mitochondrial changes are pathological hallmarks of muscles with *VCP* mutations, findings that are useful for the diagnosis of this clinically complicated disease.

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Disclosure of conflict of interest

The authors declare no financial or other conflict of interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. A list of antibodies used in this study.

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References

1. Watts GD, Wymer J, Kovach MJ, *et al.* Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat Genet* 2004; **36:** 377–381.
2. Weihl CC, Pestronk A, Kimonis VE. Valosin-containing protein disease: inclusion body myopathy with Paget's disease of the bone and fronto-temporal dementia. *Neuromuscul Disord* 2009; **19:** 308–315.
3. Schroder R, Watts GD, Mehta SG, *et al.* Mutant valosin-containing protein causes a novel type of frontotemporal dementia. *Ann Neurol* 2005; **57:** 457–461.
4. Johnson JO, Mandrioli J, Benatar M, *et al.* Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* 2010; **68:** 857–864.
5. Kim EJ, Park YE, Kim DS, *et al.* Inclusion body myopathy with paget disease of bone and frontotemporal dementia linked to VCP p.Arg155Cys in a Korean family. *Arch Neurol* 2011; **68:** 787–796.
6. Stojkovic T, Hammouda el H, Richard P, *et al.* Clinical outcome in 19 French and Spanish patients with valosin-containing protein myopathy associated with Paget's disease of bone and frontotemporal dementia. *Neuromuscul Disord* 2009; **19:** 316–323.
7. Kimonis VE, Mehta SG, Fulchiero EC, *et al.* Clinical studies in familial VCP myopathy associated with Paget disease of bone and frontotemporal dementia. *Am J Med Genet A* 2008; **146A:** 745–757.
8. Hashimoto J, Yoshikawa H. [Diagnosis and management of Paget's disease of bone]. *Nippon Rinsho* 2007; **65**(Suppl. 9): 56–64.
9. Guyant-Marechal L, Laquerriere A, Duyckaerts C, *et al.* Valosin-containing protein gene mutations: clinical and neuropathologic features. *Neurology* 2006; **67:** 644–651.
10. Nonaka I, Noguchi S, Nishino I. Distal myopathy with rimmed vacuoles and hereditary inclusion body myopathy. *Curr Neurol Neurosci Rep* 2005; **5:** 61–65.
11. Sivakumar K, Dalakas MC. The spectrum of familial inclusion body myopathies in 13 families and a description of a quadriceps-sparing phenotype in non-Iranian Jews. *Neurology* 1996; **47:** 977–984.
12. Miller TM, Layzer RB. Muscle cramps. *Muscle Nerve* 2005; **32:** 431–442.
13. Ju JS, Weihl CC. Inclusion body myopathy, Paget's disease of the bone and fronto-temporal dementia: a disorder of autophagy. *Hum Mol Genet* 2010; **19**(R1): R38–R45.
14. Ju JS, Fuentealba RA, Miller SE, *et al.* Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J Cell Biol* 2009; **187:** 875–888.
15. Neumann M, Mackenzie IR, Cairns NJ, *et al.* TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *J Neuropathol Exp Neurol* 2007; **66:** 152–157.
16. Weihl CC, Temiz P, Miller SE, *et al.* TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J Neurol Neurosurg Psychiatry* 2008; **79:** 1186–1189.
17. Neumann M, Sampathu DM, Kwong LK, *et al.* Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; **314:** 130–133.
18. Boyault C, Gilquin B, Zhang Y, *et al.* HDAC6-p97/VCP controlled polyubiquitin chain turnover. *EMBO J* 2006; **25:** 3357–3366.
19. Lee JY, Koga H, Kawaguchi Y, *et al.* HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy. *EMBO J* 2010; **29:** 969–980.
20. Hetzer M, Meyer HH, Walther TC, Bilbao-Cortes D, Warren G, Mattaj IW. Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. *Nat Cell Biol* 2001; **3:** 1086–1091.
21. Miyachi K, Hirano Y, Horigome T, *et al.* Autoantibodies from primary biliary cirrhosis patients with anti-p95c antibodies bind to recombinant p97/VCP and inhibit *in vitro* nuclear envelope assembly. *Clin Exp Immunol* 2004; **136:** 568–573.
22. Braun RJ, Zischka H. Mechanisms of Cdc48/VCP-mediated cell death: from yeast apoptosis to human disease. *Biochim Biophys Acta* 2008; **1783:** 1418–1435.
23. Ayala YM, Misteli T, Baralle FE. TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. *Proc Natl Acad Sci U S A* 2008; **105:** 3785–3789.
24. Braun RJ, Zischka H, Madeo F, *et al.* Crucial mitochondrial impairment upon CDC48 mutation in apoptotic yeast. *J Biol Chem* 2006; **281:** 25757–25767.

25. Heo JM, Livnat-Levanon N, Taylor EB, *et al.* A stress-responsive system for mitochondrial protein degradation. *Mol Cell* 2010; **40:** 465–480.
26. Xu YF, Gendron TF, Zhang YJ, *et al.* Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J Neurosci* 2010; **30:** 10851–10859.
27. Braun RJ, Sommer C, Carmona-Gutierrez D, *et al.* Neurotoxic 43-kDa TAR DNA-binding protein (TDP-43) triggers mitochondrion-dependent programmed cell death in yeast. *J Biol Chem* 2011; **286:** 19958–19972.
28. Guinto JB, Ritson GP, Taylor JP, Forman MS. Valosin-containing protein and the pathogenesis of frontotemporal dementia associated with inclusion body myopathy. *Acta Neuropathol* 2007; **114:** 55–61.

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ORIGINAL ARTICLE

Effects of enzyme replacement therapy on five patients with advanced late-onset glycogen storage disease type II: a 2-year follow-up study

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Abstract We examined the efficacy of 2-year enzyme replacement therapy (ERT) using recombinant human α-glucosidase (GAA; Myozyme®) in five long-term ventilator-dependent adults and aged patients with advanced, late-onset glycogen storage disease type II (GSDII, also known as Pompe disease). Although all patients had advanced respiratory failure and were ventilator-dependent for more than 6 years, four showed obvious improvements in muscle strength, pulmonary function, and activities of daily living after ERT. Improvement in each parameter was more prominent in the first year than in the second year. Values in the second year were still significantly better than those at study entry and indicate stabilization in the clinical status of all patients. These results suggest that ERT continues to be effective in the second year of treatment even in patients suffering from advanced late-onset GSDII disease with severe respiratory failure.

Introduction

Glycogen storage disease type II (GSDII), or Pompe disease, is an autosomal recessive lysosomal glycogen storage disease

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resulting from a deficiency in α-glucosidase (GAA) activity (OMIM #232300). The different clinical phenotypes of GSDII include classic infantile-onset; non-classic infantile-onset; childhood, juvenile, and adult forms of GSDII; and late-onset GSDII. However, GSDII presents as a broad spectrum with varying degrees of severity and rates of progression. The classic infantile-onset form is characterized by hypertrophic cardiomyopathy and generalized muscle weakness, which appear in the first few months of life (Hirshhorn and Reuser 2001; Engel et al. 2004). Late-onset GSDII is characterized by progressive skeletal muscle weakness and loss of respiratory function.

Enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) derived from transfected Chinese hamster ovary cells resulted in marked improvement in the survival rate of 18 patients with infantile-onset GSDII (Kishnani et al. 2008). Nicolino and colleagues also reported that rhGAA reduced the risk of death and invasive ventilation by 79 and 58%, respectively, in infants and children with advanced Pompe disease (Nicolino et al. 2009). The use of ERT with Myozyme® (α-glucosidase) was approved by the U.S. Food and Drug Administration (FDA) in 2006 and by the Japan Ministry of Health, Labor and Welfare (MHLW) in 2007.

Previous studies confirmed the efficacy of ERT in late-onset GSDII patients with acute respiratory failure or relatively mild respiratory dysfunction (Winkel et al. 2004; Pascual-Pascual et al. 2006; Merk et al. 2007, 2009; Case et al. 2008; Yamamoto et al. 2008; Rossi et al. 2007; van Capelle et al. 2008; Strothotte et al. 2010; van der Ploeg et al. 2010). On the other hand, ERT efficacy in advanced patients seemed to be lower than that in milder patients (Orlikowski et al. 2011). It is not clear whether ERT is continuously effective in ventilator-dependent patients with advanced disease and long-term respiratory failure. Because ERT is relatively expensive, it is important to determine whether continuous administration is effective, or whether therapy is only effective for a short duration. In the present study, we evaluated the efficacy of ERT in five patients with advanced late-onset GSDII for 2 years and analyzed factors related to its efficacy.

Patients and methods

Patients

Patients with late-onset Pompe disease diagnosed based on both muscle biopsies and fibroblast/muscle residual GAA activity, and who had undergone ERT at the National Center Hospital (National Center of Neurology and Psychiatry), were included in this study. Written informed consent was obtained before enrollment. The study protocol was approved by the National Center Hospital Ethics Committee. Patients 4 and 5 have been reported previously (Sasaki et al. 1992; Yamazaki et al. 1992). Table 1 lists the characteristics of all five patients (two men and three women).

Genomic DNA was extracted from blood or muscle biopsy samples according to standard protocols. All exons and flanking intronic regions of GAA were amplified and sequenced using an automated 3100 DNA sequencer (Applied Biosystems, Foster, CA). Primer sequences are available upon request. All patients had previously reported mutations (Tsujino et al. 2000; Tsunoda et al. 1996; Lam et al. 2003; Pipo et al. 2003; Hermans et al. 2004). The average (SD) age at ERT initiation was 47 (13.6) years (range 32–66 years), and the average duration of disease was 26 (4.5) years (range 20–31 years). The average duration of mechanical ventilatory support before ERT was 8.0 (1.9) years (range 6–11 years). Patients 1, 2, 4, and 5 had been treated with noninvasive ventilation (NIV), and patient 3 had been treated with invasive ventilation. All patients were wheelchair-bound for a mean of 7.0 (5.1) years (range 2–14 years). Only patient 4 was able stand for a few minutes or walk a few steps with assistance. Others were completely wheelchair-bound.

Methods

ERT (Myozyme®) was administered at 20 mg/kg body weight biweekly at a dose of 1 mg/kg/h for the first 30 min, 3 mg/kg/h for the second 30 min, and then increased to 5 mg/kg/h, and finally 7 mg/kg/h every 30 min. Patients were carefully monitored for infusion-related reactions during and after ERT administration. Clinical condition was assessed every 6 months, including physical examination, manual muscle test (MMT), ECG, Holter ECG, ultrasound cardiography (UCG), and pulmonary function tests [% vital capacity (%VC), % force vital capacity (% FVC), forced expiratory volume in the first second (FEV1.0), peak expiratory flow rate (PEF), peak cough flow (PCF; Bach 2004)], and lean body mass (Discovery Bone Densitometer, Hologic, Bedford, MA). Muscle strength, including grip power (Dynamometer®, TTM, Japan, for patient 1; Grip Strength Dynamometer®, Takei, Japan, for patients 2–5) and pinch power (PinchTrack™, Jtech, Japan), was assessed every 2 weeks. The Barthel index and gross motor function measure manual (GMFM) were assessed every 6 months from the second year (Hosoda and Yanagisawa 2000; Kondo and Fukuda 2000). Occlusal force in the right and left first molar was measured using the Occlusal Force Meter GM10® (Nagano Keiki, Japan) every 6 months. In this test, which was repeated three times, patients were asked to bite on a block as hard as possible. All patients rested for more than 2 h before each muscle strength test. Normal values for grip power

Table 1 Baseline patient characteristics and conditions

Patient no.	1	2	3	4	5
Sex	Male	Male	Female	Female	Female
Age at inclusion (years)	66	55	44	38	32
Age at onset (years)	35	35	25	8	7
Observation period (weeks)	104	104	104	104	104
Symptom at onset (weakness)	Lower extremities	Lower extremities	Lower extremities	Neck	Lower extremities
Ventilator since (age in years)	58	49	36	32	21
Duration of ventilator use (years)	8	7	8	6	11
Wheelchair-bound	Complete	Complete	Complete	Complete	Partial
Ventilator use (h/day)	24	10 (at night)	24	22	10 (at night)
Tracheotomy (age in years)	None	48	36	None	None
Wheelchair since (age in years)	51	48	36	36	29
Genotype	c.1585–1586TC > GT(p.S529V) homozygote	c.546 G > T(p.T182T) homozygote	c.307 T > C(p.C103R)/ c.546 G > A(p.T182T)	c.1309 C > T(p.R437C)/ c. 1857 C > G(p.S619R)	c.546 G > T(p.T182T)/ c.1798 C > T(p.R600C)
Enzyme activity[a]	1.2 (M)	0.6 (M)	1.88 (M)	0.46 (F)	3.8 (M)
Complications	Diabetes mellitus	Atrial fibrillation	Interstitial pneumonia pneumothorax	Pneumothorax subcutaneous/ mediastinal emphysema	—
Pathology	Myopathic changes	Myopathic changes	Myopathic changes	Myopathic changes	Myopathic changes
AcP- and PAS-positive vacuoles	Few	Scattered	Scattered	Stained for acid phosphatase	Many

[a] *(M)* Muscle (nmols 4MU/mg/h) (14.6±4.4), *(F)* fibroblast (mmol/pg protein) (161±32.4)

and occlusal force were provided by the manufacturer, and three healthy volunteers were tested as controls for pinch power [see Table in Electronic Supplementary Material (ESM)]. Blood cell counts and blood chemistry tests were conducted regularly. We interviewed patients and their families about activities of daily living (ADL). IgG antibodies to rhGAA were measured regularly by enzyme-linked immunosorbent assay (ELISA) (Kishnani et al. 2006).

Annual changes in quantitative parameters (pulmonary function tests, grip power, pinch power, and occlusal force) were calculated for the first and second years by subtracting old data from new data. Changes were analyzed with the Mann-Whitney *U* test. Statistical analyses were performed with SPSS for Macintosh (version 18, SPSS, Chicago, IL).

Results

Case presentation

Patient 1 suffered from limb muscle atrophy at age 35. He could not climb stairs and visited us at age 44. Muscle biopsy and acid maltase activity revealed Pompe disease. He lost ambulation at age 51. He experienced dyspnea, and %VC was 22.4 at age 58. Nocturnal NIV was initiated; he required continuous NIV from age 63 and was able to remove the NIV mask for <1 min before ERT. ERT was initiated at age 66. After 6 months of ERT, the patient was able to stop NIV for 9 min, allowing for a much easier transfer of the patient from car to wheelchair by the caregiver. This also provided the caregiver more than 5 min for shaving and/or cleaning the patient's face, compared to the 1-min limit before ERT.

Patient 2 had difficulty climbing stairs from age 36. He experienced dyspnea in the supine position at age 47 and visited a physician due to morning headache and severe dyspnea. He presented with pneumonia and CO_2 narcosis; nocturnal oxygen therapy was initiated after recovery. A muscle biopsy led to the diagnosis of Pompe disease. The patient lost ambulation during hospitalization. He visited us at age 50 and nocturnal NIV was initiated. The patient had difficulty lying down in the supine position without NIV before ERT. After ERT was initiated at age 55, he was able to lie down for 10 min at 24 weeks of ERT and for 60 min at 48 weeks without respiratory support. He was also less fatigued in the afternoons and able to drive alone for 2 h after 40 weeks.

Patient 3 noticed gait disturbance at age 22, visited a neurologist at age 26, and was diagnosed with limb-girdle

muscular dystrophy. At age 36, she complained of morning headache and drowsiness; she was intubated and tracheostomy was performed due to CO_2 narcosis and pneumonia. The patient lost ambulation during hospitalization and had recurrent pneumothorax and pneumonia. She visited us at age 39 and was diagnosed with Pompe disease by muscle biopsy and GAA activity. Recurrent pneumonia due to *Pseudomonas aeruginosa* required hospitalization with intravenous antibiotics once every 2 months before ERT. After ERT was initiated at age 44, she developed a mild fever of <38°C twice at 12 and 36 weeks after ERT, and recovered without antibiotics. She was able to open a plastic bottle unaided after 24 weeks of treatment, a task that could not be completed for 8 years prior to treatment. She was able to easily move from bed to wheelchair after 44 weeks. She also noticed less fatigue during meals, was able to pull up both legs unaided after 2 years of ERT, and could put on socks while sitting in the wheelchair.

Patient 4 had proximal weakness at age 15. She was referred to a neurologist and found to have high creatine kinase levels (1,256 U/L) and mild respiratory dysfunction (%VC: 77) at age 21. She was diagnosed with late-onset Pompe disease by muscle biopsy and fibroblast acid maltase activity. At age 32, she experienced dyspnea and initiated NIV during the night. At age 35, her %VC decreased to 18.9 and she required NIV all day. She began to use a wheelchair due to exertional dyspnea. At age 36, she presented with a right-sided pneumothorax, and %VC decreased to 15.8. She was able to turn off NIV only for 5 min to take a bath and could not comb her hair by herself before ERT. At 24 weeks after ERT initiation, pinch power increased from 48.4 N to 55.2 N, and she was able to stand with less effort. At 64 weeks of treatment, she was able to switch off NIV for 15 min while taking a bath and combing her hair. However, she experienced severe dyspnea and recurrent pneumothorax after 64 weeks of ERT and became fully dependent on NIV thereafter. She developed pneumothorax and emphysema at 80 weeks of ERT again and was completely bedridden and required cuirass ventilation in addition to NIV. She was also treated with parenteral hyperalimentation, including standard calorie and protein, for approximately 1 month due to inability to eat caused by dyspnea. After recovery from severe emphysema, she remained bedridden and consequently lost ambulation. Occlusal force was also lower after parenteral hyperalimentation.

Patient 5 could not stand without hand support and visited a pediatrician at age 13 and visited us and muscle biopsy and acid maltase activity. She initiated NIV at age 21 and required a wheelchair at age 29. After ERT was initiated at age 31, she found it easier to expectorate sputum through coughing than before ERT and could move her hip from floor to chair unaided after 44 weeks, which had been impossible for several years. She also noticed alleviation of lumbago, and after three doses of ERT, she was able to discontinue non-steroidal anti-inflammatory drugs (NSAIDS) used for back pain. The patient suffered from emaciation before ERT and was advised that this could not be resolved, but she gained 3 kg of body weight after ERT. At present, she can drive 2.5 h to go to the hospital every 2 weeks, which was impossible before ERT due to fatigue and back pain.

ERT-induced changes

Table 2 lists the results of clinical and laboratory tests before and after ERT. The mean duration of follow-up was 104 weeks. Grip power (Fig. 1a) and pinch power (Fig. 1b) showed gradual improvement in all patients. In patient 4, both grip and pinch powers continued to improve until 60 weeks after ERT initiation, but deteriorated thereafter. Occlusal force improved markedly in patients 1 and 3 (Fig. 1c), but deteriorated in patient 4. No changes in MMT were noted in any of the patients. GMFM improved slightly in patients with a score of >25, while it remained unchanged in those with a score of <5. After initiation of ERT, all patients, except patient 4 who had severe emphysema and pneumothorax, showed improvement in %VC (Fig. 2a), PEF (Fig. 2b), PCF (Fig. 2c), %FVC (Fig. 2d), and/or FEV1.0 (Fig.2e).

Creatine kinase (CK) levels decreased during treatment in patients 2, 4, and 5, and particularly in patient 4 (Table 2). CK levels were normal in patients 1 and 3 at the commencement of treatment and did not show marked changes during and after treatment. Body weight [44.4 (17.0) to 43.6 (16.1) kg, $p=0.93$)] and lean body mass [25.8 (7.9) to 25.8 (10.2) kg, $p=0.99$] did not change.

Changes in the first year were greater than in the second year (Table 3). Most data were not available for patient 4 at the first year evaluation because bed rest was required for pneumothorax therapy. Changes in %VC, %FVC, PEF, PCF, pinch power, and occlusal force were greater in the first year than in the second year ($p<0.05$). While %VC, % FVC, PEF, PCF, pinch power, and occlusal force significantly changed in the first year after ERT, changes in these parameters were not significant in the second year.

IgG antibody against Myozyme® was measured in patients 1, 3, 4, and 5 (see figure in ESM). All patients were IgG antibody positive at around weeks 12 to 16, but patients 4 and 5 became negative thereafter. Furthermore, IgG antibody titers increased to a peak level in patient 3, and increased in patient 1 to 25,600. The antibody titer of patient 2, measured once at 108 weeks after ERT, was negative. Only patient 3 developed a skin rash immediately after Myozyme® infusion at 12 weeks, but the rash disappeared completely after treatment with an antihistamine. Other patients did not experience any infusion-related reactions.

Table 2 Results of clinical and laboratory tests before and after ERT

		Patient 1			Patient 2			Patient 3			Patient 4			Patient 5		
		Pre	1 year	2 year	Pre	1 year	2 year	Pre	1 year	2 year	Pre	1 year	2 year	Pre	1 year	2 year
MMT	Neck flexion	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Shoulder flexion	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Shoulder abduction	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Elbow flexion	1	1	1	3	3	4	3	3	3	4	4	4	3	4	4
	Elbow extension	1	1	1	4	4	4	4	4	4	4	4	4	3	3	3
	Wrist flexion	4	4	4	5	5	5	5	5	5	4	4	4	5	5	5
	Hip flexion	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	Knee flexion	1	1	1	2	2	2	2	2	2	3	3	3	2	2	2
	Knee extension	1	1	1	2	2	2	2	2	2	3	3	3	2	2	2
	Ankle flexion	1	1	1	5	5	5	2	2	2	4	4	4	5	5	5
Body weight (kg)		44	43	43	73.0	70	69	42	40	42	33	31	31	30	31	33
Lean body mass (kg)		23.9	22.6	22.6	39.8	39.8	39.8	23.0	24.4	24.4	21.1	NT	19.9	21.4	22.2	22.2
Pulmonary function	%VC	4.9	10.7	9.6	45.6	62.0	67.2	12.1	15.4	17.3	17.6	NT	9.2	13.1	19.5	21.4
	%FVC	0.0	26.8	7.7	46.3	51.2	66.1	9.3	12.5	16.1	14.2	NT	7.0	10.3	17.7	20.4
	FEV1.0	0.00	0.62	0.21	1.52	1.78	1.99	0.24	0.49	0.41	0.32	NT	0.14	0.29	0.50	0.55
	PEF (L/s)	0.38	0.93	0.50	3.72	6.40	5.49	0.46	0.63	0.70	0.58	NT	0.25	1.24	1.63	1.70
	PCF (L/s)	0.34	0.74	0.69	4.87	7.26	7.16	0.60	0.82	0.85	1.52	NT	0.86	1.19	1.96	2.17
Grip power (kg)		3.4	4.1	4.4	39.6	42.7	44.1	14.2	17.4	16.5	17.0	18.0	17.7	17.5	23.9	25.0
Pinch power (N)		14.7	21.1	15.5	81.9	96.1	98.8	23.6	42.4	42.5	48.3	56.3	53.0	44.3	48.5	47.3
Occlusal force (kgf)		6.4	15	15.9	NT	50.0	55.2	24.1	42.8	46.3	16.4	NT	8.4	NT	65.8	64.0
GMFM		NT	3	3	NT	25	31	NT	5	5	NT	56	59	NT	32	35
CK (IU/l)		47	36	50	238.0	132	10	166	132	100	621	NT	154	241	161	166
Barthel index		20	20	20	75.0	75	75	55	55	55	80	80	70	80	80	80

%VC Percent vital capacity, *%FVC* percent force vital capacity, *FEV1.0* forced expiratory volume in the first second, *PEF* peak expiratory flow, *PCF* peak cough flow, *GMFM* gross motor function measure, *CK* creatine kinase, *NT* not tested

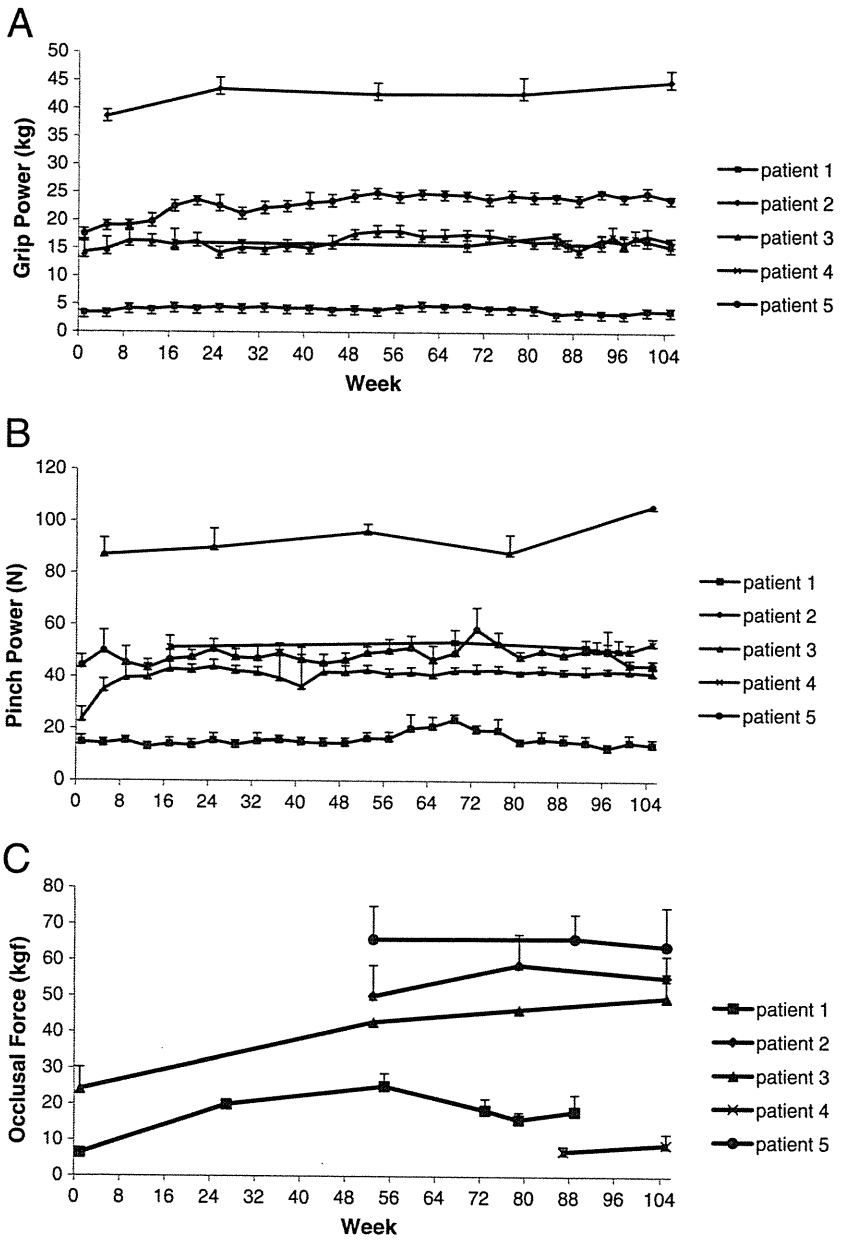


Fig. 1 Effects of ERT on grip power (**a**), pinch power (**b**), and occlusal force (**c**). Each data point represents the average of three bilateral measurements. ERT improved all of these parameters in four of five patients (with the exception of patient 4). Data are presented as mean ± SEM

Discussion

ERT is often difficult to initiate in the early stages of subclinical GSDII or in early-stage GSDII because the disease is difficult to diagnose due to heterogeneity in clinical presentation and overlapping symptoms with other neuromuscular diseases. Accordingly, it is important to gain an understanding of ERT efficacy in patients with advanced GSDII. Our study demonstrated that ERT is effective for 2 years without severe complications in adult patients who have advanced GSDII and are dependent on ventilator and wheelchair support. During the 2 years of ERT, all patients showed some improvements in muscle and pulmonary function and ADL.

All parameters improved during the first year of treatment. While the results of various tests in the second year were lower than those recorded at the end of the first year, they were still better than before ERT initiation. Although the rate of improvement differed widely among patients, our results indicate that ERT is more effective in the first year and it maintains its efficacy for 2 years. At present, there is no explanation for the better outcome in the first year compared to the second year. Taking into consideration the muscle pathology associated with GSDII, intracellular accumulation of large amounts of glycogen may cause displacement, replacement, or compression of normal cellular organelles. Thus, ERT may normalize cell function by reducing such accumulation in surviving

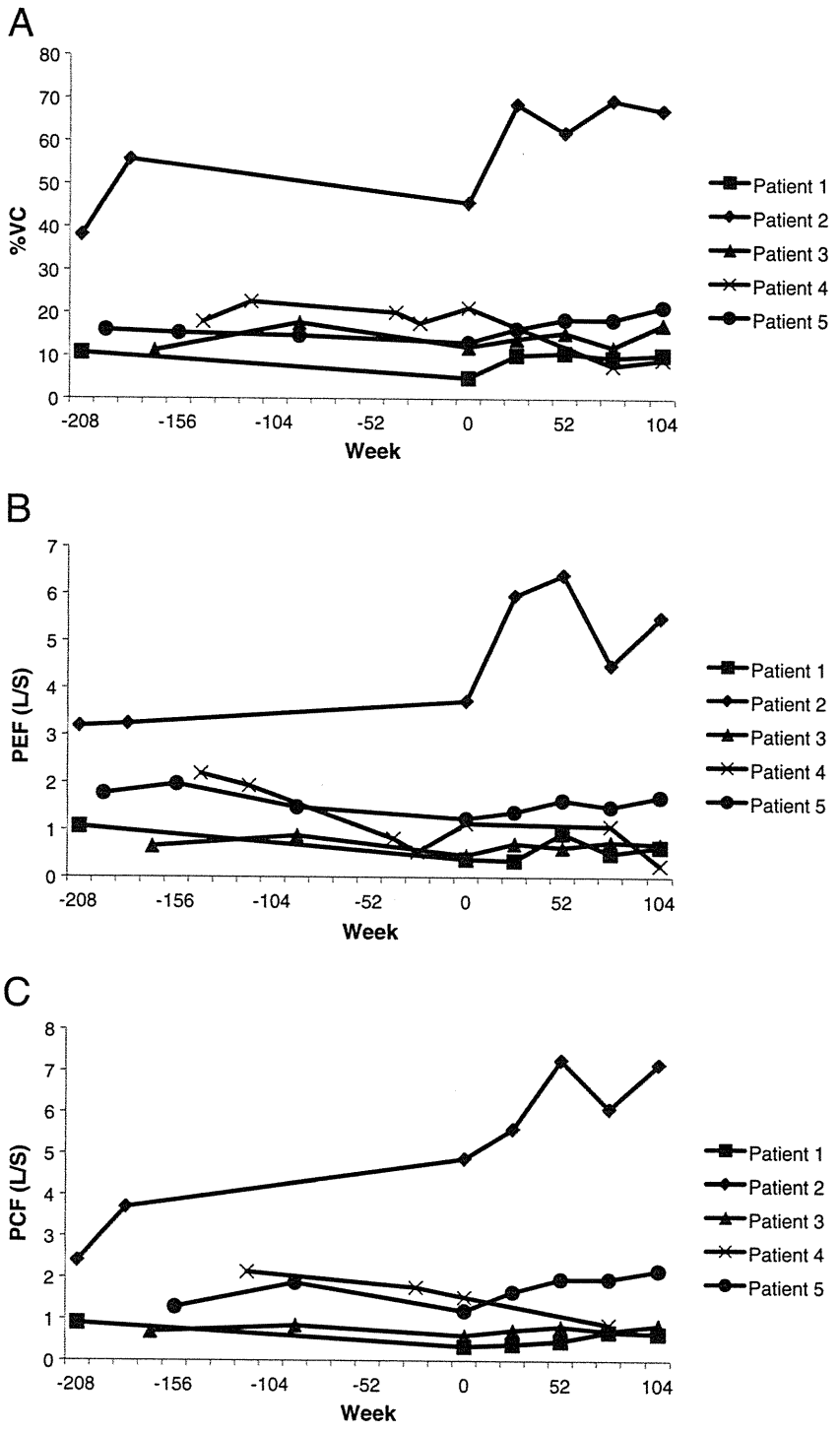
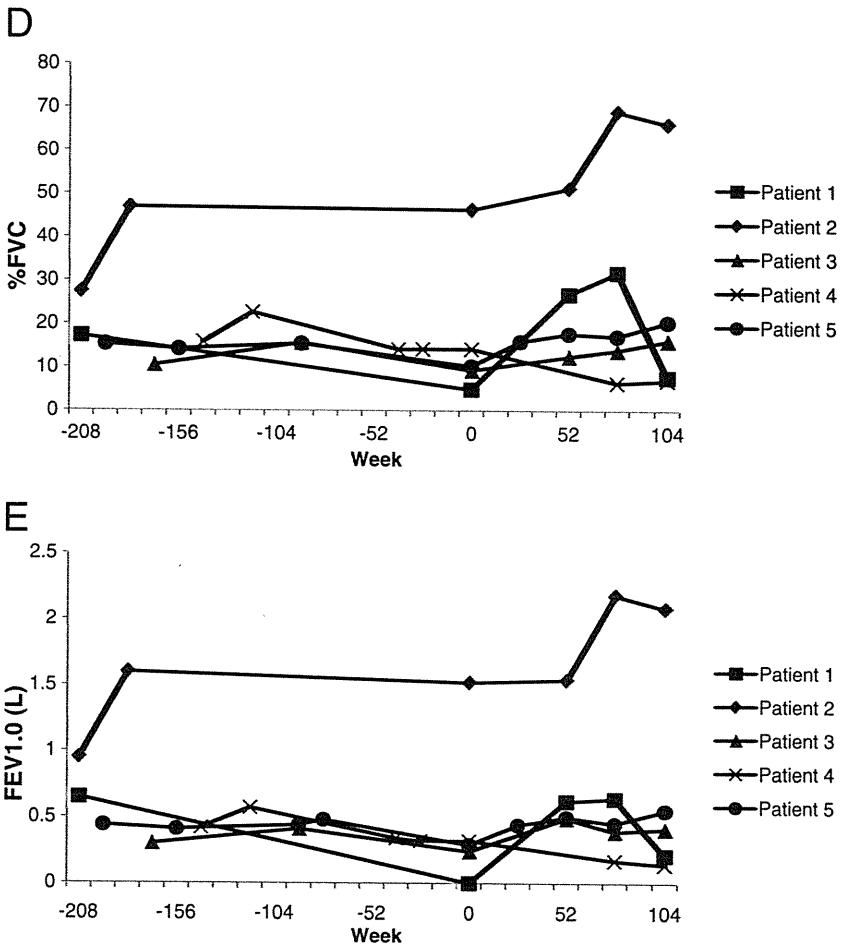


Fig. 2a–d Effects of ERT on respiratory function. Percent vital capacity (**a**), peak expiratory flow (**b**), peak cough flow (**c**), percent force vital capacity (**d**), and forced expiratory volume in the first second (**e**). Note the low values of all parameters prior to ERT and their improvement after ERT. The improvement is more pronounced in patients with spared baseline functions

myotubes, followed by a gradual regeneration of myofibers. The observed effects of ERT may represent its acute effect on intracellular glycogen accumulation.

Younger or milder cases, including those presented in a randomized controlled study of ERT, showed a greater improvement over advanced cases (Winkel et al. 2004; Pascual-Pascual et al. 2006; van der Ploeg et al. 2010). Van der Ploeg and colleagues reported on ambulant patients whose %VC was greater than 30 (van der Ploeg et al. 2010). In this clinical study, ERT elicited significant improvements in walking distance and stabilization of pulmonary function. On the other hand, efficacy of ERT in patients with advanced GSDII seemed to be milder or partial. A case report of a 67-year-old wheelchair-bound woman described alleviation of muscle symptoms following ERT, although pulmonary function tests showed no improve-

Fig. 2a–d (continued)



ment, suggesting cases with no respiratory recovery (Merk et al. 2007). Furthermore, one open-label observational study of ERT in 44 late-onset GSDII patients showed that both motor function tests and CK levels improved, and pulmonary function stabilized (Strothotte et al. 2010). Orlikowski et al. reported a 52-week follow-up of five patients (Orlikowski et al. 2011) with respiratory dysfunction as severe as in our patients, and respiratory and motor functions in all patients improved somewhat. Our data further these findings by suggesting that the improvements continue through the second year of ERT and that ERT is beneficial even for patients with advanced-stage Pompe disease.

Only patient 4 failed to show a clear recovery at the end of the follow-up period. However, grip and pinch powers increased in this patient at 60 weeks of ERT. Immobility and suspension of oral feeding resulted in reduction of muscle power, particularly in the masseter muscles. Pneumothorax also influenced the improvement in pulmonary function. Thus, we speculate that the small improvement was offset by the negative influence of pneumothorax. Because patients in similar condition at the beginning of the study responded to treatment (patients 3 and 5), one can rule out any effects of age, body weight, lean body mass, and lung dysfunction on the prognosis. Variability in the response to treatment may reflect individual differences in disease severity at treatment initiation and rate of disease progression.

The benefits conferred by ERT may not be adequate when considering ERT costs, as none of the patients exhibited an improvement in Barthel index; however, observation before ERT indicated gradual deterioration before the therapeutic intervention was initiated (Table 2). In one study, dramatic changes did not occur at the advanced stage, although certain benefits were evident (Orlikowski et al. 2011). However, we speculate that patient conditions will deteriorate if ERT is terminated after the first year, a period showing the greatest improvements. Serial pulmonary function tests indicated that the respiratory function of our patients will sequentially deteriorate (Fig. 2).

Based on our assumption that therapeutic effects of ERT cannot be measured by MMT or morbidity function in 6-min walk tests, we attempted to measure muscle power in relatively spared functions. Occlusal force is known to decrease in parallel with disease progression in Duchenne muscular dystrophy (DMD) (Ueki et al. 2007). Occlusal,

Table 3 Annual changes in parameters

	%VC						%FVC						FEV (L)						PEF (L)					
Years	1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2	1	2	1 + 2	*P*: 1 vs 2	*P* 1 vs 1 + 2	*P* 2 vs 1 + 2	1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2	1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2
Patient 1	5.8	−1.1	4.7				21.9	−19.1	2.8				0.6	−0.4	0.21				0.55	−0.43	0.1			
Patient 2	16.4	5.2	21.6				4.9	14.9	19.8				0.3	0.2	0.47				2.68	−0.91	1.8			
Patient 3	3.3	1.9	5.2	0.043	0.773	0.057	3.2	3.6	6.8	0.248	1.000	0.248	0.3	−0.1	0.17	0.200	0.306	0.043	0.17	0.07	0.24	0.020	0.468	0.430
Patient 4[a]	Not tested						Not tested						Not tested						Not tested					
Patient 5	5.4	2.9	8.3				7.4	2.7	10.1				0.21	0.05	0.26				0.39	0.07	0.46			

PCF (L)							Grip power (kg)						Pinch power (N)						Occlusal force (kgf)					
1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2		1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2	1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2	1	2	1 + 2	*P*: 1 vs 2	*P*: 1 vs 1 + 2	*P*: 2 vs 1 + 2
0.4	−0.05	0.35					0.7	0.3	1.0				6.4	−5.6	0.8				8.6	0.9	9.5			
2.39	−0.1	2.29					3.1	1.4	4.5				14.2	2.7	16.9				50	5.2	55.2			
0.22	0.03	0.25	0.028	0.885	0.020		3.2	−0.9	2.3	0.083	0.905	0.142	18.8	0.1	18.9	0.009	0.69	0.016	18.7	3.5	22.2	0.021	0.886	0.021
Not tested							Not tested		0.7				8	−3.3	4.7				Not tested					
0.77	0.21	0.98					6.4	1.1	7.5				4.2	−1.2	3				65.8	−1.8	64			

%VC Percent vital capacity, *%FVC* percent force vital capacity, *FEV1.0* forced expiratory volume in the first second, *PEF* peak expiratory flow, *PCF* peak cough flow

[a] Patient 4 could not be evaluated at 1 year after ERT initiation due to severe pneumothorax

grip, and pinch powers were relatively spared in all patients, except patient 1. Four of five patients could write, use utensils, fasten a button, or bite foods as efficiently as healthy people, although their data revealed some decrements compared to normal controls. Cranial muscle involvement is thought to be rare, but we found that occlusal force was mildly reduced in patients with advanced Pompe disease. This suggests that occlusal force is a sensitive parameter for assessing the response to ERT.

Conclusions

The present study showed that ERT improved respiratory function and muscle power for 2 years even in adult patients with advanced GSDII. Improved muscle strength resulted in better ADL and quality of life during the long follow-up period. Taking our results into consideration, we recommend the initiation of ERT in GSDII patients, irrespective of age and disease severity.

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References

Bach JR (2004) Pulmonary defence mechanisms and cough peak flow. In: Management of patients with neuromuscular disorders. Hanley & Belfus, Philadelphia, pp 193–199

Case LE, Koeberl DD et al (2008) Improvement with ongoing enzyme replacement therapy in advanced late-onset Pompe disease: a case study. Mol Genet Metab 95:233–235

Engel AG, Hirschhorn RH, Hue ML (2004) Acid maltase deficiency. In: Engel AG, Franzini-Armstrong C (eds) Myology, 3rd ed. McGraw-Hill, New York, pp 1559–1586

Hermans MM, van Leenen D et al (2004) Twenty-two novel mutations in the lysosomal alpha-glucosidase gene (GAA) underscore the genotype-phenotype correlation in glycogen storage disease type II. Hum Mutat 23:47–56

Hirshhorn R, Reuser AJJ (2001) Glycogen storage disease type II; acid alpha-glycosidase (acid maltase) deficiency. In: Scriver CR, Baudet AL, Sly WS (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3389–3420

Hosoda T, Yanagisawa K (2000) Handbook of physiotherapy, 3rd ed (in Japanese). Igaku-Shoin, Tokyo

Kishnani PS, Nicolino M et al (2006) Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. J Pediatr 149:89–97

Kishnani PS, Corzo D et al (2008) Recombinant human acid alpha-glucosidase: major clinical benefits in infantile-onset Pompe disease. Neurology 68:99–109

Kondo I, Fukuda M (2000) Gross motor functional measure manual (in Japanese). Igaku-Shoin, Tokyo

Lam CW, Yuen YP et al (2003) Juvenile-onset glycogen storage disease type II with novel mutations in acid alpha-glucosidase gene. Neurology 25(60):715–717

Merk T, Wibmer T et al (2007) Enzyme replacement therapy in Pompe's disease. Med Klin 102:570–573

Merk T, Wibmer T et al (2009) Glycogen storage disease type II (Pompe disease)-influence of enzyme replacement therapy in adults. Eur J Neurol 16:274–277

Nicolino M, Byrne B et al (2009) Clinical outcomes after long-term treatment with alglucosidasealfa in infants and children with advanced Pompe disease. Genet Med 11:210–219

Orlikowski D, Pellegrini N et al (2011). Recombinant human acid alpha-glucosidase (rhGAA) in adult patients with severe respiratory failure due to Pompe disease. Neuromuscul Disord 21:477–782

Pascual-Pascual SI, Rubio P et al (2006) Sudden deterioration in nonclassical infantile-onset Pompe disease responding to alglucosidase alfa infusion therapy: a case report. J Inherit Metab Dis 29:763

Pipo JR, Feng JH et al (2003) New GAA mutations in Japanese patients with GSDII (Pompe disease). Pediatr Neurol 29:284–287

Rossi M, Parenti G, Della Casa R (2007) Long-term enzyme replacement therapy for Pompe disease with recombinant human alpha-glucosidase derived from Chinese hamster ovary cells. J Child Neurol 22:565–573

Sasaki M, Sakuragawa N, Nonaka I (1992) A case of childhood-onset glycogen storage disease type II with 10-year-old onset (in Japanese). SyonikaRinsho 55:430–436

Strothotte S, Strigl-Pill N et al (2010) Enzyme replacement therapy with alglucosidasealfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. J Neurol 257:91–97

Tsujino S, Huie M et al (2000) Frequent mutations in Japanese patients with acid maltase deficiency. Neuromuscul Disord 10:599–603

Tsunoda H, Ohshima T et al (1996) Acid alpha-glucosidase deficiency: identification and expression of a missense mutation (S529V) in a Japanese adult phenotype. Hum Genet 97:496–499

Ueki K, Nagasawa K, Yamamoto E (2007) Bite force and maxillofacial morphology in patients with Duchenne-type muscular dystrophy. J Oral Maxillofac Surg 65:34–39

van Capelle CI, Winkel LP et al (2008) Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. Neuromuscul Disord 18:447–452

van der Ploeg AT, Clemens PR et al (2010) A randomized study of alglucosidasealfa in late-onset Pompe's disease. N Engl J Med 362:1396–1406

Winkel LP, van den Hout JM et al (2004) Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. Ann Neurol 55:495–502

Yamamoto T, Ohsaki Y, Nanba E, Tsujino S, Sakuragawa N, Martiniuk F, Ninomiya H, Oka A, Ohno K, Ravaglia S, Danesino C et al (2008) Enzyme replacement therapy in severe adult-onset glycogen storage disease type II. Adv Ther 25:820–829

Yamazaki M, Shintani M et al (1992) A case of acid maltase deficiency (juvenile type)-immunohistological and biochemical study (in Japanese). Rinsho Shinkeigaku 32:1266–1271

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Case report

Acid phosphatase-positive globular inclusions is a good diagnostic marker for two patients with adult-onset Pompe disease lacking disease specific pathology

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Abstract

Diagnosis of adult-onset Pompe disease is sometimes challenging because of its clinical similarities to muscular dystrophy and the paucity of disease-specific vacuolated fibers in the skeletal muscle pathology. We describe two patients with adult-onset Pompe disease whose muscle pathology showed no typical vacuolated fibers but did show unique globular inclusions with acid phosphatase activity. The acid phosphatase-positive globular inclusions may be a useful diagnostic marker for adult-onset Pompe disease even when typical vacuolated fibers are absent.

Keywords: Pompe disease; *GAA*; Globular inclusion; Acid phosphatase

Introduction

Pompe disease (glycogen storage disease type 2; acid maltase deficiency; OMIM #232300) is an autosomal recessive disease caused by mutations in the gene encoding acid α-glucosidase (GAA, OMIM #606800), a lysosomal enzyme involved in glycogen degradation [1]. Based on age of onset and clinical severity, which depends on residual GAA activity, the disease can be classified into infantile, childhood-onset, and adult-onset forms.

Most of the infantile and childhood-onset forms exhibit disease-specific skeletal muscle pathology, which shows fibers occupied by huge vacuoles that contain basophilic amorphous materials. However, diagnosis of the adult-onset form is sometimes challenging due to clinical similarities to muscular dystrophy and the paucity of typical vacuolated myofibers. We diagnosed 37 patients with Pompe disease including 11 infantile, 16 childhood-onset, and 10 adult-onset forms in the muscle repository of the National Center of Neurology and Psychiatry (NCNP), Japan, based on a deficiency of GAA enzyme activity assayed using biopsied muscles, as previously described [2]. Among these 37 patients, two unrelated Japanese patients did not have disease-specific vacuolated muscle fibers but did have unique cytoplasmic inclusions. Here, we report the diagnostic utility of acid phosphatase (ACP)-positive globular inclusions for adult-onset Pompe disease.

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Case report

Clinical summary

Patient 1: A 44-year-old man had been well until the age of 41 years when he started having difficulty in running. He was admitted to the hospital because of progressive muscle weakness. His parents were first cousins, but there was no family history of neuromuscular disorders. He was clinically suspected to suffer from muscular dystrophy because of slowly progressive muscle weakness and elevated creatine kinase levels of around 800 IU/L (normal, <171 IU/L). On examination, he had grade 4-muscle weakness on medical research council (MRC) scale and marked atrophy in his thighs. He did not have apparent respiratory impairment. Electromyography (EMG) showed myopathic changes with fibrillation and increased polyphasic motor unit potentials (MUPs).

Patient 2: A 62-year-old woman first noticed difficulty in climbing stairs at the age of 35 years, and needed a stick to walk at 45 years. Muscle weakness gradually worsened predominantly in her proximal limbs, and she became wheelchair-bound at 55 years. A muscle biopsy was performed at the age of 61 years. On examination, she had muscle weakness and atrophy predominantly in the proximal upper and lower limbs at the grade 3–4 on MRC scale. Serum CK level was 70 IU/L (normal, <142 IU/L). An EMG showed myopathic changes with increased polyphasic MUPs and myotonic-like repetitive discharges. She had been on non-invasive positive-pressure ventilation since the age of 62 years when the respiratory insufficiency appeared.

Skeletal muscle pathology

The skeletal muscle pathology from the vastus lateralis of patient 1 and from the biceps brachi of patient 2 showed nonspecific myopathic changes with moderate fiber size variation, mild endomysial fibrosis, and some fiber splitting (Fig. 1A). No necrotic or regenerating fibers were seen. No vacuoles containing amorphous materials were observed. Importantly, both muscles contained red–purple globular inclusions on modified Gomori-trichrome (mGT) stain (Fig. 1A and B). The average percentages of fibers with globular inclusions in the whole mGT-stained section were 0.5% in patient 1 and 2% in patient 2. These inclusions were invariably highlighted by ACP stain but not stained by periodic acid Schiff (PAS) (Fig. 1C). Inclusions were stained only faintly on menadione-linked α-glycerophosphate dehydrogenase (MAG) without substrate (Fig. 3A). Fibers with ACP-positive globular inclusions were also found in 15 of 16 childhood-onset and seven of eight adult-onset patients with disease-specific pathology in varying proportions (0.1–10%). The rate of fibers with inclusions was not significantly different between the childhood-onset and adult-onset forms. Fibers carrying inclusions did not have typical vacuoles with amorphous materials inside. In the infantile cases, more than 90% of the fibers were vacuolated, whereas non-vacuolated fibers with inclusions were hardly recognizable.

Double immunostaining was performed using primary antibodies against a lysosomal marker, lysosomal associated membrane protein-2 (LAMP-2; Developmental Studies Hybridoma Bank (DSHB), Iowa City, IA, USA) and an autophagosomal marker, microtubule-associated protein 1 light chain 3 (LC3; Novus Biologicals, Littleton, CO, USA). In fibers with ACP-positive inclusions, immunoreactivity for LAMP-2 and LC3 were accumulated focally in inclusions and surrounding area (Fig. 1D). We also examined another samples from adult-onset patients with typical vacuoles. Fibers with typical vacuoles were entirely positive for LAMP-2 and LC3 (data not shown).

On PAS staining, performed on epon-embedded sections (Epon-PAS) to detect glycogen more sensitively, PAS was negative in globular inclusions but positive in the surrounding area (Fig. 1E).

Electron micrography was performed as previously described using a Tecnai spirit transmission electron microscope (FEI, Hillsboro, OR, USA) [3]. The inclusions consisted of homogeneous electron-dense globules surrounded by increased glycogen particles and autophagic vacuoles (Fig. 1F). The globules contained neither dotted glycogen particles nor a filamentous structure.

GAA enzymatic analysis and genetic analysis

Presence of globular inclusions led us to suspect Pompe disease, and GAA enzymatic activity analyses revealed 7.5% of normal control activity in patient 1 and 12.3% in patient 2.

Genomic DNA was extracted from peripheral lymphocytes or biopsied muscle using a standard protocol for mutational analysis of *GAA*. All exons and their flanking intronic regions of *GAA* were amplified by PCR and directly sequenced with an ABI PRISM 3100 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Both patients carried the homozygous *GAA* mutation at the last codon of exon 2 (c. 546G > T). RT-PCR and direct sequencing were performed using RNA extracted from biopsied muscles. This novel mutation causes aberrant splicing by skipping exon 2 (Fig. 2). This homozygous c. 546G > T mutation was also found in another patient with the adult-onset form, whose muscle pathology showed typical skeletal muscle pathology with vacuolated fibers.

Discussion

ACP-positive globular inclusions were a good diagnostic marker for the two patients with adult-onset Pompe disease lacking typical vacuolated fibers. Among 12,103 muscle biopsies in the NCNP repository from 1979 to 2010, ACP-positive globular inclusions were not reported, except for Pompe disease.

The globular inclusions are most likely the same as "reducing body-like globular inclusions in late-onset Pompe disease" reported by Sharma et al., as the pathological features are

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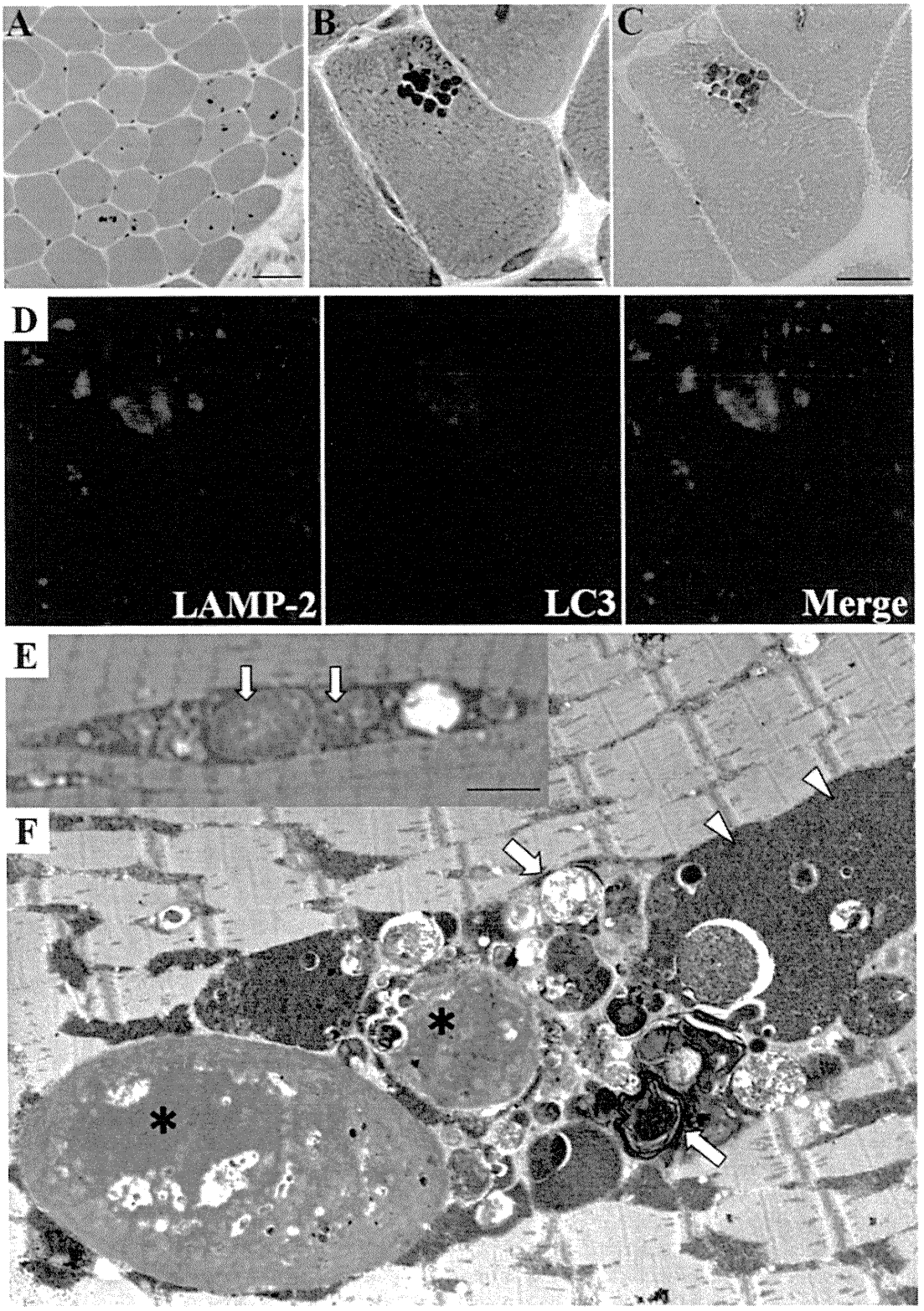

Fig. 1. Acid phosphatase-positive globular inclusions in patient 2. (A and B) Biopsied skeletal muscle showed nonspecific myopathic changes with scattered red–purple colored globular inclusions on modified Gomori-trichrome stain. (C) The inclusions have intense activity on acid phosphatase stain. Bar = 20 μm. (D) Double immunostaining for LAMP-2 (green) and LC3 (red) demonstrates colocalization of positive immunoreactions in the inclusions and surrounding area (B–D; serial sections). (E) On epon-embedded section, periodic acid Schiff stain is negative in inclusions (arrows). Bar = 5 μm. (F) On electron microscopy, globular inclusions (asterisks) lack Z-line structure, which differs from cytoplasmic bodies. Autophagic vacuoles (arrows) and glycogen particles (arrow heads) are seen in the vicinity of globular inclusions (12000×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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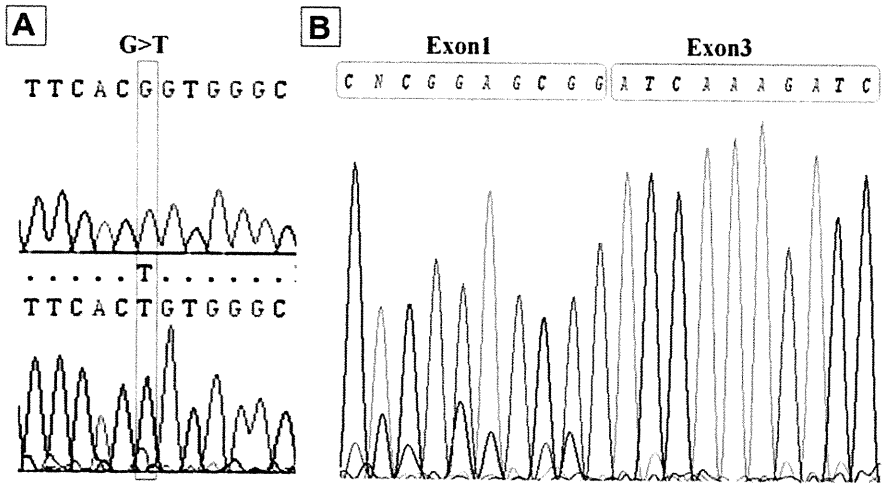

Fig. 2. Mutational analysis of *GAA*. Both patient have a homozygous c. 546G > T mutation at the last codon of exon2 (A upper: control, lower: patient), which creates mRNA with skipping exon 2 (B).

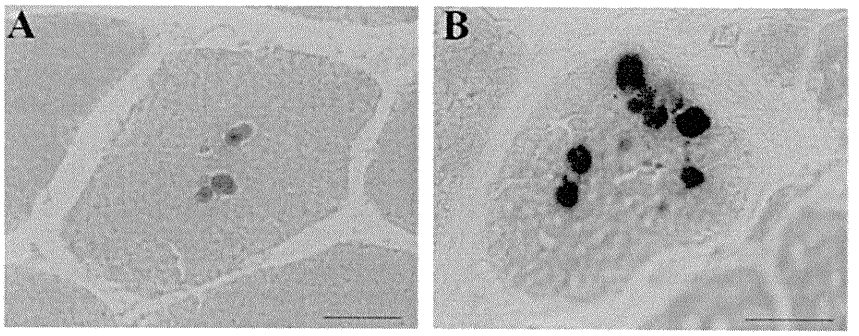

Fig. 3. Inclusions on menadione-linked α-glycerophosphate dehydrogenase (MAG) without substrate. Globular inclusions in Pompe disease (A) are only faintly stained comparing reducing bodies in reducing body myopathy with *FHL1* mutation (B). Bar = 20 μm.

rather similar [4]. However, globular inclusions showed much fainter staining on MAG without substrate than genuine reducing bodies seen in reducing body myopathy with *FHL1* mutations (Fig. 3). More importantly, ACP positivity has not been clearly described previously.

These globular inclusions are reminiscent of cytoplasmic bodies, which are nonspecific findings reflecting degeneration of the Z-disk in various neuromuscular diseases, particularly myofibrillar myopathies. However, the nature of the globular inclusions differs essentially from cytoplasmic bodies because of positive ACP staining and the lack of associated Z-disk components. Although it remains unclear how the ACP-positive globular inclusions are formed, the absence of glycogens in the globular inclusions suggest that they differ from glycogen accumulations in lysosomes. Fibers with typical vacuoles were diffusely positive for both lysosomal and autophagosomal markers as shown previously [5,6]. On the other hand, immunoreactivities of these markers accumulated more focally in fibers with inclusions. Further study should be needed to clarify what causes these pathological differences.

In conclusion, ACP-positive globular inclusions may be a hallmark of Pompe disease and a useful diagnostic marker for adult-onset Pompe disease lacking typical vacuolated fibers. Since enzyme replacement therapy is effective, albeit not fully, in adult-onset patients, early diagnosis is necessary for a better prognosis.

Ethical approval

All clinical materials used in this study were obtained for diagnostic purposes with written informed consent approved by the Ethical Committee of NCNP.

Acknowledgements

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and Mental Disorders (20B-12, 20B-13) from the Ministry of Health, Labor, and Welfare, and Intramural Research Grant (23-4, 23-5) for Neurological and Psychiatric Disorders from NCNP.

References

[1] Hers HG. Alpha-glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). Biochem J 1963;86:11–6.
[2] Shanske S, Dimauro S. Late-onset acid maltase deficiency. Biochemical studies of leukocytes. J Neurol Sci 1981;50:57–62.
[3] Park YE, Hayashi YK, Goto K, et al. Nuclear changes in skeletal muscle extend to satellite cells in autosomal dominant Emery-Dreifuss muscular dystrophy/limb-girdle muscular dystrophy 1B. Neuromuscul Disord 2009;19:29–36.
[4] Sharma MC, Schultze C, von Moers A, et al. Delayed or late-onset type II glycogenosis with globular inclusions. Acta Neuropathol 2005;110:151–7.
[5] Raben N, Ralston E, Chien YH, et al. Differences in the predominance of lysosomal and autophagic pathologies between infants and adults with Pompe disease: implications for therapy. Mol Genet Metab 2010;101:324–31.
[6] Schoser BGH, Müller-Höcker J, Horvath R, et al. Adult-onset glycogen storage disease type 2: clinico-pathological phenotype revisited. Neuropathol Appl Neurobiol 2007;33:544–59.

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CORRESPONDENCE

Myotonic dystrophy type 2 is rare in the Japanese population

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Myotonic dystrophy (DM) is the most common form of adult-onset muscular dystrophy and is characterized by autosomal dominant progressive myopathy, myotonia and multiorgan involvement. There are two distinct entities currently known: DM type 1 (DM1) and type 2 (DM2). DM2 is caused by the expansion of a tetranucleotide CCTG repeat in the first intron of the zinc finger protein 9 (*ZNF9*) gene on chromosome 3q21,[1] whereas DM1 is caused by a CTG repeat expansion in the 3′-untranslated region of the dystrophia myotonica-protein kinase gene (*DMPK*).[2] In the normal allele for *ZNF9*, the repeat sequence is a complex motif with an overall configuration of $(TG)_n(TCTG)_n(CCTG)_n$. The number of CCTG repeats is <30 in the normal allele, with interruptions by GCTG and/or TCTG motifs, and this allele is stably transmitted from one generation to the next.[1,3] However, in the expanded allele only the CCTG tract elongates and no GCTG and TCTG interruptions occur. The expanded *ZNF9* allele is extremely unstable and the size is highly variable, ranging from 75 to 11 000 repeats, with a mean of 5000 CCTG repeats. This unprecedented repeat size and somatic heterogeneity make the molecular diagnosis of DM2 difficult, and explain why the expansion yields variable clinical phenotypes.[4]

To date, DM2 mutations have been identified predominantly in European Caucasians.[3,5] Although a small number of DM2 mutations have been reported in non-European populations, including families in Morocco, Algeria, Lebanon, Afghanistan and Sri Lanka,[6,7] all reported that DM2 patients had been considered to originate from a single common founder because they shared an identical haplotype.[3,5,7] However, in 2008 we identified the first case of DM2 in an East-Asian population, in a Japanese patient with a disease haplotype distinct from that shared among Caucasians, indicating that DM2 exists in non-Caucasian populations and that there may have been separate founders.[8]

Thus, it was of interest to determine the frequency of DM2 in non-Caucasian populations. We studied a Japanese population for the presence of the DM2 mutation. We included both patients with clinically and/or electrically confirmed myotonia in which the DM1 mutation had been excluded and patients with the limb-girdle muscular dystrophy (LGMD) phenotype, because DM2 is generally proximal dominant[4] and the phenotype often lacks myotonia,[9] similar to LGMD, a heterogenous group of muscle disorders for which >60% of the genetic causes have remained undisclosed in Japan (Y.K. Hayashi *et al.*, unpublished data). It has been currently reported that the frequency of the DM2 mutation is more than DM1 in the European population:[10] 1 in 1830 in the general Finnish population, 1 in 988 Finnish patients with non-myotonic neuromuscular diseases and 1 in 93 Italian patients with undetermined non-myotonic proximal myopathy or asymptomatic hyperCKemia. Both the Finnish and Italian population are expected to be a relatively representative European population with regard to the DM2 mutation, because of a single European founder haplotype.[3,5,7]

Genomic DNA was extracted from blood leukocytes or muscle biopsy samples according to the standard protocols. The CCTG repeat size was determined by PCR, using primers flanking the repeat. When a single allele was amplified, Southern blot analysis using *Eco*RI or repeat-primed PCR specific for the DM2 expansion[1,4] was performed to distinguish homozygosity from heterozygosity involving a large CCTG expansion. All subjects included in this study gave informed consent and the protocol was approved by the Ethical Committee of Okayama University, Nagoya University and the National Center of Neurology and Psychiatry. In total, we studied 153 unrelated patients. In all, 34 were myotonic patients without the DM1 mutation and 119 showed a LGMD phenotype without identified LGMD mutations. Clinical

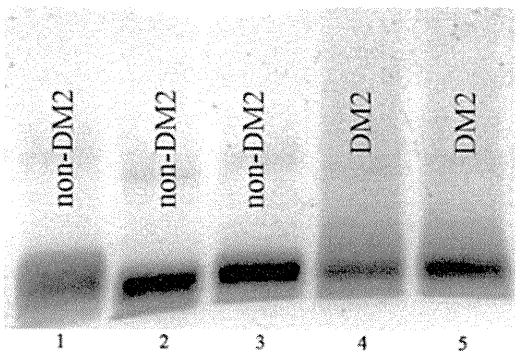

Figure 1 Repeat-primed PCR analysis. Expanded CCTG repeats in the two DM2 patients (Caucasian and Japanese DM2[9] in lanes 4 and 5, respectively) are detected as a continuous characteristic smear of products at higher molecular weight than those in non-DM2 patients (3 different individuals from the 11 patients showing a single allele by PCR amplification of the DM2 repeat in lanes 1–3).

information was assessed based on records provided by the physicians.

We identified 295 alleles ranging in length from 180 to 258 bp by PCR amplification of the DM2 repeat. Heterozygosity was identified in 142 individuals (0.93). In the remaining 11 samples showing a single allele, Southern blot or repeat-primed PCR analysis showed no expanded CCTG repeats (Figure 1), indicating that all of them are homozygous for a single allele. Thus, in our extensive survey, no DM2-related CCTG expansion was detected.

Most DM patients in Japan have been considered to have DM1 (NIH Genetics Home Reference, http://ghr.nlm.nih.gov/condition/myotonic-dystrophy). Our study confirms that DM2 is an extremely rare cause of myotonic and/or LGMD patients in Japan. Although the spectrum of clinical presentation of DM2 is variable and only one Japanese DM2 patient has been reported to date, our data have important implications concerning the indications for genetic testing and counseling for DM2 in East-Asian populations. The origin of most DM2 mutations is estimated to be 200–540 generations ago in Europe, and DM2 has since spread into several European populations.[3] The rarity of DM2 in East-Asian populations may be because of a lack of founder effects or extinction of DM2 by genetic drift or selective causes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Liquori, C. L., Ricker, K., Moseley, M. L., Jacobsen, J. F., Kress, W ., Naylor, S. L. *et al.* Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* **293,** 864–867 (2001).
2 Harper, P. S. in *Myotonic Dystrophy* (W.B. Saunders, London, 2001).
3 Liquori, C. L., Ikeda, Y ., Weatherspoon, M., Ricker, K., Schoser, B. G., Dalton, J. C. *et al.* Myotonic dystrophy type 2: human founder haplotype and evolutionary conservation of the repeat tract. *Am. J. Hum. Genet.* **73,** 849–862 (2003).
4 Day, J. W., Ricker, K., Jacobsen, J. F., Rasmussen, L. J., Dick, K. A., Kress, W. *et al.* Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. *Neurology* **60,** 657–664 (2003).
5 Bachinski, L. L., Udd, B., Meola, G., Sansone, V., Bassez, G., Eymard, B. *et al.* Confirmation of the type 2 myotonic dystrophy $(CCTG)_n$ expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. *Am. J. Hum. Genet.* **73,** 835–848 (2003).
6 Udd, B., Meola, G., Krahe, R., Thornton, C., Ranum, L., Day, J. *et al.* Report of the 115th ENMC workshop: DM2/PROMM and other myotonic dystrophies: 3rd Workshop. *Neuromuscul. Disord.* **13,** 589–596 (2003).
7 Coenen, M. J., Tieleman, A. A., Schijvenaars, M. M., Leferink, M., Ranum, L. P., Scheffer, H. *et al.* Dutch myotonic dystrophy type 2 patients and a North-African DM2 family carry the common European founder haplotype. *Eur. J. Hum. Genet.* **19,** 567–570 (2011).
8 Saito, T., Amakusa, Y., Kimura, T., Yahara, O., Aizawa, H., Ikeda, Y. *et al.* Myotonic dystrophy type 2 in Japan: ancestral origin distinct from Caucasian families. *Neurogenetics* **9,** 61–63 (2008).
9 Young, N. P., Daube, J. R., Sorenson, E. J. & Milone, M. Absent, unrecognized, and minimal myotonic discharges in myotonic dystrophy type 2. *Muscle Nerve* **41,** 758–762 (2010).
10 Suominen, T., Bachinski, L. L., Auvinen, S., Hackman, P., Baggerly, K. A., Angelini, C. *et al.* Population frequency of myotonic dystrophy: higher than expected frequency of myotonic dystrophy type 2 (DM2) mutation in Finland. *Eur. J. Hum. Genet.* **19,** 776–782 (2011).