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Characteristic findings of skeletal muscle MRI in caveolinopathies

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Abstract

Caveolinopathies, caused by *CAV3* mutations, can include several phenotypes such as rippling muscle disease, limb-girdle muscular dystrophy type 1C, distal myopathy, familial hypertrophic cardiomyopathy, and idiopathic hyperCKemia. Here we present characteristic skeletal muscle imaging findings in four patients with genetically defined childhood-onset RMD caused by *CAV3* mutations and in one patient with congenital generalized lipodystrophy type 4 with muscular dystrophy due to *polymerase I and transcript release factor (PTRF)* mutations, which may have caused secondary deficiency of caveolin-3. Muscle MRI revealed that the rectus femoris and semitendinosus muscles were most commonly affected in the rippling muscle disease patients. Peripheral changes in the rectus femoris were specific and observed even in one of the younger patients in this study. Furthermore, muscle involvement extended to the semitendinosus muscles, biceps femoris, and gracilis with disease progression or increase in its severity. Similar patterns of involvement were observed on reviewing skeletal muscle images of various previously reported phenotypes of caveolinopathy; interestingly, patients with secondary deficiency of caveolin due to *PTRF* mutations revealed the same pattern. Thus, primary caveolinopathies and secondary deficiency of caveolin demonstrated specific findings on skeletal muscle imaging, regardless of the broad phenotypic spectrum of these two conditions.

Keywords: Caveolinopathy; Caveolin-3; Rippling muscle disease; Limb-girdle muscular dystrophy 1C; Skeletal muscle magnetic resonance imaging; Polymerase I and transcript release factor.

1. Introduction

Caveolins are important components of uncoated plasma membrane invaginations that regulate both signal transduction and vesicular trafficking [1]. Mutations in the *CAV3* gene encoding caveolin-3 that maps to chromosome 3p25 can produce several phenotypes, including Rippling muscle disease (RMD), limb-girdle muscular dystrophy type 1C (LGMD1C), distal myopathy, familial hypertrophic cardiomyopathy, and idiopathic hyper creatine kinasemia (hyperCKemia) [2,3]. The clinical phenotypes may overlap each other within the same individual or in individuals within the same family and present with the same mutations in *CAV3*; thus, the genotype-phenotype correlation remains unclear [4].

RMD is a rare autosomal dominant disorder, characterized by signs of increased muscle irritability. These signs, which give RMD its name include percussion-induced rapid contraction, percussion-induced localized muscle mounding, and/or

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percussion/ stretching-induced rolling movements across a muscle group (muscle rippling) [5]. Patients with RMD may begin to suffer from muscle cramps, pain, and stiffness during the first or second decade of their lives. Despite normal muscle strength, patients with RMD are often unable to sit down on their heels, and toe walking is a common observation. Recently, congenital generalized lipodystrophy type 4 with muscular dystrophy (CGL4) due to *polymerase I and transcript release factor (PTRF*) mutations, has been reported (OMIM #613,327). Patients with PTRF deficiency present with loss of subcutaneous adipose tissues from birth, manifestations of muscular dystrophy, arrhythmia, and several other symptoms [6]. Secondary reduction of caveolin-3 may progress with age or disease progression, and percussion-induced muscle mounding is a characteristic finding in patients with PTRF deficiency as well as in patients with RMD caused by *CAV3* mutations.

Several skeletal muscle-imaging studies have recently been reported for differential diagnosis and assessment of the progression of each distinct neuromuscular disorder. T1-weighted (T1W) skeletal muscle magnetic resonance imaging (MRI) of the lower limbs in genetically distinct neuromuscular disorders have identified specific patterns characteristic to these neuromuscular diseases. To date, there have been a few reports on specific muscle MRI patterns in patients with caveolinopathies. Herein, we present characteristic skeletal MRI findings in four patients with childhood-onset RMD, who were identified with known *CAV3* mutations, and in one patient with CGL4, who developed secondary caveolin-3 deficiency due to PTRF deficiency. In addition, we reviewed skeletal muscle imaging examinations of previously reported caveolinopathies.

2. Material and methods

Muscle MRI was performed using conventional T1 weighted spin echo on a 1.5-tesla TOSHIBA or Philips MR scanner. Non-contrast images of the thighs and legs were obtained. The axial plane was selected with respect to the long axis of the body. The thickness of the slices ranged from 5 to 10 mm with a gap of 10–20 mm between each slice. A fast gradient echo was used for pilots, whereas a spin echo pulse sequence was used for the images (repetition time [TR], 424–696 ms; echo time [TE], 10–18 ms; row matrix 320–512 points; column matrix, 219–512 points; and variable field of view [FOV], 25–40 cm). The average total examination time was 30 min per patient. Skeletal muscle MRI was evaluated by a radiologist, a neurologist, and two pediatric neurologists. Individual muscles of the patients were analyzed; the involvement of 19 muscles in the leg (12 in the thigh and 7 in the lower leg) were assessed and categorized using the Mercuri score [7] as follows: Stage 0, normal appearance; Stage 1, scattered small areas of increased density; Stage 2a, numerous discrete areas of increased density less than 30% of the volume of the muscle; Stage 2b, numerous discrete areas of increased density with early confluence (30%–60% of the volume of the muscle); Stage 3, washed-out appearance due to confluent areas of increased density with muscle still present at the periphery; and Stage 4, end-stage appearance with muscle entirely replaced by areas of increased density. The patterns of each of the affected muscles were classified according to the Treat-NMD workshop report as follows: Stage 0–1, black; Stage 2a–2b, gray; and Stage 3–4, white (Figs. 1 and 2; cross sections of thigh and calf) [7,8].

3. Case description

3.1. Case 1

Case 1 was a 28-year-old woman, who had an elder brother with epilepsy, but no skeletal muscle symptoms; her parents and an elder sister were also asymptomatic (Fig. 3(a)). History revealed that she would fall down very often at 5–6 years of age. At 11 years of age, she started to complain of muscle pain in the thigh after exercise, which was followed by rapid progression of calf muscle hypertrophy. At the age of 12, she was found to have a high serum CK level (14,171 U/L). Neurological evaluation revealed moderate muscle weakness of the neck anteflexion (3/5) and mild muscle weakness (4/5) in the proximal limb muscles. She showed symptoms like weak grip and apparent percussion myotonia. Deep tendon reflexes were slightly hyperactive in all extremities. She was unable to sit down on her heels due to calf muscle hypertrophy. Muscle CT revealed atrophic and moth-eaten changes in the rectus femoris, hamstrings, and gastrocnemius, with hypertrophy of the vastus lateralis and paraspinal muscles. Fig. 4 presents her skeletal muscle CT at 18 years of age. Muscle biopsy revealed moderate muscle fiber size variation, a few necrotic and regenerating fibers, and moderate endomysial fibrosis. The number of internal nuclei was increased and immunohistochemistry revealed that the cells were negative for caveolin-3. The subject continued to experience stiffness and cramps with gradual progression of limb-girdle muscle weakness. At 28 years of age, she complained of difficulty in jumping and climbing upstairs, and thus, skeletal muscle MRI images were obtained (Figs 1 and 2). Genetic analysis revealed a *CAV3* mutation: p. Glu47Asp, which has been reported to cause RMD [2].

3.2. Cases 2 and 3

Cases 2 and 3 were siblings, an 8-year-old and a 6-year-old boy, respectively. At 9 months of age, the proband case (Case 3) was evaluated for motor development delay. He was unable to sit without support and mild muscle weakness was noted. At 17 months, elevated serum CK levels (500–800 U/L) were observed. He had been unable to jump at 2 years and 6 months of age, and experienced muscle pain after exercise and upon awakening at the age of 4 years and 8 months. He lacked the strength to unscrew bottle caps. Neurological evaluation revealed isolated muscle weakness in the anteflexion of the neck (3/5). He showed calf hypertrophy and difficulty bending the ankle joints. Symptoms similar to percussion myotonia such as mounding of his leg muscles and rippling were

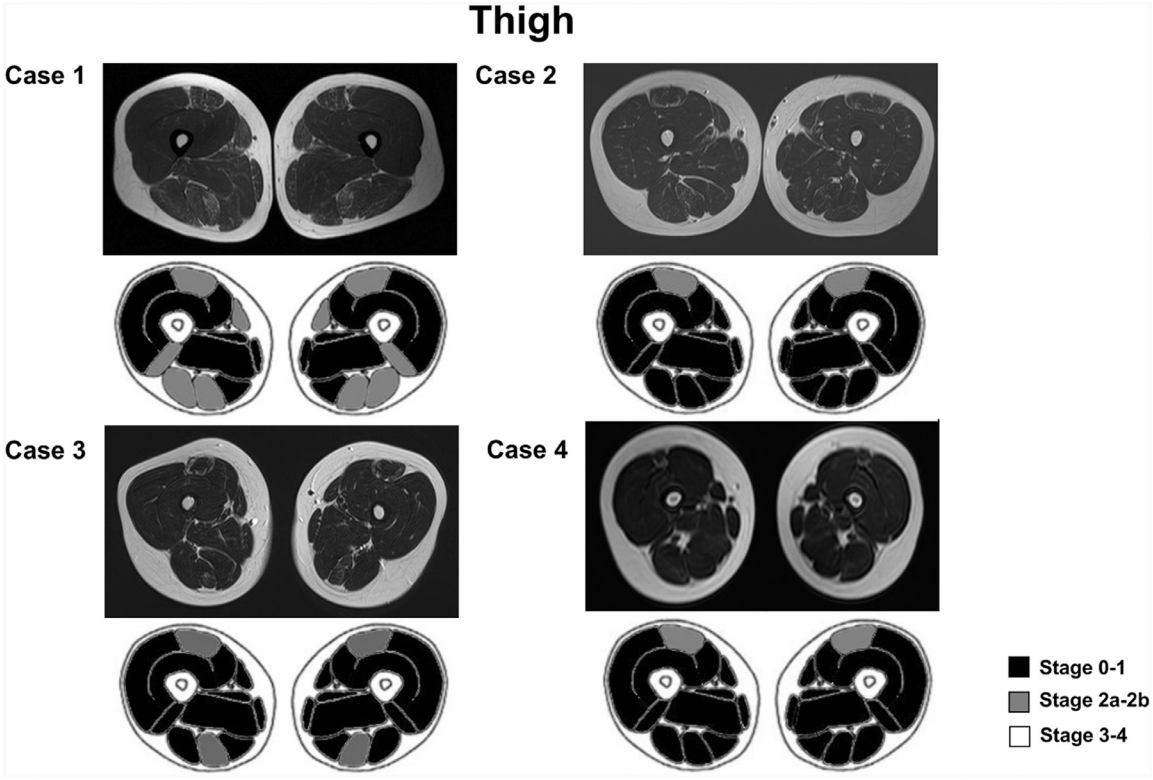

Fig. 1. Muscle MRI findings and classification according to the Mercuri score of Thigh. T1W muscle MRI imaging (upper) and the schema (lower) of the affected muscles of the thigh (a) of each of the four cases. The schema indicates the pattern of muscles affected based on the individual muscles assessed. The finding was graded according to the Mercuri score as follows: Stage 0–1, presented as black color; Stage 2a–2b, gray; and Stage 3–4, white. The rectus femoral, sartorius, and biceps femoral muscles appeared shrunken and irregular in shape with indentations between the muscles and the connective tissue resulting in a wrinkled effect on the MR images.

detected. Deep tendon reflexes were normal. Gower's sign was negative. Muscle biopsy at the age of 3 years indicated moderate muscle fiber size variation, few necrotic and regenerating fibers, and moderate endomysial fibrosis. The number of internal nuclei was increased and the cells were negative for caveolin-3. Family history revealed that both his mother, 36 years old at presentation, and his elder brother (Case 2), had muscle pain and stiffness (Fig. 3(b)). His mother had often experienced leg cramps since elementary school.

The 8-year-old boy (Case 2) presented with calf muscle hypertrophy and complained of muscle pain after school. Both he and his mother showed mounding of the lower legs. The severity of increased muscle irritability in Case 2 was more remarkable than that in Case 3. Skeletal muscle MRI was performed for Cases 2 and 3 at 8 and 4 years of age, respectively (Figs. 1 and 2). Genetic analysis revealed the presence of a mutation in *CAV3*: p. Arg27Gln in the two boys and their mother. This mutation has previously been associated with RMD [9,10].

3.3. Case 4

Case 4 was a 2-year-old girl, who was born as a very low birth weight infant with aortic coarctation (Fig. 3(c)). At 3 months of age, elevated CK levels (1417 U/L) were observed when she underwent catheter treatment for the cardiac problem. She showed mild motor developmental delay, such as gained head control at 5 months and walking at 21 months of age. The subject presented with hyperreflexia, which included the biceps and triceps reflex, patellar reflex, and Achilles tendon reflex. She showed symptoms similar to percussion myotonia and apparent mounding. Her father was also noticed to have mild mounding, rippling, and thigh and calf muscle hypertrophy. He could not sit down on his heels, although his symptoms were much milder than that of his daughter. Genetic analysis of the patient and her father revealed the presence of a mutation in *CAV3*: p. Arg27Gln, which was the same as that seen in Cases 2 and 3. Skeletal muscle MRI was performed for Case 4 at 2 years of age (Figs. 1 and 2).

3.4. Case 5

Case 5 was a 3-year-old boy with CLG4 caused by *PTRF* mutations [11]. He showed delayed motor development (started walking at 2 years and 6 months of age) and insulin resistance (at 3 years of age). He presented with a homozygous c.696_697insC mutation in *PTRF*. Muscle CT was evaluated at the age of 3 years (Fig. 5).

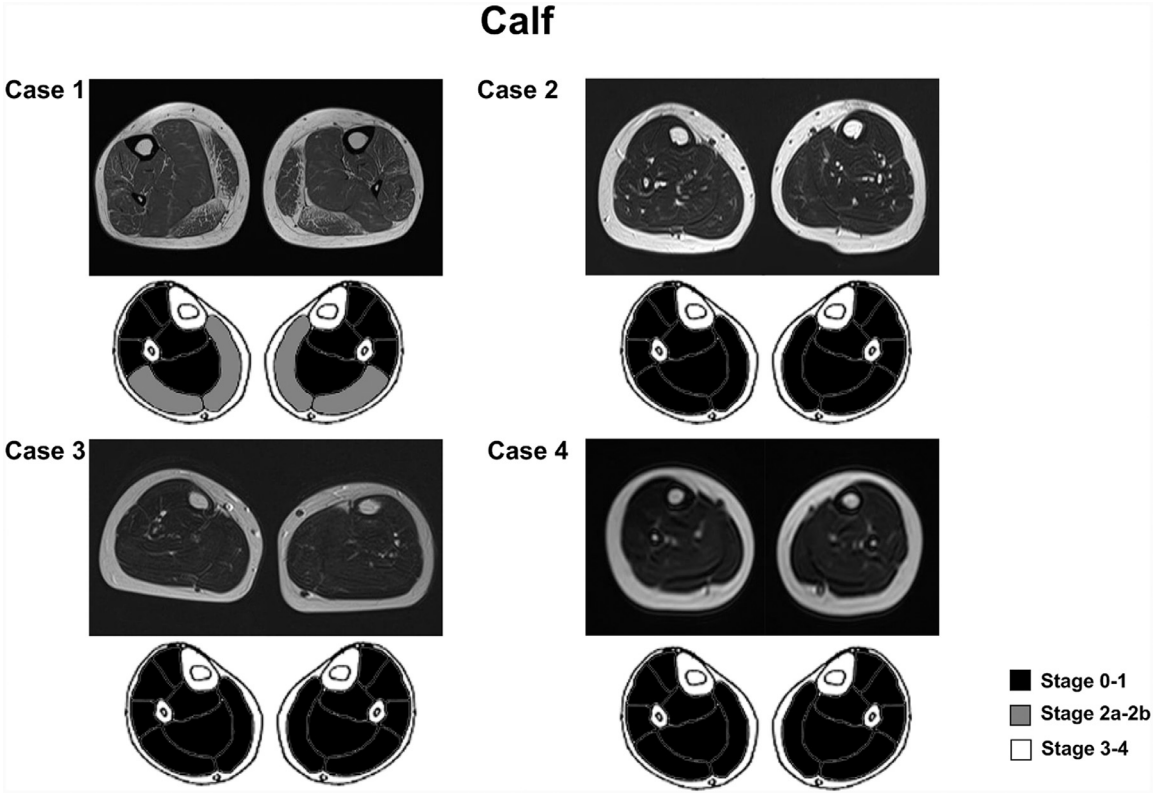

Fig. 2. Muscle MRI findings and classification according to the Mercuri score of Calf. T1W muscle MRI imaging (upper) and the schema (lower) of the affected muscles of the calf of each of the four cases, as Fig. 1. Only Case 1 showed mild changes in the gastrocnemius muscles.

4. Results

Skeletal Muscle MRI (Figs. 1 and 2) and CT scans (Figs. 3 and 4)

Muscle CT scans of Case 1 at 18 years of age revealed atrophic and moth-eaten changes in the rectus femoris, hamstrings, and gastrocnemius (Fig. 4).

Changes in T1-weighed skeletal muscle MRI scans of the thigh and calf muscles were evaluated. In Case 1, MRI scans of the skeletal muscles of the calves were obtained a few years after those of the thighs. The pattern of changes was found to be similar in all four cases (Fig. 1). Case 1 showed diffuse changes mainly in the rectus femoris, sartorius, biceps femoris, and semitendinosus muscles. In addition, mild changes were noted in the semimembranosus and gastrocnemius muscles. The rectus femoris and semitendinosus muscles were also affected in Case 2. The vastus medialis, but not the calf muscles, were also involved. The most marked changes in Case 3 included peripheral changes in the rectus femoris and atrophy of the semitendinosus muscles, whereas the biceps femoris was very mildly affected. The youngest patient in the current study (Case 4) had already demonstrated apparent peripheral changes in the rectus femoris. In fact, changes in rectus femoris were commonly noticed in all four cases in this study. The changes were more diffuse in Case 1, whereas in the other three cases, atrophic or peripheral changes forming ring-like patterns were noted in the rectus femoris.

Muscle CT images from Case 5 revealed peripheral involvement of the rectus femoris and marked reduction in subcutaneous adipose tissue (Fig. 5).

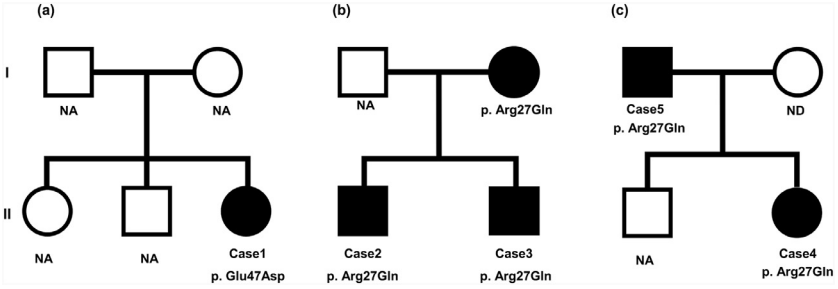

Fig. 3. Family trees of the four patients. (a) Case 1, (b) Case 2, (c) Case 4. ND: mutations were not detected. NA: patients were not analyzed.

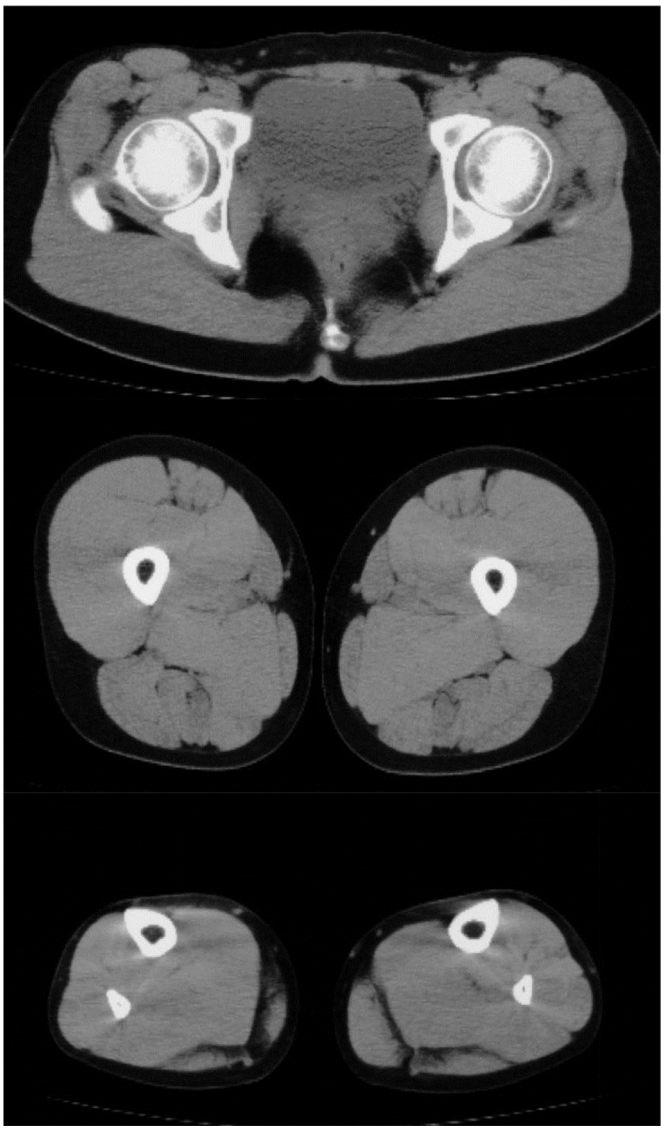

Fig. 4. Muscle CT scan images of Case 1 aged 18 years old. Muscle CT scan revealed atrophic and moth-eaten changes in the rectus femoris, hamstrings, and gastrocnemius.

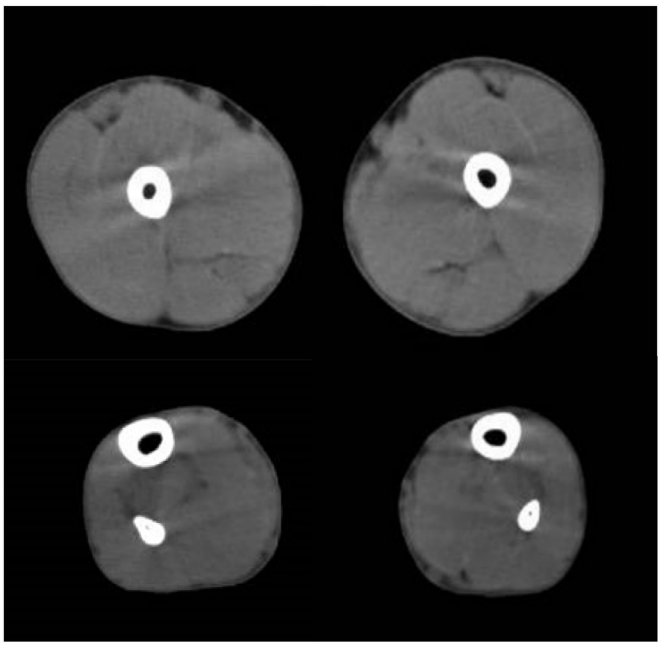

Fig. 5. Muscle CT image of the patient with PTRF deficiency. Muscle CT image of the 3-year-old boy with PTRF deficiency. Atrophic or peripheral fatty changes were observed in the rectus femoris only.

5. Discussion

In recent years, the importance of clinical muscular imaging in patients with suspected or proven inherited muscle diseases has increasingly been recognized [12]. The specific patterns of skeletal muscle imaging facilitate differential diagnosis in some muscular dystrophies. To date, there have been a few reports on skeletal muscle imaging in patients with caveolinopathies; most patients with RMD demonstrated normal skeletal muscle imaging [13]. In the present study, we reported four patients, including young children from three families, with childhood-onset RMD. The striking findings of this study using skeletal muscle MRI were that the peripheral rectus femoris and semitendinosus muscles were affected in the four childhood-onset RMD patients. The rectus femoris, in particular, showed atrophic or peripheral involvement in a ring-like pattern, which appears to be specific for this disease. Careful review of previous case reports revealed that peripheral changes in the shape of the rectus femoris muscle and diffuse changes in the semitendinosus muscle were common in patients with RMD [13,14]. Scalco et al. reported that only one (a 12-year-old boy) of the eight patients in their study had presented with abnormal MRI findings (mild involvement of the rectus femoris, sartorius, biceps femoris, gastrocnemius, and semitendinosus muscles); the remaining patients had demonstrated normal muscle images or not been tested. In another case report on a family with RMD, a 28-year-old male presented with striking symmetric lipomatous degeneration of the distal parts of the rectus femoris, and mild changes in the semitendinosus muscles, similar to Case 1 in the current study. In another report involving a 39-year-old Japanese male with RMD and extraocular muscle paresis, peripheral changes in the rectus femoris and mild changes in the semitendinosus muscles were clearly noticeable in the figure, although the authors had not commented on them in the original report [14].

We evaluated muscle involvement changes specific to various stages ranging from early infancy to adulthood in RMD and identified a correlation between the degree of muscle involvement and the age or stage. The rectus femoris and semitendinosus muscles were most affected in all four cases, although the range of involvement increased as the disease progressed with age (Case 1). Furthermore, the pattern of muscle MRI involvement was detectable even in a toddler (Case 4), where the change was detected only in the rectus femoris, whereas in school-aged children, the muscle involvement had expanded from the rectus femoris to the semitendinosus and

sartorius muscles. Interestingly, Case 1, the most severely affected adult patient with muscle weakness, showed high T1W signal intensities extending to the semimembranosus, biceps femoris, gracilis, adductor longus, and gastrocnemius muscles. In previous reports, adult patients with RMD showed abnormal MRI findings in not only the rectus femoris and semitendinosus muscles but also in the semimembranosus, biceps femoris, gracilis, and tibialis anterior muscles. Patients with prominent signs of RMD or those with overlapping LGMD 1C tended to show diffused and marked involvement in the skeletal muscle MRI [4,15]. Case 1 developed muscle weakness during adulthood, which could be classified as overlapping of RMD and LGMD1C. The widespread muscle involvement in the MRI findings might suggest the progression of the disease with aging in this patient (Case 1); in addition, it may indicate the severity of the disease or the signs of overlap with LGMD1C. However, the number of patients in this study was small. Longitudinal studies with increased number of RMD cases showing muscle involvement via MRI are necessary to establish the characteristic MRI findings of this disease.

On the other hand, the muscle CT scans in patients with CGL4 due to *PTRF* mutations showed similar patterns to those seen in patients with caveolinopathies. One of the clinical features of patients with *PTRF* mutations was percussion-induced muscle mounding, a characteristic feature of RMD. *PTRF* mutations cause PTRF deficiency, which may lead to secondary deficiency of caveoline-3 with disease progression. Interestingly, the patterns of skeletal muscle involvement in patients with secondary deficiency of caveolin-3 (due to *PTRF* mutations) were similar to that seen in patients with primary caveolinopathies. Further studies involving a larger number of cases are required to evaluate the progression of involvement patterns of the muscles in patients with secondary caveolinopathy

6. Conclusion

In conclusion, characteristic MRI findings appear to be useful for the diagnosis of caveolinopathies, especially in childhood-onset RMD. Longitudinal studies involving more cases with caveolinopathies and secondary deficiency of caveoline-3 are merited to confirm the findings of the current study.

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