a defect in the myelinating process of Schwann cells. Finally, we computationally predicted the effect of p.Thr387Asn (T387N) on protein function using two distinct pathogenicity prediction algorithms: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (www.mutationtaster.org). Both programs predicted that T387N is most likely damaging or pathogenic (data not shown). The results of the segregation studies, conservation of T387 in a variety of species, and results of the two prediction algorithms point to the pathogenicity of T387N, presumably resulting in the demyelinating neuropathy.

Up to thirteen *EGR2* mutations have been reported to date in demyelinating neuropathies, most of which have early-onset or severe phenotypes. For instance, patients with D355V, R359W, R358Q, R381H, and R409W exhibit a child-onset CMT1 phenotype, whereas patients with R381H and G412V exhibit DSN. While nine patients exhibited severe childhood-onset CMT or DSN/CHN, one patient with R381C presented with an adult-onset mild CMT1 phenotype at 59 years of age. Similar to the case presented in this report, the patient with R381C also exhibited a mild demyelinating phenotype without atrophy of the tibialis anterior muscle. The clinical findings in the proband can be explained, in part, by a weak pathogenicity T387N mutation, possibly because both threonine and asparagine are hydrophilic polarized amino acids, and thus, the mutation resulted only in mild functional loss of EGR2.

Recognition of the mild CMT phenotype can be critical for two reasons. First, some patients with CMT disease with atypical presentations, such as asymmetric symptoms, can be misdiagnosed as having chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and may be treated with unnecessary and costly therapeutic interventions such as immunoglobulin administration. Second, recognition of CMT can prevent the administration of potentially risky neurotoxic drugs, for example, vincristine, which can be toxic in the demyelinating form of CMT *(Weiss et al., 1974)*. This form of CMT has recently been found in another patient with an *EGR2* mutation *(Nakamura et al., 2012)*. Therefore, we consider that early recognition and diagnosis of CMT is crucial, even if the patient presents with a mild phenotype as in this case.

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Prominent fatigue in spinal muscular atrophy and spinal and bulbar muscular atrophy: Evidence of activity-dependent conduction block

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HIGHLIGHTS

- We showed that patients with chronic lower motor neuron disease [spinal muscular atrophy and spinal and bulbar muscular atrophy (SMA/SBMA)] frequently suffer disabling muscle fatigue.
- Single fiber electromyography with high-frequency stimulation revealed that SMA/SBMA patients might have activity-dependent conduction block phenomenon in distal motor axons.
- Activity-dependent conduction block is presumably produced by the reduced safety factor due to markedly increased axonal branching associated with collateral sprouting.

ABSTRACT

Objectives: To clarify whether patients with spinal muscular atrophy (SMA) or spinal and bulbar muscular atrophy (SBMA) suffer disabling muscle fatigue, and whether activity-dependent conduction block (ADCB) contributes to their fatigue. ADCB is usually caused by reduced safety factor for impulse transmission in demyelinating diseases, whereas markedly increased axonal branching associated with collateral sprouting may reduce the safety factor in chronic lower motor neuron disorders.
Methods: We assessed the fatigue severity scale (FSS) in 22 patients with SMA/SBMA, and in 100 disease controls (multiple sclerosis, myasthenia gravis, chronic inflammatory demyelinating polyneuropathy (CIDP), and axonal neuropathy). We then performed stimulated-single fibre electromyography (s-SFEMG) in the extensor digitorum communis (EDC) muscle of 21 SMA/SBMA patients, 6 CIDP patients, and 10 normal subjects.
Results: The FSS score was the highest in SMA/SBMA patients [4.9 ± 1.1 (mean ± SD)], with 81% of them complaining of disabling fatigue, compared with normal controls (3.5 ± 1.0), whereas patients with multiple sclerosis (4.3 ± 1.6), myasthenia gravis (4.0 ± 1.6) or CIDP (4.3 ± 1.4) also showed higher FSS score. When 2000 stimuli were delivered at 20 Hz in s-SFEMG, conduction block of single motor axons developed in 46% of patients with SMA/SBMA, and 40% of CIDP patients, but in none of the normal controls.
Conclusion: SMA/SBMA patients frequently suffer from disabling fatigue presumably caused by ADCB induced by voluntary activity.
Significance: ADCB could be the mechanism for muscle fatigue in chronic lower motor neuron diseases.

1. Introduction

Fatigue and weakness are common complaints of neurological disorder patients and significantly impair the quality of life. It is

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widely known that fatigue is one of the most disabling symptoms in patients with multiple sclerosis (MS) (Krupp et al., 1988) and those with chronic inflammatory demyelinating polyneuropathy (CIDP) (Boukhris et al., 2005; Bissay et al., 2008). Activity-induced fatigue and weakness were also described in not only patients with MS and CIDP but also with multifocal motor neuropathy (Cappelen-Smith et al., 2000; Kaji et al., 2000; Vucic et al., 2010; Straver et al., 2011).

In addition to demyelinating diseases, patients with a neurodegenerative motor neuron disorder often complain of fatigability. Persistent fatigue is a common complaint in patients with amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) (Piepers et al., 2008; McElhiney et al., 2009), and is associated with an impaired quality of life (Robbins et al., 2001). Straver et al. demonstrated that SMA patients had activity-induced weakness more often than normal subjects (Straver et al., 2011). Spinal and bulbar muscular atrophy (SBMA) is also a slowly progressive lower motor neuron disease, and SBMA patients may therefore show fatigability.

Activity-induced fatigue and weakness are caused by repetitive activity or exertion. It has been suggested that this phenomenon is attributable to activity-dependent conduction block (ADCB) arising in demyelinated axons. After repetitive firing, ionic concentration gradients in the axon are restored by increased Na^+/K^+ pump activity (Bostock and Grafe, 1985). Thus, with each pump-cycle, three Na^+ ions are expelled and only two K^+ ions enter (i.e., electrogenic pump), and the axons hyperpolarized by the pump (Schoepfle and Katholi, 1973), resulting in a decrease in the safety factor for impulse transmission. Nerve conduction is blocked if the safety factor is below unity due to leakage of the driving current caused by demyelination. In lower motor neuron disorders, the safety factor could also be reduced at the distal branching points due to collateral sprouting. Therefore, it is possible that ADCB could occur and may contribute to fatigue and weakness in SMA and SBMA patients.

We have developed a novel method to assess axonal activity-dependent hyperpolarization at a constant stimulus frequency using intra-muscular axonal stimulated-single fiber electromyography (s-SFEMG) (Noto et al., 2011). It was shown that tetanic stimulation at a constant rate (5, 10, and 20 Hz) resulted in a significant latency increase in single human motor axons, the extent of which depended on the stimulus frequency. This technique may detect ADCB if the safety factor is significantly reduced by demyelination or increased branching.

Given the recent interest in the mechanism of fatigue and weakness in demyelinating or chronic neurogenic diseases, the aim of this study was to assess the severity of fatigue in patients with SMA or SBMA, and to investigate whether ADCB contributes to fatigue in such patients.

2. Patients and methods

The study was conducted at Chiba University Hospital between October 2009 and March 2011. Informed consent was provided by each subject, and all experiments and the study protocol were conducted in accordance with the Declaration of Helsinki and with the approval of the Ethics Committee of Chiba University School of Medicine for Human Research Studies.

2.1. Subject

The present study included five different patient groups [SMA/SBMA, CIDP, MS, myasthenia gravis (MG), and axonal neuropathy] and normal subjects. The SMA/SBMA group ($n = 22$) consisted of 5 SMA patients and 17 SBMA patients; one of the 5 SMA patients had mutation of the SMN gene and the remaining SMA patients did not have genetic testing and were diagnosed based on the clinical/familial history and electrophysiological examination. All SBMA patients had expanded CAG repeats of the androgen receptor. Disease durations of SMA and SBMA patients were 22.6 ± 9.5 (mean ± SD) years and 10.2 ± 6.4 years, respectively. Modified Rankin Scale scores were 3.2 ± 0.8 and 2.2 ± 0.4.

The CIDP ($n = 16$), MS ($n = 31$), and MG ($n = 33$) groups consisted of consecutive patients in the study period. Neuropathy patients ($n = 20$) consisted of 13 patients with diabetic polyneuropathy, 6 with vasculitic neuropathy and 1 with vitamin B12 deficiency. This study also included 58 normal healthy subjects; none of whom had a neurological disorder, systematic disease, or was taking medication affecting the peripheral nerve function.

s-SFEMG was performed in 21 SMA/SBMA patients, 6 CIDP patients, and 10 normal subjects who consented to the examination protocol.

2.2. Assessment of fatigue

Fatigue was assessed by the Fatigue Severity Scale (FSS) (Krupp et al., 1989). The FSS was developed as a method of evaluating fatigue in patients with MS and other conditions such as systemic lupus erythematosus. The FSS questionnaire is composed of the following 9 statements related to patients' subjective perception of fatigue and its consequences for everyday activities: 1. My motivation is lower when I am fatigued, 2. Exercise brings on my fatigue, 3. I am easily fatigued, 4. Fatigue interferes with my physical functioning, 5. Fatigue causes frequent problems for me, 6. My fatigue prevents sustained physical functioning, 7. Fatigue interferes with carrying out certain duties and responsibilities, 8. Fatigue is among my three most disabling symptoms, 9. Fatigue interferes with my work, family, or social life. Patients are asked to rate their level of agreement (toward 7) or disagreement (toward 0) with the 9 statements. The final score represents the mean value of the 9 items.

2.3. Stimulated-single fiber electromyography

s-SFEMG was performed in the extensor digitorum communis muscle (EDC) using a Nicolet Viking 4 EMG machine (Nicolet Biomedical Japan, Tokyo, Japan), as described previously (Noto et al., 2011). The recordings were made intra-muscularly with a concentric needle electrode (30 G; TECA elite US53153). The high pass filter was set to 2 kHz and the low pass filter to 10 kHz. Intramuscular axonal stimulation was performed with a monopolar needle electrode (28 G; TECA U0809P02) and a reference surface electrode placed 2 cm laterally (Fig. 1). The stimulus duration was 0.1 ms. The distance between the stimulating and recording electrodes was 2 cm. The stimulus intensity was initially determined as 20% above the activation threshold of the target muscle action potential (MAP).

Before this study was performed, we predicted that blockings might occur due to slight movement of either the stimulating or recording electrodes produced by the muscle twitch. To avoid this phenomenon, the fingers of subjects and electrodes were fixed with a strap or a strut as shown in Fig. 1. In fact, during 20-Hz stimulation, the muscle twitch of the EDC muscle was not observed because 20-Hz axonal-stimulation produced persistent contraction of muscle bundles in all subjects. Therefore, the probability of blockings due to the movement of electrodes was low. We also observed the return of a previously blocked muscle action potential after rest in some recordings with blocking. However, we had to wait for over 15 min in each site in order to clear the effect of axonal hyperpolarization (Kiernan et al., 2004), and long time waiting

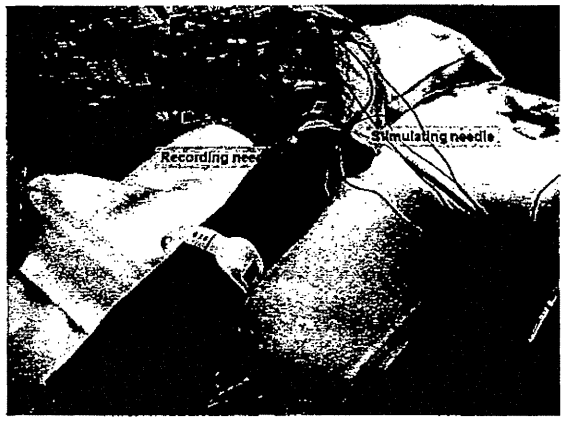

Fig. 1. The set up of stimulated-single fiber electromyography technique. To avoid slight movement of the stimulating and recording electrodes produced by the muscle twitch, fingers of the subject and electrodes were fixed with a strap or a metallic strut.

could not be tolerated for patients. For this reason, the re-stimulation after the rest was omitted.

When intermittent or persistent blockings were observed during stimulation, we increased the stimulus intensity by a further 20% of the previous intensity to secure supra-threshold stimulation. In consideration of tolerability of subjects, the extent of increasing stimulus intensity was up to 20%. A total of 2000 stimuli at 20 Hz were delivered. We examined 5 sites in the EDC in each subject and recorded the latency and shape of some MAPs, and calculated the percentage of MAPs with persistent or intermittent blockings. In this method, we defined persistent blocking as the condition whereby no MAP was evoked during the last 100 stimuli (1901–2000 times). The judgment of blocking was done by latency plotting with a special program for latency measurements (Medical Try System Co. Ltd., Tokyo, Japan).

2.4. Data analysis

The Fisher exact test was used to analyze gender ratio, the differences in proportion of subjects who have high FSS score ($\geqslant$4.0) between groups. The Wilcoxon rank-sum test was used to compare differences in FSS between genders in the normal control group. The correlation between the FSS scores and age in normal controls was tested with Spearman's rank correlation coefficient. Dunnett's multiple comparison tests were applied between normal controls and disease groups. In all comparisons, a p-value of less than 0.05 was considered to be significant. All statistical analyses were performed using STATA software (Stata Corp., Texas, USA) and R software, which is open source and freely available (see http://www.R-project.org).

3. Results

3.1. The fatigue severity scale scores

Clinical profiles and the fatigue severity scale (FSS) scores are shown in Table 1. The mean FSS score was significantly higher in the SMA/SBMA groups than in normal controls. Among the patient groups, the mean FSS score was higher in the SMA/SBMA group than in the other disease groups, although the differences were not significant. There was no significant association of the FSS scores with age or gender. When disabling fatigability was defined as an FSS score of 4.0 or above, as in previous studies (Armutlu et al., 2007; Kaynak et al., 2006), 36% of the normal subjects and 81% of the patients with SMA/SBMA had the degree of fatigue. The percentage of patients with fatigue was higher in the SMA/SBMA group than in the other disease groups.

On analyzing the correlation of the FSS scores with the age, disease duration, or modified Rankin scale score in each disease group, a positive correlation between the FSS scores and disease duration was found only in the MS group.

3.2. Blockings in stimulated-single fiber electromyography

Total numbers of MAPs examined were 41 from 10 normal controls, 85 from 21 SMA/SBMA patients (5 SMA and 16 SBMA patients), and 23 from 6 CIDP patients. The number of examined MAPs per subject ranged from 1 to 9. Table 2 shows the detection rates of intermittent/persistent blocking in normal controls, as well as SMA/SBMA and CIDP patients. Also, in this population, the mean FSS score in the SMA/SBMA group was significantly higher than in normal subjects.

In normal controls, no blocking was observed during 2000 stimuli. A representative recording and the latency scattergraph of a single MAP from a single normal subject are shown in Fig. 2A, 3A, and Supplementary Video 1. The latency prolonged linearly. Although increased jitter with intermittent blockings was found in normal subjects because of subthreshold stimulation, the jitter and blockings disappeared with an increasing stimulus intensity.

In SMA/SBMA and CIDP patients, intermittent or persistent blockings were similarly observed at the 2000th stimulus. We calculated the detection rates of persistent/intermittent blockings at the 2000th stimulus. The percentages of them in SMA/SBMA and CIDP groups were 11.8/45.8 and 13.3/40.0%, respectively (Table 2). Fig. 2B, 3B, and Supplementary Video 2 illustrate a characteristic recording and latency plot of intermittent blocking (finally leading to persistent blocking) from a single SBMA patient. Jitter and blocking were clearly visible in real-time (Supplementary Videos 2, 3). Such a phenomenon was never detected in normal controls. No significant correlation between the FSS score and percentage of intermittent/persistent blockings was demonstrated in SMA/SBMA and CIDP patients.

Table 1
Demographic, clinical findings and fatigue severity scale (FSS) score.

	Normal controls (n = 58)	SMA/SBMA (n = 22)	CIDP (n = 16)	Multiple sclerosis (n = 31)	MG (n = 33)	Neuropathy (n = 20)
Gender (M:F)	28:30	19:3**	13:3*	2:29**	16:17	9:11
Age; mean (range)	50 (20–85)	59 (37–75)	54 (23–79)	45 (20–65)	61 (24–83)**	60 (31–71)**
Disease duration (years); mean (range)	N/A	13.3 (1.5–34.0)	8.7 (0.2–22.0)	10.7 (1.3–38.0)	5.1 (2.0–6.9)	9.7 (0.2–30.0)
Modified Rankin Scale; median (range)	0	2 (2–4)**	2 (1–4)**	2 (1–4)**	1 (0–2)**	1 (1–4)**
FSS score; mean (SD)	3.5 (1.0)	4.9 (1.1)**	4.3 (1.4)	4.3 (1.6)*	4.0 (1.6)	3.6 (1.6)
FSS score $\geqslant$ 4.0 (%)	36	81**	63	65*	61*	35

*P < 0.05, **P < 0.01 vs. Normal control. SMA/SBMA, spinal muscular atrophy/spinal and bulbar muscular atrophy; CIDP, chronic inflammatory demyelinating polyneuropathy; MS, multiple sclerosis; MG, myasthenia gravis; N/A, not applicable.

Table 2
Detection rates of intermittent and persistent blockings during 2000 stimuli at 20 Hz in stimulated-single fiber electromyography and fatigue severity scale score.

	Normal controls	SMA/SBMA	CIDP
	$n = 10$	$n = 21$	$n = 6$
Gender (M:F)	6:4	18:3	5:1
FSS score; mean (SD)	3.8 (1.0)	4.9 (1.1)*	4.3 (1.5)
Intermittent blocking (%)***	0.0 (0.0)	11.8 (16.7)	13.3 (24.2)
Persistent blocking (%)***	0.0 (0.0)	45.8 (32.9)**	40.0 (23.5)*

$P < 0.05$, **$P < 0.01$ vs. Normal control, ***mean (SD); SMA/SBMA, spinal muscular atrophy/spinal and bulbar muscular atrophy; CIDP, chronic inflammatory demyelinating polyneuropathy; FSS, fatigue severity scale.

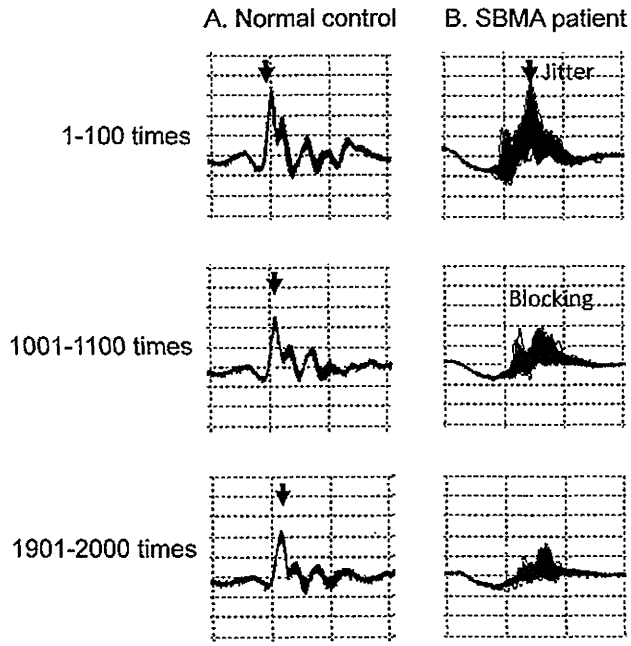

Fig. 2. Examples of superimposed single muscle action potentials during prolonged 20-Hz intramuscular microstimulation in the extensor digitorum communis muscle of a normal control (A) and a spinal and bulbar muscular muscular atrophy (SBMA) patient (B). Traces 1–100, 1001–1100, and 1901–2000 are shown separately. Note a gradual increase in latencies with no block of a muscle action potential (MAP) in a normal control (arrow in A), and jitter and subsequent blocking of a MAP in an SBMA patient (arrow in B).

4. Discussion

The present study demonstrates that patients with SMA/SBMA have prominent muscle fatigue, as well as patients with MS or CIDP. s-SFEMG studies have shown that SMA/SBMA and CIDP patients might have ADCB phenomenon induced by high-frequency axonal stimulation, and that both baseline neuromuscular transmission failure and axonal hyperpolarization could contribute to the activity-dependent changes observed in this study. The pathophysiology and mechanisms of fatigue are different among the disorders. This study firstly shows evidence that ADCB could contribute to muscle fatigue in SMA/SBMA.

4.1. Fatigue in neurodegenerative lower motor neuron disorders

In this study, "fatigue" means activity-induced muscle weakness and fatigability, not baseline muscle weakness and clinical severity. Demyelinating diseases, such as MS, MMN, and CIDP, often cause fatigability (Krupp et al., 1988; Boukhris et al., 2005; Bissay et al., 2008), and ADCB has been regarded as a mechanism of fatigue in MS and MMN (Vucic et al., 2010; Kaji et al., 2000). A recent report showed that SMA patients exhibited fatigue to the same extent as MMN and CIDP patients using FSS (Straver et al., 2011). Our study confirmed that fatigue was a frequently disabling symptom in not only SMA but also SBMA patients.

There is no widely used method to assess activity-dependent weakness or fatigue. Although FSS alone could not discriminate between psychological disinclination and fatigue due to an impairment of nerve, neuromuscular junction or muscle, some questions of FSS are to assess activity-induced fatigue, and the FSS score correlated with motor function deficits (Huisinga et al., 2011).

4.2. Mechanisms for blocking during 20-Hz stimulation

Our s-SFEMG technique detected axonal conduction failure during 20-Hz stimulation in SMA/SBMA and CIDP patients, whereas our method could not identify the site of blocking (axon, neuromuscular junction or muscle) accurately. This was a main limitation of our method. However we considered the main site of conduction failure we observed was axons. The possibility of electrode dislocation cannot be excluded as explanation of disappearance of some potentials. However we think it is possible that the change in the muscle action potential's shape is induced by hyperpolarization of the muscle fiber membrane as Bergmans (2012) emphasized in his recent commentary. This phenomenon was also consistent in this study. The change was generally observed in all subjects, and occurs not at random.

Our previous study demonstrated that tetanic stimulation at 20-Hz results in a significant latency increase in normal subjects (Noto et al., 2011). Along with previous studies, this phenomenon is believed to be induced by activation of the electrogenic Na^+/K^+ pump and resulting axonal hyperpolarization which leads to axonal conduction slowing (Vagg et al., 1998; Kuwabara et al., 2001; Kuwabara et al., 2002; Bergmans, 1970; Lin et al., 2000). Meanwhile, our previous study demonstrated the muscle action potential's shape became smaller and less sharp during stimulation. This phenomenon might be physiological, and was not due to a recording needle dislocation. Bergmans commented to this phenomenon in our previous study that the changes in waveforms and amplitudes in muscle action potentials during stimulation were caused by hyperpolarization of the muscle fiber membrane (Bergmans, 2012).

Regarding the physiological aspects of axonal firing, A 20-Hz stimulation is almost equivalent to physiological maximum voluntary contraction in EDC, and causes axonal hyperpolarization (Burke and Jankelowitz, 2009). This is the reason why we selected 20-Hz stimulation. As seen in Fig. 3B, the latency is gradually prolonged until the blocking occurs, although the dispersion of latencies (i.e., jitter) is also observed simultaneously. These findings may reflect activity-dependent hyperpolarization in the axon and the following blocking (i.e., ADCB).

This technique could not deny that blockings occur at the neuromuscular junction due to dysfunction of the synaptic terminal (e.g., the deficiency of acetylcholine in the axon). 20-Hz s-SFEMG with administration of cholinesterase inhibitor could solve this problem. However, it was difficult to perform it because of invasiveness. A further improvement in methodology is needed in future. In addition, recent studies reported that survival motor neuron protein deficiency produce neuromuscular junction dysfunction (Ling et al., 2012; Martinez et al., 2012). Although this factor might contribute to findings obtained from SMA patients, they were similar to findings in SBMA patients, and were not necessarily specific in SMA patients.

Also, our method was unable to calculate jitter values accurately. For this reason, we judged the intermittent/persistent blocking by latency plotting. Most of the blockings occurred a

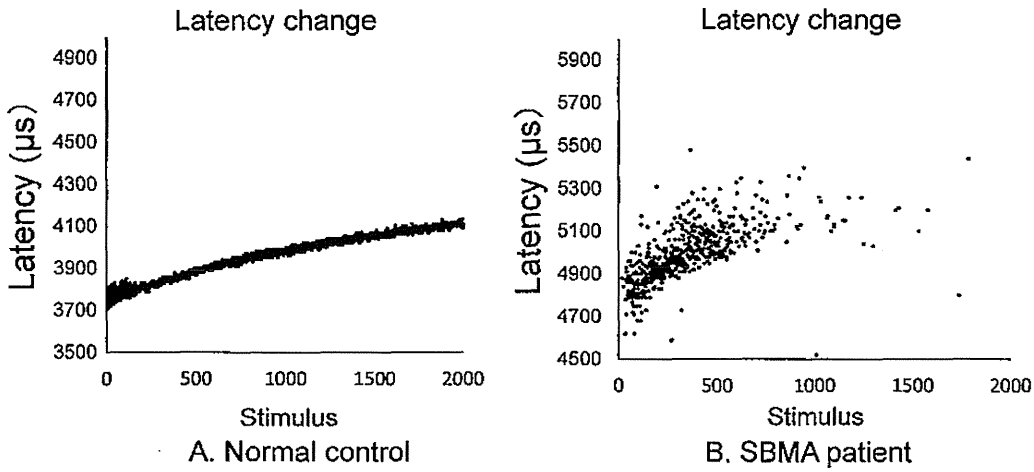

Fig. 3. Examples of the scatter plot of the latencies of single muscle action potentials during 20-Hz stimulation recorded from the extensor digitorum communis muscle of a normal control (A) and a spinal and bulbar muscular atrophy (SBMA) patient (B). The 2 subjects are the same as in Figure 2. Note a progressive increase in latencies during 2000 stimuli at 20 Hz in a normal control, and the dispersion of latencies and subsequent blocking in an SBMA patient.

few seconds after the start of stimulation, and they were judged as absolute blockings by enhancing the stimulus intensity. Enhancing level was set a further 20% of the previous intensity. Although it was difficult to ensure suprathreshold stimulation, the findings of SMA/SBMA and CIDP patients were distinctly different from that of normal controls in our method.

While there were some methodological limitations, blockings observed only during 20-Hz stimulation were likely to suggest conduction failure due to axonal hyperpolarization. Thus, these findings may reflect the existence of ADCB in physiological voluntary activity.

4.3. Activity-dependent conduction block in SMA and SBMA

The mechanism of ADCB in demyelinating neuropathy has been discussed in previous studies (Burke and Jankelowitz, 2009; Park et al., 2011). In SMA and SBMA, the main pathology is slowly progressive axonal loss. ADCB in neurodegenerative lower motor neuron disorders may be related to increased multiple axonal branches associated with collateral sprouting. Computer model by Zhou et al. simulated action potential propagation from a parent myelinated branch through a single branch point to two myelinated daughter branches (Zhou and Chiu, 2001). In the study, action potentials in daughter branches were smaller than that in the parent branch. If an axon has many sprouting branches, an axonal driving current in each nerve terminal branches will be extremely small. Thereby the safety factor could become critically lowered in the nerve terminals. This condition leads to a vulnerability to conduction failure when axons are hyperpolarized. Stålberg et al. reported that paired blocking was seen in ALS and SMA patients using a voluntary-single fiber electromyography method (Stålberg and Thiele, 1972). They proposed that this type of blocking occurred in newly formed sprouts because myelination in new branches was premature and offered a low safety factor for transmission. However, we assume that blocking could also occur in the terminals of many mature branches considering the existence of an axonal hyperpolarization mechanism induced by activity.

This reinnervation mechanism may also partly contribute to ADCB in chronic demyelinating disease, such as CIDP and MMN, because denervation and collateral sprouting are seen in the pathology of these diseases (Bouchard et al., 1999; Van Asseldonk et al., 2003). In addition to reinnervation, axons with higher firing rates might be involved in ADCB in motor neuron disorders. Vucic et al. demonstrated relatively larger increases in the threshold following activity in ALS patients than in healthy controls (Vucic et al., 2007). The central drive for higher firing rates in surviving axons would cause a greater impulse load on the axon also in SMA and SBMA. It is clinically relevant to explore the ionic mechanisms for muscle fatigue, which may provide a new treatment option by modulating the pump activity and specific ionic currents.

Disclosure

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinph.2012.12.053.

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Contrasting echogenicity in FDP-FCU: a diagnostic ultrasound pattern in sporadic inclusion body myositis

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Dr. Noto: design of the study, analysis of data, and drafting of the manuscript. Dr. Shiga and Tsuji: design of the study and revision of the manuscript. Drs. Kondo, Tokuda and Mizuno: interpretation and acquisition of data. Dr. Nakagawa: revision of the manuscript.

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Abstract

Introduction: We aimed to clarify whether muscle ultrasound (US) of the forearm can be used to differentiate between patients with sporadic inclusion body myositis (s-IBM) and those with s-IBM-mimicking diseases.

Methods: We compared the echo intensity (EI) of the flexor digitorum profundus (FDP) muscle and the flexor carpi ulnaris (FCU) muscles in patients with s-IBM (n = 6), polymyositis/dermatomyositis (PM/DM) (n = 6), and amyotrophic lateral sclerosis (ALS) (n = 6).

Results: We identified EI abnormalities in 100% of patients with s-IBM, 33% of those with PM/DM, and 33% of those with ALS. An "FDP-FCU echogenicity contrast", a US pattern involving a higher EI in the FDP than in the FCU, was observed in all patients with s-IBM, but in none of those with PM/DM or ALS.

Conclusions: "FDP-FCU echogenicity contrast" in muscle US is a sensitive diagnostic indicator of s-IBM.

Key words: ultrasound, muscle, diagnosis, inclusion body myositis, amyotrophic lateral sclerosis.

Introduction

Sporadic inclusion body myositis (s-IBM), one of the most common myopathies in older adults, is characterized by slowly progressive muscle weakness with a predilection for the quadriceps femoris and flexor digitorum profundus (FDP) muscles.[1,2] In needle electromyography (EMG), s-IBM can be confused with amyotrophic lateral sclerosis (ALS) because of the presence of so-called "neurogenic changes" such as high amplitude and long-duration motor unit potentials. These findings may be indicative of motor units with hypertrophied myofibers in chronic myopathy.[3] Hokkoku et al. reported that examination of the FDP muscle in EMG can reduce the risk of making a misdiagnosis of ALS in patients with s-IBM.[4] In addition, magnetic resonance imaging (MRI) of the forearm was also reported as useful for the diagnosis of s-IBM.[2] Selective involvement of the FDP has thus drawn attention in the clinical diagnosis of s-IBM.

Over the past few decades, high-frequency US of muscles and peripheral nerves has emerged as a non-invasive and simple tool to assist in the diagnosis of neuromuscular disorders. Therefore, we employed US of the FDP to accurately differentiate between patients with s-IBM and those with other s-IBM-mimicking diseases.

Materials and Methods

Subjects. Consecutive Japanese patients who presented with s-IBM (n = 6), polymyositis (PM) or dermatomyositis (DM) (PM: n = 2, DM: n = 4), or ALS (n = 6) at our hospital were enrolled. Informed consent was provided by all patients. The diagnosis of s-IBM or PM/DM was confirmed by muscle biopsy according to previously established criteria.[5,6] All ALS patients fulfilled Awaji criteria for probable/definite ALS, or showed progressive muscle weakness compatible with ALS.

Ultrasound. US imaging for all patients was performed by the same physician (Y.N.) using a GE LOGIQ P5 system with a 10-MHz linear array probe (GE healthcare Japan, Tokyo, Japan). Each subject lay in the supine position with the right elbow bent. The transducer was placed at 5 cm distal to the right olecranon, as shown in **Figure 1A**. The FDP could be identified as a triangular compartment adjacent to the ulna. The ulnar nerve is a landmark for identification of the 3 muscles [flexor digitorum profundus (FDP), flexor digitorum superficialis (FDS), and flexor carpi ulnaris (FCU)], because the nerve is encircled by those muscles. This transducer placement provides a favorable cross-sectional view of the FDP and FCU muscles at the same depth to enable effective comparison of echo intensity (EI) between them. Machine settings for image acquisition were preset and constant for all images without adjusting the focal point, gain, or time gain compensation settings.

Visual assessment of muscle echo intensity

The EI of the FDP muscle adjacent to the ulna was scored retrospectively by a single examiner (Y.N.), based on the Heckmatt rating scale, as follows: 1, normal; 2, slightly increased muscle EI with normal bone reflection; 3, moderately increased muscle EI with reduced bone reflection; and 4, severely increased muscle EI without bone reflection.[7] Then, the Heckmatt grade of the FCU muscle was scored by comparing it with that of the FDP muscle. A patient with increased "FDP-FCU echogenicity contrast" was defined when the EI grade of the FDP muscle was more than 1 grade higher than the FCU muscle. The proportion of patients who had increased FDP-FCU contrast was calculated in each disease group. Additionally, FDP/FCU EI ratios (EI score of the FDP muscle divided by score of the FCU muscle) by Heckmatt rating scale scoring were also calculated.

Quantitative assessment of muscle echo intensity

Quantification of muscle EI using gray scale analysis was performed to confirm the results obtained by visual assessment (i.e., Heckmatt rating scale scoring). This objective assessment was done with a standard histogram function, which was available in Adobe Photoshop (Adobe systems Inc., San Jose, CA, USA) as reported previously. [8] The mean gray values of FDP and FCU muscles were calculated respectively from the histogram, after encircling these muscles without surrounding fascia using the tracking software function. FDP/FCU EI ratios by gray scale analysis were calculated.

Assessment of muscle atrophy

At the site described above, the muscle cross-sectional areas (CSAs) of FDP and FCU were measured by continuous manual tracing of the muscle circumference excluding surrounding fascia. Measurement of the FDS muscle was omitted, because a fraction of the circumference of the FDS muscle was often out of the cross-sectional image we obtained. FDP/FCU CSA ratios were calculated.

Statistical analysis. All statistical analyses were performed using STATA software (Stata Corp., Texas, USA). Frequencies of all EI abnormalities and FDP-FCU contrast detected by US were tested with the Fisher exact test. Multiple comparisons of Heckmatt rating scale scores, mean gray values, FDP/FCU EI ratio of Heckmatt rating scale and gray scale analysis, and FDP/FCU CSA ratio between the 3 groups were tested with ANOVA and the Bonferroni procedure.

Standard protocol approvals. The local ethics committee of Kyoto Prefectural University of Medicine Graduate School of Medical science approved this study.

Results

Clinical characteristics and Echo intensity. Clinical profiles, Heckmatt rating scale scores for FDP and FCU, and detection rates of EI abnormalities and FDP-FCU contrast are shown in

Table 1. The mean Heckmatt rating scale score for the FDP and the FDP/FCU EI ratio by the Heckmatt rating scale were significantly higher in the s-IBM group than in the PM/DM or ALS groups. All s-IBM patients had EI abnormalities of the FDP muscle, whereas 2 of 6 PM/DM patients (33%) and 2 of 6 ALS patients (33%) had EI abnormalities. All EI abnormalities in the FDP muscles obtained from s-IBM arms showed a homogeneous high echoic pattern, whereas those obtained from PM/DM and ALS arms showed a rather heterogeneous high echoic pattern, as shown in **Figure 1B**. On visual assessment, none of the s-IBM patients had EI abnormality of the FCU, while 2 PM/DM patients and 2 ALS patients had increased EI in both the FCU and FDP muscles. In the PM/DM or ALS arms, no patients exhibited a higher EI in FDP than in FCU. Thus, the FDP-FCU echogenicity contrast pattern, indicating higher EI in FDP compared to the FCU muscle, was a characteristic finding in s-IBM patients (**Figure 1B**). Quantitative analysis using gray scale analysis also showed that the FDP/FCU EI ratio was significantly higher in the s-IBM group than in the PM/DM or ALS groups (P <0.01 and P <0.01, respectively). The mean FDP/FCU EI ratios of s-IBM, PM/DM, and ALS patients were 1.33, 0.88, and 1.02, respectively. No significant differences in any indices were found in comparing between the PM/DM and ALS groups.

Muscle cross-sectional area

Muscle CSAs and FDP/FCU CSA ratios are shown in **Table 2**. No significant difference in CSA of the FDP muscle was found among the 3 groups, whereas CSAs of the FCU muscles in s-IBM were significantly larger than those in ALS patients. The FDP/FCU EI ratio was significantly lower in the s-IBM group than in the PM/DM or ALS group.

Discussion

This study revealed that patients with s-IBM show "FDP-FCU echogenicity contrast", in which the EI of the FDP muscle is higher than that of the FCU muscle. This recognizable EI pattern was not seen in any of the patients with PM/DM or those with ALS in this study. These results demonstrate that muscle US of the forearm is a non-invasive and easily accessible diagnostic tool for s-IBM.

A high EI in muscle US suggests increased fibrous tissue or fatty degeneration in interstitial components of the muscle indicating chronic myopathy. A low EI reflects interstitial edema, which is often observed in the acute stage of inflammatory myopathy. [9, 10] It remains to be elucidated whether s-IBM is caused by an inflammatory or degenerative mechanism together with a secondary inflammatory process. In general, muscle biopsy of s-IBM patients reveals abundant chronic myopathic changes, such as marked variation in fiber size, endomysial fibrosis, and fatty degeneration. In this study, a visually homogeneous high echoic pattern of the FDP muscle was found in s-IBM patients, whereas EI abnormality in muscle in ALS and PM/DM patients showed a rather heterogeneous pattern. Although we found that mean gray scale values of the FDP in s-IBM were higher than those in PM/DM and ALS patients, it is unclear what determines the increased echoic pattern (i.e., homogeneous or heterogeneous). Further study related to the correlation between the visual ultrasound pattern and the muscle pathology of s-IBM is needed.

Sekul et al. reported that muscle MRI of the forearm demonstrates selective involvement of the FDP muscle in up to 95% of s-IBM patients.[2] Furthermore, they observed marbled brightness of the FCU and FDS muscles on T1-weighted MRI in 33 and 29% of patients, respectively, and concluded that the FDS muscle was spared even in late stages of s-IBM. In addition, a quantitative motor unit potential (MUP) analysis revealed decreased amplitude, short duration, and reduced MUP size index in the FDP.[4] Both the MRI study and quantitative MUP indicate

that selective involvement of the FDP can be a unique finding to identify patients with s-IBM. In our US study, the frequencies of a high EI of the FDP and FCU muscles were comparable to the MRI study (100% in FDP and 33% in FCU muscles), indicating that US image analysis is another promising technique to identify FDP muscle pathology. This study also revealed decreased FDP/FCU CSA ratios in s-IBM patients, indicating selective atrophy of the FDP. Among the various tests, muscle US is advantageous because it is not only less invasive than EMG or muscle biopsy, but it is also far less expensive than MRI. In addition, US assessment is readily available in any hospital and can be repeated easily at the bedside.

There are a few limitations in this study. First, our findings were obtained from a small population of patients. Second, the patients with PM/DM in this study were younger than those with ALS. Muscle echo intensity increases with age due to replacement by fat or fibrous tissue. [8] This could have influenced the results of echo intensity in older patients to some extent. Finally, the US examinations in this study were done by a single examiner who was not blinded to the clinical diagnoses. A blinded design with a larger number of patients including inter- or intra-rater reliability assessment are essential to confirm the results of our study.

Abbreviation

ALS = amyotrophic lateral sclerosis

CSA = cross-sectional area

DM = dermatomyositis

EI= echo intensity

FCU = flexor carpi ulnaris

FDP = flexor digitorum profundus

FDS = flexor digitorum superficialis

PM = polymyositis

s-IBM = sporadic inclusion body myositis

US = ultrasound

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Figure legends

Figure 1. Transducer position used to visualize the flexor digitorum profundus (FDP), flexor digitorum superficialis (FDS), and flexor carpi ulnaris (FCU) muscles simultaneously (A). The ulnar nerve (*) is encircled by the FDP, FDS, and FCU muscles. Patterns of muscle ultrasound images in a normal subject, sporadic inclusion body myositis (s-IBM), polymyositis, and amyotrophic lateral sclerosis (B). Note moderately high echo intensity of the FDP and normal echo intensity of the FCU in the s-IBM patient ("FDP-FCU echogenicity contrast") (arrow in B). Slightly high echo intensities of the FDP and FCU muscles in PM and ALS patients (B).

Table 1. Demographics, clinical findings, and echo intensity abnormalities.

	s-IBM (n = 6)	PM/DM (n = 6)	ALS (n = 6)
Gender (M:F)	5:1	3:3	3:3
Age; mean (range)	71.5 (68-79)	56.3 (39-72)	72.2 (62-79)
Disease duration (months); mean (range)	56.7 (14-120)	49.3 (1-215)	29.8 (2-94)
Heckmatt rating scale of FDP; mean (range)	2.7 (2-3)* †	1.3 (1-2)	1.3 (1-2)
Heckmatt rating scale of FCU; mean (range)	1.3 (1-2)	1.3 (1-2)	1.3 (1-2)
FDP/FCU Heckmatt rating scale ratio; mean	2.2* †	1.0	1.0
EI abnormality of any FDP and FCU muscle; n (%)	6 (100) ** † †	2 (33)	2 (33)
FDP-FCU echogenicity contrast; n (%)	6 (100) ** † †	0 (0)	0 (0)

*P<0.05 and **P <0.01 vs. PM/DM. †P <0.05 and † †P <0.01 vs. ALS. s-IBM, sporadic inclusion body myositis; PM/DM, polymyositis/dermatomyositis; ALS, amyotrophic lateral sclerosis; EI, echo intensity; FDP, flexor digitorum profundus muscle; FCU, flexor carpi ulnaris muscle. FDP/FCU Heckmatt rating scale ratio is defined as EI score of the FDP muscle divided by EI score of the FCU muscle. EI abnormality is defined as Heckmatt ration scale ≧2. FDP-FCU echogenicity contrast is defined as an echo intensity pattern of a higher intensity in FDP than in FCU muscle.