60% of the awake-time, we arbitrary classified the days into two categories based on this proportion; when more than 60% of the awake-time was scored as ≥3, the day was defined as a "good condition" (GC) day, and when less than 60% of the awake-time was ≥3, the day was defined as a "bad condition" (BC) day. Six of the PD patients, whose diagnoses included MRI findings, and who had not received any antiparkinsonism drugs wore the ECOLOG for more than 6 consecutive days both before the initiation of medication and after the stabilization of medication effects (Pt. 1, 2, 6, 7, 17, 18). The "after" study was conducted when the dose of the medication (2–3 mg of cabergoline or 0.45–0.75 mg of pergolide) was stable for more than 3 weeks in each patient.

We separated the data acquired during awake-time and sleep-time with Action-W, Version 2 (Ambulatory Monitors, Ardsley, NY) and the data during awake-time were used for analyses. To examine temporal autocorrelation of the physical activity time series (i.e., dynamic aspects of physical activity) we used an extended, random-walk analysis, the detrended fluctuation analysis (DFA),[13] with a recent modification[12] for various "real-world" signals including activity time series. The original DFA evaluates relationships between time scales and magnitudes of fluctuation (standard deviations) within each time scale; more correlated signals represent a greater growth of the fluctuation magnitude with increasing time scale or length of data window. It also eliminates nonstationarity in the input data (i.e., changes in the baseline and trends within the data windows at different scales) that could affect calculation of the magnitudes of fluctuation, thus making this approach suitable for the analysis of the long-term data collected in the present study. The power-law (scaling) exponent (α), obtained as the slope of a straight line fit in the double-logarithmic plot of time scales versus magnitudes of fluctuation, was used to characterize the level of such correlation. This index reflects the probability of a simultaneous increase or decrease in the variability at two distant points in time in the time series, applied to all distances up to long-range time scales, thereby probing the nature of "switching" patterns between high and low values in a statistical sense. Larger power-law exponents indicate positive temporal autocorrelation or persistency in the increase or decrease, and lower values correspond to negative autocorrelation or antipersistency.

Recently, Ohashi et al. reported that physical activity data have different power-law exponents in periods with higher and lower activity levels, corresponding to qualitatively different physiological states, (i.e., active and rest, respectively).[12] The actual procedures we used are as follows: (see Ohashi et al. for details).[12] First, a daytime physical activity time series was integrated, as in DFA, and wavelets with different time scales (S) were slid along the time series and correlated with the data to obtain the wavelet coefficients ($W(S)$) at each point. We used the third derivative of the Gaussian function as the so-called "mother wavelet":

$$\psi(t) \quad t(3 \quad t^2)e^{-0.5t^2},$$

where t is time. This is equivalent to using the Gaussian second derivative (so-called "Mexican hat") wavelet to examine the raw signals (Fig. 1), though the integration approach automatically removes the local mean and the local linear trend, as in DFA. By changing the scale of the wavelet, this "hat-shaped" template dilates or contracts in time, probing transient increases or decreases in activity records in different time scales. The transient increases (low-high-low activity patterns) yield local maxima of the wavelet coefficients at their time points, while the decreases (high-low-high activity patterns) yield local minima of the wavelet coefficients (Fig. 1). Next, the squared wavelet coefficients at the local maxima or minima were averaged for all the available days. As the coefficient gives the magnitude of local fluctuations matching the shape of $\psi(t)$ with different time scales, the squared $W(S)$ was used, again as in DFA. Finally, the power-law exponent (α) was obtained separately for local maxima and minima as the slope of a

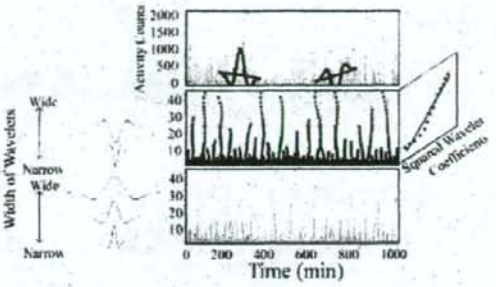

FIG. 1. Conceptual explanation of the method to obtain power-law exponents for local maxima and minima. (top) Various widths of hat-shaped wavelets are slid along the data to detect local minima (middle) and local maxima (bottom) of the wavelet coefficients. Note that the local minima and maxima appear at the transient decreases and increases of the activity, respectively. The power-law exponents are calculated from the slope of the log-log plot of squared wavelet coefficients versus the scale for local minima and maxima. In the actual analysis, we used an integrated, rather than raw, time series and $\psi(t)$, i.e., the derivative of the "hat-shaped" wavelet. This yields the same power-law exponents as those obtained by the DFA method for the same local maxima and minima as obtained in this figure (see Methods for details).

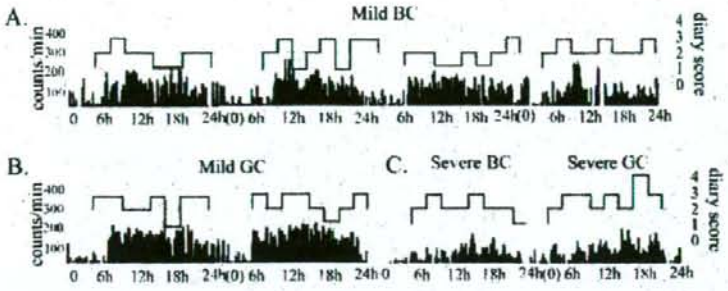

FIG. 2. Examples of daily activity profiles and the corresponding subjective, diary-based scoring on days in different conditions in patients with different disease severities. BC, bad condition day; GC, good condition day.

straight line fit in the double-logarithmic plot of S versus $W(S)^2$. In this study, the range of S corresponding to 8 to 35 minutes, where acceptable linear relationships between log S and log $W(S)^2$ were observed for all the records, was used. This range is also approximately the same as that used in Ohashi et al.[12] Note that this method yields the same α-values as does DFA,[20] but separately for periods with higher and lower activity levels. The power-law exponent α's of local maxima and minima were used to assess the quantitative disabilities during awake-time and the differences in disabilities between GC days and BC days, between before and after antiparkinsonism medication in individuals, between the severe and mild groups, and between groups with and without tremor. Records during 6 consecutive days were used in the analysis.

Wilcoxon signed rank tests were performed to compare α-values for local maxima or minima in the various group comparisons. P values < 0.05 were considered statistically significant.

RESULTS

The daily profile of physical activity exhibited robust activity-rest cycles but no apparent correspondence between daily activity profiles and diary scores (the mean activity counts vs. diary score: $r = -0.063$) (Fig. 2).

Average wavelet coefficients for local maxima and minima of the severe and mild groups provided straight lines in the range of 8 to 35 minutes (Fig. 3A), indicative of very robust α-values. When the mean α-values for local maxima and minima were compared, we found a significantly lower α-value for local maxima in the mild group than in the severe group (Fig. 3B). All the patients in both the severe and mild groups showed significantly lower α-values for local maxima on GC days than on BC days, whereas there was no significant difference in the mean α-values for local minima (Fig. 3C). When the effects of medication were examined, we found that all the patients showed lower α-values for local maxima, but not for

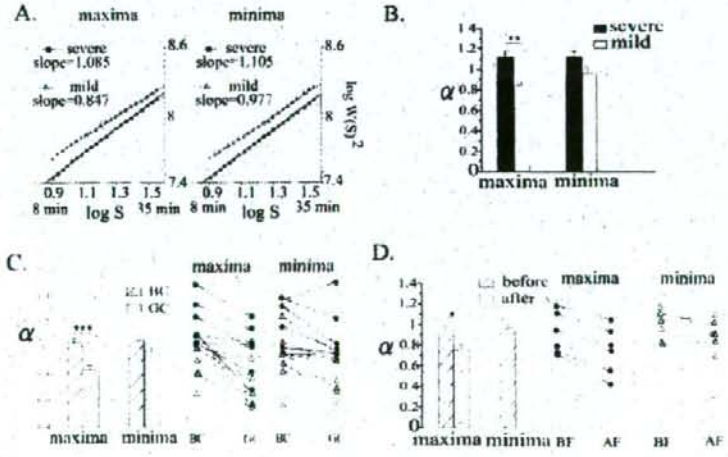

FIG. 3. Local maxima and minima of fluctuation of physical activity. (A) Average wavelet coefficients, as a function of the wavelet scale, for local maxima and minima. The slopes are power-law exponents, α. (B) Comparisons of the mean α for the severe and the mild groups, (C) for BC and GC days and for individual patients, and (D) for days before and after antiparkinsonism medication and for each patient. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

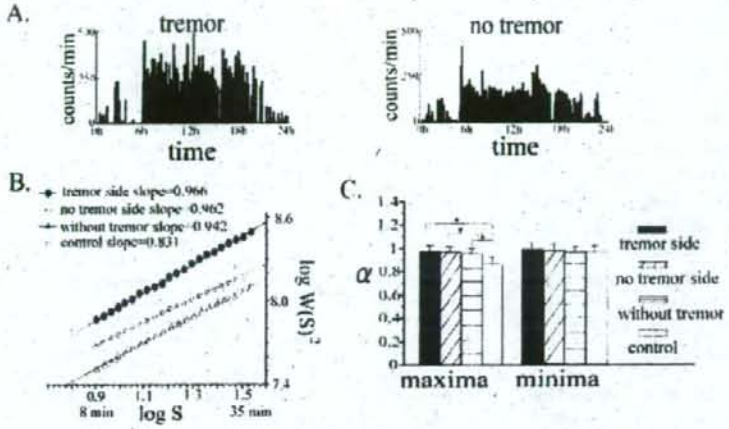

FIG. 4. Effects of tremor. (A) Daily profiles of physical activity for the arm affected with tremor and that without tremor of a patient with unilaterally predominant parkinsonism with continuous tremor on one side. (B) Average wavelet coefficients for local maxima among arms with tremor (tremor) and without tremor (no tremor). Arms of patients with tremor (n = 6), arms of patients without tremor (without tremor: n = 13), and control subjects (control: n = 6). (C) The power-law exponents for local maxima and minima. $^{*}P < 0.05$.

local minima, on days after they received antiparkinsonism medication than on those before (Fig. 3D).

We compared the activity records from the arms with tremor and without tremor from 6 patients with tremor, and arms of patients not affected with tremor (n = 13). The activity counts in the arms with tremor were significantly higher than those in the arms without tremor (Fig. 4A). Power-law scaling of the records from arms with tremor showed a linear correlation between log S and log $W(S)^2$ in the range of 8 to 35 minutes (Fig. 4B) and α-values for local maxima but not for minima were significantly higher in patient arms than in control arms irrespective of tremor (Fig. 4C).

DISCUSSION

We demonstrated that analysis of records of a custom actigraph by the power-law temporal correlation is a powerful tool for the quantitative evaluation of physical activity in patients with parkinsonism. The diary-based subjective scoring of good or bad conditions was apparently not correlated with the objective daily profiles of physical activity recorded by the accelerometer, indicating that the activity counts themselves do not represent the patient's condition.

Larger power-law exponents (α) indicate positive temporal autocorrelation, or persistency, in the increase or decrease in the variability of activity at two distant points in time in the time series, and lower values correspond to negative autocorrelation or anti-persistency.[12] In other words, a lower α for local maxima or minima of activity records reflects more frequent switching behavior from low to high or high to low physical activity, respectively, and the switching behavior from lower to higher activity levels is considered to be related to akinesia in patients with parkinsonism. We found lower α-values for local maxima during GC days than during BC days, in the mild group than in the severe group, and before medication than after medication. Thus, these results demonstrate that the power-law analyses accurately describe the well known phenomenon that under these conditions patients switch their physical activity from lower to higher levels more easily, in other words they exhibit milder akinesia, when the parkinsonism is mild than when it is severe. It is worthy to note that lower α-values for local maxima were obtained for all the patients after medication than before, and when in GC than in BC, thereby providing a temporal profile of parkinsonism in each individual patient.

We adopted Mode 13 of the ECOLOG to record the motion range during daily living. This is compatible with the same mode of the AMI Mini-Motionlogger and is said to filter out the majority of movements with frequencies outside the 2 to 3 Hz range. Although some resting tremor in the 4 to 8 Hz range, found in typical parkinsonism or in a part of the "true" movement accelerations resulting from muscle force[21] might have been filtered out of our recordings, we found higher activity counts during awake time on the arms with tremor, which erroneously indicated milder parkinsonism compared with the arms without tremor when judged from the level of activity counts. In contrast, the α-values for local maxima did not differ between the arms with tremor and those without tremor, but were significantly lower in both of the patient groups than in the control arms, indicating that although the presence of tremor

greatly influenced the actigraphic counts, the presence of tremor did not yield false positive results in the power law exponent for maxima.

In conclusion, we found that the power-law exponent for local maxima sensitively and reliably reflects disability without being influenced by the presence of tremor or the pattern of daily living. Our results thus suggest that analysis of power-law temporal autocorrelation of physical activity time series using the bidirectional extension[12] is applicable to patients with parkinsonism for the evaluation of akinesia irrespective of the presence of involuntary movements including tremor and may provide useful objective data necessary for the control of drug dosage in the outpatient clinic and also for the evaluation of new drugs for parkinsonism.

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INTERNAL MEDICINE

□ SHORT COMMUNICATION □

Gabapentin for Painful Legs and Moving Toes Syndrome

Hitoshi Aizawa

Key words: gabapentin, painful legs, moving toes syndrome

(DOI: 10.2169/internalmedicine.46.0416)

A 73-year-old woman presented with a 5-month history of continuous tingling pain in both feet. She had a past history of laminectomy at the level of the fourth lumbar vertebra due to the damage of lumbar intervertebral disc 7 years ago. Lumbar sympathetic blocks could calm the pain transiently. The patient had not an urge to move the legs or the toes. The symptoms did not change with rest or walking. On neurological examination, there were intermittent and irregular movements of the right toes, mainly of abduction and adduction. The patient could not suppress them voluntarily. There was no muscle atrophy, fasciculations nor myokymia. Muscle strength was normal. Tendon reflexes were normal except for ankle reflexes which were absent. Light touch, pinprick, cold sense and position sense were normal, however, vibration sense was slightly decreased at the distal lower extremities. Peroneal and posterior tibial motor nerve conductions were normal. Sural sensory nerve conduction was slightly decreased. MRI of the lumbar spine revealed no abnormality in the spinal cord or roots. Baclofen, clonazepam, carbamazepine and tricyclic antidepressants have been tried without success. Gabapentin (200 mg 3 times daily) was prescribed, with partial relief of the pain. Then, the pain could be controlled by 700 mg of gabapentin per day, however, movement of toes continued.

Discussion

Painful legs and moving toes syndrome may not be a homogenous entity (2, 3). At least two different physiopathologic mechanisms have been proposed: peripheral and central mechanisms (2, 3). Lesions in the posterior root ganglion, cauda equina, nerve roots, or a peripheral nerve can cause frequent impulses in afferent fibers which activate local circuits of interneuron and motoneurons resulting in local muscle movements. Pain and involuntary movement may also occur together in a central disorder (2, 3), although the precise mechanism is still under investigation.

A variety of medications, such as baclofen, benzodiazepines, tricyclic antidepressant, anticonvulsant, beta-blockers, and corticosteroids, have been tried in painful legs and moving toes syndrome previously, usually with disappointing results. Gabapentin was initially produced as an adjunctive antiepileptic drug, its indications now include diabetic neuropathy, postherpetic neuralgia, trigeminal neuralgia, migrain prophylaxis, bipolar disorder and anxiety disorders.

Gabapentin is structurally related to the neurotransmitter GABA, although it has no direct GABAergic action on GABA receptors. Gabapentin seems to enhance inhibitory input of GABA-mediated pathways. It has an inhibitory effect on voltage-dependent calcium ion channels at the postsynaptic dorsal horns and may interrupt the series of events leading to the neuropathic pain (4). Gabapentin has been clearly demonstrated to be effective for the treatment of neuropathic pain in diabetic neuropathy and postherpetic neuralgia (4). Therefore, gabapentin should be considered an important drug in the management of other neuropathic pain syndromes such as painful legs and moving toes syndrome, although there is only one previously reported case successfully treated with gabapentin (5).

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INTERNAL MEDICINE

□ CASE REPORT □

An Adult Case of Relapsing Human Herpesvirus-6 Encephalitis

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Abstract

Human herpesvirus-6 (HHV-6) is the main etiologic agent of exanthema subitum in young children. Central nervous system (CNS) infections in children due to HHV-6 have been described on many occasions. HHV-6 is also a common cause of infections in immunocompromised individuals. However, little is known concerning the impact of HHV-6 on the CNS in immunocompetent adults. We report the first case of relapsing HHV-6 encephalitis in a healthy 73-year-old female.

Key words: human herpesvirus-6 (HHV-6), relapse, encephalitis, immunocompetent

(DOI: 10.2169/internalmedicine.46.0239)

Introduction

Human herpes virus-6 (HHV-6) was first isolated in 1986 from patients with lymphoproliferative disorders or HIV infection, and it has been identified as the causal agent of exanthem subitum in young children (1, 2). Central nervous system (CNS) disorders such as febrile seizures, meningoencephalitis, and encephalopathy may complicate the course of HHV-6 primary infection in children (3, 4). HHV-6 is also a common cause of infections in immunocompromised individuals (4). However, CNS infection induced by HHV-6 is very rare in immunocompetent adults, and the pathogenesis of these cases remains unclear (5-14).

Here, we report the first case of relapsing HHV-6 encephalitis in an immunocompetent 73-year-old female.

Case Report

A 70-year-old healthy female was admitted to our hospital with disorientation after tonic-clonic seizures in June 2002. She had been well until one month earlier, when she began to feel general fatigue. She had no anamnesis and had never gone abroad. Neurological examinations revealed disturbance of consciousness, right Chaddock sign, and frontal sign. Blood cell counts were normal and blood serum and chemistry showed mild liver dysfunction and elevation of C reactive protein (CRP). Analysis of cerebrospinal fluid (CSF) showed a protein increase (85 mg/dl) with a normal leukocyte count (3 cells/mm^3). Magnetic resonance imaging (MRI) performed in early July revealed high intensity lesions at the bilateral basal ganglia on a T2-weighted image and a fluid attenuated inversion recovery image (Fig. 1A). Oral antiepileptic drugs (phenytoin: 30 mg and phenobarbital: 100 mg per day respectively) were started after admission. But drug-induced skin eruptions appeared on her hip the next day, and the rashes extended to her trunk, limbs and face with high fever on the following day. Therefore the treatment of phenytoin and phenobarbital was discontinued, and oral prednisolone (30 mg per day) was administered from the same day. Improvement of the skin rashes and fever were seen from mid-July, and the eruption was disappeared by the end of July. Her consciousness improved gradually, and she left the hospital two months after admission with mild cognitive impairment. HHV-6 IgG antibody titer of the serum which was collected three days after admission, was elevated to 80 -fold (reference value is less than 10 -fold), and decreased to 10 -fold early in July, while the serum IgM antibody was not elevated. The abnormalities on MR images disappeared by April 2003.

In January 2005, she complained of general fatigue. She became disorientated with a slight fever of 37.8℃ in early February and was readmitted to our hospital. Neurological examinations revealed disturbance of consciousness and bi-

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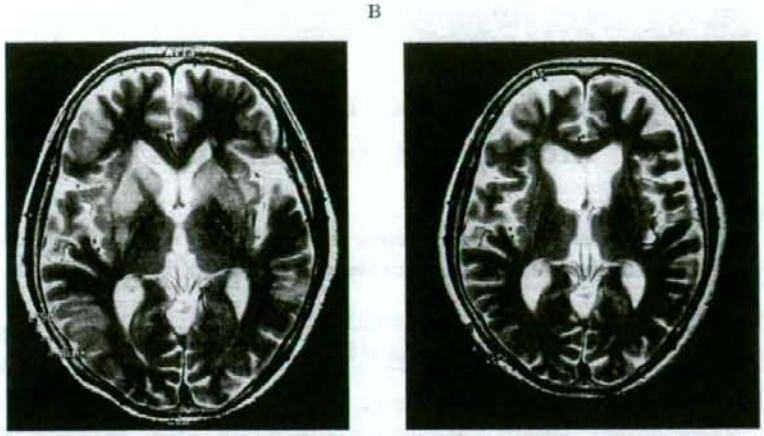

Figure 1. **MRI findings on T2-weighted image of this patient. A: There were wide high intensity lesions at bilateral basal ganglia on the 19th day of the first hospitalization. B: There was no intracranial abnormal lesion including basal ganglia on the 20th day of the second hospitalization.**

lateral patellar hyperreflexia. Signs of meningeal irritation were not observed. Her blood cell count was normal, and blood serum and chemical studies showed neither liver nor renal dysfunction. CRP was not elevated. CSF analysis performed on the admission day revealed increase in leukocytes (32 monocytes/mm^3) and protein (102 mg/dl) with a normal glucose level. Bacterial, fungal, and mycobacterial cultures of the CSF were all negative. Human herpesvirus simplex (HSV) PCR was negative, and cytology showed no malignant cells. Electroencephalography showed a diffuse slowing, but no seizure discharge. Brain computed tomography performed in early February revealed no abnormal lesion. Because viral encephalitis was suspected from the clinical findings and CSF analysis, aciclovir (ACV) was started immediately, but her consciousness disturbance did not improve. Skin rashes appeared three days after admission, and corticosteroids were administered. She could speak after a few days, and skin eruptions disappeared. After three weeks, she could walk with support. Brain MRI findings at the end of February revealed no abnormal lesion apart from mild atrophy of bilateral frontal lobe (Fig. 1B). HHV-6 IgG antibody titer of the serum which had collected in mid-February was significantly elevated (maximum value: 320-fold), and decreased to 20-fold with improvement of consciousness by mid-March, while serum IgM antibody was not elevated. Neither anti-HHV-6 IgG nor IgM antibodies were elevated in the CSF. The CSF was not examined for HHV-6 DNA because the volume collected was insufficient. Blood HHV-6 DNA was not detected. Furthermore, neither IgG nor IgM antibodies to other herpesvirus (including HSV-1, varicella-zoster virus, cytomegarovirus, and Epstein-Barr virus) and Japanese encephalitis virus were abnormally elevated. From these results, we assumed that this relapsing encephalitis might be caused by HHV-6. Finally, she was discharged without any sequelae fifty-one days after hospitalization.

Discussion

HHV-6 is the main etiologic agent of exanthema subitum in young children, and has been implicated as a possible cause of encephalitis in pediatric patients (1-4). Epidemiologic studies have shown that most people are infected with HHV-6 at an early age. The virus remains latent state in lymphocytes, salivary glands, and brain tissue after primary infection, and has been reactivated in immunocompromised patients (15, 16). Encephalitis caused by HHV-6 has occasionally been documented in immunocompromised individuals, e.g., HIV-positive patients; recipients of bone marrow transplants, liver transplants, and renal transplants; and persons with lymphoproliferative disorders (1, 17, 18). However, there are few reports on the involvement of HHV-6 in the CNS in immunocompetent adults suffering from meningitis and/or encephalitis (5-14). Furthermore, there is no report of relapsing encephalitis due to HHV-6 in an immunocompetent adult. The present non-immunocompromised patient experienced a recurrence of HHV-6 encephalitis, which is very rare.

After primary infection, HHV-6 is characterized by lifelong latency in peripheral blood monocytes, salivary glands, and brain tissue (15, 16). HHV-6 seems to be a resident virus of human brain and is able to cause a restricted or minimally productive infection of brain cells, including microglial cells, astrocytes, and oligodendrocytes (16). There have been several reports that suggest the direct invasion of HHV-6 into the CNS. The frequency of detecting the HHV-6 genome by PCR in the brain tissue of immunocompetent adults was reportedly between 15% and 85% (19, 20). One group demonstrated HHV-6 DNA in 57% of brain tissues obtained from AIDS patients (21). Reactivation of infection occurs occasionally during pharmacological immunosuppres-

sion or acquired immunodeficiency. Thus, HHV-6 may be considered an important opportunistic pathogen. In contrast, immunocompetent adults very rarely have HHV-6-induced CNS infection (5-14). In the present case, the serum HHV-6 IgG antibody was elevated in the early stage and decreased afterward, while the serum IgM antibody against HHV-6 was not elevated. The considerable increase of IgG antibodies without a positive IgM antibody titer indicates reactivation of the virus (14), but its cause is unknown. One possibility is that the pathogenic mechanism involved in HHV-6 meningitis/encephalitis in immunocompetent adults may be related to the ability of HHV-6 to evade host immune responses through various mechanisms; induction of CD4 lymphocyte depletion via apoptosis, down-regulation of CD3 expression in T cell clones infected in vitro, a decrease of peripheral blood lymphocyte proliferation by HHV-6 via transcriptional down-regulation of IL-2, and decreased generation of reactive oxygen intermediates from monocytes that were infected with HHV-6 in vitro (22). Another undeniable possibility is that the patient had an immunocompromising disease that had not been diagnosed. In the present case, ACV was administered because the etiology was uncertain at first, but ganciclovir (GCV) and foscarnet were demonstrated to be more effective than ACV for some immunosuppressed patients with HHV-6 induced encephalitis (17, 18).

According to the past literature about immunocompetent patients with HHV-6 induced encephalitis, the majority (80%) of the patients presented with an altered level of consciousness; 60% had seizures, and 55% had focal neurological signs (9). Meningeal irritation, weakness of limbs, hyperreflexia, ataxia and visual disturbance were reported as the neurological findings. Analysis of CSF revealed mild-moderate increase of leukocytes (monocytes-dominant) and proteins in almost cases. In about half cases there were CT or/and MRI abnormal findings at CNS: including basal ganglia, thalamus, cerebral white matter, brain stem, and spinal cord, but there were no lesion at CNS in the other cases (5-14).

The first symptom of our patient was disturbance of consciousness and tonic-clonic seizures, but the only recurring symptom was disturbance of consciousness. The MRI findings in the first hospitalization revealed bilateral basal ganglia lesions, but there was no obvious lesion on MRI in the second hospitalization. The reason is unknown, but we assume that the degree of second HHV-6 reactivation was more subtle, and therefore the clinical features due to HHV-6 were different in the same individual. It is reported that basal ganglia lesion on MRI is often observed in encephalitis caused by some viruses such as Japanese encephalitis virus, Nipah virus, West Nile virus (23-25). Because serum antibody levels to Japanese encephalitis virus were not elevated in our case and the patient had never gone abroad, it was thought that these viruses were not the cause of encephalitis in this patient.

Drug-induced hypersensitivity syndrome (DIHS) is characterized by a severe, potentially fatal, multiorgan hypersensitivity reaction. DIHS usually occurs 3 weeks to 3 months after starting a limited number of drugs, including carbamazepine, phenytoin, phenobarbital, dapsone, mexiletine, salazosulfapyridine, alloprinol, and minocycline. The diagnosis of DIHS is confirmed by the presence of five of the following six criteria: 1) macuropapular rash developing > 3 weeks after starting therapy with the above drugs, 2) lymphadenopathy, 3) fever, 4) leukocytosis, 5) hepatitis, 6) HHV-6 reactivation (26). HHV-6 encephalitis associated with DIHS was reported previously (27). In the present case, a skin rash with high fever appeared soon after the dosage of phenytoin and phenobarbital in the first hospitalization. Our case can not be said to satisfy this DIHS criteria, as it lacked the following criteria: lymphadenopathy, leukocytosis, and hepatitis. But the skin rash appeared in both the first and the second hospitalization, which may also be related to encephalitis by HHV-6.

It is particularly unusual for a person not in an immunocompromised state to suffer from relapsing encephalitis due to HHV-6. We should consider the possibility of HHV-6 in the differential diagnosis of encephalitis in immunocompetent adult patients when the viral etiology of meningoencephalitis is unknown.

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SHORT COMMUNICATION

Myotonic dystrophy type 2 in Japan: ancestral origin distinct from Caucasian families

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Abstract Myotonic dystrophy type 2 (DM2) is caused by expansion of a tetranucleotide CCTG repeat in intron 1 of the *ZNF9* gene on chromosome 3q21. All studied DM2 mutations have been reported in Caucasians and share an identical haplotype, suggesting a common founder. We identified a Japanese patient with DM2 and showed that the affected haplotype is distinct from the previously identified DM2 haplotype shared among Caucasians. These data strongly suggest that DM2 expansion mutations originate from separate founders in Europe and Japan and are more widely distributed than previously recognized.

Keywords Myotonic dystrophy type 2 · CCTG tetranucleotide repeat expansion · Founder haplotype

Saito and Amakusa have contributed equally to the work.

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Introduction

Myotonic dystrophy type 2 (DM2) is an autosomal dominant, myotonic multisystemic disorder caused by the expansion of a tetranucleotide CCTG repeat in intron 1 of the zinc finger protein 9 (*ZNF9*) gene on chromosome 3q21 [1]. The size of expanded alleles is extremely variable, ranging from 75 to 11,000 repeats, with a very large mean of 5,000 CCTG repeats. Because of this unprecedented size and somatic heterogeneity, molecular diagnosis of DM2 is complicated. DM2 is also clinically variable, described as proximal myotonic myopathy [2], proximal myotonic dystrophy [3], or "myotonic dystrophy with no CTG expansion" [4]. Further studies suggested that all genetically confirmed DM2 patients arose from a single ancestral origin [5, 6]. No DM2 mutation, to date, has been identified in sub-Saharan or East-Asian populations [7]. Herein, we report the first Japanese family with a DM2 mutation.

Case report

A 59-year-old Japanese woman was admitted to our hospital for weakness of all four limbs that had progressed

slowly for more than 12 years. There was no complaint of muscle pain or stiffness. At age 47, she developed type 2 diabetes mellitus. At age 52, she had a right posterior subcapsular cataract extracted. There was no known consanguinity or genetic admixture with other ethnicities in her family. Her father and mother had no history of muscle weakness before having died at ages 67 and 72 years, respectively. There was a history of undiagnosed muscle disease in her brother and sister, but they were unavailable for examination. Her two children were asymptomatic.

Neurologically, this patient had normal language, speech, and cognition on routine clinical evaluation. She showed mild facial weakness and temporal wasting as well as weakness and atrophy of sternocleidomastoid muscles. Motor examination revealed predominantly proximal muscle weakness and atrophy in all limbs. Grip myotonia was present, but percussion myotonia was not elicitable. Tendon reflexes were present, but hypoactive and sensation was intact. Serum creatine kinase was 99 IU/l (normal range, 45–163 IU/l), and serum IgG level was slightly decreased to 828 mg/dl (normal range, 870–1,700 mg/dl). Electrocardiogram revealed complete right bundle branch block, and Holter monitoring detected premature ventricular contractions. Electromyography showed small motor unit potentials with early recruitment and myotonic discharges in all muscles examined. Nerve conduction studies were normal. Muscle computed tomography revealed diffuse muscle atrophy in the trunk and proximally in all limbs, whereas forearm and distal leg muscles were well preserved (Supplementary Figs. 2 and 3). **T2-weighted brain magnetic resonance imaging demonstrated diffuse periventricular white matter hyperintensities without significant cerebral atrophy. No CTG expansion in the *DMPK* gene associated with myotonic dystrophy type 1 (DM1) [8] was detected.**

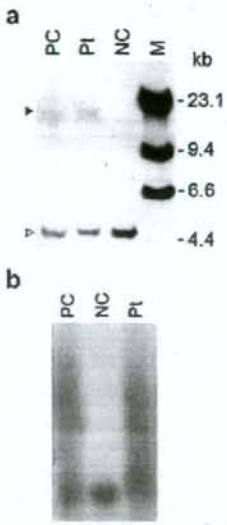
Materials and methods

Mutation analysis

Blood samples were obtained from the patient and her family members with informed consent approved by the institutional review boards of the National Dohoku Hospital and the Nagoya University Graduate School of Medicine for human research. High molecular weight genomic DNA was extracted by a standard procedure. Polymerase chain reaction (PCR) products across the DM2 repeat (marker CL3N58) in the first intron of the *ZNF9* [1] were analyzed by capillary electrophoresis using an automated DNA sequencer (ABI 310A Genetic Analyzer, Applied Biosystems). For detection of the DM2 CCTG expansion, Southern blot analysis and the repeat-primed PCR assay using an oligonucleotide primed within the DM2 CCTG repeat were performed as described elsewhere [1, 9, 10].

Haplotype analysis

To investigate the ancestral origin of Japanese DM2, we performed a haplotype analysis of our patient's family. We genotyped available family members (the patient, her spouse, and two children) for the previously described microsatellite markers: CL3N83; CL3N95; CL3N96; CL3N59; C3N116; CL3N117; CL3N118; CL3N119 [6] and a single nucleotide polymorphism (SNP): rs1871922, which is identical to TSC873597 in the report by Bachinski et al. [5]. We also analyzed three unrelated Caucasian DM2 DNA samples sharing the identical core haplotype as controls [6]. PCR products for all microsatellite markers were analyzed, and the SNP genotyping was performed by PCR amplification followed by restriction enzyme digestion (*Hae*III). The data were compared to the consensus Caucasian haplotypes reported in the previous report [6].



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Location (kb)	Marker	Our pedigree	Caucasian haplotype B	Caucasian haplotype A	Caucasian haplotype C
273	CL3N83	191	189	195	185
241	CL3N95	224	222	222	212
240	CL3N96	159	159	159	157
119	CL3N59	129	149	149	141
0	DM2 repeat	exp.	exp.	exp.	exp.
11	rs1871922	A	C	C	C
74	CL3N116	222	222	222	222
103	CL3N117	239	235	235	235
127	CL3N118	227	221	221	221
216	CL3N119	220	234	230	230

Fig. 1 **a** Southern blot analysis of myotonic dystrophy type 2 (DM2). A *closed arrowhead* points to expanded alleles in DM2. *M* λDNA/*Hind* III marker, *NC* normal control, *Pt* our patient showing an 18.1-kb expanded allele as well as a normal allele, *PC* a Caucasian positive control with a DM2 expansion (17.5 kb). **b** Repeat-primed PCR analysis. Expanded CCTG repeats are detected as a continuous characteristic ladder in the patient (*Pt*) and positive control (*PC*) lanes, the size of which exceeds the range in the normal control (*NC*). **c** Comparison of DM2-affected haplotypes between Japanese and Caucasian DM2 families. Genotypes shared among Caucasian haplotypes are *shaded*. The distance of each marker from the DM2 CCTG repeat expansion (*exp*) is denoted on the *left*

Results

Mutation analysis

PCR amplification of the DM2 repeat detected a single normal allele at 228 bp. To determine if this patient also had a disease allele too large to be amplified by PCR, Southern analysis was performed and showed an expanded DM2-mutant allele of 18.1 kb, corresponding to approximately 3,400 CCTG repeats, as well as a normal allele of 4.5 kb (Fig. 1a). The repeat-primed PCR assay also showed a smear PCR product, confirming the presence of DM2 CCTG expansion (Fig. 1b).

Haplotype analysis

As shown in Fig. 1c, we found that this patient has an expansion-associated haplotype distinct from that commonly found in Caucasian DM2 patients [5, 6], indicating a different ancestral origin. Although a short common haplotype, less than 130 kb, between CL3N59 and rs1871922, is still possible, a telomeric recombination between the mutation and the SNP (11 kb telomeric of the mutation) is unlikely.

Discussion

Consistent with the typical DM2 phenotype [7, 10, 11], our patient clinically showed a combination of adult-onset proximal muscle weakness and myotonia. To our knowledge, this is the first DM2 patient identified from an East-Asian population [7]. Although DM2 mutations were reported in non-European populations including Morocco, Algeria, Lebanon, Afghanistan, and Sri Lanka [7, 11], all reported DM2 patients were considered to originate from a single common founder because they shared an identical haplotype [5, 6]. Our data have implications for molecular genetic diagnostics and counseling of East-Asian patients with the DM clinical phenotype and their families, as well as providing insight into the evolution of this complex disease. Physicians and genetic counselors should be aware that DM2 exists in non-Caucasian populations. Further epidemiologic studies, especially collection of additional non-Caucasian DM2 patients, will be of interest. It will also be of value to determine whether DM2 patients of different ethnic backgrounds originated from separate founders and whether they have unique clinical features and differences in genetic instability.

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The experiments performed comply with current legislation in Japan.

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Evaluation of corticospinal tracts in ALS with diffusion tensor MRI and brainstem stimulation

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Evaluation of corticospinal tracts in ALS with diffusion tensor MRI and brainstem stimulation

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ABSTRACT

Objective: To assess corticospinal tract involvement in patients with amyotrophic lateral sclerosis (ALS) by correlating diffusion tensor imaging (DTI) measures with intra- and extracranial central motor conduction time (CMCT) and clinical features of the patients.

Methods: We investigated 31 patients with ALS and 31 normal volunteers by DTI and measured fractional anisotropy (FA) within the corticospinal tracts and in the extramotor white matter. We measured CMCT for the first dorsal interosseous muscle and segmented it into cortical-brainstem (CTX-BS CT) and brainstem-cervical root (BS-CV CT) conduction times by magnetic brainstem stimulation at the foramen magnum level. Clinical status of each patient was evaluated with the ALS Functional Rating Scale-Revised (ALSFRS-R) and upper motor neuron (UMN) score devised for this study.

Results: We found a significant decrease of mean FA in all regions of the corticospinal tracts in patients with ALS as compared with controls. We found that FA along the corticospinal tract decreased significantly with higher UMN scores. There was no significant correlation between FA and ALSFRS-R, to which both upper and lower motoneuron involvements contribute. FA showed a significant correlation with the intracranial part of the central motor conduction (CTX-BS CT) but not with the extracranial conduction time.

Conclusions: Fractional anisotropy reflects functional abnormality of intracranial corticospinal tracts and can be used for objective evaluation of upper motor neuron impairment in amyotrophic lateral sclerosis. *Neurology*® 2008;70:528-532

GLOSSARY

ALS = amyotrophic lateral sclerosis; **ALSFRS-R** = ALS Functional Rating Scale-Revised; **BS-CV CT** = brainstem-cervical root conduction time; **CMCT** = central motor conduction time; **CTX-BS CT** = cortical-brainstem conduction time; **FA** = fractional anisotropy; **FDI** = first dorsal interosseous; **LMN** = lower motor neuron; **ROI** = region of interest; **UMN** = upper motor neuron.

Amyotrophic lateral sclerosis (ALS) is clinically diagnosed by lower motor neuron (LMN) signs of limb and bulbar muscles associated with upper motor neuron (UMN) signs. Subclinical LMN involvement is detectable by needle electromyographic findings of denervation, which are incorporated in revised El Escorial criteria.[1] UMN involvement can be evaluated by physiologic measures or neuroimaging techniques,[2] although these have not been sufficiently well established to be incorporated into diagnostic criteria. Diffusion tensor MRI visualizes the overall orientation of the fiber tracts and their integrity in the white matter by measuring anisotropic water diffusion.[3] Decreased fractional anisotropy (FA) along the corticospinal tract has recently been reported in patients with ALS.[4,5] However, the pathophysiology of such reduced FA remains unclear. The present

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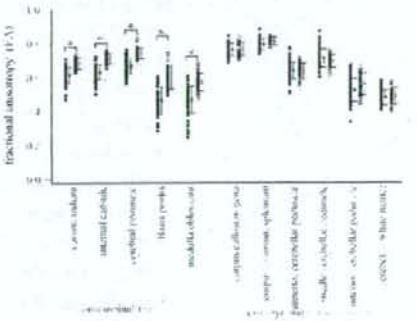
investigation was undertaken with the intent of clarifying the mechanism for reduced FA in ALS by studying correlations of the FA value with central motor conduction time segmented into intracranial and extracranial conduction times using brainstem stimulation.

Figure 1 Individual plots of fractional anisotropy (FA) at each region of interest (ROI) for patients with amyotrophic lateral sclerosis (filled circles) and controls (open circles) (mean ± SD)



METHODS **Subjects.** We recruited 31 patients with ALS and 31 age-matched normal subjects (ALS 60.7 ± 12.9 years, normal 57.1 ± 13.0 years, $p = 0.166$). The Ethical Review Committee of the University of Tokyo approved this study. All subjects gave their written informed consent to participate in the study. All patients were enrolled if they met definite, probable, or possible categories of revised El Escorial criteria.[3] The degree of abnormality was quantified using the ALS Functional Rating Scale-Revised (ALSFRS-R). The UMN score was designed to assess UMN impairment. The following neurologic signs were rated on a 0 to 2 scale according to their severity (0 = absent or normal, 1 = moderately impaired, and 2 = greatly impaired): jaw jerk, other pathologic reflexes of the cranial regions, overactive tendon reflexes in upper limbs, overactive finger flexor reflexes, overactive tendon reflexes in lower limbs, pathologic reflexes in lower limbs, spasticity, and presence of clonus. The scale generates a score from 0 to 16.

Transcranial magnetic stimulation of corticospinal pathways. Central motor conduction time (CMCT) was measured with methods described previously,[4] recorded from the first dorsal interosseous (FDI) muscles. A round coil was used for motor cortical and spinal motor root stimulation, and a double cone coil was used for brainstem stimulation. CMCT (motor-evoked potential latency difference between motor cortical and cervical root simulation), cortical-brainstem conduction time (CTX-BS CT, latency difference between motor cortical and brainstem stimulation), and brainstem-cervical root conduction time (BS-CV CT, latency difference between brainstem and cervical root stimulation) were calculated and evaluated by neurophysiologists blinded to MRI results.

Diffusion tensor MRI scanning protocol. Diffusion tensor images were acquired with 1.5-tesla Signa Horizon LX MRI system (GE Medical Systems), using single-shot spin-echo echoplanar sequences (repeat time 6,000 msec, echo time 78 msec, field of view 24 cm, NEX 4, 128 × 128-pixel matrix, diffusion gradients [b-value of 1,000 sec/mm^2], 3-mm slice thickness). Diffusion properties were measured along 13 noncollinear directions. FA was measured using a region-of-interest (ROI) method. Elliptical ROIs were placed along bilateral corticospinal tracts (corona radiata, internal capsule, cerebral peduncle, basis pontis, and medulla oblongata) and extramotor white matters (genu and splenium of the corpus callosum, superior, middle, and inferior cerebellar peduncle, and cerebellar white matter) on FA maps by one author blinded to subject clinical status, based on empirical anatomic knowledge and reference to pertinent literature.

Statistical analyses. We used a two-way analysis of variance (ANOVA) (factors of subject group and region). We used Scheffe analysis as post hoc multiple comparisons (significance level 0.05). Linear regression analyses were applied for all correlations (significance level 0.05) using StatView software (version 5; SAS Institute). Because FA is reported to decline with advancing age,[7] correlations between FA and clinical or physiologic measures are examined by using ratio of FA at each ROI to that of the splenium of the corpus callosum, to compensate for interindividual variability of absolute FA values. In evaluation of correlations between FA and CMCT as well as CTX-BS CT and BS-CV CT, we used all FA values and compatible physiologic measures, such as a FA on one side and a physiologic measure for the contralateral FDI. When a correlation between anisotropy data and ALSFRS-R or UMN score was analyzed, we averaged values from right and left sides to provide a single mean FA at a site for each individual.

RESULTS **Fractional anisotropy.** Individual plots of FA at each ROI for patients with ALS and controls are shown in figure 1. Two-way ANOVA showed an effect of the subject group (patient and control) and region (effect of subject group: $F = 205.763$, $p < 0.0001$; effect of region: $F = 395.421$, $p < 0.0001$). It also showed an interaction between the subject group and region ($F = 25.457$, $p < 0.0001$). Post hoc analyses showed that the mean FA was lower in patients with ALS than in controls in all ROI within the corticospinal tracts (the corona radiata, posterior limb of the internal capsule, cerebral peduncle, basis pontis, pyramid of the medulla oblongata) ($p < 0.0005$). No significant differences were found within extramotor white matter. FA decreased significantly with higher UMN scores at corona radiata, internal capsule, and pyramids of medulla oblongata. No correlation was apparent between UMN scores and FA in extramotor white matter. FA showed no significant correlation with ALSFRS-R in any

Table 1 Correlation between fractional anisotropy (FA) and clinical/physiologic measures

	Correlation between FA and clinical measures		Correlation between FA and physiologic measures		
Site	ALSFRS-R	UMN score	CMCT	CTX-BS CT	BS-CV CT
FA of corticospinal tract					
Corona radiata	0.070 (0.707)	0.397 (0.027*)	0.432 (0.004*)	0.409 (0.020*)	0.236 (0.201)
Internal capsule	0.292 (0.111)	0.440 (0.013*)	0.547 (<0.0001*)	0.535 (0.002*)	0.249 (0.176)
Cerebral peduncle	0.307 (0.093)	0.173 (0.353)	0.284 (0.065)	0.178 (0.330)	0.264 (0.152)
Basis pontis	0.037 (0.843)	0.210 (0.258)	0.306 (0.046*)	0.378 (0.033*)	0.073 (0.696)
Medulla oblongata	0.215 (0.245)	0.436 (0.014*)	0.408 (0.007*)	0.349 (0.051)	0.239 (0.195)
FA of extracorticospinal tract					
Corpus callosum genu	0.136 (0.465)	0.147 (0.429)	0.072 (0.751)	0.085 (0.763)	0.093 (0.741)
Superior cerebellar peduncle	0.120 (0.520)	0.071 (0.704)	0.213 (0.171)	0.083 (0.652)	0.106 (0.571)
Middle cerebellar peduncle	0.105 (0.574)	0.035 (0.853)	0.249 (0.108)	0.183 (0.315)	0.173 (0.353)
Inferior cerebellar peduncle	0.105 (0.575)	0.044 (0.814)	0.005 (0.974)	0.064 (0.727)	0.037 (0.843)
Cerebellar white matter	0.321 (0.078)	0.100 (0.591)	0.061 (0.698)	0.092 (0.616)	0.149 (0.425)

Correlation coefficient and the respective p value are shown.
*Significant.
ALSFRS-R = ALS Functional Rating Scale-Revised; BS-CV CT = brainstem-cervical root conduction time; CMCT = central motor conduction time; CTX-BS CT = cortical-brainstem conduction time; UMN = upper motor neuron.

ROI (table 1).

Transcranial magnetic stimulation of corticospinal pathways. We examined 47 limbs of 25 patients. In 16 limbs of 10 patients, no responses were obtained with motor cortical, brainstem, or motor root stimulation. The averaged ALSFRS-R of these patients was 29.7 ± 11.7, which was worse than that of the rest of the patients (36.7 ± 8.2, $p < 0.05$). There was no difference in the averaged UMN scores between the two patient groups (6.3 ± 3.8 and 6.2 ± 4.1, $p > 0.05$). Theoretically, unobtainable responses are attributable to cortical inexcitability resulting from motor cortical cell loss, severe peripheral involvement, or a combination of both. However, in the patients studied here, based on the above results of correlations, we can infer that dysfunction of LMN contributes more than that of the UMN to the lack of responses. These absent responses were excluded from the following correlation analyses because there were no measurable latencies.

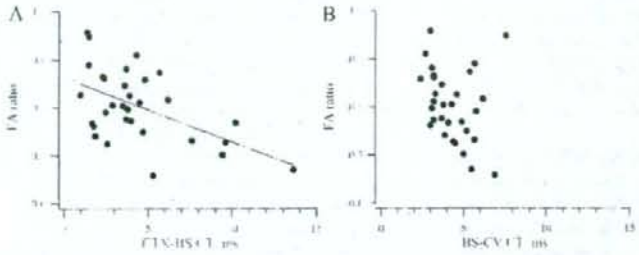
In all, we obtained 43 CMCTs and 31 CTX-BS CTs as well as BS-CV CTs. The averaged CMCT of FDI of the patients was 8.5 ± 3.4 msec (the average ± SD of the normal subjects at our facility was 7.0 ± 0.4 msec). Seventeen of 43 CMCTs were abnormally delayed (above the average + 2SD of the normal values). The average of CTX-BS CTs from the patients was 4.4 ± 3.0 msec (the normal average was 3.3 ± 0.3 msec). Twelve of 31 CTX-BS CTs were delayed. The average of BS-CV CTs of the patients was 4.3 ± 2.9 (the normal average was 3.7 ± 0.5), and 12 of 31 BS-CV CTs were abnormally prolonged. We found overall abnormal results including absent responses to either cortical or root stimulation, and delayed responses, in 44.7% of all the limbs studied. Delayed CMCT, CTX-BS CT, and BS-CV CT were found in 39.5%, 38.7%, and 38.7% of recorded responses. CMCT and CTX-BS CT correlated significantly with both ALSFRS-R and UMN scores, but BS-CV CT correlated only with ALSFRS-R (table 2).

Table 2 Correlations between central motor conduction times and clinical indices

Measures	Correlation	p Value
CMCT vs		
ALSFRS-R	0.482	0.0011*
UMN score	0.598	<0.0001*
CTX-BS CT vs		
ALSFRS-R	0.457	0.0098*
UMN score	0.595	0.0004*
BS-CV CT vs		
ALSFRS-R	0.559	0.0011*
UMN score	0.234	0.2048

*Significant.
CMCT = central motor conduction time; ALSFRS-R = ALS Functional Rating Scale-Revised; BS-CV CT = brainstem-cervical root conduction time; CTX-BS CT = cortical-brainstem conduction time; UMN = upper motor neuron.

Figure 2 Regression plots of fractional anisotropy at the internal capsule as a function of cortical-brainstem conduction time (CTX-BS CT) (A) and brainstem-cervical root conduction time (BS-CV CT) (B)



Correlation was only apparent between FA and cortical-brainstem conduction time ($|r| = 0.535$, $p = 0.002$). No correlation was observed between FA and the extracranial conduction time (brainstem-cervical root conduction time) ($|r| = 0.249$, $p = 0.176$).

Correlation between FA and CMCTs. FAs at most regions along the corticospinal tract decreased significantly with delayed CMCT and CTX-BS CT (table 1, figure 2A). However, BS-CV CT did not correlate with FA in any ROI (figure 2B). No significant correlation was found between FA of extramotor regions and CMCT or CTX-BS CT.

DISCUSSION In ALS, we have demonstrated reduced FA restricted to the corticospinal tracts. We also found that FA along the corticospinal tract decreased with higher UMN scores or delayed CTX-BS CT. The brainstem stimulation is considered to differentiate the CMCT delay due to an intracranial lesion from that due to an extracranial spinal lesion.[8] The CTX-BS CT must purely reflect UMN function, whereas the BS-CV CT must mostly reflect LMN function, and CMCT both LMN and UMN functions. Based on this theory, our present results suggest that FA measurement can evaluate UMN function in patients.

We showed that CTX-BS CT, but not BS-CV CT, delayed significantly with decreased FA at sites of the corticospinal tracts other than the cerebral peduncle where CSF has a greater partial volume effect on images. Decreased FA suggests tract degeneration that engenders the loss of organized coherent structures. FA changes are explainable by both intracellular water diffusion changes and extracellular matrix changes. Previous histopathologic studies of ALS suggest that the former corresponds to degeneration of the corticospinal tract axon itself with associated astrocytosis and accumulation of axonal spheroids, whereas the latter corresponds to extracellular matrix expansion and astrocytosis within interaxonal spaces. Both processes can cause reduced anisotropy of water diffusion. Meanwhile, slowing of the conduction time is considered to result from the loss of larger and faster conducting neurons and reduction of functioning rapidly conducting axons following corticospinal cell loss.[9] From animal experiments, the magnitude of latency delay by failed firing attributable to functioning fiber loss is estimated to be a few milliseconds at maximum from the cortex to cervical spinal cord. A striking increase of the conduction time in excess of this range would suggest slowing of conduction itself, which might be attributable to conduction through slowly conducting fibers due to degeneration of rapidly conducting fibers, or secondary demyelination when cortical neuronal loss is severe. These inferences are supported by white matter histopathology of the corticospinal tract: Myelin loss is commonly observed, especially in advanced patients, and the severity of that loss is generally related to neuronal loss of the motor cortex.[10] With the finding that the FA along the corticospinal tract decreased with intracranial motor conduction delay in ALS, we can infer that impaired axonal function, rather than extracellular factors, mainly contributes to the reduced FA. Demyelination secondary to motor axonal loss may add water diffusion changes in patients with excessive delayed motor conduction. This idea is consistent with the current view of determinants of anisotropy that the primary contributor is axonal membrane function, whereas other microstructures such as the myelin sheath, the neurofibrils (microtubules, neurofilaments), and axonal transport can play a secondary modulating role.[11] For more precise elucidation on potential determinants of anisotropic changes in ALS, thorough comparative studies of diffusion tensor imaging and postmortem specimens are necessary.

We demonstrated a significant correlation of FA with other clinical or physiologic indices. Potential applications of this method for patients with ALS include its use as an objective marker in following the natural course of the disease or modified course in therapeutic trials, or detecting a mild lesion of the corticospinal tracts at early stages.

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