manifestations of a blockade of liver blood outflow, but the elevation in inferior vena cava pressure caused by a sharp rise in intrapleural pressure using MI-E might have been a potential cause of peliosis hepatis.

In conclusion, peliosis hepatis is a rare but important fatal complication that may occur more often once genetic or other therapies for XLMTM become available with a resulting increase in life expectancy. To avoid fatal hepatic hemorrhage from peliosis hepatis, routine liver function tests and abdominal imaging studies are recommended for all XLMTM patients. In addition, it might be necessary to carefully check liver imaging tests, especially at the time of using mechanical ventilation.

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# Limb-girdle muscular dystrophy type 2I is not rare in Taiwan

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**Abstract**

Alpha-dystroglycanopathy is caused by the glycosylation defects of α-dystroglycan (α-DG). The clinical spectrum ranges from severe congenital muscular dystrophy (CMD) to later-onset limb girdle muscular dystrophy (LGMD). Among all α-dystroglycanopathies, LGMD type 2I caused by *FKRP* mutations is most commonly seen in Europe but appears to be rare in Asia. We screened uncategorized 40 LGMD and 10 CMD patients by immunohistochemistry for α-DG and found 7 with reduced α-DG immunostaining. Immunoblotting with laminin overlay assay confirmed the impaired glycosylation of α-DG. Among them, five LGMD patients harbored *FKRP* mutations leading to the diagnosis of LGMD2I. One common mutation, c.948delC, was identified and cardiomyopathy was found to be very common in our cohort. Muscle images showed severe involvement of gluteal muscles and posterior compartment at both thigh and calf levels, which is helpful for the differential diagnosis. Due to the higher frequency of LGMD2I with cardiomyopathy in our series, the early introduction of mutation analysis of *FKRP* in undiagnosed Taiwanese LGMD patients is highly recommended.
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*Keywords:* Alpha-dystroglycan; Alpha-dystroglycanopathy; Limb-girdle muscular dystrophy type 2I; *FKRP*; Dilated cardiomyopathy; Glycosylation defect; Laminin binding; Muscle imaging

## 1. Introduction

Alpha-dystroglycanopathy is a group of muscular dystrophies caused by altered glycosylation of α-dystroglycan (α-DG), which is one of the components of dystrophin–glycoprotein complex [1,2]. The clinical phenotypes form a broad spectrum, ranging from severe congenital muscular dystrophy (CMD) with or without ocular and central nervous system involvement to later-onset limb girdle muscular dystrophy (LGMD) [3–5].

A number of genes have been reported to cause α-dystroglycanopathy, including *POMT1*, *POMT2*, *POMGnT1*, *FKTN*, *FKRP*, and *LARGE* that are known to be involved in glycosylation of α-DG, and *DAG1*, which encodes DG itself [6–11]. Recently, the number of genes associated with α-dystroglycanopathy has been increasing to include *ISPD*, *TMEM5*, *GTDC2*, *B3GNT1*, *DOLK*, *DPM2* and *DPM3* [12–19]. Patients with all

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kinds of α-dystroglycanopathy are inherited with autosomal recessive trait.

Among those causative genes for α-dystroglycanopathy, *FKRP* mutations are the most frequently seen in the Caucasian population, causing LGMD2I and congenital muscular dystrophy type 1C (MDC1C). In the Asian population, on the other hand, the most common α-dystroglycanopathy is Fukuyama congenital muscular dystrophy and LGMD2M caused by the mutations in *FKTN* [20–24]. This phenomenon may be caused by the founder effect of c.826C>A substitution in *FKRP* and the ancestral insertion of a SINE-VNTR-Alu (SVA) retrotransposon in *FKTN* in different geographic areas [21,25]. Recently, an increasing number of patients having *FKTN* mutations were identified outside Asia but so far few Asian patients with LGMD2I caused by *FKRP* mutations have been reported [26–29].

In this study, we found that LGMD2I is common in the Taiwanese patients with α-dystroglycanopathy due to a common mutation, c.948delC (p.Cys317Alafs*111), which may cause more severe phenotype and cardiomyopathy.

## 2. Materials and methods

### 2.1. Patients

Forty patients clinically and pathologically diagnosed as LGMD and 10 patients with CMD who received muscle biopsy in Kaohsiung Medical University Hospital from January, 2008 to December, 2011 were enrolled. LGMD was defined as progressive proximal-dominant muscle weakness with characteristic dystrophic changes in muscle pathology. CMD was recognized as infantile floppiness with dystrophic muscle. Patients with deficiencies of dystrophin, sarcoglycans, dysferlin, merosin or collagen VI were excluded by immunohistochemistry beforehand. All merosin deficiency patients were confirmed to have *LAMA2* mutations [30]. This study was approved by the institutional review board of the Kaohsiung Medical University Hospital.

### 2.2. Histochemistry

Biopsied muscle specimens were frozen in isopentane cooled in liquid nitrogen. A serial frozen section was stained by a battery of histochemical methods including hematoxylin and eosin (H&E), modified Gomori-trichrome (mGt) and NADH-tetrazolium reductase (NADH-TR).

### 2.3. Immunohistochemistry

Frozen sections of 6 μm thickness were used for immunohistochemistry according to the standard protocols with Vantana Benchmark automated stainer. Primary antibodies used in this study were monoclonal anti-α-DG (VIA4-1; Upstate Biotechnology, Lake Placid, NY, USA) and anti-β-DG (43DAG1/8D5; Novocastra Laboratories, Newcastle upon Tyne, UK) antibodies.

### 2.4. Immunoblotting and laminin overlay assay

The detailed techniques of immunoblotting, and laminin overlay assay have been described previously [31]. The following antibodies were used for immunoblotting analysis: monoclonal anti-α-DG (VIA4-1) and polyclonal anti-α-DG (GT20ADG, kindly provided by Prof. K. Campbell, Iowa Univ.), polyclonal anti-laminin-1 (Sigma, St. Louis, MO, USA), and monoclonal anti-β-DG (43DAG1/8D5).

### 2.5. Mutation analyses of α-DGP associated genes

Genomic DNA was extracted from leukocytes in peripheral blood lymphocytes according to standard protocols. All exons and their flanking intronic regions of *FKRP* (NM_024301.4), *FKTN* (NM_001079802.1), *POMGnT1* (NM_001243766.1), *POMT1* (NM_007171.3), *POMT2* (NM_013382.5), and *LARGE* (NM_004737.4) were amplified and sequenced using an automated 3100 DNA sequencer (Applied Biosystems, Foster, CA, USA). Primer sequences are available upon request. DNA samples from 100 Taiwanese individuals without apparent neuromuscular disorders were analyzed as controls.

## 3. Results

### 3.1. Patients with α-DGP caused by FKRP mutations

Seven of 50 patients with unclassified LGMD and CMD had a reduced α-DG immunoreaction using VIA4-1antibody, which recognizes glycosylated forms of α-DG on muscles, and they were thus considered to have α-dystroglycanopathy (Fig. 1). Among these seven patients, six had LGMD phenotype and one was CMD. Mutation screening revealed that five LGMD patients from four families harbored *FKRP* mutations (Fig. 2). No mutation in *FKTN*, *POMGnT1*, *POMT1*, *POMT2* and *LARGE* was identified in these seven patients. The clinical, pathological and biochemical information of all five patients with *FKRP* mutations are summarized in Table 1 together with that of a previously reported Taiwanese LGMD2I patient who was the first reported case in East-Asia (Patient 6) [26]. The c.948delC (p.Cys317Alafs*111) mutation was found heterozygously in four newly diagnosed patients (Patients 2, 3, 4 and 5) as well as in Patient 6. Patients 2, 3, and 4 carried a c.545A>G (p.TyrY182Cys) mutation, which was previously reported in two Brazilian patients, and a c.823C>T (p.Arg275Cys) mutation was identified in Patients 5 and 6. The compound heterozygous mutations for Patients 2, 5, and 6 were also found to lie on different parental alleles. Patient 1 bears two different novel

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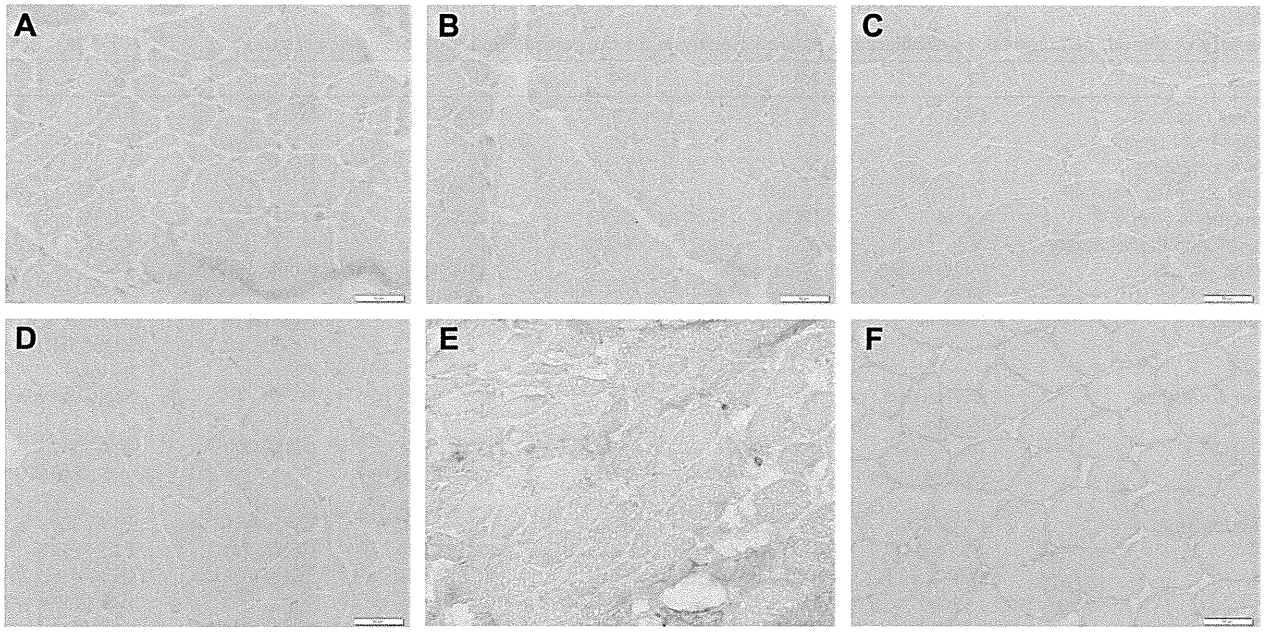


Fig. 1. Immunohistochemistry for α-DG (VIA4-I) in Patients 1, 2, 4, 5, and 6 (A–E). All patients' muscle samples showed markedly reduced staining, as compared with controls (F). Bar: 50 μm.

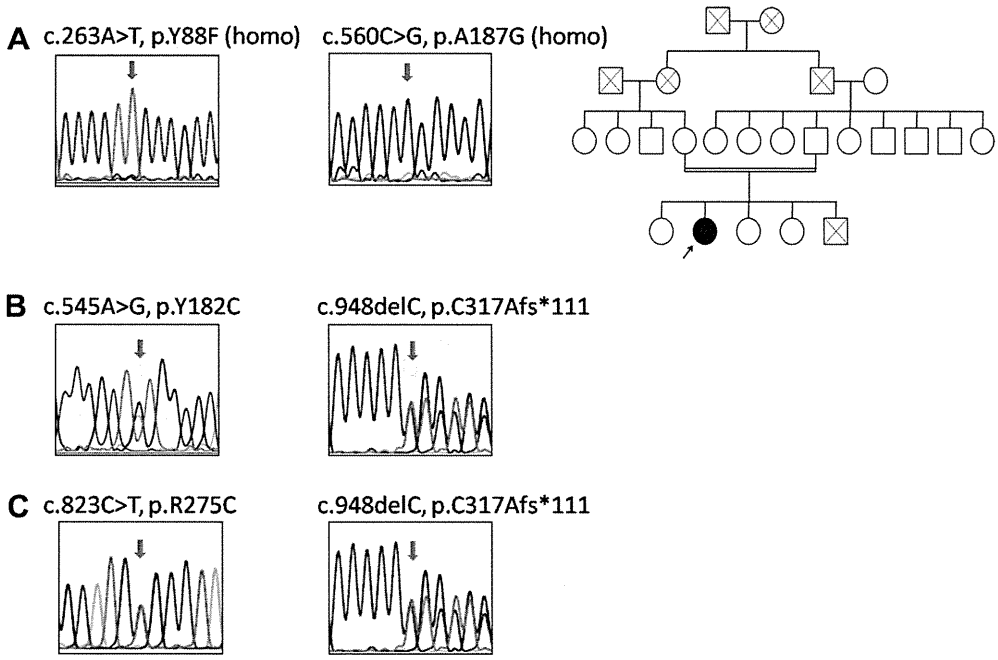


Fig. 2. Sequence analysis of *FKRP* revealed homozygous c.263A>T and c.560C>G mutations in Patient 1 (A), compound heterozygous c.545A>G and c.948delC mutations in Patients 2, 3, and 4 (B), and compound heterozygous c.823C>T and c.948delC mutations in Patients 5 and 6 (C). The pedigree of Patient 1 is also shown; the youngest brother of Patient 1 died of unknown causes at 7 months of age (A).

homozygous mutations, c.263A>T (p.Try88Phe) and c.560C>G (p.Ala187Gly), neither of which were identified in the human genome mutation database (HGMD) and 100 healthy individuals. The consanguineous healthy parents of Patient 1 carried these two missense mutations, heterozygously.

### 3.2. Reduced glycosylation of α-DG in LGMD2I patients

We further confirmed the altered glycosylation of α-DG in our LGMD2I patients (P2, 4, 5 and 6) using immunoblotting analysis and laminin overlay assay. On immunoblotting analysis using VIA4-1 antibody, skeletal

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Table 1
Summary of clinical, pathological, biochemical and molecular analyses for the patients with α-dystroglycanopathy.

| | P1 | P2 | P3[a] | P4[a] | P5 | P6[b] |
|---|---|---|---|---|---|---|
| Sex/age (years) | F/35 | M/16 | M/31 | M/30 | M/10 | F/23 |
| Age of onset (years) | 2 | 5 | 10 | 17 | 2 | 2 |
| Calf hypertrophy | Y | Y | Y | Y | Y | Y |
| Cardiomyopathy (age of diagnosis, years) | Y (28) DCM | Y (14) DCM | Y (31) DCM | Y (30) DCM | N | Y (17) DCM |
| Loss of ambulatory ability (age, years) | N 6-min walk: 76 m | Y 6-min walk: 343 m | Y (29) | N 6-min walk: not done | N 6-min walk: 210 m | Y (12) |
| Cognition | Normal | Normal | Normal | Normal | Normal | Normal Brain MRI: negative finding |
| Other anomalies | Over-active bladder | N | N | N | N | Scoliosis with op |
| CK (IU/L) | 1000–1500 max: unknown | 4000–8000 max:>10,000 | 1500–2000 max: unknown | 1500–2000 max: unknown | 6000–9000 | 200–500 max: >10,000 |
| Lung function | FVC: 32% FEV1: 33% PCF: 2.12 L/s | FVC: 45% FEV1: 53% PCF: 6.91 L/s | FVC: 43% FEV1: 36% PCF: 3.46 L/s | FVC: 62% FEV1: 73% PCF: 3.7 L/s | FVC: 64% FEV1: 76% PCF: 4.64 L/s | FVC: 10% FEV1: 12% PCF: 0.42 L/s BiPAP use at night |
| FKRP mutations | c.263A>T homo (F & M, hetero) c.560C>G homo (F & M, hetero) | c.545A>G (F, hetero) c.948delC (M, hetero) | c.545A>G c.948delC | c.545A>G c.948delC | c.823C>T (M, hetero) c.948delC (F, hetero) | c.823C>T (F, hetero) c.948delC (M, hetero) |

Y: yes; N: nil; DCM: dilated cardiomyopathy; min: minute; m: meter; op: operation; max: maximum; F: Father; M: Mother.
[a] Siblings.
[b] Previously reported (Reference [21]).

muscles from all four patients showed fainter and smaller sized bands than the control (Fig. 3A). With GT20ADG antibody for the core region of α-DG, all skeletal muscles from these patients showed fainter broadbands with smaller molecular mass than that detected in the control (Fig. 3B). Laminin overlay assay displayed greatly reduced binding ability of α-DG to laminin in all patients (Fig. 3C).

### 3.3. Clinical findings of LGMD2I patients (Table 1)

The mean age of all 6 LGMD2I patients at examination was 24.2 ± 9.7 years, and the mean disease duration was 17.8 ± 9.1 years. The disease onset was variable, ranging from early childhood to late teens (2–17 years; 6.3 ± 6.1). All patients had calf hypertrophy and proximal dominant muscle weakness, starting from lower extremities and

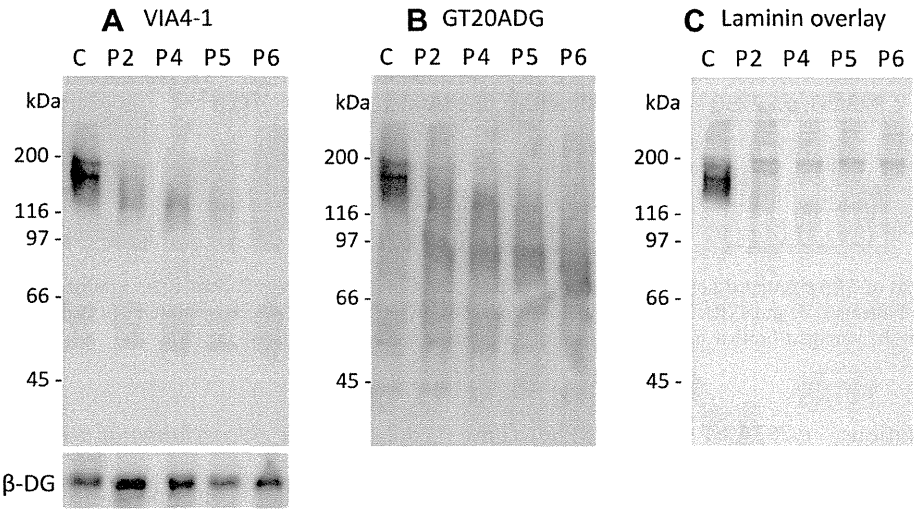


Fig. 3. Immunoblotting analysis. All 4 patients (P2, P4, P5 and P6) examined showed fainter and smaller sized bands than controls using α-DG (VIA4-1) (A). With the antibody of GT20ADG, fainter broadbands with smaller molecular weights were detected (B). Laminin overlay assay displayed greatly reduced binding ability of α-DG to laminin in all patients (C).

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then extending to shoulder girdle and arms. Patient 2 became wheelchair-bound at the age of 29 years while Patient 6 lost her ambulatory ability at 14 years of age. Dilated cardiomyopathy (DCM) was seen in five of six patients (83.3%) and they are currently under medication. DCM was diagnosed with echocardiogram in Patients 2, 3, and 4 at their first visit to our hospital, so that the exact onset age of cardiac involvement was unclear. All patients had impaired pulmonary function with different degrees of severity but only Patient 6 required ventilator assistance (1 in 6; 16.7%). All patients had normal cognitive functions and the brain MRI of Patient 6 showed no notable abnormal changes. As for other abnormalities, only Patient 6 received an operation for scoliosis at 13 years of age. Serum creatine kinase levels were usually up to 10,000 IU/L at disease onset and then declined to hundreds at a later stage.

### 3.4. Muscle CT of LGMD2I patients

On muscle CT, all assessed patients (Patients 1–5) showed similar patterns of muscle involvement (Fig. 4) Lower extremities were more severely affected than upper extremities. Gluteus maximus was the most affected muscle (Fig. 4A), followed by posterior compartment of thigh muscles, among which biceps femoris and then adductors showed marked hypodensity (Fig. 4B). In the anterior compartment of thigh, vastus muscles and rectus femoris were equally involved. At the calf level, posterior compartment muscles, especially gastrocnemius and soleus, were also more affected than anterior part (Fig. 4C). As for upper extremities, involvement of shoulder girdle muscles including subscapularis, infraspinatus and supraspinatus was more prominent than trapezium and deltoid muscles (Fig. 4D).

## 4. Discussion

Wide variability in clinical picture has been reported in LGMD2I, of which the clinical features can be Duchenne muscular dystrophy-like, late-onset LGMD phenotypic and even asymptomatic [32,33]. In European countries, homozygosity of the most common missense mutation of c.826C>A (p.Leu276Ile) has been reported to confer a relatively milder phenotype than patients with compound heterozygous mutations [34]. A homozygous mutation of c.545A>G identified in the Brazilian patients has previously been reported to cause mild clinical phenotypes and disease course [32]. In our series, Patients 2–4 harbor the same compound heterozygous mutations of c.545A>G and c.948delC while Patients 5 and 6 both carry the same c.823C>T and c.948delC mutations. The patients carrying c.823C>T and c.948delC seem to show more severe clinical features than the patients having c.545A>G and c.948delC in terms of the age at onset, disease course, motor deterioration and complications. Because only a limited number of patients were included, however, additional patients with each mutation are required to clarify the phenotype and genotype correlation more clearly.

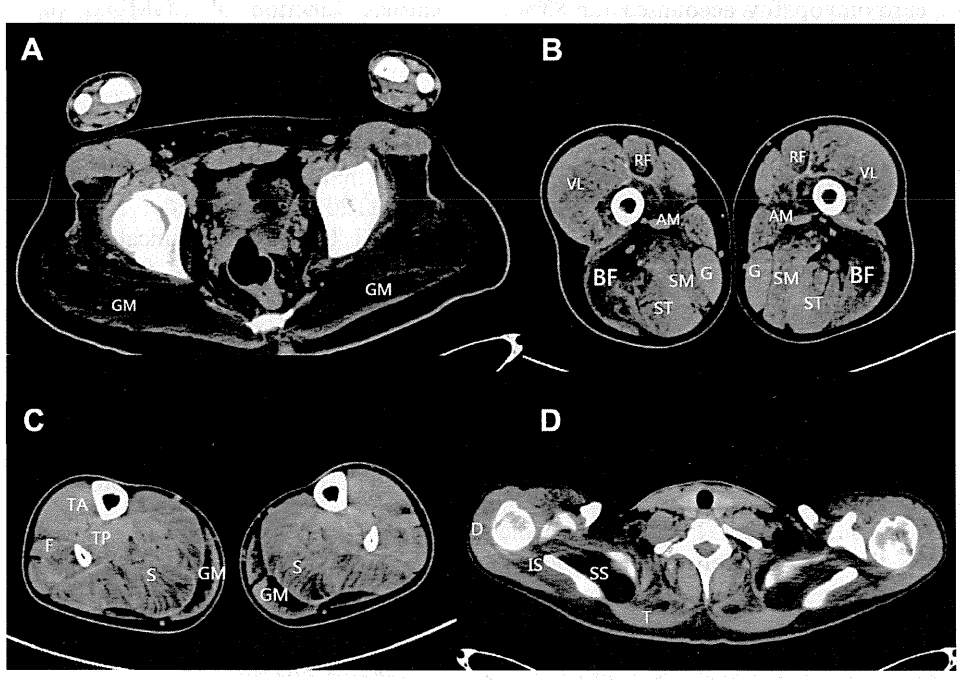


Fig. 4. Muscle CT on Patient 2. Gluteus maximus muscles were severely affected (A), followed by biceps femoris and adductors (B). At the calf level, gastrocnemius and soleus muscles were severely involved (C). In the upper extremities, involvement of subscapularis, infraspinatus and supraspinatus were more severe than trapezium and deltoid (D). (D: deltoid; IS: infraspinatus; SC: subscapularis; BF: biceps femoris; ST: semitendinosus; SM: semimembranous; S: soleus; F: fibularis; G: gastrocnemius; GM: gluteus maximus; RF: rectus femoris; VL: vastus lateralis; AM: adductor magus; G: gracilis).

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Noteworthily, c.948delC in *FKRP* is a common mutation in Taiwanese LGMD2I patients. The mutation could cause frame shift and premature termination in translation (p.Cys317Alafs*111). We further screened 300 controls without neuromuscular diseases to determine the carrier frequency of c.948delC but none carried this mutation. This result suggests that the prevalence of the homozygosity of c.948delC is at least lower than 1 in 360,000, which may be too low to identify a homozygous patient. On the other hand, this result may also indicate that the homozygosity of this frame shift mutation is too severe to survive, since none of the homozygous null mutations in *FKRP* has been reported to date and *FKRP* knockout mice also showed embryonic lethality [35].

Interestingly, two different homozygous mutations, c.263A>T (p.Tyr88Phe) and c.560C>G (p.Ala187Gly), were found in Patient 1, but not in 100 controls. Her parents were consanguineous (cousins) and both harbored these two mutations heterozygously. Compared the amino acid sequences of the FKRP protein among different species, p.Tyr88 is highly conserved in mammals while p.Ala187 is preserved among primates and some mammals, but not in rodents. Furthermore, predictions of functional effects of these two variants using software showed that p.Tyr88Phe change is probably damaging but p.Ala187Gly is benign in terms of functional impact (http://genetics.bwh.harvard.edu/pph2/index.shtml). Accordingly, c.263A>T (p.Tyr88Phe) is more likely to be pathogenic in Patient 1 although further functional studies are still necessary.

In our cohort, cardiomyopathy accounted for 83% of our patients, whereas about 10–55% of European LGMD2I patients were reported to have cardiac problems [36]. As for respiratory function, only one of our patients (Patient 6) was ventilator-dependent at night although the other five developed variable degrees of respiratory impairment. However, the proportion of respiratory aid requirement was slightly lower than other reports [20, 36–38], probably because the assessment age and disease duration of our patients were also lower. Similar to previously reported LGMD2I patients, none of our patients had overt mental retardation.

So far few papers have focused specifically on the muscle imaging of LGMD2I patients [37,39]. Based on previous related literature, gluteal muscles and posterior compartment of thigh muscles were more affected than anterior compartment in LGMD2I. In our report, similar muscle involvement was seen on CT images in which gluteal maximus was the most severely affected, followed by adductors and biceps femoris. Some of these changes may overlap with those seen in other common LGMD, especially LGMD2A [39], such as the early involvement of gluteal muscles and predominant involvement of posterior compartment. However, selective involvement of medial gastrocnemius and soleus and relative sparing of vastus lateralis are characteristic for LGMD2A [12,21,40], which suggests that muscle images are still helpful for a differential diagnosis. In addition, different clinical phenotypes including commonly-seen calf hypertrophy and cardiac involvement in LGMD2I and the presence of characteristic lobulated fibers on muscle pathology of LGMD2A are also important to make the differentiation. In our series, all patients showed calf hypertrophy and 83% had cardiac problems; lobulated fibers were not observed in skeletal muscle from any patient and molecular analysis of *CAPN3* revealed no mutation.

LGMD2I is one of the most prevalent LGMD in Europe but is very rare in Asia. Only one from Taiwan (P6), two from China and another Asian patient from North America have been reported on thus far [26–28]. Also in Japan, only one LGMD2I patient was identified by the National Center of Neurology and Psychiatry, which has the largest muscle repository in Japan. Therefore, our report discloses that LGMD2I is not rare at least in Taiwan. Considering that the glycosylation defect may be too mild to be detected by immunohistochemical screening, there must be more LGMD2I patients who are as yet undiagnosed in Taiwan. Larger scale mutation analysis for uncategorized LGMD patients may be necessary for an early diagnosis of LGMD2I to be made. One common mutation, c.948delC, in the Taiwanese population may be associated with higher frequency and early development of cardiomyopathy although a larger number of patients is required to make this conclusive. However, it is still suggested that clinicians should closely monitor the cardiac function of LGMD2I patients harboring this mutation from late childhood or their early teens.

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RESEARCH PAPER

# Rapidly progressive scoliosis and respiratory deterioration in Ullrich congenital muscular dystrophy

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## ABSTRACT

**Objective** To characterise the natural history of Ullrich congenital muscular dystrophy (UCMD).

**Patients and methods** Questionnaire-based nationwide survey to all 5442 certified paediatric and adult neurologists in Japan was conducted from October 2010 to February 2011. We enrolled the 33 patients (age at assessment, 11±6.6 years) who were reported to have collagen VI deficiency on immunohistochemistry in muscle biopsies. We analysed the development, clinical manifestations, Cobb angle and %vital capacity (%VC) in spirogram.

**Results** Cobb angle over 30° was noted at age 9.9 ±5.3 years (n=17). The maximum progression rate was 16.2±10°/year (n=13). %VC was decreased exponentially with age, resulting in severe respiratory dysfunction before pubescence. Scoliosis surgery was performed in 3 patients at ages 5 years, 9 years and 10 years. Postoperative %VC was relatively well maintained in the youngest patient. Non-invasive ventilation was initiated at age 11.2±3.6 years (n=13). Twenty-five (81%) of 31 patients walked independently by age 1.7±0.5 years but lost this ability by age 8.8 ±2.9 years (n=11). Six patients never walked independently.

**Conclusions** The natural history of scoliosis, respiratory function and walking ability in UCMD patients were characterised. Although the age of onset varied, scoliosis, as well as restrictive respiratory dysfunction, progressed rapidly within years, once they appeared.

## INTRODUCTION

Ullrich congenital muscular dystrophy (UCMD) is, after Fukuyama CMD, the second most common CMD in Japan.[1] UCMD is characterised by proximal joint contractures, distal joint hyperlaxity, proximal muscle weakness, scoliosis and respiratory failure.[1–4] The prevalence of UCMD is reported to be 1.3 per million in northern England.[5] Mutations in either *COL6A1*, *COL6A2* or *COL6A3* gene, each encoding a subunit of collagen VI (COL6), are known to cause UCMD. We have previously shown that there are two modes of COL6 deficiency: complete COL6 deficiency (CD) and sarcolemma specific COL6 deficiency (SSCD),[6 7] which are associated with recessive and de novo dominant mutations in *COL6* genes, respectively.[1]

To date, there is no cure for UCMD, and patients rely on supportive treatment of symptoms such as spinal deformity and respiratory failure. However, pathological hypotheses leading to myofibre degeneration in COL6-deficient skeletal muscle have been proposed and therapeutic targets have been suggested.[8] There is currently a clinical trial for UCMD patients based upon the theory of impaired autophagy.[9 10] Furthermore, a gene-based therapy to inhibit mutant transcripts by antisense has also been proposed because an abnormal mutated subunit can be assembled into growing supramolecular structures and sequester normal subunits into non-functional complexes, thus exerting dominant-negative effect.[11 12] Advances in such therapeutic research make knowledge of the natural course of the disease and appropriate outcome measures necessary. However, only limited information is available, especially regarding the rate of disease progression.[13] We have therefore attempted to determine the natural history of UCMD.

## PATIENTS AND METHODS

This clinical study was performed in conformity with the Declaration of Helsinki for investigation involving human subjects and was approved by the ethics committee of the National Center of Neurology and Psychiatry.

A questionnaire-based nationwide survey was conducted from October 2010 to February 2011. The questionnaire was mailed to all 5442 certified paediatric and adult neurologists by the Japanese Society of Child Neurology and the Japanese Society of Neurology, and we received 1881 (34.6%) responses. This survey consisted of questions about perinatal and developmental aspects, age and clinical manifestations at diagnosis, age at assessment, age at loss of ambulation, age at scoliosis surgery, age at initiation of non-invasive ventilation (NIV), data of Cobb angle on x-ray and % vital capacity (VC) in spirogram, in addition to pathological findings and COL6 immunohistochemistry in biopsied muscle (table 1).

Among 40 patients reported to have UCMD, we enrolled 33 patients (15 men and 18 women) with COL6 deficiency: 5 with CD and 28 with SSCD, on immunohistochemistry in skeletal muscle. Sequence analysis of *COL6* genes was performed using genomic DNA from 32 patients, 19 (59.4%) of whom carried identifiable mutations (table 2). All the 14 patients, who were not genetically confirmed, manifested clinical features compatible with UCMD in addition to COL6 deficiency (tables 2 and 3).[14] Age at muscle biopsy was 3.2±2 years

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Table 1 Summary of questionnaire items in this study

| Items | | Items | |
|---|---|---|---|
| **Patient's visit** | **Yes/No** | **Age at muscle biopsy** | **Age** |
| Age at assessment | Age | Clinical manifestation at diagnosis | |
| Sex | Male/Female | Distal joint hyperlaxity | Yes/No |
| Muscle biopsy | Yes/No | Protruding calcaneus | Yes/No |
| COL6 deficiency on IHC | Yes/No | High arched palate | Yes/No |
| Mode of COL6 deficiency | CD/SSCD | Proximal joint contractures | Yes/No |
| Perinatal history | | Scoliosis | Yes/No |
| Decreased fetal movement | Yes/No | Spinal rigidity | Yes/No |
| Poor sucking | Yes/No | Ankle joint contracture | Yes/No |
| Floppiness | Yes/No | Facial weakness | Yes/No |
| CHD | Yes/No | Skin lesions | Yes/No |
| Torticollis | Yes/No | | |
| AMC | Yes/No | Age at loss of ambulation | Age |
| Developmental history | | NIV | Yes/No |
| Head control | Age | Age at initiation of NIV | Age |
| Sitting | Age | Data of %VC | |
| Walking | Age | Age at scoliosis surgery | Age |
| Phrases | Age | Data of Cobb angle | |

AMC, arthrogryposis multiplex congenita; CD, complete collagen VI deficiency; CHD, congenital hip dislocation; COL6, collagen VI; IHC, immunohistochemistry; NIV, non-invasive ventilation; SSCD, sarcolemma-specific collagen VI deficiency; VC, vital capacity.

(mean±SD). Age at assessment was 11±6.6 years. We analysed the information on perinatal abnormalities, development, clinical features, deterioration of ability to walk, progression of scoliosis and respiratory dysfunction.

## RESULTS

Perinatal abnormalities and clinical manifestations are shown in tables 2 and 3. Congenital hip dislocation, torticollis and arthrogryposis multiplex were noted in 36.4%, 27.3% and 20% of patients, respectively. More than 50% of patients had distal joint hyperlaxity, protruding calcaneus, high arched palate, proximal joint contractures and scoliosis. Creatine kinase level at muscle biopsy was 315±110 IU/L (n=31). Among patients with CD, homozygous or compound heterozygous mutations were identified in three but a heterozygous mutation was identified in one patient. All the 15 patients with SSCD carried a heterozygous mutation (table 2).

Twenty-five (81%) of 31 patients were able to sit and walk independently. Six patients (19%) never walked, three of whom had CD by muscle immunohistochemistry (tables 2 and 3). Head control, sitting and independent ambulation were completed at median ages of 4 months, 9 months and 18 months, respectively (figure 1A). In contrast, achievement of speaking phrases was not delayed, ranging from ages 12 months to 25 months (figure 1A). Most patients became able to walk independently by age 2 years but this ability deteriorated with age (figure 1B). Loss of ambulation occurred at age 8.8±2.9 years (n=11). Patient 18 was reported to walk with knee-ankle-foot orthoses at age 11 years. Patients 20 and 30, respectively, required a wheelchair at ages 13 years and 6 years. Half of the patients became wheelchair-bound by age 11 years (figure 1B). Six patients became wheelchair-bound by age 7 years, two of whom had CD and loss of ambulation at ages 5.5 years and 6 years, respectively. Two of four patients with SSCD who carried a heterozygous c.850G>A (p.Gly284Arg) mutation in *COL6A1* did not acquire independent ambulation (tables 2 and 3).

The severity and progression of scoliosis were assessed by Cobb angle (n=23). Maximum Cobb angles are shown in table 3. Patient 28 was reported to suffer from marked scoliosis albeit no data of Cobb angle was available. Cobb angle over 30° was noted at age 9.9±5.3 years in 17 patients (table 4). Among them 13 patients had Cobb angle data available for 3 or more years and show a maximum progression rate of 16.2±10°/year. Overall, although the onset of scoliosis varied, it progressed rapidly within years once scoliosis was noted (figure 1C). Surgical intervention for scoliosis was performed in Patients 8, 9 and 21 at ages 10 years, 9 years and 5 years, respectively. Presurgical and postsurgical Cobb angle data were available in the last two patients, both of whom showed improvement from 107° to 80° and 90° to 40°, respectively.

Percent VCs at survey are shown in table 3. Patients 8 and 27 had 32% and 70.4% of predicted VC at ages 8 years and 6 years, respectively. Respiratory function, measured by %VC (n=20), decreased exponentially with age accompanied by a sharp decline below age 10 years (figure 1D). Importantly, postoperative VC was relatively well maintained in the patient who underwent surgery at age 5 years (figure 1D). The percentage of patients requiring NIV increased with age (figure 1E). Half of the patients required NIV by age 12 years. Age at initiation of NIV was 11.2±3.6 years (n=13) (table 3). On the other hand, %VC at mean age of NIV initiation was estimated at around 36%.

## DISCUSSION

This is the first nationwide survey of the natural history of UCMD in Japan. This study confirmed the previously reported clinical features of UCMD: delayed motor milestone, absence of mental retardation, distal joint hyperlaxity, proximal joint contractures, scoliosis and respiratory involvement.[1–4] Furthermore, we characterised the natural history of scoliosis, respiratory function and ambulation in this relatively large UCMD series.

UCMD is on a disease spectrum of COL6 related myopathy. Intermediate phenotypes, named mild UCMD or severe Bethlem myopathy, have been known, and currently there is no

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Table 2 Clinical, pathological and genetical findings in the 33 patients

| Pt | Age at biopsy (years) | Clinical manifestations and CK level at muscle biopsy | | | | | | | | | | Collagen VI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Distal joint hyperlaxity | Protruding calcaneus | High arched palate | Proximal joint contracture | Scoliosis | Spinal rigidity | Ankle joint contracture | Facial weakness | Skin lesion | CK | Deficiency on IHC | Gene mutation |
| 1 | 3 | (+) | ND | (+) | (–) | ND | ND | (–) | (+) | (–) | 440 | CD | *COL6A2* c.1771-3G>C<br>*COL6A2* c.1270-1G>C |
| 2 | 2 | (+) | (+) | (–) | (+) | (+) | (–) | (–) | (–) | (+) | 413 | CD | *COL6A3* c.5692delG p.Val1898fs<br>*COL6A3* c.8737delG p.Ala2913fs |
| 3 | 2 | (+) | (+) | (–) | (–) | (–) | (–) | (–) | (–) | (+) | 474 | CD | NF |
| 4 | 1 | ND | (+) | (–) | (–) | (–) | (–) | (–) | (+) | (–) | 339 | CD | *COL6A2* c.2678_2700del23<br>p.Pro893fs<br>homozygous |
| 5 | 1 | ND | (+) | (–) | (+) | ND | (+) | (+) | (+) | (–) | ND | CD | *COL6A3* c.4184G>A p.Arg1395Gln |
| 6 | 2 | (+) | (+) | (+) | (–) | ND | ND | (–) | (–) | (–) | 290 | SSCD | *COL6A1* c.850G>A p.Gly284Arg |
| 7 | 1 | (+) | ND | (+) | (+) | (+) | (+) | (+) | (–) | ND | 195 | SSCD | *COL6A1* c.850G>A p.Gly284Arg |
| 8 | 1 | (+) | (+) | ND | (+) | ND | (+) | (+) | ND | (–) | 319 | SSCD | NF |
| 9 | 3 | (+) | (–) | ND | (+) | (+) | ND | (–) | ND | (–) | ND | SSCD | NF |
| 10 | 3 | (+) | (+) | (+) | (+) | (+) | (–) | (–) | (–) | (+) | 428 | SSCD | *COL6A2* c.950_954+8del |
| 11 | 5 | (+) | (+) | (+) | (+) | (+) | (+) | (–) | (–) | (–) | 151 | SSCD | NA |
| 12 | 4 | (+) | (+) | (–) | (+) | (+) | ND | (–) | (–) | ND | 207 | SSCD | *COL6A2* c.901G>T p.Gly301Cys |
| 13 | 6 | ND | (–) | (+) | (–) | (–) | (–) | (+) | (–) | (–) | 564 | SSCD | NF |
| 14 | 7 | (–) | (+) | (+) | (–) | (–) | (–) | (–) | (–) | (–) | 329 | SSCD | *COL6A2* c.812G>A p.Gly271Asp |
| 15 | 5 | (+) | (–) | (+) | (–) | (+) | (–) | (–) | (+) | (–) | 289 | SSCD | *COL6A1* c.958_966del9<br>p.Gly320_Lys322del |
| 16 | 3 | (–) | ND | (+) | (–) | (–) | (–) | (–) | (–) | (–) | 425 | SSCD | *COL6A3* c.6210+2T>A |
| 17 | 3 | ND | ND | (+) | ND | ND | ND | ND | (–) | ND | 242 | SSCD | *COL6A1* c.850G>A p.Gly284Arg |
| 18 | 2 | (+) | (+) | (+) | (+) | (–) | (+) | (+) | (+) | (–) | 299 | SSCD | NF |
| 19 | 3 | (–) | (+) | (+) | (+) | (–) | (–) | (+) | (–) | (–) | 464 | SSCD | NF |
| 20 | 1 | ND | ND | (+) | (–) | (–) | (–) | (–) | (–) | ND | 254 | SSCD | *COL6A1* c.958_966del9<br>p.Gly320_Lys322del |
| 21 | 5 | (+) | ND | (–) | (–) | (+) | (+) | (–) | (–) | (–) | 138 | SSCD | *COL6A1* c.868G>A p.Gly290Arg |
| 22 | 6 | (+) | ND | (–) | (+) | (–) | (–) | (+) | (–) | (–) | 495 | SSCD | NF |
| 23 | 1 | (+) | (+) | (+) | (+) | (–) | (–) | (–) | (–) | (+) | 300 | SSCD | *COL6A1* c.850G>A p.Gly284Arg |
| 24 | 2 | (+) | (+) | (+) | (+) | (–) | (–) | (–) | (+) | (–) | 388 | SSCD | NF |
| 25 | 4 | (+) | (+) | (–) | (–) | (+) | (+) | (–) | (–) | (–) | 277 | SSCD | *COL6A1* c.860G>A p.Gly287Glu |
| 26 | 6 | (+) | (+) | (+) | (–) | (+) | (+) | (+) | (–) | (–) | 103 | SSCD | NF |
| 27 | 5 | ND | ND | ND | ND | ND | ND | ND | ND | ND | 262 | SSCD | *COL6A1* c.1056+1G>A |
| 28 | 8 | ND | (–) | (+) | (+) | ND | (+) | (–) | (+) | (+) | 254 | SSCD | NF |
| 29 | 2 | ND | ND | (–) | (–) | (+) | (–) | (–) | (–) | (–) | 361 | SSCD | *COL6A3* c.6210+1G>A |
| 30 | 3 | (–) | (+) | ND | (–) | (–) | (–) | (–) | ND | (–) | 278 | SSCD | *COL6A3* c.6310-2A>G |
| 31 | 1 | (+) | ND | (–) | (+) | (+) | (–) | (–) | (–) | (–) | 261 | SSCD | NF |
| 32 | 4 | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (–) | (–) | 323 | SSCD | NF |
| 33 | 0 | (+) | (–) | (+) | (+) | (+) | (+) | (–) | (–) | (–) | 214 | SSCD | NF |
| | | 21/25 (84%) | 18/23 (78.3%) | 19/29 (65.5%) | 17/31 (54.3%) | 14/ 26 (53.8%) | 11/ 27 (40.7%) | 9/31 (29%) | 7/29 (24.1%) | 5/ 28 (17.9%) | | | |

CD, complete deficiency; CK, creatine kinase; IHC, immunohistochemistry; NA, not available; ND, no data; NF, not found; Pt, patient; SSCD, sarcolemma specific collagen VI deficiency; (+); present, (–); absent.

Table 3 Perinatal, developmental history and disease progression in our series

| | | Perinatal history | | | | | | Developmental milestone (months) | | | | Motor function at survey | | Respiratory function | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pt | Age at survey (years) | Floppiness | Poor sucking | CHD | Decreased fetal movement | Torticollis | AMC | Head control | Sit | Walk | Phrases | Walk independently | Age at loss of ambulation (yrs) | %VC at survey (%) | NIV | Age at initiation of NIV (yrs) | Maximum Cobb angle (°) (yrs) |
| 1 | 14 | (+) | (+) | (+) | (+) | (+) | (+) | 6 | 30 | (–) | ND | (–) | | 29 | (+) | 8 | 45 (11) |
| 2 | 10 | (–) | (+) | (+) | (–) | (–) | ND | 5 | 12 | (–) | ND | (–) | | 22.3 | (+) | 8 | 12 (10) |
| 3 | 7 | (+) | (–) | (+) | (–) | (+) | (–) | ND | 9 | 24 | 24 | (–) | 5.5 | 55 | (–) | | ND |
| 4 | 3 | (–) | (+) | (–) | (–) | (+) | (–) | 7 | 12 | (–) | 25 | (–) | | ND | (–) | | 0 (3) |
| 5 | 23 | (+) | (+) | (+) | (–) | (–) | (–) | ND | ND | 18 | ND | (–) | 6 | 14.9 | (+) | 15 | 50 (23) |
| 6 | 23 | (+) | (+) | (+) | (+) | (+) | (–) | 5 | 18 | (–) | 18 | (–) | | 10.4 | (+) | 15 | 54 (23) |
| 7 | 14 | (+) | (+) | (+) | (–) | (+) | (–) | 3 | 11 | (–) | 23 | (–) | | ND | (+) | 5 | 99 (9) |
| 8 | 21 | (–) | (–) | (–) | (–) | (–) | ND | 5 | 12 | (–) | ND | (–) | | ND | (+) | 9 | 53 (10) |
| 9 | 20 | ND | ND | (–) | ND | (+) | (–) | 4 | 7 | 18 | 16 | (–) | 6 | 12.2 | (+) | 10 | 107 (9) |
| 10 | 6 | (–) | (–) | (+) | (–) | (–) | (–) | 3 | 12 | 24 | 18 | (–) | 6 | ND | (–) | | 20 (6) |
| 11 | 10 | (–) | (–) | (–) | (+) | (–) | (–) | 3 | 7 | 18 | 19 | (–) | 7 | 46 | (–) | | 10 (10) |
| 12 | 15 | (+) | (–) | (–) | (–) | (–) | (–) | 4 | 8 | 22 | 24 | (–) | 10 | 37.9 | (–) | | 31 (3) |
| 13 | 15 | (–) | (–) | (+) | (–) | (–) | ND | 5 | 6 | 18 | 18 | (–) | 10 | 27.6 | (+) | 12 | 60 (15) |
| 14 | 15 | (+) | (–) | (+) | (+) | (–) | (–) | 5 | 12 | 16 | 12 | (–) | 11 | 15.7 | (+) | 9.5 | 110 (15) |
| 15 | 13 | (+) | (+) | (–) | (+) | (–) | (–) | 4 | 7 | 14 | 24 | (–) | 11 | 21.9 | (+) | 11 | 94 (14) |
| 16 | 11 | (–) | (+) | (–) | (–) | (–) | (+) | 4 | 6 | 16 | 16 | (–) | 11 | 30.7 | (–) | | 70 (11) |
| 17 | 23 | (–) | (+) | (–) | (–) | (–) | (–) | 4 | 8 | 16 | 24 | (–) | 14 | 19.8 | (+) | 16 | 41 (23) |
| 18 | 10 | (+) | (+) | (–) | (+) | (–) | (–) | 7 | 9 | 27 | 24 | (+) | | 45 | (–) | | 0(9) |
| 19 | 3 | (–) | (–) | (–) | (–) | (–) | (–) | 8 | 11 | 24 | 24 | (+) | | ND | (–) | | ND |
| 20 | 16 | (+) | (–) | (–) | (–) | (–) | (+) | 4 | 8 | 20 | 12 | (+) | | ND | (+) | 10 | 90(10) |
| 21 | 12 | (–) | (–) | (+) | (–) | (–) | (–) | 6 | 15 | 30 | 14 | (+) | | 44.3 | (–) | | 90(5) |
| 22 | 10 | (–) | (–) | (–) | (+) | (–) | (–) | 4 | 8 | 14 | 24 | (+) | | 58.9 | (–) | | 0(10) |
| 23 | 5 | (+) | (–) | (–) | (–) | (–) | (–) | 2 | 7 | 14 | 21 | (+) | | ND | (–) | | 2(4) |
| 24 | 5 | (–) | (–) | (+) | ND | (–) | (–) | 3 | 8 | 22 | 18 | (+) | | ND | (–) | | 5(5) |
| 25 | 5 | (–) | (+) | (–) | (–) | (+) | ND | 4 | 6 | 15 | ND | (+) | | ND | (–) | | 60(6) |
| 26 | 7 | (+) | (+) | (–) | (+) | (–) | (+) | 3 | 12 | 24 | 18 | (+) | | 53.4 | (–) | | 46(7) |
| 27 | 11 | (–) | (–) | (–) | ND | (–) | ND | 4 | 8 | 16 | ND | ND | | ND | (–) | | ND |
| 28 | 18 | (+) | (+) | (–) | (+) | (–) | ND | ND | 20 | 36 | 24 | (+) | | ND | (+) | 17 | ND |
| 29 | 2 | (+) | (–) | (–) | ND | (+) | (–) | 4 | 8 | 19 | ND | (+) | | ND | ND | | ND |
| 30 | 7 | (–) | (–) | (–) | (–) | (–) | ND | 3 | 8 | 13 | 24 | (+) | | ND | (–) | | ND |
| 31 | 1 | (–) | (–) | (–) | (+) | (–) | (+) | 6 | 14 | ND | ND | ND | | ND | ND | | ND |
| 32 | 6 | (–) | (–) | (–) | (–) | (–) | ND | 5 | 9 | 18 | 22 | (+) | | ND | (–) | | 40(4) |
| 33 | 1 | (+) | (–) | (+) | (–) | (+) | (–) | 7 | (–) | ND | ND | ND | | ND | (–) | | 5(1) |
| | | 15/32 (46.9%) | 13/32 (40.6%) | 12/33 (36.4%) | 10/29 (34.5%) | 9/33 (27.3%) | 5/25 (20%) | | | | | | | | | | |

Pt 18 was reported to walk with knee-ankle-foot orthoses at age 11 years. Pts 20 and 30 were reported to be able to walk independently but they respectively required a wheelchair at ages 13 years and 6 years. Pts 8 and 27 showed 32% and 70.4% of predicted VC at ages 8 years and 6 years, respectively. Pt 28 was reported to suffer from marked scoliosis but no data of Cobb angle was available.
AMC, arthrogryposis multiplex congenita; CHD, congenital hip dislocation; ND, no data; NIV, noninvasive ventilation; Pt, patient; %VC, % vital capacity; (+), present; (–), absent.



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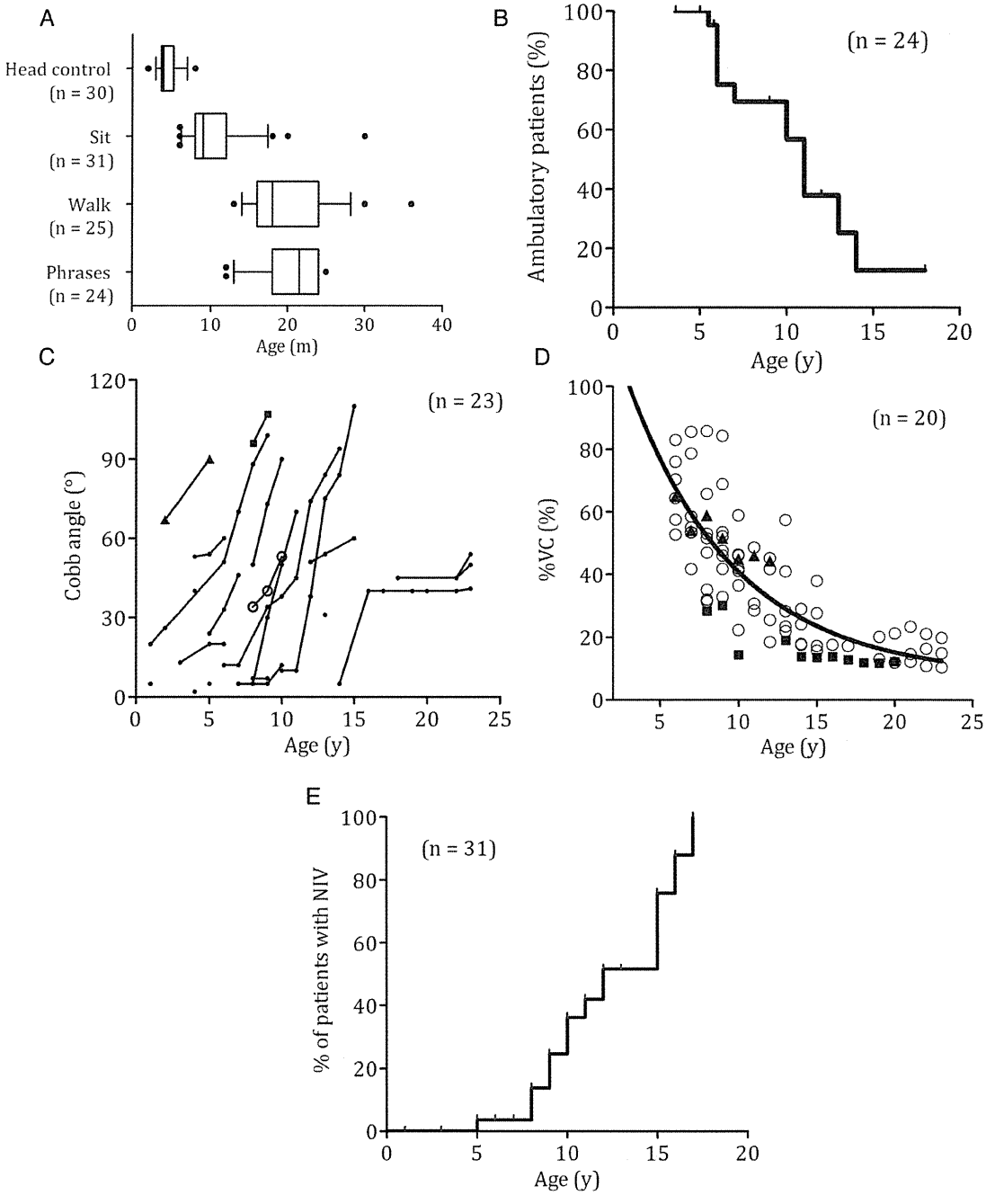


Figure 1 (A) Age ranges at completion of neck control, sit, independent ambulation and phrases. The boxes represent the range from the 25–75th percentile, while the bars span the 10–90th percentile. (B) Kaplan-Meier curve showing deterioration of walking ability in Ullrich congenital muscular dystrophy (n=24). Patients 20 and 30, respectively, become wheelchair-bound at ages 13 years and 6 years. (C) Severity and progression of scoliosis (n=23). Open circles, solid squares and triangles indicate preoperative Cobb angles from Patients 8, 9 and 21 who underwent scoliosis surgery at ages 10 years, 9 years and 5 years, respectively. (D) %Vital capacity (%VC) (n=20). Solid line represents the regression curve (%VC=144.8*exp (−0.146*Age)+7.386, $R^2$=0.6684). Solid squares and triangles respectively represent values from Patients 9 and 21 who underwent scoliosis surgery at ages 9 years and 5 years. (E) Kaplan-Meier curve showing the percentage of patients with non-invasive ventilation (NIV) (n=31).

clear-cut definition of two major phenotypes.[8 15] According to the clinical classification of early onset COL6-related myopathies, all the patients in our series can be classified into the most severe (early-severe) or moderate-progressive groups.[16 17] The age at loss of ambulation was slightly younger compared with the previous observations (10.7±4.8 years and 10.1 ±4.4 years).[13 17] Interestingly, patients with CD never walked independently or became unable to walk by age 6 years, indicating that CD is most likely to be associated with the more severe phenotype than SSCD. On the other hand, 3 (10.7%) of 28 patients with SSCD did not acquire independent ambulation. Unlike patients with CD, a great heterogeneity in the maximal motor capacity was observed in those with SSCD, ranging from no acquisition of walking ability to retaining ambulation throughout childhood. Four patients with a heterozygous c.850G>A (p.Gly284Arg) mutation in *COL6A1* showed a wide

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Table 4 Data of Cobb angle in 23 patients

| Pt | COL6 deficiency on IHC | Age at loss of ambulation (years) | Age at assessment (years) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | <3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 22 | 23 |
| 1 | CD | NW | | | | | | | | | 45 | | | | | | | | | | | |
| 2 | CD | NW | | | | | 5 | 5 | 5 | 12 | | | | | | | | | | | | |
| 5 | CD | 6 | | | | | | | | | | | | | | | | | | | 45 | 50 |
| 6 | SSCD | NW | | | | | | | | | | | | | | | | 45 | | | 45 | 54 |
| 7 | SSCD | NW | 26 (2) | | | 51 | 70 | 88 | 99 | | | | | | | | | | | | | |
| 8 | SSCD | NW | | | | | | 34 | 40 | 50 | | | | | | | | | | | | |
| 9 | SSCD | 6 | | | | | | 96 | 107 | | | | | 80 | | | | | | | | |
| 10 | SSCD | 6 | 13 (3) | | 20 | 20 | | | | | | | | | | | | | | | | |
| 11 | SSCD | 7 | | | | | | 7 | 7 | 10 | | | | | | | | | | | | |
| 12 | SSCD | 10 | | | | | | | | | | | 31 | | | | | | | | | |
| 13 | SSCD | 10 | | | | | | | | | | 51 | 54 | | 60 | | | | | | | |
| 14 | SSCD | 11 | | | | | | 0 | | 10 | 10 | 38 | 75 | 84 | 110 | | | | | | | |
| 15 | SSCD | 11 | | | | 12 | 12 | | 34 | 38 | 45 | 74 | 84 | 94 | | | | | | | | |
| 16 | SSCD | 11 | | | | | | 5 | 30 | 50 | 70 | | | | | | | | | | | |
| 17 | SSCD | 14 | | | | | | | | | | | | 5 | | 40 | 40 | | 40 | 40 | 40 | 41 |
| 20 | SSCD | W | | | | | | 50 | 73 | 90 | | | | | | | | | | | | |
| 21 | SSCD | W | 67 (2) | | 90 | 40 | | | | | | | | | | | | | | | | |
| 23 | SSCD | W | | 2 | | | | | | | | | | | | | | | | | | |
| 24 | SSCD | W | | | 5 | | | | | | | | | | | | | | | | | |
| 25 | SSCD | W | | 53 | 54 | 60 | | | | | | | | | | | | | | | | |
| 26 | SSCD | W | | | 24 | 33 | 46 | | | | | | | | | | | | | | | |
| 32 | SSCD | W | | 40 | | | | | | | | | | | | | | | | | | |
| 33 | SSCD | NW | 5 (1) | | | | | | | | | | | | | | | | | | | |

Pts 8, 9 and 21 respectively underwent scoliosis surgery at age 10, 9 and 5 years.
CD, complete deficiency; COL6, collagen VI; IHC, immunohistochemistry; NW, not walk; Pt, patient; SSCD, sarcolemma specific collagen VI deficiency; W, walk.

variety in their ability to walk (table 3). In this study we were not able to confirm recessive mutations and a heterozygous mutation in 2 with CD and 13 with SSCD, respectively. The mutation detection rate (59.4%) was comparable with those reported to be up to 60% in other groups,[15] and those patients without a putative mutation identified may carry deletions or duplications of one or more exons as well as intronic, regulatory mutations.

The onset of scoliosis preceded loss of ambulation in UCMD. This pattern of scoliosis progression was also pointed out by Nadeau *et al*.[13] Development of scoliosis in Duchenne muscular dystrophy, on the other hand, is strongly related to the loss of walking ability.[18] In Duchenne muscular dystrophy, typically, scoliosis is not evident in ambulatory patients and starts after patients become wheelchair dependent. In UCMD, in contrast, scoliosis developed even when patients were still ambulant and is characterised by marked progression from early stage. For the first time, we characterised scoliosis progression in this study. It is noteworthy that scoliosis progresses rapidly, within years, once it starts. The early-onset and rapidly-progressive scoliosis in UCMD may well accelerate physical disability, such as difficulty in sitting, standing and walking, and cause pain. More importantly, scoliosis may well compromise respiratory function by reducing chest wall compliance.

VC declined exponentially with age, with a sharp decrease by age 10 years. Nadeau *et al* showed that forced VC (%predicted) in UCMD declined by 6.6±1.9%/year from age 6 years to 10 years compared with by 0.4±3%/year from age 11 years to 15 years.[13] Although the parameters were different, both studies indicate that UCMD patients develop restrictive respiratory dysfunction rapidly in the first decade of life. This decay in VC might be associated with proximal joint and vertebral contractures together with weakness of the diaphragm. Considering the slower decline of %VC in the youngest patient after surgical correction of scoliosis, earlier surgical intervention to correct spinal deformity may be beneficial for maintaining chest wall compliance, thus preventing progressive respiratory dysfunction. Takaso *et al* successfully performed scoliosis surgery in three patients with UCMD at ages 11 years, 13 years and 17 years, respectively (not enrolled in the present study).[19] However, in these patients, surgery did not prevent deterioration of respiratory function suggesting that at such older ages pulmonary and chest wall compliance might be too severely compromised for patients to benefit from scoliosis surgery, and earlier surgical intervention may be more beneficial. However, further studies are necessary to conclude the efficacy of early scoliosis surgery.

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**Contributors** TY: designed the study, performed literature search, analysed the data and wrote the manuscript. HK, MO and YKH: supervised all aspects of this study, including the study design, interpretation and manuscript preparation. IN, KS and MS gave valuable comments for the manuscript. IN was involved in analysing and interpreting all the data and also supervised the study design, execution and manuscript preparation.

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# Rapidly progressive scoliosis and respiratory deterioration in Ullrich congenital muscular dystrophy

Takahiro Yonekawa, Hirofumi Komaki, Mari Okada, et al.

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Case report

# Congenital generalized lipodystrophy type 4 with muscular dystrophy: Clinical and pathological manifestations in early childhood

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**Abstract**

A boy with congenital generalized lipodystrophy type 4 with muscular dystrophy presented in infancy with delay in motor milestones and a persistent elevation of CK. There was no associated mental retardation. He was followed up to 3 years and 11 months; he had a homozygous c.696_697insC mutation in *polymerase I and transcript release factor* (PTRF). He started to walk at 2 years and 6 months although he did not have mental retardation. Insulin resistance appeared at 3 years and 11 months of age. PTRF immunostaining positivity was absent in the muscle but caveolin-3 was preserved in the sarcolemma at 16 months of age. Secondary deficiency of caveolins may be closely associated with disease progression.
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*Keywords:* PTRF; Generalized lipodystrophy; Muscular dystrophy; Insulin resistance; Muscle mounding

## 1. Introduction

Congenital generalized lipodystrophies are rare autosomal recessive disorders that are characterized by an almost total loss of subcutaneous adipose tissues from birth, insulin resistance, diabetes, hypertriglyceridemia, and hepatic steatosis [1,2]. Recently, we first described muscular dystrophy with generalized lipodystrophy caused by *polymerase I and transcript release factor* (PTRF) mutations [3], and this disease was categorized as congenital generalized lipodystrophy type 4 (CGL4) (OMIM #613327). Patients with PTRF deficiency can show various symptoms that include arrhythmia, atlantoaxial instability, and pyloric stenosis in addition to manifestations of congenital generalized lipodystrophy and muscular dystrophy [3–6].

Only a limited number of patients with this condition have been reported. Therefore, the accumulation of detailed clinical information, especially in early childhood, is important to understand this disease and to facilitate early diagnosis. Herein, we describe the detailed clinical course of a 3-year-old Japanese boy with CGL4 with muscular dystrophy.

## 2. Case report

Our patient was a Japanese boy aged 3 years and 11 months from healthy non-consanguineous parents. He was born via normal delivery at 39 weeks and 4 days of gestational age. His body height, body weight, and head circumstance were 47 cm (−1.0 SD), 3070 g (−0.3 SD), and 34 cm (0.3 SD), respectively. He gained body weight

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slowly and weighed 4.5 kg at 4 months of age, upon which he was diagnosed with hypothyroidism. An elevated serum creatine kinase (CK) level (812 IU/L; normal <200) was also noted. He received thyroid hormone therapy consisting of 10 μg/day levothyroxine sodium hydrate. The subcutaneous fat of his face began to decrease at 7 months of age. He was referred to our hospital at 11 months of age because of continuous elevation of serum CK levels. Although he had head control at 4 months of age, he showed delayed motor milestones. He could crawl at 14 months and sit at 16 months of age. He showed normal mental development and spoke several meaningful words at 14 months of age.

At 16 months of age, his body height and weight were 78.5 cm (−0.1 SD) and 9.6 kg (−0.5 SD), respectively. He had a saddle nose, prominent ears, curled hair, and mild macroglossia. Loss of subcutaneous fat was marked on his face and limbs, which exposed prominent blood vessels in his extremities. His abdomen was distended without evidence of hepatosplenomegaly or tumor. He did not have hypertrophic tonsils or scoliosis and facial muscle involvement or a high arched palate was not observed. He had mild hypertrophic muscles, especially in his extremities, and mild proximal muscle weakness was seen with normal deep tendon reflexes. He could crawl and stand with support. Although he demonstrated percussion-induced muscle mounding, he did not demonstrate the rippling phenomenon.

His serum CK level had increased to 2293 IU/L, but his serum immunoglobulin level was normal. Chest radiography, electrocardiography, cardiac echocardiography, and bone radiography showed normal findings. A muscle computed tomography (CT) showed decreased subcutaneous adipose tissue and hypertrophic muscles with normal intensity (Fig. 1A).

A muscle biopsy taken from his left biceps brachii showed dystrophic changes including variation in fiber size and scattered necrotic and regenerating fibers (Fig. 2A and B). Immunohistochemistry for PTRF was negative in both the sarcolemma of the muscle fibers and blood vessels (Fig. 2C). The caveolin-3 stain was slightly irregular but well preserved (Fig. 2D), whereas the immunoreactions of caveolins-1 and -2 were barely detectable in the blood vessels (data not shown). Antibodies for dystrophin and other major proteins associated with muscular dystrophies showed normal staining (data not shown). Genetic analysis revealed a homozygous mutation of c.696_697insC in the *PTRF* gene, which is a common mutation in Japanese CGL4 patients [3]. This mutation resulted in substitution of the last 158 amino acids with an unrelated 191-amino acid sequence; moreover, the mutant protein was mislocalized, as shown by a previous *in vitro* experiment [3].

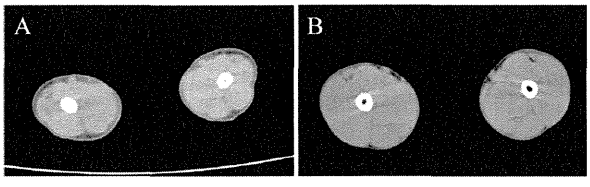


Fig. 1. Computed tomographic scan at the thigh level at 1 year (A) and 2 years and 10 months (B) of age. The thigh muscles showed hypertrophy without abnormal intensity. Note the progressive loss of subcutaneous adipose tissues.

He started to walk without support at 2 years and 6 months of age and he could speak 2-word sentences. At the age of 2 years and 10 months, his body height and weight were 92.7 cm (+0.2 SD) and 13.2 kg (−0.1 SD), respectively (Table 1). He could run and climb stairs slowly, but he could not jump. The Gowers' sign was negative. The endocrinological examination results at that time were as follows: total cholesterol (T-cho), 213 (140–220) IU/L; triglyceride (TG), 309 (30–150) mg/dL; high-density lipopolysaccharide cholesterol (HDL-C), 27 (40–76) mg/dL; low-density lipopolysaccharide cholesterol (LDL-C), 125 (70–139) mg/dL; glucose, 98 mg/dL; HbA1c, 5.1 (3.8–5.5)%; insulin, 1.5 (5.0–20.0) μU/mL; fT4, 1.42 (0.97–1.80) ng/dL; fT3, 4.36 (2.73–4.50) pg/mL; and thyroid-stimulating hormone, 4.560 (0.300–3.000) μU/mL. The oral glucose tolerance test showed a normal reaction and his blood sugar level was 123 mg/mL 2 h after administration. The calculated insulin resistance index (HOMA-R) was 1.24 (<1.6). Serum adiponectin, total PAI-1, and leptin levels were 1.2 (>4.0) μg/mL, 23 (<50) ng/mL, and 1.9 (male: 0.9–13.0, female: 2.5–21.8) ng/mL, respectively.

At 3 years and 11 months of age, his body height and weight were 99.2 cm (−0.1 SD) and 15.3 kg (−0.1 SD), respectively. The endocrinological examination results at that time were as follows: T-cho, 154 IU/L; TG, 680 mg/dL; HDL-C, 20 mg/dL; LDL-C, 57 mg/dL; glucose, 98 mg/dL; HbA1c, 5.0%; and insulin, 12.3 μU/mL. His HOMA-R was increased to 2.98. Serum adiponectin, total PAI-1, and leptin levels were 1.1 μg/mL, 96 ng/mL, and 1.6 ng/mL, respectively. His body fat percentage was 12.2% according to dual energy X-ray absorptiometry (DEXA) measurement.

## 3. Discussion

We previously reported that the clinical features observed in patients with *PTRF* mutations were closely associated with a secondary deficiency of caveolin [3].

In various types of congenital generalized lipodystrophy, body fat loss and insulin resistance are usually noticed at birth. In CGL4, however, the loss of adipose tissue in the face is observed after several months of age and insulin resistance appears from early childhood [3–6]. The boy described herein showed decreased subcutaneous fat in his face at 7 months of age. Lipodystrophy was progressive, and loss of adipose tissue in the lower legs became more apparent with age. This finding was confirmed by CT images. Metabolic

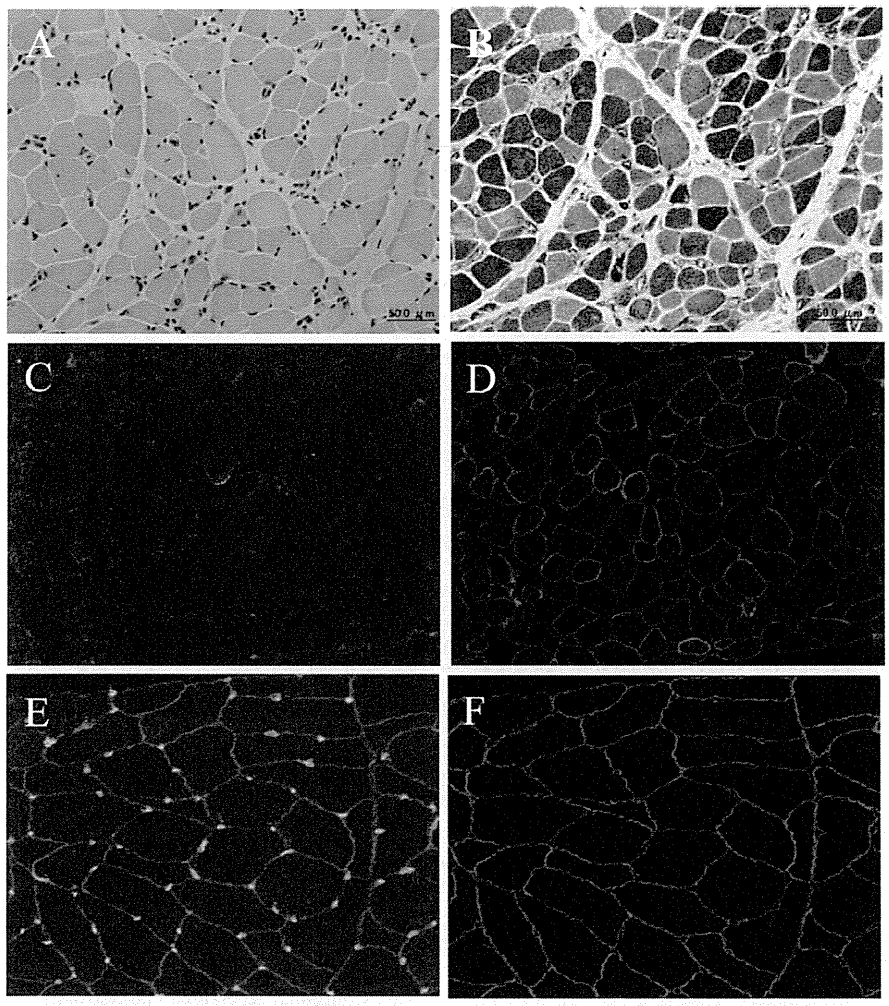


Fig. 2. The biceps brachii muscle showed moderate variation in fiber size, a few necrotic fibers, and increased endomysial and perimysial fibrosis (A, B). Negative immunoreactivity for PTRF (C) but almost normal caveolin-3 levels was observed (D). A: hematoxylin and eosin stain, B: NADH–tetrazolium reductase stain, C, E: PTRF immunohistochemistry, D, F: caveolin-3 immunohistochemistry. E, F: control muscle. Scale bar: 50 μm.

abnormalities were also progressive. Although he did not have insulin resistance at 2 years and 10 months of age, he already showed high levels of T-cho and TG. He demonstrated insulin resistance at 3 years and 11 months.

PTRF is an essential component for the stabilization of caveolae. PTRF-deficient mice do not have detectable caveolae and show decreased insulin receptor levels in fat tissue [10]. Similarly, caveolin-1-deficient mice that show loss of caveolae have been reported to show insulin resistance due to decreased insulin receptor levels in adipose tissues [7–9]. This and a previous report [3] have shown that caveolin-1 and caveolin-2 levels greatly reduced in the intramuscular blood vessels from PTRF-deficient patients. Lipodystrophy and insulin resistance can be closely associated with secondary deficiency of caveolins.

This boy had delayed motor milestones associated with dystrophic changes in his muscles. Interestingly, sarcolemmal caveolin-3 staining was well preserved in this patient compared to previously reported results in older patients, although his immunoreactivity for PTRF was defective. In the case of a 3-year-old Japanese girl who did not show muscle weakness accompanied by high serum levels of CK [5], caveolin-3 immunoreactivity was well preserved despite negative reactivity of PTRF. Secondary reduction of caveolin-3 may progress with age or disease progression [3]; and further studies are necessary to confirm this.

Percussion-induced muscle mounding is a characteristic finding in patients with PTRF deficiency as well as in some patients with caveolin-3 deficiency. Although the detailed mechanism involved in muscle mounding remains to be elucidated, it may be closely related to deficiencies of both caveolin-3 and PTRF.

CGL4 with muscular dystrophy is a progressive disorder, and cardiac problems, including arrhythmia,

Table 1
Clinical and biological summary.

| Age | Height (SD) | Weight (SD) | Clinical and biological signs | CK (normal < 200 IU/L) | T-Cho (140–220 IU/L) | TG (30–150 mg/dL) | Adiponectin (>4.0 μg/mL) | Leptin (0.9–13.0 ng/mL) |
|---|---|---|---|---|---|---|---|---|
| Birth | 47.0 cm (−1.0) | 3.1 kg (−0.3) | Normal amount of subcutaneous fat | ND | ND | ND | ND | ND |
| 4 m | 59.0 cm (−2.3) | 4.5 kg (−2.9) | Head control (+), hypothyroidism | 812 | ND | ND | ND | ND |
| 7 m | 66.4 cm (−1.2) | 5.8 kg (−2.7) | Deceased subcutaneous fat on his face | 1779 | ND | ND | ND | ND |
| 14 m | 76.2 cm (−0.3) | 8.6 kg (−1.2) | Crawl (+), stand with support (+), meaningful words (+) | 1973 | ND | ND | ND | ND |
| 16 m | 78.5 cm (−0.1) | 9.6 kg (−0.5) | Sit alone (+), loss of subcutaneous fat on his face and extremities | 2293 | 252 | ND | ND | ND |
| 2 y 6 m | 88.0 cm (−0.5) | 12.9 kg (+0.2) | Walk alone (+), 2-word sentences (+) | ND | ND | ND | ND | ND |
| 2 y 10 m | 92.7 cm (+0.2) | 13.2 kg (−0.1) | Run (+), jump (−), normal glucose tolerance and no insulin resistance, low level of adipokines | 1715 | 213 | 309 | 1.2 | 1.9 |
| 3 y 11 m | 99.2 cm (−0.1) | 15.3 kg (−0.1) | Insulin resistance (+) | 1777 | 154 | 680 | 1.1 | 1.6 |

ND: not done.

supraventricular, and ventricular tachycardia, may develop after 8–10 years of age [3,4,6]. Careful follow-up is necessary for a better prognosis.

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