

Pompe disease gene therapy

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Received March 29, 2011; Revised and Accepted April 16, 2011

Pompe disease is an autosomal recessive metabolic myopathy caused by the deficiency of the lysosomal enzyme acid alpha-glucosidase and results in cellular lysosomal and cytoplasmic glycogen accumulation. A wide spectrum of disease exists from hypotonia and severe cardiac hypertrophy in the first few months of life due to severe mutations to a milder form with the onset of symptoms in adulthood. In either condition, the involvement of several systems leads to progressive weakness and disability. In early-onset severe cases, the natural history is characteristically cardiorespiratory failure and death in the first year of life. Since the advent of enzyme replacement therapy (ERT), the clinical outcomes have improved. However, it has become apparent that a new natural history is being defined in which some patients have substantial improvement following ERT, while others develop chronic disability reminiscent of the late-onset disease. In order to improve on the current clinical outcomes in Pompe patients with diminished clinical response to ERT, we sought to address the cause and potential for the treatment of disease manifestations which are not amenable to ERT. In this review, we will focus on the preclinical studies that are relevant to the development of a gene therapy strategy for Pompe disease, and have led to the first clinical trial of recombinant adeno-associated virus-mediated gene-based therapy for Pompe disease. We will cover the preliminary laboratory studies and rationale for a clinical trial, which is based on the treatment of the high rate of respiratory failure in the early-onset patients receiving ERT.

INTRODUCTION

The concept of gene therapy for lysosomal storage disease was borne out of the finding that this category of genetic disease is exclusively due to recessive inheritance, and that a single gene is responsible for all primary disease manifestations. Of course, there are many secondary abnormalities that are associated with the main pathophysiology and would not contribute to the disease in the absence of the primary causative mutations. An important aspect of the decision to pursue a gene therapy strategy for any condition is that the pathophysiology related to gene function is fully understood, specifically: Is the condition due solely to the target gene? Is the therapeutic range large enough to allow for unregulated gene

expression? Is tissue-restricted expression required and what are the immunological consequences of transgene expression? The approach of gene augmentation or replacement therapy is likely to fit best with these criteria and therefore appropriate for a recessive condition like Pompe disease.

Pompe disease is best characterized as a metabolic myopathy in the broad category of muscular dystrophy, as the major disease manifestations are cardiac and skeletal muscle weakness. However, since the affected gene [acid alpha-glucosidase (GAA)] in Pompe disease is required to some extent in all tissues, disease manifestations are found in most organ systems. Indeed, the requirement for glycogen degradation in lysosomes is directly related to the rate of glycogen synthesis in each tissue. The recent advent of enzyme replacement

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therapy (ERT) for Pompe disease (1,2) has led to a new natural history that is being defined by a longitudinal registry of treated and untreated patients (3). Additional observations from the patient population in clinical studies has helped to define a number of second or late manifestations of Pompe disease, which were unrecognized when survival of the severe or early-onset patients was limited to 8 months of age (4).

The transition from preclinical studies (5,6) to human clinical studies offers a unique opportunity to study the pathophysiology of Pompe disease and provides important insight into human disease findings that can be evaluated in the mouse model (3,7–9). The goal of this review is to establish the basis for gene therapy in Pompe disease by discussion of the extensive preclinical data from a number of groups which is relevant to this approach as well as to review the study design and regulatory approval process for the initial clinical experience in adeno-associated virus (AAV)-mediated gene therapy for Pompe disease.

EARLY EVIDENCE OF GENE CORRECTION FOR POMPE – *IN VITRO* STUDIES

Initial proof-of-concept studies focused on the demonstration that Pompe patient-derived cells would faithfully express human GAA (hGAA) and target the recombinant protein to the lysosome (10–12). The early studies on the complementation of GAA activity in deficient cells also demonstrated the concept of cross-correction via tissue culture medium, which was further support for the concept of ERT. Subsequently, studies in the knockout mouse model of Pompe (13,14) was used to reach beyond the restoration of enzymatic activity and levels of glycogen accumulation to the important challenge of correcting the physiological consequences of Pompe disease.

CARDIAC GENE THERAPY

The majority of early-onset patients with null or severe missense mutations are found to have progressive cardiomyopathy, which is responsible for early mortality (4,15). A natural history study of the untreated patients found that the average age of death in this group was 8 months old, owing to cardiorespiratory failure (1). The cardiac findings in early-onset Pompe disease include shortened PR interval and large amplitude R-wave on ECG and severe cardiomegaly as observed by chest X-ray (16). In the pivotal trial for Myozyme approval, echocardiographic analysis demonstrated the severe increased cardiac mass and dysfunction at baseline, and following 52 weeks of Myozyme therapy there was reduction of left ventricular (LV) mass index (193.4 to 86.8 gm/M²) post-treatment and LV mass Z-scores decreased from 7.1 to 3.3 at 52 weeks (17). The observed changes in ejection fraction are complicated by the extensive remodeling of the left ventricle with glycogen depletion, and subgroup analysis has highlighted a subset of patients who can be identified at baseline to be less likely to have significant reduction in LV mass index (3,17). Therefore, earlier diagnosis and alternative solutions mediating higher cellular levels of hGAA within the myocardium would be desirable

to promote a reduction in LV mass and improved cardiac function.

A genetically modified mouse model (*Gaa*^{-/-}) displays the Pompe phenotype and has been instrumental in elucidating the consequences of absence or deficiency of GAA and has been an invaluable tool in assessing the functional improvements following gene therapy strategies (13). The mouse exhibits many features of both early- and late-onset disease; however, the degree of cardiac enlargement is not as extensive as the early-onset patient and affected mice live into adulthood. Abnormalities in cardiac function, electrocardiogram and LV mass are apparent in *Gaa*^{-/-} mice by 6 months of age (13,18–20).

High-field cardiac magnetic resonance imaging detects LV hypertrophy with diminished cardiac output and ejection fraction in *Gaa*^{-/-} mice (7,18,21–23). Our group and others have shown AAV-mediated expression of hGAA in cardiac tissue and clearance of glycogen via systemic or via cross-correction from liver-mediated production of GAA (7,9,12,18,21–29). The efficacy of a single intravenous AAV vector injection results in high transduction and therefore significant hGAA expression within the myocardium, ultimately leading to restoration of function during early and late (adult) stages of disease (18,21,26,30,31). Systemic administration of AAV1-CMV-hGAA in newborn mice resulted in ~70% reduction in cardiac glycogen content 1 year post-injection, while significantly normalizing the shortened PR interval and reducing LV mass (7,18). Subsequent experiments using alternative serotypes resulted in increased transduction efficiency of cardiac tissue when administered systemically. Quantification of gene expression following systemic delivery of AAV9-CMV-LacZ resulted in a 200-fold increase in expression when compared with transgene delivery via AAV1 (22). Initial studies showed that systemic administration of a therapeutic rAAV2/9 vector could lead to increased GAA activity and clearance of glycogen in cardiac and skeletal muscles (21,26). In addition to biochemical correction, systemic delivery of rAAV2/9-CMV-hGAA in newborn *Gaa*^{-/-} or adult mice where cardiac abnormalities and signs of muscle weakness were already present lead to correction of the shortened PR interval by an average of 5 ms. Quantitative glycogen measurement, and periodic acid schiff staining and electron microscopy all showed clearance of glycogen and healthy sub-cellular morphology in the hearts of treated mice (21). Verification of AAV9-mediated transduction and expression of hGAA was verified in rhesus macaques, where ~5 fold increase over basal levels of GAA was detected in heart tissue 6 months after dosing (22).

In summary, experiments evaluating the efficacy of systemically delivered AAV–GAA portray high-level transduction in cardiac tissue leading to the reduction of glycogen content and restoration of cardiac morphology and function. However, subtle residual effects on cardiac function are observed in the animals at the dose level to date, suggesting secondary adaptations as a result of prolonged glycogen accumulation or possible effects of lysosomal dysfunction on Ca²⁺ flux or ventricular relaxation. Subsequent studies may include adjunctive therapies to address the downstream events which occur (i.e. mitophagy, loss of calcium homeostasis, increased proteolysis).

AAV VECTORS IN MUSCULOSKELETAL GENE TRANSFER FOR POMPE

Similar to the Pompe patient population, *Gaa*^{-/-} mice have a progressive skeletal muscle weakness and loss of diaphragm contractile strength with progressive age. Isometric force–frequency relationships from diaphragm muscle isolated from untreated Pompe mice show a significant and progressive decrease in contractile strength with age from 3 months to 2 years (8). In addition, Pompe mice also exhibit attenuated ventilatory function *in vivo*, similar to juvenile and adult-onset patients (32).

Both systemic delivery of a therapeutic rAAV vector to directly transduce target tissues and delivery to a peripheral tissue in which correction of distal tissues is mediated by the uptake of secreted expressed enzyme (cross-correction) are the strategies that have mediated partial biochemical correction of the skeletal muscles and diaphragm in *Gaa*^{-/-} mice. Systemic delivery of rAAV2/1 to *Gaa*^{-/-} neonates resulted in long-term correction of skeletal muscles and diaphragm, with sustained GAA enzyme activity and glycogen clearance. Both soleus and diaphragm force mechanics were improved with approximately 90% of wild-type peak diaphragm contractile strength and corresponding improved ventilatory capabilities at 1 year post-treatment (18). In young adult *Gaa*^{-/-} mice, systemic delivery of therapeutic muscle-restricted rAAV2/8 or rAAV2/9 vectors significantly reduced glycogen content in striated muscle and diaphragm at 4.5 months post-treatment (26). In one study, *ex vivo* force mechanics on excised diaphragm sections from *Gaa*^{-/-} mice administered rAAV2/9 systemically demonstrated an improvement in the contractile force of treated mice and computed tomography/X-ray analysis showed a distinct decrease in spinal kyphosis (21). Of particular note, rAAV2/9 vectors have been shown to transduce striated muscle at equivalent or even at higher efficiency than the rAAV2/8 vector, and more importantly rAAV2/9 vectors have been shown to transduce more myofiber types, thereby giving it a potential advantage over other rAAV serotype vectors as a therapeutic vector for muscular dystrophies (22,26,30). Liver-directed delivery of rAAV2/5 and rAAV2/8 vectors using a cross-correction strategy have also resulted in significantly increased enzyme levels in the diaphragm and hindlimb muscles with concomitant reduction of glycogen content (9,24). The level of correction, however, is in part dependent on the presence or absence of a humoral immune response against the expressed and secreted rAAV-encoded GAA. Other factors such as age of the animal at the time of treatment and the prevalence of mannose-6-phosphate receptors in the affected tissues have also been implicated in the success of therapeutic interventions (8,28,29,31).

Currently, the only Food and Drug Administration-approved therapy for Pompe disease is Myozyme® recombinant ERT. Myozyme has been shown to improve ventilator-free survival rates in patients with infantile-onset disease, however long-term follow-up of subjects showed a progressive loss of independent ventilation with 22 of the original 38 subjects now either using assisted ventilation or have died. Furthermore, all subjects have demonstrated functional deficits in respiratory function and disease progression has not been

eliminated. Given these findings in the treated patient population, we have sought a further understanding of the incomplete response to ERT and identify additional therapeutic or adjunct therapy.

Direct administration of rAAV has yielded the highest degree of biochemical and functional correction of the diaphragm in the *Gaa*^{-/-} mouse model (25,33). Targeted administration of rAAV2/1-CMV-hGAA to diaphragms of *Gaa*^{-/-} mice resulted in near wild-type levels of GAA activity and the clearance of accumulated glycogen in the diaphragm tissue, both in younger animals as well as in older (2 years of age) mice with established disease. Of particular importance, targeted therapy also yielded significant improvement in the contractile strength of the diaphragm muscle. Gel-mediated delivery of rAAV2/1-CMV-hGAA to adult animals (3, 9 and 21 months of age) lead to significantly improved diaphragm muscle contractile strength at 3 months post-vector delivery and for a cohort treated at 3 and 9 months post-vector delivery when compared with age-matched untreated controls. Furthermore, all treated mice had improved *in vivo* ventilatory capabilities; however, the level of correction was affected by the level of disease progression at the time of therapy, in that the younger the age when treated (and therefore less-established pathology), the greater the functional improvement. Nonetheless, even the oldest cohort of animals showed significant biochemical and functional improvements suggesting that even patients with severe disease may benefit from targeted gene therapy (8,33).

RESPIRATORY DEFICITS IN POMPE – EVIDENCE FOR NEURAL GENE THERAPY

The severe weakness in Pompe disease patients has been attributed predominantly to muscle pathology (34), however, it has been known for sometime that glycogen accumulates in the central nervous system (CNS) of Pompe patients (32,35–39) and in animal models of the disease (32,40,41). Neurological motor symptoms have also been reported in case studies (39,42–45) and a recent report describes possible cognitive deficiencies in Pompe disease (46). Further understanding of the respiratory dysfunction in the *Gaa*^{-/-} mouse lead to the finding of considerable glycogen accumulation in phrenic motoneurons coupled with impaired ventilation (8,32) (Fig. 1). These data are particularly noteworthy since an autopsy report documented similar neuropathology in putative spinal motoneurons of a Pompe infant that had been treated with Myozyme (32). This CNS pathology is consistent with the notion that recombinant enzyme supplied into the systemic circulation does not effectively cross the blood–brain barrier (20,47) and therefore cannot mitigate GAA deficiency in the CNS. Accordingly, we suggest that variability in the success of enzyme replacement (48) could reflect persistent (untreated) CNS pathology. Consistent with this view, Muller *et al.* (49) reported that children with Pompe disease remain at high risk for speech disorders despite ongoing ERT. Our group has advanced the hypothesis that therapies targeting both skeletal muscle and the CNS may be required to fully correct respiratory-related motor deficits in Pompe disease (8,18,32). Recombinant AAV has been shown to be

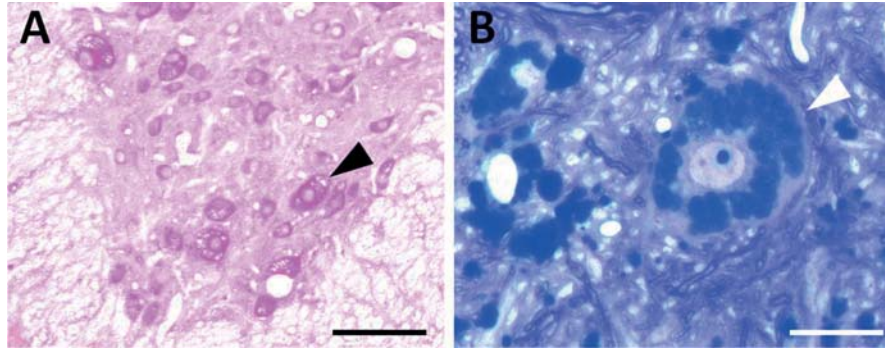


Figure 1. Histological evidence for neuronal glycogen accumulation in the spinal ventral horn of Pompe mouse model. Spinal tissues were harvested from 12-months-old *Gaa*^{-/-} mice. (A) This panel shows an example of paraffin-embedded tissue (10 μm sections) stained with the periodic acid schiff (PAS) method. The example is from the thoracic spinal cord and shows robust neuronal PAS staining (pink color), indicative of glycogen accumulation, throughout the ventral horn. The arrow indicates a large motoneuron with positive PAS stain. (B) This panel provides an example from another *Gaa*^{-/-} mouse in which tissue was plastic-embedded, cut at 2 μm and stained with toluidine blue. The neuron indicated by the arrow is located in the C4 ventral horn. Note the extensive accumulation of droplets in the cytoplasm with no accumulation in the nucleus. Scale bars indicate 100 (A) and 20 μm (B).

capable of efficiently transducing neural tissue either by direct administration or via retrograde transport of vector after intramuscular delivery (50,51). In preclinical studies, initial data show that direct administration of rAAV2/1 to the diaphragm of an adult *Gaa*^{-/-} mouse is associated with increased efferent phrenic nerve activity, in addition to the aforementioned diaphragm biochemical and functional correction, suggesting that rAAV-mediated gene therapy can also modulate CNS activity in Pompe disease (8). In this regard, gene transfer methods using AAV may be particularly attractive since a single skeletal muscle injection has the potential to transduce (and therefore ‘treat’) both muscle cells and motoneurons.

IMMUNOLOGICAL COMPLICATIONS OF THERAPY FOR POMPE DISEASE

Based on the current standard of care for the treatment of Pompe disease, ERT is administered to symptomatic patients. Treatment with ERT has led to anti-GAA antibody titers in most patients and a high rate of infusion-associated reactions (17,52). Similarly, *Gaa*^{-/-} mice generate strong humoral immune responses to recombinant human GAA. In addition to IgG, IgE formation has been observed, which likely causes the fatal anaphylactic reactions that occur in these animals after repeated intravenous infusion of the enzyme (53). This response can be suppressed with anti-histamine drugs. Studies on CD4⁺ T-helper cell responses in mice and humans are needed to (i) define the mechanism that leads to antibody formation, and (ii) uncover whether similar mechanisms are at play in both species.

In *Gaa*^{-/-} mice, tolerance induction to GAA by hepatic AAV gene transfer prevents the predisposition to anaphylactic reactions (53). Hepatocyte-derived expression induces transgene product-specific immune tolerance. This is in part via TGF- β -dependent induction of CD4⁺CD25⁺FoxP3⁺ Treg, which actively suppresses B and T cells (54–56). Alternative approaches towards immune tolerance induction can be developed using immune-suppressive drugs, such as rapamycin or B cell-depleting antibodies (57,58).

In patients, anti-rhGAA antibodies negatively affect the efficacy of ERT (59). CRIM⁻ patients in early-onset Pompe

disease are most severely affected with some CRIM⁺ patients also developing antibody responses. Combinations of Rituximab and methotrexate only or in combination with Bortuzomib and cyclophosphamide have controlled the anti-rhGAA IgG responses (57). In those subjects who developed hypersensitivity reactions owing to IgE formation, the use of anti-IgE (omalizumab) can be effective in preventing allergic/anaphylactic reactions (46). Ultimately, effective tolerance protocols should be further developed in a way that reduces the dependence on immune-suppressive reagents, and these strategies will have an important impact on future gene therapy protocols.

Gene therapy has been facilitated by the improvement of viral vector delivery systems, due to their transgene carrying capacity and effective targeting of individual mammalian cell types and organs. These vectors, however, may be targeted by the immune system that is geared towards removing offending pathogens. Activation of the innate and adaptive immune system against the vector or transgene product can limit gene therapy. AAV induces a low, transient innate response accompanied by complement activation, TLR-9 signaling and plasmacytoid dendritic cell activation. However, these responses are minimal when compared with other viral vectors (58). In humans, pre-existing neutralizing antibodies and memory responses to the capsid may result in the early exposure to wt-AAV in childhood and can reduce the efficiency of gene transfer (60). The occurrence and magnitude of adaptive responses against the GAA transgene product vary depending on the vector serotype, route of administration, dose, promoter and the targeted organ (24,31,61–63). For example, IgG1 formation against GAA has been found to occur in response to liver-directed AAV2/8- and AAV2/5-DHBV-hGAA in *Gaa*^{-/-} mice, albeit that such responses may be impacted by the vector dose and strain background (24). For example, low-dose adjunct liver-directed AAV2/8-LSP-hGAApA administration followed by ERT has been shown to reduce IgE-mediated hypersensitivity in *Gaa*^{-/-} mice (53). Transient immune suppression has been used to circumvent innate and adaptive responses in gene therapy and ERT for Pompe disease and other protein deficiency disorders (58,64). Thus, such transient immune

Time Line: Phase I/II rAAV1-hGAA

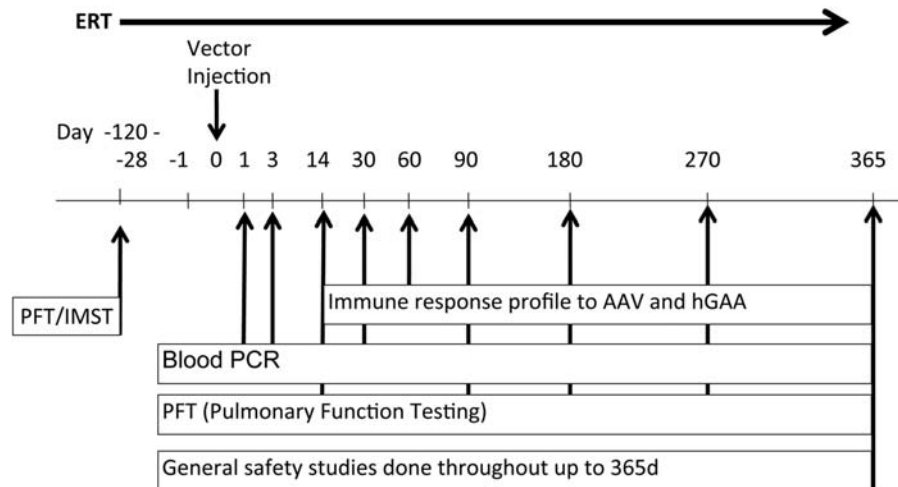


Figure 2. Timeline of phase I/II clinical trial for Pompe disease. Following subject enrollment, a period of inspiratory muscle strength training is started prior to study agent dosing. Enzyme replacement therapy is continued throughout the study. Safety and exploratory efficacy endpoints are assessed for 1 year.

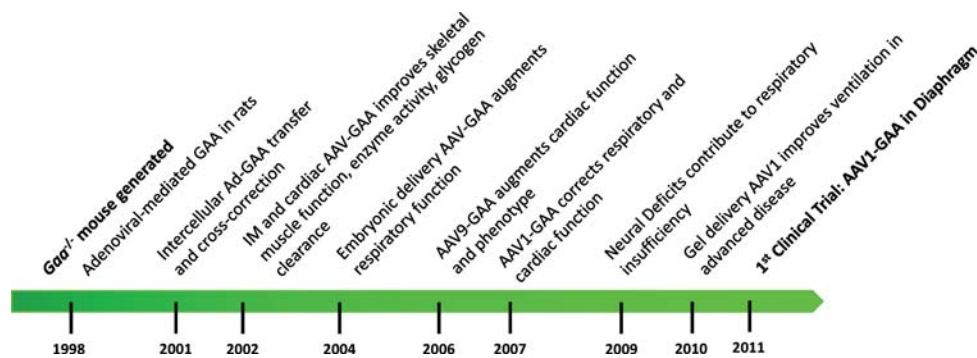


Figure 3. Development of gene therapy for Pompe disease. The mouse model for the Pompe disease was developed at the same time as *in vitro* gene transfer studies were established and has been invaluable in working towards the current clinical studies.

suppression protocols may be feasible in combination with gene therapy.

PHASE I/II CLINICAL TRIALS IN POMPE PATIENTS WITH VENTILATORY FAILURE

A successful gene therapy strategy for the treatment of Pompe disease would address many of the disease manifestations, which are defined by a new natural history with the regulatory approval of ERT. Although the ultimate goal is to simultaneously correct all affected tissues, initial clinical trial efforts will focus on establishing the safe and effective delivery of AAV vectors to dystrophic muscle using an approach that is clinically relevant. To this end, an open label, phase I/II study administering rAAV2/1-CMV-hGAA by direct intramuscular injection to the diaphragm of Pompe human subjects has been initiated (ClinicalTrials.gov Identifier: NCT00976352). This phase I/II study is designed to target respiratory insufficiency which is the most life-threatening manifestation of Pompe. The target population for this study is

children aged 3–14 who are dependent on mechanical ventilation despite ERT. The subject population for this study is well defined because of disease progression to the point of ventilatory failure. These children represent the more severely affected spectrum of Pompe patients and the population most in need of improved therapeutic strategies. Figure 2 shows the time line for the current gene therapy study.

The feasibility of this approach for the treatment of Pompe disease has been well-established by a wide range of preclinical data. Direct intramuscular injection of rAAV vectors has been assessed in other clinical trials, setting precedence for the safety of AAV-mediated gene transfer (65–67). Although gel-mediated delivery was used for direct delivery in the pre-clinical mouse model, direct injection can be used in the human subjects. Direct injection of rAAV2/1 in rabbit diaphragm resulted in detectable copies of vector throughout the diaphragm with the highest concentration of vector and gene expression being noted around the injection site. In the first cohort, we do not expect a significant clinical benefit, however in the higher dose cohort, there is an intended clinical benefit on phrenic nerve output based on preclinical studies.

CONCLUSIONS

Based on preclinical and clinical data, the important concept of adjunctive therapy for Pompe disease is being used in the current clinical study of rAAV-mediated gene therapy for Pompe (see Fig. 3 for timeline). Future studies may be directed at simultaneously correcting cardiac, respiratory and neural components of disease pathology, which may entail systemic delivery of a vector where transduction is directed by the route of delivery and tissue-restricted promoters. Ongoing preclinical studies in the mouse model will result in continually refining gene therapy strategies. Most importantly though, the first critical step in generating a successful AAV-based gene therapy for Pompe disease has been achieved by the initiation of a clinical study in a patient population most in need of additional treatment strategies.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the University of Florida Powell Gene Therapy Center Vector Core Laboratory, which produced the rAAV vectors for preclinical studies at UF, the UF Toxicology Core Laboratory for vector toxicology analysis and the Human Applications Laboratory that generated the rAAV vectors for clinical trial. Special thanks to Drs Kai Qiu and Paul Reier for assistance with the analysis of spinal cord tissue samples and Nina Raben for the original strain of *Gaa*^{-/-} mice.

Conflict of Interest statement. None declared.

FUNDING

This work was supported by grants from the National Institutes of Health [NHLBI P01 HL59412-06, NIDDK P01 DK58327-03, 1R01HD052682-01A1, NHLBI-1F32HL095282-01A1 (D.J.F.) and the NHLBI Gene Therapy Resource Program]. B.J.B., C.A.P., D.D.F., C.S.M., The Johns Hopkins University and the University of Florida could be entitled to patent royalties for inventions described in this manuscript.

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