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Short Communication

Caveolin-3 gene mutation in Japanese with rippling muscle disease

Abstract

Objectives – Rippling muscle disease (RMD) is a rare myopathy characterized by percussion-induced rapid muscle contractions, muscle mounding, and rippling. Recently a caveolin-3 gene (CAV3) mutation was identified in patients with autosomal dominant RMD. The objective of this study was to determine whether a similar mutation was present in two Japanese families with this condition. *Patients and Methods*- Clinical examination, mutational analysis, and muscle immunohistochemistry were carried out in six patients from two Japanese RMD pedigrees. *Results* - Apart from the atrophy of the intrinsic muscles in their hands and a slight muscle weakness in their fingers, the clinical features of our patients were compatible with RMD. Our investigation revealed a CAV3 missense mutation i.e., Arg26Gln in both families. Immunohistochemistry performed on a muscle biopsy specimen showed

reduced caveolin-3 surface expression. *Conclusions* - Japanese RMD also appears to result from a CAV3 mutation.

Key words; Rippling muscle disease, Caveolin-3, muscle atrophy, distal myopathy, hyperCKemia

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Introduction

Rippling muscle disease (RMD) (MIM 600332) is a rare autosomal dominant disorder characterized by mechanically-induced involuntary contractions of skeletal muscle. The key clinical symptoms of RMD are muscle stiffness, exercise-induced muscle pain, and cramp-like sensations (1,2). The hallmarks of RMD are percussion-induced rapid muscle contractions, localized mounding of muscle induced by tapping, and rolling movements across the muscle (rippling) triggered by tapping or passive muscle stretch (1,2). Calf hypertrophy was sometimes observed (1). However, muscle weakness and atrophy are not prominent features of RMD (1).

RMD is genetically heterogenous. The first RMD locus to be reported was mapped to the long arm of chromosome 1 in a single Oregon pedigree (3), but this locus was excluded in all subsequently described RMD families. In 2001, Betz and coworkers showed that RMD was linked to a second locus on chromosome 3p25 in the originally reported Norwegian family and in four

German families (4).   This locus included the caveolin-3 gene (CAV3).   Four

different missense mutations in CAV3 have been identified as the molecular

basis for expression of the RMD phenotype in these independent pedigrees (4).

Interestingly, heterozygous mutations in CAV3 have also been described in

patients with autosomal dominant limb-girdle muscular dystrophy type 1C

(LGMD-1C) (5-7), and in individuals with sporadic or familial occurrence of

elevated levels of serum creatine kinase (also called hyperCKemia) (8,9).

RMD, which was reported in patients from various European countries (1-10),

is extremely rare in Japan.   Only three sporadic cases and one family with

similar clinical features to RMD have been reported (11-14).   There have not

been any reports describing a CAV3 mutation in Japanese patients with RMD.

In the present study, we report that two Japanese families showing the

presence of an autosomal dominant RMD, also carry a mutation in CAV3 and

that this CAV3 mutation leads to a partial deficiency of caveolin-3 expression at

the muscle cell surface in one patient.

Patients and methods

The pedigrees of these families are shown in Figure 1.   Solid symbols show clinically affected subjects.   All subjects were residents of Hokkaido, the northernmost island of Japan. In Family-1, an affected grandparent of the proband (I-2) migrated from Miyagi prefecture in the Tohoku area, the northern part of Honshu Island, nearly a century ago.   Neurological exams were carried out on the proband (III-1), his father (II-1) and his two sons (IV-1,-2).   In Family-2, an affected grandparent of the proband (I-1) migrated from Shikoku Island, in south-west Japan, nearly a century ago.   Neurological exams were carried out on the proband and his son. (II-1 & III-1).

Case reports of both probands

Case 1 (the proband of Family-1).   This patient was a 31-year-old man.   At age 4, he started to complain of painful cramps in the muscles of his lower limbs that typically occurred when he started to rise from a sitting position.   At age 10, he noticed cramping and stiffness in his arms when exercising in school sports. At age 13, he noticed involuntary muscle movements in his shoulder, arms and

legs after the muscles in these areas were flexed or extended strongly.   The patient's complaints always referred to muscles that were just active as opposed to those that had been at rest.   Movement after a period of rest was particularly troublesome.   For instance, after sitting in a chair for 10 minutes, he was unable to stand up and walk immediately but rather needed to get up very slowly and walk on his toes to avoid painful stiffness.   After several minutes he was then able to walk normally.

  On neurological examination, muscle strength was moderately reduced in the palmar interossei and opponens pollicis muscles {scale of muscle manual test (MMT) was 4. (the range of MMT scale; 0-5)}, while all other muscle groups showed normal strength. Mild muscle atrophy was observed in the intrinsic muscles of his hands.   Calf hypertrophy was not observed.   His cranial nerve functions, tendon reflexes, sensory perception, cerebellar functions and autonomic system were normal, as were his cognitive functions.   He moved slowly to avoid pain, stiffness, and rippling in his muscles.   Muscle rippling could easily be induced by sudden stretching or squeezing of the muscles, and

occurred most readily in his quadriceps.　Percussion or the application of rapid

pressure on a muscle point with the examiner's fingertip produced

percussion-induced rapid contraction (PIRC).　Strong percussion of a muscle

resulted in a painful and prolonged muscle mounding.

Serum creatine kinase (CK) levels (from 2297 to 4547 U/L, normal is < 180

U/L) were found to be elevated in this patient.　Other blood constituents were

normal including the levels of anti-nuclear antibodies, lactic acid and pyruvic

acid.　Needle electromyography showed no fibrillations and no myotonic runs

in the right biceps brachii nor in both rectus femoris muscles, and the shape and

recruitment pattern of their motor units were normal.　Nerve conduction

velocities were normal.　A urine analysis was also normal, as was his cervical

MRI.　Electrocardiogram was normal.　Therapeutic trials with phenytoin and

dantrolene sodium did not alter the patient's symptoms.

Case 5 (the proband of Family-2).　This patient was a 53-year-old man who, at

age 10, first noticed cramping and stiffness in his arms when exercising.　At

age 15, he noticed involuntary muscle movements in his arms and legs after the

muscles in these areas were flexed or extended strongly.　His symptoms had since remained stable.　However, he noticed muscle weakness in his hands recently.

　On neurological examination, muscle strength was severely reduced in his palmar interossei, opponens digiti minimi, abductor digiti minimi and opponens pollicis muscles (Scale of MMT was 3), and muscle atrophy was observed in the intrinsic muscles of his hands.(Fig. 2)　Mild calf hypertrophy was recognized. Muscle rippling could easily be induced, and occurred most readily in the biceps brachii muscles. PIRC was also observed.

　Serum CK levels (412 U/L) were found to be elevated in this patient.　Nerve conduction velocities and cervical MRI were normal.

　The clinical features of the other affected members of these families are shown in the table.

　Molecular genetic studies.　After informed consents was obtained from one

patient (case 1) in Family-1 and from two patients (cases 5 & 6) in Family-2,

blood samples were obtained from these individuals and DNA studies

performed.   Genetic analysis was not performed on the other affected subjects

in the pedigrees since they declined to provide their informed consent. Genomic

DNA samples were extracted using standard procedures. The 2 coding exons of

CAV3 were amplified by the polymerase chain reaction (PCR) using the

reported primers and the method that has previously been reported (4,10).

Amplified PCR products were sequenced directly using the Big Dye terminator

cycle sequencing kit (PE Applied Biosystems, Tokyo, Japan)   on an ABI PRISM

377 DNA sequencing system (PE Applied Biosystems, Tokyo, Japan).

   Muscle biopsy studies.   An open biopsy sample from the biceps brachii

muscle was taken from the proband (case 1) in Family-1.   Muscle biopsy was

not performed on the other affected subjects again because of the lack of

informed consent.

 Routine and immunohistochemical stainings were performed on 10-μm

transverse sections of frozen muscle. Routine stainings were done with

hematoxylin and eosin, trichrome, alkaline phosphatase, NADH, non-specific

esterase, and ATPase.   In immunohistochemical staining, a mouse

monoclonal antibody recognizing caveolin-3 (Santa Cruz Biotechnology Inc.,

USA) was used at a dilution of 1:500. Sections were preincubated with a 1:10

dilution of normal rabbit serum to block nonspecific binding. Endogenous

peroxidase activity was blocked by incubating the sections with 0.3% hydrogen

peroxide in absolute methanol for 30 minutes at room temperature. Sections

were incubated with primary antibody overnight at 4 . After several washes, the

sections were incubated with biotinylated rabbit anti-mouse immunoglobulin for

20 minutes. Binding of the biotinylated antibody was then detected by stepwise

incubation with avidin-biotin-peroxidase complexes followed by development of

the colored reaction product using 3,3'-diaminobenzidine tetrahydrochloride

(DAB) solution.

Results

Genetic studies.

 Direct sequencing of the two coding exons revealed a common heterozygous

mutation within exon 1 of CAV3 in all three patients. The missense mutation was due to a G→A base transition on one allele at nucleotide position 77 causing an amino acid change at codon 26 from the positively charged arginine to glutamine (Arg26Gln; R26Q) (data not shown).

Muscle biopsy studies

There were no degenerative or regenerative changes, fibrosis, or centrally-located nuclei in the patient's muscle as determined by routine stainings. Caveolin-3 expression was reduced on the sarcolemma in this patient's muscle compared to a disease control muscle biopsy sample.(Fig.3)

Discussion

In this paper, we described the first Japanese cases of autosomal dominant RMD in which the patients carried a mutation in CAV3. The CAV3 mutation in these patients was the same as that seen in some Caucasian cases and in one reported Japanese case with distal myopathy (8,10,15). The mutation (R26Q) is in the N-terminal part of the protein and affects an amino acid that is

evolutionary highly conserved.

Apart from the muscle atrophy and weakness in adult patients, the clinical and pathological features in these patients were compatible with a diagnosis of RMD. Immunohistochemistry performed on the muscle from one of these patients showed reduced muscle caveolin-3 surface expression.

Some RMD cases with the same mutation as in our patients (R26Q) have been reported to be associated with hyperCKemia (8). In addition, a Japanese patient with distal myopathy but without rippling muscles was recently reported to have had the same mutation (15). These data imply that other genes or factors are involved in the clinical expression of this disease. Moreover, the distribution of muscle atrophy and weakness in a Japanese patient with distal myopathy was reported to be similar to that seen in our patients (15). Since muscle atrophy and weakness have not been reported in patients from other countries who had a CAV3 mutation, these clinical findings may be a reflection of the unique pathogenetic factors and genes that play a role in mediating the disease in Japanese patients. (8,10,15)

The R26Q mutation has also been found in three different de novo cases of hyperCKemia or RMD (8,10).   This region , which is included in the first exon of CAV3, is considered to be a genetic hot spot for the development of a common sporadic mutation.   Our cases and the other reported Japanese case with distal myopathy have the same mutation, further supporting an important role for this region in the N-terminal domain of caveolin-3 in disease pathogenesis (15).

Neuronal nitric oxide synthase (nNOS) activity is already known to be inhibited by caveolin. Betz and coworkers reported that increased activity of nNOS as a result of a CAV3 mutation might contribute to the development of increased mechanical muscle hyperexcitability, muscle stiffness and hyperCKemia (4). Although the absolute number of patients with RMD in Japan is very small, there are a lot of patients who have hyperCKemia with muscle stiffness (16).   It is conceivable that some of those patients whose quality of life has been diminished as a result of their muscle contractions and stiffness, may have a CAV3 mutation.   Furthermore, other muscle diseases may also be

caused by a CAV3 mutation since the myopathies associated with mutations in

this gene are known to show clinical heterogeneity.

Legend for Figure 1

The rippling muscle disease pedigrees of our patients' families.□, man; ○, woman; / ; deceased. Solid symbols show subjects affected with rippling muscle disease.

Legend for Figure 2

Muscle atrophy in the intrinsic muscles of case 6.

Legend for Figure 3

Immunohistochemical staining of muscle obtained from our patient (a) and from a control subjects (b) using a caveolin-3-specific antibody (X66). Caveolin-3 staining was absent from the muscle of our patient.

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Table 1

Clinical findings of affected members in two Japanese families with rippling muscle disease.
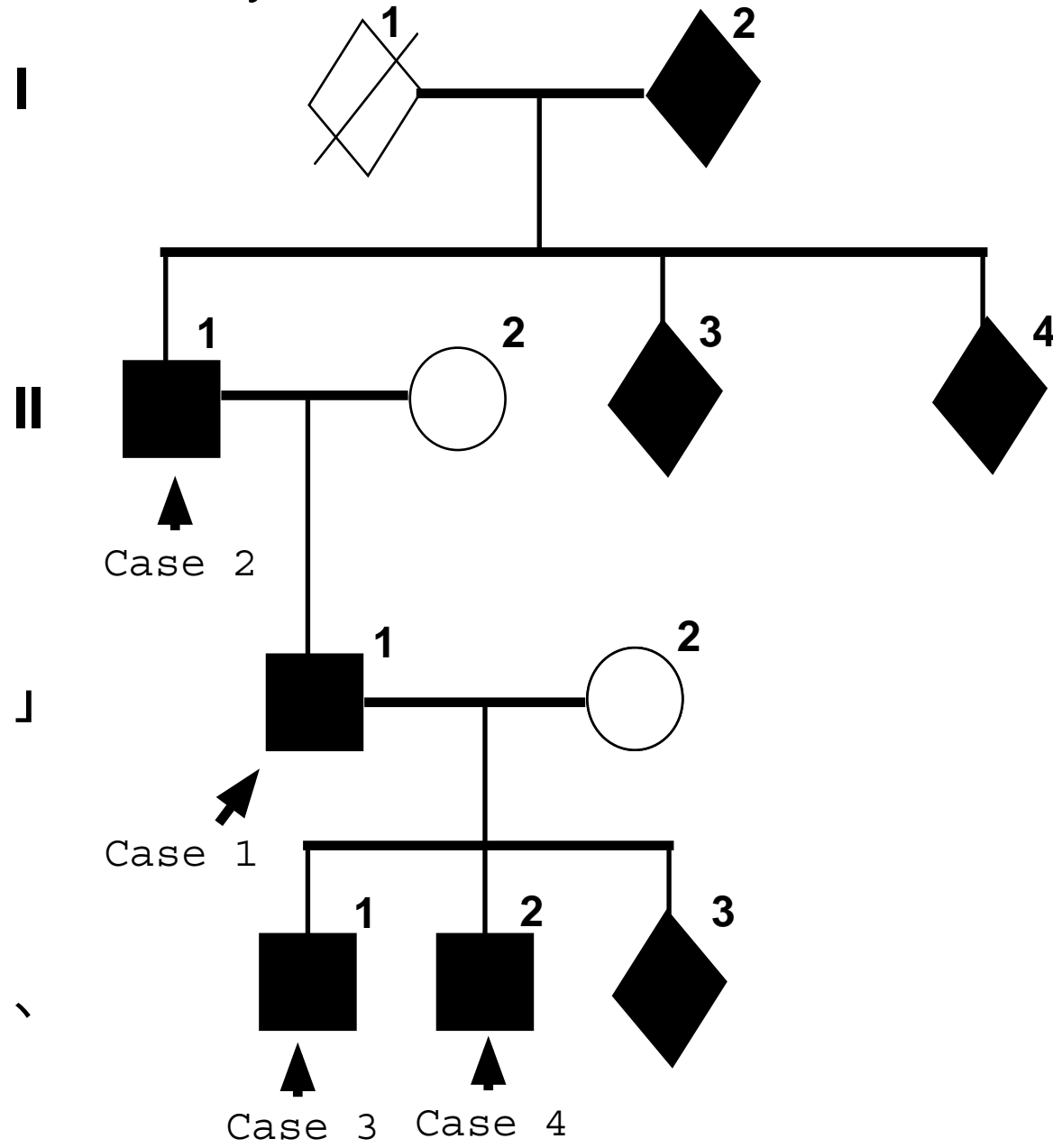
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age (years) / Gender	31 / Male	60 / Male	6 / Male	4 / Male	53 / Male	22 / Male
Age of onset (years)	4	15	3	3	10	7
Rippling in muscles	+	+	+	+	+	+
PIRC	+	+	+	+	+	+
Muscle atrophy in hands	+	+	-	-	+	+
Muscle weakness in hands	+	+	-	-	+	+
(Scale of MMT)	(4)	(4)	(5)	(5)	(3)	(4)
Calf hypertrophy	-	-	+	+	+	+
HyperCKemia	+	+	+	+	+	+
(normal is < 180 U/L)	2,297-4,547U/L	376U/L	1470U/L	1135U/L	412U/L	2350U/L

PIRC; percussion-induced rapid contraction

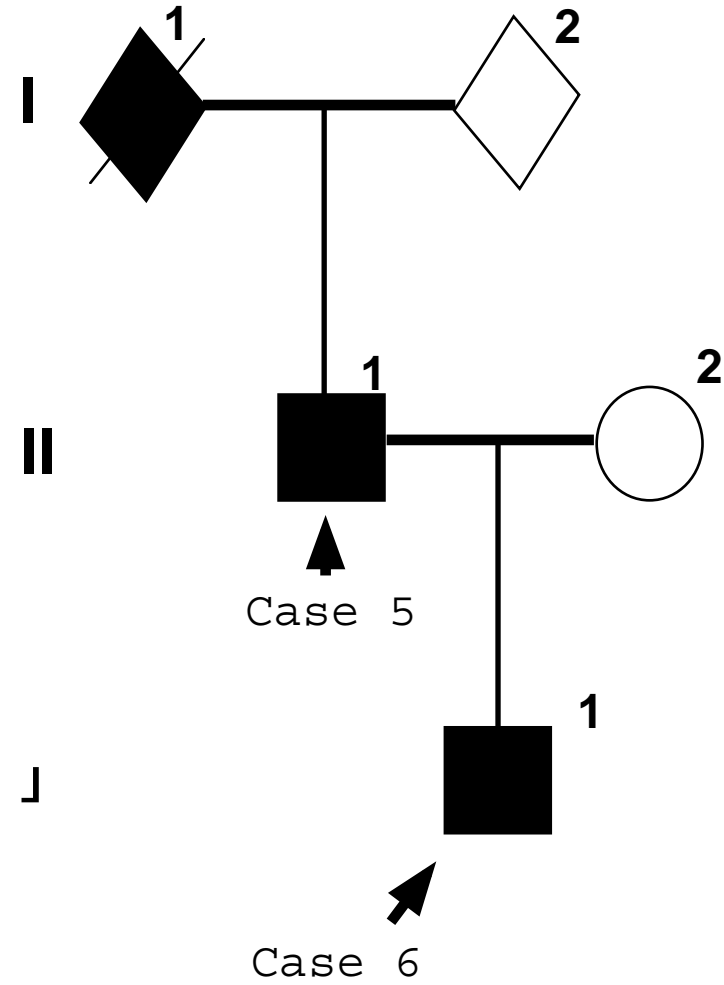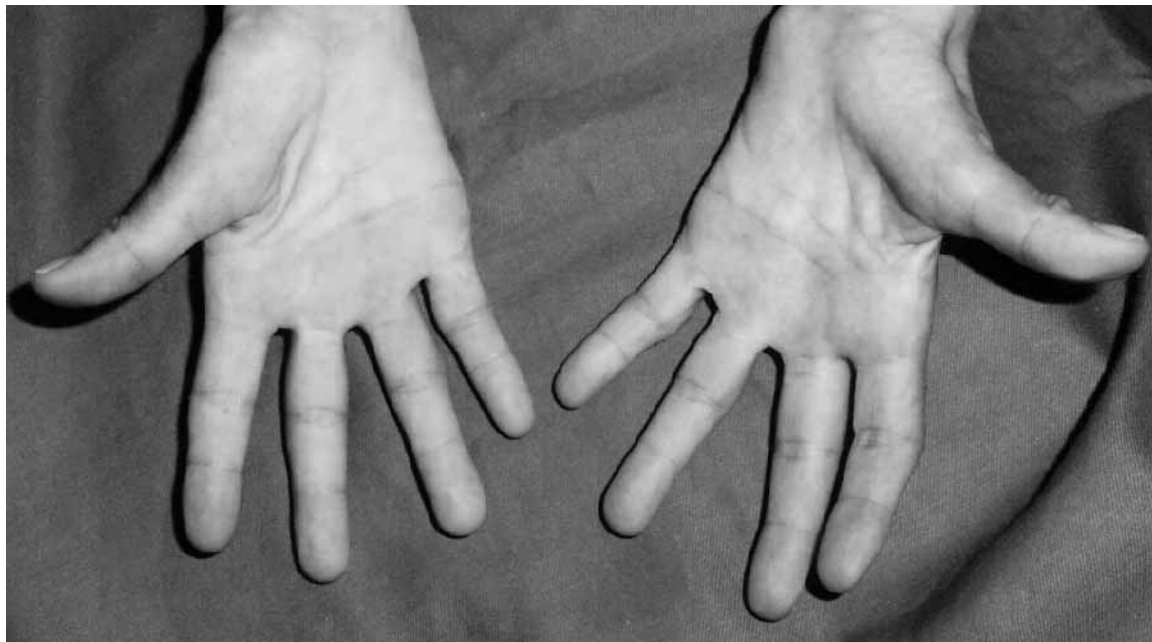MMT; manual muscle testing (the range of MMT scale; 0-5)

Figure 1

Figure 2

Figure 3

a

b