# A review of treatment of Pompe disease in infants

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**Abstract:** The glycogen storage disease type II (GSD-II), or Pompe disease, is due to the deficit of lysosomal glycogen degradation enzyme acid α-glucosidase (GAA). In infants, Pompe disease is characterized by prominent hypotonia, muscle weakness, motor delay, feeding problems, and respiratory and cardiac insufficiency. In a retrospective study, the median age at death was 8.7 months. Enzyme replacement therapy with recombinant human GAA is recently used to treat patients with Pompe disease, and has been shown to prolong survival, reverse cardiomyopathy, and improve motor function. This article briefly reviews the history and manifestations of Pompe disease, and then focuses on the development of the drug for Pompe disease, alglucosidase alfa. Current status of treatment and future developments are also discussed.

**Keywords:** alglucosidase alfa, Pompe disease, alpha-glucosidase

## History

Pompe disease was first described by Dr. Pompe almost 55 years ago in a 7-month-old girl with cardiomyopathy, in whom massive accumulation of glycogen in vacuoles was observed in all tissues examined (Pompe 1932). After identification of lysosome by de Duve in 1963 (De Duve 1963, 2005), Pompe disease became the first recognized lysosomal storage disorder (LSD) when it was found that the disease was due to the deficit of lysosomal glycogen degradation enzyme acid α-glucosidase (GAA, EC 3.2.1.20) (Hers 1963). Pompe disease is formally named glycogen storage disease type II (GSD-II), but most other types of GSDs are caused by defects in the cytosolic glycogen cleavage or synthesis pathways (Shin 2006). Pompe disease is remotely related to Danon disease, also known as X-linked vacuolar cardiomyopathy and myopathy (Danon et al 1981). In Danon disease, lysosomal glycogen storage is caused by mutation of the lysosome-associated membrane protein-2 (LAMP-2) gene, and there is no GAA deficiency (Nishino et al 2000).

## Clinical manifestation

Pompe disease is usually classified into infantile-onset and late-onset for convenience, although there is clearly a continuum in disease severity ranging from the most severe classical infantile-onset Pompe disease (IOPD), to the mildest adult-onset Pompe disease (Hirschhorn and Reuser 2001; Raben et al 2002). In the Netherlands, a combined frequency for all type of Pompe disease was estimated to be 1 in 50,000 (Poorthuis et al 1999). After screening for seven mutations in an ethnically diverse population, a study in New York suggested an incidence of 1 in 40,000 (Martiniuk et al 1998). The incidence of Pompe disease in certain ethnic groups, for example in Israel (Bashan et al 1988) and in Taiwan (Lin and Shieh 1996), may be higher. Patients of IOPD are usually recognized at the age of 3–5 months because of a respiratory infection (Hirschhorn and Reuser 2001; Marsden 2005). A chest X-ray at that time may show cardiomegaly which leads to further check-up and diagnosis of IOPD. On physical examination, head lag during a traction from the supine position is usual prominent. Although weakness of extremities or trunk can usually be confirmed retrospectively

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at the age of 2–3 months (Marsden 2005; Kishnani et al 2006a), only rarely do these signs trigger an investigation of affected children (Howell et al 2006). The lack of power for early diagnosis for hypotonia can be explained both by parental lack of experience and by children's large individual variation in normal development.

Because of the late diagnosis in most cases of IOPD, it is not clear how early signs of cardiomegaly can be detected. Pompe disease diagnosis at the newborn stage has been described, but was not found in a recent large-scale natural history study (Kishnani et al 2006a). However, recently a newborn baby was diagnosed in Hong Kong because of respiratory distress shortly after birth (personal communication to Dr. Grace Poon). Large QRS complexes and short PR interval have been the cardinal diagnostic criteria in EKG (Ansong et al 2006), but the sensitivity of short PR interval is not high. In echocardiography, the thickening of the ventricular wall and the interventricular septum are marked, the ventricular cavities are very small and the outflow tract can be obliterated. Symptomatic outflow tract obstruction may occur shortly after the diagnosis of IOPD. A careful management of this condition is critical, and an adequate use of diuretics and β-blockers can usually help the affected baby to overcome this symptom, but acute death is also possible (Kishnani et al 2006c). As the disease advances, the heart becomes more dilated and obstruction sign can disappear. Occasionally, overt cardiac failure leads to death.

According to the recent natural history review, the progression of IOPD is very fast, and the gap between the median age at diagnosis and the median age of first use of ventilator was only 1.2 months (Kishnani et al 2006a), and between median age at diagnosis and death was 2.4 months in a Dutch study (van den Hout et al 2003). The major illness beyond cardiac obstruction would be respiratory failure. Weakness of the respiratory muscles and cough reflex are the major causes of respiratory problem, and once these children are intubated, they rapidly become ventilator dependent. If the lives of patients could be prolonged, for example by ventilator support or enzyme replacement (to be discussed later), symptoms related to other organ systems may present (Kishnani et al 2006c). Weakness of the diaphragm makes hygiene of the lower pulmonary segments difficult. Paradoxical respiration, that is, elevation of the diaphragm during inspiration, is also common. Dysfunction of the gastrointestinal tract is a serious complication. Dysphagia, gastro-esophageal reflux, delayed gastric empty time, and intestinal dysfunction are all likely. If swallowing is problematic, jejunostomy rather than gastrostomy will be necessary to achieve adequate feeding without causing aspiration (Kishnani et al 2006c). The involvements of other organ systems besides the muscle are not a surprise, since glycogen storage is universal throughout the body. Four cases with IOPD revealed a hearing loss which may be due to a problem in the cochlea or middle ear (Van den Hout et al 2004). Brain development is also a concern, since excessive glycogen storage has been found in both neurons and glial cells (Gambetti et al 1971; Sakurai et al 1974). However, catch up in brain myelination after stabilization of the patients has also been reported (Chien et al 2006). In conclusion, without specific treatment, neither any of the above-mentioned efforts, nor rehabilitation or dietary management could alter the outcome of IOPD.

## Molecular basis

The GAA gene is located on chromosome 17q25. The gene encodes a peptide of 952 amino acids with nonglycosylated weight of 105 kDa (Hirschhorn and Reuser 2001). Extensive modifications of the protein occurs afterwards, which include glycosylation and proteolytic processing, resulting in the 76 and 70 kDa mature GAA protein (Hirschhorn and Reuser 2001). More than 70 mutations have been found on the GAA gene (Raben et al 2002; Hermans et al 2004). The mutations are spread over the gene. Several common mutations are found in different ethnic groups, including the IVS1 t-13g in Caucasian patients with adult-onset disease (Huie et al 1994; Boerkoel et al 1995; Kroos et al 1995), the Arg854X mutation in African Americans (Tsujino et al 2000), and the Asp645Glu mutation in Chinese patients from Taiwan (Lin and Shieh 1996; Ko et al 1999). A few of the mutations have been expressed in cultured cells and the GAA activities obtained can be correlated to the phenotypes or the severity of the disease (Hermans et al 2004).

## Laboratory diagnosis

Laboratory diagnosis depends on the measurement of GAA activity in blood or tissues. The assay used to measure GAA activity usually employs an artificial fluorogenic substrate, 4-methylumbelliferyl-α-D-glucopyranoside (4-MUG), but several α-glucosidase (E.C. 3.2.1.20) isoenzymes especially maltase-glucoamylase (MGA) in blood and hematopoietic cells contribute to the observed reaction (Taniguchi et al 1978; Shin et al 1985; Hirschhorn and Reuser 2001). Therefore the assay has to be done either in lymphocytes or in skin fibroblasts which have activity at pH 4.0 mainly due to GAA (Taniguchi et al 1978; Shin et al 1985). However, lymphocyte

preparations are often contaminated by leukocytes, and the resulting falsely high residual activity cannot be used to predict phenotype. Recently, immobilized antibodies have been used to remove the interfering enzymes (Schram et al 1979; Umapathysivam et al 2000; Umapathysivam et al 2001). GAA specific activity measured in this way, together with data from skin fibrobasts, may make it possible to predict the age of onset of Pompe disease (Umapathysivam et al 2005).

## The start of enzyme replacement therapy

The first successful attempt towards a specific pharmaceutical treatment of LSD was in Gaucher disease. The enzyme deficient in Gaucher disease, the β-glucosidase, was first purified from placenta in 1974 (Brady et al 1974; Furbish et al 1977). It was discovered later that it was necessary to modify the oligosaccharide moieties on the enzyme to facilitate its macrophage targeting. The exogenous enzyme molecules are internalized and shipped in vacuoles through receptor-mediated mechanisms. These vacuoles then fuse with the lysosomes elegantly to correct their intrinsic defect. These processes make exogenous supplementation of lysosomal enzymes, or enzyme replacement therapy (ERT), an efficient way to treat LSD (Brady 2006). Placental β-glucosidase was approved for this use by the US Food and Drug Administration (FDA) in 1991, and was later replaced by a Chinese hamster ovary (CHO) cell-produced recombinant β-glucosidase in 1994 (Barranger and O'Rourke 2001). More than 4000 patients all over the world have been treated with this drug (Rosenbloom et al 2005). ERT have been developed for several other diseases, including Fabry disease, mucopolysaccharidosis (MPS) type I, MPS type II and MPS type IV (Beck 2007).

## Recombinant human GAA (rhGAA) production

Previously, there was no effective treatment for Pompe disease. Bone marrow transplantation seems not to work. A combined treatment including dietary management and physical therapy has not shown significant effects in IOPD patients (Bembi et al 2003). Recently, two groups of researchers followed different approaches to produce a recombinant GAA protein. One group used the innovative approach of transgenic production of the therapeutic enzyme in mammalian milk (Bijvoet et al 1996). The human GAA cDNA was placed under the control of the $\alpha_{S1}$-casein promoter and expressed in mice. The recombinant GAA purified from mice milk was internalized via the mannose 6-phosphate receptor and corrected the enzyme deficiency in fibroblasts from patients (Bijvoet et al 1996). The mouse transgenic construct was later modified for higher production, and the enzyme, when injected intravenously, corrects the GAA deficiency in heart and skeletal muscles of GSD-II knockout mice (Bijvoet et al 1998). This success in producing recombinant GAA from mice milk led to the industrial manufacturing of GAA from rabbit. In the GAA knockout mice, a single intravenous infusion of the enzyme gave a full correction of GAA deficiency in all mice tissues except brain (Bijvoet et al 1999). Weekly enzyme infusions over 6 months resulted in degradation of lysosomal glycogen in the heart and muscle. The results then led to a clinical trial with rabbit milk recombinant enzyme.

Another study group chose the more traditional approach of expressing the recombinant enzyme in CHO cells (Van Hove et al 1996, 1997). This enzyme was effectively taken up by fibroblasts from Pompe patients (Van Hove et al 1996), restoring normal levels of GAA and glycogen. By intravenous infusion, the enzyme can be targeted to the heart and liver efficiently in guinea pigs (Van Hove et al 1996). Pre-clinical studies for CHO enzyme were conducted in GAA-deficient Japanese quails. Uptake and targeting of the enzyme were first tested in quail fibroblast (Yang et al 1998). The results of the treatment were very encouraging; besides improvements in muscle histology, there was clear gain in function (Kikuchi et al 1998). The quails were treated at the age of 4 weeks. After 7 injections over a 16-day period, the sham-treated quails exhibited progressive myopathy and could not lift their wings or right themselves from the supine position. However, treated birds could flap their wings, and one bird flew up more than 100 cm.

## Dosage and frequency

However, these pre-clinical studies also disclosed one truth: high doses of the recombinant enzyme were required for a positive response. For example, in the quail study, the effect of high-dose treatment (14 mg/kg) was much better than low-dose treatment (4.2 mg/kg) (Kikuchi et al 1998). There was an additional experiment with intermediate doses (5.7–9 mg/kg) and extended treatment. Despite significantly improved histopathology of affected tissues, the quails in this group showed no clinical improvement in muscle strength and were not able to flap their wings or fly (Kikuchi et al 1998). The requirement in Pompe disease of large amount of enzyme to correct muscle pathology is in contrast to the small

dose necessary to correct several other LSDs. For example, the regular dosage for Fabry disease is 1 mg/kg (Wilcox et al 2004). Currently, why a high dose of the enzyme is required in Pompe disease is not fully known. One possible explanation is that the paucity of mannose 6-phosphate receptor in the muscle tissues prevents efficient uptake of the enzyme (Wenk et al 1991; Raben et al 2003). This large requirement of enzyme should have posed a challenge in both the production and the cost of the drug, but currently these issues have been solved. Problems, such as immunologic responses, arising from the infusion of large amounts of foreign protein, will be discussed later.

## Clinical trials

Early clinical trials for rhGAA in IOPD patients revealed promising results. With the rabbit milk rhGAA, 4 patients were treated for 36 weeks and first reported in 2000 (Van den Hout et al 2000). Doses started from 15–20 mg/kg to 40 mg/kg once weekly. The most prominent effect was on the heart: the left ventricular mass index decreased to less than 30% of baseline within 36 weeks. Skeletal muscle function and strength also improved in all patients. One patient treated at the age of 2.5 months was cross-reactive-immunological-material (CRIM) negative, but her improvement continued over the study period. Only one patient gained the normal major motor development. Therefore the researchers recommended that treatment be started early, before the destruction of muscle architecture, for successful outcome of treatment (Van den Hout et al 2004). The long-term outcomes of 2 cases were further described, and survival time under therapy was 55 and 58 months (Klinge et al 2005a; Klinge et al 2005b). There were only mild infusion-associated reactions during the follow-up period. Both patients sat, and their mental development was normal. The production of milk rhGAA was later discontinued.

The first clinical trial (phase I/II) with CHO cell-produced rhGAA was done in 3 patients (Amalfitano et al 2001). They received twice-weekly infusion of rhGAA at a dosage of 5 mg/kg for 14–17 months. Adverse reactions were mild and could be resolved by a pre-infusion dose of antihistamine. Steady decrease in heart size was universal. Improvements in skeletal muscle function were also noted. One patient walked independently from 12 months of age. A high titer of anti-rhGAA antibody developed in two patients who had no detectable GAA protein by Western blot analysis (CRIM negative). This promising preliminary result triggered a scaled-up production of the enzyme and the phase II trial in 8 patients with IOPD (Kishnani et al 2006b). Dosages were either 10–20 mg/kg weekly or 20 mg/kg every 2 weeks. Safety of the drug was first confirmed. Although IgG antibodies to rhGAA developed in all 8 patients by week 8 of treatment, after 52 weeks of treatment the antibody titers either decreased or remained unchanged. Six of 8 patients were alive after the study period. Clinical improvements included ameliorated cardiomyopathy, improved growth and cognition, and 3 patients walked independently. Even though 4 patients subsequently died, in view of the fact that this is a rapidly fatal disease if left untreated, rhGAA did change the clinical course of the disease.

Finally a phase III trial with the CHO cell rhGAA started. This trial enrolled 18 patients younger than 6 months of age worldwide (Kishnani et al 2007). The dosage was 20 or 40 mg/kg every other week. After treatment for 52 weeks, all patients survived to 18 months of age. There was no clear advantage of the 40 mg/kg dose. A Cox proportional hazards analysis demonstrated that the treatment decreased risk of death by 99%. This clinical trial led to FDA approval of Alglucosidase Alfa (Myozyme®, Genyzme Corporation). The recommended dose is 20 mg/kg every other week. For late-onset forms of Pompe disease, rhGAA both from rabbit milk or from CHO cells has shown preliminary therapeutic effects, although the number of reported cases is still small (Van den Hout et al 2004; Winkel et al 2004).

## Disadvantages: high required dosage and antibody formation

The requirement of high dose of rhGAA to treat Pompe disease, almost 10 fold of other diseases, is also unique. The volume of muscle tissue to treat is large, as mentioned. Lower efficiency in enzyme uptake by the muscular system may be another factor. Both mediated by the mannose 6-phosphate receptor, the cardiac muscles are much more efficient in enzyme uptake than the skeletal muscles (Wenk et al 1991; Raben et al 2003). Infusion of large amount of foreign proteins may causes problems. One case report described the development of nephrotic syndrome in a patient receiving a daily infusion of 10 mg/kg of rhGAA (Hunley et al 2004). Life-threatening anaphylactic reactions including anaphylactic shock have been observed in patients during alglucosidase alfa treatment. Antibody development is universal in treated patients, most within the first 3 months of treatment (Kishnani et al 2006b). Antibodies to the infused enzyme have been observed in other ERTs, but rarely cause trouble (Rosenberg et al 1999; Wilcox et al 2004). In IOPD, some patients were CRIM negative. Antibody titers seemed to be higher in those cases (Amalfitano et al 2001). Although the

surge of antibody titers in some patients has been suspected to be related to the loss of therapeutic effect (Amalfitano et al 2001), the case number is too small to conclude whether CRIM status or the antibody titer will be an untoward prognostic factor in treatment of Pompe disease.

CRIM status may indeed reflect the severity of the disease. It is suggested that the better the clinical status before initiation of treatment, the better the outcome. With a milder or later-onset disease, application of alglucosidase alfa therapy promises a good likelihood of recovery. In a recent paper, the muscle pathologies of Pompe disease were classified into 5 stages (Thurberg et al 2006). Landmarks in the staging include appearance of intra-lysosomal or cytoplasmic glycogen, integrity of the mitochondria, and myofibril dissociation (Thurberg et al 2006). It is likely that muscle cells may be not able to recover when the damage is too advanced.

## Early treatment

Another view in considering the relationship between treatment effect and disease severity is the timing of treatment. That is, treatment should be started before the threshold of muscle pathology been reached (Thurberg et al 2006). A more severe disease or more rapid disease progression requires earlier treatment. Clinical conditions like respiratory failure, mechanical ventilation, or immobilization are almost fatal regardless of treatment. However, from a recent IOPD natural history survey, the median age at diagnosis was 4.7 months, while the median age of first ventilator use was 5.9 months, only one month apart (van den Hout et al 2003; Kishnani et al 2006a). On the other hand, previous experience in Pompe disease treatment has demonstrated that drug effect takes 2–3 months to appear. For example, the decrease in heart size usually takes 3 months after initiation of treatment (Amalfitano et al 2001). Therefore it is likely that a poor early clinical course will destroy the therapeutic effect. In the past few years, a number of IOPD patients have been treated in different clinical trials or by compassionate use. Because of the difficulty of getting into treatment, many cases had late treatment. Partial reversal of disease manifestations, including being bedridden or wheelchair bound, feeding problems, or even ventilator dependence were not uncommon. In those partially treated cases, complications included bone fracture, heart arrhythmia (preexisting ectopy including premature ventricular contractions, perhaps caused by fibrous tissue deposition in the myocardium), and problems in brain myelination (Cook et al 2006; Case et al 2007). After the formal launch of alglucosidase alfa, the situation should improve. However, patients receiving ERT may need a follow-up to see the long-term safety and efficacy of alglucosidase alfa.

Currently accumulating evidence suggests that early treatment for IOPD should be beneficial. However, as discussed in previously sections, early diagnosis of IOPD is rarely achieved unless there is positive family history, because early signs are non-specific. Therefore, screening for IOPD in very early ages of babies is the best and only choice. Laboratory diagnosis of Pompe disease, as discussed in a previous section, has been difficult. But in the past few years there were significant progresses in this field. First is the employment of blood spot as the source for lysosomal enzyme assays (Chamoles et al 2001). With an extended enzyme reaction time, a tiny amount of blood lysate from 1/8 inch spots could serve several assays (Chamoles et al 2002a, b). The most critical invention is the use of inhibitors (maltose or acarbose) in the assay, so the assay could be performed without blood cell separation (Chamoles et al 2004; Li et al 2004). Very important information will be generated from the screening programs, for example, what percentage of IOPD can have a satisfactory outcome if treated early, or whether CRIM-negative patients can be rescued if treated early.

## Future aspects in ERT

Lastly, the failure of the rabbit milk product has shown the difficulty of developing transgenic methods to produce large molecule therapeutic agents. It is not clear why the company withdraw the project, since early clinical trials disclosed comparable treatment effect to the other product. There may be difficulties or high cost in maintaining the herd or in purification of the product from the large quantities of milk protein. However, the method of standardization in culturing cells does look more feasible than raising animals. Several recent enzyme products are produced from human fibroblasts. It has been argued whether human cell products will be better than animal cell products, for example the agalsidase beta from CHO cells (Fabrazyme®, Genzyme Corporation) and agalsidase alfa from human fibroblasts (Replagal®, Shire) (Beck 2002). But the first and successful ERT product, the imiglucerase (Cerezyme®, Genzyme Corporation), is from CHO cells. The infusion reaction for imiglucerase is transient and mild, and rarely interferes with treatment. Therefore, contamination of animal cell proteins seems negligible. The question of the efficacy of fibroblasts enzymes cannot be easily answered because of the large differences in disease pathophysiology.

Future development in ERT therapy will need to overcome other difficulties especially in targeting the enzyme. For example, conjugation of mannose 6-phosphate-containing oligosaccharides to acid alpha-glucosidase improves the delivery to muscles and the clearance of glycogen in pompe mice (Zhu et al 2004, 2005). Also, a tag with an acidic oligopeptide may enhance drug delivery to bone (Nishioka et al 2006), and a phosphorylated rhGALNS can be delivered to multiple tissues, including bone, implying a potential enzyme replacement treatment for mucopolysaccharidosis type IVA (Tomatsu et al 2007).

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