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

Acknowledgements

This study was supported by: a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science; Research on Psychiatric and Neurological Diseases and Mental Health, Research on Measures for Intractable Diseases, Health Labour Sciences Research Grant for Nervous and Mental Disorders (20B-12, 20B-13) from the Ministry of Health, Labor, and Welfare, and Intramural Research Grant (23-4, 23-5, 23-6) for Neurological and Psychiatric Disorders of NCNP.

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.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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.

© 2011 The Author(s)

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.

© 2011 The Author(s)
European Journal of Neurology © 2011 EFNS *European Journal of Neurology*

サルコメア配列異常を主病変とする筋ジストロフィーの

病因・病態の解明と治療法の開発

J Inherit Metab Dis (2012) 35:301–310
DOI 10.1007/s10545-011-9393-6

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

Yoshihiko Furusawa · Madoka Mori-Yoshimura · Toshiyuki Yamamoto · Chikako Sakamoto · Mizuki Wakita · Yoko Kobayashi · Yutaka Fukumoto · Yasushi Oya · Tokiko Fukuda · Hideo Sugie · Yukiko K. Hayashi · Ichizo Nishino · Ikuya Nonaka · Miho Murata

Received: 26 April 2011 / Revised: 4 September 2011 / Accepted: 8 September 2011 / Published online: 7 October 2011
© SSIEM and Springer 2011

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

Communicated by: Ed Wraith

Competing interests: None declared.

Electronic supplementary material The online version of this article (doi:10.1007/s10545-011-9393-6) contains supplementary material, which is available to authorized users.

Y. Furusawa · M. Mori-Yoshimura (✉) · T. Yamamoto · Y. Oya · M. Murata
Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8551, Japan
e-mail: yoshimur@ncnp.go.jp

C. Sakamoto · M. Wakita · Y. Kobayashi
Department of Rehabilitation, National Center Hospital, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8551, Japan

Y. Fukumoto
Dental Branch, National Center Hospital, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8551, Japan

I. Nonaka
Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8551, Japan

T. Fukuda · H. Sugie
Department of Pediatrics, Jichi Medical School, 3311-1, Yakushiji, Shimotsuke-city, Tochigi 329-0498, Japan

Y. K. Hayashi · I. Nishino
Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8502, Japan

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

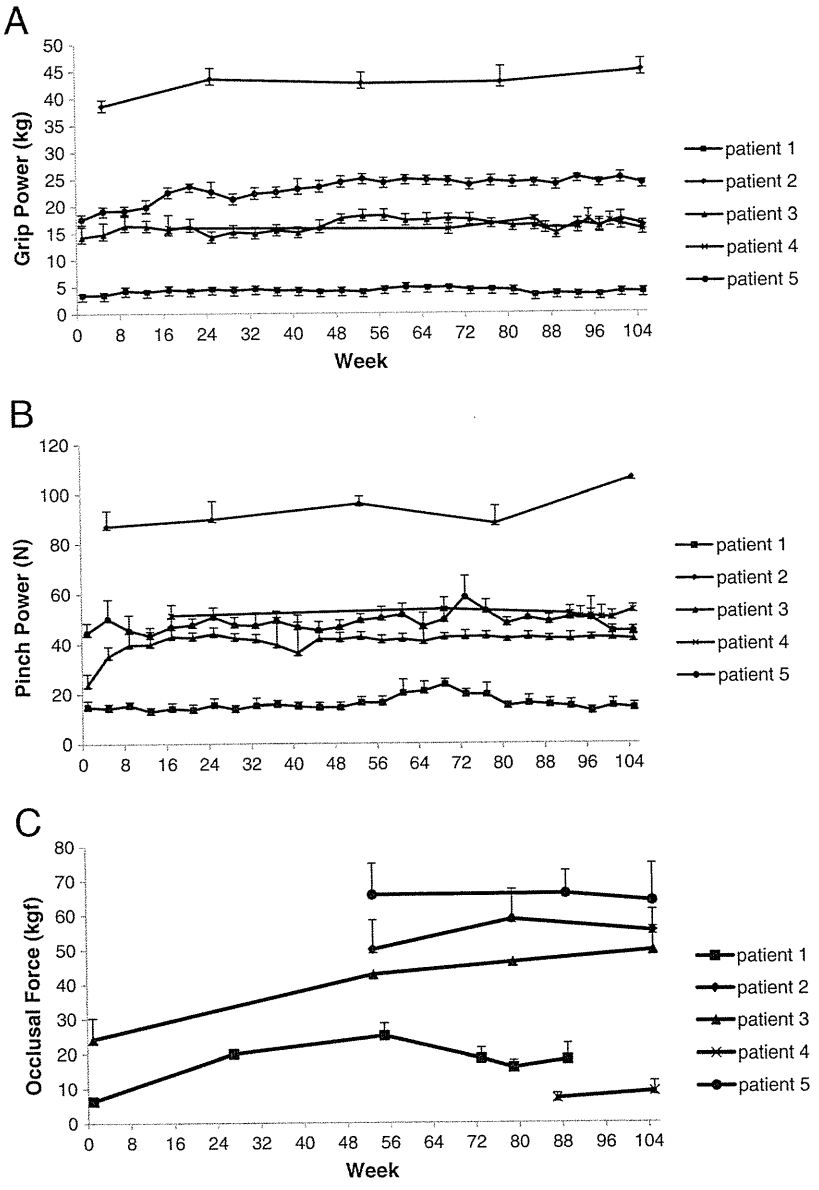


Fig. 1 Effects of ERT on grip power (**a**), pinch power (**b**), and occlusal force (**c**). Each data point represents the average of three bilateral measurements. ERT improved all of these parameters in four of five patients (with the exception of patient 4). Data are presented as mean ± SEM

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

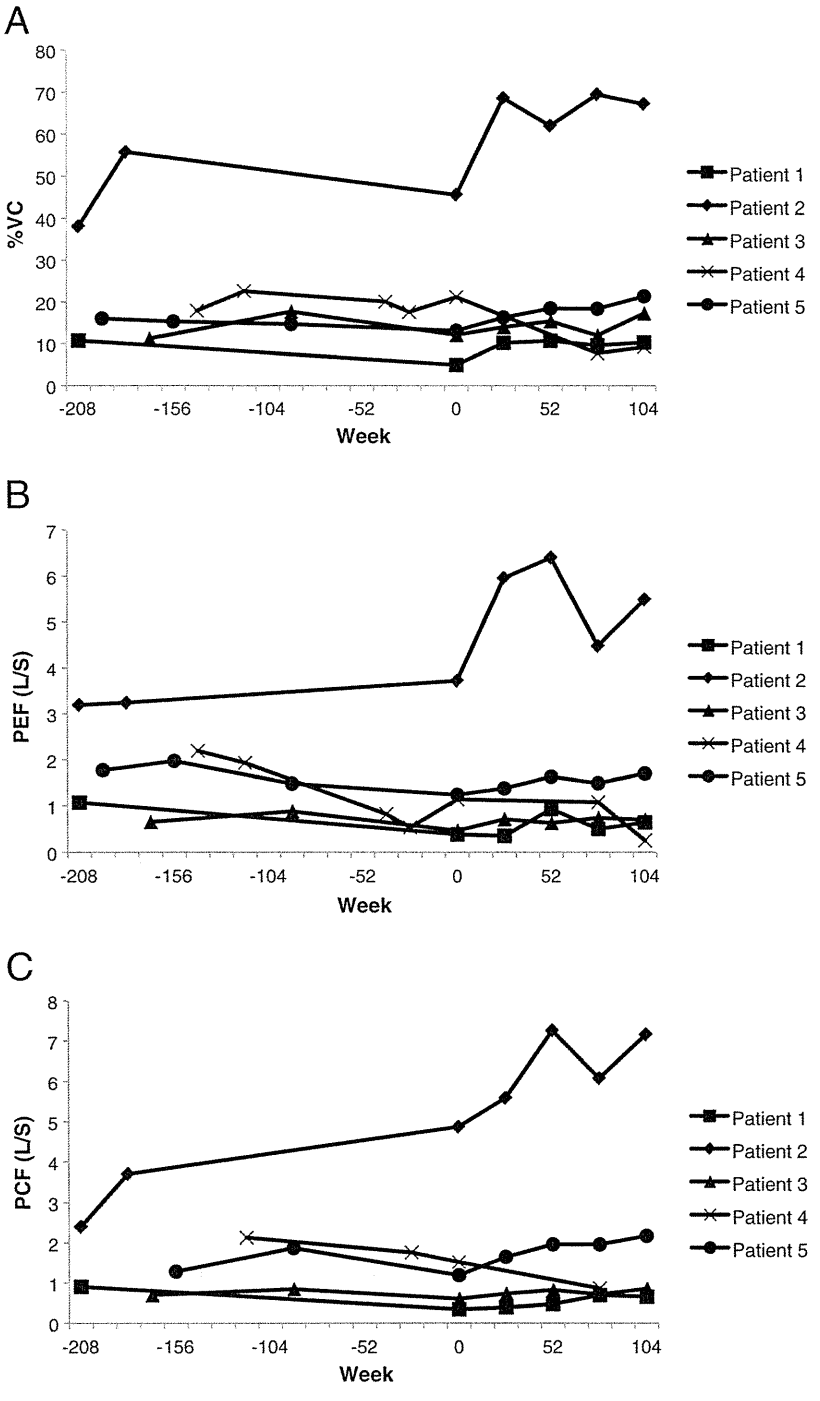
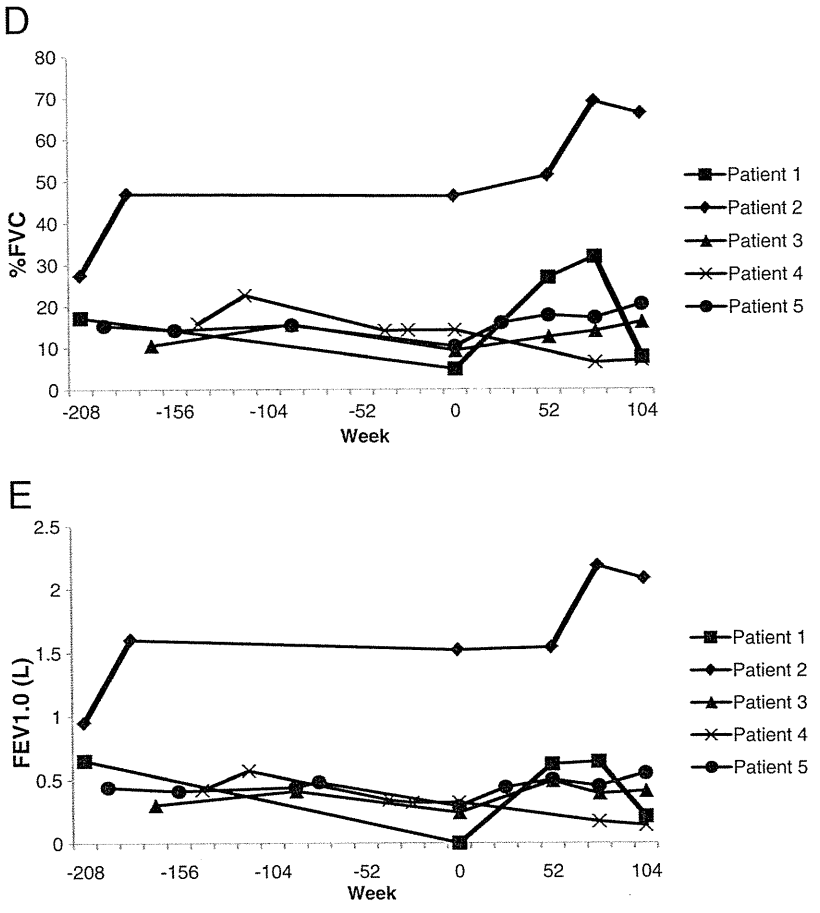


Fig. 2a–d Effects of ERT on respiratory function. Percent vital capacity (**a**), peak expiratory flow (**b**), peak cough flow (**c**), percent force vital capacity (**d**), and forced expiratory volume in the first second (**e**). Note the low values of all parameters prior to ERT and their improvement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

Acknowledgments This study was supported partly by the Research on Psychiatric and Neurological Diseases and Mental Health of Health and Labour Sciences research grants; partly by Research on Intractable Diseases of Health and Labor Sciences research grants; partly by a Research Grant for Nervous and Mental Disorders (20B-12, 20B-13) from the Ministry of Health, Labour and Welfare; partly by an Intramural Research Grant (23-4, 23-5) for Neurological and Psychiatric Disorders from NCNP; partly by KAKENHI (20390250); partly by Research on Publicly Essential Drugs and Medical Devices of Health and Labor Sciences research grants; partly by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO); and partly by a grant from the Japan Foundation for Neuroscience and Mental Health.

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

J Neurol (2012) 259:554–556
DOI 10.1007/s00415-011-6185-8

LETTER TO THE EDITORS

Increase in number of sporadic inclusion body myositis (sIBM) in Japan

Naoki Suzuki · Masashi Aoki · Madoka Mori-Yoshimura · Yukiko K. Hayashi · Ikuya Nonaka · Ichizo Nishino

Received: 28 May 2011 / Accepted: 12 July 2011 / Published online: 29 July 2011
© Springer-Verlag 2011

Dear Sirs,

Sporadic inclusion body myositis (sIBM) is the most common form of myopathy with inflammation in those over the age of 50 years in Western countries [1, 3, 5, 7]. The prevalence in Caucasians is 4.9–14.9 per million, but 1.07 in Turkey [6]. The prevalence of sIBM in Asian people including Japanese has not been examined. Several mechanisms of sIBM are proposed, for example, beta-amyloid accumulation, immune system abnormalities, viral infection, genetic background [1, 8]. However, none of these are concluded to be the specific cause of sIBM.

We have now performed a retrospective survey of Japanese patients of sIBM diagnosed at the National Center of Neurology and Psychiatry (NCNP). The increasing numbers of sIBM patients may suggest the clue to elucidate the pathomechanism of sIBM.

N. Suzuki · M. Aoki (✉)
Department of Neurology, Tohoku University School of Medicine, 1-1Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
e-mail: aokim@med.tohoku.ac.jp

M. Mori-Yoshimura
Department of Neurology, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Y. K. Hayashi · I. Nonaka · I. Nishino
Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Only patients with 'definite' or 'probable' sIBM by the clinical and biopsy criteria [7] were included in the analysis. Biopsies were re-evaluated, and were confirmed the pathological diagnosis of sIBM. We also used revised Bohan and Peter criteria for diagnosis of polymyositis (PM) [5]. In NCNP, the first patient of sIBM was diagnosed in 1989, and the number of patients diagnosed has been increasing year by year, especially after 2002 (Fig. 1). A total of 77 sIBM patients were identified between 1990 and 2007. The average age of onset in sIBM in Japan was 63.4 years old. The numbers of patients with sIBM and PM between 1999 and 2007 were 69 and 165, respectively (Table 1). Accordingly, the number of sIBM patients is estimated to be half that of PM. Given the number of PM patients in the national survey in 2003 (approximately 3,000 patients) in Japan, the number of sIBM is estimated to be around 1,250. Therefore, we assess that the prevalence of sIBM in Japan is 9.83 per million in 2003. The numbers of sIBM and PM between 1990 and 1998 were 8 and 151 patients, respectively. As the number of PM patients in the national survey of 1991 was still around 3,000, the prevalence calculated by the same method was 1.28 per million in 1991, suggesting an increase in the number of sIBM in Japan. We also examined the relationship between birth year and the number of sIBM patients diagnosed in NCNP since 1978 (Fig. 1b). The numbers of sIBM patients are increasing in a linear manner among the individuals born after 1920s.

The etiology of sIBM is not yet known and still under discussion in either a primary inflammatory myopathy or a primary degenerative myopathy with a secondary inflammatory disease. The lack of significant clinical response with various immunosuppressants is against sIBM being a primary autoimmune disorder. Accumulation of beta-amyloid in rimmed vacuoles is interpreted as a primary

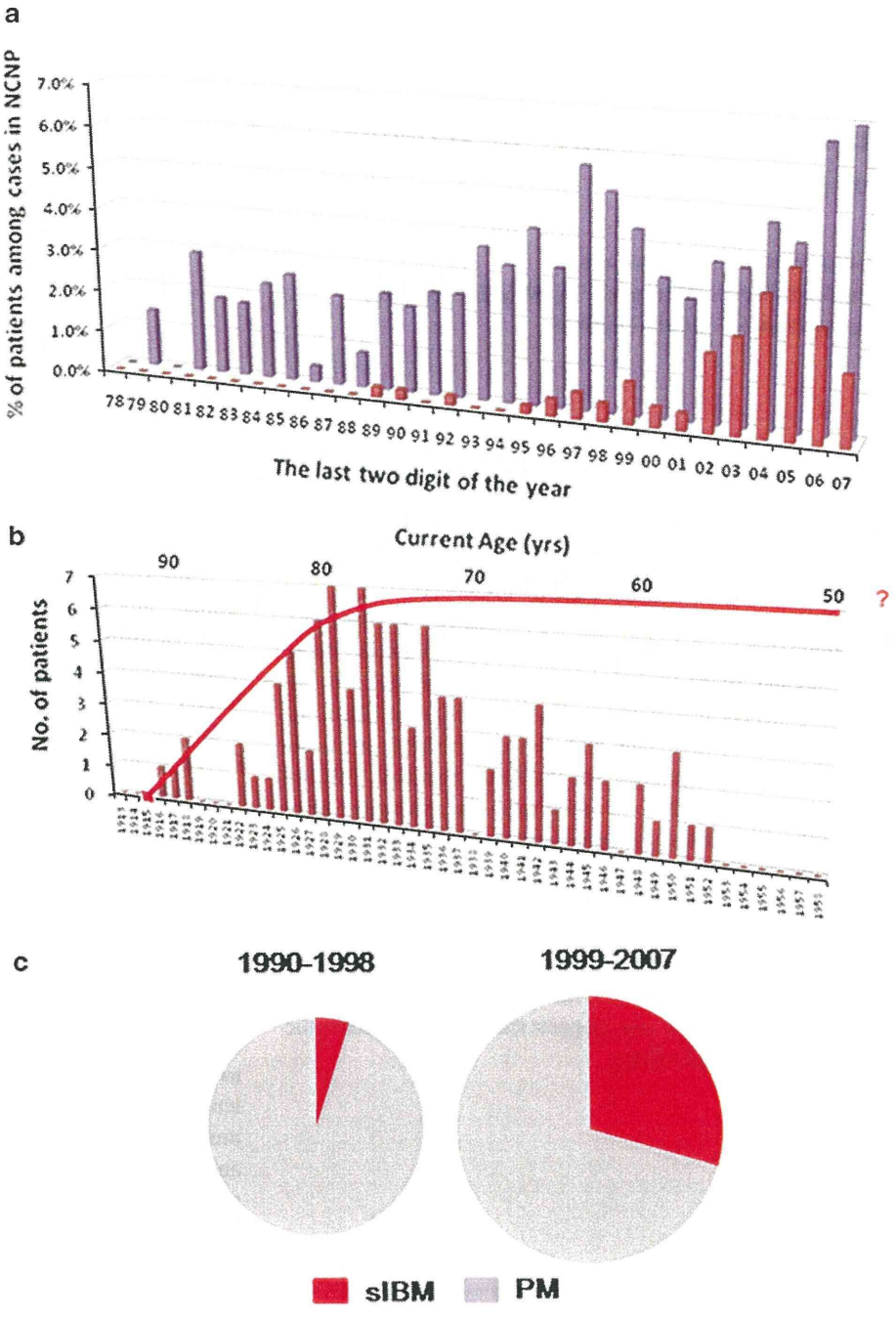


Fig. 1 **a** The number of IBM patients diagnosed in NCNP is increasing year by year. The *blue bar* represents the percentage of patients with polymyositis (PM) and the *red bar* represents sporadic inclusion body myositis (sIBM). **b** The relationship between the birth year and the number of sIBM patients diagnosed in NCNP since 1978. The *vertical axis* represents the number of sIBM patients. Note that the persons born after 1940s are now in their sixties and are at the optimal disease onset of age for sIBM. **c** The number of sIBM and PM patients diagnosed in the NCNP. Data are presented as the total of each half decade

degenerative mechanism [2]; however, some researcher pointed out that beta-amyloid is not specifically found with immunohistochemistry [9]. It was previously reported that two out of six female rabbits fed a cholesterol-enriched diet presented pathological features resembling sIBM [4]. As observed in our study, we found many patients diagnosed after 2002. The age at onset of sIBM is around 60 years old [7]. Interestingly, patients born after the 1940s were in their sixties in the 2000s and were at the optimal age of disease onset for sIBM. The increasing numbers of sIBM is followed by the rapid change of dietary habit from traditional style to a Westernized one after World War II in Japan. These data suggest that the change of dietary habit may have an influence on the increasing number of sIBM patients in Japan.

It is needed to consider the influence of prolongation of life span in Japan and also the presence of a referral filter bias for diagnostically difficult patients. Diagnostic

Table 1 The estimated number of sIBM patients in Japan

No of sIBM diagnosed at NCNP		
	1990–1998	1999–2007
PM	151	165
sIBM	8	69
PM/sIBM	18.88	2.40
No of sIBM in Japan (estimated)		
	1991	2003
PM (surveyed)	~3,000	~3,000
sIBM (estimated)	159	1,255
Total population in Japan	124,043	127,623
sIBM (estimated)/million	1.28	9.83

PM Polymyositis, *sIBM* sporadic inclusion body myositis, *PM/sIBM* the ratio of number of PM per sIBM, *NCNP* National Center of Neurology and Psychiatry

suspicion bias is also considered, but we have diagnosed distal myopathy with rimmed vacuoles since the 1980s and couldn't miss the findings of patients with rimmed vacuoles. Motorized society and sedentary lifestyle may be another possible factor after World War II in Japan. This is the first report that the number of sIBM is increasing in an Asian country. It is important to examine the other Asian countries and Asian race in Western society for elucidating the influence of food and genetic factors on the pathomechanism of sIBM.

Acknowledgments This work was supported by Research on Measures for Intractable Diseases, Research on Psychiatric and Neurological Diseases and Mental Health from the Japanese Ministry of Health Labor and Welfare. We also thank Mr. Brent Bell for reading the manuscript. Drs. Aoki, Mori-Yoshimura, Hayashi and Nishino received research support from the Research on Intractable Diseases of Health and Labor Sciences Research Grants. Dr. Hayashi also received research support from the Research on Psychiatric and Neurological Diseases and Mental health of Health and Labour Sciences Research Grants, and the Research Grant (20B-13) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare. Drs. Suzuki and Nonaka report no disclosures.

Conflict of interest None.

References

1. Amato AA, Barohn RJ (2009) Inclusion body myositis: old and new concepts. J Neurol Neurosurg Psychiatry 80:1186–1193
2. Askanas V, Engel WK (2006) Inclusion-body myositis: a myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. Neurology 66:S39–S48
3. Askanas V, Engel WK, Nogalska A (2009) Inclusion body myositis: a degenerative muscle disease associated with intramuscle fiber multi-protein aggregates, proteasome inhibition, endoplasmic reticulum stress and decreased lysosomal degradation. Brain Pathol 19:493–506
4. Chen X, Ghribi O, Geiger JD (2008) Rabbits fed cholesterol-enriched diets exhibit pathological features of inclusion body myositis. Am J Physiol Regul Integr Comp Physiol 294:R829–R835
5. Dalakas MC, Hohlfeld R (2003) Polymyositis and dermatomyositis. Lancet 362:971–982
6. Needham M, Corbett A, Day T, Christiansen F, Fabian V, Mastaglia FL (2008) Prevalence of sporadic inclusion body myositis and factors contributing to delayed diagnosis. J Clin Neurosci 15:1350–1353
7. Needham M, Mastaglia FL (2007) Inclusion body myositis: current pathogenetic concepts and diagnostic and therapeutic approaches. Lancet Neurol 6:620–631
8. Needham M, Mastaglia FL, Garlepp MJ (2007) Genetics of inclusion-body myositis. Muscle Nerve 35:549–561
9. Salajegheh M, Pinkus JL, Taylor JP, Amato AA, Nazareno R, Baloh RH, Greenberg SA (2009) Sarcoplasmic redistribution of nuclear TDP-43 in inclusion body myositis. Muscle Nerve 40:19–31

サルコメア配列異常を主病変とする筋ジストロフィーの

病因・病態の解明と治療法の開発

The American Journal of Pathology, Vol. 180, No. 4, April 2012
Copyright © 2012 American Society for Investigative Pathology.
Published by Elsevier Inc. All rights reserved.
DOI: 10.1016/j.ajpath.2011.12.040

Molecular Pathogenesis of Genetic and Inherited Diseases

In Vivo Characterization of Mutant Myotilins

Etsuko Keduka,* Yukiko K. Hayashi,* Sherine Shalaby,* Hiroaki Mitsuhashi,*† Satoru Noguchi,* Ikuya Nonaka,* and Ichizo Nishino*

From the Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; and the Division of Genetics,† Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts*

Myofibrillar myopathy (MFM) is a group of disorders that are pathologically defined by the disorganization of the myofibrillar alignment associated with the intracellular accumulation of Z-disk–associated proteins. MFM is caused by mutations in genes encoding Z-disk–associated proteins, including myotilin. Although a number of MFM mutations have been identified, it has been difficult to elucidate the precise roles of the mutant proteins. Here, we present a useful method for the characterization of mutant proteins associated with MFM. Expression of mutant myotilins in mouse tibialis anterior muscle by *in vivo* electroporation recapitulated both the pathological changes and the biochemical characteristics observed in patients with myotilinopathy. In mutant myotilin-expressing muscle fibers, myotilin aggregates and is costained with polyubiquitin, and Z-disk–associated proteins and myofibrillar disorganization were commonly seen. In addition, the expressed S60C mutant myotilin protein displayed marked detergent insolubility in electroporated mouse muscle, similar to that observed in human MFM muscle with the same mutation. Thus, *in vivo* electroporation can be a useful method for evaluating the pathogenicity of mutations identified in MFM. *(Am J Pathol 2012, 180: 1570–1580; DOI: 10.1016/j.ajpath.2011.12.040)*

Myofibrillar myopathy (MFM) is a group of neuromuscular diseases with common morphological features such as disorganized myofibrillar alignment and accumulation of Z-disk–associated proteins.[1] Mutations in genes encoding Z-disk–associated proteins are known to cause MFM. Disease-associated mutations have been identified in six genes, including myotilin, desmin, αB-crystallin, ZASP, filamin C, and BAG3.[2,3] Elucidation of their pathogenicity, however, is sometimes difficult.

Myotilin (myofibrillar protein with titin-like immunoglobulin domains) is a 57-kDa protein with 10 exons encoded by the myotilin gene (*MYOT*) on chromosome 5q31. Myotilin consists of a unique serine-rich domain at the N-terminus and two Ig-like domains at the C-terminus.[4–7] Myotilin is highly expressed in skeletal and cardiac muscle, and localizes to the Z-disk,[4] which plays important roles in sarcomere assembly, actin filament stabilization, and muscle force transmission.[8,9] Myotilin interacts with several Z-disk–associated proteins, including α-actinin,[4] filamin C,[10,11] FATZ,[11] ZASP,[12] and MuRF ubiquitin ligase.[13] Myotilin also interacts with actin monomers and filaments through its Ig-like domains, which also mediate homodimerization.[14] Previous studies have shown that myotilin can bundle actin filaments *in vitro*, acting alone or in collaboration with α-actinin and filamin C.[4,14,15] Thus, myotilin is thought to play a role in anchoring and stabilizing actin filaments at the Z-disk, and is involved in the organization and maintenance of Z-disk integrity.[12] Missense mutations in *MYOT* have been associated with MFM,[16–18] limb girdle muscular dystrophy type 1A,[17,19,20] and distal myopathy.[21,22] We have previously identified a mutation p.Arg405Lys (R405K) in exon 9 in the second Ig-like domain of myotilin. The R405K mutant myotilin exhibited defective homodimerization and decreased interaction with α-actinin in a yeast 2-hybrid (Y2H) system.[23] All of the other previously reported *MYOT* mutations are located in exon 2 [14,16–18,24], with p.Ser60Cys (S60C) being one of the most common mutations. The pathogenic effects of *MYOT* mutations and

Supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science; a Comprehensive Research on Disability Health and Welfare (20B-12, 20B-13) award from the Ministry of Health, Labor and Welfare; a Research on Intractable Diseases award from the Ministry of Health, Labor and Welfare; an Intramural Research Grant (23-4, 23-5, 23-6) for Neurological and Psychiatric Disorders, National Center of Neurology and Psychiatry; and a grant from the Japan Foundation for Neuroscience and Mental Health.

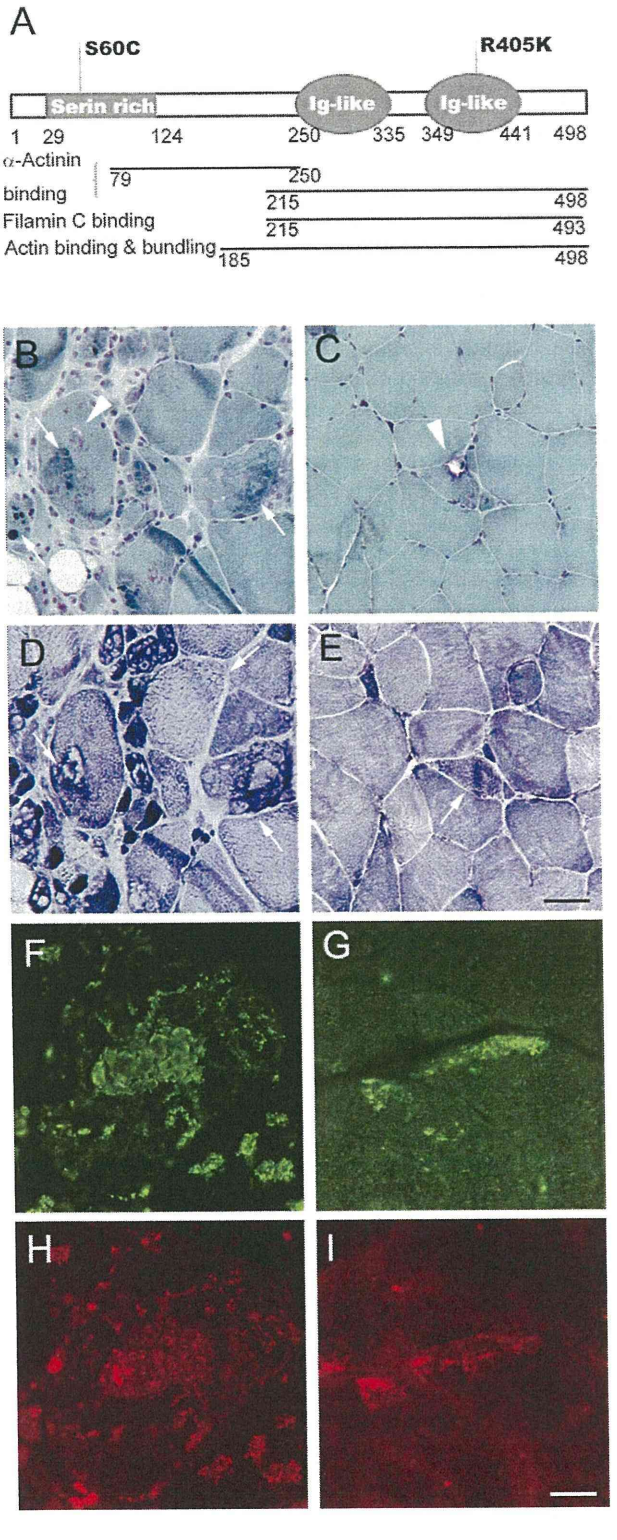
Accepted for publication December 29, 2011.

Supplemental material for this article can be found at *http://ajp.amjpathol.org* or at doi: 10.1016/j.ajpath.2011.12.040.

Address reprint requests to Yukiko K. Hayashi, M.D., Ph.D., Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashicho, Kodaira, Tokyo, 187-8502, Japan. E-mail: hayasi_y@ncnp.go.jp.

the disease mechanism involved remain poorly understood.

Model animals, such as transgenic mice, have contributed to understanding of the critical pathogenic events in MFM.[25–27] Some MFMs, including myotilinopathies, are late-onset and slowly progressive diseases.[1,3] To reproduce clinical and pathological features in model animals for such late-onset mild myopathy is both labor intensive and time consuming. Among the 10 missense mutations identified to date in patients with myotilinopathy,[14,16–18,23,24] only the Thr57Ile (T57I) mutation reproduces the pathological changes in transgenic mice after 12 months of age.[28] To screen for candidate mutations in MFM, a new method is required for demonstrating the pathogenicity of mutations. In the present study, we expressed mutant myotilin in mouse muscle by *in vivo* electroporation and were able to easily reproduce pathological changes similar to those observed in skeletal muscle from patients with *MYOT* mutations.



Materials and Methods

Clinical Materials

All clinical materials used in this study were obtained for diagnostic purposes with written informed consent. The studies were approved by the Ethical Committee of the National Center of Neurology and Psychiatry.

Genetic Analysis

Genomic DNA was isolated from peripheral lymphocytes or muscle specimens of patients, using standard techniques. Sequencing and mutation analysis of *MYOT* were performed as described previously.[23]

Plasmid Construction

We cloned full-length human myotilin cDNA and generated mutant myotilin (mMYOT) by site-directed mutagenesis, as described previously.[23] A C→G substitution at nucleotide position 179 and a G→A substitution at nucleotide 1214 were introduced to obtain p.S60C and p.R405K, respectively. A schematic of the location of these mutations in the structure of the myotilin protein is given in Figure 1A. For expression in mammalian cells, cDNAs of wild-type myotilin (wtMYOT) or mMYOT (S60C or R405K) were subcloned into pCMV-Myc vector (Ta-

Figure 1. Myotilin mutations and histopathological findings in myotilinopathy patients. **A:** Myotilin structure and disease-related mutations. p.Ser60Cys (S60C) is located in the serine-rich domain and p.Arg405Lys (R405K) is located in the second immunoglobulin (Ig)-like domain of myotilin. **B–I:** Pathological changes in muscles from patient 1 with *MYOT* S60C (**B**, **D**, **F**, and **H**) and from patient 2 with *MYOT* R405K (**C**, **E**, **G**, and **I**). **B:** Modified Gömöri trichrome (mGT) staining of biopsied skeletal muscle from patient 1 revealed markedly degenerated fibers with many spheroid protein inclusions (**arrows**). Some fibers had rimmed vacuoles (**arrowhead**). **C:** mGT staining of biopsied skeletal muscle from patient 2 revealed scattered fibers with rimmed vacuoles (**arrowhead**). **D:** NADH tetrazolium reductase (NADH-TR) staining of the serial section shown in **B** revealed markedly disorganized intermyofibrillar networks (**arrows**). **E:** NADH-TR staining of the serial section shown in **C** revealed disorganized intermyofibrillar networks (**arrow**). **F–I:** Coimmunostaining of muscles from patients using anti-myotilin (green) and anti-polyubiquitin (red) antibodies. **F:** Large accumulations of myotilin were observed in many fibers in patient 1. **G:** Small accumulations of myotilin were seen in some fibers in patient 2. Myotilin aggregates were positive for polyubiquitin in both patient 1 (**H**) and patient 2 (**I**). Scale bars: 50 μm (**B–E**); 20 μm (**F–I**).

kara Bio, Shiga, Japan). All constructs were verified by sequencing. Primer sequences are available on request.

Cell Culture, Transfection, and Immunocytochemical Analysis

C2C12 murine myoblast cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere of 5% carbon dioxide. The cells were transiently transfected using FuGENE HD transfection reagent (Roche Diagnostics, Indianapolis, IN), according to the manufacturer's instructions. Forty-eight hours after transfection, the cells were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton-X 100, and costained with anti-Myc antibody (Sigma-Aldrich) and rhodamine-labeled phalloidin (Wako Pure Chemical Industries, Osaka, Japan) to detect transfected myotilin and actin filaments, respectively, according to standard protocol.[29]

In Vivo *Electroporation*

ICR mice were purchased from CLEA Japan (Fuji, Shizuoka, Japan). Animals were handled in accordance with the guidelines established by the Ethical Review Committee on the Care and Use of Rodents in the National Institute of Neuroscience, National Center of Neurology and Psychiatry. All mouse experiments were approved by the Committee. Five-week-old male ICR mice were anesthetized with diethyl ether, and the tibialis anterior (TA) muscles of mice were injected with 80 μg of purified Myc-tagged myotilin plasmid DNA. wtMYOT was injected to one side of TA muscle and mMYOT (S60C or R405K) was injected to the other side of TA muscle. *In vivo* transfection was performed using a square-wave electroporator (CUY-21SC; Nepa Gene, Ichikawa, Japan). A pair of electrode needles was inserted into the muscle to a depth of 3 mm to encompass the DNA injection sites. Each injected site was administered with three consecutive 50 ms-long pulses at the required voltage (50 to 90 V) to yield a current of 150 mA. After a 1-second interval, three consecutive pulses of the opposite polarity were administered. At 7 or 14 days after electroporation, mice were sacrificed by cervical dislocation, and TA muscles were isolated.

Histochemical and Immunohistochemical Analyses

Biopsied human muscles or electroporated mouse TA muscles were frozen in isopentane cooled in liquid nitrogen. Serial 10-μm cryosections were stained with modified Gömöri trichrome (mGT) and NADH-tetrazolium reductase (NADH-TR) and were subjected to a battery of histochemical methods. Immunohistochemistry was performed on serial 6-μm cryosections, as described previously.[29]

Antibodies

The primary antibodies used in this study were as follows: actin (Kantoukagaku, Tokyo, Japan), α-actinin (Sigma-Aldrich), BAG3 (Abcam, Tokyo, Japan), αB-crystallin (StressGen Biotechnologies, Victoria, BC, Canada), desmin (PROGEN Biotechnik, Heidelberg, Germany), filamin C (kindly provided by A.H. Beggs),[30] c-Myc (Sigma-Aldrich), c-Myc (PROGEN Biotechnik), myotilin (Proteintech Group, Chicago, IL), polyubiquitinated protein (Biomol International-Enzo Life Sciences, Plymouth Meeting, PA), GAPDH (Advanced ImmunoChemical, Long Beach, CA), and horseradish peroxidase-labeled anti-c-Myc antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Evaluation of Aggregates

Histochemical and immunohistochemical analyses were performed on cryosections of electroporated muscles sectioned at 500-μm intervals. The section containing the highest number of Myc-positive fibers (>100 fibers) was used. Myc-positive granules >1 μm in diameter were defined as aggregates. The Myc-positive fibers containing Myc-positive aggregates were counted among all Myc-positive fibers. Five mice each from the wtMYOT-, mMYOT S60C-, and mMYOT R405K-expressing groups were examined. To compare the number and size of Myc-positive aggregates per fiber, we measured the number and area of Myc-positive aggregates in 30 myofibers from each specimen using ImageJ software version 1.43 (NIH, Bethesda, MD). The results are presented as bar graphs (±SD) and histograms. Fifteen serial sections were immunoblotted to measure the amounts of electroporated Myc-tagged myotilin protein.

Electron Microscopy

For electron microscopy, cryosections (25 μm thick) of biopsied muscle with the S60C mutation (patient 1) were fixed with 2% glutaraldehyde in 100 mmol/L cacodylate buffer for 15 minutes on ice. After a shaking with a mixture of 4% osmium tetroxide, 1.5% lanthanum nitrate, and 200 mmol/L s-collidine for 1 to 2 hours, samples were embedded in epoxy resin. TA muscles of 5-week-old ICR mice were coelectroporated with pEGFP-C1 plasmid (Clontech, Tokyo, Japan), which encodes enhanced green fluorescent protein (EGFP), and with either Myc-wtMYOT or Myc-mMYOT (S60C or R405K) plasmid (40 μg each). As a control, pEGFP-C1 plasmid was electroporated alone. TA muscles were isolated 7 and 14 days after electroporation. EGFP-positive regions were trimmed under a fluorescence microscope and fixed with 2% glutaraldehyde in 100 mmol/L cacodylate buffer for 3 hours. After a shaking with a mixture of 4% osmium tetroxide, 1.5% lanthanum nitrate, and 200 mmol/L s-collidine for 2 to 3 hours, samples were embedded in epoxy resin. Semithin sections (1 μm thick) were stained with Toluidine Blue. Ultrathin sections (100 nm thick) were stained with uranyl acetate and lead citrate, and were analyzed at 120 kV using a Tecnai Spirit transmission electron microscope (FEI, Hillsboro, OR).