

# Pompe disease: clinical perspectives

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**Abstract:** Pompe disease (acid alpha-glucosidase deficiency, OMIM 232300) is a rare lysosomal storage disorder due to autosomal recessive mutations in the *GAA* gene. It has also been called acid maltase deficiency and glycogen storage disease type II. There is a broad clinical presentation: the most severe form that presents in the first few months of life with cardiomyopathy and generalized muscle weakness that rapidly progresses to death from cardio-respiratory failure in the first year of life (infant-onset Pompe disease). A more slowly progressive disease, with little or no cardiac involvement, presents with proximal myopathy and/or pulmonary insufficiency, from the second year of life to late adulthood (late-onset Pompe disease). The recent development and introduction of enzyme replacement therapy with intravenous infusion of recombinant human acid alpha-glucosidase have made a major improvement in the morbidity and mortality of this disease. New therapies are also in development. With the availability of treatment, diagnostic methods have also improved, allowing for earlier recognition and potential early therapeutic intervention. The advent of newborn screening for Pompe disease may identify patients who can be treated before significant irreversible disease has occurred.

**Keywords:** Pompe disease, glycogen storage disease, lysosomal storage disease, enzyme replacement therapy, gene therapy, chaperone therapy, genotype/phenotype, newborn screening

## Introduction

Pompe disease is an autosomal recessive inherited disorder of glycogen metabolism due to a deficiency of the lysosomal enzyme, acid alpha-glucosidase (*GAA*) (also called acid maltase), resulting from mutations in the *GAA* gene (EC 3.2.1.20). Historically, it has been classified as a glycogen storage disease (type II), an enzyme deficiency (acid maltase deficiency), and more recently as a lysosomal storage disease. A deficiency of the enzyme<sup>1</sup> results in neuromuscular symptoms, ranging from severe early-onset disease, with involvement primarily of the heart, proximal skeletal muscles, diaphragm, and intercostal muscles, leading to cardio-respiratory failure and death in infancy, to a later onset, more slowly progressive, limb-girdle muscular dystrophy with diaphragm involvement, leading eventually to wheelchair dependence, respiratory failure, and death in adulthood.

The disease was first described in 1932 by a Dutch pathologist, Johannes Pompe, when he noted ubiquitous deposition of glycogen in vacuoles in the heart and other tissues of a 7-month-old girl who had died from hypertrophic cardiomyopathy. In 1963, HG Cori classified it as glycogen storage disease type II and, following the discovery of the lysosome by de Duve, Hers determined that the intra-lysosomal glycogen deposition was secondary to a deficiency of acid alpha-1,4 glucosidase (acid maltase), thus

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the first known lysosomal storage disorder. Currently, there are over 40 disorders known to be due to a defect of one of the lysosomal hydrolase enzymes.

## Biochemistry/molecular genetics

Acid alpha-1,4-glucosidase is a lysosomal enzyme that hydrolyzes the alpha-1,4 and alpha-1,6 linkages of its natural substrate, glycogen at acid pH. The enzyme is primarily targeted to the lysosome via mannose-6-phosphate receptors. The enzyme appears to be expressed in most tissues,<sup>1</sup> including skeletal muscle, diaphragm, heart, placenta, kidneys, and central nervous system (CNS), predominantly in the spinal cord and brain stem Schwann cells and neuronal cell, including the anterior horn cells.

To date, over 300 variations have been detected in the *GAA* gene,<sup>2</sup> which is mapped to 17q25.2-25.3, including 116 pathogenic mutations considered to be very severe, 91 potentially less severe, 23 less severe, and 18 potentially mild. The gene encodes an inactive precursor protein with a molecular mass of ~110 kD that is transported to the prelysosomal and lysosomal compartments and further processed to 95, 76, and 70 kDa fragments that can be seen on Western blots, and appear to be fully active and not produced by nonspecific proteolysis during the purification process.<sup>3</sup> The severity of the mutations largely depends on where the mutations occur in the gene and the type of mutation.<sup>4</sup> The combination of mutations in heterozygous patients may explain some of the phenotypic heterogeneity seen in this disease. Large deletions or nonsense mutations involving the coding area of the gene, or other critical areas for transcription, result in a truncated enzyme with essentially no activity. Patients who inherit two severe mutations have infant onset Pompe disease (IOPD). The combination of a severe mutation and a mutation that retains some transcriptional activity is usually associated with late-onset Pompe disease (LOPD).<sup>5,6</sup> The most severe mutations result in no detectable protein on Western blot analysis, and therefore designated cross reactive immune material-negative (CRIM-negative). A normal length precursor protein that is not fully translated can also result in a nonfunctional enzyme.

A database is maintained for all reported mutations at Erasmus Medical Center in the Netherlands.<sup>7</sup> The tables list pathogenic variants, with predicted severity. Nonpathogenic variants and variants of unknown significance are also listed. This information may be helpful in predicting genotype–phenotype correlation in some cases.<sup>4</sup>

## Pathology

In affected patients, the classic appearance on muscle biopsy is of a vacuolar myopathy with periodic-acid Schiff staining

of areas that, on electron microscopy, are seen to be intralysosomal glycogen. Accumulated glycogen may also be seen in the cytoplasm and inter-fibrillary spaces.<sup>8</sup> Massive accumulation eventually leads to rupture of the lysosome and large “glycogen lakes.” This process has been thought to contribute to the muscle pathology by disrupting the contractile elements of the muscle cell leading to fibrosis and loss of function.<sup>9</sup> More recently, it has been shown in the knockout mouse model, and also confirmed in humans, that autophagy (a normal process of degradation of lysosomal or other malfunctioning cellular components) in type II, fast twitch glycolytic muscle fibers leads to build up of autophagic granules and destruction of the fibers. Accumulation of autophagic material in these muscle fibers<sup>10,11</sup> also appears to act as a sink for therapeutic recombinant enzyme, disrupting efficient delivery to the lysosomes. The type I slow twitch oxidative fibers appear to store less glycogen, but clear it more rapidly<sup>11</sup> with enzyme replacement therapy (ERT).

In addition to autophagy, it has been recognized that mitochondrial dysfunction and abnormalities in calcium homeostasis<sup>12</sup> may also contribute to the pathology. Intramitochondrial calcium has a buffering role, and overload may result in necrosis or apoptosis. Dysfunctional mitochondria can also contribute to cellular pathology due to increased production of reactive oxygen species and consequent damage.

Recently, Falk et al<sup>13</sup> have reported that in the Pompe knockout mouse model there were both pre- and postsynaptic neuropathic changes in the neuromuscular junction tissues of skeletal muscle and diaphragm, suggesting that neuromuscular junction pathology may contribute to the decline in pulmonary and muscle function in Pompe disease. Small-fiber neuropathy may also contribute to the clinical phenotypes of both infant- and late-onset patients.<sup>14</sup>

## Epidemiology

Pompe disease is an autosomal recessive disorder with an estimated worldwide incidence of ~1/40,000,<sup>1</sup> although the precise number is not known as the disease can be difficult to diagnose and there may be larger numbers of unrecognized patients. Newborn screening programs (discussed later) may eventually be able to provide more accurate information. Data from the Pompe Registry<sup>15</sup> indicate that ~20% of patients have IOPD. There is a different incidence of Pompe disease in some populations, often with regional mutations, suggesting founder effects, especially in Taiwanese/Southern Chinese population, African-Americans, and the Druze population in Israel.

It was thought for some time that the Taiwanese population had a higher incidence of Pompe disease, as the diagnosed patients were typically children. However, early newborn screening<sup>16</sup> data suggest that the incidence of IOPD is ~1/57,000 and for LOPD 1/26,500, with an overall incidence of ~1/40,000, similar to other populations. Of the later-onset patients to date, most appear to be children. The majority of patients carry at least one copy of the severe p.Asp645Glu mutation,<sup>17</sup> which is likely a founder mutation in this population. A pseudo-deficiency allele, p.[G576S; E689K],<sup>18,19</sup> is relatively common in Asian populations and leads to falsely low measured enzyme activity. It was recently reported in 3.9% of Japanese. It has also been found in the Taiwanese population.

In African-Americans,<sup>1</sup> the incidence is ~1/14,000, with a common severe mutation p.Arg854X, which is CRIM-negative.

In late-onset Caucasian patients, there is a high incidence of a leaky splice-site mutation, c.336-13T>G, present in ~70% of alleles.<sup>6</sup> It has been found in combination with many different mutations with a wide spectrum of clinical presentations.

## Clinical phenotypes

Pompe disease can present anytime from the newborn period through late adulthood. Historically, it has been classified as infant-onset, presenting in the first year of life, juvenile-onset presenting from the second year of life through adolescence, and adult-onset later in life. In reality, however, it is a continuum of symptoms involving multiple systems. Patients who may be diagnosed later in life, because of neuromuscular and/or pulmonary symptoms, often recall symptoms in childhood that were not recognized as being pathologic.<sup>20</sup> Other symptoms may be related to generalized involvement of smooth muscle, including vascular and intestinal systems (Table 1).

There is no definitive genotype–phenotype correlation; there have been rare reports of variable ages of presentation within the same family carrying the same mutations,<sup>21</sup> such as one family with severe infant-onset symptoms in one

generation and milder late-onset symptoms in another. The severity of symptoms and rate of progression of the disease may also depend on other epigenetic variables;<sup>22</sup> however, in general, earlier onset of symptoms is associated with more severe and more rapidly progressive disease.<sup>20</sup>

Affected infants have been detected prenatally, especially in families with a previously affected infant, by finding cardiac enlargement on ultrasound. The average age of diagnosis is ~4 months.<sup>23</sup> There is <1% measureable enzyme activity,<sup>1</sup> with ~10% having no detectable enzyme activity (CRIM-negative), although the CRIM-negative rate may be higher in some consanguineous populations, such as the south Asians in the UK,<sup>40</sup> where it can be almost 50%.

Presenting symptoms are related to muscle weakness and/or cardiac involvement (Table 2). The infants appear to be cognitively normal. The majority of untreated infants (discussed below) die of cardio-respiratory failure by 1 year of age. A few atypical infants may survive into later infancy or childhood, with progressive muscle weakness, less severe heart involvement, but often requiring ventilator support.

The more common LOPD, also traditionally referred to as acid maltase deficiency,<sup>1</sup> is essentially a form of limb-girdle muscular dystrophy. There is little or no cardiac involvement. These patients usually have residual enzyme activity of 10%–30%. Symptoms can present any time after the first year of life and progress slowly. Surprisingly, ~13% of late-onset patients have been shown to have <1% residual enzyme activity,<sup>24</sup> so there is not always a direct correlation between clinical phenotype and residual enzyme activity. The initial symptoms (Table 1) are usually related to proximal muscle weakness,<sup>20</sup> with the lower limbs being more affected than the upper limbs. The weakness becomes progressively worse with later involvement of the intercostal muscles and the diaphragm, leading to respiratory failure and ventilator-dependence. Patients typically survive through the fourth or fifth decades. Occasionally, pulmonary symptoms can precede significant muscle weakness.

Table 3 provides the differential diagnosis.

**Table 1** Common features in late-onset Pompe disease

Proximal muscle weakness
Gait instability, falling
Difficulty climbing stairs
Neck weakness
Scapular winging
Paraspinal muscle atrophy
Swallowing difficulty
Facial weakness, ptosis

**Table 2** Common features in infant-onset Pompe disease

Hypotonia
Head lag
Respiratory distress
Macroglossia
Cardiomegaly
Cardiac failure
Feeding difficulty
Failure to thrive
Motor delay

**Table 3** Differential diagnosis of Pompe Disease

Infant-onset	Common signs and symptoms
Spinal muscular atrophy (Werdnig–Hoffmann)	Hypotonia, progressive limb-girdle muscle weakness, absent reflexes, feeding difficulties, elevated creatine kinase (CK)
Congenital muscular dystrophy	Severe hypotonia and muscle weakness
Mitochondrial disorders	Hepatomegaly, cardiomyopathy, myopathy, elevated CK
Peroxisomal disorders	Hypotonia, hepatomegaly
Prader–Willi Syndrome	Dysmorphic features, severe feeding difficulties, absence of cardiomegaly
Idiopathic hypertrophic cardiomyopathy	Biventricular hypertrophy
Myocarditis	Inflammation of the myocardium contributing to cardiac enlargement
Endocardial fibroelastosis	Breathlessness, feeding difficulties, cardiomegaly, heart failure
Carnitine deficiency	Cardiomyopathy, muscle weakness
<b>Late-onset</b>	
Proximal limb-girdle muscular dystrophies	Progressive muscle weakness in the pelvis, legs, or shoulders, abnormal gait, elevated CK
Danon disease	Skeletal muscle myopathy, limb-girdle muscle weakness, scapuloperoneal muscular weakness, elevated CK
Becker muscular dystrophy	Progressive limb-girdle muscle weakness, respiratory impairment, difficulty walking, elevated CK
Myasthenia gravis	Daily fluctuation, fatigue, ocular involvement
Polymyositis	Progressive, often symmetrical, muscle weakness, difficulty swallowing, elevated CK

## Diagnosis

Until recently, the diagnosis of Pompe disease has been made by measuring enzyme activity using a fluorometric assay with the artificial substrate, 4-methylumbelliferyl-D-glucosidase,<sup>1</sup> in tissue obtained by muscle biopsy or cultured skin fibroblasts. In the past, measurement of GAA activity in the blood has been problematic. The enzyme is expressed in lymphocytes, but mature neutrophils contain another enzyme, maltase glucoamylase, which has some overlapping activity with GAA. As it can be very difficult to obtain a pure lymphocyte sample, false negatives can occur. It was recognized that acarbose inhibits maltase glucoamylase and its addition to a whole blood sample results in a reliable measurement of GAA activity.<sup>25</sup> This has led to the development of a simple blood filter paper sample, which can be mailed directly to the reference laboratory, where the blood is extracted and processed for the enzyme assay. This same method has been adapted for newborn screening.

In the later-onset patients, the diagnostic evaluation has typically been by muscle biopsy;<sup>26,27</sup> however, there is a significant false negative rate because muscle fibers

sporadically accumulate glycogen, so it is possible to sample normal tissue. On microscopy, glycogen is periodic-acid Schiff stain-positive, though glycogen can be washed out during processing, and the empty vacuoles may not be readily recognized. Molecular testing may be used to confirm the diagnosis after an abnormal enzyme assay result or after an abnormal muscle biopsy. Magnetic resonance imaging studies<sup>28</sup> have shown that there is a consistent sequence of muscle changes in patients with LOPD, and may help distinguish Pompe disease from other limb-girdle muscular dystrophies. In patients with known disease,<sup>29</sup> nuclear magnetic resonance imaging is able to quantitate the rate of fatty degenerative changes per year.

Genotype information can be used for family counseling and potentially for prenatal diagnosis when the mutations in the family are known, such as from having a previously affected child. The presence of known severe or mild mutations may inform the phenotype and prognosis, for example, patients with at least one copy of the common splice-site mutation generally have a milder disease while those with CRIM-negative mutations have severe disease.

A diagnostic workup depends on the age of presentation.

Where newborn screening is available, it is fairly straightforward. A chest X-ray will show cardiomegaly and follow-up two-dimensional (2D)-echocardiography will show evidence of left ventricular hypertrophy in IOPD. The GAA deficiency can be confirmed on leucocyte assay; a skin biopsy for fibroblast culture and CRIM status determination is advisable.

The evaluation of hypertrophic cardiomyopathy in the first few months of life, especially with associated neck and truncal hypotonia, should have GAA activity measure (leucocytes or filter paper sample). Similarly, infants being evaluated for hypotonia and poor feeding should have a chest X-ray and further evaluation with GAA activity if there is evidence of cardiomegaly.

Older patients with evidence of proximal limb-girdle weakness and/or pulmonary dysfunction or disordered breathing should have GAA activity measured. Muscle magnetic resonance imaging may also be helpful to support the diagnosis and determine the degree of muscle involvement.

## Therapy development

Early attempts at ERT<sup>1</sup> were not successful. A trial in three infants with Pompe disease with an enzyme derived from the fungus *Aspergillus niger* showed uptake of the enzyme in the liver, but not clearance from skeletal muscle. The patients also developed an immune response. Similarly, treatment with human placenta-derived GAA did not provide a sustained

effect. Following the cloning of the GAA cDNA, and the recognition of the importance of enzyme uptake via the mannose-6-phosphate receptors, recombinant human GAA (rhGAA) was able to be produced in Chinese hamster ovary cell lines and from the milk of transgenic animals, including rabbits. The earliest clinical trials<sup>1</sup> were carried out in infants over 36 weeks, using intravenous infusion of rabbit milk-derived GAA. Results showed an increase in skeletal muscle enzyme activity and improved muscle function. Ultimately, a Chinese hamster ovary-derived enzyme, which was able to be produced more efficiently, was used for further clinical development, regulatory approval studies, and subsequent commercial supply.

Myozyme (alglucosidase alpha) was approved in 2006 in the USA and EU, and is now commercially available in many industrially developed countries. Later, in the USA, Lumizyme (GAA), made in a scaled up manufacturing process, was not considered by the US Food and Drug Administration to be equivalent to Myozyme. It was initially approved for use in patients over 8 years of age, and in 2014 was approved for all ages.

The clinical development of rhGAA was unique in that the first clinical trials were in infants because of the great unmet medical need and high mortality rate in untreated infants. Typically, new drugs are initially developed and gain regulatory approval for adult patients before studies are done in children.

## Infant-onset Pompe disease

Two open-label studies were carried out to determine the efficacy of rhGAA (Myozyme). One study was of 18 infants,<sup>30</sup> aged 6 months or less at the time enrollment, treated with bi-weekly infusions for 52 weeks, at either 20 or 40 mg/kg/day. The primary clinical endpoint was survival, compared to a historical control group. At 18 months of age, all of the infants were alive, a reduction in risk of death by 99%. By the end of the study, however, six infants required ventilator support, representing a reduction by 88%. The second study<sup>31</sup> was in 21 infants, aged between 3 and 43 months at the time of enrollment; therefore, some of these infants had more advanced disease at enrollment. At study end, 71% (15/21) were alive and 44% (7/16) were free of invasive ventilation, showing a reduced risk of death by 79% and ventilator-dependence by 58%. No benefit was demonstrated for a dose of 40 mg/kg dose over the 20 mg/kg dose, the ultimately approved dose.

## Late-onset Pompe disease

A Phase III pivotal randomized double-blind placebo-controlled study in LOPD<sup>32</sup> was completed in 2007 in 90 patients aged

8 years and older and who were ambulatory and free of invasive ventilation; 60 patients received the drug and 30 placebo. After 78 weeks of treatment (20 mg/kg), subjects showed improvement in the primary endpoints, 6-minute walk test and forced vital capacity. Hypersensitivity reactions occurred in 5%–8%. Antibody sero-conversion occurred in all patients with available complete data (59/60); median peak titers were at 12 weeks of treatment and tended to decrease thereafter; no patients tested positive for inhibition of enzyme activity, but 18 tested positive for inhibition of enzyme uptake.

## Treatment

Currently, there is one approved drug for the treatment of Pompe disease: ERT with intravenous infusion of rhGAA (Myozyme, Lumizyme). The development of this drug, noted earlier, followed the precedent of other ERTs for lysosomal storage diseases (eg, recombinant glucocerebrosidase for Gaucher disease).

In some countries, ERT may be available for treatment of IOPD, but not for LOPD, possibly because of concern about the cost of treating a slowly progressive disease.

## Outcomes

Treatment outcomes depend largely on CRIM status, age at initiation of treatment, severity of the muscle involvement (fibrotic muscle cannot regenerate), muscle fiber distribution, and degree of autophagy in muscle cells, as discussed in the “Pathology” section.

A subset of the most severely affected infants (CRIM-negative) have less successful response to ERT.<sup>33</sup> In a retrospective review of eleven CRIM-negative and 21 CRIM-positive infants, Kishnani et al<sup>33</sup> showed that by 27 months of age, all of the CRIM-negative infants had died or became ventilator dependent but only 4/21 (19%) of the CRIM-positive infants had a similar outcome. Anti-GAA immunoglobulin (IgG) antibody levels were increased earlier and reached a higher level in the CRIM-negative infants; analysis of neutralizing antibody by assessing inhibition of enzyme activity was positive in 2/5 and inhibition of enzyme uptake was positive in 3/5 of these infants, suggesting that outcome is related to the immune-mediated response to the exogenous protein. Immune modulation therapy<sup>34</sup> with retuximab and methotrexate has resulted in rapid clearance of antibody in a CRIM-negative infant and some clinical improvements with continued ERT. Two additional patients with elevated antibody titers after initiation of ERT and two patients<sup>35</sup> treated concomitantly with the initiation of ERT were reported to be immune-tolerant and making clinical progress.

In patients with established highly elevated antibody titers, response to the standard immune modulation regimen may not be successful.<sup>36</sup> Some CRIM-positive infants and late-onset patients<sup>37</sup> can develop high-sustained antibody levels that may impact ERT efficacy. The addition of bortezomib,<sup>38,39</sup> a protease inhibitor that depletes plasma cells, has resulted in reduction of high-sustained antibody titers in both CRIM-negative and CRIM-positive patients.

In a recent report of 33 IOPD patients in the UK,<sup>40</sup> 13/29 (45%) were CRIM-negative, of whom nine underwent immunomodulation therapy (one CRIM-positive patient was treated because of a sibling history of severe infusion-associated reactions) prior to initiation of ERT. The mean follow-up time was 4 years, 1.5 months (range: 6 months–13.5 years). At baseline, 79% had heart failure, 66% were failing to thrive, and 70% had evidence of focal pulmonary collapse. At follow-up, 60% were ventilator-free and 30% were ambulatory. The higher than previously reported number of CRIM-negative patients in this cohort may explain the heterogeneity of outcomes. Details of patients identified by newborn screening and treated presymptomatically are discussed below.

In LOPD, a retrospective and prospective study of 62 patients (59 treated)<sup>41</sup> in the UK, followed for up to 3 years, there was a significant improvement in the 6-minute walk test and muscle strength; there was no correlation between time on ERT and respiratory function or body mass index.

Van der Meijden et al<sup>42</sup> reported the largest cohort of patient-reported outcomes, from the International Pompe Association, from 408 patients (seven primary participating countries) during 2002–2013. They describe the natural history of the disease with impact on daily functioning and quality of life. In 268 untreated patients, the 5-year survival rate from diagnosis was 98% and the respective 10-, 20-, and 30-year survival rates were 83%, 65%, and 40%. In a survival model in 283 patients on ERT, it was estimated that at any given time, a patient on ERT had a 59% less risk of dying than an untreated patient.

## Other therapies

### Diet therapy

Supportive treatment with a low carbohydrate, high protein diet, and aerobic exercise<sup>43</sup> may help prevent decline by decreasing glycogen storage and improving fatty acid oxidation but will not impact irreversible muscle damage. Treatment with a high protein diet and l-alanine supplementation in late-onset patients<sup>44</sup> did not have a significant effect.

## Gene therapy

Early attempts at gene therapy with adenovirus and adeno-associated virus (AAV) vectors in the Pompe disease mouse model were mostly unsuccessful due in part to nonsustained expression of enzyme over time and/or immune responses. Further modification of the AAV vectors with specific serotypes that have less immunogenicity and the addition of tissue specific promoters, such as for the liver,<sup>45</sup> have resulted in a high degree of efficacy in mouse models. There are currently many ongoing clinical trials<sup>46</sup> utilizing AAV gene delivery systems.

A Phase I/II clinical trial of intra-diaphragmatic delivery of recombinant AAV1-human GAA (rAAV1-human GAA)<sup>47</sup> was carried out in five ventilator-dependent children. Following injection, all subjects showed improvements in respiratory function, including increased length of time tolerated off assisted ventilation.

In humans, however, prior exposure to the AAV in early life<sup>45</sup> can result in a potent humoral immune response when treated with an AAV therapeutic, inducing neutralizing antibodies that can impact efficacy and may compromise repeat dosing if necessary. In order to mitigate this risk, Corti et al<sup>48</sup> have developed a strategy to utilize an immune modulation protocol with pre- or coadministration of rituximab and sirolimus (previously shown to ablate B-cells in Pompe patient who had a high antibody response to ERT) with adeno-associated virus-mediated gene transfer of wild-type desmin cDNA (AAV-DES)-human GAA in a GAA mouse model. This strategy is being evaluated on single and repeat vector dosing preclinical studies (rodents and nonhuman primates) to assess the production of neutralizing antibodies, bio-distribution of the vector, and whether this strategy might enable readministration of the same AAV vector over time. Presumably, if shown to be safe, they will proceed with a proposed Phase I/II study in patients with LOPD, injecting the tibialis anterior muscle.

Other viral vectors have also been explored. Recently,<sup>49</sup> lentiviral-mediated therapy has shown some promise in neonatal murine models of mucopolysaccharidosis type I. A similar approach using the lentiviral vector encoding human GAA in a neonatal murine Pompe disease model<sup>50</sup> has resulted in sustained expression after 24 weeks with clearance of glycogen and no significant immune response.

Another approach<sup>51</sup> has been to genetically modify hematopoietic stem cells *ex vivo* using a lentiviral-mediated vector to overexpress human GAA. The cells were then transplanted back to the donor mice with ~35% engraftment, glycogen clearance, and increased exercise tolerance.

Douillard-Guilloux et al<sup>52</sup> used lentiviral vector-mediated short hairpin RNA to target the muscle form of the glycogen synthase enzyme and reduce endogenous production of glycogen. The mice showed decreased accumulation of glycogen and improved exercise tolerance.

### Enhanced ERT

While the currently approved rhGAA can significantly improve cardiac disease, there may still be progression of weakness, particularly in the skeletal muscle, which primarily utilizes the cation-independent mannose-6-phosphate receptor for enzyme uptake.<sup>53</sup> In order to take advantage of this fact and provide improved delivery of rhGAA to the lysosome, new technologies have been explored.

Neo-rhGAA has been chemically engineered<sup>54</sup> to increase enzyme uptake by the addition of mannose-6-phosphate residue-bearing synthetic oligosaccharides, resulting in increased cellular uptake and glycogen clearance in the Pompe disease mouse model. A clinical trial<sup>55</sup> is currently underway to assess the safety and efficacy in repeat dosing of neoGAA in patients with LOPD.

A fusion protein<sup>56</sup> has been developed that utilizes glycosylation-independent lysosomal targeting. The protein contains rhGAA fused with a portion of insulin-like growth factor II, which has a higher affinity for the cation-independent mannose-6-phosphate receptor. In the Pompe mouse model, there has been a significant increase in glycogen clearance in skeletal muscle.

A Phase I/II study in 21 patients with LOPD<sup>57</sup> treated for 24 weeks showed improvement in pulmonary function (maximum inspiratory and expiratory pressures) and endurance (6-minute walk test). These improvements were maintained in an extension study. Hypoglycemia was noted (due to increased insulin-like growth factor II activity), but was transient, and managed with increased calorie intake. Two patients withdrew from the study due to infusion-related reactions. A Phase III study of safety and efficacy is underway.

### Chaperone therapy

Small molecule pharmacologic chaperones are currently<sup>58–60</sup> being evaluated as therapeutic options in several single gene disorders, including lysosomal storage disorders, where the pathology can be attributed to a misfolded but still functional enzyme, albeit, with reduced activity. The misfolded mutant enzyme is recognized as aberrant by the quality control system in the endoplasmic reticulum of the cell, and is subsequently tagged for degradation, or, in some cases, retention in the endoplasmic reticulum.

The chaperones thus far studied in Pompe disease<sup>61</sup> are the imino sugars, deoxynojirimycin and N-butyldeoxynojirimycin, which bind to the active site of the enzyme. The chaperone acts by stabilizing the enzyme after translation so that it is able to exit the endoplasmic reticulum, rather than be tagged for degradation and can then be targeted normally to the end organelle, in the case of Pompe disease, the lysosome. There, the chaperone dissociates, allowing the enzyme to clear substrate. The mutations that result in a misfolded enzyme are typically missense or small insertions or deletions.

In preclinical studies in the Pompe disease murine model and in human fibroblasts, the chaperone, deoxynojirimycin (DNJ), was able to enhance the activity of the mutant enzyme. Subsequently, a phase II study of DNJ (Amicus Therapeutics AT2220, NCT00688597) was initiated in adult patients with Pompe disease.<sup>62,63</sup> Unfortunately, the first three patients enrolled showed an increase in muscle weakness and biomarkers, consistent with disease progression; the study was prematurely terminated. In order to understand the results of this study, a study of the muscle pharmacokinetics of AT2220 was carried out in normal healthy volunteers and showed that the intramuscular half-life of the chaperone was greater than anticipated compared to plasma and thus would effectively cause prolonged inhibition of GAA. It was also observed that the pharmacologic chaperone could stabilize the recombinant enzyme, and when used as an adjunct to ERT, could potentially increase the plasma half-life and allow for greater tissue penetration.

## Newborn screening

Newborn screening for inborn errors of metabolism has been one of the great public health successes since its introduction in the 1960s in most of the industrialized world. In the last 10 years, there has been a significant increase in the number of disorders that can be screened because of advancements in the technology used for high-throughput screening of newborn blood filter paper samples. In 2004, the American College of Medical Genetics<sup>64</sup> surveyed physicians with expertise in these disorders and other interested parties to determine a consensus for a uniform panel. Two of the major criteria for inclusion were availability of a suitable test and treatment. At that time, ERTs were available or in development, but a suitable test was not. Currently, methods are available and are being evaluated in pilot studies<sup>65</sup>: a fluorometric enzyme assay (a miniaturization of the method discussed earlier); a tandem mass spectrometry method for measuring residual enzyme activity, multiplexed with assays for four other lysosomal storage disorders<sup>66</sup>; a protein quantification method has also been developed,<sup>67</sup> but not

introduced; and a digital microfluidics enzyme assay<sup>68</sup> method where discrete micro droplets of blood are manipulated under an electric field on a microchip.

The data from a pilot study, utilizing the fluorometric assay in Taiwan,<sup>16</sup> are impressive. Chien et al<sup>69</sup> were able to show that four infants with IOPD (evidence of cardiomegaly, left ventricular hypertrophy, and generalized accumulation of quadriceps muscle glycogen) detected and treated early (before the age of 1 month) showed reversal of cardiac disease and normal growth and development at the time of reporting. A more recent report<sup>70</sup> of these four patients and an additional six IOPD children, followed for 28–90 months, confirmed the rapid response of the cardiomyopathy by 6 months of treatment, remaining stable. All were ventilator-free. All, however, showed slower motor development after approximately 2 years of age, with proximal muscle weakness and orofacial symptoms (facial weakness, nasal speech, hearing loss); three patients had mild cognitive delays (requiring special help with language and mathematics). All patients developed low titers of anti-rhGAA antibody that declined over time. All of these patients were CRIM-positive.

Thirteen later-onset Pompe disease patients<sup>71</sup> were also detected by newborn screening (no evidence of cardiac involvement) and followed for up to 4 years. Four patients were started on ERT between the ages of 1.5 and 3 years because of onset of hypotonia and delayed motor milestones (no outcomes were reported). The other nine patients continue to be followed every 3–6 months.

In the USA, Pompe disease was recently evaluated and recommended, in March 2015, to be added to the universal screening panel by the federal Secretary's Advisory Committee on Heritable Disease in Newborns and Children, in the Department of Health and Human Services. The final decision, however, is made by individual states. Pompe disease is currently screened or in the process of being implemented, in several states. Several other states are considering adding it to their current newborn screening panels, but have not yet been approved. Two different methods are being used, tandem mass spectrometry assays multiplexed with assays for other lysosomal storage disorders and digital microfluidics.

Results were recently published from the state of Missouri, USA,<sup>72</sup> for the first 6 months of screening by digital microfluidics for Pompe, Gaucher, and Fabry diseases, and mucopolysaccharidosis type I. A total of 43,701 samples were screened, with 18 positive for Pompe disease; six were subsequently confirmed to have Pompe disease, three infant-onset and three late-onset disease; two were found to carry a pseudo-deficiency mutation, three were carriers, two

had genotypes of unknown significance, and four were false positives. The incidence rate of 1:5,463 is much higher than the generally accepted published incidence of 1:40,000, but similar to the reported incidence in a Hungarian study<sup>73</sup> of 40,024 samples in which nine cases were confirmed, 25 were carriers, 27 were normal (false positives), and three had a genotype of unclear significance.

## Summary

Pompe disease is a rare autosomal recessive genetic disorder due to a deficiency of the lysosomal hydrolase enzyme, GAA, caused by mutations in the *GAA* gene. It presents with a broad phenotypic spectrum. The advent of ERT with recombinant GAA has resulted in significant improvements in morbidity and mortality, although some patients continue to decline despite therapeutic intervention. New strategies are being developed to improve delivery of enzyme through various methods to alter the natural history of the disease. Newborn screening has shown that early therapeutic intervention, before irreversible muscle damage has occurred, may provide the greatest clinical benefit.

## Disclosure

The authors report no conflicts of interest in this work.

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