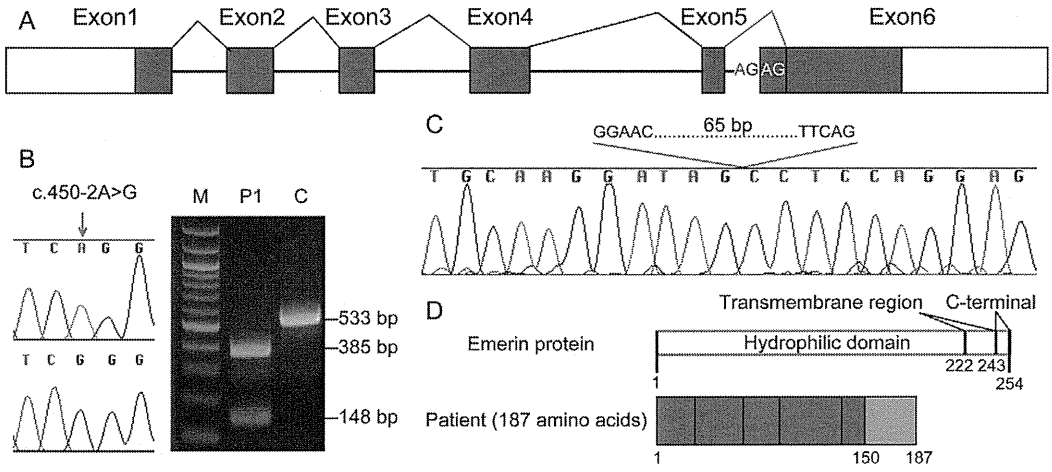


**Figure 3. Genetic studies and an ideogram of the aberrant splicing. A: The splice acceptor site mutation located at the second nucleotide before exon 6 (red: c.450-2A) of the *EMD* gene. B: Sequencing chromatogram of the c.450-2A>G mutation in *EMD* (red arrow) and the amplicon (533 bp) covering exon 6, which was divided into 385-bp and 148-bp fragments by AvaI. C: This aberrant splicing eliminated 65 nucleotides in the transcript of exon 6. D: The mutation shifted the reading frame (gray column) and produced a truncated transcript of 187 amino acids without the transmembrane region and C-terminal tail.**

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Total RNA was isolated and purified from the biopsied skeletal muscle specimen using a RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion, Austin, USA). Complementary DNA (cDNA) was generated by reverse transcription PCR (RT-PCR) of the isolated RNA (1 μg) using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. The cDNA was subsequently amplified with the forward (5'-ACCAGAGCAAGGGCTACAATGACG-3') and reverse primers (5'-GGTGGCCTTTGGTTAATCCCCTC-3') using KOD FX (Toyobo, Osaka, Japan). No band was detected at the expected position of the full-length emerin transcript, and a unique shorter band was extracted from the gel (QIAquick Gel Extraction Kit, Qiagen, Venlo, Netherlands) and sequenced. The first 65 nucleotides in exon six were found to be absent, and the reading frame was shifted. The protein was changed because the frame was shifted from position 150 to a premature stop codon at 187 (p.Arg150fs), producing a truncated emerin protein lacking the transmembrane region and C-terminal tail (Fig. 3C, D).

## Discussion

We studied the clinical, pathological, and genetic features of a Japanese patient with X-linked EDMD. We observed a partial deficiency of emerin in the skeletal muscle with severe arrhythmia and continuous serum CK elevation, but he only exhibited slight muscular involvement and mild joint contracture. A splice acceptor site mutation, c.450-2A>G (p.Arg150fs), was identified in the *EMD* gene of this patient.

The *EMD* gene on chromosome Xq28 contains six exons and encodes an emerin protein with 254 amino acids. Emerin consists of a large hydrophilic nucleoplasmic domain (residues 1-222), a transmembrane region (residues 223-243), and a short C-terminal tail (residues 244-254) (7). It is a ubiquitously expressed protein located at the inner nuclear membrane of most cells in the body with the highest mRNA expression in skeletal and cardiac muscle, and it has been proposed to have functions in gene expression, RNA processing, cell signaling, and chromatin dynamics (2, 8).

The 22-year-old patient in this study suffered a complete atrioventricular conduction block, and a permanent pacemaker was implanted to prevent lethal cardiac arrest. His mother and sisters were also affected with arrhythmia, but they did not show evidence of skeletal muscle abnormalities. Previous reports have documented that such symptomatic female carriers have very low emerin levels (<5% of normal) due to skewed X-inactivation (9). A pathological study of cardiac muscle performed by local doctors revealed nonspecific mild interstitial fibrosis. Slight muscular involvement was observed during both the clinical and imaging examinations. The cause of this proband was unclear until his persistent high CK level was noticed and cardioskeletal myopathy suspected, at which point a skeletal muscle specimen was obtained.

The histochemical pathology of the skeletal muscle indicated fiber size variation with scattered internalized nuclei. An immunohistochemical study with the NCL-Emerin monoclonal antibody targeted to 220 amino acids near the N-terminus of emerin revealed a significant reduction of nuclear emerin, and a diagnosis of X-linked EDMD was made. After direct sequencing of the *EMD* gene, an A>G transversion at 1,644 (c.450-2A>G) was observed, which should eliminate the splice acceptor site before exon six. In order to

identify the candidate acceptor site, we performed RT-PCR using mRNA extracted from the patient's frozen skeletal muscle tissue. A truncated transcript with 187 residues generated by aberrant splicing was detected. The new AG splice acceptor site, which occurs 65 bp after the wild-type site in the *EMD*, shifted the frame from position 150 to a premature stop codon at position 187. Most of the truncated emerin lacked the transmembrane segment and the C-terminal tail, thus suggesting that it might fail to target the endoplasmic reticulum and would thus be rapidly degraded (10). Interestingly, this c.450-2A>G splice site mutation was reported in another unrelated patient in Japan in 1999 (6). The initial symptom of the first patient was neck contracture, but our patient initially experienced cardiac involvement. In fact, immunostaining and immunoblotting studies performed in the previous patient revealed the absence of emerin and a premature stop codon generated at residue 235. The differences between these two patients with same mutation suggest that there could be other potential mechanisms involved in protein expression and mRNA splicing.

In an additional immunohistochemical study, we employed two additional emerin-targeted antibodies, H-12 (targets residues 3 to 254) and C-20 (targets a peptide near the C-terminus). In the present patient, emerin expression was detected by H-12 staining (but at a much lower level than normal) but not by C-20 staining. In this case, a skin sample could have been helpful in further verifying the status of emerin. We concluded that the truncated emerin in the present patient could react with antibodies targeting a peptide near the N-terminus (NCL-Emerin and H-12) at much lower levels, but it could not bind the antibody targeted to the C-terminus (C-20). In addition, the nucleoplasm showed stronger staining than the cytoplasm, but this was not as strong as that observed at the nuclear edge, thus indicating that emerin may have been expressed in the nucleoplasm. An immunoelectron microscopy study might be more reliable for identifying emerin expression. The reason why the mutated version of emerin without the transmembrane segment was detectable on the nuclei requires further research.

According to the UMD-EMD mutations database (http://www.umd.be/EMD/), 94 different mutations of the *EMD* gene have been reported in 298 records, and 15 of these are splice site mutations (15.96%). It is notable that some other X-linked EDMD patients with truncated emerin longer than 187 amino acids presented with complete absences of emerin as detected with the NCL-Emerin antibody from the same company (11-13). To evaluate the emerin protein expression, it would be more reliable to use various antibodies targeted to different domains. An antibody targeting the C-terminus could therefore be useful for detecting truncated emerin, which accounts for most *EMD* mutations.

Mutations in emerin can cause different phenotypes, even within the same family. In patients with distal premature stop codons, which do not evoke a nonsense-mediated decay mechanism, the phenotypes present as a typical triad (6, 12-16) or cardiac phenotype (11, 17). Rare cases of a reduction in emerin due to a missense mutation may have a milder phenotype (18). The cardiac muscle is supposed to be less tolerant of emerin deficiency than the skeletal muscle (19). A lack of or decrease in emerin levels in the heart may alter electrical resistance and cardiomyocyte adhesion, which could lead to conduction delay or block (17). Emerinopathy also includes a limb-girdle muscular dystrophy phenotype (20, 21). In the present case with partial emerin deficiency, although a complete atrioventricular conduction block was observed, there was only slight muscle weakness and joint contracture. The truncated protein remaining in the nucleoplasm may have contributed to the relatively benign phenotype, especially in the skeletal muscle.

In conclusion, we identified a splice site mutation before the last exon of *EMD* in an X-linked EDMD patient, and he showed evidence of a truncated protein at both pathological and genetic levels. We detected partial expression of a truncated emerin protein without the transmembrane region and C-terminal domain by immunohistochemical staining with antibodies against different emerin domains. We recommend the use of multiple antibodies that bind different emerin domains in order to obtain a comprehensive view of the protein. The anti-emerin antibody C-20 may be useful for detecting the absence of the C-terminus. Our findings also suggest that all young patients with significant cardiac conduction defects of unknown etiology be assessed to exclude the presence of EDMD, even if they have either no skeletal muscle or only slight skeletal muscle or joint involvement.

**Author's disclosure of potential Conflicts of Interest (COI).**
Hiroshi Takashima: Patent royalties/licensing fees, Achena Diagnosis

**Acknowledgement**

The authors thank Ms. N. Hirata of our department for her excellent technical assistance. We also wish to thank the Joint Research Laboratory, Kagoshima University Graduate School of Medical and Dental Sciences for the use of their facilities.

**Financial Support:** This project was funded by the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant 21591094, IH); Intramural Research Grant (23-5) for Neurological and Psychiatric Disorders of NCNP Applying Health and Technology, from the Ministry of Health, Labour and Welfare of Japan; and a research grant (23300201) from the Ministry of Health, Labour and Welfare of Japan.

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Pediatric Neurology 48 (2013) 59–62

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Pediatric Neurology

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Case Report

# Congenital Hypomyelinating Neuropathy Attributable to a De Novo p.Asp61Asn Mutation of the Myelin Protein Zero Gene

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ARTICLE INFORMATION

*Article history:*
Received 31 July 2012
Accepted 13 September 2012

ABSTRACT

We describe a boy aged 2 years and 11 months with congenital hypomyelinating neuropathy attributable to a de novo heterozygous missense mutation of c.181G>A (p.Asp61Asn) in the myelin protein zero gene. A nerve conduction study indicated markedly reduced motor conduction velocities in the upper and lower extremities. Stimuli of up to 50-100 mA were necessary for nerve activation, suggesting diseased nerves with greatly decreased excitability. A sural nerve biopsy revealed a marked loss of large myelinated fibers, the absence of myelin breakdown products, occasional basal lamina onion-bulb formations, and tomacula-like structures. The p.Asp61Asn mutation is novel in congenital hypomyelinating neuropathy, but was previously reported in a patient with Charcot-Marie-Tooth disease type 1.



## Introduction

Congenital hypomyelinating neuropathy constitutes a rare congenital neuropathy characterized by onset prenatally, neonatally, or during early infancy, with hypotonia, nonprogressive weakness, markedly reduced nerve conduction velocities, and hypomyelination. Molecular genetic analyses revealed that several mutations in five genes encoding proteins involved in peripheral nerve myelination (i.e., *MPZ*, *PMP22*, *ERG2*, *MTMR2*, and *SOX10*) can cause congenital hypomyelinating neuropathy [1].

Myelin protein zero is a transmembrane protein of the immunoglobulin family and an important myelin structural protein required for normal peripheral nerve myelination. More than 120 mutations in the *MPZ* gene have been detected in patients with various forms of hereditary motor and sensory neuropathies [1]. Recent clinical and laboratory investigations provided insights into the pathogenesis of neuropathies associated with these *MPZ* mutations. Myelin protein zero contains a large glycosylated immunoglobulin-like extracellular domain that endows proteins with adhesion properties, and a smaller basic intracellular domain that participates in electrostatic interactions [2]. To our knowledge, eight mutations (i.e., p.Arg98Cys, p.Ser111Phe, p.Gly123_Cys127del, p.Thr124Lys, p.Asn131Ser, p.Gly167Arg, p.Leu184fs, and p.Gln215X) in the *MPZ* gene have been demonstrated to segregate with congenital hypomyelinating neuropathy [1,3-5]. Among these, five and two mutations were identified in the extracellular and intracellular domains, respectively, of *MPZ* gene products.

We report on a Japanese boy with congenital hypomyelinating neuropathy attributable to a de novo heterozygous mutation of c.181G>A (p.Asp61Asn) in the *MPZ* gene. This patient presented with clinical, electrophysiologic, and morphologic features consistent with congenital hypomyelinating neuropathy.

## Case Report

A boy aged 2 years and 11 months was evaluated for gross motor delay and generalized hypotonia. No family history of neuromuscular disease was reported. The parents were not consanguineous. The patient's older brother was healthy and developmentally normal. The pregnancy was reportedly normal, with no history of decreased fetal

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http://dx.doi.org/10.1016/j.pediatrneurol.2012.09.011

movements. The patient was delivered uneventfully at 37 weeks of gestation. His birth weight was 2884 g. He sat independently at age 8 months, but was unable to stand with support at 12 months of age. He began crawling at age 15 months, and walked independently at age 2 years and 3 months. He was unable to run. His language development and fine motor development were normal.

He was alert and bright, but his face appeared myopathic. Physical examination revealed a mildly high-arched palate, but neither thenar plus hypothenar atrophy nor pes cavus. A cranial nerve examination revealed normal ocular movements and pupillary responses to light. Mild facial muscle weakness was evident. Tongue fasciculations were absent. He was hypotonic, but his muscle strength was only mildly reduced in the four extremities. Hyperextension was observed in the finger, wrist, and knee joints. His muscle bulk was normal. Deep tendon reflexes were absent at the biceps, triceps, patella, and ankles. No sensory disturbance was evident. The Romberg sign was demonstrated as negative. Truncal titubation and dysmetria were absent. He was able to stand up from a supine position using a modified Gowers maneuver. His gait, with bilateral genu recurvatum, was wide-based and ataxic.

His creatine kinase level was measured at 166 IU/L (normal range, 62-287 IU/L). Cerebrospinal fluid testing demonstrated mildly elevated protein content (54 mg/dL; normal range, 10-40 mg/dL) and a few mononuclear cells. Cranial magnetic resonance imaging indicated no intracranial abnormalities. A nerve conduction study revealed very prolonged distal latencies, markedly reduced motor conduction velocities (3.0-4.0 m/second), and the temporal dispersion of compound muscle action potentials from his upper and lower extremities (data not shown). To evoke motor nerve responses, electrical stimuli greater than 50 mA were necessary. Sensory responses were not evoked (data not shown). Magnetic resonance imaging of the lumbar plexus demonstrated no enlarged nerve roots. A sural nerve biopsy revealed a severe loss of large myelinated fibers in all fascicles. Well-organized onion-bulb formations were not evident (Fig 1a). No inflammatory infiltrates, edema, or storage materials were observed. Intramuscular nerves rarely included large myelinated fibers (data not shown). A teased fiber analysis demonstrated thin, myelinated segments and tomacula-like structures (Fig 1b). Electron microscopic examination revealed occasional onion-bulb formations consisting of multilayered empty basal lamina (Fig 1c). Occasional fibers with thin or abnormally compacted myelin (Fig 1d) were also observed. Unmyelinated fibers were well preserved. A peroneus brevis muscle biopsy indicated mild variations in fiber size, without necrotic or regenerating fibers. We detected no evidence of group atrophy. Each fiber type was distributed in a mosaic pattern, without evidence of fiber type grouping. However, type 1 fibers were mildly atrophic (data not shown). Gene resequencing according to the DNA chip technique revealed one allele to possess a de novo point mutation, c.181G>A (p.Asp61Asn), in the extracellular domain of *MPZ*. A purpose-built GeneChip CustomSeq Custom Resequencing Array (Affymetrix, Santa Clara, CA) was designed to screen for mutations of 28 disease-causing genes in Charcot-Marie-Tooth disease, congenital hypomyelinating neuropathy, and related diseases such as ataxia with oculomotor apraxia type 1, ataxia with oculomotor apraxia type 2, spinocerebellar ataxia with axonal neuropathy type 1, and hereditary motor neuropathies [6]. The p.Asp61Asn mutation was confirmed by the conventional Sanger DNA sequencing method. The patient's healthy parents and brother did not manifest this mutation.

## Discussion

Inherited neuropathy with hypomyelination remains controversial nosologically, and congenital hypomyelinating neuropathy can be difficult to differentiate from the early-onset form of hereditary motor and sensory neuropathy

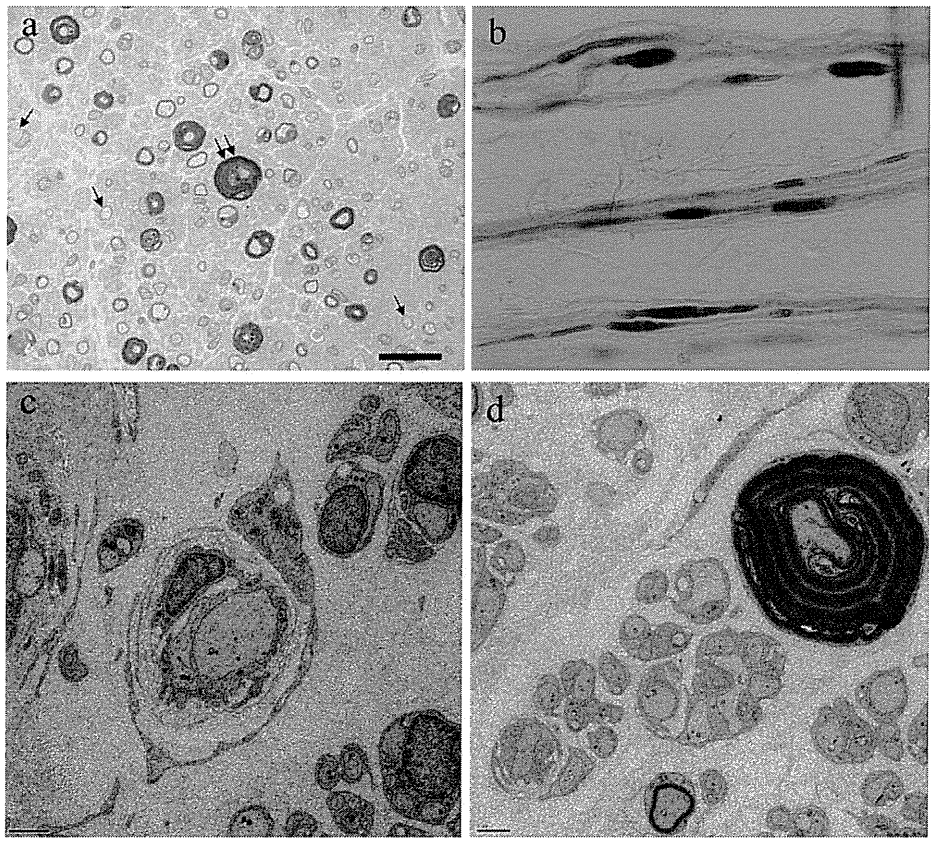


**Figure 1.** (a) Epoxy-embedded and toluidine blue-stained semithin section of the sural nerve reveals the endoneurium, including nonmyelinated (arrows), hypomyelinated, and hypermyelinated (double arrows) fibers. Bar, 20 μm. (b) Teased fiber analysis demonstrates thin, myelinated segments and tomacula-like structures. A lack of myelination is evident in the nerves. (c) Electron microscopy reveals an absence of myelin and onion-bulb formations consisting of multilayered empty basal lamina. Bar, 2 μm. (d) Electron microscopy indicates abnormally compacted myelin. Bar, 2 μm.

type 3 (Déjérine-Sottas syndrome) on a clinical basis alone. These phenotypes, it has been argued, are distinct entities with unique clinical and morphologic features, e.g., congenital hypomyelinating neuropathy is a disorder of myelin formation, whereas Déjérine-Sottas syndrome is a demyelinating disorder. On the other hand, they may occur within a continuum of "myelinopathies," differing in severity but with a common underlying defect in myelination [2]. Because repeated demyelination and remyelination are known to be responsible for the onion-bulb formation in Charcot-Marie-Tooth disease or the Déjérine-Sottas syndrome of hereditary motor and sensory neuropathies, nerve pathology can assist in differentiating between congenital hypomyelinating neuropathy and Déjérine-Sottas syndrome in the absence of myelin breakdown, well-organized onion-bulb formation, and inflammation in congenital hypomyelinating neuropathy [7].

Our patient was a young boy with sensorimotor congenital neuropathy presenting as gross motor delay, nonprogressive weakness, and hypotonia. Histopathologic images of the sural nerve were compatible with congenital hypomyelinating neuropathy. According to a nerve conduction study, stimuli as strong as 50-100 mA were necessary for nerve activation, indicating diseased nerves with markedly decreased excitability [8]. No evidence of myelin breakdown products was detected, indicating that little, if any, demyelination and remyelination were occurring. According to muscle pathology, group atrophy was absent, and the mosaic pattern of each fiber type was well-preserved. These findings suggest that the axons were most likely intact during muscle fiber growth and fiber type differentiation. In contrast with patients manifesting Déjérine-Sottas syndrome, our patient presented no evidence of repeated demyelination, but did demonstrate occasional, atypical onion-bulb formations consisting of multilayered empty basal lamina. Mild type 1 fiber atrophy was present in our patient.

Interestingly, previous reports demonstrated that muscle pathology in congenital hypomyelinating neuropathy included type 2 fiber atrophy with type 1 fiber predominance and type 1 fiber hypotrophy and predominance [4,7], although the pathogenesis underlying type 1 fiber atrophy in our patient remains unknown.

The p.Asp61Asn mutation in the *MPZ* gene was reported by Bellone et al. to be a pathogenic mutation in a patient with Charcot-Marie-Tooth disease type 1 [9,10]. This mutation is also responsible for congenital hypomyelinating neuropathy. Similarly, the p.Gly167Arg mutation was reported to contribute to congenital hypomyelinating neuropathy, Déjérine-Sottas syndrome, and Charcot-Marie-Tooth disease type 1 [1]. More than 120 mutations in the *MPZ* gene (most of which are localized within the extracellular domain of the protein) are known to cause various hereditary motor and sensory neuropathies [1]. The *MPZ* gene encodes a transmembrane protein of 219 amino acids, and contains a single extracellular domain, a single transmembrane domain, and a single cytoplasmic domain [11]. Crystallographic analysis of the *MPZ* extracellular domain demonstrates that it forms homotetramers within the plane of the membrane, and that each of them interacts with a similar homotetramer on the opposing membrane surface [12]. Furthermore, the absence of *MPZ* expression in animals, such as *Mpz* knockout mice, causes myelin to lose its normal compact state [13]. According to these data, myelin protein zero plays an essential role in the myelination of the peripheral nervous system, probably by holding together adjacent wraps of the myelin membrane via homotypic interactions mediated by myelin protein zero. Distinct phenotypes associated with different mutations at the same position may be attributable to a primary role played by amino acid changes [14]. However, the pathogenic mechanisms by which a single mutation in the *MPZ* gene can cause two hereditary motor and sensory neuropathic phenotypes remain unknown. Molecular modeling indicates that the substitution of p.Asp61Asn produces a variation in polarity, consequently affects the network of hydrogen bonds responsible for the correct folding and dimerization of the protein, and is associated with severe early-onset demyelinating neuropathy [10]. The presence of the p.Asp61Asn heterozygous mutation in our patient suggests that a mutated allele of the *MPZ* gene exerts a dominant-negative effect. Further functional studies are required to understand the pathogenic impacts of the p.Asp61Asn mutation on clinical phenotypes.

The authors thank Ichizo Nishino, MD, PhD, for the critical evaluation of muscle pathology, Yasushi Oya, MD, PhD, for helpful discussions, Yoriko Kojima for technical support with electron microscopic studies, and Akiko Yoshimura, BS, of Kagoshima University for excellent technical assistance. This work was supported by a Grant-in-Aid from the Research Committee of Spinal Muscular Atrophy at the Ministry of Health, Labor, and Welfare of Japan, and by Intramural Research Grant 23-6 for Neurologic and Psychiatric Disorders of the National Center of Neurology and Psychiatry (to T.Y. and H.K.). This study was also supported in part by grants from the Nervous and Mental Disorders and Research Committee for Charcot-Marie-Tooth Disease, Neuropathy, Ataxic Disease, and Research on Applying Health Technology at the Japanese Ministry of Health, Welfare and Labor (to H.T.).

## Supplementary material

Supplementary material associated with this article is available, in the online version, at doi:10.1016/j.pediatrneurol.2012.09.011.

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PLOS ONE

# Familial Clusters of HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

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## Abstract

***Objective:*** HTLV-1 proviral loads (PVLs) and some genetic factors are reported to be associated with the development of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). However, there are very few reports on HAM/TSP having family history. We aimed to define the clinical features and laboratory indications associated with HAM/TSP having family history.

***Methods:*** Records of 784 HAM/TSP patients who were hospitalized in Kagoshima University Hospital and related hospitals from 1987 to 2012 were reviewed. Using an unmatched case-control design, 40 patients of HAM/TSP having family history (f-HAM/TSP) were compared with 124 patients suffering from sporadic HAM/TSP, who were admitted in series over the last 10 years for associated clinical features.

***Results:*** Of the 784 patients, 40 (5.1%) were f-HAM/TSP cases. Compared with sporadic cases, the age of onset was earlier (41.3 vs. 51.6 years, $p<0.001$), motor disability grades were lower (4.0 vs. 4.9, $p=0.043$) despite longer duration of illness (14.3 vs. 10.2 years, $p=0.026$), time elapsed between onset and wheelchair use in daily life was longer (18.3 vs. 10.0 years, $p=0.025$), cases with rapid disease progression were fewer (10.0% vs. 28.2%, $p=0.019$), and protein levels in cerebrospinal fluid (CSF) were significantly lower in f-HAM/TSP cases (29.9 vs. 42.5 mg, $p<0.001$). There was no difference in HTLV-1 PVLs, anti-HTLV-1 antibody titers in serum and CSF, or cell number and neopterin levels in CSF. Furthermore, HTLV-1 PVLs were lower in cases with rapid disease progression than in those with slow progression in both f-HAM/TSP and sporadic cases.

***Conclusions:*** We demonstrated that HAM/TSP aggregates in the family, with a younger age of onset and a slow rate of progression in f-HAM/TSP cases compared with sporadic cases. These data also suggested that factors other than HTLV-1 PVLs contribute to the disease course of HAM/TSP.

**Citation:** Nozuma S, Matsuura E, Matsuzaki T, Watanabe O, Kubota R, et al. (2014) Familial Clusters of HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis. PLoS ONE 9(5): e86144. doi:10.1371/journal.pone.0086144

**Editor:** Steven Jacobson, National Institutes of Health, United States of America

**Received** June 6, 2013; **Accepted** December 5, 2013; **Published** May 6, 2014



**Funding:** This study was supported by Health and Labour Sciences Research Grants from the Ministry of Health Labour and Welfare and JSPS KAKENHI Grant Numbers 25293205 and 24133701. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by slow progressive spastic paraparesis and positivity for anti-HTLV-1 antibodies in both serum and cerebrospinal fluid (CSF) [1,2]. Worldwide, at least 10–20 million people are infected with HTLV-1 [3]. However, although the majority of infected individuals remain lifelong asymptomatic carriers, approximately 2%–5% develop adult T-cell lymphomas [4,5] and another 0.25%–3.8% develop HAM/TSP [1,2]. Although the mechanisms underlying the development of HAM/TSP are not fully understood, several risk factors are closely associated with HAM/TSP. In particular, HTLV-1 proviral loads (PVLs) are significantly higher in HAM/TSP patients than in asymptomatic carriers and are also higher in genetic relatives of HAM/TSP patients than in non-HAM-related asymptomatic carriers [6]. Host genetic factors, including human leukocyte antigen (HLA) and non-HLA gene polymorphisms affect the occurrence of HAM/TSP [7], indicating that HTLV-1 PVLs and genetic backgrounds may influence individual susceptibility to HAM/TSP. Although several reports of familial adult T-cell lymphoma have been published [8,9], to our knowledge, there is only one case report of patient with HAM/TSP having family history (f-HAM/TSP) [10]. Hence, little is known about the prevalence and character of f-HAM/TSP cases. In this study, the characteristic clinical and laboratory features of f-HAM/TSP cases are defined and compared with those of sporadic cases.

## Methods

### Ethics Statement

This study was approved by the Institutional Review Boards of Kagoshima University. All participants provided written informed consent.

### Design

We used an unmatched case-control design to identify the phenotypic features of f-HAM/TSP. f-HAM/TSP cases were identified as patients with multiple family members suffering from HAM/TSP. Controls were defined as HAM/TSP patients who were not genetically related to other HAM/TSP patients.

### Subjects

f-HAM/TSP cases were extracted from our database of individuals diagnosed with HAM/TSP in Kagoshima University Hospital and related hospitals from 1987 to 2012. Controls included consecutive patients with sporadic HAM/TSP who were evaluated in our department between January 2002 and June 2012. HAM/TSP was diagnosed according to the World Health Organization diagnostic criteria, and the updated criteria of Castro-costa Belem [11]. Clinical information was obtained from the medical records of patient attendance at our hospital. In other cases, clinical data were obtained from the clinical records of patients or directly from the referring clinicians. Clinical variables included sex, age, age of onset, and initial symptoms. Neurological disabilities were assessed using Motor Disability Grading (MDG), modified from the Osame Motor Disability Scale of 0 to 10, as reported previously [12]. Motor disability grades were defined as follows: 5, needs one-hand support while walking; 6, needs two-hand support while walking; and 7, unable to walk but can crawl. We used a different assessment for the subgroup of more than grade 6 because their disease state significantly interfered with their lifestyle and necessitated the use of wheelchairs in daily life. The subgroup of patients with rapid progression was defined by deterioration of motor disability by more than three grades within two years. Anti-HTLV-1 antibody titers in serum and CSF were detected using enzyme-linked immunosorbent assays and particle agglutination methods (Fijirebio Inc, Tokyo, Japan). HTLV-1 PVLs in peripheral blood mononuclear cells (PBMCs) were assayed using quantitative PCR with the ABI PRISM 7700TM sequence detection system as reported previously [6].

## Statistical Analysis

Data were analyzed using SPSS-20 (SPSS, Chicago, Illinois). Statistical analyses were performed using parametric (t-test) and non-parametric tests (Mann–Whitney test) for continuous variables and $\chi^2$ (Pearson$\chi^2$ test/Fisher exact test) for categorical variables. Significant differences were then adjusted for potential confounders (age and sex) using multiple linear regression analysis. Survival was estimated according to the Kaplan–Meier method. The final endpoint was defined by a MDG score of 6. Patients with MDG scores of 6 almost wheelchair bound in daily life. The log rank test was used in Kaplan–Meier analyses. Differences were considered significant when $p<0.05$.

## Results

### Clinical characteristics of f-HAM/TSP

Of the 784 patients diagnosed with HAM/TSP between January 1987 and June 2012, 40 (5.1%) were f-HAM/TSP. The sex ratio was 33 males : 7 females. Of these 40 cases, 10 had parents or children (25.0%), 27 had siblings (67.5%), and three had other relatives (7.5%) diagnosed with HAM/TSP. Three individuals from one family were diagnosed with HAM/TSP, whereas only two individuals were diagnosed with HAM/TSP in all other families. In f-HAM/TSP cases, the age of onset was earlier (41.3 vs. 51.6 years, $p<0.001$), cases with rapid progression were fewer (10.0% vs. 28.2%, $p=0.019$), motor disability grades were lower (4.0 vs. 4.9, $p=0.043$) despite longer duration of illness (14.3 vs. 10.2 years, $p=0.026$), and time elapsed between onset and wheelchair use in daily life was longer (18.3 vs. 10.0 years, $p=0.025$) compared with sporadic cases. Sex and initial symptoms did not differ significantly between f-HAM/TSP and sporadic cases (Table 1). Twelve patients of f-HAM/TSP, and 38 of the 128 sporadic cases reached endpoint MDG scores of 6. Significant differences were then adjusted for potential confounders (age and sex) using multivariate analysis. Age of onset, duration of illness, MDG scores, and time elapsed between onset and wheelchair use in daily life remained significantly different after multivariate analysis (Table 1). The proportion of patients with rapid progression did not differ significantly between the groups, although there was a trend toward a higher proportion in sporadic cases. Kaplan–Meier analyses revealed that approximately 30% of both f-HAM/TSP and sporadic cases needed a wheelchair in daily life in 15 years after onset, and approximately 50% of patients from both groups needed it in 20 years after onset (Figure 1). Although sporadic patients needed wheelchairs earlier in most cases, the difference in the ratio of the patients with MDG score above six was not statistically significant between the groups. Finally, we compared differences in the age of onset between parent–child and sibling cases in f-HAM/TSP cases. Age of onset in parent–child f-HAM/TSP cases was significantly younger than that in sibling f-HAM/TSP cases ($29.9\pm10.0$ vs. $45.1\pm13.0$ years, $p=0.002$).

### Laboratory parameters and PVLs in f-HAM/TSP cases

Protein levels in CSF were significantly lower in f-HAM/TSP cases than in sporadic cases (29.9 vs. 42.5 mg/dl, $p<0.001$). This difference in CSF protein level remained significant after multivariate analysis. Anti-HTLV-1 antibody titers in serum and CSF, and cell numbers and neopterin levels in CSF were not significantly different between two groups. Moreover, HTLV-1 PVLs did not differ significantly. (Table 2).

### Clinical and laboratory findings in patients with rapid disease progression

Previous studies suggest that an older age of onset is associated with rapid disease progression. Similar findings are found in the present study. The percentage of rapid progression tended to increase with older age of onset in both f-HAM/TSP and sporadic groups (Figure 2). We compared the characteristics of 124 sporadic HAM/TSP patients with rapid and slow progression who were admitted to Kagoshima University Hospital in series during the last 10 years (Table 3). Patients with rapid progression were significantly older at onset than those with slow progression (62.3 vs. 47.4 years, $p<0.001$), although sex and initial symptoms did not differ significantly between rapid and slow progression groups. However, the time elapsed between onset and wheelchair use in daily life was markedly shorter among patients with rapid progression (1.5 vs. 14.4 years, $p<0.001$). Cell numbers, protein levels, and anti-HTLV-1 antibody titers in CSF were significantly higher in patients with rapid progression than in those with slow progression (11.6 vs. 3.2, $p<0.001$; 55.3 vs. 36.7 mg/dl, $p<0.001$; 1,251 vs. 416, $p<0.014$, respectively). Interestingly, HTLV-1 PVLs were significantly lower in patients with rapid progression than in those with slow progression (370 vs. 1,245 copies, $p<0.001$). Furthermore, we compared the differences between women and men in patients with rapid progression because the reason remains unknown why HAM/TSP is common in female

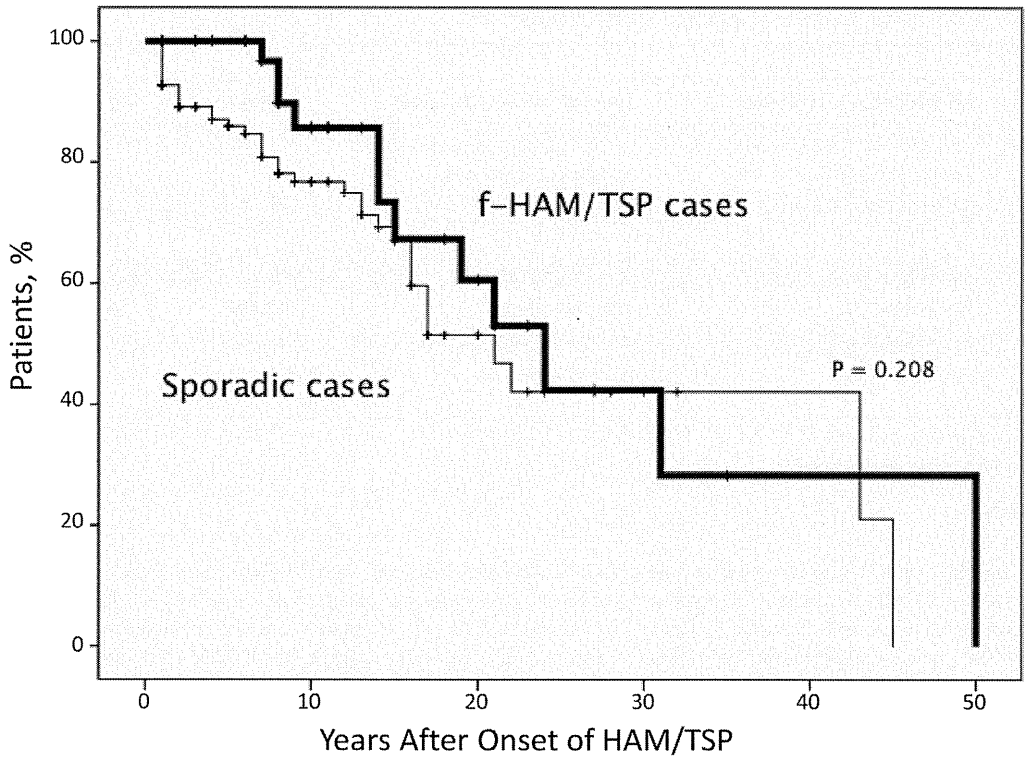


**Figure 1. Kaplan–Meier estimates of the time from disease onset to assignment of motor disability scores of 6.** In sporadic cases, more patients reached the score of six at an early stage; however, the difference was not significant. Approximately 30% of both f-HAM/TSP cases and sporadic cases needed a wheelchair in daily life in 15 years after onset and approximately 50% of patients from both groups needed a wheelchair in 20 years after onset.
doi:10.1371/journal.pone.0086144.g001

than in male. There was no significant difference between women and men in the age of onset (61.5 y.o.±12.6 vs. 62.7 y.o.±12.5), in the incidence of rapid progression (26.3% vs. 32.3%) and in MDG score (5.4 vs. 5.0; mean).

## Discussion

We demonstrated that among 784 HAM/TSP patients, 40 (5.1%) had family members with the disease. The lifetime risk of developing HAM/TSP is 0.25% of HTLV-1 carriers in Japan

**Table 1.** Clinical features of f-HAM/TSP cases or sporadic cases of HAM/TSP.

| | f-HAM/TSP cases (40 cases) | Sporadic cases (124 cases) | p value | p value† |
|---|---|---|---|---|
| Female ratio (%) | 78.8% (7 males : 33 females) | 66.4% (31 males : 93 females) | NS | |
| Age | 55.6±13.0 (23–79) | 61.8±12.5 (15–83) | **0.008** | |
| Age of onset | 41.3±13.9 (14–65) | 51.6±15.9 (13–78) | **<0.001** | **0.017** |
| Duration of illness (years) | 14.3±11.4 (1–49) | 10.2±9.6 (0–45) | **0.026** | **0.017** |
| Initial symptoms | | | | |
| Gait disturbance | 50.0% | 52.4% | NS | |
| Urinary disturbance | 32.5% | 26.6% | NS | |
| Sensory disturbance | 12.5% | 14.5% | NS | |
| Others | 5% | 6.5% | NS | |
| Rapid disease progression | 4 cases (10.0%) | 35 cases (28.2%) | **0.019** | 0.069 |
| Motor disability score | 4.0±2.0 (0–7) | 4.9±1.5 (0–8) | **0.043** | **0.036** |
| Score more than 6 | 12 cases (30.0%) | 38 cases (30.7%) | NS | |
| Time elapsed between onset and wheelchair use in daily life (years) | 18.3±12.4 (7–50) | 10.0±10.4 (1–45) | **0.025** | **0.020** |

Data are presented as mean values ± s.d., (range),
†Adjusted for age and sex.
doi:10.1371/journal.pone.0086144.t001

**Table 2.** Laboratory findings of familial clusters or sporadic cases of HAM/TSP.

| | f-HAM/TSP cases (40cases) | Sporadic cases (124 cases) | p value | p value† |
|---|---|---|---|---|
| Anti-HTLV-1 antibodies* | | | | |
| Titer in Serum | 20,787±31,004, N=37 | 31,009±36,075, N=109 | NS | |
| Titer in CSF | 2,310±11,741, N=31 | 672±1,274, N=111 | NS | |
| Cerebrospinal fluid | | | | |
| Cell number (/mm$^3$) | 3.0±2.5, N=25 | 5.7±10.0, N=109 | NS | |
| Protein (mg/dl) | 29.9±9.4, N=22 | 42.5±19.3, N=109 | **<0.001** | **0.007** |
| Neopterin (pmol/ml) | 83.2±118.1, N=18 | 38.3±56.8, N=35 | NS | |
| HTLV-1 proviral loads (Copies/10$^4$ PBMCs) | 930±781, N=32 | 968±1,746, N=101 | NS | |

* Particle Aggregation Method.
Data are presented as mean values ± s.d., N=sample number,
†Adjusted for age and sex.
doi:10.1371/journal.pone.0086144.t002

[13]. Although clustering of familial adult T-cell lymphomas has been reported [8,9], to our knowledge the prevalence of familial clusters of HAM/TSP has not been described. A study in Peru showed that 30% of HAM/TSP patients have family members with paralytic neurological disorders, but the cause of paralysis was not evaluated [14]. In the present study, we included f-HAM/TSP diagnosed in medical institutions and excluded cases with a family history of neurological disorders. Thus, the actual incidence rates of f-HAM/TSP may be higher than those reported here. Interestingly, although HTLV-1 PVL has been associated with the development and clinical progression of HAM/TSP [15–17], there was no significant difference between f-HAM/TSP and sporadic cases in the present study. Because previous studies reported that HTLV-1 PVLs of asymptomatic carriers in relatives of HAM/TSP patients were higher than those in non-HAM-related asymptomatic carriers [6], relatives of HAM/TSP are believed to be at a higher risk of developing HAM/TSP. Interestingly, our data suggest that HAM/TSP patients aggregate in families and factors other than HTLV-1 PVLs may contribute to HAM/TSP.

Compared with sporadic HAM/TSP, the clinical characteristics of f-HAM/TSP have a younger age of onset and longer time elapsed between onset and wheelchair use in daily life. Although we were unable to identify the reason for earlier onset among f-HAM/TSP cases, one can speculate that mild symptoms, such as urinary and sensory disturbances, may be identified earlier by family members who are familiar with HAM/TSP symptoms. However, the present data show no difference in initial symptoms

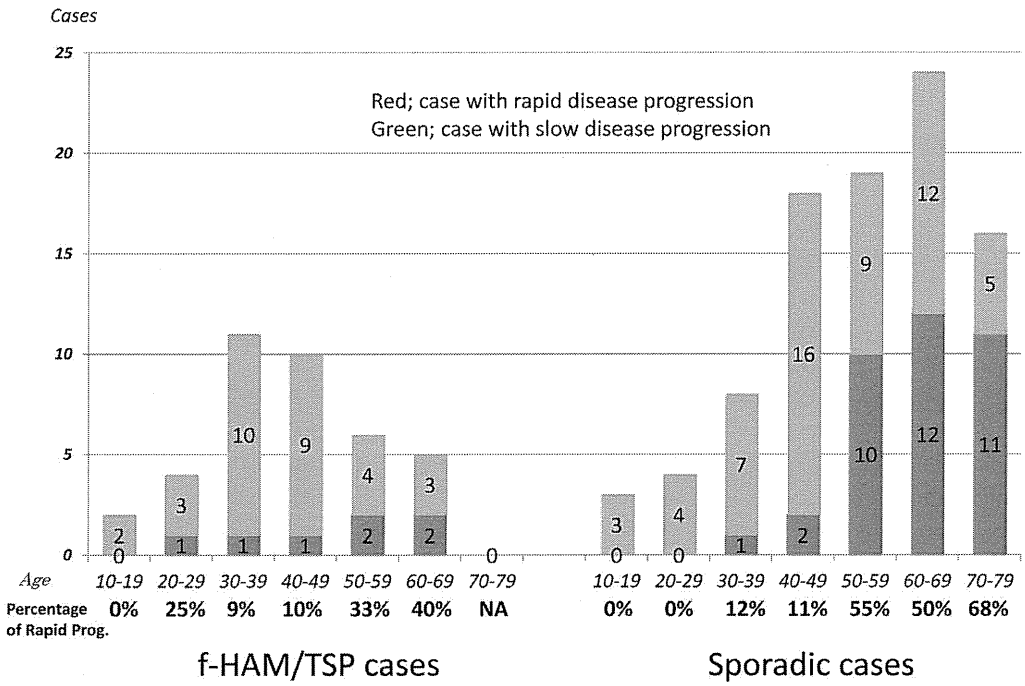


**Figure 2. Age-specific proportions of rapid disease progression.** The proportion of cases with rapid disease progression tended to increase with the older age of onset.
doi:10.1371/journal.pone.0086144.g002

**Table 3.** Clinical and laboratory findings of sporadic HAM/TSP with rapid/slow disease progression.

| Type of disease progression | Rapid progression | Slow progression | p value |
|---|---|---|---|
| Female ratio (%) | 71.4% (10 males : 25 females) | 76.4% (21 males : 68 females) | NS |
| Age of onset | 62.3±9.6, N = 35 | 47.4±15.9, N = 89 | **<0.001** |
| Age of onset of f-HAM/TSP cases | 60.5±3.7, N = 4 | 39.2±12.9, N = 36 | **0.002** |
| Duration between onset and inability to walk alone (years) | 1.5±0.9, N = 13 | 14.4±10.4, N = 25 | **<0.001** |
| Anti-HTLV-1 antibodies* | | | |
| Titer in Serum | 31,894±36,845, N = 34 | 30,608±35,965, N = 75 | NS |
| Titer in CSF | 1,251±1,800, N = 34 | 416±852, N = 77 | **0.014** |
| Cerebrospinal fluid | | | |
| Cell number (/mm$^3$) | 11.6±16.6, N = 34 | 3.2±3.5, N = 75 | **<0.001** |
| Protein (mg/dl) | 55.3±24.3, N = 34 | 36.7±13.0, N = 75 | **<0.001** |
| Neopterin (pmol/ml) | 74.9±107.9, N = 8 | 27.4±23.4, N = 27 | 0.255 |
| HTLV-1 proviral loads (Copies/10$^4$ PBMCs) | 370±327, N = 32 | 1,245±2,046, N = 69 | **<0.001** |

* Particle Aggregation Method.
Data are presented as mean values ± s.d., N = sample number.
doi:10.1371/journal.pone.0086144.t003

between f-HAM/TSP and sporadic cases. In all cases, the age of onset and initial symptoms of HAM/TSP were evaluated by the neurologists during hospitalization. Because inflammatory processes are less marked in f-HAM/TSP cases, as indicated by significantly lower protein levels in CSF, f-HAM/TSP cases may show slow progression of disease.

We need to discuss the possibility that the two groups compared represent different mode of HTLV transmission, i.e. vertical vs. sexual transmission. To clarify genetic backgrounds, sporadic HAM/TSP with seropositive carrier family members may be a more appropriate control, but are not available at present. The incidence of female cases showing no significant differences between f-HAM/TSP and sporadic cases, and between rapid and slow disease progression, might suggest less possibility of sporadic cases due to sexual transmission.

Although the subgroup of patients with rapid progression has not been clearly defined, previous studies suggest that rapid progression occurs in 10%–30% of all patients with HAM/TSP [12,14,16], and is associated with an older age of onset [14–16]. In the present study, the age of onset in patients with rapid progression was significantly older than that in patients with slow progression between f-HAM/TSP and sporadic cases, and the proportion of patients with rapid progression increased with the older age of onset (Figure 2). Among sporadic cases, cell numbers and protein levels in CSF were significantly higher in patients with rapid progression, suggesting that inflammation is more active in the spinal cords of patients with rapid progression and that cytotoxic T-lymphocyte (CTL) immune responses may be more intensive. Therefore, lower PVLs in PBMCs of patients with rapid disease progression may be attributed to the strong killing ability of the CTL. However, PVLs were higher in PBMCs of patients with HAM/TSP than in asymptomatic carriers [6]. In addition, the killing ability of CTLs in patients with HAM/TSP does not differ from that in asymptomatic carriers [18]. Hence, strong immune responses may be associated with the disease course. The onset of disease may require other factors that lead to strong immune responses. A late onset may also be associated with alterations of the immune function in HTLV-1-infected patients. Indeed, an increased age has been associated with autoimmune disorders, such as myasthenia gravis and rheumatoid arthritis, and may be partly explained by immune intolerance and accumulation of autoantibodies in older individuals [19,20].

In conclusion, we demonstrated that patients with HAM/TSP aggregate in some families. Compared with sporadic cases, the age of onset was younger and rates of disease progression were slower among familial cases, whereas HTLV-1 PVLs did not differ between f-HAM/TSP and sporadic groups. The present data suggest that factors other than HTLV-1 PVLs contribute to the disease course of HAM/TSP. Our data also suggested strong immune responses in the spinal cord of HAM/TSP patients with rapid progression. Further studies on HTLV-1, immune response to HTLV-1 and genetic factor in patients with rapid progression might provide new insights into HAM/TSP pathogenesis.

## Acknowledgments

We would like to express the deepest appreciation to Yuka Komai for collecting information from patients and Dr. Moe Moe Aye for reading our manuscript.

## Author Contributions

Conceived and designed the experiments: HT SI OW. Performed the experiments: SN EM. Analyzed the data: SN EM. Contributed reagents/materials/analysis tools: SN EM TM RK. Wrote the paper: SN EM.

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J Infect Chemother 20 (2014) 387–389

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Case report

# Exacerbation of microcytic anemia associated with cessation of anti-retroviral therapy in an HIV-1-infected patient with beta thalassemia

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ARTICLE INFO

*Article history:*
Received 5 January 2014
Received in revised form
20 January 2014
Accepted 21 January 2014

*Keywords:*
HIV infection
Exacerbation of anemia
Cessation of ART
Beta thalassemia

ABSTRACT

We report a patient with Japanese minor β thalassemia and HIV-1 infection.

The patient showed prolonged anemia, which was originally attributed to chronic parvovirus B19 infection. Twelve years later, the patient presented with exacerbation of microcytic anemia following cessation of anti-retroviral therapy; the exacerbation resolved when anti-retroviral therapy was resumed. Sequencing of the β globin gene revealed heterozygosity for a four-nucleotides deletion at codon 41/42 and minor β thalassemia was confirmed.

Because HIV-1-infected patients frequently show anemia due to nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow, it is likely to overlook other causes of anemia.

Thalassemia should be considered in the differential diagnosis of anemia even in HIV-1 infected patients, when microcytic anemia without iron deficiency is observed.

Our case suggested that active HIV infection may have worsened β thalassemia, and early introduction of anti-retroviral therapy is beneficial for the recovery of anemia.



## 1. Introduction

HIV-1 infected patient frequently manifest anemia [1]. Anemia prior to anti-retroviral therapy (ART) is often caused by amebic or cytomegalovirus colitis, parvovirus B19 infection, and HIV-1 infection itself [2]. After anti-retroviral therapy, anemia is mainly due to ART therapy itself, especially using Zidovudine (ZDV or AZT), which resulting in macrocytic changes.

In this paper, we present an HIV-1-infected β-thalassemia patient who showed exacerbation of microcytic anemia along with the cessation of ART, and the anemia resolved when ART was resumed. Hemoglobinopathy should be considered in the differential diagnosis of anemia even in HIV-1 infected patient, especially where there is microcytic anemia without iron deficiency. Early re-introduction of anti-retroviral therapy is beneficial for the recovery of anemia in β-thalassemia patient with HIV infection.

## 2. Case report

In March 2000, the patient, in his early forties, was admitted to the Kyushu Medical Center with Pneumocystis pneumonia. Since two months before the admission, severe anemia had continued. The HIV-1 RNA copy number in the plasma was 90,000 copies/ml (Fig. 1) and CD4 positive T cell count was 70/μl. Acquired immune deficiency syndrome (AIDS) was diagnosed. At the time of admission (March 2000), the hemoglobin concentration [Hb] was 5.8 g/dl and mean corpuscular volume (MCV) was 72.4 fl. On April 7,

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http://dx.doi.org/10.1016/j.jiac.2014.01.008

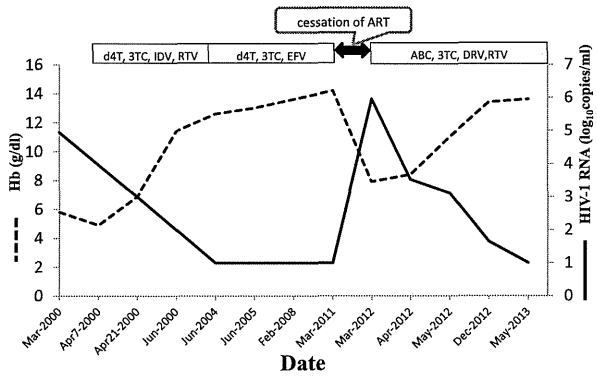


**Fig. 1.** Clinical course of Hemoglobin (Hb) and HIV RNA copy number along with the cessation of anti-retrovirus therapy (ART) and re-administration of ART. Correlation between HIV-1 viremia and exacerbation of anemia was observed. d4T sanilvudine, 3TC lamivudine, IDV indinavir, RTV ritonavir, EFV efavirenz, ABC abacavir, DRV darunavir.

following the administration of antibiotics, [Hb] declined to 4.7 g/dl without any hemorrhagic lesion, and the white blood cell (WBC) count declined to 900/μl. On April 13, bone marrow aspiration showed hypoplasia, and antiretroviral therapy [Sanilvudine (d4T), Lamivudine (3TC), Indinavir (IDV), and Ritonavir (RTV); later switched to d4T, 3TC, and Efavirenz (EFV)] was started on the same day. PCR of the bone marrow fluid revealed parvovirus B19 infection which suggested that the pancytopenia was caused by the bone marrow suppression due to antibiotics administration, or by HIV-1 infection itself, and parvovirus B19 infection-accelerated severe anemia. On April 21, HIV RNA was reduced to 1000 copies/ml and [Hb] was 7.6 g/dl. He discharged on May 2000 and his [Hb] continued to recover. On July 2000, his [Hb] was 12.5 g/dl: however, MCV was 82.7 fl and remained microcytic. The cause of continued anemia of the patient was attributed to chronic parvovirus B19 infection at that time and was reported elsewhere [3].

Five years later in June 2005, he attended Kagoshima University Hospital. At that time, the CD4 positive T cell count was 465/μl and the HIV-1 RNA copy number in the plasma was undetectable (<50 copies/ml). RBC was 5 million/μl, [Hb] was 13 g/dl, MCV was 79 fl and the reticulocyte count was 15‰. For three years he continued the same antiretroviral therapy (d4T, 3TC, EFV) during which the HIV-1 RNA copy number was always undetectable and the CD4 count ranged between 441 and 790/μl. At that time, his [Hb] level ranged between 11.4 and 13.9 g/dl and MCV ranged between 77 and 79 fl.

In Feb 2008, he moved to another prefecture. Four years later, in March 2012, he again visited Kagoshima University Hospital due to job re-relocation, and complained of easy fatigue. He had discontinued antiretroviral therapy of his own will eight months prior to this visit. His HIV-1 RNA copy number in the plasma was 920000 copies/ml, CD4 count was 101/μl, [Hb] was 7.9 g/dl, MCV was 64 fl and reticulocyte count was 19‰. He showed no evident opportunistic infection at this time. Four days later, antiretroviral therapy [3TC, Abacavir (ABC), Darunavir (DRV), RTV] was resumed. Ten days after re-administration of ART, his [Hb] was 7 g/dl, but 24 days after re-administration of ART (April 2012), his [Hb] increased to 8.4 g/dl and MCV was 68 fl, HIV-1 RNA copy number decreased to 3300 copies/ml and CD4 count recovered to 308/μl. In May 2012 (52 days after re-administration of ART), his [Hb] increased to 11 g/dl, HIV-1 RNA copy number decreased to 1300 copies/ml and CD4 count recovered to 422/μl. One year later in May 2013, his [Hb] increased to 13.6 g/dl along with the complete inhibition of HIV RNA copy number in the plasma (Fig. 1). The serum iron in March 2012 was 37 μg/dl (normal range 44–192) and UIBC was 161 μg/dl (normal range 111–255). However, 24 days after re-administration of ART, serum iron was 92 μg/dl and UIBC was 112 μg/dl without iron administration, which suggested his microcytic anemia was not from iron deficiency. Because the [Hb] in March 2012 was so low with microcytic change and there was no hemorrhagic lesion or opportunistic infection, another reason for the anemia was suspected.

Target cells were observed in the peripheral blood (Fig. 2). Hemoglobin analysis revealed a HbA2 of 9% and HbF of 4%, which suggested the existence of a hemoglobinopathy. Further tests for hemoglobinopathies showed a prolongation of the glycerol lysis time (107 s, compared to the normal control of 22–55sec) which implies elevated osmotic resistance. Finally, DNA sequencing revealed heterozygosity in the β globin gene, with the deletion of 4 nucleotides at codon 41/42 (TTCTTT to TT) in one allele (Fig. 3), and β-thalassemia minor was diagnosed.

## 3. Discussion

Anemia is a common clinical finding in HIV-1-infected patients. Many factors may contribute to the development of anemia in HIV-1-infected patients including nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow [2].

Our case showed severe anemia ([Hb] 5.8 g/dl) when AIDS was first diagnosed, when he had a high HIV-1 RNA in the plasma. The patient's anemia improved after anti-retroviral therapy, but mild anemia continued. At this time the anemia was attributed to chronic parvovirus B19 infection [3,4]. However, even after the recovery of the CD4+ cell count, mild anemia with microcytic change continued for years. Because his anemia was mild ([Hb] 13 g/dl), it was not investigated further at that time. Twelve years later, when he ceased ART, he again showed moderate microcytic anemia (Hb 7.9 g/dl), and this anemia resolved when ART was resumed. Because there was no hemorrhagic lesion, or opportunistic infection, we sought another cause of anemia. First, the hemoglobin fraction was measured, and both Hb-A2 and Hb-F were elevated, suggesting a hemoglobinopathy. Finally, sequencing of the β globin gene revealed a four-nucleotide deletion at codon 41/42 in one allele of the β globin gene, leading to the diagnosis of β thalassemia minor.

Even in non-thalassemic HIV-1 carriers, higher values of Hb-A2 have been observed during ART, especially with Zidovudine (ZDV) [5–7]: that is increased HbA2 alone is not a sufficient reason to suspect thalassemia in HIV-1 patients receiving ART. However, treatment with anti-retroviral drugs such as ZDV often results in

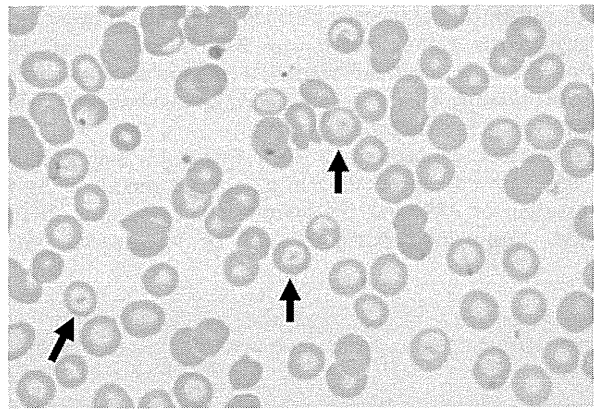

**Fig. 2.** Target cells were observed in the blood film (arrows).

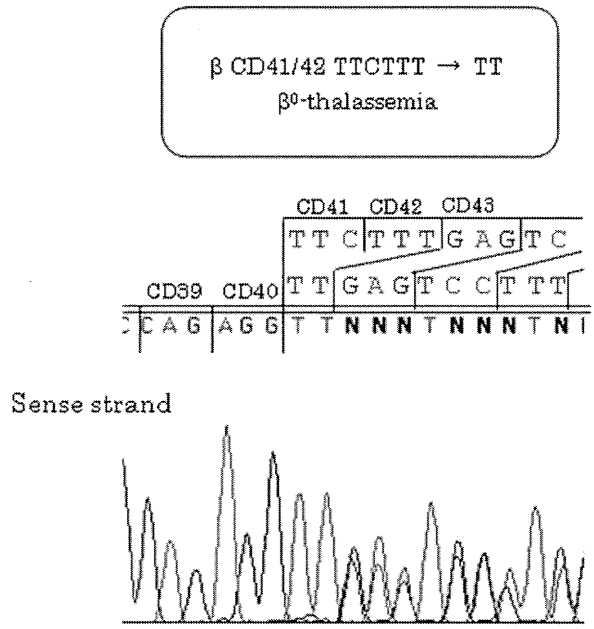


**Fig. 3.** DNA sequencing revealed four-nucleotide deletion at codon 41/42 in one allele of the β-globin gene.

macrocytosis [8]. It has also been reported that mean corpuscular volume (MCV) of HIV-1 patients with thalassemia after ART increased from microcytic levels to normocytic levels, and ART did not worsen anemia in patients with thalassemia [9,10]. Therefore, HIV-1-infected patients with non-iron-deficient microcytic anemia, in whom a hemoglobinopathy is suspected from abnormal hemoglobin fractions should be subjected to gene analysis to make a concrete diagnosis of thalassemia.

Thalassemia is relatively rare in Japan, where malaria is uncommon. The frequency of β-thalassemia in Japan is one in 600 to 1000 of the general population [11].

Most β-thalassemia patients in Japan are heterozygote and present with thalassemia minor. They are prone to be misdiagnosed as having iron deficiency anemia.

The four-nucleotide deletion at codon 41/42 in β globin gene found in this patient is the fourth most frequent mutation found in Japanese β thalassemia patients [11]. It is not known whether active HIV-1-infection (i.e. not controlled by ART) exacerbates all types of β thalassemia, but in the present case there was a strong temporal association between exacerbation of microcytic anemia when HIV-1 infection worsened following cessation of ART, and resolution of the anemia when ART was resumed (Fig. 1). Although most β-thalassemia in Japanese is heterozygous and shows no overt hemolysis but mild anemia with macrocytosis, it is reported that some of the mutant including four-nucleotide deletion at codon 41/42 observed in our case occasionally do have acute exacerbation by acquired factors such as pregnancy and infection [11].

Effect of HIV replication on erythropoiesis is not well understood. The pathogenesis of anemia in HIV-positive patients could be multifactorial [2]. Dysfunction of erythroid differentiation related to bone marrow (BM) microenvironment damage and stromal cell impairment by HIV-1 infection is reported [12]. It is also reported that IL-1β, IFN-γ, TGFβ1 and TNFα, which are elevated in BM as a result of chronic inflammation that may be associated with HIV-1 viremia, suppress the growth of progenitor cell *in vitro* and may play an important role in the induction of HIV-associated anemia [13]. Moreover, unbalanced hemoglobin chain synthesis during HIV-1 infection has been reported [14]. These multiple factors may be involved in the temporal correlation between HIV-1 viremia and exacerbation of microcytic anemia observed in the present case.

In conclusion, thalassemia should be considered in the differential diagnosis in an HIV-1-infected patient who presents with microcytic anemia without iron deficiency.

And early introduction of anti-retroviral therapy is beneficial for the recovery of anemia in β thalassemia.

## Conflict of interest

The authors declare that there have no conflict of interest.

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＜レクチャーシリーズ　06-1＞　診療に役立つ遺伝性ニューロパチーの話

# Charcot-Marie-Tooth 病の診療ポイント

中川　正法[1)]

要旨：Charcot-Marie-Tooth 病（CMT）はもっとも頻度の高い遺伝性ニューロパチーあり，その有病率は約 10 人 / 人口 10 万人と推定される．次世代シークエンサーによる解析で原因遺伝子が 50 種類以上同定されている．CMT の遺伝子診断は，予後予測，治療研究への展開などで重要である．外科的治療，リハビリテーション，装具療法，日常生活上の工夫が機能維持・改善に有用である．CMT の根治的な治療法の開発は不十分であるが，新たな治療の試みが始まっている．CMT 患者会と協力した研究班が組織されている．CMT の診療においては，他の希少性神経難病と同様に患者の訴えに真摯に耳を傾けることが必須である．

（臨床神経 2014;54:950-952）
Key words：シャルコー・マリー・トゥース病，エクソームシークエンス，臨床病型，治療

## はじめに

CMT はもっとも頻度の高い遺伝性ニューロパチーであり，世界の患者数は約 260 万人と推定され，わが国でも 10.8 人 / 人口 10 万人との報告がある[1)]．次世代シークエンサーによるエクソームシークエンスにより，CMT 関連の原因遺伝子は 50 種類以上が特定されている（http://www.molgen.ua.ac.be/CMTMutations）[2)]．CMT の根治的な治療法の開発は不十分であるが，新たな治療の試みが始まっている．しかし，CMT の遺伝子診断，治療法開発，リハビリテーションなどの情報が医療関係者，CMT 患者に十分に普及しているとはいいがたい現状である．CMT の診療においては，他の希少性神経難病と同様に患者の訴えに真摯に耳を傾けることが必須である．

## CMT の臨床症状

CMT は，一般的に四肢，とくに下肢遠位部の筋力低下と感覚障害を示す緩徐進行性の遺伝子異常による末梢神経疾患の総称である．発症年齢は，若年発症（0～20 歳）と中年期発症 の二相性分布を示す．軽症の高齢 CMT 患者を見逃さないように注意が必要である．典型的には，凹足（時に扁平足），ハンマー趾，足関節の変形，歩行・走行困難，たれ足・鶏歩，筋萎縮・筋力低下，下肢優位の感覚障害，腱反射の消失，手指振戦，筋けいれん，疼痛，下肢皮膚温低下（cold feet），先端チアノーゼを示す．非典型的症状として，脳神経障害，声帯麻痺，緑内障，視神経乳頭萎縮，錐体路障害，上肢優位障害，感覚または運動神経優位障害，近位筋優位障害などを示す例もある[3)]．

CMT は正中神経の運動神経伝導速度（MNCV）を基準に，脱髄型（CMT1/CMT4），軸索型（CMT2），中間型（intermediate-CMT）に大別される．脱髄型 CMT では，一般的に正中神経の MNCV は 38 m/s 以下，活動電位はほぼ正常または軽度低下を示し，腓腹神経所見では節性脱髄，onion bulb の形成をみとめる．軸索型 CMT では，MNCV は正常または軽度低下を示すが活動電位は明らかに低下し，腓腹神経所見では有髄線維の著明な減少を示す．しかし，いずれとも分けられない intermediate-CMT も存在する．CMT の臨床的重症度は，その原因遺伝子の局在にかかわらず，軸索障害の進行に相関しており，早期診断，早期治療が予後に大きく影響する．CMT の早期診断，早期治療を考えるばあい，着床前診断，発症前診断などの遺伝子診断の倫理的問題は避けられない．CMT 患者を対象とした自己記入式アンケート調査では，アンケート回答総数 131 名（3 歳～81 歳，中央値 52 歳，男：女＝ 71：60）中，遺伝子検査を受けている患者は，31.3％に止まっており[4)]，適切な遺伝カウンセリングを踏まえた遺伝子診断を進める必要がある．CMT の遺伝子診断は，疾患の予後，合併症，治療研究への展開などを検討する上で重要である．

CMT と慢性炎症性脱髄性多発ニューロパチー（CIDP）との合併例の検討から，CMT 患者 250 人に 1 人が CIDP 様の炎症性ニューロパチーを発症すると推定されている．CMT 患者で臨床症状の急性悪化をみとめたばあいには，CIDP 様の炎症性ニューロパチーの治療法に準じた対応を考慮してもよいと考える[5)]．

## 診断と鑑別診断

CMT の診断は，問診，神経学的診察，電気生理学的検査，神経超音波検査，家系調査，遺伝子検査でおこなわれる．CMT がうたがわれたばあいには，神経伝導検査をおこない，必要に応じて，針筋電図検査，神経超音波検査，腓腹神経生検を

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（受付日：2014 年 5 月 23 日）

Table 1　CMT の診断において注意すべき鑑別疾患.

- ●慢性炎症性脱髄性多発根神経炎（CIDP）
- ●薬剤性ニューロパチー：抗癌化学療法，HIV 薬など
- ●中毒性ニューロパチー：鉛，シンナー
- ●代謝性ニューロパチー：糖尿病，尿毒症，アルコールなど
- ●虚血性ニューロパチー：血管炎，拘やく性
- ●自己免疫性ニューロパチー：anti-MAG neuropathy, paraproteinaemic neuropathy
- ●他の遺伝性ニューロパチー：家族性アミロイドニューロパチー，HNPP
- ●係留脊髄症候群（Tethered cord syndrome）
- ●脊髄小脳変性症：Friedreich 失調症
- ●遠位性ミオパチー・先天性ミオパチー
- ●脊髄空洞症，運動ニューロン疾患，脊髄性筋萎縮症，白質ジストロフィー

MAG; myelin-associated glycoprotein, HNPP; henditory neuropathy with liability to pressure palsies.

おこなう．さらに，遺伝子検査にて確定診断となる．PMP22 の FISH 法検査は健康保険が適用される．鹿児島大学神経内科などで CMT 遺伝子診断がおこなわれている（cmtdiag@m3.kufm.kagoshima-u.ac.jp）．CMT の約半数は PMP22 重複による CMT1A と考えられている．同一遺伝子の異常であっても，ことなる臨床型・重症度を示す遺伝的多様性がある．

鑑別すべき疾患として，CIDP，抗 MAG 抗体をともなうニューロパチー，薬剤性ニューロパチー，虚血性ニューロパチー，POEMS 症候群，ビタミン B1 欠乏ニューロパチー，アルコール性多発ニューロパチー，アミロイドーシス，脊髄小脳変性症にともなうニューロパチー，傍腫瘍症候群，Refsum 病，異染性白質ジストロフィー，Krabbe 病，Tangier 病，遠位型ミオパチー，先天性ミオパチー，筋萎縮性側索硬化症，係留脊髄症候群などがある（Table 1）．CMT に特異的な血液検査所見はないが，前述の疾患との鑑別に重要である．脱髄型 CMT では，髄液蛋白の上昇や脊髄 MRI で神経根の肥厚をみとめることがある．

## 治療

CMT に対する薬物治療として，アスコルビン酸，neurotrophin-3，クルクミン，PXT3003 に関する報告がある．アスコルビン酸は PMP22mRNA 発現量を低下させる可能性がある．CMT1A に対するアスコルビン酸の無作為化比較対照試験（randomized controlled trial; RCT）またはオープン臨床試験が国内外でおこなわれたが，いずれの研究でもアスコルビン酸の有効性は確認できなかった[6]．クルクミンは秋ウコンやカレー粉に多くふくまれている自然の黄色色素である．クルクミンが CMT1A や CMT1B のモデル動物に対して有効であるとの報告がある[7]．Pharnext 社が CMT1A 80 例を対象に PXT3003（バクロフェン，ナルトレキソン，ソルビトールの合剤）の RCT を 2010～2012 年末にかけておこなった．PXT3003 は，CMT1A ラットの PMP22 の発現を抑制し，第 2 相臨床試験でも安全性と有効性が示された[8]．

## 投与注意が必要な薬物

CMT 患者が他の内科疾患などに罹患したばあい，必要に応じて使用される薬剤が末梢神経障害を悪化させるばあいがある．とくに抗腫瘍薬であるビンクリスチン・シスプラチン・タキソール・サリドマイド・ベルケードなどが CMT の症状を悪化させる可能性のある薬剤として有名である（http://www.charcot-marie-tooth.org/med_alert.php）．CMT の臨床症状を示さない潜在的な CMT 患者がいる可能性があり，抗腫瘍薬（ビンクリスチンなど）投与前の神経伝導検査の実施は，末梢神経障害の重症化を防ぐ点で可能なかぎり推奨される．

## 外科的治療，リハビリテーション，装具療法

関節変形が進行し，装具をもちいても足を適切な位置に保てず歩行に支障が出てきたばあい，関節の安定性を図るために筋延長術や骨切り術などの整形外科手術が適応となるばあいがある．CMT 33 例の凹足に対する骨切り術と軟部組織術の短期～中期の手術成績の後方視的検討では，疼痛，歩行障害が有意に改善し，90％の患者が足変形の矯正に満足していたとの報告がある[9]．内反尖足の外科治療は CMT 患者により安定した歩行をもたらすと考えられるが，その手術適応や外科的治療施行時期についてのより明確な基準が必要である．CMT 患者が手術や出産などのために麻酔を受ける際には，嚥下反射の減弱・声帯麻痺・胸鎖乳突筋の筋力低下，自律神経障害による不整脈・低血圧，側彎症による拘束性換気障害，悪性高熱症，術後呼吸不全などの合併に注意すべきである．

「過労による筋力低下 overwork weakness」については論議が多い．CMT の症状が軽症である例では利き手の握力とピンチ力が非利き手より強い傾向があるが，重症例では，利き手のピンチ力が非利き手よりも有意に低下していると報告されている．CMT の関節可動域制限の予防のために，発症早期から下腿三頭筋の持続伸張訓練をおこなう必要がある．日々の生活に運動療法を組み込むことで，疾患の自然経過による進行以上の悪化をおさえる効果が期待できる．

装具使用においては，機能障害にあった装具を使用目的と使用時間帯を明確にして，装着することが大切である．短下肢装具の使用，足関節の夜間固定などが有効との報告がある[10]．厚労省難治性疾患克服研究事業として，下肢装着型補助ロボット（HAL-HN01）に関する 医師主導治験（代表者　中島孝先生）が CMT をふくむ希少性難治性神経疾患に対して進行中である．

## CMT の経過

CMT の経過は原因となっている遺伝子異常によってことなるが，厚生労働科学研究費補助金難治性疾患克服研究事業

CMT研究班（CMT研究班，http://www.cmt-japan.com/index.html）の調査では，短下肢装具使用31.4%，長下肢装具使用1.3%，車椅子使用12.6%，気管切開1.0%，補助呼吸1.1%であった[4]．CMTに対する有効な薬物療法は未だ開発されていないが，少しでもよい健康状態を維持することは重要である．手足のケアによる四肢遠位の冷感・浮腫・外傷・胼胝・潰瘍形成の予防，深部静脈血栓症とそれに関連する肺塞栓症の注意なども重要である．CMT患者は消費カロリー/日が健常者より有意に少なく，メタボリック症候群が多い傾向がみられる．日常的な運動習慣と食事療法により，「現在の体重を維持する」ことが肝要である．

## CMT関連する研究班

CMT患者会（http://j-cmt.org/）と協力して，厚労科研費補助金「シャルコー・マリー・トゥース病の診断・治療・ケアに関する研究」班（班長　中川正法）などがあり，これまでにCMT市民公開講座，シャルコー・マリー・トゥース病診療マニュアルの作成などの啓発活動をおこなっている．文部科学省　再生医療実現化プロジェクト「疾患特異的iPS細胞を活用した難病研究」拠点である京都大学iPS細胞研究所との共同研究が進行中である．

**謝辞**：厚生労働科学研究費補助金「希少難治性神経疾患の疫学，病態解明，診断・治療法の開発に関する研究」班，「次世代遺伝子解析技術をもちいた希少難治性疾患の原因究明および病態解明に関する研究」班（班長　高嶋　博先生），アンケート調査にご協力いただいたCMT患者の皆様に深謝いたします．

本研究は，厚生労働科学研究費補助金「希少難治性神経疾患の疫学，病態解明，診断・治療法の開発に関する研究」班によっておこなわれた．

※本論文に関連し，開示すべきCOI状態にある企業，組織，団体はいずれも有りません．

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**Abstract**

## Diagnosis and care of Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth disease (CMT) is the most common form of inherited peripheral neuropathy and the prevalence rate is about 10/100,000 population in Japan. Next-generation sequencing techniques discovered more than 50 genes for CMT. Genetic diagnosis of CMT with genetic counseling is critical to speculate the prognosis, complications and therapeutic research development. Surgical therapy, rehabilitation, casting therapy, and weight control are useful to keep health-related quality of life for CMT. Although the disease-modifying therapy for CMT is not available now, new clinical trials to improve QOL are now on going. CMT research groups, which consist of researchers and CMT patient association, are organized in Japan with support of Grants-in-Aid from the Ministry of Health, Labour and Welfare of Japan. It is the most important to listen to what afflicted patients may want to share with medical doctors.

(Clin Neurol 2014;54:950-952)

**Key words:** Charcot-Marie-Tooth disease, exome sequencing, clinical phenotype, therapy


RESEARCH PAPER

# Spreading of amyotrophic lateral sclerosis lesions—multifocal hits and local propagation?

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2013-305617).

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Received 16 April 2013
Revised 27 June 2013
Accepted 17 July 2013
Published Online First 11 September 2013

## ABSTRACT

**Objective** To investigate whether or not the lesions in sporadic amyotrophic lateral sclerosis (ALS) originate from a single focal onset site and spread contiguously by prion-like cell-to-cell propagation in the rostrocaudal direction along the spinal cord, as has been hypothesised (the 'single seed and simple propagation' hypothesis).

**Methods** Subjects included 36 patients with sporadic ALS and initial symptoms in the bulbar, respiratory or upper limb regions. Abnormal spontaneous activities in needle electromyography (nEMG)—that is, fibrillation potentials, positive sharp waves (Fib/PSWs) or fasciculation potentials (FPs)—were compared among the unilateral muscles innervated by different spinal segments, especially between the T10 and L5 paraspinal muscles, and between the vastus medialis and biceps femoris. Axon length and the proportion of muscle fibre types, which are both related to motoneuronal vulnerability in ALS, are similar in the paired muscles.

**Results** Fourteen of 36 patients showed a non-contiguous distribution of nEMG abnormalities from the onset site, with skipping of intermediate segments. In eight of them, the non-contiguous pattern was evident between paired muscles with the same motoneuronal vulnerability. The non-contiguously affected lumbosacral lesions involved motoneuron columns horizontally or radially proximate to one another, appearing to form a cluster in four of the eight patients. FPs, known to precede Fib/PSWs, were shown more frequently than Fib/PSWs in all the lumbosacral segments but L5, suggesting that 2nd hits occur at L5 and then spread to other lumbosacral segments.

**Conclusions** In sporadic ALS, the distribution of lower motoneuron involvement cannot be explained by the 'single seed and simple propagation' hypothesis alone. We propose a 'multifocal hits and local propagation' hypothesis instead.

## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an incurable progressive neurodegenerative disease in which both the upper (UMN) and lower motoneurons (LMN) are diffusely involved at the end. Recent biological studies have demonstrated the remarkable concept of 'prion-like propagation' of pathogenic proteins, such as tau or α-synuclein, in neurodegenerative diseases.[1 2] According to this hypothesis, the pathogenic proteins are transferred from diseased cells to neighbouring healthy cells; this intercellular transfer then leads to spreading of the lesion. In ALS, in vitro studies have indicated that newly formed aggregates of SOD1, TDP-43 or toxic RNA conformation can act as templates for the subsequent misfolding of the respective native proteins,[3–5] and that aggregated SOD1 can be intercellularly transferred in cultured cells.[6] These suggest that the mechanism of prion-like cell-to-cell propagation also underlies the progression of ALS.

The clinical symptoms of most ALS patients start focally, which had already been confirmed both electrophysiologically[7] and pathologically.[8 9] As we have reviewed in the previous article,[10] recent clinical observations have demonstrated that the clinical symptoms spread contiguously from the onsets into the following broadly divided body regions: the bulbar region, upper limbs, trunk and lower limbs.[11–14] This has prompted us to suppose that ALS lesions simply propagate from a single 'seed' to adjacent cells in a domino-like manner (ie, the 'single seed and simple propagation' hypothesis). Alternatively, it can rest on anatomical proximity with the spreading of ALS lesion from the onset site by diffusion of soluble toxic factors in the extracellular matrix.[15] On the other hand, up to about 30% of sporadic ALS patients have also been found to show non-contiguous spread of symptoms from the bulbar region to the lower limbs or vice versa, skipping the upper limbs and trunk.[14 16] However, compensatory re-innervation by the remaining motoneurons can mask the manifestation of clinical signs until more than one-third of the LMNs for a given muscle are lost.[17] Therefore, whether the lesions actually spread non-contiguously among the spinal segments remains unclear.

Needle electromyography (nEMG) can sensitively detect LMN involvement from each segment separately, even in the presymptomatic stage. For this reason, it is a powerful method for investigating whether or not ALS lesions spread contiguously along the spinal segments. In this study, we used nEMG in the early stage of ALS to demonstrate that LMN involvement cannot be necessarily explained by the 'single seed and simple propagation' hypothesis. We then propose a hypothesis of 'multifocal hits and local propagation.'

**To cite:** Sekiguchi T, Kanouchi T, Shibuya K, *et al*. *J Neurol Neurosurg Psychiatry* 2014;**85**:85–91.