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.

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Conflict of interest None.

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Characterization of the Asian myopathy patients with *VCP* mutations

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Keywords:
amyotrophic lateral sclerosis, cytoplasmic inclusion, inclusion body myopathy with Paget's disease of bone and frontotemporal dementia, rimmed vacuolar myopathy, nuclear inclusion, transactivation response DNA-binding protein 43, ubiquitin, valosin-containing protein

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Background and purpose: Mutations in the valosin-containing protein (*VCP*) gene are known to cause inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD) and familial amyotrophic lateral sclerosis (ALS). Despite an increasing number of clinical reports, only one Asian family with IBMPFD has been described.

Methods: To characterize patients with *VCP* mutations, we screened a total of 152 unrelated Asian families who were suspected to have rimmed vacuolar myopathy.

Results: We identified *VCP* mutations in seven patients from six unrelated Asian families. Five different missense mutations were found, including a novel p.Ala439Pro substitution. All patients had adult-onset progressive muscle wasting with variable involvement of axial, proximal, and distal muscles. Two of seven patients were suggested to have mild brain involvement including cerebellar ataxia, and only one showed radiological findings indicating a change in bone. Findings from skeletal muscle indicated mixed neurogenic and myogenic changes, fibers with rimmed vacuoles, and the presence of cytoplasmic and nuclear inclusions. These inclusions were immunopositive for VCP, ubiquitin, transactivation response DNA-binding protein 43, and also histone deacetylase 6 (HDAC6), of which function is regulated by VCP. Evidence of early nuclear and mitochondrial damage was also characteristic.

Conclusions: Valosin-containing protein mutations are not rare in Asian patients, and gene analysis should be considered for patients with adult-onset rimmed vacuolar myopathy with neurogenic changes. A wide variety of central and peripheral nervous system symptoms coupled with rare bone abnormalities may complicate diagnosis.

Introduction

Mutations in the valosin-containing protein (*VCP*) gene on chromosome 9p13-p12 are known to cause an autosomal dominant multisystem disorder referred to as inclusion body myopathy with Paget's disease of bone (PDB) and frontotemporal dementia (IBMPFD) [1]. Myopathy is the most common clinical symptom observed in 90% of affected individuals, and this usually appears when patients are in their 40s. About 30% of IBMPFD patients show only muscle symptoms. Characteristic pathology findings include the presence of VCP- and ubiquitin-positive cytoplasmic and nuclear inclusions together with rimmed vacuoles in skeletal muscle. Accumulation of transactivation response DNA-binding protein 43 (TDP-43), a VCP-interacting protein, is also characteristic. PDB is observed in about a half of the IBMPFD patients at approximately the same age that the myopathy typically appears, whereas frontotemporal dementia (FTD) is seen in 32% with an age of onset that is nearly 10 years later than either the myopathy or PDB [2]. Nuclear VCP- and ubiquitin-positive inclusions are also seen in neurons [3]. Recently, *VCP* mutations were identified in five families

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with amyotrophic lateral sclerosis (ALS) [4]. Although nearly 50 families with *VCP* mutations have been reported worldwide, only one such family has been recently identified from an East Asian population [5]. Here, we identified seven Asian patients in six unrelated families with mutations in *VCP* and performed detailed clinical and pathological analyses.

Methods

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

Patients

The presence of rimmed vacuoles is a characteristic pathological finding for IBMPFD. We performed *VCP* mutation screening in a total of 152 unrelated Asian families who were suspected to have rimmed vacuolar myopathy. Eighty-seven patients had distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy (DMRV/hIBM) with no glucosamine (UDP-*N*-acetyl)-2-epimerase/*N*-acetylmannosamine kinase (*GNE*) mutations. Twenty-five cases of limb-girdle muscular dystrophy (LGMD) of unknown cause and 40 other undiagnosed myopathy cases were also included in which patients' muscle contained rimmed vacuoles.

Mutation analysis

Genomic DNA was isolated from peripheral lymphocytes or muscle specimens by using standard techniques. All 17 exons and their flanking intronic regions of *VCP* were sequenced directly using an ABI PRISM 3130 automated sequencer (PE Applied Biosystems, CA, USA). Primer sequences are available on request. For the identification of novel nucleotide changes, 100 control chromosomes were screened.

Muscle pathology

Biopsied skeletal muscles were frozen with isopentane cooled in liquid nitrogen. Frozen serial sections of 10 μm thickness were stained using various conventional histochemical methods, including hematoxylin and eosin, modified Gomori trichrome, and cytochrome *c* oxidase (COX), which reflect a mitochondrial electron transport enzyme activity. To know the fiber type distribution and their composition, ATPase stains under different pH were performed.

Immunohistochemistry was performed using standard protocols. Antibodies using in this study were listed in Table S1. The sections were observed with epifluorescence using an Axiophoto2 microscope (Carl Zeiss, Oberkochen, Germany). To detect apoptotic nuclei, a fluorometric terminal dUTP nick-end labeling (TUNEL) detection kit (Takara Bio Int., Shiga, Japan) was used according to the manufacturer's instructions.

Electron microscopy

Biopsied specimens were fixed in 2.5% glutaraldehyde and post-fixed with 2% osmium tetroxide. Semithin sections stained with toluidine blue were examined by light microscopy. Ultrastructural analysis was carried out on ultrathin sections of muscles after staining with uranyl acetate and lead citrate, using a transmission electron microscope (JEM 1400; Jeol, Tokyo, Japan).

Results

Mutation analysis of *VCP*

We identified five different heterozygous missense mutations in seven patients, including c.277C > T (p.Arg93Cys) in Patient 1, c.463C > T (p.Arg155Cys) in Patients 2 and 3 (unrelated), c.464G > A (p.Arg155His) in Patient 4, c.572G > A (p.Arg191Gln) in Patient 5, and c.1315G > C (p.Ala439Pro) in Patients 6 and 7 (from the same family). The novel c.1315G > C mutation was not found in 100 Japanese control chromosomes, and p.Ala439 is conserved among species (Fig. 1).

Clinical findings

Clinical information of each patient is summarized in Table 1. All seven patients had adult-onset slowly progressive muscle weakness and atrophy with variable involvement of axial, proximal, and distal muscles. Two patients (Patients 5 and 6) showed asymmetrical involvement at the onset of the disease. Muscle pain, cramps, and fasciculations were often observed. Serum creatine kinase (CK) levels were normal to mildly elevated. Electromyography (EMG) showed mixed findings with neurogenic and myogenic changes, and nerve conduction velocity was decreased in two patients (Patients 3 and 4).

Only one patient (Patient 7) had an irregular sclerotic region in the 5th lumbar vertebral body with normal serum alkaline phosphatase level. Increased urine deoxypyridinoline level, a specific marker for bone resorption, was observed in Patient 2 with normal bone images. The other patients showed no signs suggesting bone involvement.

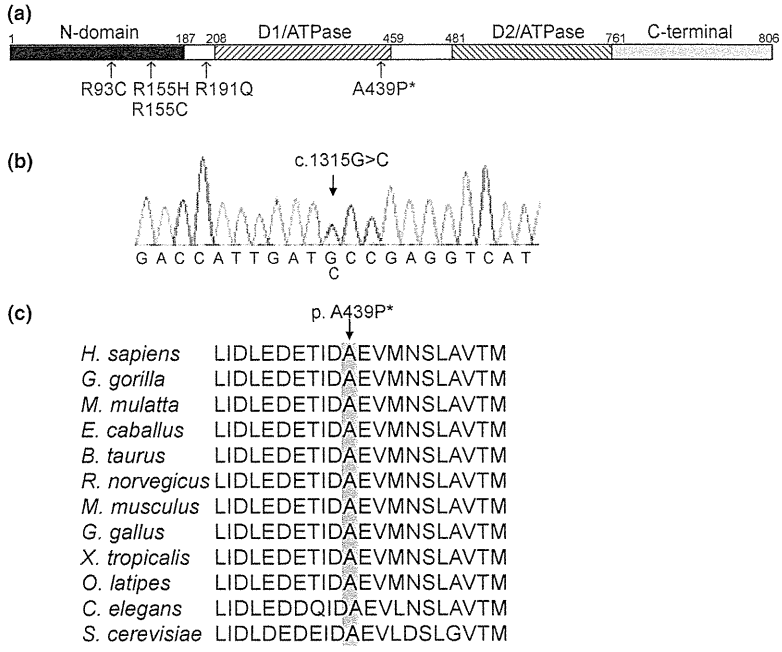

Figure 1 Result of valosin-containing protein (VCP) mutation screening. (a) The domain structure of human *VCP* (modified from Guinto *et al.* [28]) and position of the mutations identified in our series. (b) A novel heterozygous c.1315G > C substitution (p. Ala439Pro) is seen in Patients 6 and 7. (c) The alanine residue at position 439 (orange) is well preserved among the species including *Saccharomyces cerevisiae*.

Two of seven patients (Patients 2 and 7) showed mild cognitive impairment. Importantly, Patient 2, whose deceased brother had a diagnosis of spinocerebellar degeneration, showed signs of cerebellar involvement prior to impairment of frontal function, including dysarthria, symmetrical muscle hypotonia, and mild ataxia.

Muscle pathology

Skeletal muscle tissues from all seven patients with *VCP* mutations (Patients 2–7) showed mixed changes indicating myopathy and neuropathy. Scattered fibers with rimmed vacuoles were commonly seen (Fig. 2a and b). Cytoplasmic bodies were also seen in some fibers, with or without rimmed vacuoles (Fig. 2b). In addition, small angular fibers, groups of atrophic fibers, and fiber type grouping were seen. An increased number of type 2C fibers suggested presence of immature fibers or active fiber type conversion (Fig. 2a and c). Some fibers showed deficiency of COX stain, which reflect a mitochondrial electron transport enzyme activity (Fig. 2d). Succinate dehydrogenase (SDH) is a mitochondrial enzyme complex which demonstrates the relative proportions of mitochondria in muscle fibers. SDH staining of these COX-deficient fibers was variable from irregularly intense to negative (data not shown).

Immunohistochemical analysis was performed in muscle tissue from four patients with *VCP* mutations (Patients 2, 3, 4 and 6), together with samples from 10 DMRV, and eight sporadic inclusion body myositis (sIBM) patients. In normal skeletal muscle, TDP-43 is clearly detected in the nuclei (Fig. 3a). Nuclei in DMRV/hIBM and sIBM muscles were also strongly stained with TDP-43 (Figs 3c and d). In contrast, samples taken from patients with *VCP* mutations showed many nuclei with a deficiency of TDP-43 staining (Figs 3b, 4a and d). Besides, some TDP-43-positive nuclei were enlarged and costained with ubiquitin (Fig. 4a–d). These findings were commonly seen in all four patients with *VCP* mutations. Some ubiquitin- and TDP-43-positive myonuclei were also seen in DMRV/hIBM and sIBM muscles (data not shown).

The presence of nuclear inclusions stained with VCP is a characteristic finding of muscle from patients with *VCP* mutations. These VCP-positive nuclei were observed in all four patients with *VCP* mutations from 1.0 to 6.6% of myonuclei and costained with ubiquitin (Figs 3f and 4e–h). Some nuclei were also positive for histone deacetylase 6 (HDAC6) (Fig. 4j–k). The VCP-positive nuclei were not seen in muscle from patients with DMRV/hIBM or sIBM. Only a few nuclei were positive for TUNEL in all of the diseased muscle specimens examined (data not shown).

On the other hand, ubiquitin-positive cytoplasmic inclusions were observed in muscles from all patients with *VCP* mutations we examined varying from 6 to 25% of the muscle fibers (Fig. 4b). These cytoplasmic inclusions were often seen beside the nucleus and costained with TDP-43 (Fig. 4a–d), VCP (Fig. 4i), HDAC6 (Fig. 4j–l), p62, and SMI-31 (data not shown). In muscle tissue from patients with DMRV/hIBM or sIBM, scattered ubiquitin-positive, and a few

Table 1 Clinical summary of the patients

Pt No.	Sex/age (years)	Age at onset (years)	Clinical diagnosis	Affected relatives (diagnosis)	Initial symptom	Muscle weakness	CK (IU/L)	EMG	Muscle biopsy	*VCP* mutation	Bone Involve	Brain Involve
1	M/70	58	DMRV	Brother (DMRV)	Dragging gait	Four limbs (P > D, L = U), neck flexion	286	Myo/ Neuro	ND	R93C	No	No
2	F/57	47	DMRV/ IBMPFD	Brother (SCD)	Weakness of lower limbs	Paraspinal, four limbs (D > P, L > U)	82	Neuro	RVs, neurogenic changes	R155C	DPD↑	Mental disorder, cerebellar signs
3	F/47	45	Myopathy	Brother (muscle weakness)	Fall down frequently, weakness of arms	Four limbs (P > D, L = U); neck flexion	94	Myo/ Neuro	RVs, neurogenic changes	R155C	No	No
4	M/51	38	LGMD	Father (muscle wasting, cramps)	Back pain	Paraspinal, four limbs (P > D, L > U)	490	Myo/ Neuro	RVs, neurogenic changes	R155H	No	No
5	M/44	32	DMRV	Father (SMA)	Numbness of left arm	Generalized, SW	44	Neuro	RVs, neurogenic changes	R191Q	No	No
6	M/43	39	DMRV	Father (MND) Sister (P7)	Atrophy of left shoulder girdle muscles	Four limbs (D > P, L > U), SW	215	Myo/ Neuro	RVs, neurogenic changes	A439P[a]	No	No
7	F/49	46	Myopathy	Father (MND) Brother (P6)	Weakness of lower limbs	Generalized	88	Neuro	RVs, neurogenic changes	A439P[a]	Osteo-sclerosis	Mental: borderline

F, female; M, male; D, distal; P, proximal; U, upper limb; L, lower limb; SW, scapular winging; Myo, myogenic changes; Neuro, vacuoles; CK, creatine kinase; EMG, electromyogram; DPD, deoxypyridinoline; VCP, valosin-containing protein; ND, not done; IBMPFD, inclusion body myopathy with Paget's disease of bone and frontotemporal dementia; LGMD; limb-girdle muscular dystrophy; DMRV, distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy. [a]Novel mutation.

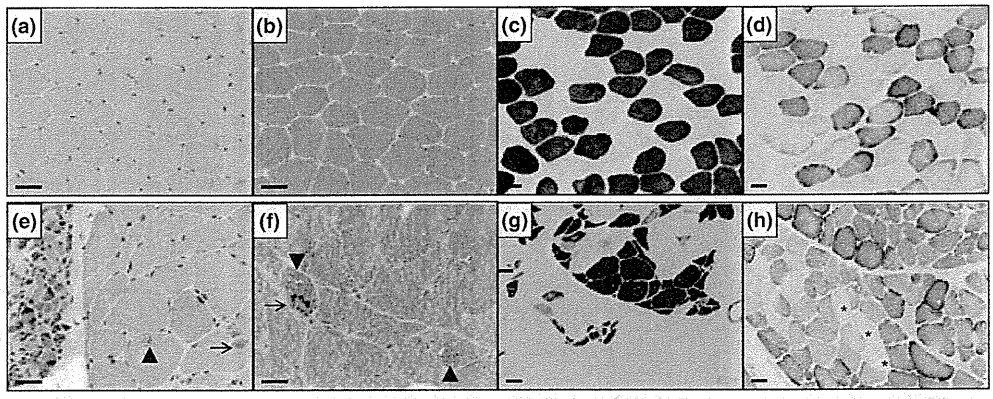

Figure 2 Histological analyses of muscle. (a–d: control. e–h: Patients 2 or 3, a and e: Hematoxylin and eosin (HE), b and f: modified Gomori trichrome (mGt), c and g: ATPase (pH 10.6), d and h: cytochrome *c* oxidase (COX). (e) HE staining of Patient 2 showed a group of atrophic fibers together with rimmed vacuoles (arrowheads) and a cytoplasmic inclusion (arrow). (f) A mGt stain of Patient 3 revealed rimmed vacuoles (arrowheads) and cytoplasmic bodies (arrow). (c) An ATPase stain of Patient 2 revealed grouped atrophy of darkly stained type 2 fibers and a large group of brightly stained type 1 fibers. Presence of scattered intermediate-colored type 2C fibers suggests immature fibers or fiber type conversion. (d) COX staining, which reflects mitochondrial electron transport enzyme activity, of Patient 3 showed some COX-deficient fibers (*). Bar = 50 μm.

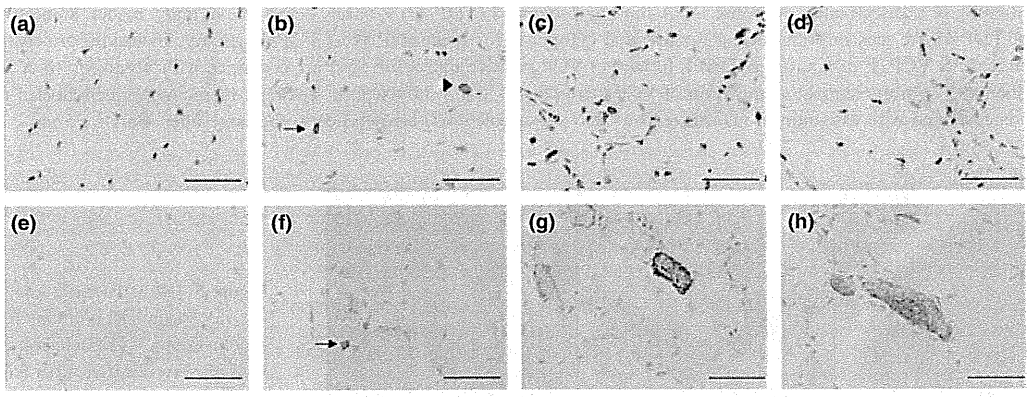

Figure 3 Immunostaining of transactivation response DNA-binding protein 43 (TDP-43) and valosin-containing protein (VCP). (a–d; TDP-43, e–h; VCP) In control muscle, clear nuclear staining of TDP-43 is seen (a), whereas VCP staining is barely detectable (e). In Patient 4, many nuclei show deficient TDP-43 staining, but scattered, strongly stained nuclei (arrow) and cytoplasmic aggregate (arrowhead) can be seen (b). VCP staining is seen in an enlarged nucleus (arrow) and subsarcolemma (f). In DMRV/hIBM (c) and sporadic inclusion body myositis (sIBM) (d) muscles, a smaller number of nuclei showing reduced staining of TDP-43 associated with cytoplasmic aggregations are seen. Some atrophic fibers show diffuse increased cytoplasmic staining of VCP in both distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy (DMRV/hIBM) (g) and IBM (h). Bar = 50 μm.

VCP-positive cytoplasmic inclusions were seen, whereas no such inclusions were seen in control muscles (data not shown). Fibers with diffuse cytoplasmic staining of VCP were also seen in the patients with *VCP* mutations, DMRV/hIBM, or sIBM (Figs 3f–h and 4i).

Ultrastructural observations

Electron microscopic observations of muscles from Patients 2 and 4 revealed many abnormally shaped nuclei with condensed or scanty irregular heterochromatin, even in those muscle fibers with well-preserved myofibril structures (Fig. 5a and c). Some degenerating nuclei were surrounded by variable-sized membranous structures (data not shown). Filamentous inclusions that were 15–20 nm in diameter were also seen in both nuclei (Fig. 5b) and subsarcolemma (Fig. 5d and e). Subsarcolemmal accumulations of mitochondria, the presence of enlarged mitochondria, and paracrystalline inclusions were prominent in some muscle fibers (Fig. 5f).

Discussion

The number of the clinical reports of IBMPFD/ALS patients with *VCP* mutations is increasing; however, a

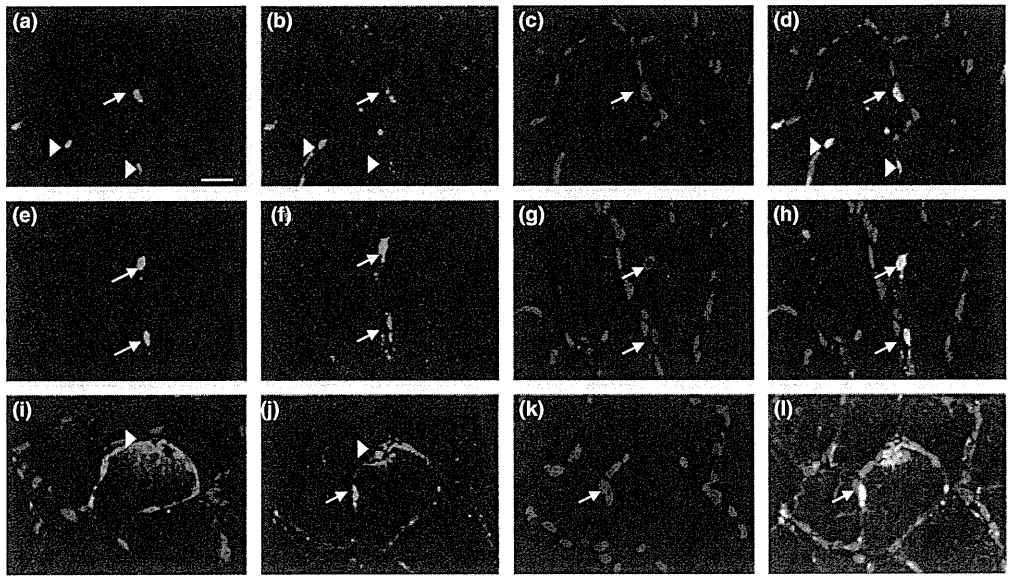

Figure 4 Immunohistochemical analyses of muscle. (a–d; a: TDP-43, b: ubiquitin, c: DAPI, d: merge) In the muscle from Patient 3, nuclear staining of transactivation response DNA-binding protein 43 (TDP-43) is barely detectable in many nuclei. Some strong positive signals of TDP-43 are seen in both nucleus (arrow) and cytoplasm (arrowheads). Most TDP-43-positive inclusions are costained with ubiquitin. (e–h; e: VCP, F: ubiquitin, g: DAPI, h: merge) VCP-positive nuclei (arrows) are costained with ubiquitin. (i) A VCP-positive muscle fiber with subsarcolemmal aggregation of VCP (arrowhead). (j–l; j: HDAC6, k: DAPI, l: merge with green-labeled ubiquitin) HDAC6 is costained with ubiquitin in nucleus (arrow) and subsarcolemma (arrowhead) in the same fiber. Bar = 25 μm.

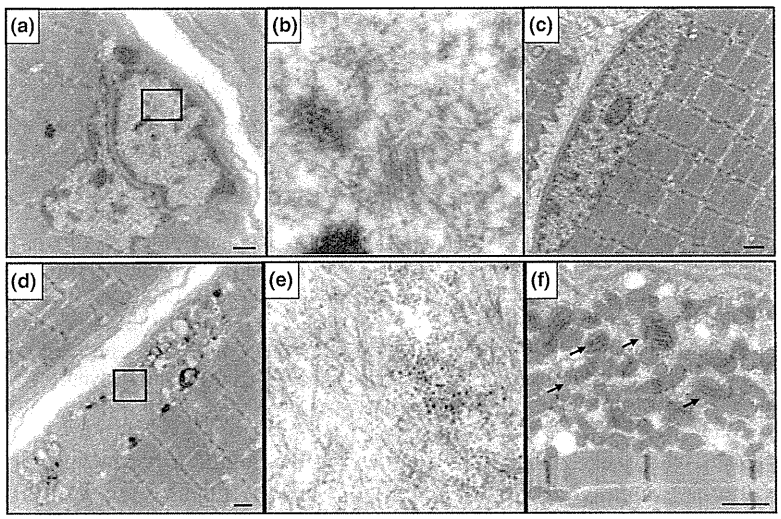

Figure 5 Ultrastructural analysis of muscle. (a) Myonuclei contains irregular heterochromatin with inclusion (square) in a well-preserved muscle fiber from Patient 2. (b) Magnified image of the region covered by the square in panel (a). A filamentous nuclear inclusion is seen. (c) A nucleus with well-preserved myofibrils in normal muscle. (d) A subsarcolemmal cytoplasmic inclusion containing filamentous structure. (e) Magnified image of the region covered by the square in panel (d). (f) A subsarcolemmal accumulation of enlarged mitochondria with paracrystalline inclusions (arrows) is seen in Patient 2. Bar = 1 μm.

Korean IBMPFD family is the only one to have been reported among Asian people [5]. Here, we show that VCP-related myopathy is not rare in an East Asian sample. Among 152 families with rimmed vacuolar myopathy, six families (4%) carried a heterozygous missense mutation including a novel p.Ala439Pro in exon 11. From the previous results that 39–64% of patients with *VCP* mutations have no rimmed vacuoles in their muscle biopsy [6,7], the incidence of VCP-opathy could be greater in myopathy patients. VCP is a member of ATPase associated with a variety of activities (AAA+) protein family and the alanine residue at position 439, located in the D1 ATPase domain, and is highly conserved among species. Furthermore, the p.Ala439Ser mutation was previously identified in a patient with IBMPFD [6].

In previous reports, more than half of the patients with *VCP* mutations have been reported to have PDB

[2]. Interestingly, only one of seven patients in our series showed a bone sclerotic region that was suggestive of PDB. PDB is reported to be rare in Asian populations and its frequency in Japan is 2.8 per 1 000 000 individuals, an incidence that is nearly 10 000 times less than that observed in Western countries [8]. The rare involvement of bone disease in Asian patients with *VCP* mutations might be related to ethnicity.

Frontotemporal dementia is another characteristic clinical symptom associated with *VCP* mutations and is observed in one-third of patients [2]. In our series, including elder affected relatives, mild mental disorder was noticed in only two patients. The cerebellar signs observed in Patient 2 are of note. Actually, the deceased elder brother of this patient had spinocerebellar degeneration. Although no patients with ataxia have been reported previously, we could not exclude the possibility of cerebellar involvement in this multisystem disorder.

Most of our patients and their symptomatic family members show isolated muscle involvement. Distribution of the affected muscles was variable, representing limb-girdle type, distal dominant, or scapuloperoneal type. Two patients showed asymmetrical involvement at the onset of the disease, which was also previously described in some IBMPFD patients [2,7,9]. Early involvement of the tibialis anterior muscles accompanied by rimmed vacuoles is indistinguishable from patients with DMRV/hIBM caused by *GNE* mutations [10]. Frequent involvement of the quadriceps femoris observed in patients with *VCP* mutations is important and helpful for differential diagnosis, because DMRV/hIBM is known as a quadriceps-sparing myopathy [11]. A combination of myogenic and neurogenic changes is an important and characteristic finding of VCP-related myopathy. Muscle cramps, pain, and fasciculation were often seen in our patients, which are also common findings in patients with motor neuron disease [12]. Pathological findings of grouped atrophy and fiber type grouping strongly suggest involvement of motor neurons and peripheral nerves. Electrophysiological results can support these findings. Like previous reports [6,8], the initial diagnosis of some affected family members in our series was motor neuron disease. The presence of these different diagnoses in the same family may be one of the characteristics of VCP-opathy.

Valosin-containing protein is involved in protein degradation by both the ubiquitin-proteasome system and the autophagic degradation system [13]. VCP is also reported to be involved in the maturation process during autophagosome formation [14]. Rimmed vacuoles, a common pathological change of VCP-related myopathy, are accumulations of membranous structures originating from autophagic vacuoles. Altered degradation of ubiquitinated proteins and autophagosome maturation may be closely associated with rimmed vacuolar formation. Consistent with this, ubiquitinated cytoplasmic and nuclear aggregations are another pathological hallmark of VCP-related myopathy. In this study, we demonstrate cytoplasmic and nuclear accumulations of ubiquitin, TDP-43, VCP, and also HDAC6. Accumulation of TDP-43 in the ubiquitinated inclusions is a characteristic pathological finding in brain and muscle from patients with *VCP* mutations as well as other neurodegenerative disorders including frontotemporal lobar degeneration with ubiquitin-positive inclusions and ALS without *VCP* mutations [15–17]. HDAC6, a cytoplasmic deacetylase, can transport ubiquitinated aggregates to the aggresome, the function of which is regulated by VCP [18]. HDAC6 is also known to involve maturation of autophagosomes [19]. Mutant VCP may influence the function of HDAC6, resulting in an accumulation of ubiquitinated proteins and insufficient protein degradation by autophagy.

Observations of electron microscopic images showed many abnormal nuclei, with or without filamentous inclusions that were seen in those muscle fibers with well-organized myofibril structures. This result suggests early nuclear damage as a key event of myopathy associated with *VCP* mutations. VCP is known to be involved in the maintenance and assembly of the nuclear envelope [20,21] and has been reported to have antiapoptotic effects [22]. Although the number of TUNEL-positive myonuclei in our samples was relatively small, mutant VCP can cause nuclear disorganization and dysfunction in skeletal muscle. Deficiency of nuclear localization of TDP-43 may also be closely associated with nuclear damage [23].

Prominent changes in mitochondria, including their localization, shape, deficiency in COX activity, and the presence of paracrystalline inclusions, strongly suggest mitochondrial dysfunction in VCP-related myopathy. Consistent with this, mutant VCP/cdc48 was reported to cause mitochondrial enlargement and dysfunction in yeast [24]. VCP has an important role in ubiquitin-dependent mitochondrial protein degradation, together with VCP/cdc48-associated mitochondrial stress-responsive 1 (Vms1) and Npl4 [25]. Further, abnormal cytoplasmic aggregations of TDP-43 are also known to cause mitochondrial damage and cell death [26,27]. Dysfunction of mitochondria in skeletal muscle could account for the muscle wasting observed in these patients.

Our study revealed clinical variability among Asian patients with *VCP* mutations. The rimmed vacuoles and ubiquitinated cytoplasmic aggregations, mixed myopathic and neuropathic changes, nuclear inclusions stained with VCP and HDAC6, and early nuclear and

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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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

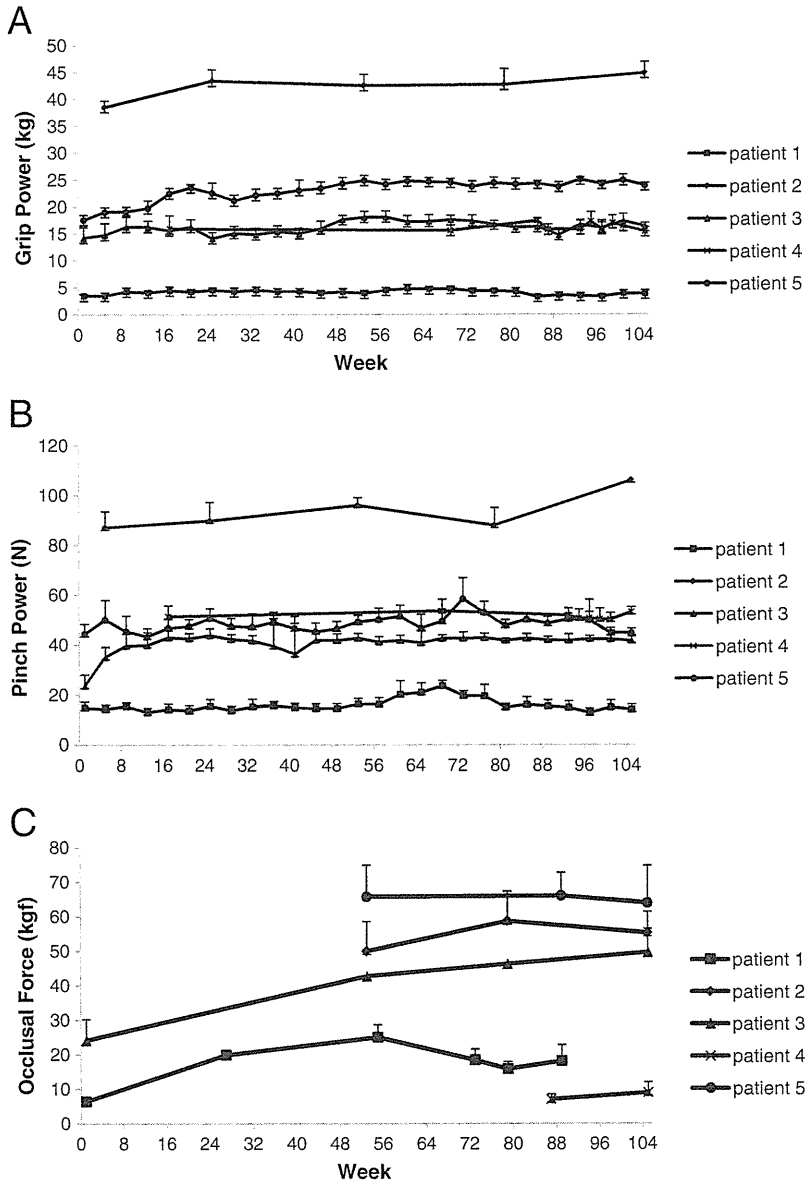


Fig. 1 Effects of ERT on grip power (**a**), pinch power (**b**), and occlusal force (**c**). Each data point represents the average of three bilateral measurements. ERT improved all of these parameters in four of five patients (with the exception of patient 4). Data are presented as mean ± SEM

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

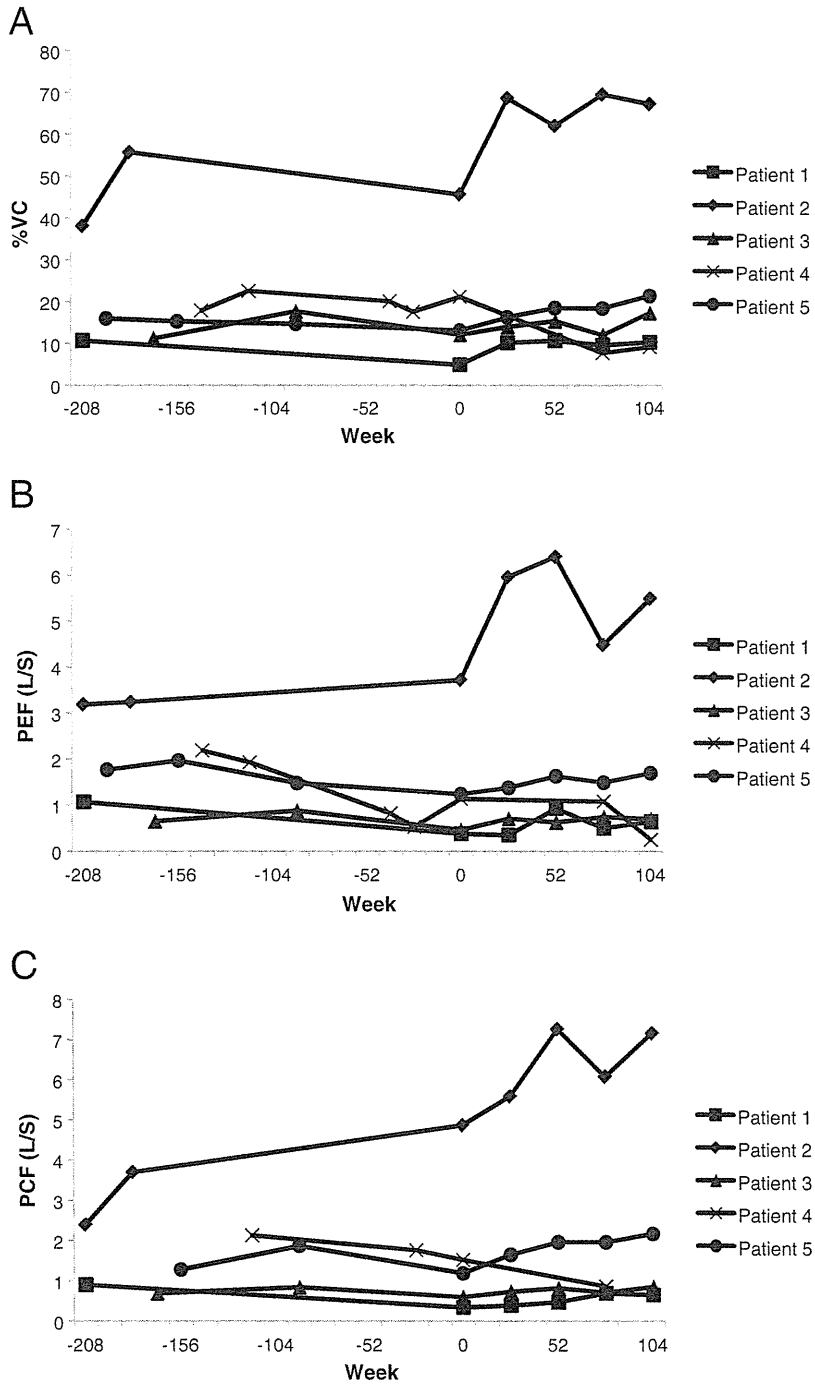
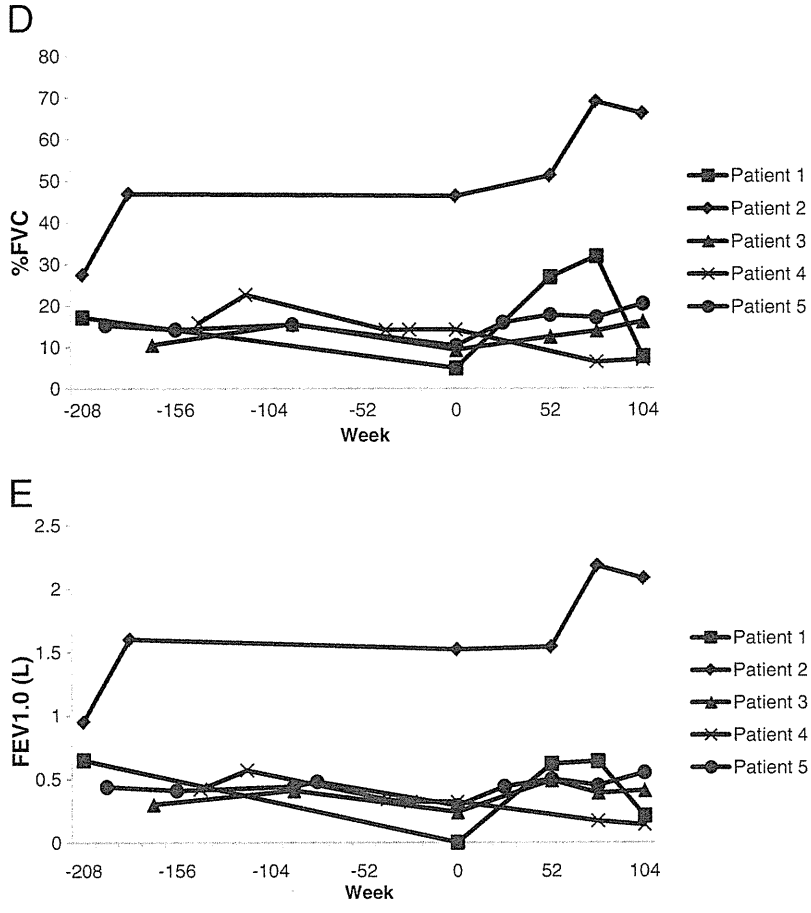


Fig. 2a–d Effects of ERT on respiratory function. Percent vital capacity (**a**), peak expiratory flow (**b**), peak cough flow (**c**), percent force vital capacity (**d**), and forced expiratory volume in the first second (**e**). Note the low values of all parameters prior to ERT and their improvement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