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Inspiratory muscle conditioning exercise and diaphragm gene therapy in Pompe disease: clinical evidence of respiratory plasticity

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Abstract

Pompe disease is an inherited disorder due to a mutation in the gene that encodes acid α -glucosidase (GAA). Children with infantile-onset Pompe disease develop progressive hypotonic weakness and cardiopulmonary insufficiency that may eventually require mechanical ventilation (MV). Our team conducted a first in human trial of diaphragmatic gene therapy (AAV1-CMV-GAA) to treat respiratory neural dysfunction in infantile-onset Pompe. Subjects (aged 2-15 years, full-time MV: n=5, partial/no MV: n=4) underwent a period of preoperative inspiratory muscle conditioning exercise. The change in respiratory function after exercise alone was compared to the change in function after intramuscular delivery of AAV1-CMV-GAA to the diaphragm with continued exercise. Since AAV-mediated gene therapy can reach phrenic motoneurons via retrograde transduction, we hypothesized that AAV1-CMV-GAA would improve dynamic respiratory motor function to a greater degree than exercise alone. Dependent measures were maximal inspiratory pressure (MIP), respiratory responses to inspiratory threshold loads (load compensation: LC), and physical evidence of diaphragm activity (descent on MRI, EMG activity). Exercise alone did not change function. After AAV1-CMV-GAA, MIP was unchanged. Flow and

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volume LC responses increased after dosing ($p < 0.05$ to $p < 0.005$), but only in the subjects with partial/no MV use. Changes in LC tended to occur on or after 180 days. At Day 180, the four subjects with MRI evidence of diaphragm descent had greater maximal voluntary ventilation ($p < 0.05$) and tended to be younger, stronger, and use fewer hours of daily MV. In conclusion, combined AAV1-CMV-GAA and exercise training conferred benefits to dynamic motor function of the diaphragm. Children with a higher baseline neuromuscular function may have greater potential for functional gains.

Keywords

Pompe disease; Diaphragm; Gene therapy; Ventilatory insufficiency

INTRODUCTION

Pompe disease is an inherited disorder due to a mutation in the gene that encodes acid α -glucosidase (GAA), the enzyme needed to degrade lysosomal glycogen. In the most severe infantile form of Pompe disease, patients produce no appreciable GAA, which results in profound accumulation of lysosomal glycogen, particularly in cardiac, hepatic, muscular and neural tissue.(DeRuisseau et al., 2009; Raben et al., 2002) Infants present weeks to months after birth with failure to thrive, severe hypotonia, macroglossia, and hypertrophic cardiac failure.(Raben et al., 2002; van den Hout et al., 2003) This cardiopulmonary failure historically resulted in death in infancy.(van den Hout et al., 2003)

An FDA-approved recombinant enzyme-replacement therapy (ERT) intravenously delivers the missing GAA enzyme and has significantly reduced mortality of infantile-onset patients, with accompanying reductions of hypertrophic cardiomyopathy and skeletal muscle glycogenosis.(Kishnani et al., 2006; Van den Hout et al., 2000) Despite the cardiac and skeletal muscle improvements, slowly progressive neuromuscular weakness persists.(Nicolino et al., 2009; Prater et al., 2012) The phrenic motor system is preferentially affected in Pompe disease, and the majority of infantile-onset survivors have eventually required external ventilatory support.(Nicolino et al., 2009; Van den Hout et al., 2004) It is thought that progressive neural pathology contributes to the gradual loss of function, since commercial ERT does not cross the blood-brain barrier.(Kikuchi et al., 1998; Raben et al., 2003) These data suggest that additional therapies may be needed to address phrenic dysfunction in Pompe.

In an effort to address this phrenic neuropathology, our group has implemented a phase I/II clinical trial of intramuscular gene therapy to the diaphragm, in children with the severe Pompe phenotype.(Smith et al., 2013) Our interim results illustrated the safety of the test agent and revealed a potential tidal volume and weaning benefit in the first five ventilator-dependent subjects. In contrast to the observed gains in dynamic respiratory function, we noted there was no change in maximal inspiratory pressure (MIP), a standard clinical estimate of static inspiratory muscle strength. However, static tests do not fully reflect a patient's ability to dynamically shorten and sustain minute ventilation over time.

Tests of dynamic respiratory function are advantageous over static tests because they account not only for tension but also the timing of the respiratory pump contractions. We noted previously that flow and volume compensatory responses to inspiratory threshold loads (termed load compensation, LC) differed between critically ill patients who were able to wean from MV and those who remained ventilator-dependent.(Smith et al., 2014) We are also interested in whether LC responses differ in partial and full-time MV users with neuromuscular disease, and whether the LC responses could be used to document a therapeutic effect. Additionally, dynamic magnetic resonance imaging (MRI) of the thorax offers the ability to visualize the actions of the diaphragm and chest wall during breathing. While MRI has documented early, preferential diaphragmatic paresis in adults with Pompe disease,(Gaeta et al., 2015) MRI is little-used in children. Finally, the presence of chronic indwelling diaphragm electrodes in one subject offered a unique opportunity to longitudinally measure changes in diaphragm activity after AAV1-CMV-GAA dosing.

Here we report the changes in dynamic respiratory function in children with severe, infantile-onset Pompe disease enrolled in a phase I/II clinical trial of AAV1-CMV-GAA gene therapy to the diaphragm. We hypothesized that, since AAV1-mediated gene therapy may reach phrenic motoneurons via retrograde transduction,(Mah et al., 2010) AAV1-GAA would improve result in improved inspiratory flow and volume to a greater degree than exercises and ERT alone. Further, we theorized that children with partial MV dependence would have a higher respiratory function at baseline and therefore have greater potential to improve phrenic neuromuscular performance.

METHODS

Design

This was a prospective analysis of the secondary study outcomes from a phase I/II clinical trial of diaphragmatic gene therapy in infantile-onset Pompe disease (NCT: 00976352, IND: BB14131). The Institutional Review Board at the University of Florida approved the study design and procedures. Subjects were enrolled during a Screening visit, and then a period of inspiratory muscle conditioning exercise was implemented as a pre-operative control. Baseline function was recorded the day prior to dosing. After dosing with rAAV1-CMV-GAA, exercise was continued post-operatively. We compared the pre-operative period of exercise, which lasted approximately 90 days, to post-dosing tests conducted every 90 days for one year. The study design and specifics of dosing were published previously (Byrne et al., 2014).

Subjects

Participants had a confirmed diagnosis of infantile Pompe disease by GAA assay blood test, fibroblast culture or mutational analysis and chronic respiratory insufficiency despite long-term use of ERT. Chronic respiratory insufficiency was defined as a requirement for one or more hours of MV assistance for a minimum of three months prior to enrollment. The first five subjects required 24-hour MV through an invasive airway. Then, when safety reports from the first five subjects were satisfactory (Smith et al., 2013), children with part-time and

non-invasive MV interfaces were eligible to participate. Informed consent was obtained from the parents or guardians, and school-aged children provided their assent to participate.

Exclusionary characteristics included a body mass under 10 kg, history of low platelet count or elevated INR, abnormal chemistry profile (elevated transaminases, alkaline phosphatases, bilirubin, or gamma-glutamyl transpeptidase), acute requirement for intravenous antibiotic therapy or corticosteroids, and/or having gene transfer agents six months prior to the study. Participants received ERT infusions every 1-2 weeks for >1 year and remained on ERT throughout the study. Subjects who participated in other therapeutic studies were not eligible.

Inspiratory Muscle Conditioning Exercises

Upon enrollment, participants were prescribed an inspiratory muscle conditioning exercise program, based upon MIP and their ability to breathe without support. Inspiratory muscle strength training (IMST) exercises were three days per week and consisted of 3-4 sets of 6-10 inspiratory efforts, using a modified threshold-training device (Threshold PEP or Accu-PEEP). The inspiratory port of the training devices remained closed until subjects generated a minimum (threshold) inspiratory pressure. Training devices were connected either directly to the tracheostomy tube, or to a facemask sealed over the mouth and nose, for patients without an invasive MV interface. Subjects trained at the highest tolerated load, where they generated at least 50% of their unassisted tidal volume while maintaining $SpO_2 > 92\%$. Subjects with an MIP of $< 3\text{ cm H}_2\text{O}$ initially used no training device. In addition to IMST, short periods of breathing with reduced or no ventilator support (endurance exercises) were prescribed two days per week for children who used the ventilator full-time. Parents of the participants were instructed in the exercise prescription and provided with a training diary to monitor progress at home. Additionally, investigators communicated regularly with families to monitor compliance, answer questions, and progress training.

Gene Therapy

Study vector was a clinical-grade adeno-associated virus vector serotype 1, with a cytomegalovirus promoter and human GAA cDNA (rAAV1-CMV-GAA) manufactured at The Powell Gene Therapy Center's Human Applications Laboratory, University of Florida. For each subject, three injections of AAV1-CMV-GAA were administered by direct visualization of each hemi-diaphragm into anterior, medial, and posterior regions via video assisted thorascopic procedure. Subjects 1-3 received 1×10^{12} vg (low dose), while subjects 4-9 received 5×10^{12} vg (high dose) of vector. Subjects were discharged from the hospital within three days. Further details on vector production, dosing, and safety profile are available in a previous publication. (Smith et al., 2013)

Respiratory Outcomes

Resting Breathing Pattern—Respiratory variables (inspired tidal volume (V_T), peak inspiratory flow (PIF), inspiratory pressure (P_I), inspiratory time (T_I), expiratory time (T_E), and duty cycle (T_I/T_{TOT})) were captured with a respiratory monitor (CO₂SMO, Philips-Respironics, Murrysville, PA) and data acquisition software (Analysis Plus, Respironics Inc, Murrysville, PA). The breathing pattern was recorded for 5 minutes after the child became

acclimated to the testing device, while distracted by a cartoon. Values obtained during the third minute were averaged and reported.

Maximal Inspiratory Pressure—Static pressure generation of the inspiratory muscles was tested with maximal inspiratory pressure (MIP) test. We used the one-way inspiratory occlusion method validated in pediatric ICU patients.(Harikumar et al., 2008) To summarize, the tracheostomy cuff was inflated for invasively ventilated subjects prior to the test. Then, subjects were removed briefly from the ventilator, and a pressure manometer and one-way valve were attached directly to the tracheostomy tube. For subjects who were not invasively ventilated, the manometer and one-way valve were connected to a mask that fully covered the nose and mouth. Exhalation was unrestricted, but the one-way valve blocked inspiration. The test lasted 15 seconds for subjects aged 3 years and under, and 20 seconds for subjects 4 years and older. The MIP test was repeated three times with at least two minutes of rest between repetitions, and the best effort was reported.

Load Compensation—Volume, flow and timing compensatory responses to inspiratory loads were evaluated using Threshold training devices while subjects breathed spontaneously without MV support. Respiratory sensors (Capnostat Plus, Philips Respironics, Murrysville, PA) were attached to the training devices and then connected directly to either the tracheostomy tube or a tightly-fitting facemask. Higher tension on the IMST device required a greater pressure effort from subjects to open the valve and receive air. At each LC pressure load (0, 5, 10 cm H₂O), subjects made 12-15 best-effort breaths, with visual feedback from a computer and strong encouragement from study staff. An estimated inspiratory workload was calculated for each LC breath (defined as the product of the inspiratory pressure * inspired volume), and the 6 breaths with the highest imposed workload were averaged for statistical analysis.

Dynamic MRI of diaphragmatic descent—T2-weighted gradient echo sequences (true fast imaging with steady-state free precession, TRUFI) of the chest without contrast were obtained from a 1.5T MR scanner (Siemens Avanto). The following parameters were used: TR = 84 ms; TE = 1.04 ms; thickness = 8 mm. The field of view ranged from the mid-cervical to lower thoracic spinal levels and took approximately 30 minutes to acquire. An MR-safe ventilator (LTV 1150) was brought in for full-time MV users. Two twenty-second scans were acquired during the following conditions: free-breathing ventilation with usual MV settings (full-time MV subjects only), free-breathing without MV (full-time and partial/no MV subjects), and deep, fast breathing without MV (partial/no MV subjects). Localizer scans visualized the costal diaphragm at end exhalation. Scans were obtained in the right and left sagittal planes, at the dome of the costal diaphragm as visualized by the localizer. MRI was conducted on the day before dosing and on Day 180.

To exclude effects of the A-P position or cardiac interference on diaphragm motion, we compared diaphragm descent in the right sagittal plane, during the free-breathing condition without MV. Diaphragm descent was analyzed with open-source imaging software (OsiriX Lite, Pixmeo, Geneva, Switzerland). The distance was measured between a fixed position at the apex of the lung and the attachment of the dorsal diaphragm to the posterior chest wall. The descent of the diaphragm was defined as the difference between the distance at end-

inhalation and the distance at end-exhalation. All breaths from the free-breathing acquisitions were used to obtain an average diaphragm descent. The inter-observer reliability of the diaphragm descent measurements was established between two independent observers blinded to the study design ($n = 5$ subjects, $ICC = 0.975$, $p < 0.001$). These observers remained blinded until the motion analysis was completed.

Diaphragm EMG—Two years prior to enrollment in the gene therapy clinical trial, one subject (#8) received a diaphragm pacemaker and weaned from fulltime MV dependence to nighttime support. The subject was maintained chronically on diaphragm pacing 30-60 minutes per day for 3-5 days/week, and continued this regimen in lieu of IMST exercises. Additionally, the implanted pacing electrodes were connected to data acquisition equipment (Power Lab 16/30, AD Instruments, Colorado Springs, Colorado) to record diaphragm activity during spontaneous and loaded breaths as well as MIP. Diaphragm activity at baseline was compared to D180 post-dosing.

Data analysis

First, the distribution of the data was tested. Subject descriptors were evaluated with either the independent t test (mean, SD) or Mann-Whitney U test (median, interquartile range). Relationships between demographics, MIP, and ILC responses were assessed with Pearson correlation. To evaluate the effects of baseline function and therapeutic interventions on LC responses, we used two-way repeated-measures ANOVA for MV support (full or partial dependence) and time (Screening/enrollment visit, Baseline at dosing, Day 90, 180, 365). Significant interactions were then subjected to Tukey's method of cell means contrasts. Row means were contrasted for significant main effects, using Tukey's corrections. All calculations were conducted using commercially available software (Prism v6, GraphPad, La Jolla, CA), and statistical significance was $p < 0.05$.

RESULTS

Demographics

All subjects experienced profound generalized hypotonia, developmental delay and chronic ventilatory insufficiency before age two. While all were diagnosed with infantile-onset Pompe disease and experienced chronic respiratory insufficiency, there was considerable variability between the participants (**Table 1**). At enrollment, subjects ranged in age from 2 to 15 years of age. Five required full-time MV via tracheostomy, two used nighttime noninvasive support, one used nighttime invasive MV and diaphragm pacing, and one did not use any ventilatory support. Since subjects #1 and #5 did not complete the 270-day tests, this time point was excluded from the group analysis.

Age of enrollment, age of dx, age of ERT

The age at enrollment was very strongly correlated to the duration of full-time MV dependence ($r = 0.963$, $p < 0.001$) and to the age at which ERT was initiated ($r = 0.832$, $p < 0.01$). There was also significant correlation between the age at which ERT was initiated and the duration of full-time MV dependence ($r = 0.815$, $p < 0.01$).

Baseline Respiratory Muscle Function and Relationship to MIP

Figure 1 depicts the MIP of full-time and part-time MV users. The initial residual respiratory muscle function in the tracheostomized, full-time MV users was significantly lower than the part-time/no MV users (median (IQR) of Full-time MV: 6.9 (1.8, 30.8) cm H₂O, Part-time/no MV: 72.8 (57.9, 83.6) cm H₂O, Mann-Whitney U, $p < 0.05$). MIP on enrollment significantly negatively correlated to both the hours of MV required daily ($r = -0.897$, $p < 0.005$) and the total duration since MV was started ($r = -0.738$, $p < 0.05$).

Most full-time MV users could not complete LC tests with a 10 cm H₂O load. Therefore, the LC responses were evaluated only with 0 and 5 cm H₂O loads. Significant correlations between MIP and LC responses were present upon enrollment. MIP correlated strongly with volume LC of 0 and 5 cm H₂O loads (volume at 0 cm H₂O: $r = 0.941$, $p < 0.001$; volume at 5 cm H₂O: $r = 0.859$, $p < 0.01$), and it was significantly related to inspiratory timing (TI at 0 cm H₂O: $r = 0.753$, $p < 0.05$; TI at 5 cm H₂O: $r = 0.800$, $p < 0.05$). MIP was correlated to PIF during 0 cm H₂O load, but not to the 5 cm H₂O load.

Effect of inspiratory muscle conditioning exercises on LC

One subject completed IMST exercises for 7 weeks prior to AAV1-GAA dosing (subject #3), while the remaining subjects completed 12 weeks of training. The changes in LC with 0 and 5 cm H₂O loads are illustrated in **Figures 2 and 3**; differences between the screening and baseline time periods represent the effects of exercise alone. Upon enrollment, both MIP and the LC varied considerably between the individual subjects, but within-subject day to day testing of subjects was consistent for MIP and volume. After the training intervention, partial MV users generated significantly higher PIF using the 5 cm H₂O load between screening and baseline (significant interaction effect, $F(4,28) = 5.992$, $p < 0.005$, **Figure 3A**). No other significant differences in VT, PIF, TI, TE, or TI/TTOT were detected between the screening and baseline test periods (**Figures 2 and 3**).

Effect of inspiratory muscle conditioning plus gene therapy on LC

Following AAV1-GAA dosing, significant group-time interactions were detected for flow ($F(4,28) = 3.419$, $p < 0.05$) and volume ($F(4,28) = 3.160$, $p < 0.05$) LC responses to 0 cm H₂O loads (**Figure 2A and B**). Cell means contrasts revealed that PIF was significantly higher at Day 180 than Screening and Baseline, and it was higher at Day 365 compared to Screening. Changes in PIF occurred only in the partial/no MV group. Additionally, volume was significantly higher in the partial MV group at Day 180, when compared to Screening and Baseline. The timing of LC breaths was not significantly different during 0 cm H₂O ILC.

Figure 3 depicts the LC responses for the 5 cm H₂O loaded breaths. With a 5 cm H₂O load, a significant load-time interaction was detected for PIF ($F(4,28) = 5.992$, $p < 0.005$, **Figure 3A**). For partial MV users, PIF was significantly higher at Day 365, when compared to Screening, Baseline, and Day 90. The body weight-adjusted ILC volumes remained stable over the study duration, but volume LC was significantly larger in partial MV users (main effect for MV use, $F(1,7) = 25.76$, $P < 0.005$, **Figure 3B**). Compared to the Screening visit, the duty cycle of all subjects' LC breaths was significantly longer at Day 180 and Day 365

(main effect for time, $F(4,28)=2.872$, $p<0.05$, **Figure 3E**). **Figure 3** depicts the LC responses for the 5 cm H₂O loaded breaths.

Effect of inspiratory muscle conditioning plus gene therapy on diaphragmatic movement

MRI was obtained in six subjects at baseline and in eight subjects at Day 180. Subject #8 could not be evaluated in MRI, due to preexisting indwelling diaphragmatic pacemaker electrodes. At Day 180, diaphragm motion was detected during free breathing, in four of eight subjects (**Figure 4**). Subjects with diaphragm movement at Day 180 tended to be younger, stronger, and less reliant on MV (Mann-Whitney U , $p>0.05$). The maximum voluntary ventilation achieved at Day 180 was significantly greater in subjects with observable diaphragm descent ($p<0.05$), but nevertheless remained <40% of predicted values for age, gender, and height.

Effect of inspiratory muscle conditioning plus gene therapy on diaphragm

EMG—Although MRI was not possible in subject #8 due to indwelling diaphragm electrodes, we recorded the spontaneous diaphragm activity during resting tidal breathing, 0 and 5 cm H₂O LC, and MIP maneuvers, both at baseline and on Day 180. The diaphragm EMG activity of subject #8 was progressively increased during the loaded breathing and MIP maneuvers (**Figure 5**). For absolute EMG, there was a significant load-time interaction (2-way RM ANOVA, $p<0.05$). At Day 180, the absolute RMS of the diaphragm was larger during the 0 cm H₂O LC ($p<0.005$) and MIP ($p<0.01$) conditions. When expressed as a percentage of the average MIP activity, the time-load interactions were strengthened (2-way RM ANOVA, $p<0.001$). During 0 cm H₂O LC, the relative diaphragm activity was significantly higher at Day 180 than at baseline. No differences in the relative EMG activity of the diaphragm were recorded during tidal breathing or the 5 cm H₂O LC condition.

DISCUSSION

The primary finding of this study was the dynamic motor function of the diaphragm appeared improved after AAV1-CMV-GAA gene therapy, as suggested by the LC, MRI, and EMG data. At 180 Days after dosing, inspiratory flow and volume generation increased over the pre-operative conditions. The ability of some participants to take a deeper breath was supported by diaphragm movement, as documented by MRI. The neuromuscular output generated by diaphragm contractions must translate to sufficient breath volume and flow, in order to maintain ventilation and maintain CO₂ homeostasis. The gains in flow and volume LC observed at Day 180 could be especially beneficial when patients must maintain ventilation during periods of increased breathing demands, such as during bronchoconstriction or mucus obstruction.(Sassoon, 2014; Smina et al., 2003) However, flow and volume LC improved only in the subjects with partial or no MV dependence upon enrollment. Since ventilatory independence is typically progressively lost in children maintained with standard doses of ERT,(Nicolino et al., 2009) we interpret these findings to suggest a potential benefit of AAV1-CMV-GAA for some of the subjects.

We did not measure a large benefit of inspiratory conditioning exercises alone on static or dynamic inspiratory muscle function. Our results differ somewhat from those reported by Jones et al, who reported a potential benefit of IMT on respiratory muscle strength, in at

least one of two children with Pompe disease.(Jones et al., 2014) In that case report, the children completed both inspiratory and expiratory exercises. Further, the exercise stimulus consisted of 5 sets of 50 breaths, which can only be sustained by using a lower-intensity resistance. These investigators also previously noted an IMT benefit in eight adults with mild to moderate functional impairment due to Pompe.(Jones et al., 2015) Importantly, none of the patients in either report was dependent on external ventilation or was tracheostomized.

Since adaptations to strength training require both ability to upregulate efferent neural drive and an appropriate motor response by effector muscle groups, we speculate there could exist an ideal therapeutic window for exercise interventions. Patients with more severe phrenic neuropathology manifested as extensive reliance on MV(Smith et al., 2016a) may then require alternative means to improve neuromuscular function, such as functional electrical stimulation (Smith et al., 2016b) or gene therapy.

In conjunction with the primary findings on dynamic respiratory function, maximal inspiratory pressure, the traditional measure of static inspiratory muscle function, did not appreciably change after AAV1-mediated therapy. Since the MIP maneuver typically elicits strenuous efforts in children for ~20 seconds, often while crying, it is unlikely the lack of change was due to an insufficient effort. Rather, the observation could be related to the properties of AAV1, which may have a somewhat higher affinity for oxidative fibers. (Kessler et al., 1996) We also appreciate that differences in intracellular trafficking of GAA and autophagy exist in Pompe, according to fiber type.(Fukuda et al., 2006; Shea and Raben, 2009) Maximal voluntary contractions including MIP recruit oxidative fibers as well as glycolytic fibers,(Mantilla et al., 2010) which produce a larger specific tension.(Geiger et al., 2000) MIP is a respiratory equivalent of a maximal voluntary contraction of the inspiratory pump, in the absence of inspiratory flow. This means that influences of airway and pulmonary mechanics as well as the force-velocity properties of muscle are minimized. However, MIP does not measure the ability of diaphragmatic contractions to rhythmically generate airflow and volume, which makes it a less functional maneuver.

In addition to measuring static and dynamic actions of the inspiratory muscle pump, we had the rare opportunity to record spontaneous diaphragmatic activity longitudinally in one child with chronic indwelling electrodes. These electrodes produce stable EMG recordings for up to one year in dogs,(Peterson et al., 1994) but the signal stability in humans is unknown. We identified increases in the RMS of the signal from the Baseline condition to Day 180, but longitudinal EMGs can be difficult in humans. Therefore, the EMG were normalized to the within-session RMS obtained during MIP. At Day 180, neither the absolute nor the relative diaphragm activity was enhanced at rest. Clinically, the diaphragm should use only a small fraction of its capacity to generate tidal breathing,(Mulreany et al., 2003) and a lower resting work of breathing is associated with a significantly greater ability to wean from MV. (Carlucci et al., 2009) On the other hand, it is desirable to maintain or increase respiratory output, when the work of breathing increases. The 180-day gains in normalized EMG during the 0 cm H₂O ILC condition suggested the subject had improved recruitment of local diaphragm fibers in the vicinity of the electrodes, with modestly increased breathing demands. However, a different approach or animal model(Falk et al., 2013; Mah et al., 2010)

is needed to distinguish between any potential neural, NMJ, or muscular mechanisms of remodeling that may have occurred after AAV1-CMV-GAA.

As the first in human investigation of muscle-directed AAV therapy, the study design was influenced by its potential risks and the sample size. Ethical concerns exist for including placebo or pure control groups in greater-than-minimal-risk clinical trials involving children. (de Melo-Martin et al., 2011) We also did not include a group that received gene therapy exclusively, without exercise. While standardizing the pre- and post-dosing exercise can help to minimize a potential confounding influence of activity on the AAV1-GAA therapeutic effect, it is a limitation of the study design.

Another limitation of this pivotal AAV1-GAA study is that the extent and durability of the study agent's therapeutic benefit is still not well-understood. The therapeutic benefit of AAV1-GAA in multi-nucleated cells will be dependent upon the % of nuclei transduced per muscle fiber, as well as the number of copies of AAV1-GAA present per nucleus. Myonuclei are added to muscle fibers with linear growth and with cycles of injury and regeneration. In growing children with a progressive muscle disease, both growth and regeneration should be expected in muscle fibers. Our data suggested that, for the dose and delivery of AAV1-CMV-GAA, a therapeutic benefit was achieved in subjects with partial MV dependence at Day 180, and some of this functional performance was maintained at Day 365. However, we acknowledge there is much to learn about the durability of the therapeutic benefit of muscle-directed gene therapy, and recognize the likelihood that re-dosing may be required.

CONCLUSIONS

The results from this phase I/II clinical trial of AAV1-CMV-GAA indicate that respiratory motor function improved in some children with infantile-onset Pompe disease and ventilatory insufficiency after one year. The combined study agent and exercises appeared to counteract the slow decline in ventilatory independence typical of the severe Pompe phenotype. LC, MRI, and EMG data suggested that the dynamic motor function of the diaphragm improved after gene therapy. Flow and volume LC improved in the subjects with higher baseline respiratory function and were maintained for at least 6-12 months after dosing. Further study in a larger sample is needed to determine the repeatability of these findings. Additionally, the functional benefits and durability of systemic dosing remains to be determined over the long term.

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ABBREVIATIONS

ERT	Enzyme replacement therapy
GAA	α -glucosidase

IMST	Inspiratory muscle strength training
LC	Load compensation
MIP	Maximal inspiratory pressure
MRI	Magnetic resonance imaging
MV	Mechanical ventilation

REFERENCES

- Byrne PI, Collins S, Mah CC, Smith B, Conlon T, Martin SD, Corti M, Cleaver B, Islam S, Lawson LA. Phase I/II trial of diaphragm delivery of recombinant adeno-associated virus acid alpha-glucosidase (rAAAV1-CMV-GAA) gene vector in patients with Pompe disease. *Human gene therapy. Clinical development*. 2014; 25:134–163.
- Carlucci A, Ceriana P, Prinianakis G, Fanfulla F, Colombo R, Nava S. Determinants of weaning success in patients with prolonged mechanical ventilation. *Crit Care*. 2009; 13:R97. [PubMed: 19549301]
- de Melo-Martin I, Sondhi D, Crystal RG. When ethics constrains clinical research: trial design of control arms in “greater than minimal risk” pediatric trials. *Hum Gene Ther*. 2011; 22:1121–1127. [PubMed: 21446781]
- DeRuisseau LR, Fuller DD, Qiu K, DeRuisseau KC, Donnelly WH Jr, Mah C, Reier PJ, Byrne BJ. Neural deficits contribute to respiratory insufficiency in Pompe disease. *Proc Natl Acad Sci U S A*. 2009; 106:9419–9424. [PubMed: 19474295]
- Falk DJ, Mah CS, Soustek MS, Lee KZ, Elmallah MK, Cloutier DA, Fuller DD, Byrne BJ. Intraleural administration of AAV9 improves neural and cardiorespiratory function in Pompe disease. *Mol Ther*. 2013; 21:1661–1667. [PubMed: 23732990]
- Fukuda T, Ewan L, Bauer M, Mattaliano RJ, Zaal K, Ralston E, Plotz PH, Raben N. Dysfunction of endocytic and autophagic pathways in a lysosomal storage disease. *Ann Neurol*. 2006; 59:700–708. [PubMed: 16532490]
- Gaeta M, Musumeci O, Mondello S, Ruggeri P, Montagnese F, Cucinotta M, Vinci S, Milardi D, Toscano A. Clinical and pathophysiological clues of respiratory dysfunction in late-onset Pompe disease: New insights from a comparative study by MRI and respiratory function assessment. *Neuromuscul Disord*. 2015; 25:852–858. [PubMed: 26410244]
- Geiger PC, Cody MJ, Macken RL, Sieck GC. Maximum specific force depends on myosin heavy chain content in rat diaphragm muscle fibers. *J Appl Physiol*. 2000; 89:695–703. [PubMed: 10926656]
- Harikumar G, Moxham J, Greenough A, Rafferty GF. Measurement of maximal inspiratory pressure in ventilated children. *Pediatr Pulmonol*. 2008; 43:1085–1091. [PubMed: 18846557]
- Jones HN, Crisp KD, Moss T, Strollo K, Robey R, Sank J, Canfield M, Case LE, Mahler L, Kravitz RM, Kishnani PS. Effects of respiratory muscle training (RMT) in children with infantile-onset Pompe disease and respiratory muscle weakness. *Journal of pediatric rehabilitation medicine*. 2014; 7:255–265. [PubMed: 25260508]
- Jones HN, Crisp KD, Robey RR, Case LE, Kravitz RM, Kishnani PS. Respiratory muscle training (RMT) in late-onset Pompe disease (LOPD): Effects of training and detraining. *Mol Genet Metab*. 2015
- Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ. Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. *Proc Natl Acad Sci U S A*. 1996; 93:14082–14087. [PubMed: 8943064]
- Kikuchi T, Yang HW, Pennybacker M, Ichihara N, Mizutani M, Van Hove JL, Chen YT. Clinical and metabolic correction of pompe disease by enzyme therapy in acid maltase-deficient quail. *J Clin Invest*. 1998; 101:827–833. [PubMed: 9466978]
- Kishnani PS, Nicolino M, Voit T, Rogers RC, Tsai AC, Waterson J, Herman GE, Amalfitano A, Thurberg BL, Richards S, Davison M, Corzo D, Chen YT. Chinese hamster ovary cell-derived

- recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr*. 2006; 149:89–97. [PubMed: 16860134]
- Mah CS, Falk DJ, Germain SA, Kelley JS, Lewis MA, Cloutier DA, DeRuisseau LR, Conlon TJ, Cresawn KO, Fraites TJ Jr. Campbell-Thompson M, Fuller DD, Byrne BJ. Gel-mediated delivery of AAV1 vectors corrects ventilatory function in Pompe mice with established disease. *Mol Ther*. 2010; 18:502–510. [PubMed: 20104213]
- Mantilla CB, Seven YB, Zhan WZ, Sieck GC. Diaphragm motor unit recruitment in rats. *Respir Physiol Neurobiol*. 2010; 173:101–106. [PubMed: 20620243]
- Mulreany LT, Weiner DJ, McDonough JM, Panitch HB, Allen JL. Noninvasive measurement of the tension-time index in children with neuromuscular disease. *J Appl Physiol*. 2003; 95:931–937. [PubMed: 12909595]
- Nicolino M, Byrne B, Wraith JE, Leslie N, Mandel H, Freyer DR, Arnold GL, Pivnick EK, Ottinger CJ, Robinson PH, Loo JC, Smitka M, Jardine P, Tato L, Chabrol B, McCandless S, Kimura S, Mehta L, Bali D, Skrinar A, Morgan C, Rangachari L, Corzo D, Kishnani PS. Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease. *Genet Med*. 2009; 11:210–219. [PubMed: 19287243]
- Peterson DK, Nochomovitz ML, Stellato TA, Mortimer JT. Long-term intramuscular electrical activation of the phrenic nerve: safety and reliability. *IEEE Trans Biomed Eng*. 1994; 41:1115–1126. [PubMed: 7851913]
- Prater SN, Banugaria SG, DeArmev SM, Botha EG, Stege EM, Case LE, Jones HN, Phornphutkul C, Wang RY, Young SP, Kishnani PS. The emerging phenotype of long-term survivors with infantile Pompe disease. *Genet Med*. 2012; 14:800–810. [PubMed: 22538254]
- Raben N, Danon M, Gilbert AL, Dwivedi S, Collins B, Thurberg BL, Mattaliano RJ, Nagaraju K, Plotz PH. Enzyme replacement therapy in the mouse model of Pompe disease. *Mol Genet Metab*. 2003; 80:159–169. [PubMed: 14567965]
- Raben N, Plotz P, Byrne BJ. Acid alpha-glucosidase deficiency (glycogenosis type II, Pompe disease). *Curr Mol Med*. 2002; 2:145–166. [PubMed: 11949932]
- Sassoon CS. What are the implications of blunted load compensation responses in prolonged-weaning patients? *Respir Care*. 2014; 59:129–132. [PubMed: 24375262]
- Shea L, Raben N. Autophagy in skeletal muscle: implications for Pompe disease. *Int J Clin Pharmacol Ther* 47 Suppl. 2009; 1:S42–47.
- Smina M, Salam A, Khamiees M, Gada P, Amoateng-Adjepong Y, Manthous CA. Cough peak flows and extubation outcomes. *Chest*. 2003; 124:262–268. [PubMed: 12853532]
- Smith BK, Collins SW, Conlon TJ, Mah CS, Lawson LA, Martin AD, Fuller DD, Cleaver BD, Clement N, Phillips D, Islam S, Dobjia N, Byrne BJ. Phase I/II Trial of Adeno-Associated Virus-Mediated Alpha-Glucosidase Gene Therapy to the Diaphragm for Chronic Respiratory Failure in Pompe Disease: Initial Safety and Ventilatory Outcomes. *Hum Gene Ther*. 2013; 24:630–640. [PubMed: 23570273]
- Smith BK, Corti M, Martin AD, Fuller DD, Byrne BJ. Altered activation of the diaphragm in late-onset Pompe disease. *Respir Physiol Neurobiol*. 2016a; 222:11–15. [PubMed: 26612101]
- Smith BK, Fuller DD, Martin AD, Lottenberg L, Islam S, Lawson LA, Onders RP, Byrne BJ. Diaphragm Pacing as a Rehabilitative Tool for Patients With Pompe Disease Who Are Ventilator-Dependent: Case Series. *Phys Ther*. 2016b; 96:696–703. [PubMed: 26893511]
- Smith BK, Gabrielli A, Davenport PW, Martin AD. Effect of training on inspiratory load compensation in weaned and unweaned mechanically ventilated ICU patients. *Respir Care*. 2014; 59:22–31. [PubMed: 23764858]
- Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet*. 2000; 356:397–398. [PubMed: 10972374]
- van den Hout HM, Hop W, van Diggelen OP, Smeitink JA, Smit GP, Poll-The BT, Bakker HD, Loonen MC, de Klerk JB, Reuser AJ, van der Ploeg AT. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics*. 2003; 112:332–340. [PubMed: 12897283]

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Van den Hout JM, Kamphoven JH, Winkel LP, Arts WF, De Klerk JB, Loonen MC, Vulto AG, Cromme-Dijkhuis A, Weisglas-Kuperus N, Hop W, Van Hirtum H, Van Diggelen OP, Boer M, Kroos MA, Van Doorn PA, Van der Voort E, Sibbles B, Van Corven EJ, Brakenhoff JP, Van Hove J, Smeitink JA, de Jong G, Reuser AJ, Van der Ploeg AT. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics*. 2004; 113:e448–457. [PubMed: 15121988]

HIGHLIGHTS

- Children with Pompe disease and ventilatory insufficiency received AAV1-CMG-GAA.
- Exercise alone did not change respiratory muscle function in any subjects.
- In less-affected children, dynamic respiratory function improved after gene therapy.

Figure 1. Baseline maximal inspiratory pressure of the sample

Maximal inspiratory pressure was significantly greater in subjects with part-time/no mechanical ventilation (MV) use than those with full-time MV use. (Median, Inter-quartile Range depicted) * $p < 0.05$

Figure 2. Load compensation responses to a 0 cm H₂O load before and after gene therapy Significant gains in peak inspiratory flow (A) and volume (B) load compensation were detected in the partial/no MV users, at Day 180 and beyond. While inspiratory time (C) and duty cycle (E) remained unchanged, expiratory time (D) was briefly lower at Day 90. However, the clinical significance of this transient change is unclear. (Results are shown as Mean, SD) * p<0.05, # p<0.01, ^ p<0.005

Figure 3. Load compensation responses to a 5 cm H₂O load before and after gene therapy
 In the partial/no MV users, significant gains in peak inspiratory flow (**A**) occurred between Screening and Baseline, and further increased at Day 180 and beyond. Volumes (**B**) were greater in partial/no MV users. No differences were found for inspiratory (**C**) and expiratory (**D**) times, but duty cycle (**E**) significantly increased after Screening. (Results are shown as Mean, SD) * $p < 0.05$, # $p < 0.01$, ^ $p < 0.005$, ** $p < 0.001$

Figure 4. Diaphragm descent measured in subjects, 180 days after AAV1-CMV-GAA dosing Using dynamic MRI of the right sagittal plane, the diaphragm was classified as having detectable descent, or no descent (average excursion <1 cm). Subjects with diaphragm descent had significantly higher maximal voluntary ventilation (**D**) (Mann-Whitney U). Other measures (**A-C**) approached but did not reach statistical significance. (Results are shown as Median, Interquartile Range) * $p < 0.05$

Figure 5. Diaphragm activity in a part-time MV user before (gray) and after (black) AAV1-CMV-GAA

Using chronic indwelling electrodes, absolute EMG activity (**A**) at Day 180 was significantly greater during the 0 cm H₂O load compensation breaths and during maximal inspiratory pressure maneuvers. When expressed as a percentage of maximal inspiratory pressure (**B**), the diaphragm activity remained significantly greater during 0 cm H₂O load compensation. (Results are shown as Mean, SD) # $p < 0.01$, ** $p < 0.001$

Table 1

Demographic characteristics of the sample.

Subject number	1	2	3	4	5	6	7	8	9
Age at enrollment (months)	96	108	66	180	30	42	26	72	54
Age at diagnosis (months)	7	5	17	18	6	5	12	5	4
Age ERT started (months)	8	15	18	108	6	6	13	6	5
Daily assist requirement (hours)	23.9	24	24	24	24	8	8	10	0
Mode of ventilatory assist	MV	MV	MV	MV	MV	CPAP	BiPAP	MV-P	N/A
Duration of MV assist (months)	54	101	37	152	12	0	0	30	0

BiPAP: Bilevel positive airway pressure; CPAP: continuous positive airway pressure; MV-P: mechanical ventilation; MV: alternating mechanical ventilation and diaphragm pacing

Table 2

Correlations between measures of baseline respiratory motor function.

	Max Volume (mL/kg)	MIP (cm H ₂ O)	Enrollment Age (months)	Age of Dx (months)	Age ERT Began (months)	Daily Hours MV Use	Duration MV Use (Months)
Max Volume (mL/kg)		0.927 **	-0.681 *	-0.446	-0.571	-0.875 ^	-0.804 #
MIP (cm H ₂ O)	0.927 **		-0.597	-0.583	-0.519	-0.897 ^	-0.738 *
Enrollment Age (months)	-0.681 *	-0.597		0.443	0.832 ^	0.499	0.963 **
Age of Dx (months)	-0.446	-0.583	0.443		0.710 *	0.420	0.470
Age ERT Began (months)	-0.571	-0.519	0.832 ^	0.710 *		0.366	0.815 #
Daily Hours MV Use	-0.875 ^	-0.897 ^	0.499	0.420	0.366		0.639
Duration MV Use (Months)	-0.804 #	-0.738 *	0.963 **	0.470	0.815 #	0.639	

*
p<0.05#
p<0.01^
p<0.005**
p<0.001