

Familial adult-onset Pompe disease associated with unusual clinical and histological features

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The adult-onset form of Pompe disease had a wide clinical spectrum, ranging from asymptomatic patients with increased CK to muscle cramps and pain syndrome or rigid-spine syndrome. In addition clinical severity and disease progression are greatly variable. We report on a family with 3 siblings characterized by an unusual adult-onset Pompe disease including dysphagia and weakness of tongue, axial and limb-girdle muscles, in association with atypical globular inclusions in muscle fibres. Our study confirms the great clinical and histological variability of adult-onset Pompe disease and further supports the need of careful evaluation of bulbar function in patients affected by this pathology.

Key words: Pompe disease, globular inclusions; bulbar symptoms.

Glycogen storage disease type II (Pompe disease or acid maltase deficiency) is a rare autosomal recessive muscular disorder characterized by deficiency of acid- α glucosidase (GAA), determining accumulation of glycogen in the lysosomes, mainly in cardiac and skeletal muscle cells. Typical phenotypes of glycogenosis type II include the severe classic infantile form, characterized by severe muscle weakness and hypertrophic cardiomyopathy, almost invariably fatal by 12 months, a "non-classic" form presenting between 1 and 2 years of age and the late-onset form, presenting at any time after the age of 1 year, including juvenile and adult-onset subtypes, which are considered as part of a continuous clinical spectrum (1). In particular the adult-onset form presents with slowly progressive proximal lower limb and/or paraspinal muscle weakness, often followed by restrictive respiratory failure, which could be life-threatening, as it is in infants

and children (2). However the clinical spectrum of adult-onset form is wide, ranging from asymptomatic patients with increased CK to muscle cramps and pain syndrome or rigid-spine syndrome (2, 3). Furthermore clinical severity and disease progression is greatly variable.

We report on a family with 3 siblings with an unusual adult-onset Pompe disease clinically characterized by weakness of bulbar, axial and limb-girdle muscles in association with atypical histopathological changes.

Case report

Clinical features

Patients were siblings born from non-consanguineous parents.

Patient 1 is a 47 year-old male, who came to our attention because of difficulty in moving tongue and lips and swallowing, occurring since the age of 43. Furthermore he noticed mild limb muscle wasting and weakness during the disease course. Neurological examination at the age of 45 years showed tongue hypotrophy and weakness without fasciculations (Fig. 1), moderate orbicularis oculi and oris weakness, waddling gait with knee hyperextension and marked spine lordosis, mild neck flexor, moderate proximal upper and lower limb muscle weakness and mild thoracic scoliosis. Electromyography showed neurogenic changes in all examined limb muscles, myopathic at genioglossum and neuromyogenic at orbicularis oculi and oris. Sensory and motor nerve conduction studies were normal.



Figure 1. Tongue hypotrophy in patient 2 (A) and tongue weakness against moderate resistance by the examiner in patient 1 (B). Photographs are printed with permission of the patients.

Patient 2 is a 56-year-old woman, having CK mildly increased (range 564-634 U/L; normal value 24-195) since the age of 38, when it was assessed for the first time, and not further investigated. At the age of 48 she noticed lower limb weakness and at the age of 53 respiratory problems and mild dysphagia. On examination at first admission in our institute at the age of 54 years she displayed marked head flexors and thigh extensor muscle weakness with waddling gate, tongue hypotrophy and weakness (Fig. 1).

Patient 3 is a 53-year-old woman, complaining of dysphagia since the age of 41 and experiencing walking difficulty since the age of 46, followed by mild upper limbs weakness and respiratory difficulties. However she has never been able to run properly. Neurological examination at the age of 51 years revealed marked waddling gait and increased lumbar lordosis, moderate to marked head flexors, lower and upper limb girdle muscle weakness. Tongue was hypotrophic and weak.

Histological features

Muscle biopsies were performed after informed consent, on the quadriceps muscle in all three patients. In patient 1 the biopsy, taken at age 45, showed a few vacuolated fibres containing Periodic-Acid-Schiff (PAS) and acid phosphatase positive material that, on haematoxylin-eosin (H&E) preparations, appeared constituted of round or oval bluish globules (Fig. 3). Oxidative enzymes and ATPase staining were normal. Globular inclusions appeared blue with menadione-linked alpha-glycerophosphate dehydrogenase (menadione-ni-

troblue tetrazolium). Similar findings, although vacuoles were more numerous but smaller, were observed in patient 2 (Fig. 3). In patient 3, PAS and acid phosphatase-positive material was more abundant and almost totally substituted the cytoplasm of several fibres (Fig. 3). Muscle biopsies in patient 2 and 3 were performed at the age of 57 and 51, respectively.

Immunostaining of EEA1, LC3, LAMP2 showed that the inclusions were mainly positive for LAMP2 (marker of lysosomes), and variably positive for EEA1 and LC3 (markers of early and late autophagy, respectively); several autofluorescent lipofuscin bodies were also present. Desmin, dystrophin, dystrophin-associated proteins, laminin- α 2, caveolin 3 were normally expressed in all 3 patients.

Electron microscopy of muscle tissue showed diffuse intramyofibrillar increase of glycogen particles and, in some fibres, small membrane-limited round bodies containing densely packed glycogen particles (Fig. 4). Large areas occupied by autophagic, lysosomal and lipofuscin material intersperse with glycogen particles were also observed in a few fibres. Small globular bodies were more frequent in patient 2.

Biochemical analysis on muscle tissue of patient 1 and 2 revealed severely reduced GAA activity (both with 0.6 nmol/h/mg of protein, normal value 2.7-15). Patient 3 had 1.0 nmol/h/mg residual acid maltase activity on muscle tissue.

Genetic features

Molecular analysis through gene direct sequencing in all patients revealed two mutations in *GAA* gene, c.-32-13T>G in intron 1 leading to aberrant splicing of exon 2,

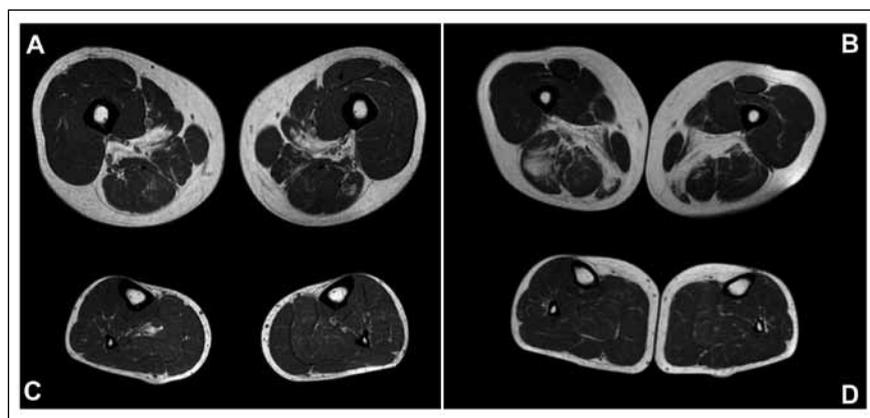


Figure 2. T1-weighted muscle MRI axial images at thigh (A, B) and leg level (C, D) in patient 1 (A, C) and 2 (B, D). At thigh level fatty degenerative changes of adductor magnus and longus and minimal of left biceps femoris have been observed in patient 1 (A), whereas patient 2 revealed a more diffuse involvement, in particular with marked fatty substitution of adductor magnus, semitendinosus and right biceps femoris (B). No relevant finding has been detected in leg muscles (C, D).

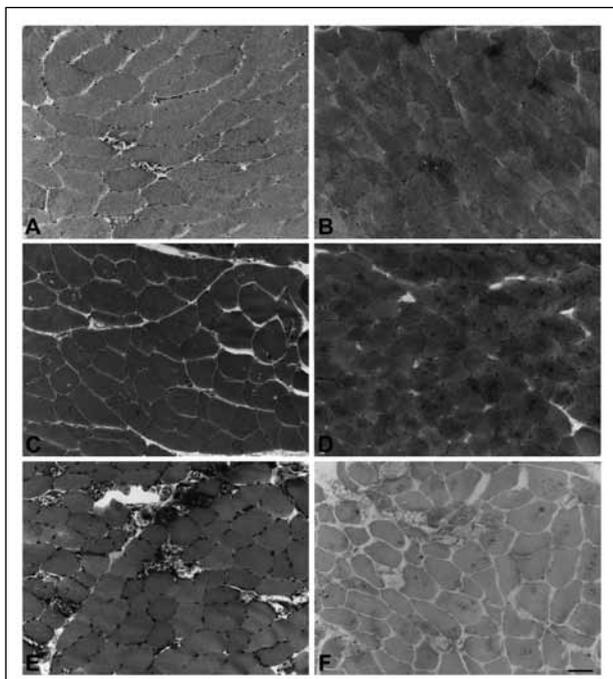


Figure 3. Histological stainings of muscle biopsies from patient 1 (A, B), 2 (C, D) and 3 (E, F). H&E (A,E) and Gomori Trichrome show grossly vacuolated fibres in patients 1 and 3 (more numerous in the latter) and finely vacuolated fibres in patient 2. Vacuoles contain PAS (B,F) and acid phosphatase-positive material. Bar = 50 µm

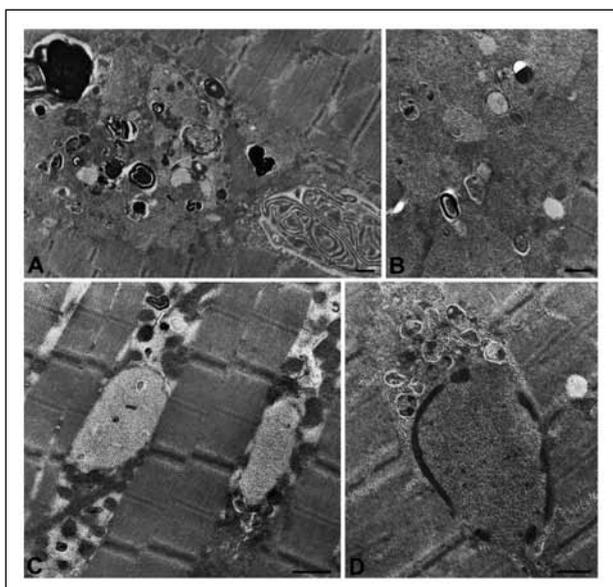


Figure 4. Electron micrographs muscle showing large non membrane-bound vacuoles containing autophagic material (A) and free glycogen particles (B) and lysosomes filled with glycogen particles (C, D) in the muscle of patient 1 (A, B) and 2 (C, D). Bar = 1 µm

associated with the juvenile/adult onset (5, 6) and the intron 10 mutation c.1551+1G>C (p.Val480-Ile517del) associated in some patients with a more severe disease (7), consistent with a diagnosis of Pompe disease. Both mutations have been reported (5-9). A brother and the mother, both carrying the c.1551+1G>C mutation in heterozygosis, were asymptomatic. The other 2 brothers were negative for both mutations and healthy. Father died several years ago, hence molecular analysis was not available.

Clinical course and treatment

Once obtained the diagnosis of Pompe disease, the enzyme-replacement therapy (ERT) at standard dose (20 mg/kg every two weeks) was started in our patients at an age ranging from 45 to 54 years, while nocturnal non invasive ventilation (NIV) was started at an age ranging from 46 to 54 years, due to reduced nocturnal oxygen saturation. All patients had a reduction of forced vital capacity (FVC) which was further reduced in the lying position, from 17 to 29 % of predicted values.

At last follow-up visit, after ERT, patient 1 and 2 had a further FVC reduction in the lying position of 34% and 22%, respectively, whereas in patient 3 this value was not available. Heart clinical evaluation and ECG were normal in all affected patients. Patients' clinical data, including assessment at baseline and at the end of the follow up period, are showed in Table 1.

The videofluoroscopic swallowing examination performed in patient 1 and 2, showed a mild impairment due to delayed tongue motion and slow oral transit, a moderate post-swallow pharyngeal residue and no penetration or aspiration. A facial CT scan performed in patient 1 at the age of 46 and a facial MRI scan performed in patient 2 at the age of 54, showed fat replacement of the tongue muscles.

Muscle MRI - performed at the age of 46 in patient 1 and 54 in patient 2 - obtained T1-weighted axial images at thigh and leg level according to standard protocol (4). As shown in figure 2, patient 1 had fatty degenerative changes of adductor magnus, longus and minimal of left biceps femoris, whereas patient 2 displayed a more diffuse involvement of posterior thigh muscles. No relevant findings were observed in leg muscles.

Bulbar symptoms did not change during follow-up period. On the contrary all patients had clinical benefit from starting ERT and/or NIV, as revealed by a better performance at 6MWT that improved from 18 to 100 metres.

Discussion

Clinical presentation of late-onset Pompe disease is largely variable, ranging from asymptomatic patients to severe proximal and diaphragmatic myopathy, with need

Table 1. Patients' clinical data.

	Patient 1	Patient 2	Patient 3
Age at onset (years) of symptoms	43	48	41
Main symptoms at onset	bulbar	↑ CK	bulbar
Predominant muscle weakness	proximal	axial/proximal	axial/proximal
Dysphagia	+	+	+
Age at first assessment	45	54	51
Tongue weakness/hypotrophy	+/+	+/+	+/+
WGMS grade	2	3	3
FVC while sitting (%)	76	54	42
FVC while lying (%)	47	37	22
Nocturnal sO ₂ <90% time (%)	20	34	49.2
BMI	19.8	25.8	27
Muscle GAA activity (nmol/h/mg)	0.6	0.6	1.0
Age at ERT beginning (years)	45	54	51
Age at NIV beginning (years)	46	54	52
Age at last F-up (years)	47	56	52
WGMS grade	2	3	3
6MWT range last-1 st F-up (metres)	+100	+32	+18
FVC while sitting (%)	76	47	49
FVC while lying (%)	42	25	na
Nocturnal SO ₂ <90% time (%)	0.8	23	na
BMI	20.9	25.8	26.6

Legenda. FVC: forced vital capacity (% of predicted normal values); F-up: follow-up; WGMS: Walton and Gardner-Medwin scale; 6MWT: 6 minute walking test; sO₂: oxygen saturation; BMI: body mass index; ERT: enzyme replacement therapy; NIV: non invasive ventilation; na: not available.

of ventilation support; however an early diagnosis could be relevant due to the chance to improve or at least stabilize the disease course through enzymatic replacement therapy (10, 11). Feeding and swallowing difficulties are quite common in infantile Pompe disease (12, 13). However, apart from few scattered single case reports (14, 15), poor attention has been generally paid to facial and bulbar symptoms in adult-onset Pompe disease and only recently few studies focused their attention on them (16-18). A vacuolar myopathy in genioglossus and proximal esophagus has also been described through autopsy study in Pompe disease (19) Furthermore, according to a nationwide prospective observational study in adults with Pompe disease in Netherlands, bulbar muscle weakness was detected in about one quarter of patients and was significantly associated with scapular winging (20).

We report on 3 family members with atypical late-onset Pompe disease, characterized by bulbar symptoms, in particular swallowing difficulty and tongue weakness, clinically relevant in all our patients and requiring assisted ventilation. In patient 1 and 3 bulbar symptoms were

reported as first symptoms and in particular patient 1 was first investigated elsewhere for disease of central nervous system. Patient 2 – presenting increased CK values – was asymptomatic for many years and presented bulbar symptoms only 5 years after the onset of lower limb weakness, confirming the great phenotypic variability of the disease. Patient 1 complained also difficulty in moving lips; facial muscle involvement was confirmed by neurological examination and electromyography. Tongue involvement with macroglossia – traditionally described in infants with classic phenotype – was considered a rare finding in late-onset disease. However tongue weakness has been reported in 19 patients affected by late-onset Pompe disease (17), one third of them complaining for swallowing difficulties, such as impairment of oral bolus control, and not further investigated. In that series tongue weakness was mild and only detected on neurological examination, being usually underestimated by the patients. On the contrary in our patients tongue weakness was more marked according to criteria established by Dubrovski and colleagues and reported as first symptom by patient 1. Fur-

thermore tongue weakness had a main role in swallowing difficulties as showed by videofluoroscopy swallowing examination performed in our patients. Differently from data reported by Dubrovski and colleagues, all our patients displayed also tongue hypotrophy. As a matter of fact tongue involvement detected in our patients was also supported by facial CT and MRI findings in patients 1 and 2, respectively, that showed fatty degeneration, according to previously reported studies (16, 17). Recently Hobson-Webb and colleagues reported that 3/12 patients affected by late-onset Pompe disease showed oropharyngeal dysphagia, although none of them as first symptom (18). These 3 patients had tongue weakness and, to be noticed, dysphagia was confirmed through videofluoroscopic swallowing examination. In this respect patient 1 and 2 of our series underwent a videofluoroscopic swallowing examination, which resulted mildly impaired exclusively due to tongue weakness. Dysphagia remained stable over the years in our patients; this is also confirmed by BMI, which did not substantially change during the follow-up period.

Our data confirm that tongue weakness and dysphagia may occur in adult-onset Pompe disease more frequently than expected and need adequate investigations for early detection and management, being the most relevant symptoms in some cases. Bulbar involvement in patients affected by Pompe disease seems to be not associated with specific mutations, hence no genotype–phenotype correlation can be found.

Globular inclusions detected in our patients represent a rare finding. Their appearance with menadione-linked alpha-glycerophosphate dehydrogenase was in accordance with reducing bodies definition. However both the location in autophagic vacuoles and their electron density were not typical of classical reducing bodies, as observed also by Sharma and colleagues. Positivity to LAMP2 immunostaining suggests that globular inclusions should be considered mainly of lysosomal nature. However autophagic process could be concomitant, as several inclusions were also mildly positive to the markers of autophagy EEA1 and LC3.

Globular inclusions in glycogenosis type II have already been described in 6 unrelated patients (21–23), 3 in infancy and 3 in adult life. Two of them, including one adult-onset case, carried the c.IVS1-13T>G mutation, the same detected in our patients; in one infantile case molecular characterization was not available (21). The 3 patients with onset in infancy presented with delay in motor development, followed by mild to moderate muscle weakness, while patients with adult onset had mild-to-moderate proximal lower limb weakness, except one patient who was wheelchair bound and required NIV at the end of follow-up period (21–23). However no bulbar

symptom was reported in these patients. In conclusion our study confirms the great clinical and histological variability of adult-onset Pompe disease and further supports the need of a careful evaluation of bulbar function in patients affected by this pathology

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