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Clinicopathological and virological analyses of familial human T-lymphotropic virus type I–associated polyneuropathy

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Human T-lymphotropic virus type I (HTLV-I) is known to be the causative agent of the chronic myelopathy, HTLV-I–associated myelopathy (HAM), and on rare occasions infection is also associated with the development of polyneuropathy. Here the authors present an HTLV-I–positive family of whom four members developed a chronic demyelinating polyneuropathy without HAM. Four female patients in a family from Hokkaido in Japan developed distal dominant paresthesia and muscle weakness in the second and third decades of their life. Neurological findings at ages ranging from 50 to 65 years included mild painful sensorimotor disturbances with atrophy of the distal parts of the extremities but without pyramidal signs or hyperactive tendon reflexes. Magnetic resonance imaging (MRI) findings of brain and spinal cord were unremarkable. Serum HTLV-I antibody levels were elevated at 1:8,192 to 1:32,768, whereas those in cerebrospinal fluid were low at 1:4 to 1:8. Electrophysiological studies revealed polyphasic compound muscle action potentials with denervation potentials on nerve conduction studies and neurogenic patterns by electromyography, which were consistent with signs of chronic motor dominant demyelinating polyneuropathy. Sural nerve biopsy showed decreased myelinated fibers, occurrence of globule formation, myelin ovoid and remyelinated fibers, and an infiltration of CD68-positive macrophages with occasional CD4-positive T cells in the nerve fascicles. The polyneuropathy was responsive to steroid therapy. Analyses of serological human leukocyte antigen (HLA) types indicated that none of the patients possessed a high-risk HLA type known to be associated with adult T-cell leukemia (ATL), whereas they did have high responsive alleles to HTLV-I *env* similar to that observed in HAM. Nucleotide sequence analysis of the HTLV-I tax region demonstrated the B subgroup in all patients. This study suggests that HTLV-I infection can result in the development of a familial form of polyneuropathy that is associated with distinct HLA class I alleles, which might possibly involve a distinct virus subtype. *Journal of NeuroVirology* (2005) **11,** 199–207.

Keywords: familial polyneuropathy; HTLV-1; immunostaining

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Introduction

Human T-lymphotropic virus type I (HTLV-I) is known to be associated with a range of lymphoproliferative and inflammatory disorders. The former, adult T-cell leukemia/lymphoma (ATLL) is a spectrum of malignancies of mature CD4-positive T lymphocytes (Johnson *et al*, 2001). The major inflammatory disorder is neurological, and is a chronic encephalomyelopathy known both as tropical spastic paraparesis (TSP) and HTLV-I–associated myelopathy (HAM) (Gessain *et al*, 1985; Osame *et al*, 1986). These two disorders are the most representative of HTLV-I–associated processes affecting the blood and the central nervous system (CNS), respectively (Nagai and Jacobson, 2001; Yoshida, 2001). In recent years, additional inflammatory disorders that have been associated with HTLV-I infection have included uveitis, arthropathy, alveolitis, dermatitis, Sjögren's syndrome (Yamaguchi and Takatsuki, 1993), and myositis (Douen *et al*, 1997; Vernant *et al*, 1990).

Involvement of the peripheral nerves has been reported in association with both the smoldering and acute forms of ATLL (Cabrera *et al*, 1991; Hori *et al*, 1998; Kasahata *et al*, 2000; Kuroda *et al*, 1989; Mitsui *et al*, 1999; Murata *et al*, 1990; Nakano *et al*, 1991; Okamura *et al*, 1984), and polyneuropathy has also been described in a number of patients with TSP/HAM (Arimura *et al*, 1987; Bhigjee *et al*, 1993; Funamoto *et al*, 1989; Johkura *et al*, 1995; Kohriyama *et al*, 1992; Kyuno *et al*, 1993; Nakazato *et al*, 1989; Said *et al*, 1988; Sugimura *et al*, 1990; Vallat *et al*, 1993; Vernant *et al*, 1990; Yanagihara *et al*, 1999). However, there have only been a few reports of chronic polyneuropathy occurring in HTLV-I–asymptomatic carriers (Arakawa *et al*, 1990; Douen *et al*, 1997; Hori *et al*, 1998; Kanzaki *et al*, 1995). In this study, we have identified four HTLV-I–seropositive patients in a family, all of whom developed a chronic demyelinating polyneuropathy without HAM/TSP. The results of the clinical evaluation, together with immunological, histopathological, and molecular genetic studies, suggest the existence of a rare form of a familial polyneuropathy associated with HTLV-I infection.

Results

The clinical features of all four patients are summarized in Table 1. The exact age of onset of symptoms could not be accurately determined as the initial neurological symptoms were often vague, involving numbness or painful weakness of the lower extremities. However, in two patients (cases 1 and 3) initial complaints seemingly began at the end of their 2nd decade of life, and these had progressed slowly without significant disabilities in that the patients were able to work normally. The other two patients (cases 2 and 4) developed neurological abnormalities at 46 and 45 years old, 7 and 5 years, respectively, prior to their initial neurological examination.

Serological studies demonstrated high levels of antibody against HTLV-I in all four patients, with titers ranging from 1:8,192 to 1:32,768 (positive: greater than 1:16), whereas cerebrospinal fluid (CSF) were low from 1:4 to 1:8. Other viral antibody titers including human immunodeficiency virus (HIV) were all negative both in sera and CSFs. There were no high protein levels in the CSF.

Electrophysiological studies with proximal stimulation demonstrated that the compound muscle action potentials (CMAPs) exhibited a temporal dispersion in all the nerves but primarily in the tibial nerve, as exemplified in case 3 (Figure 1a). Sensory as well as motor nerve conduction studies (NCSs) and F-wave were grossly within normal ranges and as was the somatosensory evoked potential (SSEP).

Table 1 Clinical summary of four cases in a family

Family A			*HTLV-I titers (PA)*						
Case	*Age*	*Sex*	*Neurologic signs*	*Onset (y)*	*Serum*	*CSF*	*NCS/EMG*	*HLA*	*Note*
1	61 y	F	Paresthesia, distal motor weakness	2nd decade	16,384×	4×	Motor dominant CDP, neurogenic pattern	A33, 68 B44, 51 DR9, 12	Uveitis, thyroid Ca, deafness
2	53 y	F	Paresthesia, distal	4th decade	8,192×	8×	Motor dominant CDP, neurogenic pattern	A2, 68 B51 DR4, 9	43 y, uterus Ca
3	52 y	F	Paresthesia, distal	2nd decade	32,768×	8×	Motor dominant CDP, neurogenic pattern	A2, 33 B39, 48, Cw7 DR2, 11	Arthralgia, HBV(+)
4	50 y	F	Paresthesia, distal motor weakness	3rd decade	8,192×	8×	Motor dominant CDP, neurogenic pattern	A24, 33 B54, 39, Cw1, 7 DR2, 4	12 y pulmonary tuberculosis

CSF: cerebrospinal fluid; NCS: nerve conduction studies; EMG: electromyography; CDP: chronic demyelinating polyneuropathy; PA: particle agglutination method; HBV: hepatitis B virus; Ca: carcinoma.

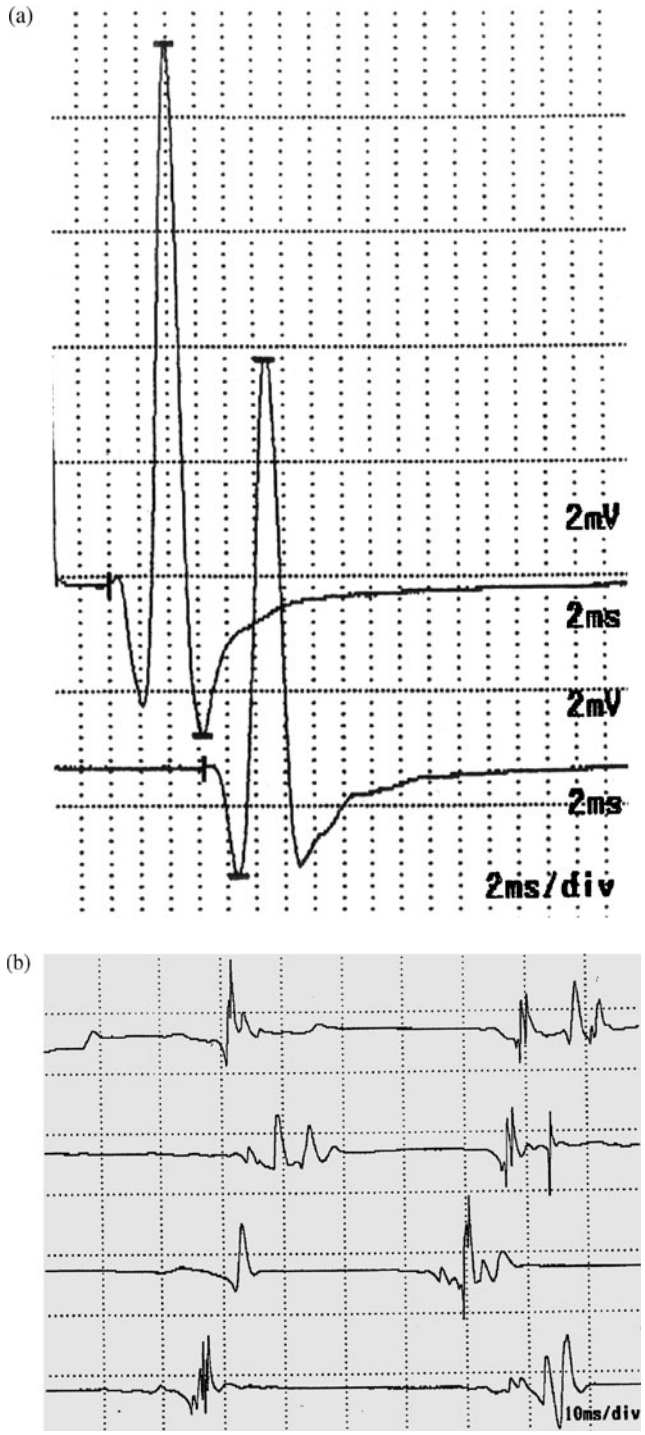

Figure 1 Electrophysiological findings of case 3 (**a**) and case 1 (**b**). **a**, Compound muscle action potentials (CAMPs) of right tibial nerve of, showing temporal dispersion. **b**, EMG of the right anterior tibial muscle, showing polyphasic potentials with occasional long duration potentials.

On electromusculographic (EMG) recordings at mild contraction, polyphasic potentials with decreased interference were detected predominantly in the lower extremities, which indicated a chronic motor dominant demyelinating polyneuropathy (case 1) (Figure 1b). There were no myopathic changes on EMG.

Histopathological findings of the sural nerves revealed decreased myelinated fibers with round bodies containing degenerated material compatible with so-called myelin ovoid, and thinly myelinated axons, in case 1 by toluidine blue (TB) staining (Figure 2a and b). Myelin globules were also occasionally encountered (Figure 2c). In cases 2 and 3, a slight decrease of nerve fibers was evident with a few thinly myelinated axons, but globular bodies were not observed. Aggregation of thinly myelinated axons was observed in all three cases examined (Figure 2d), suggestive of remyelination. No axonal changes were observed. Lymphocytic infiltration was not observed in TB-staining preparations. The sural nerve of case 4 was not examined by TB staining. By immunostaining, there was rare infiltration of CD45RO-positive T cells into the nerve fascicles and/or vessels around the nerve in all four cases. These T cells were mostly characterised as the CD4 positive (Figure 3a). CD68-positive macrophages were almost exclusively found among the nerve fibers (Figure 3b). There were no inflammatory features such as perivascular lymphocytic infiltration or neoplastic T cells in the skin and muscles in any of the biopsy samples.

The serological human leukocyte antigen (HLA) subtype is summarized in Table 1. All four patients did not have such an HLA type as A26 that have been reported to be associated with ATLL. Mutations in the peripheral myelin protein-22 (PMP22) gene and myelin protein zero (P0) gene were not detected in any of the patients.

Nucleotide sequence analysis of the HTLV-I tax region following amplification using polymerase chain reaction (PCR) demonstrated that the nucleotide position (np) and amino acid change were C in np7898 and np7959, G in np8208, and A in np8344, confirming that all four patients were infected with the tax B subgroup, which belongs to the cosmopolitan B subgroup of HTLV-I (Furukawa *et al*, 2000).

Discussion

HTLV-I–related neuropathies have been reported by a number of authors following the initial description of TSP/HAM. A summary of cases described in the literature is shown in Table 2. It can be seen that in most cases a diagnosis of HAM had been initially made before signs of neuropathy were evident. Arimura *et al* (1987), using electrophysiologic studies, first described the involvement of peripheral nerves in HAM patients. In a familial case of HAM, Nakazato *et al* (1989) described sural nerve pathology, and proposed that HLTV-I infection need not be limited to disorders involving the spinal cord but could also involve peripheral nerves. Polyneuropathy in the absence of HAM has been rarely reported. In the first report Arakawa *et al* (1990) described

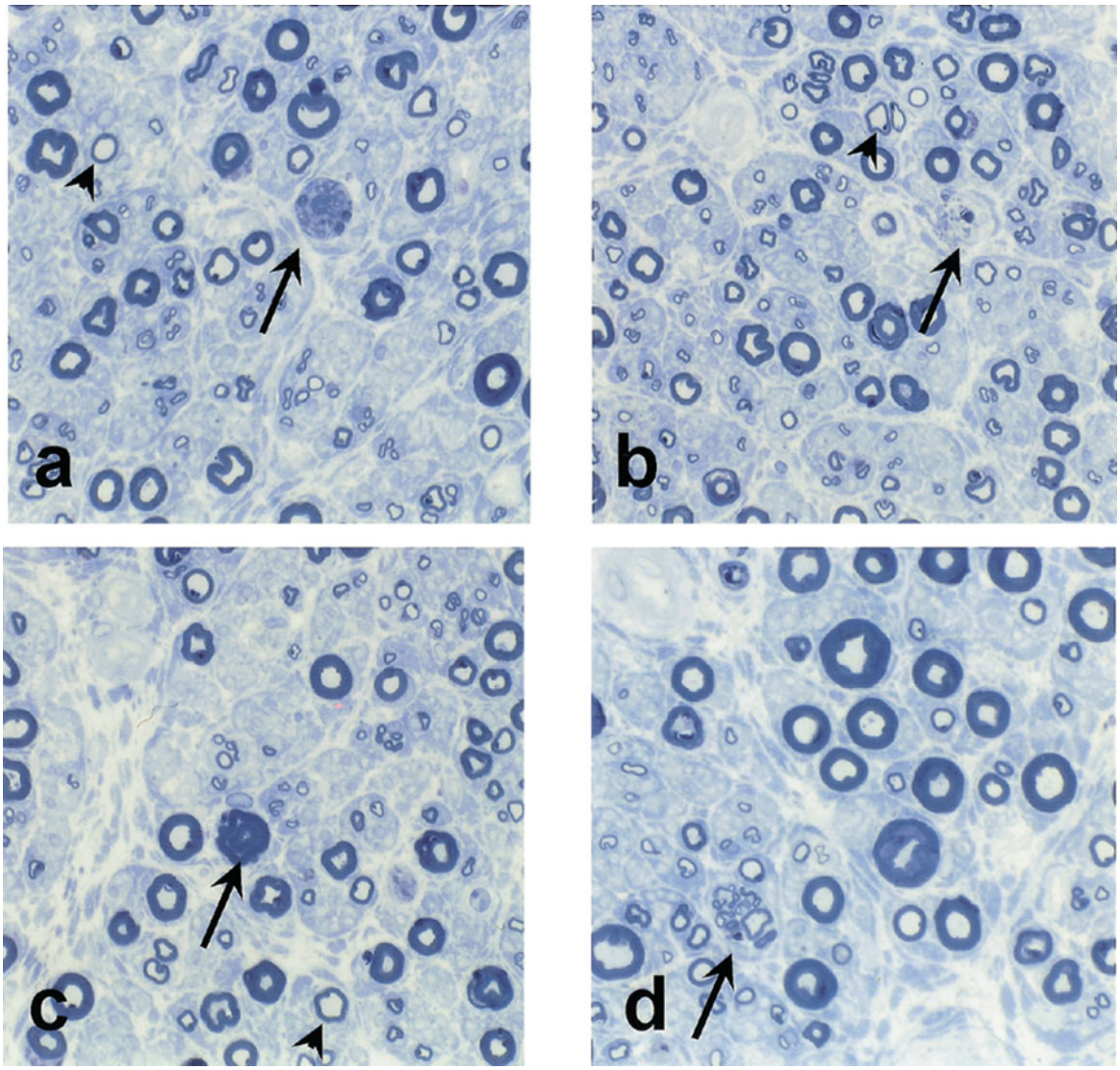

Figure 2 Sural nerve findings of case 1 (**a** and **b**), case 2 (**c**), and case 3 (**d**). Toluidine blue staining (×800). **a** and **b**, Macrophages (*arrows*) containing degenerated myelin sheaths, so-called myelin ovoid. Arrowhead shows thinly myelinated axons. **c**, Arrowhead shows thinly myelinated axons. Myelin globule (*arrow*). **d**, Aggregation of thinly myelinated axons (*arrow*).

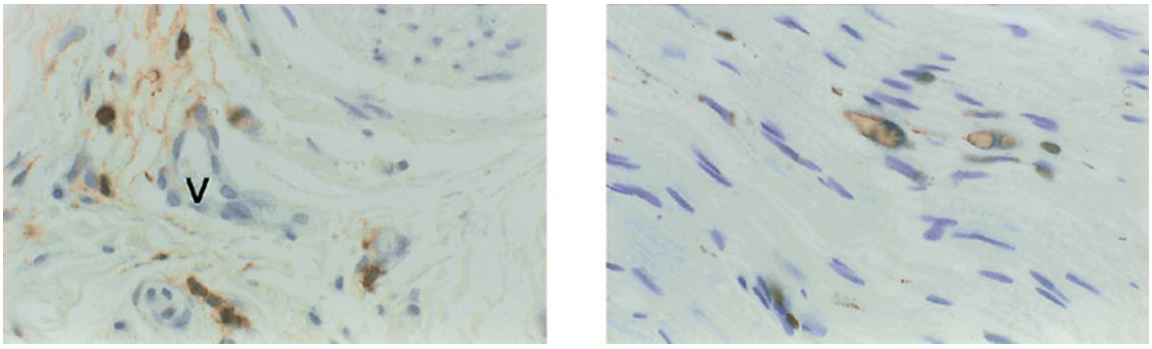

Figure 3 Immunohistochemical findings of the sural nerve of case 2. **a**, Lymphocytes around the nerve fascicles show T-cell markers (CD45R). v, vessel. **b**, Cells found in the nerve fascicles show macrophage markers (CD68).

Table 2 Summary of previously reported cases of HTLV-I–related polyneuropathy

First author	*Year*	*Cases*	*Basic disease*	*NCV/EMG*	*Histopathology*[a]	*Diagnosis/comments*
Arimura	1987	6	HAM	SSEP delay in lower limb	ND	Posterior nerve root/anterior cell lesion?
Said	1988	1	TSP	Axonal/demyelinating PN	Inflammatory neuropathy	Resemblance to CNS lesion in TSP
Funamoto	1989	1	HAM	Axonal/demyelinating PN	De- and remyelination	Slowly progressive myeloneuropathy
Nakazato	1989	2	HAM	Denervation, loss of SNP	Loss of myelinated fibers	Familial HAM with polyneuropathy
Arakawa	1990	1	Carrier	CDP, slow F-wave	ND	Chronic polyradiculoneuropathy
Sugimura	1990	3	HAM	Slight decrease in SCV	Re- and demyelination and globules	Distinct demyelinating process in HAM
Murata m	1990	1	HAM/ATL	Decrease in SCV	Axonal and demyelinating changes	Sensory neuropathy in HAM?
Vernant	1990	5	HAM and PN	ND	Clustered muscular atrophy	PN + myositis with or without HAM
Kohriyama	1992	1	HAM	Delay in MCV/FCV	Axonal and demyelinating changes	Steroid effective; HAM + PN?
Vallat	1993	5	TSP (5/18)	ND	Nonspecific re-, demyelination 3/11[a]	No association with HTLV-I/PN
Bhigjee	1993	6	HAM	Delay in SCV	Axonal/demyelinating change & globule	Primary PN associated with HTLV-I
Kyuno	1993	1	HAM	Delay in SSEP	Neurogenic muscular atrophy	Myopathy
Johkura	1995	1	HAM	Axonal PN, neurogenic	ND	Severe axonal neuropathy in HAM
Kanzaki	1995	1	Carrier	Delay in NCV	Axonal/segmental demyelination; T-cell[b]	PN and myositis in HTLV-I (3% atp-T)
Douen	1997	1	Carrier	Axonal PN, neuromyopathy	Polymyositis (muscle)	PN + myositis + brain lesion in HTLV-I
Hori	1998	1	Smoldering ATL	Delay in FCV and SSEP	ND	Radiculoneuritis + brain lesion in ATL-L
Mitsui	1999	1	HAM, CLL	Abnormal SCV and MCV	Decrease of large myelinated fibers	IgM binding and anti-gangliosides Ab
Yanagihara	1999	1	HAM	Delay in FCV and SSEP	Globular change	Anti-GM1-positive IgG and IgM
Kasahata	2000	1	ATL + HAM	Delay in SSEP	ND	Sensory/motor ataxic PN + meningitis
Nagashima	2001	4	non-HAM	Motor dominant CDP	Demyelination: T-cell	Familial PN without HAM

HAM: HTLV-I–associated myelopathy; ATL: adult T-cell leukemia/lymphoma; PN: peripheral neuropathy; TSP: tropical spastic paraparesis; CLL: chronic lymphocytic leukemia; NCV: nerve conduction velocity; EMG: electromyography; SSEP: short-latency somatosensory evoked potentials; SNP: sensorimotor neuropathy; CDP: chronic demyelinating polyneuropathy; SCV: sensory conduction velocity; FCV: F wave conduction velocity; ND: not described; CNS: central nervous system; Ab: antibody; IgG: immunoglobulin G; IgM: immunoglobulin M; atp-T: atypical T cell.
[a]Sural nerve biopsy.
[b]muscle biopsy.

an HTLV-I–infected 53-year-old female who had a predominantly lower motor neuron disorder. However, the authors could not rule out the possibility of coincidental occurrence of chronic inflammatory demyelinating polyneuropathy. Subsequently, however, similar clinical presentations were reported by Kanzaki *et al* (1995) in an HTLV-I carrier, Douen *et al* (1997) in a patient with myositis, and Hori *et al* (1998) in a patient with the smouldering form of ATLL (Hori *et al*, 1998), thus clearly establishing that polyneuropathy can certainly occur in the setting of HTLV-I infection in the absence of HAM. However, there have been no report of familial cases of polyneuropathy associated with HTLV-I infection. The present study identified four cases over two generations, and supports the proposal that HTLV-I infection is associated with a unique form of familial-associated polyneuropathy. All the patients were intensely seropositive for HTLV-I, but had negative or a lower limit of normal titers in the CSF. General physical examination excluded underlying hematological disorders, and neurological evaluation clearly ruled out the coexistence of HAM. Lack of a high protein level of the spinal fluid ruled out a concomitant association with chronic inflammatory demyelinating disease. Moreover, mutation analyses and the inherited pattern observed in the family tree excluded the inherited peripheral neuropathies (Saito *et al*, 2000). Electrophysiological studies demonstrated a motor dominant chronic demyelinating polyneuropathy without myopathic changes. Although there have been several reports of cases of polymyositis or myopathy complicated by polyneuropathy (Douen *et al*, 1997; Johkura *et al*, 1995; Vernant *et al*, 1990; Yanagihara *et al*, 1999), none of our patients had myositis on muscle biopsy.

Histopathological findings of biopsied sural nerves in HTLV-I infection have been reported as having

decreased numbers of myelinated fibers, axonal degeneration, combined axonal degeneration and segmental demyelination, occurrence of myelin globules, and the presence of myelin ovoid (Funamoto *et al*, 1989; Hori *et al*, 1998; Kanzaki *et al*, 1995; Murata *et al*, 1990; Nakazato *et al*, 1989; Said *et al*, 1988; Sugimura *et al*, 1990; Yanagihara *et al*, 1999). No vasculitis or lymphocytic infiltration has been detected. In our cases, mild-to-moderate loss of myelinated fibers was found in three cases examined and, in addition, features of remyelination were evident in two cases. Myelin globules and myelin ovoids were observed in one case, but axonal degeneration was not evident. Although an inflammatory cell infiltration was not observed in TB staining, a few lymphocytes were found in the endoneurium and perineurial connective tissues by immunostaining. The infiltrating cells expressed the CD45RO T-cell marker and were classified as the CD4 subtype. Peripheral nerves are usually examined using epoxy resin specimens or by the teased fiber method, and immunostaining using lymphocytic markers readily reveals the presence of lymphocytes. To derermine whether the small number of lymphocytes detected in the endo- and perineurium is of significance in HTLV-I polyneuropathy, examination of additional cases will be necessary. CD68-positive macrophages were found in all cases, suggesting the presence of degenerative processes in the nerves.

To begin to investigate possible pathomechanisms involved in HTLV-I–associated familial polyneuropathy, we examined both the viral subtypes of HTLV-I and the HLA alleles of the patients. Recently, nucleotide sequence and phylogenetic analysis of HTLV-I in Japanese patients with HAM/TSP, ATLL, and healthy carriers has revealed a slightly higher incidence of tax subgroup A in patients with HAM/TSP (Yashiki *et al*, 2001). In the present study, we focused our attention on four nucleotide positions in tax which has been shown to serve as useful markers to differentiate tax A and tax B (Yashiki *et al*, 2001). Sequencing of HTLV-I tax gene by PCR method demonstrated that all four patients were infected with HTLV-I tax B subgroup. However, in Japan most HTLV-I infections identified in Hokkaido and Honshu islands are in fact tax B (cosmopolitan B) (Vidal *et al*, 1994), and even the areas where both tax A and tax B are present, tax B is dominant. Therefore, the presence of the HTLV-I subtype tax B in our patients might simply be a reflection of the background population, and may not be related to development of polyneuropathy. Further familial cases are needed to define potential virological factors that might be involved in the development of polyneuropathy.

In our family all patients were female, but as the pedigree shows that there is a female bias in the family, it is unclear if this is significant. The mother of cases 3 and 4 has been free from HTLV-I–associated disorders, but as we have not been able to determine her serological status, it is unknown if she is an asymptomatic carrier. Investigation of the HLA types of all HTLV-I patients has shown significant associations with high immune responsiveness against HTLV-1 *env* (Manns *et al*, 1998; Yashiki *et al*, 2001). Although our patients did not have such alleles as HLA-A26 that have been reported to be associated with ATLL (Manns *et al*, 1998; Yashiki *et al*, 2001), one of them (case 4) does have similar alleles (A24, A33, B54, B39, Cw1, Cw7, DR4, and DR2), which are known to be associated with the risk for development of HAM/TSP. Moreover, in a previously report on two cases with HAM, the HLA haplotype was described as A24, Bw54, CW1, DR4, DQ− (Kawai *et al*, 1989). To determine whether genetic host factors are associated with the development of HTLV-I polyneuropathy, further analysis of the parents of the patient and similar cases will be necessary.

Recently, antibodies against gangliosides have been detected in the patients who have developed polyneuropathy associated with HTLV-I (Mitsui *et al*, 1999; Yanagihara *et al*, 1999). The pathogenetic importance of antiganglioside antibodies in sensory and motor neuropathy has been established in non-HTLV-I–infected patients (Oka *et al*, 1996; Pestronk *et al*, 1990; Sadiq *et al*, 1990; Umehara *et al*, 1997). In this study, among the family members (see Figure 4), we have examined anti-HTLV-I antibody titer only in cases 1, 2, 3, 4, and II-1. As cases I-1, I-2, II-4, and II-5 were deceased and cases II-2, III-3, and III-4 were healthy, we had no information relating to their blood analysis. We excluded case II-1 who had relatively high titers against HTLV-I (1,024×), because we did not have detailed clinical information on this patient. We hypothesized that high antibody titers against HTLV-I virus proteins could result in cross reactions against peripheral nerve glycolipids and/or proteins, resulting in an autoimmune response and may have caused the disease. This hypothesis is supported by the fact that most of patients

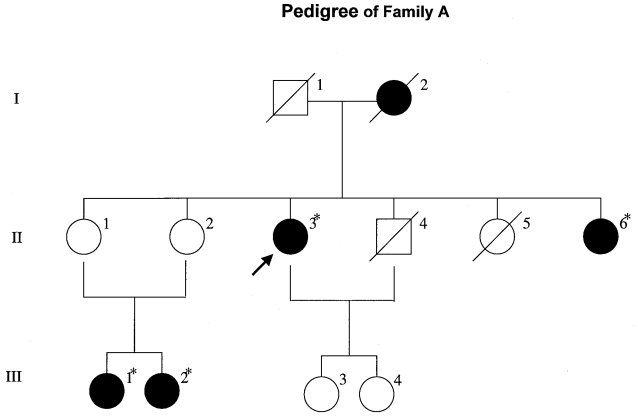

Figure 4 Pedigree of Family A. I-1: death of esophageal cancer; I-2: death of malignant lymphoma. II-1: suffering from sick sinus syndrome; II-3: case 1; II-4: sudden death; II-5: death of subarachnoid hemorrhage; II-6: case 2. III-1: case 3; III-2: case 4. *Cases examined.

with HTLV-I–associated polyneuropathy recovered or improved with steroid therapy. However, further studies will be necessary to clarify this and should help in our understanding of the pathogenesis of this form of familial polyneuropathy.

Patients and methods

Case histories

Clinical summaries of the four patients are shown in Table 1. The family pedigree (Figure 4) shows that the first generation individuals (I-1, -2) were born and spent their entire lives in the city in Hokkaido in Japan, where the average seropositivity for HTLV-I antibody of adult blood donors measured in 1984 was the second lowest (1.0%) in Japan (Tajima, 1990). The patients' mother (I-2) died of malignant lymphoma but apparently also developed numbness of the feet late in life.

Case 1 (II-3, the proband), a 61-year-old woman had a history of recurrent uveitis and hearing difficulties since childhood. At age 18, she noticed a gait disturbance and hoarseness that was followed by the gradual development of a distal sensorimotor abnormality, generalized edema, and exertional dyspnea. At age 54, she was diagnosed with a thyroid cancer by lymph node biopsy, which disclosed metastatic papillary carcinoma. After surgery and subsequent treatment with thyroxin, she noticed the progression of a sensorimotor paresis of the extremities with rectourinary disturbances. Seven years later, her thyroid cancer relapsed and required further surgery but not chemotherapy. On admission she was found to be seropositive for HTLV-I. Neurological examination demonstrated a sensory and motor paresis with pain and numbness affecting mainly the lower extremities with urinary urgency. Deep tendon reflexes were decreased or negative without pyramidal or any pathological signs. Neuroradiological studies, including brain and spinal cord magnetic resonance imaging (MRI) and skeletal muscle computed tomography (CT), revealed no metastatic mass lesions or abnormal enhancing lesions in the CNS, whereas moderate amyotrophy with fatty replacement was noticed mainly in the muscles of the lower extremities.

Case 2 (II-6) was a 53-year-old woman and the second younger sister of the proband. At age 43, she had a hysterectomy following a diagnosis of uterine cancer but did not receive blood transfusions or any postoperative chemotherapy. Three years later, she developed hoarseness and transient numbness of the lower extremities. Examination revealed generalised arthralgia, paresthesia, urinary urgency, and unsteady gait. Muscle weakness with numbness was noted in the lower extremities. Pyramidal signs and pathological reflexes were negative. Tumor markers and general radiological studies for the recurrence of her gynecological malignancy were negative.

Case 3 (III-1) was the first niece of the proband, a 52-year-old woman who had been suffering from several minor illness, including keratoconjunctivitis and hemolytic diathesis during her childhood, migraine and chronic hepatitis (hepatitis B virus [HBV] antibody positive) since the second decade of life, and paresthesias with pain in the calf muscle and weakness of extremities since her late teenage years. On the initial examination in our hospital at age 52, she presented with a mild kyphosis of the spine, distal dominant sensorimotor paresis with moderate amyotrophy and positive Barre signs in the legs. Deep tendon reflexes were decreased and her gait was unsteady. There was no urinary disturbance. Routine laboratory examination, including blood chemistry, peripheral blood count, and autoantibodies, was unremarkable except for high serum titer of HTLV-I and a positive HBV antibody titer.

Case 4 (III-2), the younger sister of case 3, noticed an unsteady gait, numbness of the feet, and headache at age of around 45 years. She had a history of pulmonary tuberculosis in her adolescence. At age 50, she tested seropositive for hepatitis C virus and also HTLV-I. Neurologically, she was noted to have paretic gait with mild hyperactive patellar tendon reflexes, painful paresthesia below the knee joint, mild motor weakness of legs, and subjective urinary urgency. Chest x-ray showed that the left lung was shrunken as a result of a previous lobectomy for tuberculosis, and moderate hypoventilation was confirmed by a spirogram. Neuroradiological studies were unremarkable except for moderate muscle atrophy in the lower extremities.

Cytological examination of CSF in all four patients revealed neither a high protein level nor atypical or malignant cells in the CSF.

Virological studies

Viral antibody titers for HTLV-I were examined by the Kishimoto Clinical Laboratory (Tomakomai, Japan) using the particle agglutination (PA) method. Serological assays for HIV-1 and-2 and other conventional viruses were examined in sera and CSF of all four patients.

HLA analysis

Serological typing for HLA of the patients was examined by the SRL Inc. (Tokyo, Japan) using a usual procedure.

Mutational analysis

Heteroduplex analysis and single strand conformation polymorphism analysis (Nelis *et al*. 1996) were performed to detect the mutation in the PMP22 gene and the P0 gene, which have been associated with the autosomal dominant type of Charcot-Marie-Tooth neuropathies (Keller and Chance, 1999). Primer sets used for PCR amplification of PMP22 and P0 genes were as previously described (Nelis *et al*, 1994).

Electrophysiological studies
Routine motor/sensory NCSs, studies of F-wave and SSEPs, and needle EMG were performed using standard procedures.

Pathological examination
Histological and immunohistochemical analyses were carried out on biopsies of skin, muscle (hamstring muscle), and sural nerves of all patients. Materials were divided into two: one for paraffin sections, the other for epoxy resin preparations. Paraffin sections including hematoxylin-eosin (H&E) and Elastica van Gieson staining with Masson trichrome were used for routine histological examination and for immunohistochemistry. Antibodies used for immunostaining were CD3 (Dako, Glostrup, Denmark), CD45RO (UCHL-1; Dako), CD4 (Nichirei, Tokyo, Japan), CD8 (Nichirei), CD20 (L-26; Dako), and CD68 (Dako). Epoxy resin preparations were stained with TB.

Nucleotide sequencing analysis
DNA samples were extracted from white blood cells of all patients, and were analyzed by the method by Furukawa *et al* (2000). PCR was performed to amplify the tax region of the provirus using primer sets PX01+ and PX02− and amplified products were extracted and purified from agarose gels. Purified DNAs were sequenced with the primers PX01+, TaxF 286, TaxF 566, and PX01p and analyzed as previously described (Furukawa *et al*, 2000).

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