnosed as HAM/TSP. The p12 sequence was identical, with a destroyed initiation codon of p12, among these three sisters.

Discussion

Recently, the function of the $p12^{I}$ of HTLV-I has been under debate, and natural variants of p12 at position 88 (Arginine; p12R and Lysine; p12K) were reported.[21] Lysine at position 88 in $p12^{I}$ is an ubiquitylation site and a shorter life of p12K compared to p12R was reported. P12K was previously reported to be frequent and specific in HAM/TSP patients,[21] but there was also a conflicting report.[22] The main purpose of the present study was to elucidate if variations in the p12 gene were associated with HTLV-I related diseases, especially with respect to the distribution of p12 R/K variation in HAM/TSP, ATL and in ACs. We also examined whether the p12R/K variation was associated with the HTLV-I *tax* viral genotype, which we previously reported to influence the risk of HAM/TSP development.[8]

The p12K variant was observed only in two HAM/TSP patients but did not

occur either in ATL patients, or in ACs. However, the frequency of p12K was very low (2 out of 144; 1.4%). Regarding the association of p12R/K alleles and the *tax* subgroup, two HTLV-I seropositive subjects with p12K were classified to have *tax* B. However, another HTLV-I seropositive individual with *tax* B (119 HAM patients) had p12R. Also, of the 142 HAM/TSP patients who carried p12R, 23 HAM/TSP patients had *tax* A and 119 had *tax* B. Trovato also reported that p12R/K alleles were found regardless of the geographical origin.[21] These findings suggest that the p12R/K variation is not specifically associated with the HTLV-I subgroup. Also, the very low frequency of p12K variant observed among HAM/TSP patients in Japan cannot be explained by the particular distribution of HTLV-I subgroup in Japan: most of the HTLV-I in Japan belongs to Cosmopolitan B, while Cosmopolitan A is widely distributed through the world.

Although the p12K variant, which may have a decreased biological effect because of its shorter life, was found at a very low frequency in HAM/TSP patients, other variations in p12 gene that can affect the function of p12 were found in HAM/TSP patients and in ATL patients. A premature stop codon was found in 8 HAM/TSP patients and in 2 ATL patients but not in ACs. Trovato et al. also reported this termination codon immediately preceding the lysine in one ATL patient who also had HAM/TSP.[21] Because the *p12*^{I} sequence with a premature stop codon immediately preceding the p12R/K allele had arginine in our study, and the p12R/K allele was lysine in Trovato's study, this stop codon does not appear to be associated with the p12R/K variation. Regarding the association with the termination codon immediately preceding the p12R/K variation and the *tax* subgroup, there were 6 HAM/TSP patients with *tax* B and one HAM/TSP patient with *tax* A. Thus, this termination codon

immediately preceding the p12R/K variation was also not specifically associated with the *tax* subgroup.

To test whether HTLV-I having a *p12* sequence with either arginine (p12R) or lysine (p12K) at position 88 and HTLV-I with truncated $p12^{I}$ could persist stably over time, we compared HTLV-I *p12* sequences at different time points. The observed *p12* nucleotide substitutions were the same in each person on each occasion (Table 3), suggesting that neither p12R/K and not a premature stop codon in $p12^{I}$ influences the course of HTLV-I infection. To examine whether HTLV-I with such a truncated $p12^{I}$ was transmissible, we further compared the p12 sequence between HAM/TSP patients with a termination codon in the p12 gene and their family members infected with HTLV-I. Interestingly, the p12 sequences with a premature stop codon at either position 82 and 87 in HAM/TSP patients were identical in asymptomatic family members of HAM/TSP patients in each family. These findings also suggest that truncations of $p12^{I}$ at position 82 or 87 do not influence the infectivity of HTLV-I, although it was possible that the truncated $p12^{I}$ retained its function. However, in our study there was a HAM/TSP patient (HAM271) with $p12^{I}$ in whom the $p12^{I}$ initiation codon was destroyed. In this case, the nucleotide at position 6836 was substituted from G to A, changing the initiation codon ATG to ATA. We were interested to determine whether such a p12 with a destroyed initiation codon was transmissible in the patient's family. We examined HTLV-I sequences from two other HTLV-I infected sisters in this family. One of the sisters was a HAM/TSP patient, while the other was an AC. The p12 sequences were identical among these three sisters, with the same substitution at nt. 6836 (G $\rightarrow$ A) which destroyed the initiation codon of p12. This observation indicates that the destroyed p12 was not a de novo mutation that happened

in patient HAM271, but rather was transmitted from their mother to the three sisters in this family. This contrasts with the observation that a putative immune escape mutation in the *tax* gene was observed in an ATL patient but not in the respective consensus sequence of asymptomatic HTLV-I carriers in the same family.[28] These findings strongly suggest that HTLV-I $p12^I$ is dispensable for HTLV-I transmission and the maintenance of HTLV-I infection. Regarding the relation between sequence variations in the HTLV-I *p12* gene and their association with HTLV-I-related diseases, lysine at position 88 (p12K) itself was not frequently observed in HAM/TSP patients (2 out of 144 HAM/TSP patients; 1.4%). However, both the p12K variant, premature stop codons and the destruction of the initiation codon were observed in 11 patients out of 144 HAM/TSP patients (7.4%), whereas none of these variations were observed in ACs. These variations in p12 gene may reduce the function of $p12^I$. Because p12K has a shorter life compared to p12R, and a premature stop codon in $p12^I$ may reduce its function, the destruction of the initiation codon in p12 gene presumably ablates the function of $p12^I$. At the beginning of this study, we expected that alterations that reduce the function of $p12^I$ would be less frequent in HAM/TSP, because HAM/TSP patients usually have a higher provirus load than asymptomatic carriers (ACs), and $p12^I$ had been suggested to confer a proliferative effect on HTLV-I infected cell in an in-vitro study. It is possible that the effects of $p12^I$ in vivo differs from its effects in vitro. However, the observed difference between HAM/TSP patients and ACs in the prevalence of these alterations that may reduce the function of $p12^I$ did not reach statistical significance, and it is therefore premature to conclude that mutations of *p12* gene are implicated in the pathogenesis of HTLV-I associated diseases. Further study with a larger number of ACs is necessary to clarify this point.

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Sequence data

DDBJ accession numbers

The accession numbers of pX sequence including the entire p12 gene in HAM/TSP cases are successively from AB127436 through AB127579. The accession numbers of pX sequence including the entire p12 gene in ATL cases are successively from AB154777 through AB154817. The accession numbers of pX sequence including the entire p12 gene in ACs are successively from AB158146 through AB158191. The accession numbers of pX sequence including the entire p12 gene at different occasions in Table 3 are AB127439 for HAM57 at Jul4/1990, AB158267 for HAM57 at Aug29/1997, AB127442 for HAM 70 at Jun27/1990, AB158266 for HAM 70 at Feb27/1998, AB127447 for HAM79 at Apr17/1991, AB158268 for HAM79 at Oct11/2000, AB127458 for HAM105 at Mar11/1992 and AB158270 for HAM105 at Jul12/2001, AB127485 for HAM181 at Mar27/1991 and AB158269 for HAM181 at Jan15/2000. The accession numbers of pX sequence including the entire p12 gene of HAM/TSP patients and family members in Table 4 are AB127485 for HAM181, AB158271 for the husband of HAM181, AB127493 for HAM201, AB158272 for the

wife of HAM201, AB127521 for HAM271, AB158275 for the asymptomatic sister of HAM271, AB158276 for the HAM/TSP sister of HAM271, AB127562 for HAM790, AB158273 for the sister of HAM 790 and AB158274 for the mother of HAM 790.

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Table 1. Summary of amino acid changes in $p12^I$ protein that may influence the function

Amino acid position (Change in amino acid)		HAM/TSP (N=144)	ATL (N=41)	ACs (N=46)
1	(M to I)	1 (0.7%)	0	0
82	(W to Stop)	1 (0.7%)	0	0
87	(W to Stop)	7 (4.9%)	2 (4.9%)	0
88	(R to K)	2 (1.4%)	0	0
Total		11 (7.6%)	2 (4.9%)	0

Number of cases that have amino acid change at position described in the left column is stated.
Amino acid change of $p12^I$ is shown in the parenthesis in the left column.
Percentage of cases are shown in the parenthesis in HAM/TSP, ATL, and ACs column.

Table 2. Characterization of HAM/TSP patients with destroyed initiation codon, premature stop codon and p12R/K allele in p12 gene

Amino acid position (Change in amino acid)		No. of Cases	Age	Anti HTLV-I antibody§(PA)	HTLV-I provirus load§
1	(M to I)	1	43	8192	1954
82	(W to Stop)	1	63	32768	1343
87	(W to Stop)	7	41¶	8192	641
88	(R to K)	2	69¶	9216	146
All HAM/TSP patients		144	56¶	8192	586

§ Anti-HTLV-I antibody titer and HTLV-I provirus load are stated as median value.

¶ Mean age is shown.

Table 3. Comparison of HTLV-I *p12* sequences at different time points with the grand consensus sequence

		Nucleotide change at position (Amino acid position)					
	Date	6900(23)	6905(24)	7078(82)	7086(85)	7093(87)	7096(88)
Consensus		C (P)	T (P)	G (W)	C (L)	G (W)	G (R)
HAM 57	Jul 4 / 1990				T (F)		G (R)
	Aug 29 /1997				T (F)		G (R)
HAM 70	Jun 27 / 1990	T (S)	G (S)				G (R)
	Feb 27 / 1998	T (S)	G (S)				G (R)
HAM 79	Apr 17 / 1991						A (K)
	Oct 11 / 2000						A (K)
HAM 105	Mar 11 / 1992					A (Stop)	G (R)
	Jul 12 / 2001					A (Stop)	G (R)
HAM 181	Mar 27 / 1991			A (Stop)			G (R)
	Jan 15 / 2000			A (Stop)			G (R)

Amino acid of $p12^{I}$ is shown in the parenthesis.

Table 4. Comparison of HTLV-I *p12* sequences within pedigree with the grand consensus sequence

		Nucleotide change at position (Amino acid change at position)						
	Sex	6836(1)	6840(3)	6900(23)	6905(24)	7078(82)	7094(87)	7134
Consensus		G (M)	T (L)	C (P)	T (P)	G (W)	G (W)	G
HAM 181	F					A (Stop)		
Husband of HAM 181	M					A (Stop)		
HAM 201	M		C (L)	T (S)	G (S)		A (Stop)	C
Wife of HAM 201	F		C (L)	T (S)	G (S)		A (Stop)	C
HAM 790	F						A (Stop)	
Sister of HAM790	F						A (Stop)	
Mother of HAM790	F						A (Stop)	
HAM 271	F	A (I)						
Sister of HAM271 (AC)	F	A (I)						
Sister of HAM271 (HAM)	F	A (I)						

In HAM 271 family, one sister was an asymptomatic HTLV-I carrier (HAM271 (AC)), and one other sister was a HAM/TSP patient (HAM271 (HAM)). These three cases had the identical p12 sequence with a destroyed initiation codon in the p12 gene.
Amino acid of $p12^{I}$ is shown in the parenthesis.

Figure legend

Fig. 1. Summary of the nucleotide and amino acid alterations. A: Summary of the nucleotide alterations in pX that covers $p12^{I}$ protein. Upper column; HAM/TSP patients. Middle column; ATL patients. Lower column; Asymptomatic carriers. X-axis: Nucleotide position. Y-axis: Percentage of nucleotide alteration at one nucleotide position. B: Summary of the amino acid alterations in $p12^{I}$ protein. Upper column; HAM/TSP patients. Middle column; ATL patients. Lower column; Asymptomatic carriers. X-axis: Amino acid position of the $p12^{I}$ protein. Y-axis: Percentage of amino acid alteration at one amino acid position.