Table 3
Frequency of *HOXD* gene polymorphisms between SDG and NSDG patients with or without autism.

| Gene | dbSNP ID | Genotype | Autistic patients | | | Normal | Non-autistic | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | SDG | NSDG | Control | SDG | NSDG |
| *HOXD11* | | GG | 94 | 8 | 17 | 89 | 8 | 8 |
| | | GT | 4 | 3 | 0 | 0 | 0 | 0 |
| *HOXD12* | | CC | 90 | 8 | 17 | 84 | 8 | 8 |
| | | AC | 8 | 3 | 0 | 5 | 0 | 0 |
| *HOXD12* | rs847151 | GG | 90 | 8 | 17 | 84 | 8 | 8 |
| | | AG | 8 | 3 | 0 | 5 | 0 | 0 |

heterozygous for *HOXD11* G-112T were observed among the 16 non-autistic disease controls including the eight patients with SDG.

## 4. Discussion

In genetic research for autism, some studies have been conducted that focused mainly on language development skills (e.g., age at first word, age at first phrase, onset of first phrase >36 months, and nonverbal communication) skill. Other studies have focused on the establishment of motor language development, bladder and bowel control milestones, developmental regression, repetitive/stereotyped behavior, restricted behavior, interest, and activity [2–4,11–13].

Manning et al. [6] reported that 2D/4D is low in autism and Asperger syndrome. In Japan, Osawa et al. [7] reported a higher incidence of low 2D/4D in autism patients than in healthy children. From their report, we assumed that it is possible to consider a low 2D/4D as a specific feature in some autism patients. Such patients formed part of a group of subjects (SDG) for investigation in our study. It was assumed that SDG in autism may express one of the common features; hence, 2D/4D may be associated with one of the etiological genes of autism. Manning et al. [14] reported the findings of their 2D/4D measurement as follows: (1) there is a gender difference in 2D/4D measurements (2D/4D is lower in males than in females); (2) a low 2D/4D is observed across races and countries; (3) 2D/4D is closely related to fetal growth, sperm count, family size, myocardial infarction, and breast cancer; and (4) 2D/4D is related to sexual differentiation, the production of sex hormones in the fetal stage, and disease programming in the fetal stage. In addition, there is an inverse correlation between 2D/4D and testosterone concentration at the fetal stage, and 2D/4D correlates with the CAG repeat number in the androgen receptor gene [15].

A study of female twins conducted by Paul et al. [16] showed that the concordance rate of 2D/4D is higher in monozygotic twins than in dizygotic twins, that the heritability of 2D/4D is approximately 66%, and that the genetic contribution to 2D/4D in females may be more influential than the effects of prenatal environmental factors. Although it is uncertain whether these findings differ significantly between males and females in the absence of any report for males, it seems possible that 2D/4D is affected by both hereditary and secondary perinatal environmental factors.

One study showed that the mean 2D/4D did not change with gestational age from the 9th week to the 40th week [17]. In addition, there was a small increase in 2D/4D with age, which was lowest in the right hand [18]. This study indicates that 2D/4D is probably established in the uterus and that this ratio remains almost constant until adult life.

Because 2D/4D, an easily measurable physical feature, is already determined in utero and remains constant until adult life, it can be used regardless of age differences among subjects and is universal; moreover, its measurement is noninvasive. Therefore, 2D/4D is an excellent parameter for evaluating a group of autistic patients.

In genomic scans of families having more than one member with autism, the susceptibility loci for autism were investigated, and identified; these included 2q21–q33 [3,4]. In the candidate genes located here, the NRP2 gene is reported as one of the genes related to autism [19]. In addition, specific polymorphism has been found in distal-less 2 (DLX2) and cAMP guanine nucleotide exchange factor II (cAMP-GEFII) in a few cases of autism [20]. On the other hand, no significant correlation has been reported between autism and distal-less 1 (DLX1) [20,21] and DLX2 [20]. With regard to *HOXD* genes, Bacchlli et al. reported that there is no relationship between *HOXD1* and autism [20]. There has been no report on *HOXD11*, *HOXD12* or *HOXD13* to date.

It seems that these genes may be found to be significant in the development of autism when cases as a study subject have been carefully chosen and classified by the specific characteristics of presenting behavior or phenotypic clinical presentations.

The present study has limitations because it is a case-control study, rather than a family study, with a small number of subjects enrolled. However, in this study,

*HOXD11* SNP -112G/-112T heterozygosity was specifically observed in autism patients with low 2D/4D. On the basis of this result, we expect that the relationships between autism and the *HOXD* genes or other candidate genes located in 2q will be clarified by studying a larger population with low 2D/4D, that is, by studying patients heterozygous for -112G/-112T in the *HOXD11* promoter.

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of p62 positive aggregates correlate pretty well with myofiber atrophy. In general the degradation systems appear to be still functioning in these patients and seem to contribute positively to counteract disease progression. In conclusion, present data underline the role of unproductive autophagy and accumulation of aggregate-prone ubiquitinated proteins in the pathogenesis of GSDII, especially in more severely affected patients.

## SM202. An exploratory analysis of scoliosis in 182 children and adults with Pompe disease from the Pompe Registry

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The prevalence of scoliosis and its relationship with respiratory function are explored in patients enrolled in the Pompe Registry. Scoliosis status was reported for 575 patients, 182 of whom had scoliosis (25 children age 0 to < 2 years or ≥ 2 to < 13 years; 24 teenagers age ≥ 13 to < 20 years, and 133 adults age ≥ 20 years).

Children age ≥ 2 years with scoliosis had a mean age at Pompe symptom onset of 1.1 years, identical to children without scoliosis. Teenagers with scoliosis had a mean age at symptom onset of 5.8 years compared with 9.1 years in teenagers without scoliosis. Adults with scoliosis had mean age at symptom onset of 25.3 years compared with 32.8 years in adults without scoliosis.

Among the subset of patients with FVC data, children age ≥ 2 years (n = 6) and teenagers (n = 9) with scoliosis had lower % predicted forced vital capacity (FVC) upright median scores (68.0% and 59.0%, respectively) than those in similar age groups without scoliosis (15 children, 5 teenagers; 77.0% and 91.1%, respectively). Children age ≥ 2 years with scoliosis (n = 3) had lower median % predicted FVC supine scores than those in similar age groups without scoliosis (n = 5) (47.0% versus 70.0%, respectively). Supine scores for teenagers without scoliosis were unavailable. Among adults, FVC % predicted upright and supine median scores were similar regardless of scoliosis status.

Further analysis and collection of detailed scoliosis and respiratory function data is needed to better understand this relationship and how scoliosis affects quality of life in patients with Pompe disease.

## SM203. Quantitative metabolome profiling of biopsied muscle in the patients with glycogen storage diseases using capillary electrophoresis mass spectrometry

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Metabolome analysis has lately been applied for the characterization of disease-specific metabolism. Recently developed capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) has enabled quantitative analysis of charged metabolites by the simultaneous measurement of their levels in tissues. In order to characterize the metabolism of muscular glycogen storage diseases (M-GSD), and also to evaluate whether CE-TOFMS could be a valuable diagnostic tool for M-GSD, we applied CE-TOFMS to measure the metabolites involved in energy production in the muscles of M-GSD. Biopsied muscles were obtained from each patient with GSDIIa, IIb, III, V, VII, and phosphoglycerate kinase (PGK) deficiency. Histologically normal muscles from three myopathy patients with normal CK values were used as controls. We identified 10 metabolites involved in glycolysis, 8 in TCA cycle, and 4 in pentose phosphatase pathway. The amounts of glycolytic intermediates locating downstream of G-1-P in the glycolytic pathway were much less in muscles of GSD III and V than in control muscles, while the amounts of glycolytic intermediates locating upstream of FDP (G-6-P, G-1-P and F-6-P) and those locating upstream of 3-phosphoglycerate were significantly high in muscles of GSD VII and in PGK deficiency, respectively. There was no difference in the amounts of glycolytic intermediates between GSD II and controls. The amounts of the metabolites in TCA cycle were higher in muscles of GSD II than in controls. The metabolome analysis of biopsied muscles had clearly determined the blockage of the metabolic pathway. We conclude that this method could be a high through-put and good method for diagnosis in M-GSD.

## SM204. Adult Pompe disease: bone mineral density before and after enzyme replacement therapy

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Pompe disease is an autosomal recessive disorder caused by lysosomal α-glycosidase deficiency. The infantile form is characterized by cardiomegaly and severe muscle weakness with an early fatal outcome, while the adult form is usually milder with progressive muscle weakness and respiratory dysfunction. Bone mineral density (BMD) seems to be decreased in the infantile form leading to osteopenia and fractures, but data concerning the adult form of the disease are still limited. The aim of the present study is to evaluate BMD in patients with the adult form of Pompe disease before and after enzyme replacement therapy (ERT).

Body composition was examined by means of dual x-ray absorptiometry at baseline and after 9-12 months of ERT in five patients with the adult onset form of Pompe disease.

One patient had reduced BMD in total body, L2-L4 spine and femoral neck in the range of osteopenia, one other had reduced L2-L4 spine BMD and two patients had slightly reduced femoral neck BMD. After 9-12 months of ERT, BMD was not considerably altered in any patient.

A slight reduction of BMD among patients with the adult form of Pompe disease might be occasionally found. The short-

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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

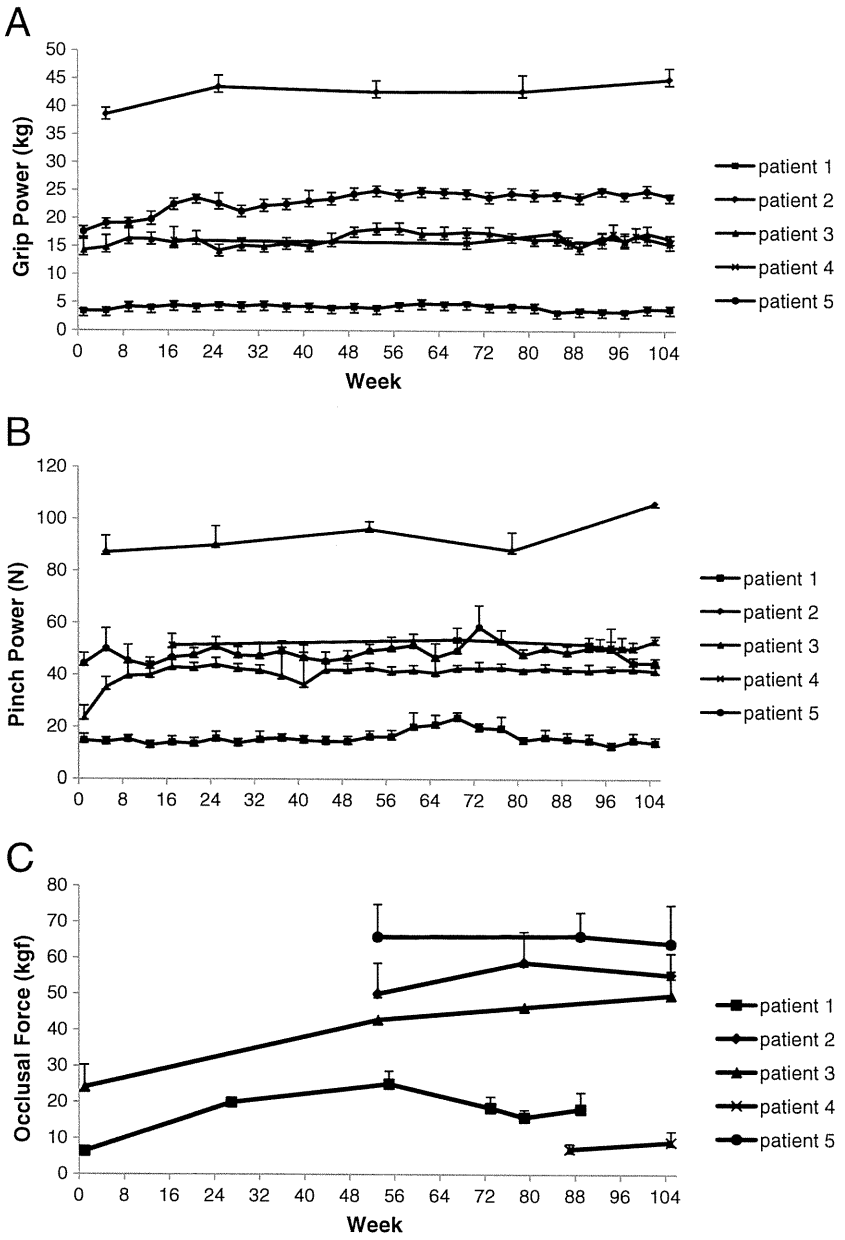



**Fig. 1** Effects of ERT on grip power (**a**), pinch power (**b**), and occlusal force (**c**). Each data point represents the average of three bilateral measurements. ERT improved all of these parameters in four of five patients (with the exception of patient 4). Data are presented as mean ± SEM

## 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

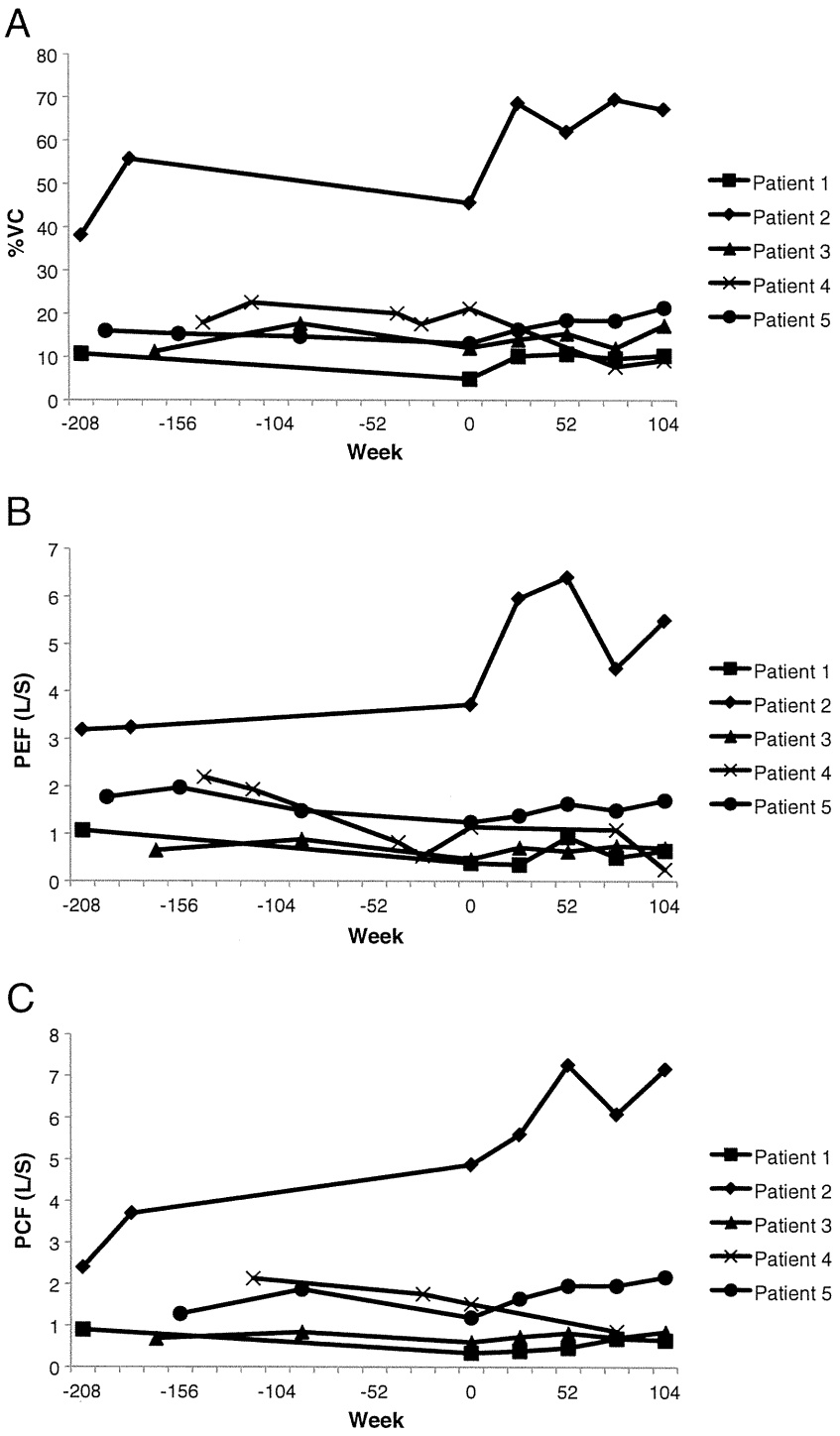
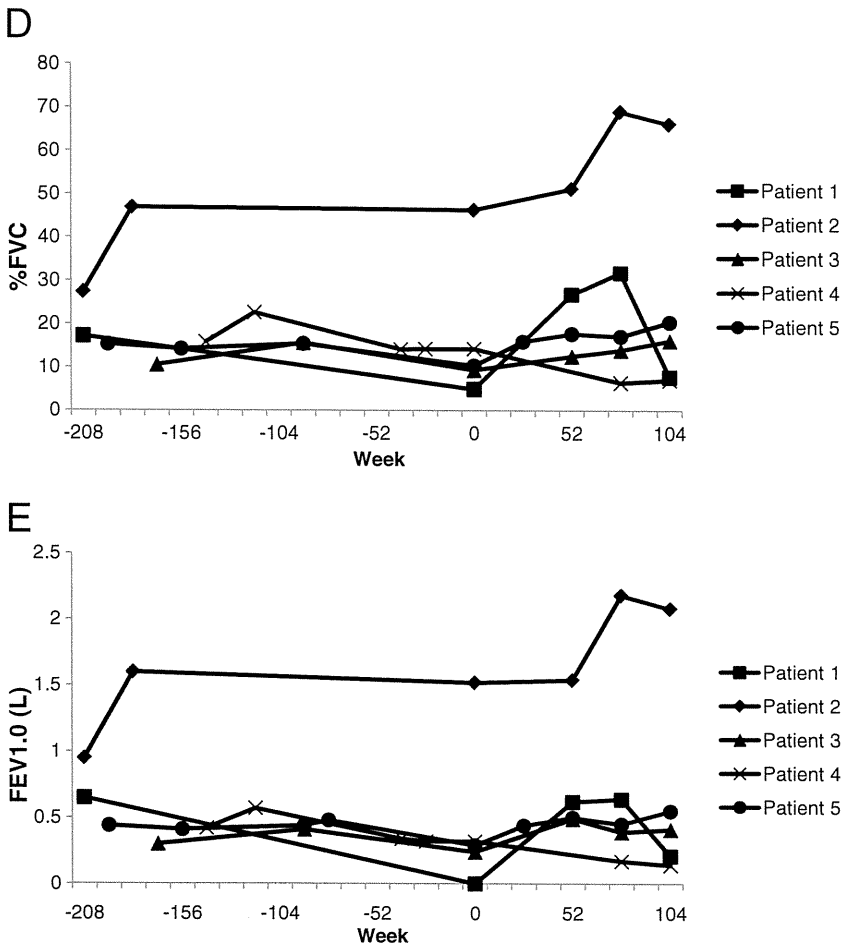



**Fig. 2a–d** Effects of ERT on respiratory function. Percent vital capacity (**a**), peak expiratory flow (**b**), peak cough flow (**c**), percent force vital capacity (**d**), and forced expiratory volume in the first second (**e**). Note the low values of all parameters prior to ERT and their improvement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

# A case of ADEM with atypical MRI findings of a centrally-located long spinal cord lesion

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**Abstract**

The patient was a 14-year-old male diagnosed with acute disseminated encephalomyelitis (ADEM) with acute onset of multifocal central nervous system symptoms. He showed increased cerebrospinal fluid cell counts and high myelin basic protein levels, which responded well to steroid pulse therapy. Spinal MRI showed a centrally-located long spinal cord lesion (LCL) involving 17 vertebral bodies from C2 to T11 that later expanded into the white matter, and lesions on the ventral side of the medulla. The cause of LCL has been reported to be heterogeneous. In this case, LCL is considered to be associated with ADEM, an acute autoimmune response to myelin, and vascular inflammation of the gray matter of the spinal cord.


*Keywords:* ADEM; Centrally located long spinal cord lesion (LCL); NMO

## 1. Introduction

A centrally-located long spinal cord lesion (LCL) is defined as a lesion that involves more than three vertebral bodies, located in the central area of the spinal cord on MRI images. LCL has attracted attention since being reported as a characteristic MRI finding in cases of neuromyelitis optica (NMO) [1–3]. The underlying diseases associated with it vary widely, however, and include not only NMO but also infections, tumors, vascular diseases, and autoimmune diseases. LCL without white matter lesions in patients with acute disseminated encephalomyelitis (ADEM) has never been reported. Lesions are typically asymmetric and variable in number and size in ADEM [4]. We report a pediatric case of a male with an ADEM who showed LCL and bilateral lesions on the ventral side of the medulla after an infection.

## 2. Case report (Fig. 1)

The patient was a 14-year-old male. A few days after an upper respiratory infection, he developed acute lower back pain, weakness and numbness of both legs, and a feeling of residual urine, resulting in difficulty in walking and urinary retention over 7 days. At 7 days from onset, his height was 168 cm (+1.0 SD), and weight was 50.0 kg (−0.2 SD). Vital signs were normal. He was fully conscious. He presented no nuchal rigidity. On neurological examination, cranial nerves were intact. A manual muscle test (MMT) of the four limbs revealed grade 5/5. Grasping power had bilaterally decreased (25/25 kg) compared with 3 months earlier (32/32 kg). Deep tendon reflexes were normal in the upper and lower limbs. Cerebellar sign was not observed, but

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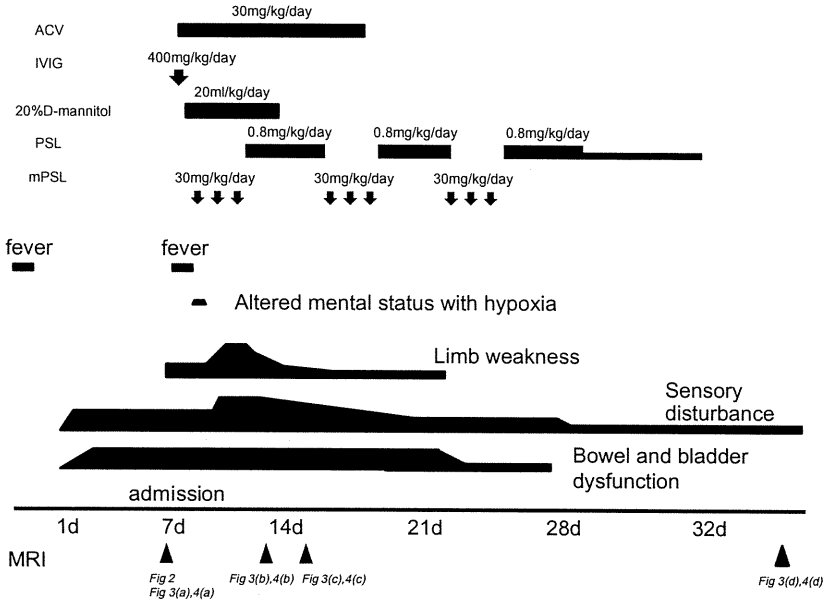


Fig. 1. Clinical course of the patient.

tactile sensation and proprioception were reduced in the lower bilateral extremities under the T10 level. He presented urinary retention and bowel dysfunction, and the cremasteric reflex was not detected.

Laboratory results were as follows: white blood cell count, 16,200/μl; C-reactive protein, 0.24 mg/dl; erythrocyte sedimentation rate, 27 mm/h. Anti-double-stranded DNA IgG antibody was 4.7 IU/ml (<10 IU/ml). Serum anti-aquaporin 4 (AQP4) antibody was negative. In the cerebrospinal fluid (CSF), cell count was 109/mm$^3$ (polycyte 6 mm$^3$, monocyte 103 mm$^3$), protein was elevated to 130 mg/dl, glucose was 66 mg/dl (serum glucose was 138 mg/dl), myelin basic protein (MBP) was over 2000 pg/ml (>102 pg/ml), and oligoclonal bands were negative, as were IgG index and AQP4. CSF culture was negative. Serum anti-*Mycoplasma* antibody and viral antibodies to *Human immunodeficiency virus*, *polio*, and *Varicella zoster virus* were all negative. *Herpes simplex virus* (HSV)-DNA was negative in the CSF by PCR, and *Epstein–Barr virus* was identified as having been a past infection. T2-weighted MRI (Fig. 2) showed high signal intensity in the central gray matter from C2 to T11 and lesions on the ventral side of the medulla and the pons that were not continuous with the spinal cord.

Intravenous acyclovir (ACV) injection (30 mg/kg/day for 7 days) and γ globulin (IVIG) administration (400 mg/kg/day for 2 days) were performed. Despite

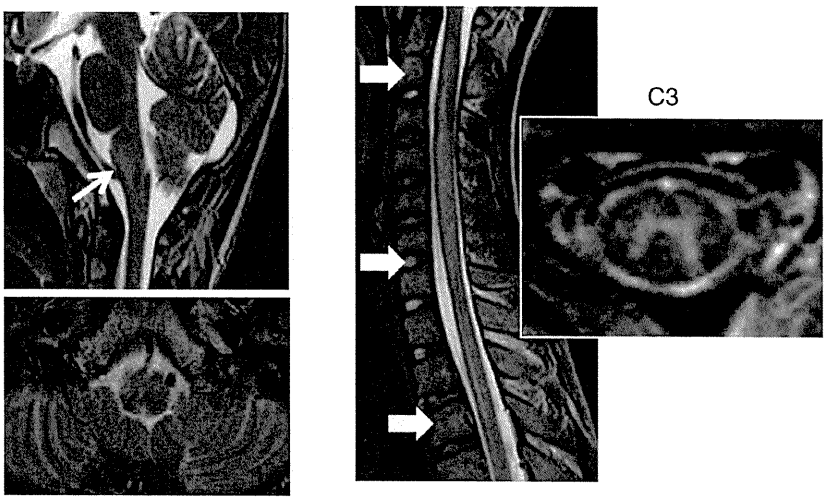


Fig. 2. T2-weighted MRI at 7 days from onset: high spinal lesion from C2 to T11, centering on the gray matter and lesions, not continuous with the spinal cord on the ventral side of the medulla.

these treatments, at 8 days from onset, he developed somnolence with hypoxia ($SpO_2$ was 88–90 in room-air), MMT of the bilateral lower limbs declined to 4/4, and tactile sensation and proprioception loss progressed from under T10 to under T5. At 9 days after onset, a treatment with 20% D-mannitol injection (20 ml/kg/day) and three courses of steroid pulse therapy (intravenous 30 mg/kg/day of methylprednisolone (mPSL) for 3 days) was started. Two days after the treatment, mental status was intact and, sensation and muscle strength were improved, although the T2 intensity of the lesion at the C2–T11 level was increased compared with the initial findings on MRI (Fig. 3a), and the lesion had expanded into the white matter (Fig. 3b). The patient could walk at 4 days after steroid treatment was begun, and the bladder and rectal dysfunction disappeared at 28 days after treatment. Bilateral numbness of the lower limbs at the L4 level persisted until 4 months after onset. During the entire course, neither relapse nor aggravation was observed. The level of T2-weighted high intensity in the spinal cord and brainstem lesions began decreasing at 15 days (Figs. 3c and 4c) and had disappeared at 41 days (Figs. 3d and 4d).

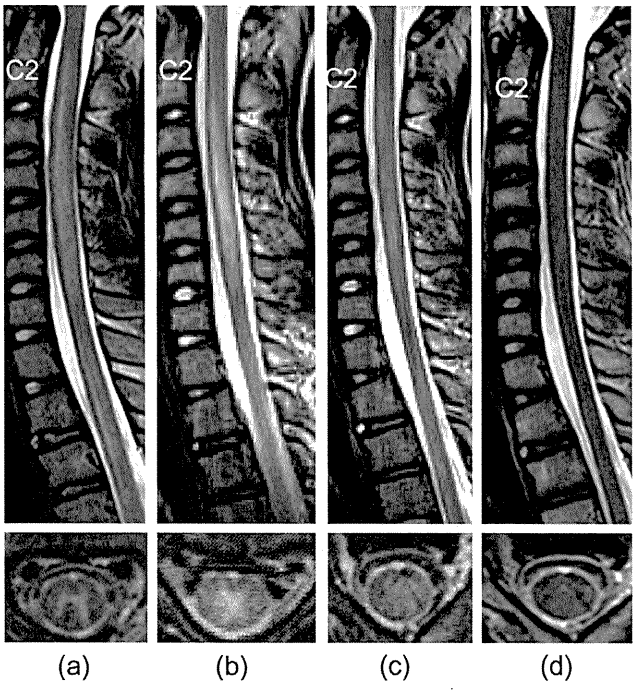


Fig. 3. T2-weighted spinal cord lesions. (a) 7 days, (b) 12 days, (c) 15 days, (d) 41 days, after neurological symptoms developed.

## 3. Discussion

In 2007, the International Pediatric Multiple Sclerosis (MS) Study Group proposed that ADEM was defined as an initial clinical event with a presumed inflammatory and demyelinating cause, with acute or subacute onset affecting multifocal areas of the central nervous system [5]. Further, its onset is associated with various symptoms and signs of multiple neurological deficits and disturbance of consciousness. The patient presented altered mental status, although the lesion contributing to it was not seen on MRI, the bilateral lesions on the ventral side of the medulla and a spinal cord lesion on MRI, increased CSF cells, and a high MBP level. These results confirmed the diagnosis of ADEM.

Differential diagnosis includes clinical isolated syndrome (CIS) and NMO. CIS can be excluded since CIS has been considered to be not associated with altered mental status [5]. The NMO feature resembled the spinal cord MRI lesion in this patient, but NMO is characterized by medullary lesions involving the pericanal region, area postrema, and nucleus tractus solitaries [3]. In addition, we considered that the diagnosis of NMO can be excluded because the serum anti-AQP4 antibody was negative, and clinically isolated findings of optic neuritis were not detected in this case. However visual evoked potentials should be conducted to detect

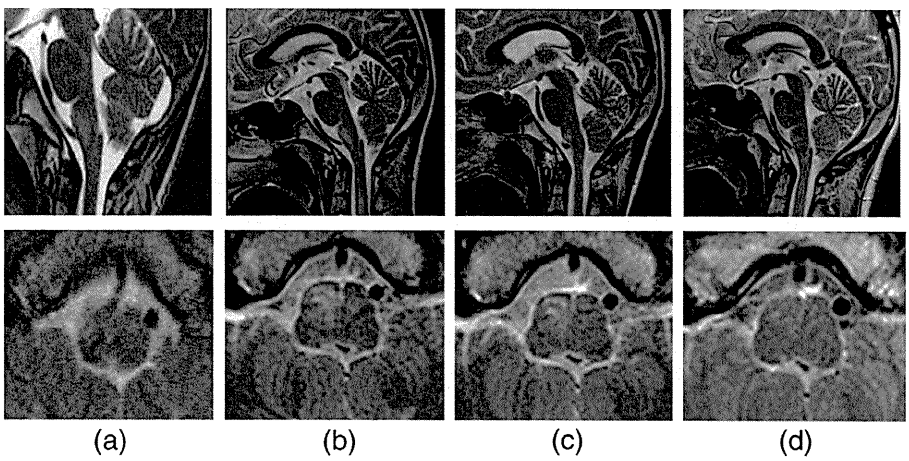


Fig. 4. T2-weighted brain stem lesions. (a) 7 days, (b) 12 days, (c) 15 days, (d) 41 days, after neurological symptoms developed.

clinically silent lesions of the visual pathway to confirm the diagnosis.

ADEM with LCL has not been reported except for one Japanese patient. The majority of ADEM cases [6] have shown asymmetrically-patchy lesions involving the gray matter and the white matter simultaneously. Reported ADEM case [7] with LCL showed a single lesion, which might have a differential diagnosis of clinically isolated syndrome.

The mechanism leading to the formation of a limited gray matter lesion is unclear. A high serum MBP level was observed from an early stage, suggesting myelin sheath damage in the gray matter in the formation of the LCL or a white matter lesion undetectable on MRI. The distinguishing histopathologic feature of ADEM is demyelination with perivascular, particularly perivenous, inflammation in CNS lesions [8], which is thought to cause vasogenic edema and brain and spinal cord swelling. It is significant that the inflammatory process involves both the white and gray matter [9]. In this case, mPSL elicited a rapid response to some neurological symptoms and resolved spinal cord swelling, in which the steroid is considered to play a role in reducing vasogenic edema in addition to reducing the inflammation.

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Case report

# Another promising treatment option for neuroblastoma-associated opsoclonus–myoclonus syndrome by oral high-dose dexamethasone pulse: Lymphocyte markers as disease activity

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**Abstract**

A one-year-old boy with neuroblastoma (NBoma)-associated opsoclonus–myoclonus syndrome (OMS) was treated by oral high-dose dexamethasone (DEX) pulses (20 mg/m$^2$/day of DEX for three consecutive days) every 28 days for 6 months after resection of the tumor. All OMS symptoms improved after the first course of DEX pulse therapy and disappeared after the last course. No adverse effects were observed. Minor deterioration of his developmental quotient was noted 33 months after the onset of the disease. NBoma remission has been maintained since treatment. Before DEX pulse therapy, frequency of T lymphocyte, in particular CD4-positive cell decreased markedly resulted in low CD4/8 ratio in the peripheral blood (PB). The frequency of B lymphocyte increased, especially in cerebrospinal fluid. These aberrant values in PB were reversed by DEX pulse therapy and correlated well with the neurological symptoms. A prospective study that assesses the efficacy of this promising and inexpensive treatment for OMS is warranted.


*Keywords:* Opsoclonus–myoclonus syndrome; Neuroblastoma; Dexamethasone; CD4/CD8 ratio

## 1. Introduction

The paraneoplastic neurologic opsoclonus–myoclonus syndrome (OMS) is characterized by ataxia, myoclonus and opsoclonus (multidirectional, chaotic eye movement) [1]. It occurs mainly in 1- to 2-year-old infants and most (50–80%) are associated with neuroblastoma (NBoma) [1]. It may be induced by autoantibodies that recognize both tumor and neural cells [2], which may be associated with derangement of lymphocyte subsets [3,4]. Most OMS-associated NBomas are stage I or II, and their oncological prognosis is excellent [2]. However, their neurological prognosis is unfavorable: 60–100% later have impaired intellectual or motor functions [2,5]. Infections often exacerbate OMS symptoms [1]. Immunosuppressive therapies including adrenocorticotropic hormone, corticosteroids, intravenous immunoglobulin, and rituximab has been used for OMS [1,6]. While these treatments can generate good initial responses, most of patients with OMS take relapsing-remitting courses and their long-term effects are generally unsatisfactory, with the exception of rituximab.

Recently, high-dose dexamethasone (DEX) pulse therapy was reported to treat NBoma-associated OMS [7,8]. We describe here a patient with NBoma-associated OMS who was treated by oral high-dose DEX pulse

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therapy; this patient also showed no serious long-term neurological sequelae. The aberrant lymphocyte subsets correlated well with the neurological symptoms.

## 2. Case report

A previously healthy 11-month-old boy with normal developmental milestone achievements presented with ataxia and abnormal eye movement. Episodes of upward eye deviation lasting about one second started 3 weeks before admission; thereafter, myoclonic movement of the extremities and trunk ataxia appeared. These symptoms worsened gradually.

On admission, he was awake and alert but irritable. He exhibited rapid and random eye movement and myoclonus of the extremities characterized by irregular flickering muscle contractions; these contractions occurred spontaneously and were easily provoked by rapid passive body movement. He showed trunk ataxia and sat without support with difficulty. His muscle tonus was hypotonic and his deep tendon reflex was normal. MRI brain scans found no abnormalities. His urinary vanillylmandelic acid and homovanillic acid levels were elevated (16.6 μg/mg Cr [normal range: 1.2–4.9] and 26.3 μg/mg Cr [normal range: 1.6–5.5], respectively). Abdominal computed tomographic scanning revealed a 3-cm diameter mass in the paraaortic area on the left renal hilus (Fig. 1). Iodine-123-metaiodobenzylguanidine scintigraphy showed this region accumulated isotopes. Immunophenotype analyses revealed total lymphocyte count of 10,600/μl with high B-cell frequency (25.3% [normal range: 9.5–18.9]), low T-cell frequency (38.8% [normal range: 65.0–81.4]), and a normal CD4/CD8 ratio (1.74 [normal range: 1.0–2.2]) in the peripheral blood (PB), and total cell count of 3/μl with high B-cell frequency (9.1% [normal range: 0.4–1.3]) in the cerebrospinal fluid (CSF). The CSF/PB B-cell ratio was also high (0.36 [normal range: 0.02–0.06]).

Stage I NBoma-associated OMS was diagnosed. The total neurological score (calculated by the assessment scale of Sugie et al. [9]), was six before OMS onset and 19 at diagnosis (Table 1).

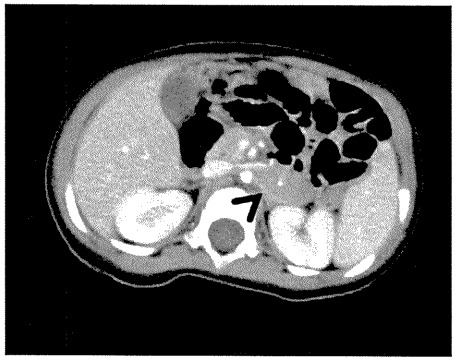

Fig. 1. Abdominal computed tomographic scan of the patients. A mass that was 3 cm in diameter was observed in the paraaortic area on the left renal hilus (arrowhead).

Four weeks after OMS onset, the patient received 2 mg/kg/day PSL orally. One week later, the opsoclonus and ataxia were partially improved, he could stand with support, and the total neurological score improved to 11 (Table 1). The PSL was tapered and the tumor was removed surgically. Pathology confirmed MYCN non-amplified NBoma. No chemotherapy was provided. After tumor resection, the myoclonus disappeared, and the total neurological score improved to six. However, 7 weeks later, an episode of upper respiratory tract infection aggravated all OMS symptoms, and the PB CD4-positive cell (19.4%) frequency dropped further resulted in reductions of T cell frequency (31.0%) and CD4/CD8 ratio (0.92).

Oral DEX pulse therapy (three consecutive days of 20 mg/m$^2$/day DEX every 28 days) was commenced and continued for 6 months. Three weeks after the first course, all OMS symptoms improved, and the patient could walk with support. After four courses, the myoclonus disappeared almost completely, he could stand without support, walk a few steps alone. After completing six courses, all OMS symptoms had subsided, he could walk alone. No adverse effects of treatment were observed. Babbling started at 15 months (after the first course) and he could speak several words at two years of age. The PB T-cell and CD4-positive cell frequencies and CD4/CD8 ratios rose with every DEX pulse therapy cycle.

At 2 years and 8 months of age, on the occasion of recurrence of mild OMS symptoms followed by respiratory infection, the patient received three courses of DEX pulse therapy. At the age of 3 years and 8 months, his development was evaluated a using Kyoto Scale of Psychological Development (KSPD) which is a standardized developmental test for Japanese children [10]. His total developmental quotient (DQ) was 76, and the details showed postural-motor 83, cognitive-adaptive 79, and language-social 76. Although it cannot be defined as distinct development retardation, his DQ level was regarded as borderline development, especially showing minor decline in language development.

At present he shows no typical presentation suggesting autism or attention deficit hyperactivity disorder (AD/HD). NBoma remission has been maintained. PB lymphocyte markers returned to almost normal.

## 3. Discussion

Here, a patient with NBoma-associated OMS was treated by six courses of oral high-dose DEX pulse therapy that completely resolved all OMS symptoms and prevented long-term serious neurological impairments with no adverse treatment effects. In two previous reports on NBoma-associated OMS treated with the

Table 1
Effect of DEX pulse therapy on neurological score* and peripheral blood lymphocyte immunophenotype.

| Age<br>Status | 10 m<br>Before onset | 11 m<br>Before PSL | 11 m<br>1 week after PSL | 1 y 0 m<br>4 weeks after surgery | 1 y 2 m<br>Before DEX pulse | 1 y 3 m<br>After 1st course of DEX pulse | 1 y 5 m<br>After 4th course of DEX pulse | 2 y 0 m<br>4 months after 6th course of DEX pulse | 3 y 3 m<br>1.5 years after six courses of DEX pulse |
|---|---|---|---|---|---|---|---|---|---|
| *Neurological signs* | (0, none; 1, mild; 2, moderate; 3, severe) | | | | | | | | |
| Opsoclonus | 0 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| Ataxia | 0 | 3 | 2 | 1 | 2 | 1 | 1 | 0 | 0 |
| Myoclonus | 0 | 3 | 3 | 0 | 2 | 1 | 0 | 0 | 0 |
| Irritability | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Motor functions* | (0, possible; 1, possible with difficulties; 2, impossible) | | | | | | | | |
| Rolling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sitting | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Crawling | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Standing with support | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Walk with support | 2 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Standing without support | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| Walk alone | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 0 |
| Total (0–36) | 6 | 19 | 11 | 6 | 10 | 6 | 2 | 0 | 0 |
| *Immunophenotype* | (normal range) | | | | | | | | |
| CD20 (9.5–18.9%) | ND | 25.3 | ND | ND | 24.3 | 24.4 | 30.4 | 28.5 | 24.6 |
| CD3 (65.0–81.4%) | ND | 38.8 | ND | ND | 31.0 | 38.3 | 46.9 | 42.8 | 57.6 |
| CD4 (32.6–44.2%) | ND | 29.5 | ND | ND | 19.4 | 24.8 | 28.8 | 25.9 | 35.7 |
| CD8 (20.0–33.4%) | ND | 17.4 | ND | ND | 21.0 | 19.7 | 20.2 | 13.0 | 16.2 |
| CD4/CD8 (1.0–2.2) | ND | 1.74 | ND | ND | 0.92 | 1.26 | 1.43 | 1.99 | 2.20 |

* Calculated by the assessment scales of Sugie et al. [9]; ND: not determined.