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Variable clinical features and genotype-phenotype correlations in 18 patients with late-onset Pompe disease

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Background: Pompe disease is a lysosomal storage disorder caused by the deficiency of enzyme acid alpha-glucosidase (*GAA*) which results in accumulation of glycogen, particularly in the skeletal, cardiac, and smooth muscles. The late-onset form with symptoms presenting in childhood through adulthood, is characterized by proximal muscle weakness, respiratory insufficiency, and unlike the infantile-onset form often with no cardiac involvement.

Methods: We report our experience with 18 adult patients (14 males/4 females) with Pompe disease, several of whom had unique findings and novel pathogenic variants. Patients ranged in ages from 22–74 years (mean 53.7 years) and were diagnosed at an age range of 11–65 years (mean 43.6 years), often after a history of progressive muscle disease of several years' duration. All 18 patients were treated with alglucosidase alfa (Lumizyme) and their response to treatment was monitored by measurements of their pulmonary function and muscle weakness, six-minute walk test (6MWT), and other functional studies.

Results: Genetic sequencing revealed that 16 out of 18 individuals had the common c.-32-13T>G splicing variant, and six patients, including two sibships had four novel pathogenic variants: c.1594G>A, c.2655_2656delCG, c.1951-1952delGGinsT, and c.1134C>G. A male with the c.1594G>A variant developed an intracerebral aneurysm at the age of 43 years treated with surgery. Two siblings with the c.2655_2656delCG developed very high antibody titers, one of whom developed a severe infusion reaction. Other clinical features included BiPAP requirement in twelve, tinnitus in seven, scoliosis in five, cardiomyopathy in three, one individual was diagnosed with a cerebral aneurysm who underwent successful Penumbra coil placement, and another individual was diagnosed with both Graves' disease and testicular cancer.

Conclusions: Our study illustrates significant variability in the range of clinical features, and the variable clinical response to enzyme replacement therapy. It also alerts us to the importance of careful monitoring and early management of complications. Possible genotype-phenotype associations with the novel mutations identified may emerge with larger studies.

Keywords: Pompe disease; enzyme replacement therapy (ERT); pulmonary function studies; novel *GAA* variants; genotype-phenotype correlation; six-minute walk test (6MWT); aneurysm; testicular cancer; newborn screening

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Introduction

Glycogen storage disease type II (GSD), also known as Pompe disease (OMIM #232300), was first described by the Dutch pathologist Joannes Cassianus Pompe in 1932 (1). It is a rare, autosomal recessive disease with an overall reported population frequency ranging from approximately 1 in 40,000 to 1 in 57,000 (2,3). Pompe disease is caused by a deficiency in the lysosomal enzyme α-glucosidase (*GAA*) which causes pathologic glycogen accumulation in all tissues. This glycogen excess leads to multisystemic degenerative effects, most frequently affecting cardiac muscle, proximal limb muscles and diaphragm which causes earlier demise due to respiratory failure (4). Pompe disease has a wide variety of phenotypic expression with considerable overlap with other neuromuscular diseases, making diagnosis often a challenge (5). Newborn screening studies in several states however have addressed this delay in diagnosis and have indicated a significantly higher incidence of Pompe disease. Pompe disease can be associated with infantile onset and late onset myopathy, the phenotype sometimes correlating with the specific pathogenic variant in the *GAA* gene located at 17q25.3, and resulting residual enzyme level (6). Patients with the most severe form of Pompe disease, classic-infantile Pompe disease, have onset of symptoms within the first few weeks to months of life, with progressive cardiac hypertrophy typically leading to cardiorespiratory failure and death between 1–2 years of age (7-9). In other patients, onset of symptoms, including a slower progressive skeletal muscle weakness that affects mobility and respiratory function, presents ≤12 months of age without cardiac involvement or later in life during early childhood through late adulthood, sometimes as late as the sixth decade of life (10-14). Definitive diagnosis is made by molecular testing of the *GAA* gene and testing of enzymatic activity from blood or tissue (15).

Enzyme replacement therapy (ERT) utilizing recombinant human α-glucosidase (rhGAA) became commercially available in 2006 and has been used to help stabilize or improve patient outcomes (16-18). Early trials that included infantile patients demonstrated that ERT was safe, effective, and generally well-tolerated. Infants showed a reduction in cardiac hypertrophy as well as an improvement in cardiac and pulmonary function, muscle motility, and strength (19,20). In the late onset patients, several studies have shown an improvement in the muscle function measurements and pulmonary function studies compared to the natural history progression of untreated Pompe patients (21-26).

Methods

Patients

The study comprises of 18 adult patients with Pompe disease all of whom had documented deficiency of acid alpha-glucosidase enzyme activity and pathogenic variants in the *GAA* gene confirmed through molecular testing. Informed consent was obtained from all patients to use data from clinical evaluations performed and from their medical records for this natural history study. An overview of the patient demographics is provided (*Table 1*) and details of each patient are provided in *Table S1*.

Currently, all patients are receiving enzyme replacement therapy with alglucosidase alfa and are evaluated at six-month intervals by a medical geneticist, neuromuscular neurologist, genetic counselor, nutritionist, physical therapist, respiratory therapist, social worker, and a representative from the Muscular Dystrophy Association.

During the clinical visits, the following information was gathered: (I) demographics; (II) presence of specific clinical features including aneurysm, tinnitus, scoliosis, and osteoporosis; (III) ambulatory status and the type of assistive device being used if applicable; (IV) motor function—six-minute walk test (6MWT) (27), MRC scale and dynamometry; (V) respiratory status—the type of ventilatory support required; (VI) pulmonary function—forced vital capacity (FVC) both in upright seated and supine position, maximum inspiratory pressure (MIP), and sniff nasal inspiratory pressure (SNIP).

6MWT

Motor function was measured through the 6MWT according to the American Thoracic Society guidelines (27) in 16 patients. The distance covered by the patients were recorded in meters. Two patients (patient 5 and patient 12) were unable to do the 6MWT due to their partial or non-ambulatory status.

Pulmonary function test and respiratory muscle strength

Pulmonary function testing was measured in upright and supine positions in accordance with the ATS/European Respiratory Society guidelines (28). Percentages of the predicted FVC for both upright and supine positions were recorded. MIP and SNIP were recorded in cmH$_2$O.

Table 1 Patient demographics

Variable	Statistics
Gender, n (%)	
Male	14 (77.78)
Female	4 (22.22)
Race, n (%)	
Caucasian	17 (94.44)
Native Hawaiian/Pacific Islander	1 (5.56)
Time points	
Current age (years)	
Median	56.50
Range	22–74
Mean ± SD	53.72±14.09
Age during onset of symptoms (years)	
Median	30.00
Range	5–58
Mean ± SD	29.56±15.80
Age at diagnosis (years)	
Median	44.00
Range	11–65
Mean ± SD	43.61±15.82
Lag time interval (years)	
Median	11.00
Range	2–32
Mean ± SD	14.06±9.31
Age at start of ERT (years)	
Median	46.50
Range	12–66
Mean ± SD	47.00±14.35

Statistical analyses

Descriptive statistics were provided for all categorical data, including patients' gender and the associated clinical features seen in our cohort. For continuous variables (i.e., age), summary statistics (mean, median, range) were calculated. Multivariate linear mixed effect model was used to assess association between time and outcome measurements before and after treatment initiation. Random effect for each subject was included in the model to account for correlation of repeated measures within each patient. For measurements FVC upright and FVC supine, interaction of before *vs.* after treatment and time from ERT was examined to see whether patients' outcome changes at different rate before and after treatment. However, for 6MWT, MIP, and SNIP, pre-treatment slope could not be examined because of the limited number of measurements in the pre-treatment period (less or equal to 3 patients had more than 2 time points at pre-treatment period). As a result, only post-treatment time trend was assessed for these measurements. Age at ERT initiation was adjusted as covariate in all models. Because there were five outcomes in this analysis, false discovery rate (FDR) P value adjustment was used to account for multiple comparisons.

GAA analysis

To confirm diagnosis, *GAA* sequencing for GSD II (Pompe disease) was done for all patients through peripheral blood sample. All our patients had done their genetic testing at Duke University Health System (DUHS) Molecular Diagnostics Laboratory or Emory Healthcare Laboratory Services by standard methodology (29). Also, changes to protein function are predicted based on algorithms that predict the effect of amino acid changes on protein structure and function based on the alignment of similar protein motifs (PolyPhen and SIFT).

Results

Group description

The gender, race, and age at varying time points throughout the course of the disease for the 18 patients are summarized in *Table 1*. The population was 77.78% (14/18) male and 22.22% (4/18) female, 17 patients are of Caucasian origin, including one mixed Chinese-Caucasian, and one patient is of Filipino descent. At the end of data collection, our cohort of patients had a mean age of 53.72 years (SD ±14.09), ranging from 22 to 74 years. For all patients, a history of progressive muscle disease of several years' duration preceded their diagnosis at a mean age of 43.61 years (SD ±15.82), ranging from 11 to 65 years. The mean age at ERT initiation was 47.00 years (SD ±14.35), ranging from 12 to 66 years. Also, our cohort had a mean lag time of 14.06 years (SD ±9.31), ranging from 2 to 32 years, between their onset of symptoms to their confirmatory Pompe disease diagnosis. Patients were followed up to 10.7 years

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Figure 1 Angiogram of the cerebral aneurysm in Patient 2 diagnosed at 42 years. Brain MRI revealed the aneurysm shortly after the patient experienced dizziness and vomiting while exercising. He subsequently underwent Penumbra coil placement.

after treatment initiation; the pre-treatment data (over a period of up to 22.4 years) were available for a few individuals.

Disease manifestations

Table 1 provides a summary of the main demographic and clinical features and *Table 2* provides the genotypes of all 18 patients in the study. Detailed clinical features of each individual patient are provided in *Table S1*. Among our cohort of patients, 94.4% (17/18) are currently ambulatory with 50% (9/18) not needing any assistive devices (cane/walker/wheelchair). Additionally, 12 use non-invasive respiratory support (66.6%), specifically BiPaP and one required tracheostomy and intermittent ventilation. A young adult with childhood onset and two adults [3/18 (16.7%)] had developed cardiomyopathy which improved with ERT. Hearing loss was present in 7/18 patients (38.9%) four of whom had tinnitus. Additional features seen were scoliosis in 5 (27.8%) and osteoporosis in 3 individuals (16.7%). Endocrinological features included hypothyroidism in 3 patients (16.7%); one patient (Patient 13) had both Graves' disease and testicular cancer. Patient 2 at 42 years of age developed a cerebral aneurysm, which was treated with a Penumbra coil placement with no further incidents (*Figure 1*). Peripheral neuropathy was seen only in Patient 12 (5.6%).

Infusion reaction

Patient 9 (1/18, 5.6%) with the novel c.2655_2656delCG mutation experienced severe infusion reactions approximately 5 years after initiating ERT. In order to continue with her treatment, she took a regimen of 20 mg of famotidine (Pepcid) twice daily and 180 mg of the antihistamine fexofenadine (Allegra) on the day before, the day of and the day after her infusions. Additionally, she was premedicated with 20 mg dexamethasone (Decadron) and 50 mg diphenhydramine (Benadryl) and ranitidine HCl (Zantac) 50 mg intravenously. Elevations in her antibody titer (1/12,800) coincided with this reaction with subsequent improvement in the antibody titers as she was eventually able to immunotolerate her ERT with no further reactions.
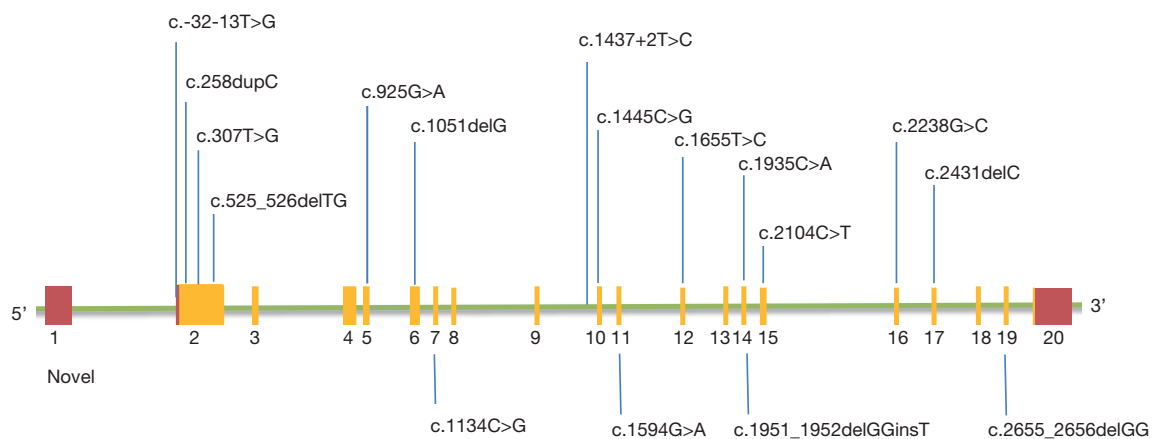
GAA mutations

Our cohort of 18 Pompe patients include 3 sets of sibships in 14 unrelated families including: Patients 5, 6, & 7, Patients 9 & 10, and Patients 11 & 12. Genetic sequencing revealed that all 18 patients are compound heterozygotes for the *GAA* gene variants (*Table S1*, *Table 2*, *Figure 2*). The 36 alleles from our 18 patients consisted of 17 splice site variants (17/36, 47.2%), 10 missense variants (10/36, 27.8%), 8 frameshift variants (8/36, 22.2%), and 1 nonsense variant (1/36, 2.8%). By far, the most common variant observed was the common splicing variant c.-32-13T>G detected in 12/14 (85.7%) families. All the other mutations occurred in individual patients in this cohort.

Thirteen of the seventeen *GAA* gene variants have previously been reported as pathogenic (http://www.pompecenter.nl). The four variants: c.1594G>A, c.2655_2656delCG, c.1951-1952delGGinsT, and c.1134C>G are novel. Variants c.2655_2656delCG, seen in siblings 9 & 10, and c.1951-1952delGGinsT, seen in siblings 11 & 12 are both indel (insertion/deletion) variants resulting in a premature termination codon, and thus a truncated protein product. Variant c.1134C>G seen in Patient 15 results in a stop codon in exon 7. Variant c.1594G>A, seen in Patient 2 was determined to be deleterious to protein function by PolyPhen and MutationTaster.

Table 2 List of mutations in our cohort of 18 patients

Nucleotide change	Protein change	Location	Effect	No. of alleles	Reference
Missense variants					
c.1655T>C	p.Leu552Pro	Exon 12	Potentially less severe	1	(30)
c.1935C>A	p.Asp645Glu	Exon 14	Potentially less severe	1	(31)
c.2238G>C	p.Tryp746Cys	Exon 16	Potentially mild	1	(32)
[N]c.1594G>A	p.Gly532Ser	Exon 11	Moderately deleterious	1	Novel
c.925G>A	p.Gly309Arg	Exon 5	Potentially less severe	3	(33)
c.1445C>G	p.Pro482Arg	Exon 10	Potentially less severe	1	(34)
c.307T>G	p.Cys103Gly	Exon 2	Potentially less severe	1	(35)
c.2104C>T	p.Arg702Cys	Exon 15	Potentially less severe	1	(36)
Frameshift variants					
c.2431delC	p.Leu811Trpfs*37	Exon 17	Very severe	1	(37)
c.258dupC	p.Asn87Glnfs*9	Exon 2	Very severe	1	(38)
[N]c.2655_2656delCG	p.Val886Glufs*2	Exon 19	Severe	2	Novel
[N]c.1951-1952delGGinsT	p.Gly651Serfs*45	Exon 14	Severe	2	Novel
c.525_526delTG	p.Asn177Profs*11	Exon 2	Very severe	1	(39)
Nonsense variants					
[N]c.1134C>G	p.Tyr378Ter	Exon 7	Severe	1	Novel
Splice cite errors					
c.-32-13T>G	p.=, p.?	Intron 1	Mild	16	(40,41)
c.1437+2T>C	p.Asp443_Lys479del	Intron 9	Very severe	1	(42)

Figure 2 A total of 17 *GAA* mutations including 4 novel variants in 18 late-onset Pompe patients. Also depicted are the untranslated regions (UTR) (red), intronic (green), and exonic (orange) regions. Previously reported mutations are indicated above, and the novel ones are below.

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Respiratory function studies

FVC: upright seated and supine

Unfortunately, even with detailed ascertainment of medical records for each patient, data prior to the start of ERT were unavailable for the majority of patients. For those individuals for whom data were available, FVC upright before the start of ERT showed a significant decline with an estimated slope of –2.07 per year compared to predicted values (P<0.0001). In contrast, for all data points after the start of ERT, a slope of –0.17 was observed (P=0.23). The difference between these slopes, before and after start of ERT, was found to be highly significant (P<0.0001) (*Figure 3A*).

For FVC supine, the estimated decline before ERT was –2.67% per year (P=0.23), while the estimated decline of FVC supine after ERT was –0.55% per year (P=0.047). Although a –2.12 difference was observed in the slopes before and after ERT, this difference did not reach statistical significance (P=0.3656) (*Figure 3B*).

MIP and SNIP

For measures of MIP and SNIP, only slopes after ERT were examined. Post ERT, patients' MIP declined at 0.92 per year (P=0.0169) and SNIP declined at 1.93 per year (P=0.0226). *Figure 3C,D* show the details of the fitted line respectively.

6MWT

While limited data on the 6MWT was available prior to ERT, deterioration in the slope post ERT was seen— the patients' 6MWT was declining by 11.6 m per year (P<0.0001) as indicated by the detailed fitted line after ERT initiation (*Figure 3E*).

Summary of results of clinical findings

The mean age at diagnosis was 43.6 years (SD ±15.8), ranging from 11 to 65 years, and a mean lag time before diagnosis was 14.06 years (SD ±9.31). The mean age at ERT initiation was 47 years (SD ±14.35), ranging from 12 to 66. The most common pathogenic variant observed was c.-32-13T>G splicing variant detected in 16/36 alleles (44.4%) in 16/18 compound heterozygotes in 14 families. We report four novel mutations in trans with the c.-32-13T>G splicing variant associated with late onset Pompe disease. In our cohort 3/18 (16.7%) developed cardiomyopathy which improved with ERT. One male with the novel mutation c.1594 G>A had a cerebral aneurysm at the age of 42 years. Scoliosis was seen in 5/18 of the patients (28%). Tinnitus and hearing loss were noted in seven individuals (38.9% respectively). BiPAP is being used by 12 individuals (66.7%), additionally one individual had a tracheostomy and was ventilator and wheelchair dependent. One female with a severe infusion reaction and high antibody titers was treated successfully with a desensitization regimen (5.6%). One male had testicular cancer that was responsive to chemotherapy and Graves' disease.

Discussion

This study reports the clinical variations and genotypes in 18 adult Pompe patients, and response to the initiation of ERT over a period of 25–90 months. We found that the mean age at diagnosis was 43.61 years with a mean diagnostic delay/lag time interval of 14.06 years. On average, patients in our cohort were diagnosed at a later stage than those reported in other studies (12,43). This delay, however, is now being addressed by newborn screening in many states.

The prevalence of scoliosis in our cohort (5/18, 27.78%) is comparable to the 24.8% (87/251) (44) and 23% (22/94) (45) found in other studies.

A higher incidence of the use of walking aids (9/18, 50%) was observed in our cohort compared to the incidences of 15% (14/94) (45), 38% (23/60) (25) and 54.5% (12/22) (46) reported in other studies. Similarly, we also observed higher incidence of respiratory support use in our patients (13/18, 72.2%) compared to that reported in other studies, 29% (27/94) (45), 33% (23/60) (25) and 40.1% (9/22) (46).

We report mild cardiomyopathy in 3/18 patients (16.7%), 2 having the common c.-32-13T>G variant. Our observation supports a recent study noting that severe cardiomyopathy is rare in patients with late-onset Pompe disease especially with the common c.-32-13T>G variant (47); all three patients showed improvement with ERT indicating that unlike the skeletal muscle, cardiac muscle is responsive to ERT. Nevertheless, we recommend regular surveillance for the cardiac manifestations of Pompe disease because of the 17% incidence of cardiomyopathy in our population.

We report one patient with a cerebral aneurysm at 42 years, which was successfully treated with a Penumbra coil placement. Cerebral arteries have been previously found to be affected by Pompe disease, and cerebral
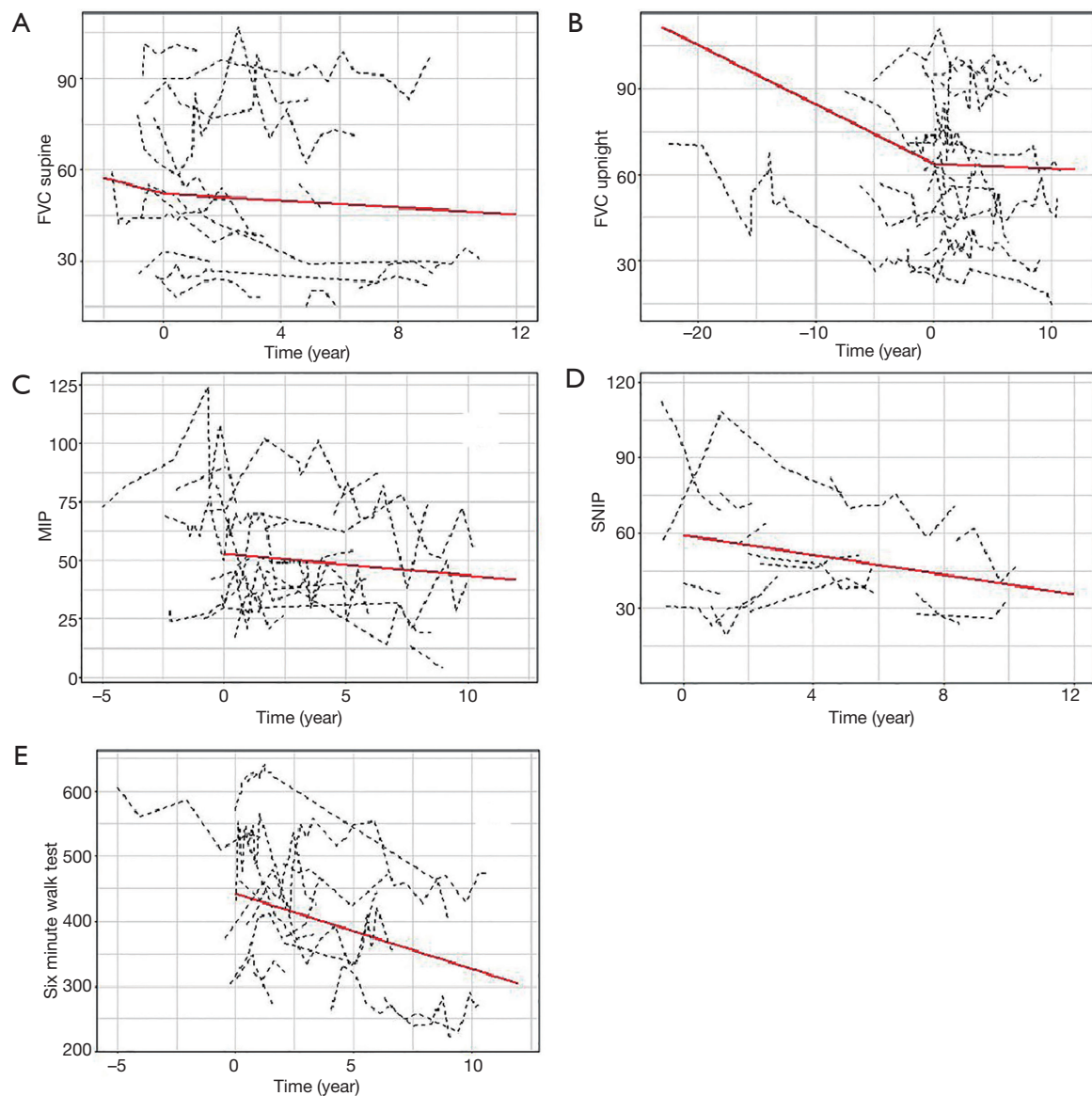
Figure 3 Longitudinal measurements of forced vital capacity (FVC) upright and supine, maximum inspiratory pressure (MIP), sniff nasal inspiratory pressure (SNIP), and six-minute walk test (6MWT) after ERT. Each dotted line represents longitudinal measurements of individual patients. The red solid lines represent the fitted slope before and after ERT from a piecewise linear mixed effect model.

aneurysms are a complication. Subarachnoid hemorrhage secondary to rupture of the MCA aneurysm was reported in a 50-year-old man with late-onset Pompe disease. The patient was admitted for an acute onset of severe occipital thunderclap headache. His aneurysm was successfully clipped, and the patient had no neurological deficit on discharge (48). On review of the literature four late-onset Pompe patients with the c.-32-13T>G allele were reported with basilar artery dolichoecstasia or internal carotid dilatative

arteriopathy (49). In a recent study, 16/23 infantile patients (70%) had ventricular enlargement and/or extra-axial cerebrospinal fluid accumulation at baseline neuroimaging; delayed myelination was detected in two of them. Follow-up neuroimaging (n=8) after 6–153 months showed marked improvement, with normalization of the baseline changes (50). However, two of three patients imaged after age 10 years demonstrated white matter changes, and one was noted to have a basilar artery aneurysm (50). More

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recently 13/21 patients (62%) showed intracranial arterial abnormalities of whom: 9.5% showed an unruptured intracranial aneurysm and 47% had a vertebrobasilar dolichoectasia (12). We also recommend surveillance by regular neuroimaging using computed tomography angiography (CTA) or magnetic resonance angiography (MRA) for cerebrovascular malformations in order to avoid sub-arachnoid hemorrhages.

We report one patient who was diagnosed with testicular cancer in his teens who was treated with excision and chemotherapy. We are aware of one other young male with Pompe syndrome with testicular cancer who has also responded well to standard treatment (personal communication with VK). To our knowledge, the association with testicular cancer has not been previously reported. Interestingly, a Pompe patient has been reported with thymic neuroendocrine carcinoma (51) and another patient, treated with ERT, was reported with a benign muscle lingual pseudohypertrophy (52). While the extent of the relationship between Pompe disease and the risk for malignancies is unknown, another lysosomal storage disorder, Gaucher disease, has long been associated with cancer, specifically multiple myeloma and lymphoma (53,34). Larger studies may determine if there is an increased risk for testicular malignancy or other types of malignancies in Pompe disease.

The most common c.-32-13T>G variant was seen in 16/36 (44.44%) alleles, in 17/18 (94.44%) patients and 12/14 (85.71%) families in our study cohort. It is well documented that c.-32-13T>G is the most common pathogenic variant identified in the *GAA* gene in Caucasian patients with late onset Pompe disease. The T>G transversion within intron 1 alters mRNA splicing resulting in the formation of alternative mRNA transcripts lacking exon 2. The altered splicing site is leaky with a small fraction of the normal RNA transcripts produced resulting in the late onset Pompe phenotype (55,56).

We also identified 17 unique *GAA* variants including four novel variants (3 in trans with the common mutation c.-32-13T>G) not previously reported in the literature in association with Pompe disease. The novel missense mutation c.1594G>A (p.Gly532Ser) is found in the only Asian patient from the Philippines in our cohort who also had a cerebral aneurysm. Recently, an additional patient has been identified with the same variant through newborn screening in 2016. This patient was a 3-week-old African-American male identified to have this variant along with the common splicing variant c.-32-13T>G (personal

communication with VK).

Two patients with the novel frameshift mutations c.2655_2656delCG had high antibody titers and one of them had an adverse event to ERT that was treated successfully with medication. The patient with novel variant c.1951-1952delGGinstT had peripheral neuropathy, whereas the patient with another novel variant c.1134C>G did not have any atypical features.

Since data prior to the initiation of ERT was limited, a comparison of the slopes pre ERT and post ERT was challenging. In our most complete parameter studied, upright FVC showed significant improvement in the patients' decline after starting ERT (–2.07 per year before ERT to –0.17 per year after starting ERT, P<0.0001). A similar difference is observed in supine FVC showing that patients declined –2.67 per year before ERT to –0.55 per year after starting ERT (P=0.047). This positive response to ERT especially in the rates of FVCs upright and supine was also noted by other authors (25,26). However, we noticed continued gradual decline in our patients' 6MWT post ERT which may be partly attributed to the normal aging process. On the other hand, this decline may reflect limited efficacy of the ERT on muscle strength in contrast to the striking benefits on cardiac function. This was also reported by Kuperus who noted that distance walked on the 6MWT increased during the initial years of treatment, followed by a gradual decline. Compared to baseline, the median 6MWT distance increased from 376 to 416 m at 5 years of treatment and compared to baseline, 69% of patients improved their walking ability at 5 years of treatment (26).

Our patient cohort illustrates significant variability in the range of clinical features and alerts us to the importance of careful monitoring and early management of these complications. Possible genotype-phenotype associations with the novel variants identified may emerge with larger studies.

Acknowledgments

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Informed consent was obtained from all patients to use data from clinical evaluations performed and from their medical records for this natural history study.

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Table S1 Clinical features in 18 patients with Pompe disease

Subject number	Sex	Age (years)	Age at symptom onset (years)	Age at diagnosis (years)	Age at start of ERT (years)	Mutation 1	Mutation 2	Aneurysm	Hearing loss	Tinnitus	BiPAP	Scoliosis	Osteoporosis	Cardio-myopathy	Infusion reaction	Peripheral neuropathy	Tracheostomy	Graves' disease	Testicular cancer	Hypothyroidism	Ambulatory status (assistive device if applicable)	Respiratory support status (type)
1	Male	22	9	11	12	c.1655T>C	c.2238G>C	–	–	–	+	+	–	+	–	–	–	–	–	–	+	+ (non-invasive ventilation)
2	Male	48	39	43	44	c.1935C>A	c.1594G>A	+	–	–	+	–	–	–	–	–	–	–	–	–	+	+ (non-invasive ventilation)
3	Male	56	30s	48	48	c.-32-13T>G	c.2431delC	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–
4	Male	34	5	30	31	c.-32-13T>G	c.258dupC	–	–	–	+	+	–	–	–	–	–	–	–	–	+	+ (non-invasive ventilation)
5	Male	59	10	33	47	c.-32-13T>G	c.925G>A	–	+	+	–	–	+	–	–	–	+	–	–	–	– (wheelchair)	+ (non-invasive ventilation)
6	Female	57	20s	31	46	c.-32-13T>G	c.925G>A	–	–	–	+	+	–	–	–	–	–	–	–	–	+ (walker)	+ (non-invasive ventilation)
7	Male	50	32	38	43	c.-32-13T>G	c.925G>A	–	+	–	+	–	–	–	–	–	–	–	–	–	+	+ (non-invasive ventilation)
8	Female	49	20s	42	42	c.-32-13T>G	c.1437+2T>C	–	–	–	–	–	–	–	–	–	–	–	–	+	+ (quad cane, wheelchair)	–
9	Female	74	30s	62	63	c.-32-13T>G	c.2655_2656delCG	–	+	+	–	–	–	–	+	–	–	–	–	–	+ (cane for long distance)	–
10	Male	70	58	65	66	c.-32-13T>G	c.2655_2656delCG	–	+	–	+	–	–	–	–	–	–	–	–	–	+ (walking stick occasionally)	+ (non-invasive ventilation)
11	Male	68	30s	55	61	c.-32-13T>G	c.1951-1952delGGinsT	–	–	+	+	+	+	–	–	–	–	–	–	+	+ (walker, cane)	+ (non-invasive ventilation)
12	Male	63	35	55	56	c.-32-13T>G	c.1951-1952delGGinsT	–	–	–	–	–	–	–	–	+	–	–	–	–	+ (cane, wheelchair)	–
13	Male	50	42	45	45	c.-32-13T>G	c.525_526delTG	–	–	+	+	+	–	+	–	–	–	+	+	+	+	+ (non-invasive ventilation)
14	Male	63	47	58	58	c.-32-13T>G	c.1445C>G	–	+	+	+	–	–	–	–	–	–	–	–	–	+ (cane)	+ (non-invasive ventilation)
15	Male	42	15	23	30	c.-32-13T>G	c.1134C>G	–	–	–	+	–	–	–	–	–	–	–	–	–	+	+ (non-invasive ventilation)
16	Male	64	56	60	61	c.-32-13T>G	c.307T>G	–	–	–	+	–	–	–	–	–	–	–	–	–	+ (cane)	+ (non-invasive ventilation)
17	Female	33	14	24	31	c.-32-13T>G	c.2104C>T	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–
18	Male	65	40	62	62	c.-32-13T>G	c.1051delG	–	+	–	+	–	+	+	–	–	–	–	–	–	+	+ (non-invasive ventilation)