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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Case report

Fatal hepatic hemorrhage by peliosis hepatis in X-linked myotubular myopathy: A case report

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Abstract

We report a 5-year-old boy with X-linked myotubular myopathy complicated by peliosis hepatis. At birth, he was affected with marked generalized muscle hypotonia and weakness, which required permanent ventilatory support, and was bedridden for life. He died of acute fatal hepatic hemorrhage after using a mechanical in-exsufflator. Peliosis hepatis, defined as multiple, variable-sized, cystic blood-filled spaces through the liver parenchyma, was confirmed by autopsy. To avoid fatal hepatic hemorrhage by peliosis hepatis, routine hepatic function tests and abdominal imaging tests should be performed for patients with X-linked myotubular myopathy, especially at the time of using artificial respiration.

Keywords: X-linked myotubular myopathy; Hepatic hemorrhage; Peliosis hepatis; Mechanical in-exsufflator

1. Introduction

X-linked myotubular myopathy (XLMTM) is one of the most serious types of centronuclear ("myotubular") myopathies, which is pathologically characterized by a high proportion of small myofibers with centrally placed nuclei [1]. With recent advances in molecular analysis, centronuclear myopathy has been classified into three genetic subtypes. XLMTM is a severe form of centronuclear myopathy presenting with symptoms from birth, including respiratory failure, ophthalmoplegia, and muscle weakness [2]. XLMTM is caused by genetic aberration of the *MTM1* gene on chromosome Xq28 [3]. *MTM1* encodes myotubularin, a dual-specificity 3-phosphoinositide phosphatase, which plays an important role in the regulation of signaling pathways involved in muscle growth and differentiation [3].

Although XLMTM is considered to be a fatal disorder within the first year of life, it has been recently shown that more than half of XLMTM patients achieve prolonged survival, and most of the long-term survivors suffer from several complications in several organ systems [4]. Among them, peliosis hepatis is a rare condition that can affect children and cause fatal hepatic hemorrhage. A few reports have suggested that XLMTM patients might be at risk for development of peliosis hepatis [4–7]. We report a 5-year-old patient with XLMTM who suffered

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from fatal peliosis hepatis. In addition, the clinical features and prevention approach of fatal hepatic hemorrhage in XLMTM are also discussed.

2. Case report

A full-term male was born at 39 weeks of gestational age by normal spontaneous vaginal delivery and weighed 2728 g. The Apgar score was 5 at 1 min and 7 at 5 min. There was no abnormal antenatal symptom (e.g. polyhydramnios, reduced fetal movements, and thinning of the ribs). And neither family history of genetic disorders nor medical problem during perinatal period was observed. At birth, however, marked generalized muscle hypotonia and weakness, which required ventilatory support, appeared in the patient. On physical examination, facial muscle weakness and a high-arched palate were detected, and extraocular muscle involvement was not detected. The hypotonia did not improve with conventional management. The karyotype of peripheral blood was normal. A muscle biopsy from the biceps branch was performed under the possible diagnosis of neuromuscular disease. All muscle fibers were small and round (Fig. 1a), and a peripheral halo was observed in most fibers (Fig. 1b), compatible with the diagnosis of myotubular myopathy. Type 1 fiber predominance was remarkable (90%) (Fig. 1c). Genetic analysis of XLMTM revealed a splice-acceptor-site mutation of *MTM1* in intron 6 (c.445-1G>A), resulting in skipping of exon 7 at the cDNA level (Fig. 1d) [8]. The patient received respiratory support using non-invasive positive pressure ventilation, and underwent a tracheotomy at 8 months of age because of frequent asphyxia caused by aspiration.

At 5 years old, he was admitted to the Uwajima City Hospital for treatment of massive pneumonia and atelectasis in the left lung. Laboratory studies on admission showed that hemoglobin was 14.6 g/dL, white blood cell count was 11,400/μL, platelet count was 338,000/μL, aspartate aminotransferase was 69 IU/L, alkaline phosphatase was 73 IU/L, and C-reactive protein was 0.46 mg/dL. Bacterial blood and sputum cultures showed negative results. Fibrinolytic activity test on four days after admission remained within normal limits (prothorombin time was 11.9 s, fibrin degradation products was 8.6 μg/ml and D-dimer was 0.8 μg/ml). The patient gradually improved with a course of antibiotics (cefotaxime sodium) and lung physical therapy. Nine days after admission, a mechanically assisted coughing system was used as a mechanical in-exsufflator (MI-E) because of difficulty of sputum expectoration. The next day, he suffered from abrupt tachycardia and cyanosis. He had a peripheral coldness and his abdomen was gradually distended, especially the right costal margin, because of hepatic enlargement. Laboratory studies

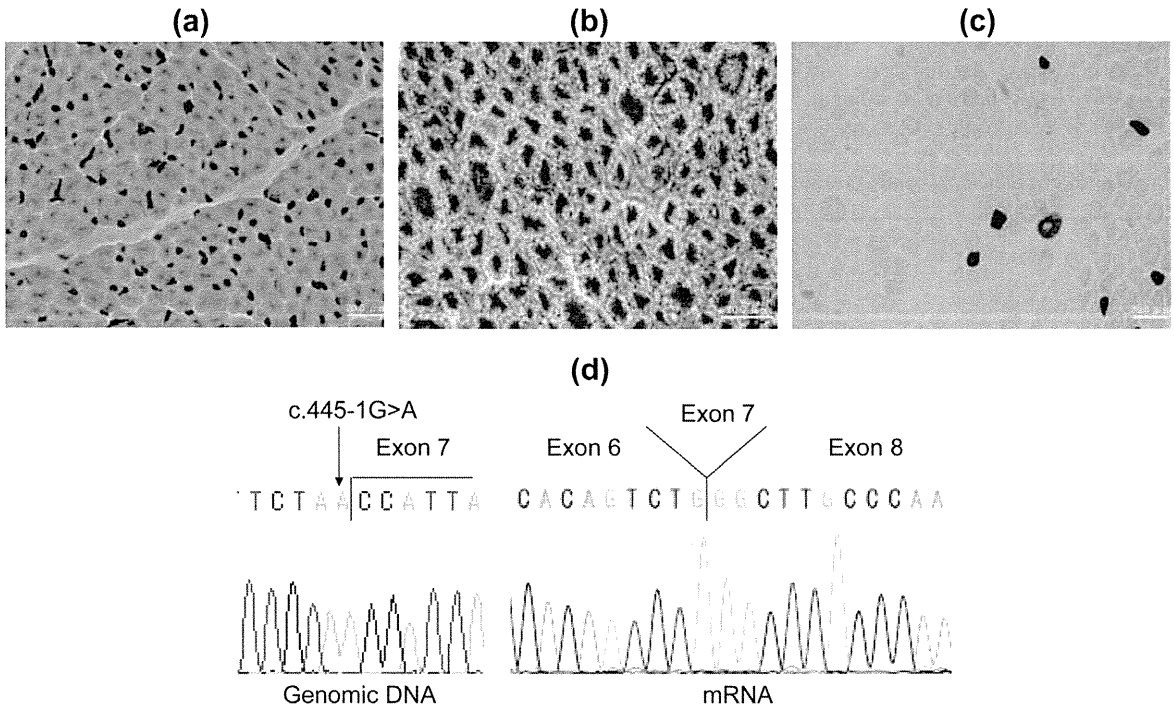

Fig. 1. Histological findings of muscle biopsy and genetic analysis. (a) Hematoxylin and eosin (H & E) staining shows that all muscle fibers are small and round. Marked perimysial fibrosis and scattered pyknotic nuclear clumps can be seen. Muscle fibers with central nuclei comprise 40% of the biopsy specimen. (b) As shown by nicotinamide adenine dinucleotide tetrazolium reductase staining, the intermyofibrillar network is markedly disorganized with peripheral halo features seen in most fibers. (c) On ATPase, types 1, 2A–C comprise 90%, 8%, 2%, and 0%, respectively. Type 2 fiber atrophy can be seen. (d) *MTM1* analysis revealed a splice-acceptor-site mutation in intron 6 (c.445-1G>A) at the genomic DNA level and skipping of exon 7 at the cDNA level.

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showed that the hemoglobin level and platelet count were decreased to 6.4 g/dL and 82,000/μL, respectively. An abdominal computed tomography (CT) scan showed hepatomegaly with a heterogeneous low density area expanding from the medial segment to the right lobe of the liver, and the leakage of contrast material into parenchyma during the arterial phase, suggesting the diagnosis of liver hemorrhage (Fig. 2a). There was little intraperitoneal bleeding. With rapid transfusion of red cells, a hepatic angiogram was performed via the common hepatic artery. Because active bleeding was observed within liver parenchyma (Fig. 2b), obstructing material (Gelpart® Molecular Devices, Nippon Kayaku Co. Ltd., Tokyo, Japan) was injected into the anterior and posterior branches of the hepatic artery to control active bleeding. Despite these treatments, hemoglobin levels gradually decreased from 11.1 to 3.8 g/dL 8 h after the obstruction therapy. The abdomen was further distended and pitting edema appeared in the lower body. Repeated abdominal CT showed massive intraperitoneal bleeding compared with that of the previous day, and a narrowed inferior vena cava caused by hepatic enlargement was detected. He died 4 days after the onset of acute hepatic hemorrhage. The autopsy indicated

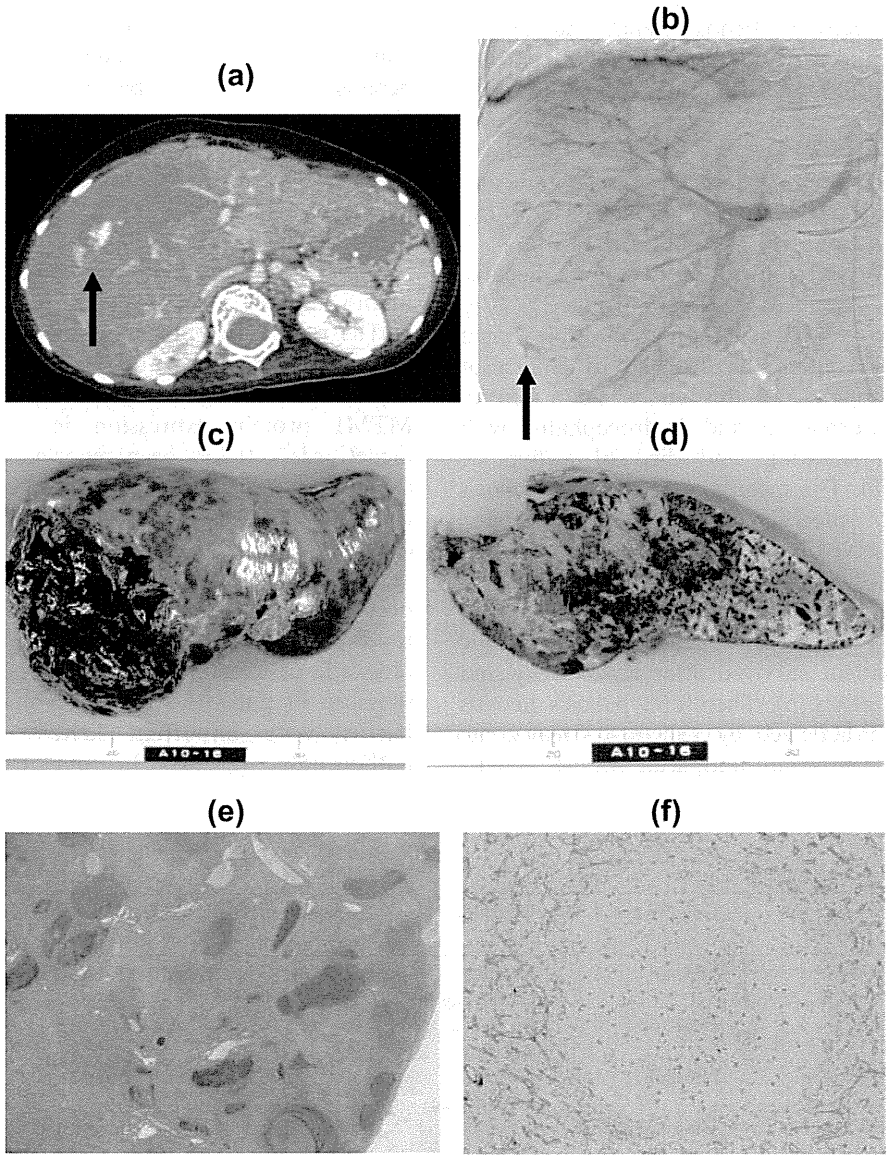

Fig. 2. Radiological and pathological findings of periosis hepatis. (a) Contrast abdominal CT shows hepatomegaly and a heterogeneous low density area extending from the medial segment to the right lobe of the liver. Contrast dye had leaked into the parenchyma (arrow) during the arterial phase. (b) A hepatic angiogram shows a number of retained contrast agents and some active bleeding (arrow) within liver parenchyma. (c and d) Autopsy findings show expansive intraperitoneal bleeding caused by rupture at the right hepatic capsule, and multiple variable-sized cystic blood-filled spaces were found in a section of the liver. (e) Multiple blood-filled spaces within the liver parenchyma can be seen (H & E). (f) Immunohistochemically, the inner surface of the blood-filled space is devoid of CD31-positive endothelial lining in contrast to the sinusoidal endothelia.

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expansive intraperitoneal bleeding caused by rupture of the right hepatic capsule (Fig. 2c). Multiple variable-sized cystic blood-filled spaces and hemorrhagic necrosis were found in the liver (Fig. 2d). Histopathologically, the liver contained multiple blood-filled spaces (Fig. 2e) that were devoid of CD31-positive endothelial lining (Fig. 2f), which was compatible with peliosis hepatis.

3. Discussion

Peliosis hepatis is a rare fatal disorder with a number of causes, and is defined as multiple, variable-sized, cystic, blood-filled spaces through the liver parenchyma, in which spaces are not covered by endothelium of blood vessels histopathologically [9]. Peliosis hepatis has mostly been reported in adult patients associated with chronic wasting disorder [10], human immunodeficiency virus infection [11–12], oral contraceptives [13], androgenic steroids [14], and *Bartonella henselae* infection [15], and is idiopathic [16]. In children, peliosis hepatis is rare and has only been reported with androgenic steroids [17–18], *Escherichia coli* pyelonephritis [19], and XLMTM [4–7].

In XLMTM, only six children with peliosis hepatis, including our case, have been reported (Table 1) [4–7], and five of them developed acute onset multiple organ failure. The remaining child was detected by chance at the time of the autopsy, and this child had a central nervous system malformation and hydrocephalus with ventriculo-peritoneal shunting, and died of a hypoxic episode at 4 years old. The mean age at onset of peliosis hepatis was 4 years. Antecedent infection before hepatic hemorrhage was observed in three patients, including our patient. An elevated liver function test was observed in three patients, including our patient, but was normal in two. Five of six patients died of acute fatal hepatic hemorrhage. One patient survived after laparotomy and transarterial embolization [5]. Therefore, peliosis hepatis with XLMTM is characterized by difficult-to-treat acute onset. Some adult patients with idiopathic peliosis hepatis have received successful emergent hepatectomy, liver transplantation, and arterial embolization [16,20]. However, once hepatic hemorrhage from peliosis hepatis occurs, it is usually difficult to control bleeding, as observed in our patient. Therefore, reducing the incidence and prompt recognition of hepatic hemorrhage are mandatory for XLMTM patients.

The diagnosis of peliosis hepatis is difficult and often missed or delayed because of the atypical findings on standard radiological tests. A previous report indicated that ultrasonic examination is useful to detect abnormal findings according to various liver conditions, and can show perinodular and intranodular vascularity in patients with peliosis hepatis [21]. Other imaging systems, including CT, magnetic resonance imaging, and angiography can also be helpful for diagnosis of peliosis hepatis [18,21]. Our patient showed persistent mild elevations in a serum liver function test before the episode of acute hemorrhage. He had not received a routine ultrasonic examination, and CT findings on admission indicated no hepatic lesion, suggesting hemorrhage or peliosis hepatis. Therefore, fatal hepatic hemorrhage from peliosis hepatis was induced by an unknown cause after admission.

The mechanism of peliosis hepatis remains to be fully elucidated. And the causal relationship between peliosis hepatis and XLMTM is poorly understood, although MTM1 protein expression in liver are reported in GeneCards®. It has been reported that a mechanical in-exsufflator (MI-E) is safe and effective for respiratory infections of pediatric patients with neuromuscular disorders [22]. MI-E was initially used for removing secretions in our case, and fatal hepatic hemorrhage occurred on the next day of MI-E adoption. In some reports describing the mechanism of peliosis hepatis, blockade of liver blood outflow and increased sinusoidal pressure in patients with abnormality of the sinusoidal barrier were important factors contributing to the pathogenesis [23,24]. In our case, there were no

Table 1
Summary of peliosis hepatis in XLMTM patients.

No.	Age	Severity of XLMTM	Cognitive development	Detection of PH	Known liver dysfunction	Infection at the onset of PH	Diagnosis	Status
1[4]	5 y	Severe	Normal	Hepatic hemorrhage	Yes	Un-documented	Autospy	Deceased
2[4]	4 y	Severe	Normal	By chance (autospy)	No	Un-documented	Autospy	Deceased
3[5]	3 y	Severe/ moderate	Normal	Hepatic hemorrhage	No	URI otitis media	CT	Improved
4[6]	2 y 6 m	Severe	Un-documented	Hepatic hemorrhage	(Un-documented)	Un-documented	Autospy	Deceased
5[7]	5 y	Severe	Un-documented	Hepatic hemorrhage	Yes	Fever	Autospy	Deceased
6 (present patient)	5 y	Severe	Slightly retarded	Hepatic hemorrhage	Yes	Pneumonia	Autospy	Deceased

PH: peliosis hepatis, URI: upper respiratory infection, CT: computed tomography.

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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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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

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 *COL6A2* c.1270-1G>C
2	2	(+)	(+)	(–)	(+)	(+)	(–)	(–)	(–)	(+)	413	CD	*COL6A3* c.5692delG p.Val1898fs *COL6A3* c.8737delG p.Ala2913fs
3	2	(+)	(+)	(–)	(–)	(–)	(–)	(–)	(–)	(+)	474	CD	NF
4	1	ND	(+)	(–)	(–)	(–)	(–)	(–)	(+)	(–)	339	CD	*COL6A2* c.2678_2700del23 p.Pro893fs 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 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 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

Pt	Age at survey (years)	Perinatal history						Developmental milestone (months)				Motor function at survey		Respiratory function			Maximum Cobb angle (°) (yrs)
		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)	
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.

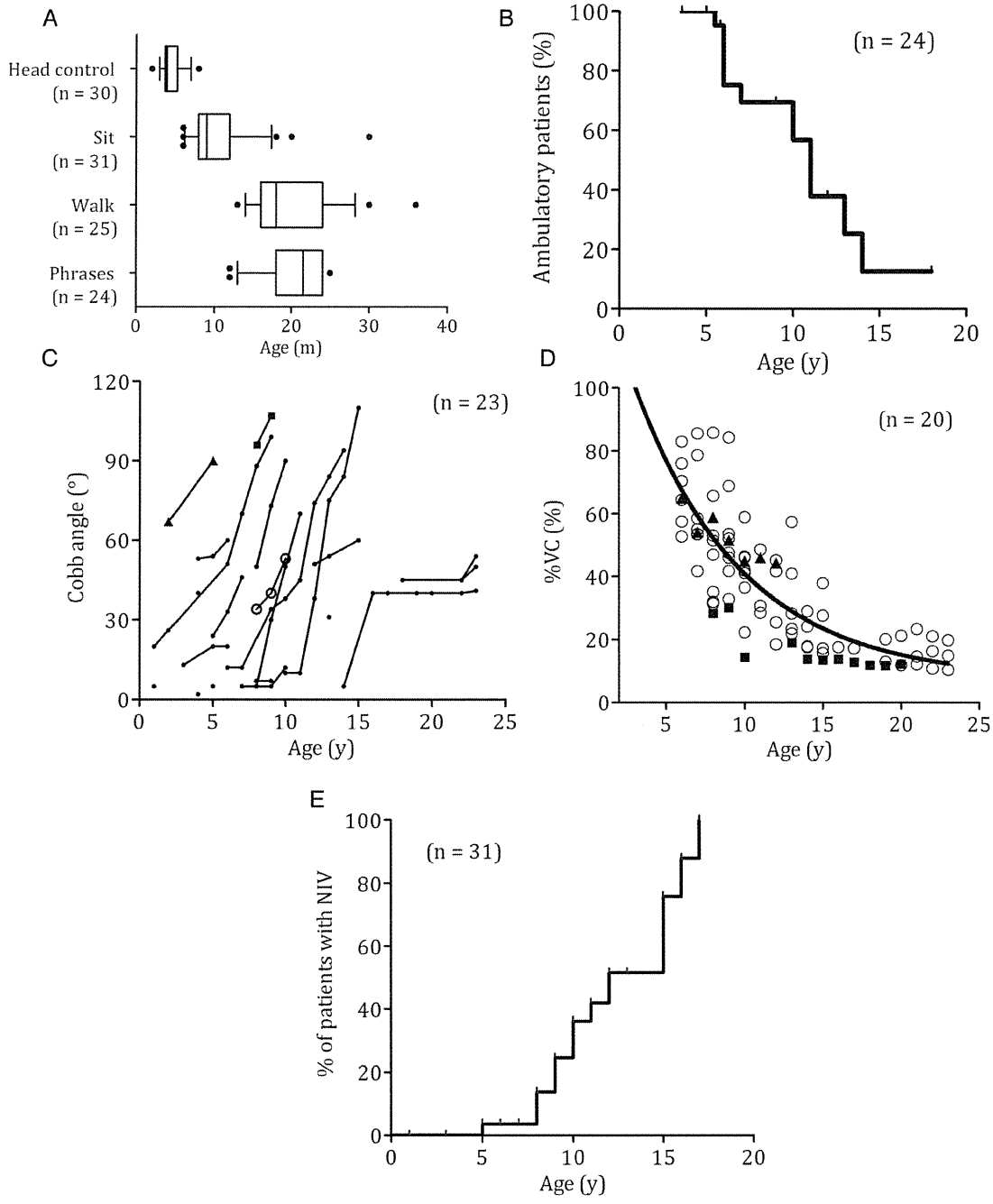

Figure 1 (A) Age ranges at completion of neck control, sit, independent ambulation and phrases. The boxes represent the range from the 25–75th percentile, while the bars span the 10–90th percentile. (B) Kaplan-Meier curve showing deterioration of walking ability in Ullrich congenital muscular dystrophy (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

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

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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.

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].

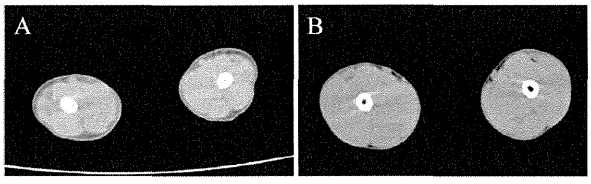

Fig. 1. Computed tomographic scan at the thigh level at 1 year (A) and 2 years and 10 months (B) of age. The thigh muscles showed hypertrophy without abnormal intensity. Note the progressive loss of subcutaneous adipose tissues.

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

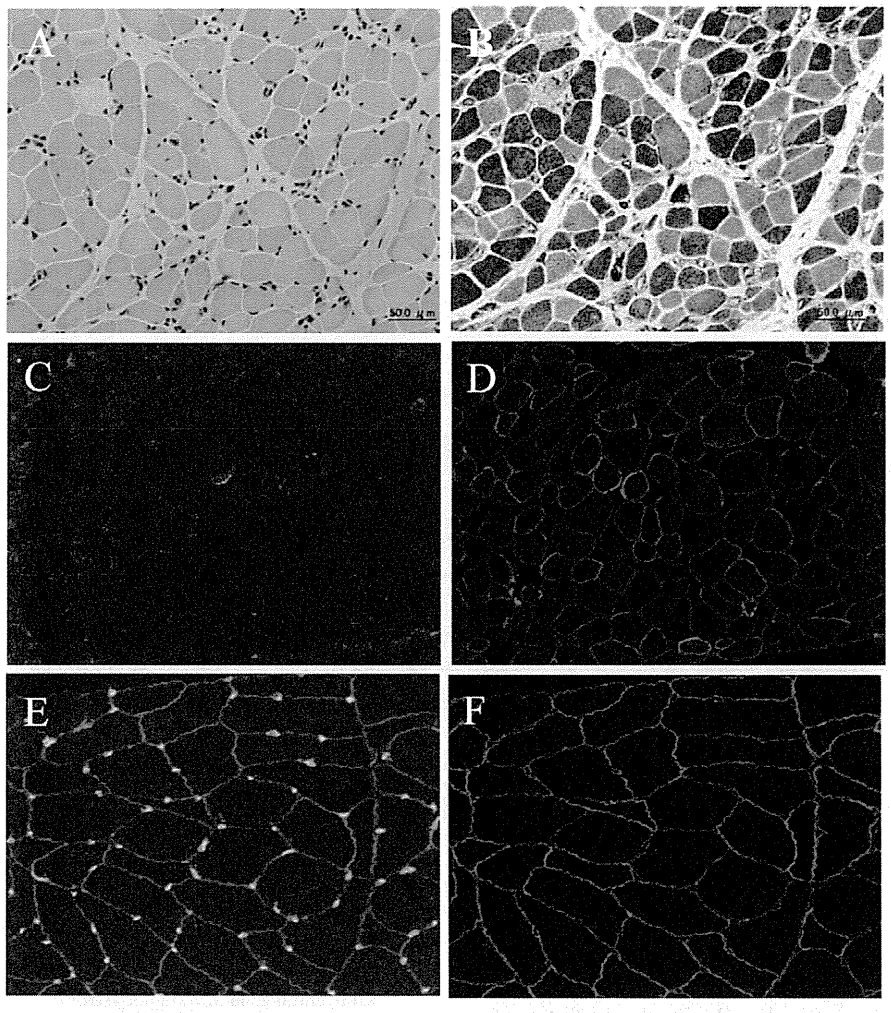

Fig. 2. The biceps brachii muscle showed moderate variation in fiber size, a few necrotic fibers, and increased endomysial and perimysial fibrosis (A, B). Negative immunoreactivity for PTRF (C) but almost normal caveolin-3 levels was observed (D). A: hematoxylin and eosin stain, B: NADH–tetrazolium reductase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