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Case report

Muscle glycogen storage disease 0 presenting recurrent syncope with weakness and myalgia

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Abstract

Muscle glycogen storage disease 0 (GSD0) is caused by glycogen depletion in skeletal and cardiac muscles due to deficiency of glycogen synthase 1 (GYS1), which is encoded by the *GYS1* gene. Only two families with this disease have been identified. We report a new muscle GSD0 patient, a Japanese girl, who had been suffering from recurrent attacks of exertional syncope accompanied by muscle weakness and pain since age 5 years until she died of cardiac arrest at age 12. Muscle biopsy at age 11 years showed glycogen depletion in all muscle fibers. Her loss of consciousness was gradual and lasted for hours, suggesting that the syncope may not be simply caused by cardiac event but probably also contributed by metabolic distress.

Keywords: Glycogen storage disease; Glycogen synthase; Glycogen; Syncope; Sudden death

1. Introduction

Glycogen is a high molecular mass polysaccharide that serves as a repository of glucose for use in times of metabolic need. It is stored in liver, cardiac and skeletal muscles, and broken down to glucose to produce ATP as energy as needed. For the synthesis of glycogen, at least two proteins, glycogenin (GYG) and glycogen synthase (GYS), are known to be essential. GYG is involved in the initiation reactions of glycogen synthesis: the covalent attachment of a glucose residue to GYG is followed by elongation to form an oligosaccharide chain [1]. GYS catalyzes the addition of glucose monomers to the growing glycogen molecule through the formation of alpha-1,4-glycoside linkages [2].

Defect in either GYG or GYS can cause glycogen depletion. Recently, muscle glycogen deficiency due to a mutation in a gene encoding muscle GYG, *GYG1*, was reported [3] and named as glycogen storage disease type XV. In contrast, glycogen depletion caused by the *GYS* gene mutation is called glycogen storage disease type 0 (GSD0). GSD0 was first reported in 1990 in patients with type 2 diabetes who had a defect in glycogen synthesis in liver, which was caused by a defect in liver GYS, GYS2, and the disease was named as liver GSD0 (or also called GSD0a) [4,5].

The disease of muscle GYS, GYS1, was first described in 2007 in three siblings and named muscle GSD0, which is

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also called GSD0b [6]. One of the patients initially manifested exercise intolerance, epilepsy and long QT syndrome since the age of 4 years, then died of sudden cardiac arrest after exertion when he was 10.5-year-old. The other two siblings were then genetically confirmed as muscle GSD0 with mutations in *GYS1* and cardiac involvement was also found in both. The second muscle GSD0 family was reported in 2009 [7]. The 8-year-old boy had been healthy before collapsing during a bout of exercise, resulting in death. Post-mortem examinations and studies verified the diagnosis of muscle GSD0. He had a female sibling who died at 6 days of age of undetermined cause. Here we report the first muscle GSD0 patient in Asia with some distinct clinical manifestations from other reported cases.

2. Case report

An 11-year-old Japanese girl with repeated episodes of post-exercise loss of consciousness, weakness, and myalgia since age 5 years, was admitted to the hospital. She was the first child of unrelated healthy parents. She was born uneventfully and was normal in psychomotor development. At age 2 years, she developed the first episode of generalized tonic-clonic seizure while she was sleeping. At age 4 years, she had the second episode of generalized tonic-clonic seizure when she was under general anesthesia for tonsillectomy, whose cause was thought to be hypoglycemia due to prolonged fasting. In both episodes, seizure was followed by strong limb pain. At age 5 years, she suffered from the first episode of syncope while climbing up stairs. She recovered after a few hours. One year later, she had the second syncopal attack after running 50 m, which was accompanied by subsequent limb muscle weakness and myalgia. Since then, similar episodes were repeated several times a year. For each bout, she first developed leg muscle weakness immediately after exercise, making her squat down, and gradually lost the consciousness. She recovered her consciousness after a few hours but always experienced strong myalgia in legs which lasted for several hours. Blood glucose level was not decreased during these attacks.

On admission, general physical examination revealed no abnormal finding. On neurological examination, she had mild proximal dominant muscle weakness and mildly limited dorsiflexion of both ankle joints. T1-weighted images of skeletal muscle MRI showed high signal intensities in gluteal and flexor muscles of the thigh, which were assessed to be fatty degeneration (Fig. 1). Systemic investigations including electrocardiography, echocardiography, stress cardiac catheterization, stress myocardial scintigraphy, brain imaging, electroencephalography, and screening tests for metabolic diseases revealed no abnormality except for a mild ischemic finding on exercise electrocardiography. Ischemic and non-ischemic forearm exercise tests [8] showed the lack of lactate elevation, raising a possibility of glycogen storage disease. A few months later, resting electrocardiography, 24-h holter monitoring and resting echocardiography were re-evaluated and again revealed normal findings.

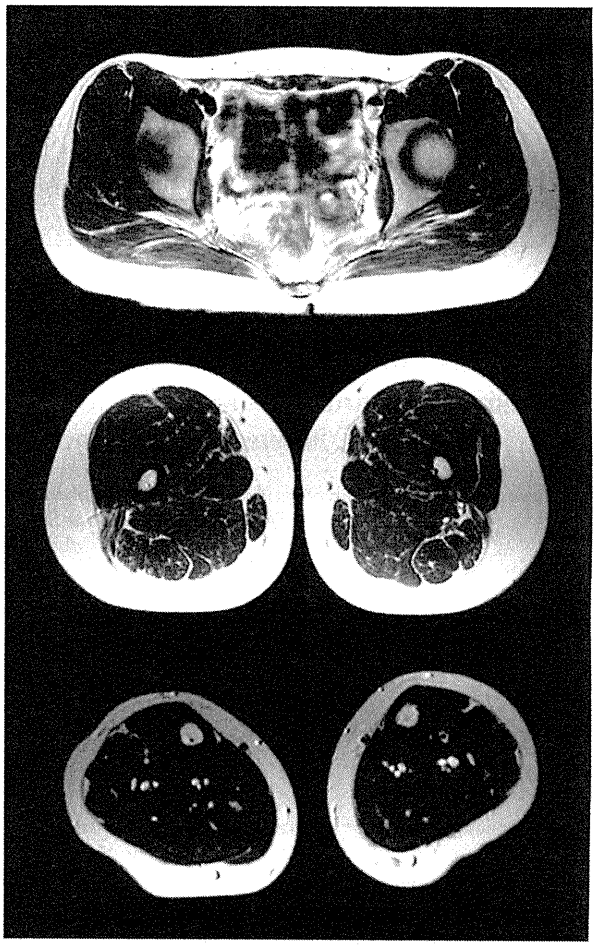

Fig. 1. Muscle MRI, T2WI, axial. It shows high intensity in gluteus maximus and biceps femoris muscles.

3. Histological analysis of skeletal muscle

Muscle biopsy was performed from biceps brachii. Serial frozen sections were stained with hematoxylin and eosin, modified Gomori trichrome, and a battery of histochemical methods. The most striking finding was depletion of glycogen in all muscle fibers but not in the interstitium on periodic acid-schiff (PAS) staining (Fig. 2A). Phosphorylase activity was also deficient in all fibers (Fig. 2B). Mitochondria especially at the periphery of muscle fibers were prominent on modified Gomori trichrome (Fig. 2D). ATPase staining revealed type 2 fiber atrophy. Electron microscopic analysis showed mitochondrial proliferation at the periphery of muscle fibers with no notable intramitochondrial inclusions (Fig. 2E).

4. Biochemical and molecular analysis

Both the activity of GYS1 and the amount of glycogen in the skeletal muscle were markedly reduced (Table 1). On western blotting, GYS1 in the patient's skeletal muscle was undetectable (Fig. 2F). The *GYS1* gene sequence analysis revealed compound heterozygous mutation of

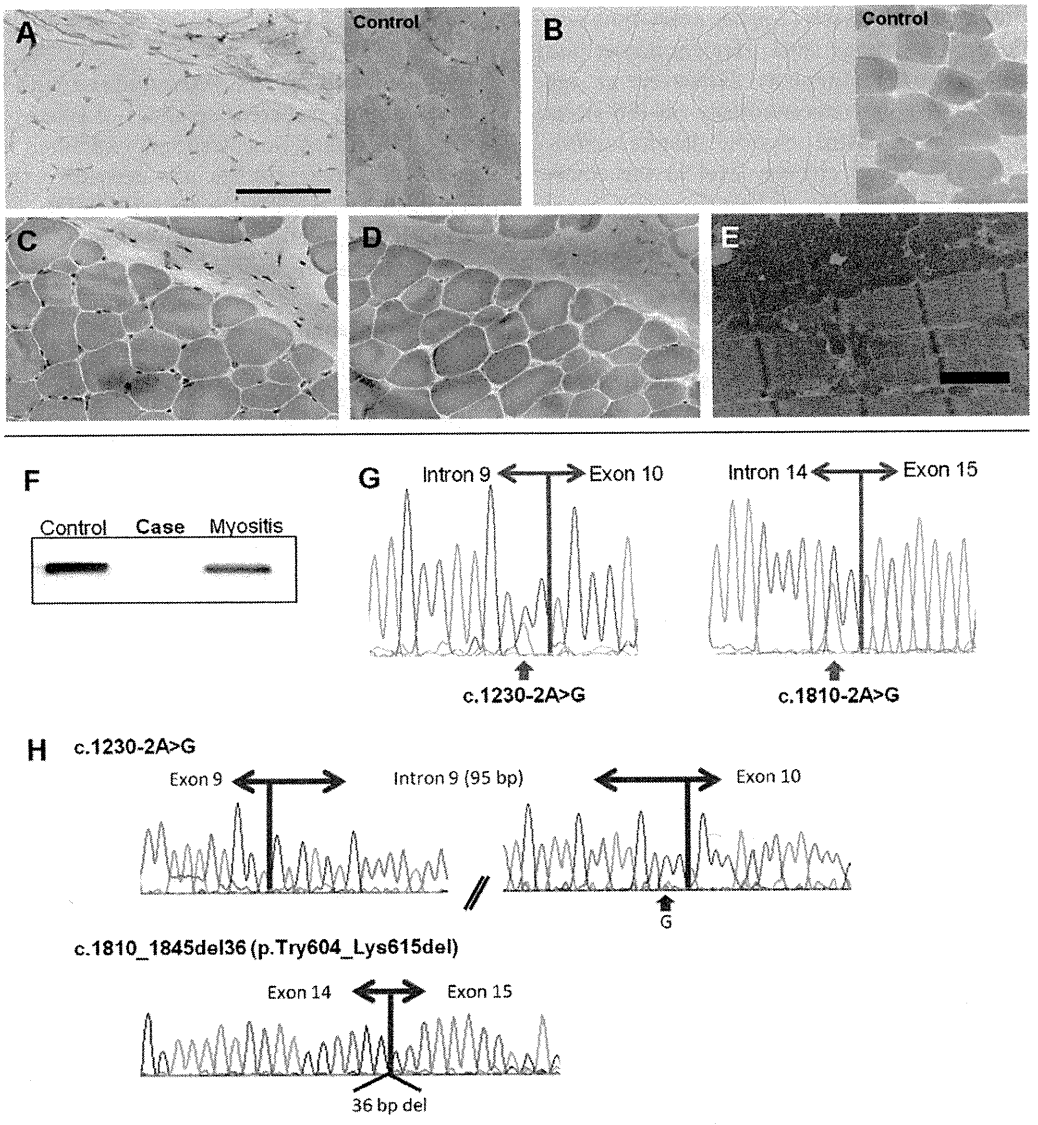

Fig. 2. Histological, genetic and protein analyses. Periodic acid-schiff (PAS) staining shows marked depletion of glycogen in muscle fibers but not in the interstitium (A). Phosphorylase activity is also deficient in all fibers (B). Hematoxylin and eosin staining shows mild fiber size variation (C). On modified Gomori trichrome, mitochondria are prominent especially at the margin of each muscle fiber (D). On electron microscopy (EM), mitochondria are increased in number at the periphery of muscle fibers (E). Bars represent 100 μm for histochemistry and 7 μm for EM. On western blotting using anti-GYS1 antibody (Abcam), GYS1 protein is absent in skeletal muscle from the patient (F). Sequence analysis for the *GYS1* gene reveals a compound heterozygous mutation of c.1230-2A > G and c.1810-2A > G (G). cDNA analysis showed insertion of intron 9 between exon 9 and 10 and 36-bp deletion from the beginning of exon 15 (H).

Table 1
Analyses of enzymatic activity and glycogen content. The activity of GYS and glycogen content in skeletal muscle were markedly reduced.

	Glycogen synthase (mol/min/mg)	UDPG-pyrophosphorylase (nmol/min/mg)	Glycogen contents (% of wet weight)
Patient	*0.9*	30.5	*0.03*
Control	42.0 ± 11.2	31.2 ± 3.5	0.94 ± 0.55

Italicized values: lower than control range.

c.1230-2A > G in intron 9 and c.1810-2A > G in intron 14 (Fig. 2G). cDNA analysis confirmed the insertion of the full-length intron 9 between exons 9 and 10 and a 36-bp deletion in the beginning of exon 15 (Fig. 2H).

5. Clinical course after diagnosis

Upon the diagnosis of GSD0, exercise was strictly limited to avoid syncope resulted from glucose depletion. In

addition, oral intake of cornstarch (2 g/kg, every 6 h) was started to maintain blood sugar level. Her condition had been stable for 1 year after diagnosis. However, at age 12 years, she was found lying unconsciously on the stairs at her school. She had persistent asystole despite ambulance resuscitation. The blood glucose level in the emergency room was above 100 mg/dl.

6. Discussion

We identified the first Asian patient with muscle GSD0, who manifested recurrent episodes of syncope with subsequent muscle weakness and myalgia, and eventually developed cardiac arrest.

Findings in our patient seem to be similar to previous reports, but some differences indicated the possibility of another pathogenesis of the disease. Our patient repeatedly suffered from episodes of syncope. In contrast to two earlier reports, those patients never had syncope, although the last attack led to sudden death [6,7]. In support of this notion, most muscle glycogen synthase knock-out mice died soon after birth due to impaired cardiac function [8]. However, the pattern of loss of consciousness in our patient cannot be explained by simple cardiac dysfunction, as she lost her consciousness gradually after exercise and took hours to regain, which is different from typical cardiac syncope, usually showing sudden loss of consciousness and rapid recovery. Alternatively, defective glycogen synthesis in brain may be related to syncope, as GYS1 is also expressed in brain, albeit not so much as in cardiac and skeletal muscles. Another possibility may be intermittent arrhythmia. However, electrocardiogram during the episode was never obtained. Further studies are necessary to answer this question.

On muscle pathology and electron microscopy, we found profound deficiency of glycogen in all muscle fibers accompanied by mitochondrial proliferation, which is similar to previous reports. The mitochondrial proliferation may reflect a compensatory mechanism for supplying ATP to glycogen-depleted muscles. Interestingly, phosphorylase activity on histochemistry seemed deficient. This is consistent with the fact that endogenous glycogen is used as a substrate of phosphorylase on histochemistry. Previous reports described the reduced number of type 2 fibers. In our patient, type 2 fiber atrophy, but not type 2 fiber deficiency, was seen. Although type 2 fiber atrophy is a nonspecific finding, this picture might also reflect the dysfunction of glycogen-dependent muscle fibers.

7. Conclusion

We identified the first Asian patient with muscle GSD0. In our patient, recurrent episodes of syncope and eventual sudden death may not be simply explained by cardiac dysfunction. Further studies are necessary to elucidate the mechanism of syncope in muscle GSD0 and to establish appropriate guideline of management for these patients to prevent sudden death.

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Original article

Severe muscle damage following viral infection in patients with Fukuyama congenital muscular dystrophy

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Abstract

Fukuyama congenital muscular dystrophy (FCMD), which is characterized by cortical migration defect and eye abnormalities, is the most common subtype of CMD in Japan. *Fukutin* (*FKTN*), the responsible gene for FCMD, encodes a protein involved in the glycosylation of alpha-dystroglycan. We have experienced some patients with FCMD who showed sudden exacerbation of muscle weakness with marked elevation of serum creatine kinase (CK) and urinary myoglobin levels a few days after a febrile episode of viral infection, occasionally leading to death. To describe this peculiar phenomenon, we focused on 12 patients who developed a sudden exacerbation of muscle weakness among 96 genetically defined FCMD patients and hospitalized because of a febrile illness at Tokyo Women's Medical University between 1997 and 2008. All the 12 patients were homozygous for a 3-kb insertion mutation of *FKTN*. The patients developed exacerbation of muscle weakness ranging from paralysis to loss of head control. The onset was concentrated in summer, and coxsackieviruses and enteroviruses were most often detected, especially in infantile patients. Eight of the 12 patients were treated with corticosteroids and recovered within 2 weeks. Four patients were treated without steroid, and needed 18.5 days on mean for improvement. None developed renal failure. The reason for muscle damage induced by viral infection remains unknown; however, physicians should consider its risk, sometimes leading to death, and draw it to parents' attention, especially in the defervescent stage.

Keywords: Fukuyama congenital muscular dystrophy; Herpangina; Viral infection; Rhabdomyolysis; *FKTN*

1. Introduction

Fukuyama congenital muscular dystrophy (FCMD) is a congenital muscular dystrophy characterized by mental retardation accompanied by a cortical migration defect and eye abnormalities [1]. *Fukutin* (*FKTN*), the responsible gene for FCMD, encodes a protein involved in the glycosylation of alpha-dystroglycan, which compromises maintenance of sarcolemmal stability [2–4].

Malignant hyperthermia induced by general anesthesia and rhabdomyolysis after exercise are well-known causes of sudden exacerbation of muscle weakness in the clinical course of patients with muscular dystrophy [5,6]. Likewise, exacerbation of muscle weakness is often clinically observed in association with infectious disease, but there are few reports on its sudden onset in defervescence. We report patients with FCMD who developed a sudden exacerbation of muscle weakness accompanied by elevated serum creatine kinase (CK) and urinary myoglobin levels, sometimes even in the defervescence stage. Since this phenomenon sometimes occurs during the defervescence stage when the parents and physicians

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feel somewhat relieved, its diagnosis can be delayed, which could even lead to death; thus, the importance of timely recognition of this condition cannot be overemphasized.

Although not included in this study, a few patients have died of respiratory depression due to sudden exacerbation of muscle weakness accompanied by elevated serum CK and urinary myoglobin levels a few days after a febrile episode by viral infection. We would thus like to draw attention to the need for prompt recognition of any sudden change in symptoms that might occur after defervescence in these patients.

2. Subjects and methods

Among 96 FCMD patients with a 3-kb insertion mutation of *FKTN* who were hospitalized at the Department of Tokyo Women's Medical University for the treatment of febrile illness between 1997 and 2008 (Table 1), 12 patients (1 year 4 months to 6 years 2 months old; mean, 3 years 7 months old; 10 female and 2 male; Table 2) who showed transient exacerbation of muscle weakness of sudden onset during a febrile illness or in the defervescence stages, were selected. The medical records were retrospectively reviewed to obtain data on age, diagnosis of febrile illness, causative virus for fever, interval (in days) between occurrence of symptoms and exacerbation of muscle weakness, daily motor skills and lowest motor skill level at exacerbation of muscle weakness, laboratory data, treatment, period between onset of muscle weakness exacerbation and recovery to previous level, and *FKTN* genotype of patients. The causative virus was determined by virus isolation from pharyngeal swabs and genetic analysis using polymerase chain reaction (PCR), as well as by blood tests for measurement of antibody titers. The causative virus could not be identified in four patients diagnosed as having herpangina. With regard to the genotyping of *FKTN*, a 3-kb insertion founder mutation was confirmed using PCR [7].

3. Results

The phenomenon tends to occur in infants with FCMD. The diagnosis on admission was herpangina in six patients, upper respiratory tract infection in two patients and pharyngitis in four patients. The causative virus was identified in eight out of the 12 patients; coxsackievirus in four patients, enterovirus in three patients and echovirus in one patient. When fever is defined as a body temperature of 37.5 °C or higher (axillary temperature), the duration of fever during the viral illness was 1–5 days (3.25 days, on mean). The interval between the occurrence of symptoms suggestive of viral infection, such as fever, cough or nasal discharge, and the exacerbation of muscle weakness, which started in defervescence in seven patients, was 2–6 days (3.42 days, on mean). Even though all initially had the ability to hold their head up and sit without support, one patient became unable to hold up his head (Table 2) and 11 were in an almost paralytic state. Three patients developed hypercapnia and required mechanical ventilatory support. The period between the onset of muscle weakness exacerbation and recovery to the previous level was 3–27 days (14.8 days, on mean). Seven patients were treated with intravenous prednisolone 1.5 mg/kg/day, and it was gradually tapered and discontinued, according to the improvement of motor abilities and urinary myoglobin level of the patients. The duration of administration of steroid was 3–5 days (4.3 days, on mean). One patient was treated with methylprednisolone (30 mg/kg/day) pulse therapy. The resting four patients did not require steroids, and all had improved to the previous level of muscle strength at discharge. The mean period between onset and recovery was 18.5 days in patients without steroids. There was a tendency towards earlier muscle recovery in patients treated with steroids than in those without them, although no statistically significant difference was noted between the two groups (Table 2). Serum CK level at the onset of exacerbation of the muscle weakness was markedly elevated by up to 2.7 to 37.8 (10.8 times, on mean) than that of the recovery stage (Table 3), and the CK-MM isoform fraction of CK was predominant. Urinary myoglobin was checked in nine patients, showing high levels from 58 to 3000 ng/ml (normal control: less than 10 ng/ml) (Table 3). All 12 patients were homozygous for the 3-kb insertion founder mutation.

Table 1
Summary of patient distribution.

	Homozygote	Heterozygote	Total (number of patients)
3-kb insertion mutation of FKTN			
Girls	52	5	57
Boys	36	3	39
	88	8	96

4. Discussion

Malignant hyperthermia induced by anesthesia and rhabdomyolysis after intense exercise are well-known life-threatening causes of muscle weakness of sudden onset in patients with Duchenne muscular dystrophy (DMD) [5,6]. However, there are few reports on exacerbation of muscle weakness during the course of a viral infection. We previously reported that 23 patients with congenital muscular dystrophy showed exacerbation of muscle weakness during the course of an infectious disease [8]. In this report, the patients were not only clinically diagnosed based on Fukuyama's classification [1], by typical signs such as cerebral dysgenesis, mental

Table 2
Clinical summary of patients.

Patient	Age	Sex	Month of year of development of infection	Diagnosis of febrile illness	Virus	Duration of fever (days)	Interval from fever to onset of muscle weakness (days)	Period from onset of muscle weakness to recovery (days)	Daily motor skill level	Lowest motor skill level	Steroid treatment	Use of ventilator
1	1 year 4 months	F	7	URI	Coxsackievirus	2	3	17	Able to maintain sitting position	Complete paralysis	–	–
2	8 years 9 months	F	8	Pharyngitis	Echovirus	5	2	24	Able to maintain sitting position	Complete paralysis	–	○
3	3 years 3 months	M	7	Herpangina	N.D.	4	3	3	Walking with support	Maintaining sitting position	PSL 1.5 mg/kg/d for 4 days	–
4	2 years 8 months	F	7	Pharyngitis	Coxsackievirus	5	2	25	Standing with support, crawling	Complete paralysis	PSL 1.5 mg/kg/d for 7 days	–
5	4 years 3 months	M	7	Herpangina	Coxsackievirus	3	2	22	Walking with support	Complete paralysis	PSL 1.5 mg/kg/d for 5 days	–
6	2 years 11 months	F	7	Pharyngitis	Enterovirus	4	5	8	Standing with support, crawling	Complete paralysis	PSL 1.5 mg/kg/d for 5 days	–
7	6 years 2 months	F	7	Herpangina	N.D.	4	5	16	Able to maintain sitting position	Complete paralysis	–	–
8	1 year 10 months	F	7	Herpangina	N.D.	3	6	3	Sliding on buttocks	Complete paralysis	PSL 1.5 mg/kg/d for 4 days	–
9	2 years 4 months	F	10	Pharyngitis	Enterovirus	2	3	17	Able to maintain sitting position	Complete paralysis	–	○
10	3 years 8 months	F	4	URI	Coxsackievirus	1	3	27	Able to maintain sitting position	Complete paralysis	mPSL 30 mg/kg/d for 3 days	○
11	1 year 10 months	F	7	Herpangina	Enterovirus	3	2	5	Sliding on buttocks	Complete paralysis	PSL 1.5 mg/kg/d for 4 days	–
12	5 years 5 months	F	8	Herpangina	N.D.	3	5	11	Able to maintain sitting position	Complete paralysis	PSL 1.5 mg/kg/d for 3 days	–
Mean	3 years 7 months					3.25	3.42	14.83			4.37	

N.D., not detected; PSL, prednisolone; mPSL, methylprednisolone; URI, upper respiratory tract inflammation.

Table 3
Clinical data of patients.

Patient	Age	Sex	Urinary myoglobin (ng/ml)	Peak CK level (IU/L)	CK level during recovery stage (IU/L)	Ratio of peak CK level to that during recovery stage
1	1 year 4 months	F	390	27,420	4981	6
2	8 years 9 months	F	N.E.	14,791	1040	14
3	3 years 3 months	M	3000	57,800	3524	16
4	2 years 8 months	F	87	45,860	4470	10
5	4 years 3 months	M	N.E.	35,020	1764	20
6	2 years 11 months	F	N.E.	25,250	2815	9
7	6 years 2 months	F	3000	12,060	4023	3
8	1 year 10 months	F	3000	24,600	5835	4
9	2 years 4 months	F	3000	43,000	1137	38
10	3 years 8 months	F	58	14,872	3546	4
11	1 years 10 months	F	3000	13,610	4935	3
12	5 years 5 months	F	N.E.	18,240	1388	13
Average	3 years 7 months		1942	27,710	3288	12

CK, creatine kinase; N.E., not examined.

retardation and muscular dystrophy, but also all of them had a definitive diagnosis of FCMD with genetic evidence.

From our results of muscle biopsy performed in two of the present subjects in the middle of the exacerbation showing no evidence of myositis [8], we think this phenomenon is suggestive of rhabdomyolysis rather than myositis. None of the patients, who had sufficient intelligence to communicate, complained of myalgia or muscle swelling. The level of myogloburinuria was mild to moderate, and none of the patients developed renal failure. This suggests that the muscle damage was not so severe, but it could be crucial for patients with FCMD who originally have muscle weakness and atrophy.

Herpangina caused by coxsackieviruses and echoviruses is a common disease in general pediatric outpatient clinics, but patients who show transient muscle weakness during defervescence are rarely seen [9]. Different from influenza virus, the most common causative virus for rhabdomyolysis, coxsackieviruses sometimes and echoviruses rarely cause rhabdomyolysis. The mechanisms of muscle breakdown in the course of a viral illness are still unknown, but it has been proposed that the muscles of the whole body may be affected by direct invasion of toxins into muscle or blood [10]. However, no patients with muscular dystrophy other than FCMD required hospitalization or outpatient care for transient muscle weakness after an infectious disease during the same observation period for about 10 years. Based on this finding, it was speculated that the abnormality of the *FKTN* gene may be involved, at least in part, in the mechanisms underlying the development of rhabdomyolysis. In a report by Muntoni et al., exacerbation of transient muscle weakness after an infectious disease was observed in patients with limb-girdle muscular dystrophy 2 M, which is also caused by a *FKTN* gene abnormality [11]. Some patients with dilated cardiomyopathy 1X with the *FKTN* gene mutation [12], also showed rhabdomyolysis or exacerbation of heart failure symptoms during defervescence from a febrile illness diagnosed as herpangina or upper respiratory tract infection.

Considering the recent aspect that many viruses recognize and bind to carbohydrate chains on host cell membranes as specific receptors [13], we can hypothesize that changes due to the *FKTN* gene abnormality may cause an increase in affinity for specific viruses.

Regarding treatment, there was a tendency towards earlier improvement in the group in which steroids were used than in the group in which steroids were not used, although no statistical significance was observed between the two groups. Recent papers have reported that steroid use maintained and improved motor abilities in patients with *FKTN* gene mutations [11], suggesting that steroid use may contribute to sarcolemmal stability, as in steroid therapy for DMD [14]. In addition, steroid therapy is considered to be curative for rhabdomyolysis, via its effect of suppressing secondary responses by cytokine secretion and inflammatory cell infiltration due to muscle breakdown [15]. Also, during the period of muscle weakness after infection in FCMD patients, in whom sarcolemmal fragility is increased, it is very likely that the recovery of the muscle strength is promoted by suppression of secondary responses after muscle breakdown, as well as stabilization of the sarcolemma. We propose to investigate this by accumulating further cases in the future.

5. Conclusion

Exacerbation of muscle weakness associated with viral infection could occur at all ages in patients with FCMD, but was most often observed in infants and young children who suffered from coxsackievirus or echovirus infection, such as herpangina. It could sometimes lead to severe respiratory failure requiring mechanical ventilation support. Since it could occur in defervescence, when attention to patients is relaxed, we

should continue careful observations on these patients especially after a viral infection, and educate their families of its risk.

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Case report

High-density CT of muscle and liver may allow early diagnosis of childhood-onset Pompe disease

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Abstract

Pompe disease is classified into infantile-, childhood- and adult-onset forms based on onset age and the degree of organ involvement. Differing from the infantile-onset form which is characterized by marked organ involvement, the childhood-onset form usually presents with muscle weakness and elevation of serum creatine kinase (CK), mimicking those of progressive muscular dystrophy. We report our successful early diagnosis and initiation of enzyme replacement therapy (ERT) in a young girl with childhood-onset Pompe disease before the development of skeletal muscle symptoms. She was referred to our hospital at the age of 2 years 4 months because of hyperCKemia detected incidentally. She was active and lacked developmental delay and muscle weakness; however, hepatomegaly was noted. The combination of high-density changes in the liver and skeletal muscle on computed tomography (CT) images was suggestive of glycogen storage disorder, especially childhood-onset Pompe disease. Low alpha-glucosidase (GAA) activity on dried blood spots facilitated the diagnostic process, and genetic analysis of GAA allowed a definitive diagnosis, without performing muscle biopsy. We promptly started ERT at the age of 2 years 6 months. After 1 year, she still had not developed any skeletal muscle symptoms, and serum CK level was almost normal. Since the efficacy of ERT is thought to depend on the extent of muscle damage at its commencement, we expect that ERT may have prevented the manifestation of skeletal muscle involvement in this patient.

Key words: Enzyme replacement therapy (ERT); Childhood-onset; Pompe disease; Hepatomegaly; Computed tomography (CT)

1. Introduction

Pompe disease is an inherited myopathy characterized by lysosomal storage of glycogen caused by acid alpha glucosidase (GAA) deficiency, and is classified into infantile-, childhood- and adult-onset forms based on the onset age and degree of organ involvement [1]. The infantile form has a rapidly progressive course with prominent cardiomegaly, hepatomegaly and muscle weakness [2]. The childhood-onset form usually presents with muscle weakness and a high level of serum creatine kinase (CK), like those of progressive muscular dystrophy, later than the infantile-onset form, and typically does not include severe cardiomyopathy [3]. Since the availability of enzyme replacement therapy (ERT) with recombinant human acid alpha glucosidase, Myozyme

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(alglucosidase alpha, Genzyme Corporation), the benefits of ERT for cardiac pathological changes in the infantile form have been confirmed in many studies [4,5]. However, its efficacy varied for skeletal muscle involvement, which is the main manifestation in the childhood- and adult-onset forms [4,6]. One reason for the variation in efficacy is thought to depend on the extent of muscle damage at the start of ERT [4–6]. Therefore, it is important to make a definitive diagnosis as early as possible. In this case report, we report our successful early diagnosis and initiation of ERT in a young child with childhood-onset Pompe disease, before the development of skeletal muscle symptoms. We suggest that the combination of high density in the liver and skeletal muscle on CT images could be helpful for early diagnosis and follow up.

2. Patient

Our patient was a 3-year, 6-month-old girl who was born from non-consanguineous healthy parents. Her motor milestones were completely normal; head control at 3 months, sitting at 7 months, walking without support at 11 months, and running before 2 years of age. At age 1 year, a high level of serum CK was incidentally detected. At 2 years and 4 months of age, she was referred to our hospital for evaluation of persistent hyperCKemia. Her height was 84.3 cm (−0.7SD), weight was 11.6 kg (±0SD). She was rather active and powerful compared to other children of about her age. She had no history of hypoglycemia or severe respiratory infection. She was intellectually normal and could speak 2-word sentences. Her face was not myopathic, but a bit specific, as if it were doll-like with long and thick eyelashes and thick eye blow. She had neither an enlarged tongue nor a nasal voice. She had no heart murmur or arrhythmia. Her abdomen was distended, with a soft and dull-edged liver palpable 4 cm below the costal margin. Hypotonicity was apparent, but no muscle weakness was recognized. She showed neither Gower's maneuver nor a waddling gait; however, she could neither jump perfectly nor keep squatting posture because of her distended abdomen. She could climb stairs, but not with alternating feet, and used a handrail for support. Examination showed a high CK level of 640 U/l (reference range 52–214 U/l). Aspartate amino transferase (AST), alanine amino transferase (ALT), and lactate dehydrogenase (LDH) were increased to 124 U/l (reference range 22–49 U/l), 127 U/l (reference range 8–28 U/l) and 493 U/l (reference range 167–383 U/l), respectively, but blood anmonia and serum gamma- glutamyl transpeptidase (GTP) were normal and coagulation factors were also all normal. Cardiac echography suggested normal wall thickness, size and function. Abdominal echography showed a high-echoic, enlarged liver. Simple plane abdominal and skeletal muscle CT scans were performed at scanning conditions of 120 kVp and 75 mAs, and 120 kVp

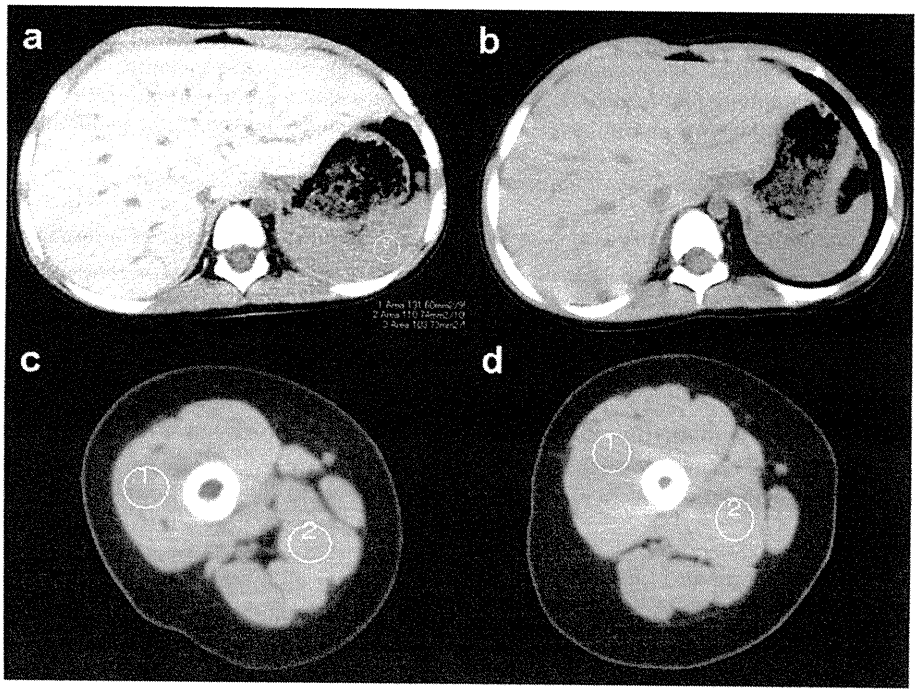

Fig. 1. Abdominal and skeletal muscle CT scans at first admission (a and c) and 3 months after starting ERT (b and d). Note, the high density of the liver with high CT number of 106 HU at first admission (a) had improved markedly after 3 months with 73 HU (b). Whole muscle showed an increased CT number over 94 HU at first admission (c). There was no remarkable visible change, but CT number improved to 83 HU after ERT (d).

and 40 mAs, respectively using Toshiba Aquilion 4 Detector. Abdominal CT images suggested an enlarged liver with an extremely increased density with CT number of 106 Hounsfield Units (HU) (normal control: 50–60 HU) (Fig 1a). Skeletal muscle CT also suggested high density of the thigh and calf muscles with a high CT number, 94 HU (normal control: 30–40 HU) (Fig 1c).

Following the finding of low GAA activity on screening of dried blood spots, deficient GAA activity in lymphocytes was confirmed (0.3 nM/mg protein/h, Control 30.7 ± 10.3). Finally, detection of compound heterozygous mutations, a reported mutation p.R437C and a novel mutation p.P726R, in the GAA gene allowed a definitive diagnosis of childhood-onset Pompe disease. At 2 years and 6 months of age, ERT was initiated with IV alglucosidase alfa (Myozyme, Genzyme Corporation) at a dosage of 20 mg/kg every other week. Written informed consent was obtained from her parents. The patient regularly received antihistamine medication beforehand.

After 1 month, efficacy on hepatomegaly was immediately apparent. The liver decreased in size within 1 month and was hardly palpable. Abdominal CT revealed a marked decrease in its density, from 106 to 73 HU (Fig 1b), 3 months after ERT initiation. ALT and LDH levels decreased to their normal ranges. AST improved to around 50–60 U/l, but then remained at this level. Following the reduction in hepatomegaly, she gained the ability to jump. This was not due to skeletal muscle involvement, but due to hepatomegaly that had caused her to lose her balance by disturbing the body curvature. The CT number of skeletal muscle also improved after 3 months, from 94 to 83 HU (Fig 1d), but not as markedly as that of liver. After 1 year, her motor function was almost normal. Her serum CK level improved, stabilizing, at 220–250 U/l, despite her increased activity level.

3. Discussion

We reported our early diagnosis of childhood-onset Pompe disease and the initiation of ERT in a young child with hepatomegaly and hyperCKemia. Hepatomegaly was the first major clue in making the diagnosis. Hepatomegaly, which was reported in 90% of Dutch patients and in 29% of cases in the literature, is one of the main signs in infantile-onset Pompe disease [2]. On the other hand, it is not frequently seen in the childhood- or adult-onset form as the first sign (1.0%), being reported in 16% of cases with onset under 1 year, 13% of those between 1 and 6 years, and none of those over 6 years [3]. Abdominal echography showing a high-echoic liver was not helpful, since the findings could not be distinguished from fatty liver change. High density of the liver on abdominal CT images was highly informative, being completely different from fatty liver change which shows a low density. Several reports have already confirmed that glycogen storage in the liver causes increased density on CT images, as seen in patients with von Gierke disease [7]. As we reported previously, high density on skeletal muscle CT is seen in glycogen storage disorders involving skeletal muscle, like McArdle disease and childhood-onset Pompe disease [8]. Excess glycogen in skeletal muscle itself increases the density, but calcium accumulation was proved to further raise the density in childhood-onset Pompe disease [8]. Therefore, the combination of high density in the liver and skeletal muscle prompted us to suspect childhood-onset Pompe disease, which may involve both organs. In such a case, CT is an informative tool, serving as the first step in differential diagnosis. CT is less invasive than muscle biopsy, facilitating both diagnosis and assessment of the efficacy of ERT. Of course, the risk of radiation in CT should always be kept in mind, especially for children. Radiation dose could be reduced by the scan parameters such as mA, kVp and imaging times (s), and simple plane CT may give sufficient information.

Our observation that the effect of ERT on the liver appeared in the very early stage of treatment was in agreement with previous studies in the infantile-onset form [9]. The improvement was clearly visible on abdominal CT 3 months after the initiation of ERT (Fig 1b). The liver is thought to have more efficient mechanisms to uptake enzyme in cells [10].

One of the main reasons for the variation in efficacy of ERT in childhood- and adult-onset forms was thought to depend on the extent of muscle damage at the start of ERT [4–6]. In this case, ERT was started before the development of skeletal muscle symptoms such as muscle weakness, motor developmental delay or myalgia. One year after the initiation of ERT, the CK level had improved and there was no clear sign of skeletal muscle involvement. We speculate that ERT may have successfully prevented disease progression since it was fortunately started before severe damage of skeletal muscle.

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Case report

Close monitoring of initial enzyme replacement therapy in a patient with childhood-onset Pompe disease

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Abstract

Pompe disease is classified into infantile and late-onset (childhood and adult) forms based on onset age and degree of organ involvement. While benefits of enzyme replacement therapy (ERT) for the infantile form have been confirmed, efficacy for late-onset forms reportedly varies. We report close monitoring of initial ERT, focusing especially on the first year, in a 12-year-old boy with childhood-onset Pompe disease. At age 10, he started ERT at 20 mg/kg every other week. Respiratory and motor functions were evaluated at each infusion, and by skeletal muscle computed tomography (CT) and cardiac echography every 4 months. He gained the ability to climb stairs without a rail and % vital capacity improved just 1.5 months after starting ERT. Grip power, manual muscle testing (MMT) and the timed and 6-min walking distance tests (6MWT) improved promptly, paralleling improvements in clinical symptoms. However, this steady improvement stopped around 8 months, with deterioration to the initial level by about 24 months. Antibody against recombinant human alpha-glucosidase was very low at 15 months; therefore, the lack of treatment response did not completely correspond to antibody production. On the other hand, cardiac wall thickening worsened after 4 months, then improved to better than baseline after 8 months, and this improvement was well maintained. Among our set parameters, the timed test results corresponded better to his changing clinical course than did grip power, MMT or 6-min walking test results.

1. Introduction

Pompe disease is an inherited myopathy caused by acid alpha glucosidase (GAA) deficiency, classified into infantile-, childhood-, and adult-onset forms based on the onset age and severity of organ involvement [1]. The infantile form has a rapidly progressive course with marked cardiomegaly and muscle weakness, and death in the first year. The childhood-onset form usually presents with muscle weakness and elevated creatine kinase (CK), mimicking progressive muscular dystrophy, later than the infantile onset form, and typically does not include severe cardiomyopathy. The adult-onset form exhibits a more slowly progressive course with limb-girdle myopathy after the second decade of life [2,3]. In 2006, enzyme replacement therapy (ERT) with recombinant human GAA, Myozyme (alglucosidase alfa, Genzyme Corporation), was approved. While the benefits of ERT for the infantile form have been confirmed by several clinical studies showing reversal of cardiac pathology and better survival [4–7], its efficacy for childhood and adult-onset forms varied widely [4,5,8–10]. Trials have shown mostly stabilized pulmonary and improved motor functions, but improvement was limited in some patients. Since ERT effects on skeletal muscle, the main tissue involved in the late-

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onset forms, are anticipated to be less than those on cardiac muscle, due to relatively inefficient transfer of recombinant GAA to lysosomes and resistance of type IIb muscle fibers to therapy [4,5], and the volume of muscle being too large a target for ERT. Furthermore, effects are thought to depend on the extent of muscle damage at ERT start and the formation of antibodies to GAA. Herein, we focused on the initial reaction to ERT in a childhood-onset Pompe disease patient by close monitoring every 2 weeks.

2. Case report

Our patient, a 12-year-old boy, was born to non-consanguineous, healthy parents. At age 2 years, he developed markedly nasal speech. He was clumsier when exercising than other children. At 4 years, elevated serum CK, around 700 U/l, was detected on preoperative examination for nasopharyngeal incompetence. At age 5, biopsied muscle showed characteristic findings and low GAA activity (0.2 nmol = 4 MU/mg/30 min, control: 7.3 ± 2.2) allowing a definitive diagnosis of childhood-onset Pompe disease. Genetic analysis revealed compound heterozygous GAA gene mutations, p.S619R and p.E579K, which have already been reported. At 10 years and 3 months of age, ICAP (International Charitable Access Program) support allowed him to start ERT. He was treated with IV alglucosidase alfa (Myozyme) at 20 mg/kg every other week. Written informed consent was obtained from his parents. He regularly received antihistamine premedication. He was assessed at every infusion during the first year, then every 3 months during the second. To assess respiratory and pulmonary functions, SpO2 was monitored overnight. To assess motor function, we evaluated (1) grip power, (2) manual muscle testing (MMT) according to the Medical Research Council (MRC) score, (3) the time required to change position, such as rolling, sitting and modified Gower's maneuver and the time to climb stairs, with video recording, and (4) 6-min walking distance test (6MWT). These parameters were examined under the same conditions; after resting on a bed for 1 h, by the same examiners. Serum CK was also measured at every infusion. Anti-recombinant human GAA antibodies were analyzed every 3 months by enzyme-linked immunosorbent and radioimmunoprecipitation assays (Genzyme). Skeletal muscle computed tomography (CT) and cardiac echography were performed every 4 months. Skeletal muscle CT scans were obtained at a window level (WL) of 30–45 Hounsfield Units (HU) and window width (WW) of 300–350 HU.

At baseline, he had a poor build (height 131 cm (−1.0 SD), weight 24 kg (−1.3 SD)). He had chronic diarrhea or incontinence, especially before school and after stressful events, and was occasionally late for school due to morning lethargy. A myopathic face with open mouth and nasal speech were noted. He had neither heart murmur nor hepatomegaly. Deep tendon reflexes were absent. There was proximal dominant muscle weakness with MMT scores of 3+/5 in proximal and 4/5 in distal muscles, especially in the neck and trunk with MMT scores of 2/5. Bilateral grasp power was 4–5 kg (reference range of healthy controls at age 10 years: 17.15 ± 3.82 kg) [11]). He showed Gower's maneuver and a waddling gait. He could not jump and needed to hold a rail to climb stairs. Serum CK was elevated; 870 U/l. Percent vital capacity (%VC) was 57% in a sitting position, with mild restrictive respiratory failure. Cardiac echography suggested normal cardiac function (left ventricular shortening fraction: 0.38), but mild cardiac hypertrophy (diastolic left ventricular posterior wall thickness: 6.4 mm (healthy control: 6.1 mm), interventricular septum 7.1 mm (healthy control: 6.1 mm)). Overnight saturation monitoring showed 2–3 episodes of diminished oxygen to around 90% during sleep. Skeletal muscle CT showed high-density areas in thigh and calf muscles.

The patient experienced no infusion-related adverse events. After 1.5 months (3rd infusion), there was an obvious clinical response. He was able to climb steps without a rail. After 4 months (8th infusion), he could climb in an almost normal manner with alternate swinging of his arms. He also gained the ability to jump slightly, with his feet actually leaving the floor. MMT improved, with a high score of 4/5 in most proximal muscles and 4–5/5 in distal muscles, except the neck, and trunk flexion remained at 2/5. Grip power increased from 4 to 8 kg, and the 6MWT result changed from 320 to 500 m after 4 months (reference range at 6–8 years: 577.8 ± 56.1 m, at 9–11 years: 672.8 ± 61.6 m) [12]. The time required to change all positions was much shorter than at baseline (Fig. 1a (reference range for 10-year-old boys: 1.08 ± 0.25 s [13])). %VC increased from 57% to 65% after 4 months (Fig. 1b). Overnight saturation monitoring also showed episodes of diminished oxygen during sleep to have decreased. He gained the ability to attend most physical education classes due to amelioration of fatigability. His appetite increased and he gained weight, 3.2 kg in 4 months. The incontinence and chronic diarrhea resolved completely after starting ERT. Up to 4 months (8th infusion), the incremental improvements were large. %VC peaked at 71% after 8 months (17th infusion) (Fig. 1b).

However, these steady improvements stopped around 8 months, followed by deterioration. The rate of weight gain slowed, and after 12 months had almost reached a plateau. He began supporting his knees with his arms to climb stairs even though he was still not using a rail after 18 months, and had deteriorated to baseline after 2 years. He lost the ability to jump after 18 months. The timed tests corresponded well to the clinical course

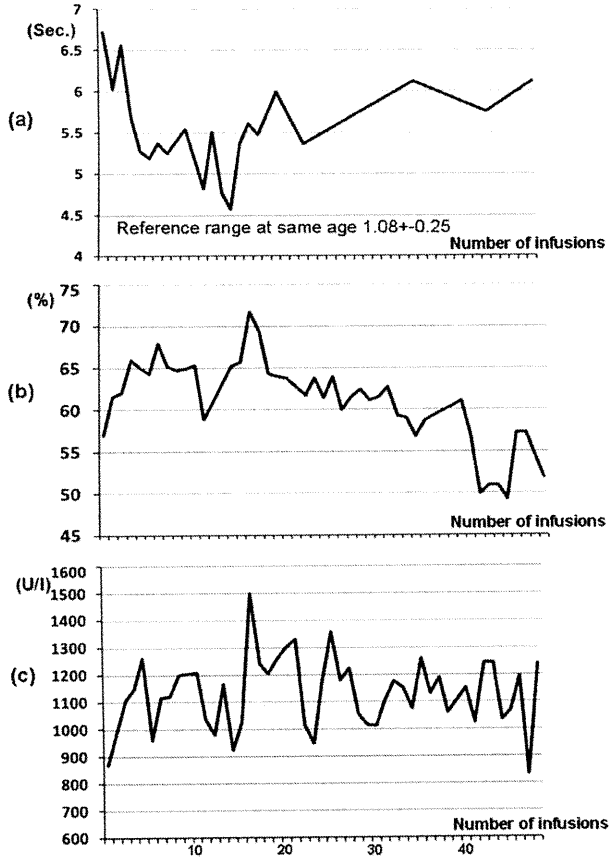

Fig. 1. Timed test from supine to standing (modified Gower's maneuver) (a), % vital capacity (b), changes in serum creatine kinase levels (c) over 2 years. (a) Time required for modified Gower's maneuver gradually became shorter up to 8 months (15th–16th infusion) and then showed prolongation again.

(Fig. 1a), but not the results of grip power, MMT or 6MWT (Fig. 2). The rate of respiratory function deterioration was far greater than that of motor function (Fig. 1b). At 18 months, %VC had deteriorated to baseline and continued to decline thereafter. Diminished oxygen episodes during sleep again increased, in parallel with worsening morning lethargy.

Serum CK increased from 800 to 1200 U/l after 1.5 months (3rd infusion) and remained at this level (Fig. 1c). Skeletal muscle CT showed high-density areas in the rectus femoris, vastus lateralis and gracilis muscles. The CT number, with a maximum of 138 HU and minimum of 88 HU (138/88) at ERT start, slightly improved to 100/64 HU after 4 months but returned to the original 136/77 HU 8 months after and 140/80 1 year after starting ERT. Cardiac wall thickness increased after 4 months. This was only a transient change, and values improved after 8 months and normalized after 20 months (Fig. 3). Even though ERT lost efficacy for motor and respiratory functions, it improved cardiac wall thickening and still has an effect on the heart in this patient.

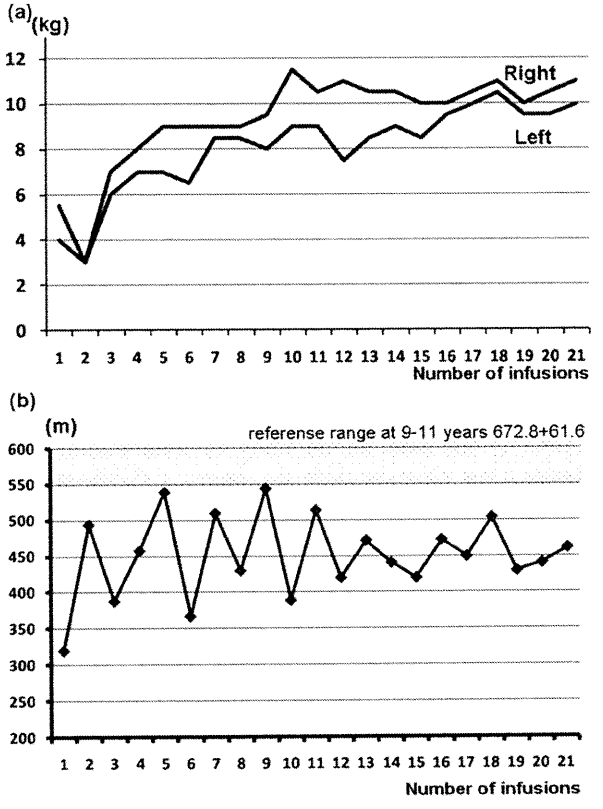

Fig. 2. Grasp power (a), six-min walking test (b) results over 1 year.

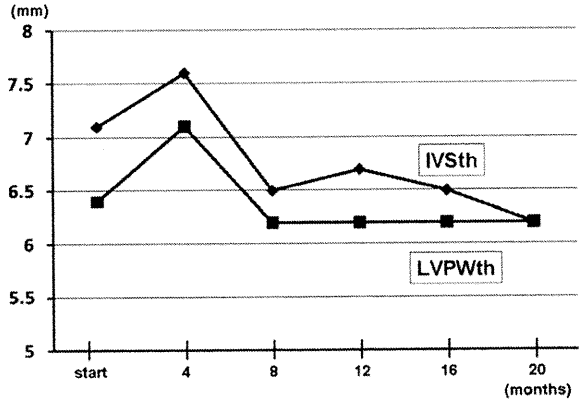

Fig. 3. Cardiac wall thickness. Interventricular septum (IVS) (healthy control: 6.1 mm). Diastolic left ventricular posterior wall thickness (LVPWTd) (healthy control: 6.1 mm).

Over 1 year, anti-recombinant human GAA antibodies remained negative. At 15 months, antibodies were finally detectable at a very low titer (1 0 0), remained at this level and then became negative at 2 years after initiating ERT.

3. Discussion

Our 2-year close monitoring of a patient with childhood-onset Pompe disease on ERT revealed initial effi-

cacy, in only 1.5 months, for clinical symptoms after a few infusions and also a rapid respiratory function response. This initial good response to ERT could be explained by relatively early therapeutic intervention, as previous reports showed improvement in younger patients [4,5,8,9]. However, our results raise another question regarding rapid deterioration despite marked initial improvement. The patient started to deteriorate after 8 months of ERT, and was in his initial condition after 2 years. Besides potential individual modifiers such as the extent of muscle damage [8,9], number of skeletal muscle mannose 6-phosphate receptors and fiber type proportions possibly blunting ERT effects [4,5], we must consider subsequent factors triggering deterioration during ERT in our case. In the study by Amalfitano et al., infantile-form patients with no residual GAA protein (cross-reactive immunological material-negative) showed transient motor function improvement and subsequent deterioration coinciding with rising titers of antibodies against recombinant human GAA (rhGAA) [6]. In Phases II and III studies, patients with the highest antibody titers showed the poorest effect [7]. In our patient, deterioration preceded antibody detection by more than 6 months, and antibody titers were much lower than average. We found no clear association between his deterioration and anti-rhGAA antibody production. However, we can hypothesize that deterioration is accelerated by antibody production, even if very low, or overload due to increased exercise.

With early stage clinical symptom and motor function improvements, cardiac wall thickness and CK elevation worsened. Improved cardiomegaly was the most significant efficacy in the infantile form [4–7]. In our patient, the cardiac wall thickening worsened once after 4 months, but then improved to less than that at baseline after 8 months. We speculate that the effects on cardiac muscle might be more delayed than those on skeletal muscle, and cardiac muscle failure might catch up with increasing exercise as motor function improved in the early stage. Before ERT, he often missed physical education classes because of fatigability and exercise limitations. With clinical improvement, he gained the ability and confidence to attend all classes, which may have overloaded cardiac muscle that had not sufficiently responded to ERT. Our results suggest that we must closely monitor patients for worsening cardiac hypertrophy in the early stage, especially those with prompt and marked improvements of motor function and fatigability.

Increased exercise may also be the reason for greater CK elevation in the early stage. Although serum CK has been recognized as a useful parameter reflecting ERT efficacy, its value has recently been considered controversial. Recent reports found CK change to be unrelated to efficacy in some patients [10]. In our case, CK elevation worsened independently of other improvements in other parameters. The peak level, 1500 U/l, corresponded to the time when the patient's clinical symptoms and motor function were best. CK may fluctuate depending on activity, such that ERT efficacy should not be predicted only from CK changes.

We previously reported a patient with childhood-onset Pompe disease showing high-density areas on CT in severely affected skeletal muscle [14]. A moderate increase in CT density is caused by increased glycogen in organs, as seen in von Gierke's disease, and calcium accumulation. Therefore, we assumed that CT density would diminish with histopathological improvement in skeletal muscle with ERT. In this study, mean CT number improved at 4 months, quickly returned to baseline at 8 months and thereafter slowly deteriorated. The change in CT imaging results roughly coincided with the clinical course, improving as clinical symptoms improved, but worsening earlier than did clinical symptoms.

Close monitoring with assessment of the patient's course every 2 weeks, showing phases of both improvement and deterioration within a short period, enabled us to gain insights as to appropriate parameters for pediatric patients with late-onset Pompe disease. Grasp power seemed to progress consistently over 2 years of ERT, but its improvement was completely independent of overall symptom deterioration. This discrepancy is explained by grip power, one of the parameters of distal muscle function, being well preserved in late-onset Pompe disease [3] or distal muscles responding better to ERT than proximal muscles [8]. MMT scores also improved as clinical symptoms initially responded to ERT, but changed as deterioration started. Since each MRC score grade, especially grade 4, covers a wide range depending on the patient and examiner, it is difficult to detect a decline based on this grade. Quantitative muscle testing may be more suitable. 6-MWT is frequently used to assess treatment efficacy in patients with neuromuscular disorders like Duchenne muscular dystrophy and Pompe disease. In trials in adult patients with late-onset Pompe disease, it reflects the efficacy of ERT [10]. We repeated this test every 2 weeks for a year. The results showed marked fluctuations of 100–200 m on each occasion, and did not reflect his clinical course. Since 6MWT changes with aging, development, learning and especially motivation of patients, it is not suitable for detecting changes during short-term follow-up. Moreover, the reliability of this test is not high in children with inconsistent motivation.

As motor and respiratory functions deteriorated, chronic diarrhea, which had completely stopped after ERT, recurred. The patient had a few watery stools, without visible blood or pus, but only before going school in the morning and after stressful events. He often suffered incontinence before going to the toilet. After starting ERT, these symptoms were relieved

completely. Around 18 months after starting ERT, he had diarrhea occasionally, and after 2 years it was just as frequent as before. A recent report also suggested ERT to be effective for gastrointestinal dysfunction in patients with Pompe disease [15]. The mechanism underlying this symptom, which is similar to irritable bowel syndrome, in our patient remains unknown. Considering that ERT was effective for these symptoms, we hypothesize that ERT may also provide some benefits for smooth muscle or autonomic nervous system disorders.

After 2 years, ERT lost its effect on motor and respiratory functions, but did clearly prevent progression for these years, and is still effective for cardiac hypertrophy.

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