Molecular Genetics and Metabolism 135 (2022) 179–185

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Early clinical phenotype of late onset Pompe disease: Lessons learned from newborn screening

Erin Huggins [a], Maggie Holland [b], Laura E. Case [c], Janet Blount [a], Andrew P. Landstrom [d], Harrison N. Jones [e], Priya S. Kishnani [a,*]

[a] Division of Medical Genetics, Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA
[b] Department of Physical and Occupational Therapy, Duke University Health System, Durham, NC, USA
[c] Doctor of Physical Therapy Division, Department of Orthopedic Surgery, Duke University School of Medicine, Durham, NC, USA
[d] Department of Pediatrics, Division of Cardiology and Department of Cell Biology, Duke University School of Medicine, Durham, NC, USA
[e] Department of Head and Neck Surgery & Communication Sciences, Duke University School of Medicine, Durham, NC, USA

ARTICLE INFO

Article history:
Received 14 January 2022
Accepted 14 January 2022
Available online 23 January 2022

Keywords:
Newborn screening
Pompe disease
Late-onset Pompe disease

ABSTRACT

Purpose: Thoroughly phenotype children with late-onset Pompe disease (LOPD) diagnosed via newborn screening (NBS) to provide guidance for long-term follow up.
Methods: Twenty infants ages 6–21 months with LOPD diagnosed by NBS underwent systematic clinical evaluation at Duke University including cardiac imaging, biomarker testing, physical therapy evaluation, and speech-language pathology evaluation.
Results: Of the 20 infants, four were homozygous for the "late-onset" IVS1 splice site variant c.-32-13 T > G, fourteen were compound heterozygous, and two did not have any copies of this variant. None of the patients had evidence of cardiomyopathy or cardiac rhythm disturbances. Biomarker testing showed an increase in CK, AST, and ALT in 8 patients (40%) and increase in Glc4 in two patients (10%). All patients demonstrated postural and kinematic concerns. Three patients (17%) scored below the 10%ile on the Alberta Infant Motor Scale (AIMS) and 15 patients (83%) scored above the 10%ile. Speech-language pathology assessments were normal in all patients and mild feeding/swallowing abnormalities were noted in nine patients (45%).
Conclusion: Our data show high variability among children with LOPD diagnosed via NBS. Careful physical therapy evaluation is necessary to monitor for subtle musculoskeletal signs that may reflect early muscle involvement. Patients should be monitored closely for symptom progression.

1. Introduction

Pompe disease, or Glycogen Storage Disease (GSD) Type II, is an autosomal recessive metabolic disorder that is both a GSD and a lysosomal storage disorder (LSD), caused by a deficiency of the lysosomal glycogen-hydrolyzing enzyme acid alpha-glucosidase (GAA) [1]. GAA plays a vital role in the breakdown of lysosomal glycogen into the simple sugar glucose, and its deficiency leads to toxic accumulation of glycogen in multiple tissues, primarily skeletal, cardiac, and smooth muscle [1,2]. Severity of the disease is inversely related to levels of residual GAA activity, primarily influenced by *GAA* genotype. The clinical spectrum ranges from rapidly progressive, classic infantile-onset disease (IOPD) at the most severe end of the spectrum, to a highly variable, later-onset form (LOPD). Classic IOPD is characterized by hypertrophic cardiomyopathy (HCM) in the first year of life. Infants have significant muscle weakness, failure to thrive, and respiratory distress. In untreated patients, death typically occurs due to cardiorespiratory failure by one to two years of age [3]. LOPD represents all cases of Pompe disease without cardiomyopathy in the first year of life and thus represents a spectrum of involvement and age of symptom onset. LOPD progresses more slowly and involves muscle weakness, decreased pulmonary function, and a host of other systemic manifestations [4]. While HCM is characteristically absent in patients with LOPD, there may still be a cardiac phenotype in the form of rhythm disturbances, such as Wolff-Parkinson-White syndrome [4,5]. Historically, it was thought LOPD presented later in life and represented a more mild disease course; however, more recent literature has shown that LOPD can present in the first year of life and may become evident throughout childhood and into adulthood, with the disease course ranging significantly in severity [2,6–9]. The currently approved treatment for Pompe disease consists of enzyme replacement therapy (ERT) with alglucosidase alfa (rhGAA). Recently, a second ERT, avalglucosidase alfa, was approved by the

* Corresponding author.
E-mail address: priya.kishnani@duke.edu (P.S. Kishnani).

https://doi.org/10.1016/j.ymgme.2022.01.003
1096-7192/

United States Food and Drug Administration (FDA) for treatment of LOPD patients 1 year of age and older. There are other late-stage therapies in development for additional treatment options for Pompe disease. In IOPD, treatment with alglucosidase alfa ERT is life-saving and significantly increases ventilator-free survival, and reverses or improves HCM. Very early initiation of treatment with ERT leads to the best possible outcomes and even a matter of days can influence outcomes [8,10,11]. In LOPD, treatment may or may not be initiated at the time of diagnosis, depending on symptom onset. Treatment in symptomatic LOPD patients has been shown to improve or stabilize motor function and respiratory status [8,12].

In 2015, Pompe disease was the first LSD added to the recommended uniform screening panel (RUSP) in the USA. At the time of this publication, 28 U.S. states and Washington, D.C., have added Pompe disease to their respective state NBS panels. Evidence of the success of NBS for Pompe disease emerged from Taiwan's newborn screening program, initiated in 2005 [11,13–15]. While data from Taiwan have been invaluable in demonstrating the utility of NBS in IOPD cases, data are limited for LOPD cases, in part due to the lack of a particular variant in the Taiwanese population. None of the cases in the Taiwan NBS cohort have a well-known "late-onset" variant (c.-32-13 T > G, formerly called IVS1-13 T > G and herein referred to as the IVS1 variant), a splice site variant present in compound heterozygosity or homozygosity in 68–90% of Caucasians with LOPD [7,12,16,17]. This variant is located in intron 1 and results in aberrant splicing of exon 2 with low levels of normally spliced mRNA [16,18]. Patients with this variant are not expected to develop HCM [19,20], thus the classification of this as a "late-onset" variant. However, symptoms such as muscle weakness have been reported in this patient population across the lifespan, including during infancy in those diagnosed clinically [7,20–22].

In 2017, guidelines were published by the Pompe Disease Newborn Screening Working Group outlining recommendations for diagnostic confirmation and early management of infants with IOPD and symptomatic LOPD after positive NBS [23,24]. While these guidelines provide an excellent framework for disease management in IOPD, there are gaps in understanding the classification of a symptomatic vs. asymptomatic LOPD patient, thus making application of these guidelines a challenge for clinicians. Patients with LOPD represent the majority of cases of Pompe disease identified on NBS at this time [25], yet there are no established criteria for how to classify these patients as symptomatic vs. asymptomatic. Further, there is not yet a consensus on determining when a patient with LOPD identified via NBS requires intervention, such as treatment with ERT.

We aim to identify the early phenotypic features of patients with LOPD (with and without the IVS1 variant) within the first two years of life after NBS in order to provide a framework for continued clinical evaluation and identification of those at risk for earlier symptom progression.

2. Methods

Twenty patients were enrolled in a Duke University Institutional Review Board-approved study (Pro00100223, ClinicalTrials.gov: NCT03694561). Patients met the following inclusion criteria: patient had a confirmed and documented diagnosis of Pompe disease via NBS without cardiac involvement, and had predicted "late-onset" *GAA* variants in homozygosity or compound heterozygosity (such as c.-32-13 T > G, c.2188G > T, c.1953C > A, or c.118C > T). Each patient's NBS results and molecular testing results were reviewed in detail to confirm eligibility. Enrolled patients underwent systematic clinical evaluation including the following: cardiac evaluation, plasma and urine biomarker testing, motor assessment, and speech-language pathology assessment. Please see Supplementary Methods for complete details of each evaluation.

3. Results

3.1. Demographics and genotype

All patients (12 male and 8 female) were classified as having LOPD following positive NBS in their respective states based on *GAA* variants and absence of HCM on baseline echocardiogram performed in the newborn period. The median age at evaluation was 9.5 months (range: 6–21 months). None of the patients had a known family history of Pompe disease and all were born to non-consanguineous parents. All patients were Caucasian except Patient 2 (Chinese descent), and Patients 6 and 14 (both mixed race - Caucasian and Black/African-American). Patient 14 had begun treatment with ERT under the care of his local geneticist, approximately two weeks prior to his evaluation at Duke University. All other patients were treatment-naïve.

All patients had *GAA* sequencing and confirmatory GAA enzyme analysis in blood prior to their evaluation; all patients' results were reviewed in detail by a geneticist and genetic counselor and were consistent with a diagnosis of LOPD (Table 1). Four patients were homozygous for the IVS1 variant. Fourteen patients were compound heterozygous for the IVS1 variant with a second *GAA* variant. Parental testing confirmed trans configuration of variants in all except two heterozygous patients (8 and 16) whose parents did not undergo *GAA* sequencing. Three of these patients (5, 13, and 17) had a missense variant of uncertain significance (VUS) in trans with the IVS1 variant. In all three patients, no pseudodeficiency alleles were identified that could explain their deficient GAA enzyme. All three VUS were either absent from a large population database (https://gnomad.broadinstitute.org/) or reported in very low frequency (c.316C > T minor allele frequency = 0.0004%). Based on this evidence and deficient GAA enzyme in blood in all three patients, these patients were included in our study with a diagnosis of LOPD. Patient 20 had a maternally inherited VUS, c.2330_2331 + 4dup, inherited in cis with the IVS1 variant and in trans with a second likely pathogenic variant. This variant is not expected to impact phenotype. Two patients (2 and 12) did not have any copies of the IVS1 variant and parental testing confirme trans configuration of disease-causing variants in both patients.

3.2. Cardiac status

None of the patients had evidence of HCM on echocardiogram. No major abnormalities were observed on electrocardiogram (ECG) in the patients that underwent this study ($n = 12$). Possible LVH was initially reported on ECG for one patient (3) with follow up echocardiogram showing no evidence of hypertrophy. This patient was also reported to have high QRS voltages in precordial leads with normal repolarization on ECG. Additional ECG findings reported include possible left axis deviation (LAD) in two patients (1 and 16), and non-specific T-wave abnormality in one patient (12). On subsequent review by a pediatric cardiologist (APL), these were felt to be normal ECG variations representative of common findings in children. All remaining patients' ECGs were interpreted as normal.

3.3. Biomarkers

Plasma creatine kinase (CK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) collected at the time of the baseline analysis were all consistently elevated above the upper limits of normal in eight patients (40%), all of whom were compound heterozygous for the IVS1 variant (Table 2). Of these, five patients had a frameshift or nonsense variant in trans with the IVS1 variant. Nine patients (45%) including all four IVS1 homozygotes had normal CK, AST, and ALT values. One patient had elevated AST alone with normal CK, and one patient had elevated AST and ALT with normal CK. Two patients had elevations in urine Glc4. One patient (3) had a Glc4 value at the upper limit of normal for age (8.3 mmol/molCr, upper limit ≤8.3), with normal CK and AST

Table 1
Demographics and genotype information. (VUS = variant of uncertain significance).

	Patient	Age (months)/Sex	GAA Enzyme Activity in Blood (cutoff)	Variant 1	Variant 1 Classification	Variant 1 Type	Variant 2	Variant 2 Classification	Variant 2 Type
IVS1 Homozygotes	4	10/F	7.76 mcmol/L/h (not provided)	c.-32-13 T > G	Pathogenic	Splice Site	c.-32-13 T > G	Pathogenic	Splice Site
IVS1 Compound Heterozygotes	1	7/F	2.00 umol/ml/h (>3.88)	c.2219_2220del (p.Val740Glyfs*55)	Pathogenic	Frameshift			
	3	21/F	1.37 nmol/mL/h (<10% of daily mean, 1.54)	c.2242dup (p.Glu748Glyfs*48)	Pathogenic	Frameshift			
	5	10.5/M	0.42 umol/L/h (≥2.10)	c.1103G > A (p.Gly368Asp)	VUS	Missense			
	7	13/M	14 uM/L (>22% of daily mean)	c.1839G > A (p.Trp613*)	Pathogenic	Nonsense			
	8	19/M	1.31 umol/L/h (>2.36)	c.1552-3C > G	Pathogenic	Splice Site			
	10	7.5/F	2.7 nmol/mg protein/h (RR: 20.9–140.7)	c.29del (p.His10Profs*33)	Likely Pathogenic	Frameshift			
	11	10/M	0.64 umol/L/h (>1.0)	c.2608C > T (p.Arg870*)	Pathogenic	Nonsense			
	13	10/M	1.59 umol/h (>15% daily mean)	c.316C > T (p.Arg106Cys)	VUS	Missense	c.-32-13 T > G	Pathogenic	Splice Site
	14	6.5/M	0.56 umol/L/h (≥2.10)	c.1655 T > C (p.Leu552Pro)	Pathogenic	Missense			
	15	9/M	2.6 pmol/punch/h (>3.88)	c.766_785delinsC p.Tyr256Argfs*6)	Pathogenic	Frameshift			
	16	6/F	8.39% daily median (>15% daily median)	c.437del (p.Met146Argfs*7)	Pathogenic	Frameshift			
	17	13/M	0.61 umol/L/h (≥2.10)	c.1721 T > C (p.Leu574Pro)	VUS	Missense			
	19	9/F	1.45 umol/L/h (<18% of daily median,13.45)	c.546G > C (p.Thr182=)	Likely Pathogenic	Silent			
	20	7.5/F	2.2 nmol/mg protein/h (RR: 20.9–140.7)	c.2459_2461del (p.Ala820del)	Likely Pathogenic	In-frame deletion			
Non-IVS1 Patients	2	8/M	3.7% of daily mean (>15%)	c.1843G > A (p.Gly615Arg)	Pathogenic	Missense	c.858G > A (p.Thr286=)	Likely Pathogenic	Silent
	12	12/M	2.9 pmol/punch/h (>3.88)	c.2238G > C (p.Trp746Cys)	Pathogenic	Missense	c.2242dup (p.Glu748Glyfs*48)	Pathogenic	Frameshift

and an elevated ALT. A second patient (11) had an elevated Glc4 for age (19.6 mol/molCr, upper limit ≤14.0) with elevated CK, AST, and ALT. These patients had a frameshift and a nonsense variant in trans with the IVS1 variant, respectively. See Supplementary Table S1 for complete biomarker data.

3.4. Motor assessment

Kinematic analysis showed 11 frequent gross motor findings (defined as present in at least 50% of all patients) (Fig. 1). These included tightness of iliotibial bands in 80% (16/20); excessive positional hip external rotation in sitting in 80% (16/20); posterior pelvic tilt in sitting in 70% (14/20); increased excessive positional hip abduction in sitting and supine in 70% (14/20); excessive positional hip external rotation in supine in 65% (13/20); scapular winging in 55% (11/20); head lag on pull to sit - greater than expected for age in 50% (10/20); and a rounded back in 50%, (10/20). Seventy percent (14/20) of patients did not demonstrate age-appropriate use of abdominal oblique muscles and 85% (17/20) did not demonstrate age appropriate use of hip extensor muscles. Additional findings present in less than 50% of patients are listed in Supplementary Table S2.

Table 2
List of patients with elevated plasma biomarkers, from highest to lowest CK value.

Patient	CK (u/L)	AST (u/L)	ALT (u/L)	Genotype
11	771	288	190	IVS1 + Nonsense
20	709	312	141	IVS1 + In frame deletion
*14	668	156	103	IVS1 + Missense
1	640	179	125	IVS1 + Frameshift
5	588	212	155	IVS1 + Missense VUS
15	572	215	153	IVS1 + Frameshift
7	443	90	68	IVS1 + Nonsense
*16	415	125	67	IVS1 + Frameshift

Reference Ranges provided by DUHS Clinical Laboratories (u/L = units per liter)
CK:
< 10 Years: 70–320 u/L
AST:
< 1 Year: 30–120 U/L
> 1 Year: 15–41 U/L
ALT:
< 1 Year: 5–45 U/L
1–3 Years: 5–40 U/L
* Patients 14 and 16 lab values were collected and interpreted outside of DUHS.

The AIMS was completed in 18/20 patients and scores were reported as percentile ranges. Scores were highly variable among patients, ranging from the 5th percentile to the 90th percentile (Table 3). Three patients scored below the 10th percentile – two IVS1 heterozygotes (1 and 15) and one non-IVS1 patient (2), which would be considered concerning in terms of acquisition of gross motor skills. Patient 1 and 2 both scored between 1 and 2 standard deviations (SDs) below the mean, which would be considered a "suspicious" motor performance. Patient 15 scored over 2 SDs below the mean, which would be considered an "abnormal" motor performance. All IVS1 homozygotes scored in the 50-75th percentile, which would be considered expected for age. The patient with the highest number of gross motor findings (11/11) scored in the 50-75th percentile range on the AIMS; the patient with the least gross motor findings (3/11) also scored within the 50-75th percentile range.

3.5. Speech-language pathology assessment

The mean REEL-3 receptive language ability score was 97.1 (range: 82–107), mean expressive language ability score was 95.9 (range: 78–110), and mean composite ability score was 95.8 (range: 76–107) (standard: 90–110). Oral motor examination results were within

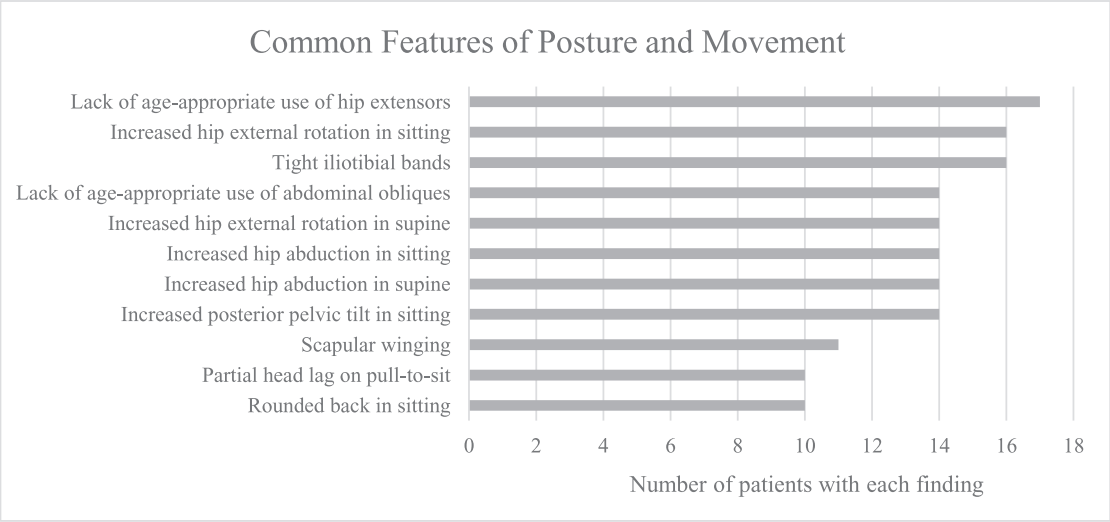

Fig. 1. Common features of posture and movement present in ≥ 50% of all patients ($n = 20$).

normal limits in 17/20 (85%). Abnormal oral motor examination in three patients included limited lingual range of motion, open mouth posture, and a square-shaped tongue. Dysphonia was present in one subject (11) but concerns for hypernasality or reduced loudness were not noted in any patients. Feeding/swallow function was found to be within normal limits in 11/20 (55%), while the remaining nine patients (45%) were found to have mild feeding/swallowing impairments (Table 4). In these nine patients, clinical signs of aspiration were noted in six (e.g., coughing after swallowing, changes in vocal quality). However, signs of dysphagia/aspiration were mild in all cases and an instrumental swallowing assessment was not recommended for any patients. Other signs of feeding/swallowing impairments included anterior bolus loss, decreased lingual lateralization, and decreased labial clearance from spoon. FOIS level for infants and children was a level five in all patients, indicating an expansion of oral diet reached for infants and a total oral diet without special preparations/compensations for children.

Table 3
AIMS score categories and number of frequent gross motor findings by patient.

Patient	Number of Frequent Gross Motor Findings ($n = 11$)	Genotype
10% or below		
1	8	IVS1 + Frameshift
2	10	Missense+Silent
15	8	IVS1 + Frameshift
10–25%		
10*	10	IVS1 + Frameshift
16	5	IVS1 + Frameshift
25–50%		
5	10	IVS1 + Missense
12	7	Missense+Frameshift
13	8	IVS1 + Missense
14	10	IVS1 + Missense
17	9	IVS1 + Missense
20	3	IVS1 + In frame deletion
50–75%		
6	10	IVS1 + IVS1
9	11	IVS1 + IVS1
11	3	IVS1 + Nonsense
18	4	IVS1 + IVS1
19	8	IVS1 + Silent
75% and above		
4	5	IVS1 + IVS1
7	7	IVS1 + Nonsense

* AIMS score adjusted for prematurity.

Caregiver questionnaires to assess observable symptoms of problematic feeding were administered for all patients including the PediEAT in 18 and the NeoEAT-Bottle in two. In all cases, the total scores indicated no concerns regarding problematic feeding and no parental growth concerns were reported in any patients. Complete scores by patient for the PediEAT and NeoEAT-Bottle can be found in Supplementary Table S3.

4. Discussion

With the adoption of NBS across the US, documentation of the phenotypic spectrum of newborns with LOPD after positive NBS is key to determining the most effective management post-diagnosis. Guidelines published by the Pompe Disease NBS Working Group [24] provide a framework for management of confirmed IOPD patients and "symptomatic" LOPD patients after positive NBS; yet there is not a consensus on what defines a "symptomatic" LOPD patient. Data from other countries with successful NBS programs for Pompe disease, such as Taiwan, have limited application to patients in the US due to the overall lack of the IVS1 variant in non-Caucasian populations. Herein, we report

Table 4
Abnormal findings on speech-language pathology feeding/swallowing assessment. All patients with signs of dysphagia were rated mild.

Patient	Dysphagia signs	Clinical signs of aspiration
2	Mild anterior loss with bottle; occasional tongue protrusion with solids; audible gulping; coughing after swallowing	+
7	Drooling during meals; decreased tongue lateralization with solids that required recruitment of fingers during mastication; audible gulping	−
8	Wet vocal quality after swallowing	+
10	Mild anterior loss with bottle and pureed; wet vocal quality and coughing 1× after swallowing	+
11	Decreased suck-swallow-breathe coordination; coughing 1× after swallowing	+
12	Atypical oral sensory responses limiting intake of soft/chewy solids; difficulty advancing to cup drinking; coughing 2× after swallowing	+
14	Decreased upper lip movement for bolus clearance during spoon feeding; decreased tongue lateralization; slow oral transit with pureed	−
17	Occasional overstuffing/large bite sizes; occasional coughing after swallowing	+
19	Decreased upper lip movement for bolus clearance during spoon feeding; audible gulping	−

on the phenotypic spectrum that is observed among patients with LOPD under 2 years of age, most of which possess at least one copy of the IVS1 variant, the most common LOPD variant in Caucasians [7,12,16,17,26]. Patients in our cohort with this variant demonstrated a spectrum of clinical manifestations of disease, similar to what has been documented in the literature [9,22]. Our findings also support previous literature documenting absence of HCM in patients with at least one copy of the IVS1 variant. Due to the presence of residual functional enzyme activity [18,27], patients with the IVS1 variant in heterozygosity or homozygosity are expected to present as LOPD. This applies even in patients who have this variant in compound heterozygosity with a variant seen primarily in infantile-onset patients, such as c.525del (p.Glu176Argfs*45) or nonsense variants that result in absent or very little enzyme activity. However, an exception to this should be considered when there are two additional disease-causing variants present; for example, it is possible that a patient could have one copy of the IVS1 variant in cis with another pathogenic variant plus a third pathogenic variant in trans; this could potentially lead to an IOPD presentation. While this scenario was not observed in our cohort, this combination is possible due to the high frequency of the IVS1 variant in the United States.

Newborns with a positive NBS for Pompe disease should always have an echocardiogram as early as possible to distinguish between IOPD and LOPD. Once it has been established that a patient has LOPD and lack of HCM, the current guidelines recommend that patients with at least one copy of the IVS1 variant should have a follow up echocardiogram at age 6 months; if normal, the frequency of these can be reduced as clinically indicated. Evidence up until this publication suggests the IVS1 variant is cardioprotective based on residual functional GAA enzyme levels [20], and our data continues to support this. Patients with no copies of the IVS1 variant should undergo annual echocardiogram after their 6-month confirmatory evaluation, as there have not been other established genotype-phenotype correlations that demonstrate cardioprotective nature of any other LOPD variants. ECGs should be performed on a routine basis every 12 months regardless of genotype, as rhythm disturbances have been reported in patients with LOPD, even as an isolated symptom [4,28]. Of our patients that had an abnormal initial read on ECG, these findings were further reviewed by a pediatric cardiologist and felt to be representative of normal variations that can be observed in healthy children. Thus, it is imperative that abnormal ECG findings are reviewed by a pediatric cardiologist and followed up with an echocardiogram.

With the absence of HCM, assessment of symptom onset should heavily focus on the musculoskeletal profile of these patients. Muscle weakness may not be clearly evident on routine physical exams and there is a risk of missing subtle musculoskeletal symptoms that may progress over time. The NBS Working Group guidelines recommend monitoring developmental progress using a variety of tools including the Denver Developmental Screening test (Denver II), the AIMS, the Test of Infant Motor Performance (TIMP) and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND). However, there are no specific recommendations for utilization of these tests to determine whether or not a patient is symptomatic from a musculoskeletal perspective. The AIMS was utilized in our study and revealed a wide range of scores, particularly among those that were compound heterozygous for the IVS1 variant. Most patients had normal or even above average AIMS scores and may have been classified as developing typically when motor status is determined by skill acquisition alone. Although our analysis was limited by lack of a control group, all patients in our cohort had some kinematic or postural features which are considered classic musculoskeletal features of posture and movement in IOPD and in older LOPD patients [6,9,22,29] and considered abnormal when compared to established developmental norms [22,30]. These features are considered signs of weakness and musculoskeletal involvement that could lead to symptom progression requiring close monitoring. Further, children that scored within the same percentile range on the AIMS demonstrated a wide range of kinematic concerns; the patient with the least kinematic concerns (patient 11; IVS1 + Nonsense) and the patient with the most kinematic concerns (patient 9, IVS1 homozygote) both scored within the 50-75th percentile range on the AIMS. Notably, patient 11 also had the highest CK, AST, and ALT values in the cohort. This is a limitation of using standardized testing alone; standardized test scores such as the AIMS should be interpreted in the context of the clinical phenotype of these patients. Not only do these findings highlight that the specific features of posture and movement known to be characteristic of LOPD and IOPD can be present in the first year of life in those with LOPD, it also demonstrates that these can be present in even in the absence of apparent motor delay measurable by standardized assessment. Careful phenotyping by an experienced physical therapist is imperative along with standardized testing such as the AIMS in order to obtain a complete physical therapy evaluation for these patients. Patients should continue to be monitored closely per published guidelines, to identify even subtle musculoskeletal symptoms for monitoring over time.

While outward signs of musculoskeletal involvement may not always be apparent, serum biomarkers such as CK and AST/ALT may be indicative of disease progression. While these are not specific to Pompe disease, elevated muscle enzymes in the setting of a known progressive muscle disease warrants continued monitoring and evaluation. Caution should be exercised when interpreting these values. They may be falsely elevated, especially if collected during a period of illness or after a long period of physical activity (such as a physical therapy evaluation). For this reason, biomarkers should be collected prior to physical therapy evaluation, if possible, on the day of a clinical visit. Biomarker values should be trended over time, and single data points should not be used as the basis for recommending treatment with ERT. Current guidelines recommend these labs should be collected every 3 months within the first year and every 3–12 months thereafter to monitor trends [24]. Should values remain persistently elevated, these should be interpreted in the context of the clinical phenotype gleaned from the musculoskeletal evaluation.

On the other hand, urine Glc4 is a more sensitive and specific Pompe disease biomarker [31]. Only two of our patients had elevated or borderline elevated values, which is typical given that patients with LOPD diagnosed via NBS are expected to have normal Glc4 values at diagnosis. [32]. Normal Glc4 values at this age in LOPD patients may be representative of intra-lysosomal glycogen, which would not be detectable in urine. Glc4 is also utilized as a biomarker to monitor disease progression over time [33] and may increase above the upper limits of normal as patients age and with disease progression.

Finally, speech-language pathology assessment indicated speech and language function to be within normal limits in all participants. However, due to the age of the participants, these assessments were inherently limited and ongoing assessment of communication function is warranted. While clinical feeding/swallowing evaluation results revealed mild oropharyngeal dysphagia and clinical signs of aspiration in some cases, these clinical signs of aspiration were mild and instrumental assessment of swallowing was not deemed necessary. Additionally, based on FOIS level, none of the participants presented with limitations to their oral intake and formal and informal parent reports indicated no concerns regarding problematic feeding or growth. Referral to speech-language pathology for assessment of speech-language and feeding/swallowing should be considered for infants and children with LOPD if there are concerning clinical features.

A major limitation of our study is that it represents a single time point for each patient, which results in challenges with interpretation of its significance. Thus, long-term follow up is needed for these patients to determine trends over time, specifically biomarkers and musculoskeletal features. At this time, genotype alone is not sufficient to predict the disease course for children with LOPD, especially in the setting of an increase in novel variants due to adoption of NBS. However, genotype can be an important indicator of LOPD vs. IOPD when known LOPD variants are detected, such as the IVS1 variant. Further, molecular testing

can identify potential modifier variants, such as the c.510C > T polymorphism, which is predicted to modify phenotype by modulating the aberrant splicing caused by the IVS1 variant [34]. In our cohort, molecular reports in seven patients indicated absence of this polymorphism; however, as we were unable to confirm this information for the remaining patients in the study, potential effects of this variant were not formally analyzed as part of this study.

While clinicians may experience challenges in caring for patients with LOPD detected on NBS, parents and families also may experience a significant burden associated with these challenges. Parents of children with LOPD diagnosed via NBS are at risk for feeling as though their child is a "patient in waiting," a phenomenon describing patients diagnosed with a "late-onset" disorder before clinically significant symptoms have occurred [35]. This phenomenon has been described in this patient population, and is known to increase fear and anxiety in parents [36]. In addition to anticipating symptom onset, parents of children with LOPD may also experience anxiety related to the absence of specific guidelines that determine when and if their child will need treatment with ERT. Our purpose is to provide insights into the early phenotype of this patient population that allow clinicians to identify potential symptoms and monitor their progression over time decisions based on clinical evidence and give families direction in advocating for the care of their child. *CRediT category:* Conceptualization (E.H., M.H., L.E.C., P.S.K.), Data Curation (E.H., J.B.), Formal Analysis (E.H., M.H., L.E.C., A.P.L., H.N.J., P.S.K.), Project Administration (J.B.), Writing-original draft (E.H.), Writing-review and editing (M.H., L.E.C., A.P.L., H.N.J., P.S.K.)

Ethics declaration

The study was reviewed and approved by Duke University Health System Institutional Review Board (IRB, Pro00100223). The authors attest that (1) written informed consent was obtained for all subjects enrolled in the study (2) informed consent included consent for both study participation and publication of de-identified data and (3) the consents forms are kept in a safe and secure location and are accessible by request if needed.

Data availability

All relevant clinical data are included in this manuscript and supplementary materials. Any additional data requested for the use of replicating methods and results presented in this manuscript can be shared by request from any qualified investigator, contingent on the study protocol.

Acknowledgements

The authors would like to thank the patients and their families who participated in this study, and acknowledge Amicus Therapeutics which provided funding in part for this research. The authors would also like to thank Sophia's Pompe Strong Team, Gabriel's Gale, the Harris family, the Spaulding/Sinks family, Marshall's Mountain, Ryker's Foundation, and the Landreth family for their generous contributions to research efforts in newborn screening for Pompe disease.

Appendix A. Supplementary data

Supplementary methods, data, and literature cired in this article can be found online at https://doi.org/10.1016/j.ymgme.2022.01.003.

References

[1] Rochelle Hirschhorn, Arnold J.J. Reuser, Glycogen Storage Disease Type II: Acid α-Glucosidase (Acid Maltase) Deficiency | The Online Metabolic and Molecular Bases of Inherited Disease | OMMBID | McGraw-Hill Medical, Accessed March 13, 2020 https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225890450.

[2] P.S. Kishnani, A.A. Beckemeyer, N.J. Mendelsohn, The new era of Pompe disease: advances in the detection, understanding of the phenotypic spectrum, pathophysiology, and management, Am. J. Med. Genet. C Semin. Med. Genet. 160 C (1) (2012) 1–7, https://doi.org/10.1002/ajmg.c.31324.

[3] P.S. Kishnani, W.L. Hwu, H. Mandel, M. Nicolino, F. Yong, D. Corzo, A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease, J. Pediatr. 148 (5) (2006) https://doi.org/10.1016/j.jpeds.2005.11.033 671-676.e2.

[4] J. Chan, A.K. Desai, Z.B. Kazi, et al., The emerging phenotype of late-onset Pompe disease: a systematic literature review, Mol. Genet. Metab. 120 (3) (2017) 163–172, https://doi.org/10.1016/j.ymgme.2016.12.004.

[5] C.I. Van Capelle, J.C. Van Der Meijden, J.M.P. Van Den Hout, et al., Childhood Pompe disease: clinical spectrum and genotype in 31 patients, Orphan. J. Rare Dis. 11 (1) (2016)https://doi.org/10.1186/s13023-016-0442-y.

[6] P.S. Kishnani, R.D. Steiner, D. Bali, et al., Pompe disease diagnosis and management guideline, Genet Med. 8 (5) (2006) 267–288, https://doi.org/10.1097/01.gim.0000218152.87434.f3.

[7] M.A. Kroos, R.J. Pomponio, M.L. Hagemans, et al., Broad spectrum of Pompe disease in patients with the same c.-32-13 T → G haplotype, Neurology. 68 (2) (2007) 110–115, https://doi.org/10.1212/01.wnl.0000252798.25690.76.

[8] B. Schoser, A. Stewart, S. Kanters, et al., Survival and long-term outcomes in late-onset Pompe disease following alglucosidase alfa treatment: a systematic review and meta-analysis, J. Neurol. 264 (4) (2017) 621–630, https://doi.org/10.1007/s00415-016-8219-8.

[9] M. Herbert, L.E. Case, M. Rairikar, et al., Early-onset of symptoms and clinical course of Pompe disease associated with the c.-32–13 T > G variant, Mol. Genet. Metab. 126 (2) (2019) 106–116, https://doi.org/10.1016/j.ymgme.2018.08.009.

[10] Y.H. Chien, N.C. Lee, B.L. Thurberg, et al., Pompe disease in infants: improving the prognosis by newborn screening and early treatment, Pediatrics 124 (6) (2009) https://doi.org/10.1542/peds.2008-3667.

[11] C.F. Yang, C.C. Yang, H.C. Liao, et al., Very early treatment for infantile-Onset Pompe disease contributes to better outcomes, J. Pediatr. 169 (2016) https://doi.org/10.1016/j.jpeds.2015.10.078 174-180.e1.

[12] J.M. De Vries, N.A. Van Der Beek, W.C. Hop, et al., Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study, Orphan. J. Rare Dis. 7 (1) (2012) 1–10, https://doi.org/10.1186/1750-1172-7-73.

[13] Y.H. Chien, S.C. Chiang, X.K. Zhang, et al., Early detection of pompe disease by newborn screening is feasible: Results from the Taiwan screening program, Pediatrics 122 (1) (2008) https://doi.org/10.1542/peds.2007-2222.

[14] Y.H. Chien, W.L. Hwu, N.C. Lee, Pompe disease: Early diagnosis and early treatment make a difference, Pediatr. Neonatol. 54 (4) (2013) 219–227, https://doi.org/10.1016/j.pedneo.2013.03.009.

[15] Y.H. Chien, N.C. Lee, C.A. Chen, et al., Long-term prognosis of patients with infantile-onset pompe disease diagnosed by newborn screening and treated since birth, J. Pediatr. 166 (4) (2015) https://doi.org/10.1016/j.jpeds.2014.10.068 985-991.e2.

[16] M.L. Hule, A.S. Chen, S. Tsujino, et al., Aberrant splicing in adult onset glycogen storage disease type II (GSDII): Molecular identification of an IVS1 (− 13 T → G) mutation in a majority of patients and a novel IVS10 (+ 1GT → CT) mutation, Hum. Mol. Genet. 3 (12) (1994) 2231–2236, https://doi.org/10.1093/hmg/3.12.2231.

[17] M.A. Kroos, M. Van Der Kraan, O.P. Van Diggelen, et al., Glycogen storage disease type II: Frequency of three common mutant alleles and their associated clinical phenotypes studied in 121 patients, J. Med. Genet. 32 (10) (1995) 836–837, https://doi.org/10.1136/jmg.32.10.836-a.

[18] A. Dardis, I. Zanin, S. Zampieri, et al., Functional characterization of the common c.-32-13 T > G mutation of GAA gene: identification of potential therapeutic agents, Nucleic Acids Res. 42 (2) (2014) 1291, https://doi.org/10.1093/NAR/GKT987.

[19] N.A. van der Beek, O.I. Soliman, C.I. van Capelle, et al., Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13 T > G genotype rarely reveals abnormalities, J. Neurol. Sci. 275 (1–2) (2008) 46–50, https://doi.org/10.1016/j.jns.2008.07.013.

[20] M. Herbert, H. Cope, J.S. Li, P.S. Kishnani, Severe cardiac involvement is rare in patients with late-Onset Pompe disease and the common c.-32-13 T > G variant: implications for newborn screening, J. Pediatr. 198 (2018) 308–312, https://doi.org/10.1016/j.jpeds.2018.02.007.

[21] O. Musumeci, A. Thieme, K.G. Claeys, et al., Homozygosity for the common GAA gene splice site mutation c.-32-13 T > G in Pompe disease is associated with the classical adult phenotypical spectrum, 25 (9) (2015) 719–724, https://doi.org/10.1016/J.NMD.2015.07.002.

[22] M.V. Rairikar, L.E. Case, L.A. Bailey, et al., Insight into the phenotype of infants with Pompe disease identified by newborn screening with the common c.-32-13 T > G "late-onset" GAA variant, Mol. Genet. Metab. 122 (3) (2017) 99–107, https://doi.org/10.1016/j.ymgme.2017.09.008.

[23] B.K. Burton, D.F. Kronn, W.L. Hwu, P.S. Kishnani, The initial evaluation of patients after positive newborn screening: Recommended algorithms leading to a confirmed diagnosis of pompe disease, Pediatrics 140 (Suppl. 1) (2017) https://doi.org/10.1542/peds.2016-0280D.

[24] D.F. Kronn, D. Day-Salvatore, W.L. Hwu, et al., Management of confirmed newborn-screened patients with pompe disease across the disease spectrum, Pediatrics 140 (Suppl. 1) (2017) https://doi.org/10.1542/peds.2016-0280E.

[25] B.K. Burton, J. Charrow, G.E. Hoganson, et al., Newborn screening for lysosomal storage disorders in illinois: the initial 15-month experience, J. Pediatr. 190 (2017) 130–135, https://doi.org/10.1016/J.JPEDS.2017.06.048.

[26] P. Laforêt, K. Laloui, B. Granger, et al., The French Pompe registry. Baseline characteristics of a cohort of 126 patients with adult Pompe disease, Rev. Neurol. (Paris) 169 (8–9) (2013) 595–602, https://doi.org/10.1016/j.neurol.2013.07.002.

[27] C.F. Boerkoel, R. Exelbert, C. Nicastri, et al., Leaky splicing mutation in the acid maltase gene is associated with delayed onset of glycogenosis type II, Am. J. Hum. Genet. 56 (4) (1995) 887–897 Accessed April 8, 2021. /pmc/articles/PMC1801206/?report = abstract.
[28] M. Mori, L.A. Bailey, J. Estrada, et al., Severe cardiomyopathy as the isolated presenting feature in an adult with late-Onset Pompe disease: a case report, JIMD Rep. 31 (2017) 79–83, https://doi.org/10.1007/8904_2016_563.
[29] L.E. Case, P.S. Kishnani, Physical therapy management of Pompe disease, Genet Med. 8 (5) (2006) 318–327, https://doi.org/10.1097/01.GIM.0000217789.14470.C5.
[30] L. Bly, Motor Skills Acquisition in the First Year: An Illustrated Guide to Normal Development, Therapy Skill Builders 1994.
[31] S.P. Young, M. Piraud, J.L. Goldstein, et al., Assessing disease severity in Pompe disease: the roles of a urinary glucose tetrasaccharide biomarker and imaging techniques, Am. J. Med. Genet. C Semin. Med. Genet. 160 C (1) (2012) 50–58, https://doi.org/10.1002/ajmg.c.31320.
[32] Y.H. Chien, J.L. Goldstein, W.L. Hwu, et al., Baseline urinary glucose tetrasaccharide concentrations in patients with infantile- and late-onset pompe disease identified by newborn screening, JIMD Reports, Vol 19, Springer 2015, pp. 67–73, https://doi.org/10.1007/8904_2014_366.
[33] S.P. Young, H. Zhang, D. Corzo, et al., Long-term monitoring of patients with infantile-onset Pompe disease on enzyme replacement therapy using a urinary glucose tetrasaccharide biomarker, Genet Med. 11 (7) (2009) 536–541, https://doi.org/10.1097/GIM.0b013e3181a87867.
[34] A.J. Bergsma, in't Groen SLM, van den Dorpel JJA, et al., A genetic modifier of symptom onset in Pompe disease, EBioMedicine. 43 (2019) 553–561, https://doi.org/10.1016/j.ebiom.2019.03.048.
[35] S. Timmermans, M. Buchbinder, Patients-in-waiting: Living between sickness and health in the genomics era, J. Health Soc. Behav. 51 (4) (2010) 408–423, https://doi.org/10.1177/0022146510386794.
[36] B. Pruniski, E. Lisi, N. Ali, Newborn screening for Pompe disease: impact on families, J. Inherit. Metab. Dis. 41 (6) (2018) 1189–1203, https://doi.org/10.1007/S10545-018-0159-2.