

Title	Rippling is not always electrically silent in rippling muscle disease.
Author(s)	Maki, Takakuni; Matsumoto, Riki; Kohara, Nobuo; Kondo, Takayuki; Son, Insuk; Mezaki, Takahiro; Nishino, Ichizo; Ikeda, Akio; Takahashi, Ryosuke
Citation	Muscle & nerve (2011), 43(4): 601-605
Issue Date	2011-03-14
URL	http://hdl.handle.net/2433/196888
Right	This is the peer reviewed version of the following article: Maki, T., Matsumoto, R., Kohara, N., Kondo, T., Son, I., Mezaki, T., Nishino, I., Ikeda, A. and Takahashi, R. (2011), Rippling is not always electrically silent in rippling muscle disease. Muscle Nerve, 43: 601–605, which has been published in final form at http://dx.doi.org/10.1002/mus.21947
Type	Journal Article
Textversion	author

Title

Rippling is not always electrically silent in rippling muscle disease: a case report

Takakuni Maki, MD,¹ Riki Matsumoto, MD, PhD,¹ Nobuo Kohara, MD, PhD,²

Takayuki Kondo, MD,¹ Insuk Son, MD,¹ Takahiro Mezaki, MD, PhD,³ Ichizo Nishino, MD, PhD,⁴

Akio Ikeda, MD, PhD,¹ and Ryosuke Takahashi, MD, PhD¹

¹ Department of Neurology, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto

606-8507, Japan

² Department of Neurology, Kobe City Medical Center General Hospital, 4-6 Minatojima-Nakamachi,

Chuo-ku, Kobe, Hyogo 650-0046, Japan

³ Department of Neurology, Sakakibara Hakuho Hospital, 5630 Sakakibara-cho, Tsu, Mie

514-1251, Japan

⁴ Department of Neuromuscular Research, National Institute of Neuroscience, National Center of

Neurology and Psychiatry (NCNP), 4-1-1 Ogawa-Higashi-cho, Kodaira, Tokyo 187-8502, Japan

Acknowledgements:

We thank Professor Jun Kimura for his insightful comments on this manuscript. This work was partly supported by Grants-in-Aid for Scientific Research (C) 20591022 from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

Correspondence to: Riki Matsumoto; Department of Neurology, Graduate School of Medicine,

Kyoto University, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan; E-mail:

matsumot@kuhp.kyoto-u.ac.jp

Running title: Electromyography of RMD

Total words: 2980 words

Abstract

Rippling muscle disease (RMD) is a myopathy with hyperirritability, the pathophysiology for which is uncertain. We report electromyographic findings in a 30-year-old man with RMD. Clinical features included muscle rippling and percussion-induced rapid muscle contractions. Both were associated with bursts of short duration and low amplitude spikes which resembled single muscle fiber discharges. Our case stands in contrast to previously reported cases which showed either electrical silence or motor unit potential discharges associated with rippling, and it may represent muscle fiber hyperexcitability.

Key words: caveolin-3; rippling muscle disease; electromyography; percussion-induced rapid muscle contraction, electrically silent

Introduction

Rippling muscle disease (RMD), a myopathy with signs and symptoms of muscular hyperirritability, characteristically shows muscle stiffness, percussion-induced rapid muscle contractions (PIRCs), local muscle mounding, and involuntary rolling muscle contractions or muscle rippling. They are elicited by mechanical muscle stimulation like exercise, tapping, or stretch. Both genetic and sporadic forms have been described.[1-3]

In most reported cases, genetic analyses documented homo- or heterozygotic mutations in the gene encoding caveolin-3 (*CAV3*), a membrane-associated protein localized to the sarcolemma, transverse (T)-tubule system,[4] and neuromuscular junction[5] of the skeletal muscle fibers. The mislocalization of *CAV3* leads to increased activity of neuronal nitric oxide synthase (nNOS), which, in turn, may cause muscle hyperexcitability.[6,7] Previous studies of genetic RMD disclosed structural disorganization of T-tubule systems potentially disturbing normal kinetics of excitation-contraction coupling.[3,8]

The electromyographic findings of the involuntary muscle activities in RMD have been inconsistent among previous reports. Here we report detailed needle and surface electromyography (EMG) findings in a case with familial RMD. The EMG findings demonstrated unique electrical activities associated with muscle rippling.

Methods

Case report

We evaluated a 30-year-old man with exercise-induced involuntary muscle contractions. He had shown muscle hypertrophy of bilateral thighs and calves since 4 or 5 years old, and had noticed rolling muscle movement in his shoulders and legs at age 8. Painful stiffness occurred in leg muscles already in his childhood, particularly during exercise without warm-up. He had to run on the toes to avoid this stiffness. Although the episodes of painful stiffness became less frequent after adolescence, he still has had easy fatigability.

Thigh and calf muscles were bilaterally hypertrophic. The muscle strength was normal, although mild contracture of Achilles tendons prevented full ankle dorsiflexion. Tendon reflexes were normal. Sudden muscle stretch induced rippling in the quadriceps femoris and gastrocnemius muscles, and, to a lesser degree, in the pectoralis major and deltoid muscles. Rippling muscle contractions spread diagonally or transversally to the axis of muscle fibers, occasionally showing a to-and-fro wavelike appearance. The rippling abated rapidly with repeated trials, but they could be elicited again after ten or more minutes of rest. Percussion induced rapid muscle contractions in the sternocleidomastoid, trapezius and all limb muscles tested, without habituation by repeated trials. Strong grasping of the biceps brachii muscle resulted in slight muscle mounding that lasted for about one second.

Cranial nerves, cerebellar function and sensation were all normal. Serum creatine kinase (CK) was elevated (522 U/L; normal <180 U/L). Other laboratory findings were normal. Cardiopulmonary function was normal. Therapeutic trials with dantrolene sodium had no impact on

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5 his symptoms.
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8 We also examined the patient's 59 year-old father and a 27 year-old sister, both having had
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10 exercise intolerance, muscle stiffness, and rippling since their childhood. The proband's sister had
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12 been diagnosed as Thomsen disease at age 3. Since her school age, she had also noticed rippling
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14 movements, and had to run on her toes to avoid painful stiffness. On examination, she had well
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16 developed musculature and normal strength, but Achilles tendon contractures had restricted the range
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18 of dorsiflexion of her feet. She had PIRCs and muscle rippling, to a lesser extent as compared with
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20 the proband. Her serum CK was 313 U/L. Their 59-year-old father also had been forced to walk on
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22 his toes since age 5 with muscle stiffness and rippling, and complained of muscle fatigue after
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24 exercise. His condition had not changed over years after his adolescence.
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39 **Muscle biopsy study and mutation analysis**

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41 An open biopsy from the left vastus medialis muscle was done after written, informed consent was
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43 obtained from the patient. Using standard techniques, immunohistological analyses were performed
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45 as previously described.[9]
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51 Genomic DNA extracted from peripheral blood lymphocytes of the patient was used as a
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53 template for PCR amplification after informed consent. Direct sequence analysis of PCR products for
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55 *CAV3* was performed as previously described.[9]
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Electrophysiological study

Following routine nerve conduction study and needle EMG (Viking Select, Nicolet, WI), concentric needle and surface EMG studies (MEP-2204, Nihon Kohden, Tokyo) during PIRCs and muscle rippling were performed to clarify the electrophysiological nature of abnormal muscle contractions.

Mechanomyogram was also monitored with a one-dimensional accelerometer (MP110-10-101, Medi Sens Inc., Tokyo) with surface electrodes placed on the skin above the muscles.

PIRCs were induced and recorded by tapping the vastus medialis or gastrocnemius muscle with reflex hammer. To distinguish PIRCs from the reflex contractions, electrical activities of the gastrocnemius muscle by tapping the lower part of the muscle belly were compared with those by tapping the Achilles tendon. As a control, the same maneuver was performed in a healthy 27-year-old man.

Rippling muscle movement was also induced by a sudden stretch of the quadriceps femoris muscle, i.e., an abrupt knee flexion following its forceful extension for 15 seconds. EMG and the accelerogram were recorded from the vastus medialis muscle, as it showed the most robust rippling phenomenon.

Results

Muscle biopsy study and mutation analysis

Muscle biopsy revealed a moderate degree of fiber size variation with abundant hypertrophic fibers and scattered fibers with centrally-placed nuclei. Mild disorganization of intermyofibrillar networks was observed in hypertrophic fibers. There were no necrotic or regenerative fibers.

Immunohistochemistry revealed that the expression of caveolin-3 at the sarcolemma was markedly reduced (Fig. 1B), as compared with the control (Fig. 1A).

The mutation analysis revealed a heterozygous G→A substitution on one allele at position 80 in the first exon of *CAV3* with amino acid replacement from arginine to glutamine at position 27 (Fig. 1C).

Electrophysiological study

On routine investigation, needle EMG of the quadriceps femoris, deltoid and gastrocnemius muscles showed no spontaneous activities at rest, like fibrillations or myotonic discharges. The shape and the recruitment pattern of motor unit potentials were normal. Nerve conduction studies were normal in all limbs.

PIRCs were elicited by tapping the lower part of the belly of the gastrocnemius muscle near the Achilles tendon. During PIRCs, irregular and decrementing bursts of activities were observed by needle EMG, lasting for more than 100 ms, and their onset-latency appeared less than 10 ms although the tapping artifact obscured their precise onset (Fig. 2B, C). Surface EMG demonstrated, if any, less

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5 prominent irregular titubations of the baseline (Fig. 2B). On the other hand, tapping the Achilles
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8 tendon elicited a synchronous spike on both surface and needle recordings, and its onset-latency was
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10 approximately 35 ms (Fig. 2A). The abnormal EMG activities were much more robust after muscle
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12 grasping, with longer-lasting abundant activities by needle EMG but, in comparison, only strikingly
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14 smaller activities were obtained with surface EMG (Fig. 3). The pattern of mechanomyogram during
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16 PIRCs and after muscle grasping was more irregular compared to that by Achilles tendon tapping. In
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18 contrast, muscle percussion or grasping in a control subject did not show any asynchronous or
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20 synchronous electrical activities.
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30 Figure 4 shows surface and needle EMG during rippling movements. Rapid bursts of electrical
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32 activities with short duration (<2 ms) were clearly recorded only by needle EMG (Fig. 4A, B).
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34 Surface EMG demonstrated only very low amplitude activities. The duration of each spike recorded
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36 by needle EMG was much shorter than that usually observed in normal motor unit potentials, and
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38 their waveforms resembled those of fibrillation potentials aside from larger amplitude. These EMG
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40 activities were not recorded by the thigh stretching once the maneuver failed to produce muscle
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42 rippling.
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51 Electrical activities observed by needle EMG during PIRCs, grasping-induced contractions, and
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53 rippling produced a characteristic high-pitched irregular sputtering on loud speaker like end plate
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55 spikes.
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Discussion

In this report, we demonstrated electrical activities during PIRCs and muscle rippling in a patient with RMD. The EMG features can be summarized as i) bursts of asynchronous activities, ii) short duration of individual potentials (<2 ms), and iii) low amplitude of surface EMG activities. We do not think that they are artifacts, because no corresponding activities were recorded by tapping Achilles tendon in our patient, and not at all by any task in a control subject. As another evidence, these activities did not appear when thigh stretching failed to produce muscle rippling. The shape and duration of individual potentials resembled those of fibrillation potentials, indicating that these activities originated from single muscle fibers. Phase cancellations among many, short duration potentials might have obscured appearance in surface EMG.

The electrical activities during muscle rippling, PIRCs or muscle mounding in genetic form of RMD remain under controversy. While there are several reports arguing electrical silence during involuntary muscle contractions,[3,10-14] two reports have demonstrated electrical activities during PIRCs and/or muscle rippling recorded by needle EMG in cases with genetic RMD.[1,7] As one of them, Vorgerd et al.[7] presented “rapid bursts of fiber potentials” elicited by slight translocation of the needle electrode within the muscle or mild tapping close to the point of needle insertion, being consistent with our findings.

There is no consensus either in acquired RMD. Muscle rippling was reported to be electrically silent[15] or to be associated with repetitive motor unit discharges as shown in a case with RMD with

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5 myasthenia gravis,[16] the latter suggesting hyperexcitability of the motor nerve or at its terminal. It
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8 seems discordant with ours, because the electrical activities of muscle rippling in our patient most
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11 likely represent single muscle fiber discharges rather than motor unit discharges.
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14 The precise molecular basis of the abnormally propagating contractions induced by mechanical
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16 stimuli or stretch remains unknown. The most commonly suggested mechanism is that local
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18 contraction in one portion of a fiber stretches neighboring sarcomeres, eliciting contraction there,
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21 which in turn stretches and activates sarcomeres further along the fiber.[1,17] A large amount of Ca^{++}
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24 is required, however, to produce a rapid contraction in skeletal muscles by this mechanism, and it is
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27 hard to explain the propagation of rolling movements and tapping-induced contractions in clinical
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30 cases unless action potentials is somehow involved in the process.[17] Lamb discussed that abnormal
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33 muscle contractions become “electrically silent” because no action potentials occur on the
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36 sarcolemma, while action potentials are actually generated and travelling within the aberrant tubular
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39 system, being isolated from the sarcolemma.[17] However, in the circumstances where large electric
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42 currents are produced within the T-tubular system due to fiber hyperexcitability, these large currents
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45 could be detected with the recording needle electrode, if the electrode is butted right up against the
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48 muscle fiber such that a large surface area of the fiber is very close to the electrode.[18] This would
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51 be possibly the case with our patient as well as those reported by Ricker and Vorgerd et al.[1,7]
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55 Muscle fiber excitability may vary among RMD patients given that there are phenotypical variations
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58 including RMD, limb-girdle muscular dystrophy type 1C, asymptomatic elevated serum CK, and
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5 distal myopathy within the family with the same *CAV3* mutation,[19] or that there are variations of
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8 cellular behavior among different pathogenic *CAV3* mutations.[20,21] Variability of the muscle fiber
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11 excitability as well as the location of the EMG needle may explain the discordance of
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14 electromyographic findings in RMD patients. Investigations of electrophysiological variability linked
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17 with genetic and phenotypic heterogeneity are warranted to clarify the mechanism of abnormal
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20 muscle contractions in RMD.
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57 **Abbreviations:** *CAV3*, caveolin-3; CK, creatine kinase; EMG, electromyogram; nNOS, neuronal
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60 nitric oxide synthase; PIRC, percussion-induced rapid muscle contractions; RMD, rippling muscle

disease; T-tubule, transverse-tubule

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Figure legend

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Figure 1.

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5 (A, B) Immunohistochemical analysis of caveolin-3. Caveolin-3 was markedly reduced at the
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8 sarcolemma in the present patient (B), as compared with control muscle (A). Bars in A and B: 100
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11 μm . (C) Direct sequence analysis of the caveolin-3 gene. The heterozygous G→A substitution on one
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14 allele at position 80 in the first exon of *CAV3* was disclosed with an amino acid change from arginine
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17 to glutamine at position 27.
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24 Figure 2.

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26 Surface EMG, needle EMG and mechanomyogram recorded from the gastrocnemius muscle during
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29 Achilles tendon reflex (A), percussion-induced rapid muscle contractions (PIRCs) (B). A part of the
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32 needle EMG recording during PIRC (bold line) was enlarged in a shorter time-window (C). The
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35 latency between tapping the muscle belly (arrow) and the onset of muscle contractions, i.e., PIRC,
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38 was less than 10 ms (B), while the latency between tapping the Achilles tendon (arrow) and the onset
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41 of Achilles tendon reflex, was about 35 ms (A). During PIRC (B, C), needle EMG demonstrated
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44 rapid bursts of asynchronous activities of short duration for each spike (<2 ms). The activities were
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48 very small in amplitude by surface EMG.
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58 Figure 3.

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60 Surface EMG, needle EMG and mechanomyogram recorded from the gastrocnemius muscle during

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5 grasping-induced contractions (A). A part of the needle EMG recording during grasping-induced
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8 contractions (bold line) was enlarged in a shorter time-window (B). During grasping-induced
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11 contractions, needle EMG demonstrated rapid bursts of asynchronous activities of short duration for
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14 each spike (<2 ms). The activities were very small in amplitude by surface EMG.
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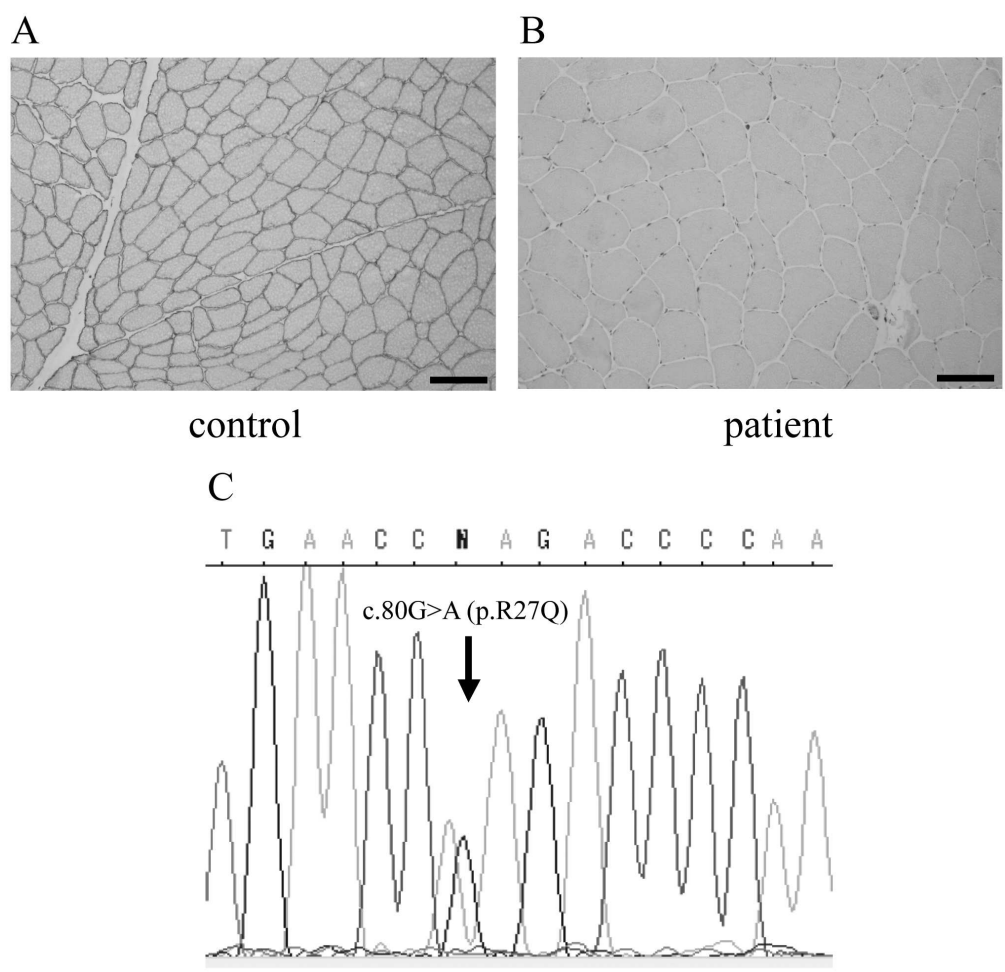
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21 Figure 4.

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23 Surface and needle EMG from the quadriceps femoris muscle during muscle rippling. In surface
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EMG, there were no robust activities recorded during rippling contractions. In contrast, rapid bursts of spiky activities were recorded by needle EMG (A). A part of the needle EMG recording during muscle rippling (bold line) was enlarged in a shorter time-window at (B). Short duration potentials (<2 ms) resembled fibrillations in shape, although mostly larger in amplitude.

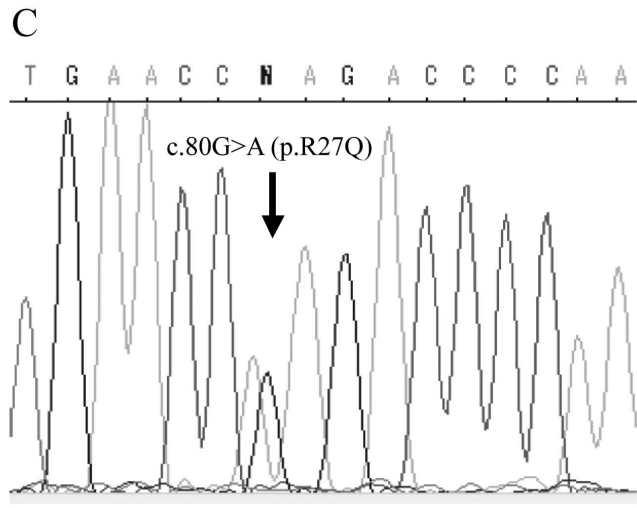
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Figure 1



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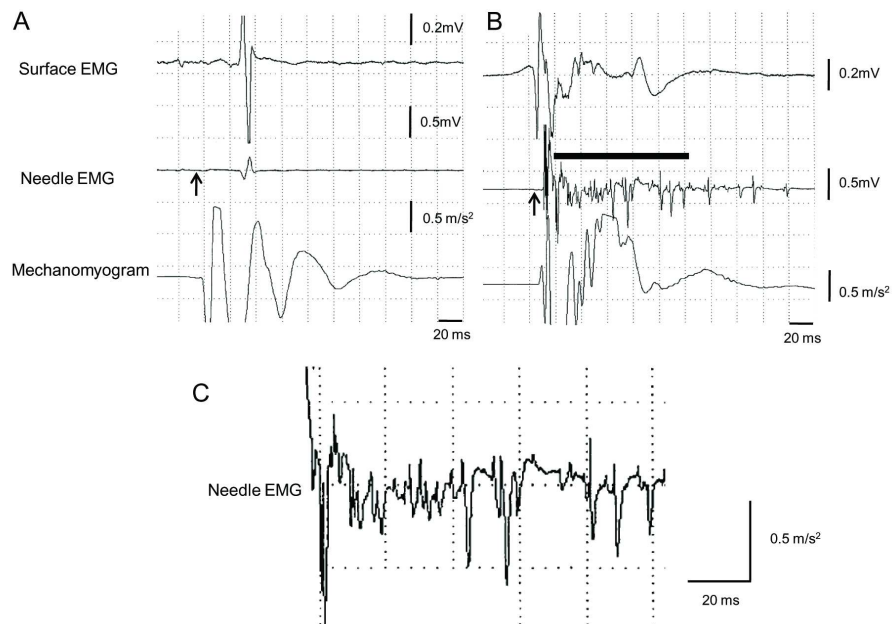
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Figure 2

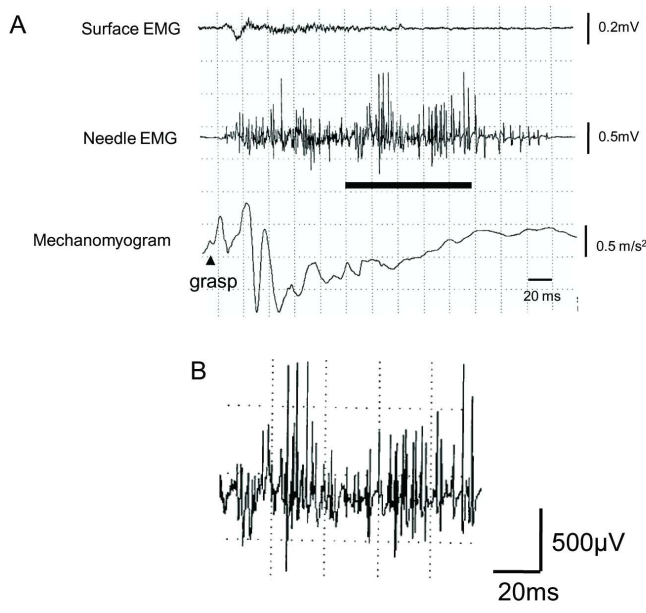


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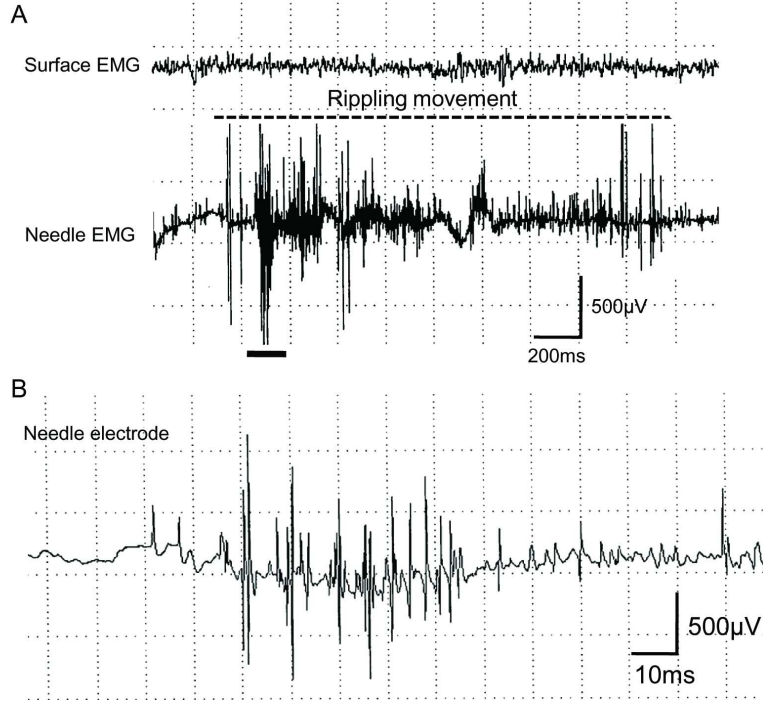
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Figure 3



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Figure 4



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