# Outcome of Later-Onset Pompe Disease Identified Through Newborn Screening

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**Objective** To determine the outcomes of patients with later-onset Pompe disease (LOPD) identified through newborn screening (NBS).

**Study design** A prospective observational cohort study was conducted from the initiation of Pompe disease NBS by following subjects every 3-12 months for motor development and biochemical markers.

**Results** Between 2005 and 2018, 39 of 994 975 newborns evaluated were classified as having LOPD based on low acid α-glucosidase (GAA) activity but no cardiac involvement at the time of screening. As of December 2020, 8 of these 39 infants (21%) were treated with enzyme replacement therapy owing to persistent elevation of creatine kinase (CK), cardiac involvement, or developmental delay. All subjects' physical performance and endurance improved after treatment. Subjects carrying c.[752C>T;761C>T] and c.[546+5G>T; 1726G>A] presented a phenotype of nonprogressive hypotonia, muscle weakness, and impairment in physical fitness tests, but they have not received treatment.

**Conclusions** One-fifth of subjects identified through NBS as having LOPD developed symptoms after a follow-up of up to 15 years. NBS was found to facilitate the early detection and early treatment of those subjects. *GAA* variants c.[752C>T;761C>T] and c.[546+5G>T; 1726G>A] might not cause Pompe disease but still may affect skeletal muscle function. *(J Pediatr 2022;244:139-47)*.

Pompe disease, or glycogen storage disease type II, is a lysosomal storage disorder in which deficiency of acid α-glucosidase (GAA; EC 3.2.1.20) causes intralysosomal accumulation of glycogen in all tissues, most notably in skeletal muscles.[1] Pompe disease exhibits a continuum of disease severity and can be classified as infantile-onset Pompe disease when cardiac involvement and weakness occur before age 12 months and as later-onset Pompe disease (LOPD) if symptoms occur after age 12 months. Enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA)[2,3] is currently the sole available treatment. Newborn screening (NBS) for Pompe disease[4] identifies patients with infantile-onset Pompe disease. NBS also identifies newborns with genetic risk factors but no cardiac involvement at birth; these infants are considered to have LOPD, and some develop symptoms after follow-up.[5]

ERT improves the outcomes of patients with infantile-onset Pompe disease, especially those who are treated early.[2,6] In contrast, the reported clinical benefit from ERT treatment in individuals with LOPD is variable,[7,8] although a delay in treatment is an unfavorable prognostic factor.[9] An obstacle to initiating ERT in LOPD is the difficulty in predicting the pathogenicity of *GAA* variants. To date, 648 pathogenic or likely pathogenic *GAA* variants and numerous variants of unknown significance (VUSs) have been reported.[10,11] Another obstacle is the lack

| | | | |
|---|---|---|---|
| 4-MU | 4-methylumbelliferone-α-D-glucopyranoside | PDMS-2 | Peabody Developmental Motor Scales, 2nd Edition |
| 6MWT | Six-minute walk test | Pompe-PEDI | Pompe Pediatric Evaluation of Disability Inventory |
| AIMS | Alberta Infant Motor Scale | | |
| CK | Creatine kinase | QMFT | Quick Motor Function Test |
| ERT | Enzyme replacement therapy | rhGAA | Recombinant human acid α-glucosidase |
| GAA | Acid α-glucosidase | RT-PCR | Reverse-transcription polymerase chain reaction |
| GMFM-66 | Gross Motor Function Measure 66 | uGlc4 | Urinary glucose tetrasaccharide |
| LOPD | Later-onset Pompe disease | | |
| NBS | Newborn screening | VUS | Variant of unknown significance |

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Sanofi Genzyme partially funded this long-term follow-up study. The study funder had no involvement in the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the manuscript for publication. Y-H.C. has received compensation as a member of the scientific advisory boards of Sanofi Genzyme, Amicus Therapeutics, Takeda, and AskBio; has undertaken contracted research for Sanofi Genzyme and Biogen; and has received honoraria and consulting fees from Sanofi Genzyme, Takeda, Amicus, BioMarin, Biogen, and Novartis. W-L.H. has received compensation as a member of the scientific advisory boards of Sanofi Genzyme, PTC Therapeutics, Takeda, Amicus Therapeutics, and Biogen; has undertaken contracted research for Sanofi Genzyme, BioMarin, and Takeda; and has received honoraria and consulting fees from Sanofi Genzyme, Biogen, BioMarin, and Takeda. N-C.L. has received compensation as a member of the scientific advisory board for Sanofi Genzyme and has received honoraria and consulting fees from Sanofi Genzyme, Takeda, and BioMarin. The other authors declare no conflicts of interest.


https://doi.org/10.1016/j.jpeds.2021.12.072

of knowledge regarding the early manifestations of LOPD, especially childhood- or juvenile-onset Pompe disease. The currently published consensus for starting ERT in adult patients with Pompe disease is based on skeletal muscle weakness.[12] The data from published studies generally lack details on initial musculoskeletal involvement. Early signs of LOPD, such as subtle muscular weakness, are often mistaken for nonspecific hypotonia or developmental delay.[13,14] We and others have reported the subsequent occurrence of motor delay and increased creatine kinase (CK) in a segment of these patients.[5,15,16]

In the present study, we analyzed the molecular and biochemical data of subjects with LOPD identified through NBS and correlated these data to their clinical manifestations. We also summarized the outcomes of those who had been treated with ERT.

## Methods

The NBS Center at National Taiwan University Hospital initiated a program for Pompe disease in 2005 involving measuring GAA activity from dried blood spots. Newborns with low GAA activity were referred for confirmatory diagnosis of Pompe disease, including lymphocyte GAA activity and *GAA* whole-gene sequencing. GAA deficiency was defined as lymphocyte GAA activity <3% of the normal mean and *GAA* variants on both the maternally and paternally inherited chromosomes. Subjects with only the pseudodeficiency variant c.1726G>A [p.(G576S)] were excluded. Infantile-onset Pompe disease refers to patients with cardiac involvement at the time of diagnosis; all other cases were classified as LOPD. *GAA* haplotypes were defined according to a previous report.[17] Treatment with ERT was provided for subjects with infantile-onset Pompe disease and those with LOPD who developed signs and symptoms of Pompe disease during follow-up. Subjects classified as LOPD were followed up every 3 months during the first year of life and every 6-12 months thereafter.[5,18] Laboratory assessments included CK and urinary glucose tetrasaccharide (uGlc4).[19] Developmental assessments included qualitative assessment of posture and movement, as well as age-specific standardized assessments including the Alberta Infant Motor Scale (AIMS), the Peabody Developmental Motor Scales 2nd Edition (PDMS-2), the Pompe Pediatric Evaluation of Disability Inventory (Pompe-PEDI), the Gross Motor Function Measure (GMFM-66), the 6-minute walk test (6MWT), the Movement Assessment Battery for Children, the Quick Motor Function Test (QMFT),[20] and Taiwanese student physical fitness tests (sit-ups in 30 seconds and broad jump). All standardized assessments were administered and scored in accordance with standardized, task-specific test procedures. Data from the PDMS-2, Pompe-PEDI, Movement Assessment Battery for Children, 6MWT,[21,22] and Taiwanese student physical fitness tests are provided relative to the norms. Norms for sit-ups and the broad jump are available starting at age 7 years. The GMFM-66 provides information regarding the child's individual development. The QMFT, a valid tool for muscle function assessment in patients with Pompe disease, consists of 16 motor tasks that are especially difficult for these patients, and a higher QMFT score indicates better motor function.[20] The follow-up program was approved by the hospital's Institutional Review Board (200612017R, 200703045R, and 200804012R) and registered at ClinicalTrials.gov (identifiers NCT00713245 and NCT02399748).

### Mutagenesis and in Vitro Assays

**Cell Culture.** Fibroblasts and HEK293T cells were cultured using proliferation medium (Dulbecco's Modified Eagle Medium; Gibco) supplemented with 10% fetal bovine serum (Westburg) and 1% penicillin-streptomycin-glutamine (Life Technologies). Transfections were performed at 80% confluence using Lipofectamine 2000 (Invitrogen) as the transfection reagent; the reagent was used according to the manufacturer's instructions. Cells were harvested using TrypLE (Life Technologies) at 72 hours after transfection. RNA was extracted using the RNeasy Mini Kit (QIAGEN), including DNase I (QIAGEN) treatment, and protein extracts were used to assess GAA activity.

**Mutagenesis.** To generate an expression construct, *GAA* cDNA was reverse-transcribed from healthy control RNA using iScript (Bio-Rad) and amplified with PFU Ultra Hotstart polymerase (Agilent Technologies). This product was cloned into the NheI and AflII restriction sites of the pcDNA3.1(−) Myc-His A vector. The variants c.752C>T, c.761C>T, and c.[752C>T;761C>T] were introduced using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies) and verified by Sanger sequencing.

**GAA Activity Assays.** Measurements using 4-methylumbelliferone-α-D-glucopyranoside (4-MU) as a substrate were performed as a 1-step protocol using substrate dissolved in citrate-phosphate buffer for 1 hour at 37°C, after which fluorescence was measured at 365/448 nm using the Varioskan system (Thermo Fisher Scientific), as described previously.[23] Measurements with glycogen as a substrate were performed using a 2-step protocol: the protein extract was first incubated with 7.5% glycogen dissolved in citrate-phosphate buffer for 2 hours, then incubated with glucose-detection reagent (exact composition[24]) for 75 minutes, after which fluorescence was measured at 420 nm using the Varioskan system, as described previously.[24] Enzyme activity measurements performed in fibroblasts were corrected for total protein and measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).[23] Measurements based on *GAA* cDNA expression constructs were corrected for transfection efficiency using neomycin mRNA expression, as described previously.[23]

### Prediction of Splicing

To assess the presumptive effect of the c.546+5G>T variant on splicing, we used 4 different in silico prediction tools: Splice Site Finder,[25] MaxEntScan,[26] Splice Site Prediction by Neural Network,[27] and the GeneSplicer program.[28] The output score

**Table. Genotypes and phenotypes of the study subjects**

| No. | GAA | Genotype | Associated phenotype |
|---|---|---|---|
| Group 1 | | | |
| 3* | 0.8 | c.[2238G>C;1726G>A]/c.2662G>T | Childhood or adult/classic infantile |
| 9* | 0.46 | c.[1935C>A;1726G>A]/c.[2238G>C;1726G>A] | Classic infantile/childhood or adult |
| 15* | 1.14 | c.872T>C/c.1798C>T | Classic infantile/classic infantile |
| 16* | 0.74 | c.[1935C>A;1726G>A]/c.1634C>T | Classic infantile/childhood or adult |
| 18 | 2.14 | c.[1935C>A;1726G>A]/c.671G>A | Classic infantile/adult |
| 20 | 5.51 | c.-32-13T>G/c.[2214G>A;1726G>A] | Childhood or adult/predicted severe |
| 25* | 1.01 | c.[1935C>A;1726G>A]/c.[2238G>C;1726G>A] | Classic infantile/childhood or adult |
| 30* | 4.59 | c.1579del/c.1222A>G/ | Predicted severe/classic infantile |
| 36 | 2.64 | c.[1935C>A;1726G>A]/c.2236T>G | Classic infantile/predicted less severe |
| 37 | 1.88 | c.[1935C>A;1726G>A]/c.546G>A | Classic infantile/adult |
| Group 2 | | | |
| 1* | 1.65 | c.424_440del/c.[811A>G;1726G>A] | Classic infantile/predicted nonpathogenic |
| 4 | 1.27 | c.2662G>T/c.1574T>A | Classic infantile/predicted mild |
| 5 | 1.45 | c.424_440del/c.[533G>A;1726G>A] | Classic infantile/uncertain prediction |
| 6* | 1.49 | c.[1935C>A;1726G>A]/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 7 | 1.93 | c.1080C>G/c.[546+5G>T;1726G>A] | Predicted severe/uncertain prediction |
| 10 | 0.35 | c.1843G>A/c.[1324G>A;1726G>A] | Classic infantile/uncertain prediction |
| 12 | 0.84 | c.[1935C>A;1726G>A]/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 13 | 0.69 | c.[1935C>A;1726G>A]/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 14 | 1.54 | c.[1935C>A;1726G>A]/c.[546+5G>T;1726G>A] | Classic infantile/uncertain prediction |
| 17 | 2.18 | c.[1935C>A;1726G>A]/c.1958C>A | Classic infantile/uncertain prediction |
| 19 | 5.97 | c.[1935C>A;1726G>A]/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 21 | 0.91 | c.[1935C>A;1726G>A]/c.1958C>A | Classic infantile/uncertain prediction |
| 22 | 2.89 | c.2662G>T/c.1757C>T | Classic infantile/uncertain prediction |
| 23 | 1.14 | c.2185del/c.1958C>A | Classic infantile/uncertain prediction |
| 24 | 1.11 | c.2815_2816del/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 26 | 1.38 | c.[2238G>C;1726G>A]/c.[752C>T;761C>T] | Childhood or adult/predicted nonpathogenic |
| 27 | NA | c.671G>A/c.[752C>T;761C>T] | Adult/predicted nonpathogenic |
| 29 | 2.64 | c.1843G>A/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 32 | 1.41 | c.2236T>G/c.[752C>T;761C>T] | Predicted less severe/predicted nonpathogenic |
| 33 | 2.7 | c.[1935C>A;1726G>A]/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 34 | 4.64 | c.[1935C>A;1726G>A]/c.[546+5G>T;1726G>A] | Classic infantile/uncertain prediction |
| 35 | 1.98 | c.1577T>C/c.[752C>T;761C>T] | Classic infantile/predicted nonpathogenic |
| 39 | 0.86 | c.[1935C>A;1726G>A]/c.[546+5G>T;1726G>A] | Classic infantile/uncertain prediction |
| Group 3 | | | |
| 2 | 0.75 | c.[752C>T;761C>T]/c.[752C>T;761C>T] | Predicted nonpathogenic/predicted nonpathogenic |
| 8 | 6.36 | c.[546+5G>T;1726G>A]/c.[546+5G>T;1726G>A] | Uncertain prediction/uncertain prediction |
| 11 | 0.58 | c.[752C>T;761C>T]/c.1958C>A | Predicted nonpathogenic/uncertain prediction |
| 28 | 0.99 | c.[752C>T;761C>T]/c.[752C>T;761C>T] | Predicted nonpathogenic/uncertain prediction |
| 31 | 2.54 | c.[752C>T;761C>T]/c.1048G>A | Predicted nonpathogenic/uncertain prediction |
| 38 | 0.36 | c.[752C>T;761C>T]/c.[752C>T;761C>T] | Predicted nonpathogenic/predicted nonpathogenic |

*NA*, not analyzed.
*Subjects on enzyme replacement therapy.

of each tool indicates the splicing signal strength, with a higher value indicating a greater possibility of being a splicing site in vivo. Primary skin fibroblasts were obtained from subject 7 (c. [546+5G>T;1726G>A]/c.1080C>G) and subject 8 (c.[546+5G>T;1726G>A], homozygous). RNA was extracted from the fibroblasts, reverse-transcribed, amplified by reverse-transcription polymerase chain reaction (RT-PCR), and verified using Sanger sequencing.

## Results

Between 2005 and 2018, we screened 994 975 newborns and identified 55 with Pompe disease, for a total incidence of 1 in 18 090 (95% CI, 13 970-23 664). Sixteen newborns were found to have hypertrophic cardiomyopathy at the time of diagnosis and were classified as having infantile-onset Pompe disease,[19] with an incidence of 1 in 62 186 (95% CI, 38 470-101 530). The newborns with infantile-onset Pompe disease had been treated with ERT starting at age 5-34 days. Thirty-nine newborns were found to have no cardiac involvement at birth and thus were classified as having LOPD at the time of diagnosis (Table), with an incidence of 1 in 25 512.

The most common *GAA* haplotypes were *03 (31 of 78; 40%), *09 (28%), and *08 (12%). Haplotype *03 contains the pseudodeficiency variant c.1726G>A [p.(G576S)], which decreases the activity of the enzyme.[29] Variants related to haplotype *03 are associated with c.1726G>A and expressed by square brackets indicating that the 2 variants are located on the same chromosome. The 3 most common *GAA* variants in NBS-LOPD were c.[752C>T;761C>T] (p.[(S251L);(S254L)]) (19 of 78; 24%), c.[1935C>A;1726G>A] (p.[(D645E);(G576S)]) (21%), and c.[546+5G>T;1726G>A] (8%) (Table). Variant c.[752C>T;761C>T] was related to haplotype *09 and was not associated with c.1726G>A.

We divided NBS-LOPD into 3 groups according to the pathogenicity of the variants, as listed in the Pompe disease GAA variant database (Table)[10,11]: group 1, with 2 known

pathogenic/likely pathogenic variants (n = 10; 9 with biochemical data and 8 with follow-up data); group 2, with 1 known pathogenic/likely pathogenic variant and 1 VUS (n = 23; 22 with biochemical data and 15 with follow-up data); and group 3, with 2 VUSs (n = 6; all with biochemical data and 4 with follow-up data). Biochemical data, available for 37 subjects, revealed that 41% of them had an initial CK > 200 U/L (22% with CK > 300 U/L), and 49% had elevated uGlc4 (>12 mmol/mol Cre) (**Figure 1**; available at www.jpeds.com). In group 1 (n = 9), 78% had CK > 200 U/L (56% with CK > 300 U/L), and 67% exhibited uGlc4 elevation. In group 2 (n = 22), 36% had CK > 200 IU/L (13% with CK > 300 U/L), and 50% had elevated uGlc4. In group 3, only 1 subject displayed uGlc4 elevation, and none had CK elevation. Compared with group 2, group 1 subjects had a higher incidence of CK > 200 U/L ($P = .036$) and CK > 300 U/L ($P = .015$) but not of elevated uGlc4 ($P = .397$, $\chi^2$ test). The distribution of these values among the different groups is depicted in **Figure 2**.

## Follow-Up

**Onset of Signs and Symptoms.** As of December 2020, 6 subjects in group 1 (60%) and 2 subjects in group 2 (8.7%) had been treated with ERT between age 1.6 months and 3 years. Five of these subjects (1, 3, 6, 9, and 15) have been described previously.[15] The indication for ERT was persistent CK elevation in subjects 3, 9, 16, and 25; elevation of CK and the occurrence of hypertrophic cardiomyopathy in subject 15; hypotonia and delay in gross motor development in subjects 1, 3, and 6; and an unrecorded indication in subject 30. Subjects 3, 9, and 25 carried 1 c.[2238G>C;1726G>A] (p.[(W746C);(G576S)]) variant and 1 severe variant. The c.[2238G>C;1726G>A] variation, also modified by the c.1726G>A pseudodeficiency variant with *GAA* haplotype *03, is usually associated with juvenile-onset Pompe disease.[30] Overall, 8 subjects (8 of 39; 21%) were undergoing ERT at the time of this report. The other 4 subjects in group 1 were still asymptomatic at age 3-9 years. All 4 of these subjects had a variant that was either predicted as milder or has been observed in adult-onset Pompe disease. Taken together, the conservative estimation of those who would have symptoms was 12 in 994 975 (1 in 82 915).

## ERT Outcomes

After ERT, the biochemical variable (serum CK and uGlc4) of all subjects improved, although the improvement was not sustained in some subjects. Motor performance (GMFM-66, PDMS-2 gross motor, and Pompe-PEDI mobility) also improved in all subjects, and they could maintain average

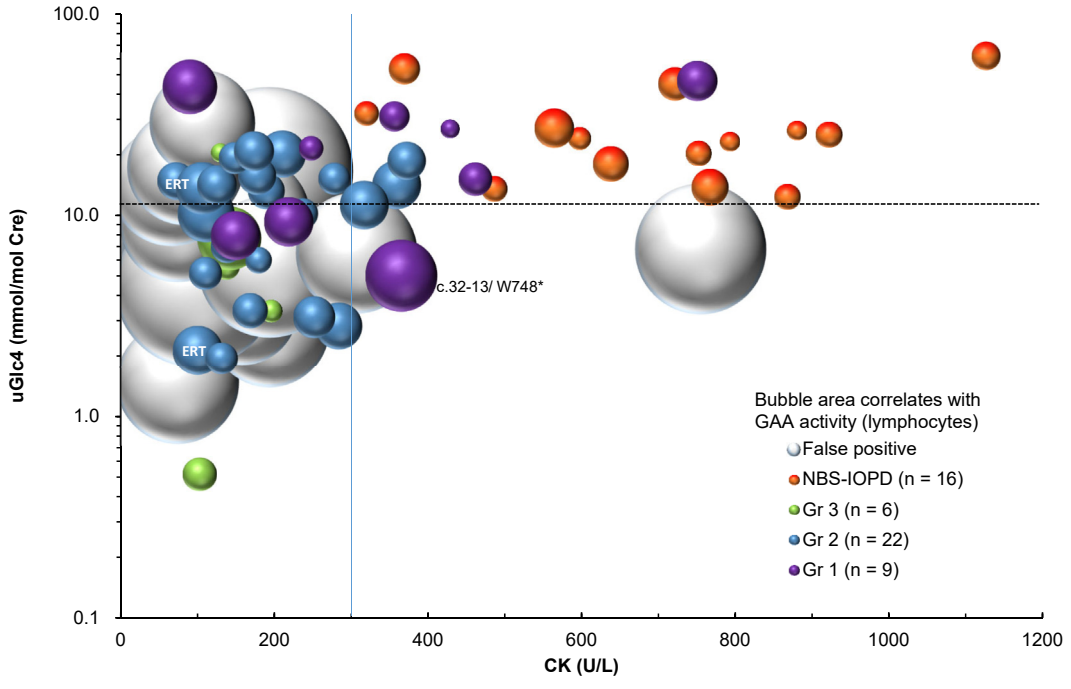


**Figure 2.** Correlation between initial biochemical markers and lymphocyte GAA activity. Lymphocyte GAA activity is represented by the *area of each bubble*. The *y*-axis represents the urinary glucose tetrasaccharide (uGlc4) level, and the *x*-axis represents the serum CK level. Screening-diagnosed patients with infantile-onset Pompe disease (NBS-IOPD; *orange dots*) all had low GAA activity and high CK and uGlc4 levels at birth (upper right quadrant, delineated by uGlc4 >12 mmol/mol Cre and CK > 300 U/L). Patients with later-onset Pompe disease are divided into 3 groups. A portion of group 1 subjects (with 2 pathogenic/likely pathogenic *GAA* variants; *purple*) are also located in the upper right quadrant. Three subjects in group 2 (with 1 pathogenic/likely pathogenic *GAA* variant; *blue*) and none in group 3 (with 2 VUSs in GAA; *green*) are in the upper right quadrant. *Light-gray dots* represent false-positive NBS cases.

physical endurance as measured by the 6MWT (Figure 3). The elder sibling of subject 9 (9a), who already had lordosis and elevated CK levels at age 7 years,[15] was also stabilized (Figure 3, E). In contrast, subject 25, who started ERT at age 4 months but withdrew at 10 months due to family issues, showed worsening biochemical variables and GMFM-66 after that (Figure 3, F). However, all patients treated with ERT performed poorly in the sit-up test, suggesting weakness of the abdominal and pelvic muscles. Therefore, we reviewed the AIMS evaluation videos taken at age 1 year in 5 patients from group 1 (subjects 3, 20, 25, 36, and 37) who were not on ERT at that time. We found that all 5 (100%) had hip extensor weakness, 2 (40%) had back extensor weakness, 4 (80%) had abdominal and pelvic weakness, and 3 (60%) had hypotonia. Subject 3 later had CK elevation and was treated (Figure 3, A). Subject 36 had a PDMS-2 gross motor quotient at the 16th percentile at age 12 months; the parents enforced training and reported a later improvement in gross motor development, and the subject remained under observation. Subjects 20 and 37 did not develop gross motor delay or elevation of CK and remained under observation.

### Role of c.[752C>T;761C>T]

**GAA Activity of c.[752C>T;761C>T].** Primary skin fibroblasts were obtained from subject 2, who was homozygous for c.[752C>T;761C>T], and subjects 1 and 3, who were compound heterozygous for c.[752C>T;761C>T] and 1 severe variant. All 3 fibroblast samples revealed GAA deficiency when tested using 4-MU as a substrate, and the values were even lower than those in fibroblasts from an adult patient with Pompe disease with a c.-32-13T>G/c.525del genotype (Figure 4, A).

To examine the pathogenicity of c.[752C>T;761C>T] independent of the genetic background, 4 *GAA* cDNA constructs were generated by mutagenesis (Figure 4, B). One of these constructs contained a "healthy" copy of *GAA*, 1 construct contained a combination of c.[752C>T;761C>T],

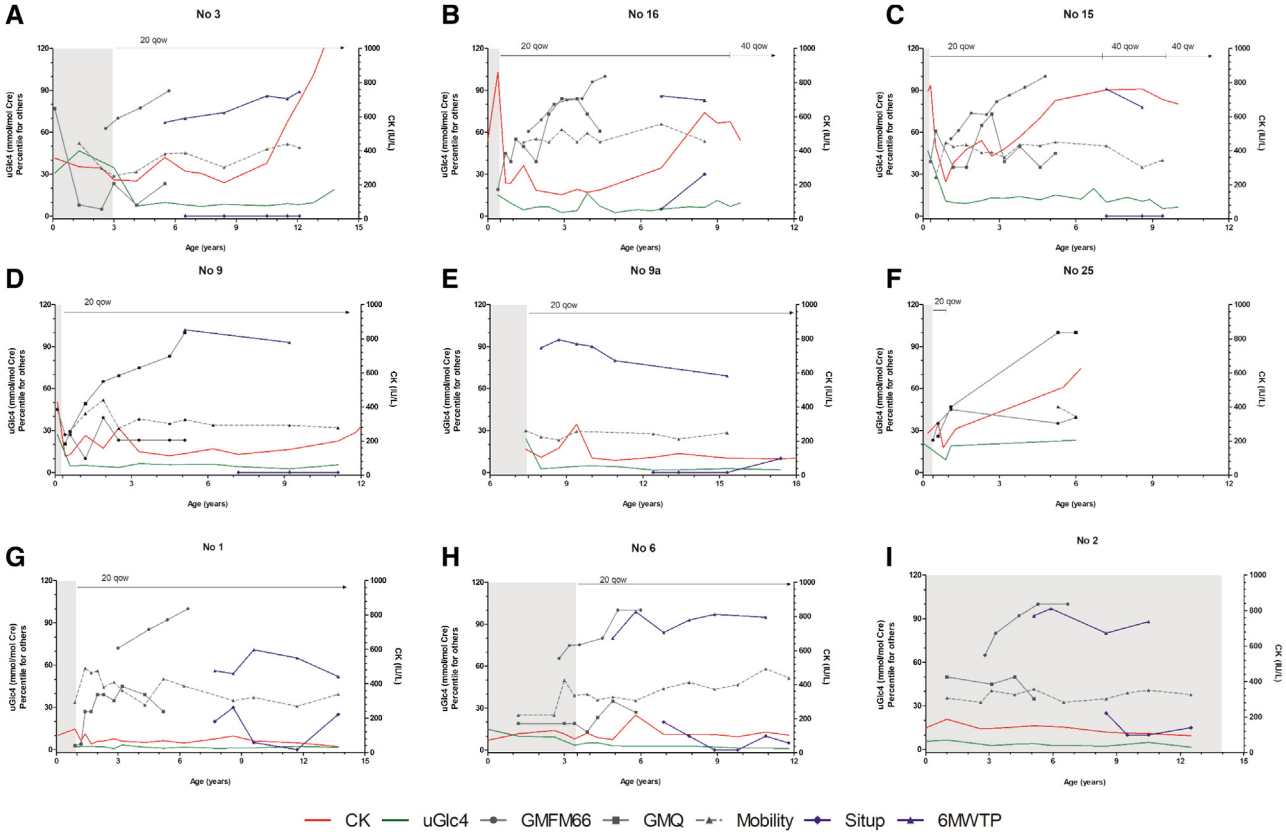


**Figure 3.** Clinical courses of patients under ERT. **A-D** and **F-H,** Subjects identified through screening or **E,** cascade family screening. **I,** Subject 2, who was identified through screening but was not on ERT, is plotted for comparison. *Shaded areas* represent periods before the start of ERT. ERT dosages are labeled on top of each panel: 20 qow (20 mg/kg every 2 weeks), 40 qow (40 mg/kg every 2 weeks), or 40 qw (40 mg/kg every week). **F,** Subject 25 was on ERT for only 6 months and then discontinued it for family reasons. **A-F,** The indication for ERT was persistent elevation of CK, **B,** cardiomegaly, or **G** and **H,** gross motor delay. In all patients, biochemical abnormalities improved after ERT, and gross motor performance improved or stabilized; however, all of them still showed impairment in sit-up performance. *GMQ*, gross motor quotient of the PDMS-2, shown as a percentile. Mobility refers to the mobility domain of the Pompe-PEDI, shown relative to the normative scores; sit-ups refer to the number of sit-ups in 30 seconds, shown as a percentile.

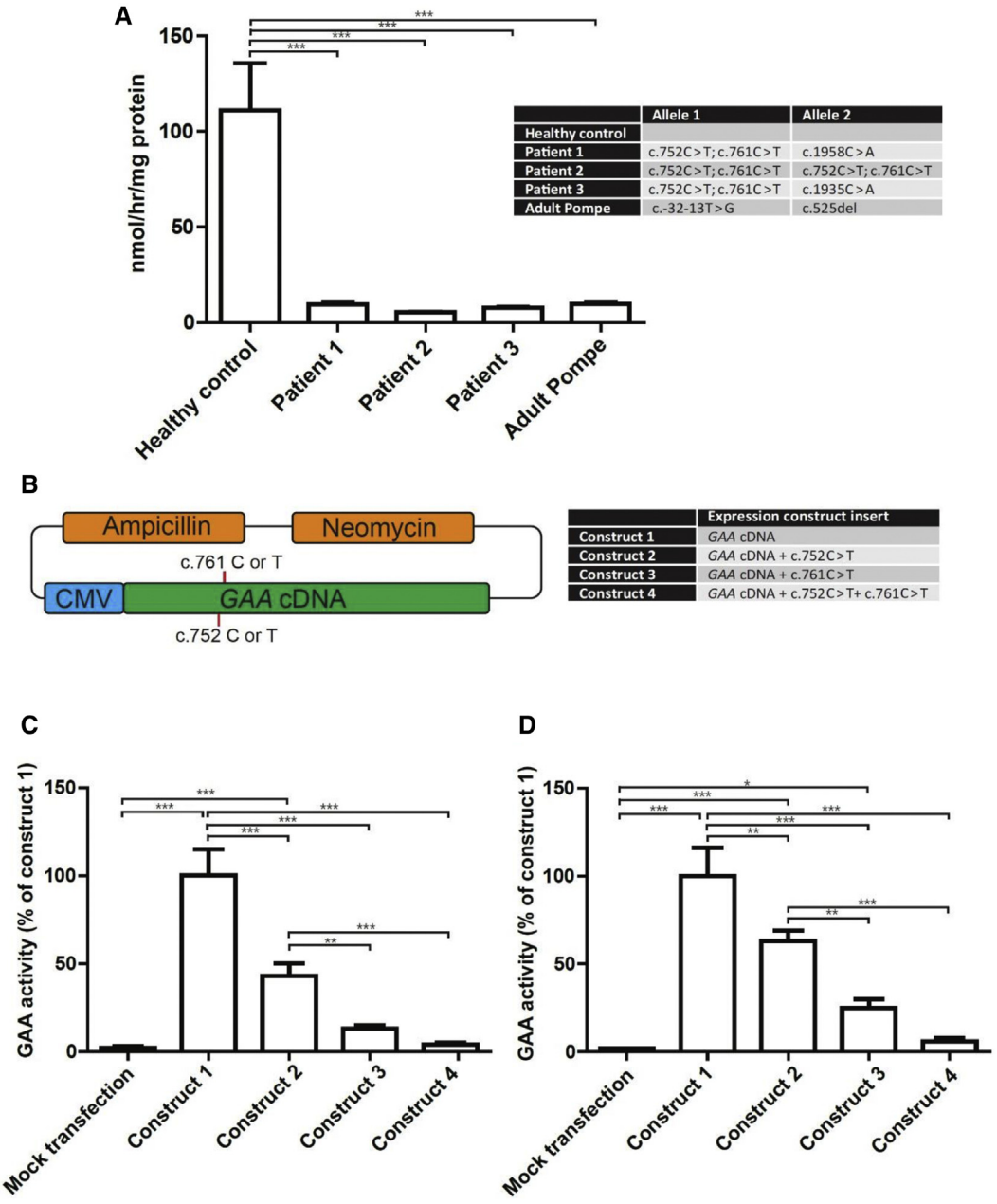


**Figure 4.** GAA activity of the c.[752C>T;761C>T] variant. **A,** GAA activity in primary fibroblasts of 3 subjects carrying the c.[752C>T;761C>T] variant and 1 adult with Pompe disease. The genotypes of these subjects are provided in the table (right inset). **B,** Schematic of the expression construct (*left*) and an overview of the introduced mutations (*right*). **C,** GAA activity of HEK293T cells transfected with *GAA* expression constructs, as measured using 4-MU as the substrate. **D,** As in C, but measured using glycogen as the substrate. Data are presented as mean ± SD. The significance of the results was determined using one-way ANOVA of independent samples with a post hoc Tukey test.

and each of the last 2 constructs contained 1 of the individual variants alone. These constructs were expressed in HEK293T cells. With 4-MU as the substrate, the GAA translated from the c.752C>T-containing cDNA had 43% of the activity of wild-type cDNA, the product of the c.761C>T cDNA had 13%, and the product of the c.[752C>T;761C>T] cDNA had only 4% (Figure 4, C). Similar results were obtained using glycogen as the substrate (Figure 4, D).

**Outcomes of c.[752C>T;761C>T] Heterozygotes.** Twelve subjects, including 1 subject homozygous for c.[752C>T;761C>T], 9 subjects compound heterozygous for c.[752C>T;761C>T] and 1 severe variant,[31] and 2 subjects compound heterozygous with a VUS, were followed (Figure 1). Four subjects showed a transient elevation in CK and/or uGlc4 at birth. Several subjects had PDMS-2 scores <20th percentile during follow-up (Figure 5;

available at www.jpeds.com). AIMS videos of the 9 subjects with compound heterozygous for c.[752C>T;761C>T] and 1 severe variant taken at age 1 year revealed that 4 (44%) had hypotonia, 5 (56%) had hip extensor weakness, 2 (22%) had back extensor weakness, and 4 (44%) had abdominal and pelvic weakness. One subject (subject 24; 11%) showed an initial delay in gross motor development (based on both AIMS and PDMS-2 results) but was normal by age 2 years. Subject 2 (group homozygous for c.[752C>T;761C>T]) at age 13 years had performance <20th percentile in sit-ups (**Figure 3**, J) and the broad jump. However, all 12 subjects exhibited normal gross motor performance at the latest evaluation. Therefore, subjects with c.[752C>T;761C>T] and another severe variant revealed a phenotype of hypotonia, nonprogressive muscle weakness, and impairment in school physical fitness tests. Owing to the nonprogressive nature of the symptoms, these subjects have not yet been classified as having LOPD and are not currently undergoing ERT.

**c.[752C>T;761C>T] Compound Heterozygotes at Older Ages.** We incidentally encountered 2 adults harboring the c.[752C>T;761C>T] variant. Adult case 1, whose other allele was c.2024_2026del [p.(N675del)], displayed normal muscle power, normal CK, and normal uGlc4 at age 43 years. Adult case 2, whose other allele was c.1958C>A (p.(T653N)), presented with multiple joint edema and muscle pain at age 40 years. Although EMG was suggestive of myopathy, a muscle biopsy from the left quadriceps revealed no glycogen accumulation (examination by Dr Ichizo Nishino, National Center of Neurology and Psychiatry, Tokyo, Japan). Serum CK and uGlc4 values were normal.

**Effects of the Splicing Variant c.[546+5G>T; 1726G>A].** The predicted effects of c.546+5G>T revealed a loss of the original splicing donor site and the creation of a potential new donor site at c.546+3. Subject 7 was compound heterozygous for c.[546+5G>T; 1726G>A], with a predicted deleterious variant for the second allele. RT-PCR of RNA extracted from primary fibroblasts revealed 2 fragments (**Figure 6**, A; available at www.jpeds.com). The normal-sized RT-PCR fragment contained 2 sequences, 1 normal and 1 with a 3-bp insertion (**Figure 6**, B and D); the aberrantly short fragment was found to be the result of exon 2 deletion (**Figure 6**, C and D). Subject 7 had an initial CK elevation and a slight delay in gross motor development at age 1 year and had generalized hypotonia, and weakness in the hip extensor, knee extensor, and abdominal and pelvic muscles on his AIMS video taken at age 1 year. Motor development was normal at age 4 years, although slight hypotonia was still present. According to the family, he was normal at age 13 years.

## Discussion

In our prospective follow-up of LOPD identified through NBS by sensitive developmental/motor and biochemical tests, we delineated early manifestations of these patients. In the present study, the indications of ERT for the 8 treated subjects were persistent CK elevation (n = 4), CK elevation with cardiomyopathy (n = 1), and hypotonia with delayed gross motor development (n = 2). CK elevation in Pompe disease indicates myocyte injury and should trigger the initiation of ERT; however, the normal range of CK is broad, and any rise above the baseline CK value should be noted even if the value is in the normal range. In addition, a transient CK elevation is often seen in the neonatal period or after minor injuries. Mild hypotonia and motor delay also can be missed because of the wide individual variation in motor capacity; therefore, longitudinal follow-up and a good baseline, as in the present study, are indispensable.

We attempted to correlate patients' initial biochemical profiles, including GAA activity, CK, and uGlc4, with their genotypes and final diagnoses. Patients with infantile-onset Pompe disease had CK > 300 U/L and abnormal uGlc4 levels (upper right quadrant in **Figure 2**). Unfortunately, 4 of the 9 subjects in group 1 (44%) were also located in the upper right quadrant, whereas 2 of the 9 (22%) had completely normal values. In addition, 3 of 22 NBS-LOPD group 2 patients (14%) were located in the upper right quadrant, implying false-positives predicted by the initial biochemical marker profile. The poor predictive power of initial biochemical markers for these genotypes may be related to the slow progression of the disease and the wide variation in marker values during the newborn stage. Nevertheless, measurement of these biomarkers is critical during follow-up to determine the appropriate timing of ERT initiation, as well as its effect (**Figure 2**).

Predicting the pathogenicity of *GAA* variants may be problematic for NBS programs. In our program, we used different in vitro functional studies to verify the pathogenicity of VUSs. Nonetheless, we still cannot predict the time of symptom onset in a subset of subjects. After a follow-up of up to 15 years, we know that subjects with c.[752C>T;761C>T] or c.[546+5G>T; 1726G>A] could be asymptomatic or could develop late adulthood symptoms. The inability to predict phenotype by molecular findings and biomarkers is of concern in other NBS programs, and so different terms have been used, such as "later-onset" in Missouri,[32] "possible LOPD" in Illinois,[33] "suspected LOPD" in Pennsylvania,[34] and "not otherwise specified Pompe disease" in California.[35] The incidence of LOPD is likely overestimated in all NBS programs, and further classification and terminology alignment may be necessary.

In the present study, 8 subjects received ERT starting at age 1.4 months to 3 years, and all maintained their physical endurance during follow-up. This finding is in contrast to our previous clinical experience in 15 patients with LOPD diagnosed at age 10-38 years, in which 8 patients (53%) required mechanical ventilation at the time of diagnosis or shortly thereafter.[14] Our group also treated a 13-year-old boy with a genotype of c.[1935C>A;1726G>A]/c.[2238G>C;1726G>A] who had weakness only in the abdominal muscles. Unfortunately, after 11 years of ERT,

his CK level fluctuated between 1273 and 2336 U/L, muscle degeneration progressed, and forced vital capacity, 6MWT performance, and endurance decreased.[36] Subject 25, as the same genotype untreated comparator, showed worsening of biological variables and low motor performance during follow-up, whereas the treated comparators 9 and 9a, who received ERT at a much younger age, were stabilized after the appearance of minimal signs and symptoms. Our data support that patients with LOPD identified through NBS who were genotypically similar to the clinical cases improved biochemically and clinically after early ERT. However, abdominal and pelvic muscle weakness, resulting in poor performance in the sit-up test, was present in most of the treated patients with LOPD; in fact, weakness was already present before age 1 year in group 1 subjects, even without ERT. We speculate that the abdominal and pelvic muscles of patients with LOPD were already damaged early in life, and that the function of these muscles was not fully restored by ERT. Nevertheless, we cannot use these early signs as criteria for initiating ERT, because they were not progressive in subjects carrying nonpathogenic GAA variants.

The most common variant in our subjects with NBS-LOPD, c.[752C>T;761C>T], also has been found in Japanese[37] and California[35] programs. Although this variant is classified as presumably nonpathogenic in the Pompe disease GAA variant database,[10] our present findings show that fibroblasts with this variant have GAA activity in the patient range. A mutagenesis study further found that *GAA* cDNA containing this variant caused cells to display only 4% as much GAA activity as control cells. Similarly, c.[546+5G>T; 1726G>A] fibroblasts exhibited GAA activity in the patient range, and RNA analyses revealed aberrant splicing caused by the variant. Subjects harboring these variants may show a phenotype of nonprogressive hypotonia, muscle weakness, and impairment in physical fitness tests. The GAA activity of all these subjects was lower than that of patients with LOPD with the c.-32-13T>G variant, which is associated with adult patients with Pompe disease. Therefore, it is reasonable to assume that c.[752C>T;761C>T] and c.[546+5G>T; 1726G>A] also may cause Pompe disease in adults. However, we are unaware of any patient clinically diagnosed with LOPD who carried either c.[752C>T;761C>T] or c.[546+5G>T; 1726G>A] variants. In contrast, we encountered 2 adults, aged 40 and 43 years, with the c.[752C>T;761C>T] variant but no signs of muscular glycogen accumulation, supporting the argument that this variant is nonpathogenic. Regardless, a conservative approach is advised, as patients with Pompe disease with the c.-32-13T>G variant may develop symptoms in late adulthood. It is still likely that we underdiagnosed elderly patients with Pompe disease.

Another hypothesis for the underdiagnosis of adult Pompe disease in subjects carrying c.[752C>T;761C>T] or c.[546+5G>T; 1726G>A] variants is based on atypical manifestations, for example, only abdominal and pelvic muscle weakness with no other muscle involvement. Abdominal and pelvic muscle weakness is reminiscent of sarcopenia, a condition marked by the loss of core abdominal muscles in old age,[38,39] with largely unknown etiology.[40,41] Further studies, such as targeted screening of subjects with sarcopenia, may help elucidate the roles of these GAA variants.

This study highlights the importance of long-term follow-up of NBS data. In this way, we have demonstrated the early clinical and biochemical manifestations of LOPD and may be able to predict LOPD after NBS. We also found favorable early treatment outcomes in patients with LOPD, even though residual pathology, characterized by abdominal and pelvic muscle weakness, remains. Given their association with muscle weakness, the correlation between Pompe disease and several GAA variants requires further investigation. ■

Submitted for publication Apr 25, 2021; last revision received Dec 24, 2021; accepted Dec 30, 2021.

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## References

1. Hirschhorn R, Reuser AJ. Glycogen storage disease type II: acid alphaglucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle MD, Kinzler KW, Vogelstein B, eds. The metabolic and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001. p. 3389-420.
2. Amalfitano A, Bengur AR, Morse RP, Majure JM, Case LE, Veerling DL, et al. Recombinant human acid α-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. Genet Med 2001;3:132-8.
3. 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;356:397-8.
4. Chien YH, Chiang SC, Zhang XK, Keutzer J, Lee NC, Huang AC, et al. Early detection of Pompe disease by newborn screening is feasible: results from the Taiwan screening program. Pediatrics 2008;122:e39-45.
5. Chien YH, Lee NC, Huang HJ, Thurberg BL, Tsai FJ, Hwu WL. Later-onset Pompe disease: early detection and early treatment initiation enabled by newborn screening. J Pediatr 2011;158:1023-7.e1.
6. Chien YH, Lee NC, Thurberg BL, Chiang SC, Zhang XK, Keutzer J, et al. Pompe disease in infants: improving the prognosis by newborn screening and early treatment. Pediatrics 2009;124:e1116-25.
7. Berli S, Brandi G, Keller E, Najia N, Vitale J, Pagnamenta A. Clinical efficacy of the enzyme replacement therapy in patients with late-onset Pompe disease: a systematic review and a meta-analysis. J Neurol 2021. https://doi.org/10.1007/s00415-021-10526-5 [Epub ahead of print].
8. Wenninger S, Gutschmidt K, Wirner C, Einvag K, Montagnese F, Schoser B. The impact of interrupting enzyme replacement therapy in late-onset Pompe disease. J Neurol 2021;268:2943-50.
9. Papadimas GK, Anagnostopoulos C, Xirou S, Michelakakis H, Terzis G, Mavridou I, et al. Effect of long term enzyme replacement therapy in late onset Pompe disease: a single-centre experience. Neuromuscul Disord 2021;31:91-100.
10. Nino MY, In 't Groen SL, Bergsma AJ, van der Beek NA, Kroos M, Hoogeveen-Westerveld M, et al. Extension of the Pompe mutation database by linking disease-associated variants to clinical severity. Hum Mutat 2019;40:1954-67.
11. de Faria DOS, In 't Groen S, Hoogeveen-Westerveld M, Nino MY, van der Ploeg AT, Bergsma AJ, et al. Update of the Pompe variant database for the prediction of clinical phenotypes: novel disease-associated variants, common sequence variants, and results from newborn screening. Hum Mutat 2021;42:119-34.
12. van der Ploeg AT, Kruijshaar ME, Toscano A, Laforêt P, Angelini C, Lachmann RH, et al. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. Eur J Neurol 2017;24:768.e31.

13. Kishnani PS, Amartino HM, Lindberg C, Miller TM, Wilson A, Keutzer J, et al. Timing of diagnosis of patients with Pompe disease: data from the Pompe registry. Am J Med Genet A 2013;161A:2431-43.
14. Yang CC, Chien YH, Lee NC, Chiang SC, Lin SP, Kuo YT, et al. Rapid progressive course of later-onset Pompe disease in Chinese patients. Mol Genet Metab 2011;104:284-8.
15. Chien YH, Lee NC, Huang PH, Lee WT, Thurberg BL, Hwu WL. Early pathologic changes and responses to treatment in patients with later-onset Pompe disease. Pediatr Neurol 2012;46:168-71.
16. Rairikar MV, Case LE, Bailey LA, Kazi ZB, Desai AK, Berrier KL, et al. Insight into the phenotype of infants with Pompe disease identified by newborn screening with the common c.-32-13T>G "late-onset" GAA variant. Mol Genet Metab 2017;122:99-107.
17. Labrousse P, Chien YH, Pomponio RJ, Keutzer J, Lee NC, Akmaev VR, et al. Genetic heterozygosity and pseudodeficiency in the Pompe disease newborn screening pilot program. Mol Genet Metab 2010;99:379-83.
18. Chien YH, Hwu WL, Lee NC. Newborn screening: Taiwanese experience. Ann Transl Med 2019;7:281.
19. Chien YH, Tsai WH, Chang CL, Chiu PC, Chou YY, Tsai FJ, et al. Earlier and higher dosing of alglucosidase alfa improve outcomes in patients with infantile-onset Pompe disease: evidence from real-world experiences. Mol Genet Metab Rep 2020;23:100591.
20. van Capelle CI, van der Beek NA, de Vries JM, van Doorn PA, Duivenvoorden HJ, Leshner RT, et al. The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients. J Inherit Metab Dis 2012;35:317-23.
21. Chen CA, Chang CH, Lin MT, Hua YC, Fang WQ, Wu MH, et al. Six-Minute Walking Test: normal reference values for Taiwanese children and adolescents. Acta Cardiol Sin 2015;31:193-201.
22. Ulrich S, Hildenbrand FF, Treder U, Fischler M, Keusch S, Speich R, et al. Reference values for the 6-minute walk test in healthy children and adolescents in Switzerland. BMC Pulm Med 2013;13:49.
23. Bergsma AJ, In 't Groen SL, Verheijen FW, van der Ploeg AT, Pijnappel W. From cryptic toward canonical pre-mRNA splicing in Pompe disease: a pipeline for the development of antisense oligonucleotides. Mol Ther Nucleic Acids 2016;5:e361.
24. Okumiya T, Keulemans JL, Kroos MA, Van der Beek NM, Boer MA, Takeuchi H, et al. A new diagnostic assay for glycogen storage disease type II in mixed leukocytes. Mol Genet Metab 2006;88:22-8.
25. Shapiro MB, Senapathy P. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res 1987;15:7155-74.
26. Yeo G, Burge CB. Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. J Comput Biol 2004;11:377-94.
27. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. J Comput Biol 1997;4:311-23.
28. Pertea M, Lin X, Salzberg SL. GeneSplicer: a new computational method for splice site prediction. Nucleic Acids Res 2001;29:1185-90.
29. Chien YH, Hwu WL, Lee NC. Pompe disease: early diagnosis and early treatment make a difference. Pediatr Neonatol 2013;54:219-27.
30. Sawada T, Kido J, Nakamura K. Newborn screening for Pompe disease. Int J Neonatal Screen 2020;6:31.
31. Kroos M, Pomponio RJ, van Vliet L, Palmer RE, Phipps M, Van der Helm R, et al. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. Hum Mutat 2008;29:E13-26.
32. Klug TL, Swartz LB, Washburn J, Brannen C, Kiesling JL. Lessons learned from Pompe disease newborn screening and follow-up. Int J Neonatal Screen 2020;6:11.
33. Burton BK, Charrow J, Hoganson GE, Fleischer J, Grange DK, Braddock SR, et al. Newborn screening for Pompe disease in Illinois: experience with 684,290 infants. Int J Neonatal Screen 2020;6:4.
34. Ficicioglu C, Ahrens-Nicklas RC, Barch J, Cuddapah SR, DiBoscio BS, DiPerna JC, et al. Newborn screening for Pompe disease: Pennsylvania experience. Int J Neonatal Screen 2020;6:89.
35. Tang H, Feuchtbaum L, Sciortino S, Matteson J, Mathur D, Bishop T, et al. The first year experience of newborn screening for Pompe disease in California. Int J Neonatal Screen 2020;6:9.
36. Chien YH, Lee NC, Hwu WL, Fang JY. Disease progression in a pre-symptomatically treated patient with juvenile-onset Pompe disease—need for an earlier treatment? Eur J Neurol 2018;25:e111.
37. Momosaki K, Kido J, Yoshida S, Sugawara K, Miyamoto T, Inoue T, et al. Newborn screening for Pompe disease in Japan: report and literature review of mutations in the GAA gene in Japanese and Asian patients. J Hum Genet 2019;64:741-55.
38. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412-23.
39. Jones K, Gordon-Weeks A, Coleman C, Silva M. Radiologically determined sarcopenia predicts morbidity and mortality following abdominal surgery: a systematic review and meta-analysis. World J Surg 2017;41:2266-79.
40. Urzi F, Pokorny B, Buzan E. Pilot study on genetic associations with age-related sarcopenia. Front Genet 2020;11:615238.
41. Palmio J, Udd B. Borderlines between sarcopenia and mild late-onset muscle disease. Front Aging Neurosci 2014;6:267.

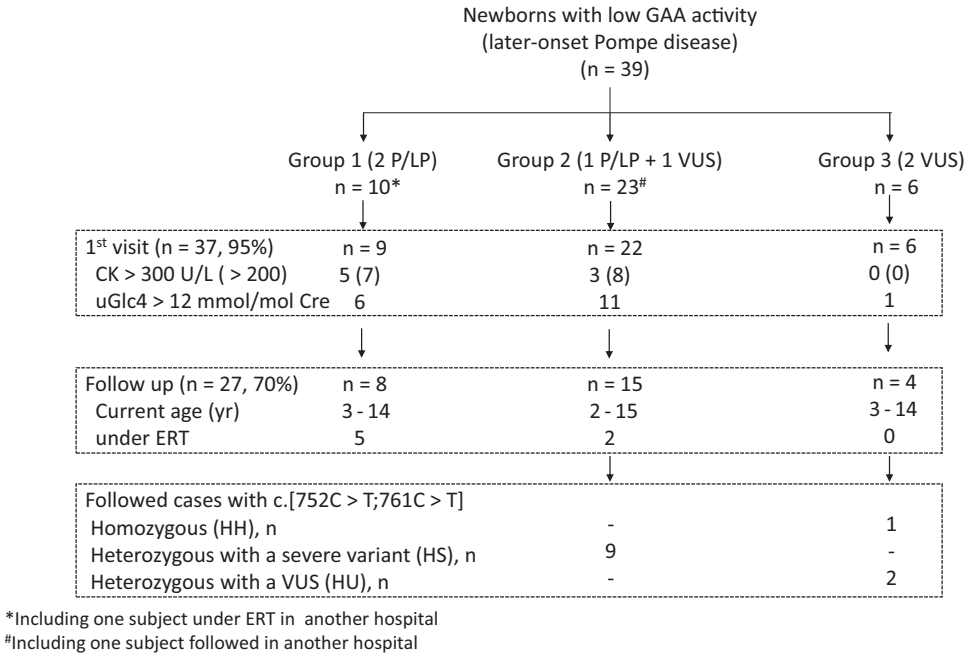


**Figure 1.** Patient flow in this study. *LP*, likely pathogenic; *P*, pathogenic.

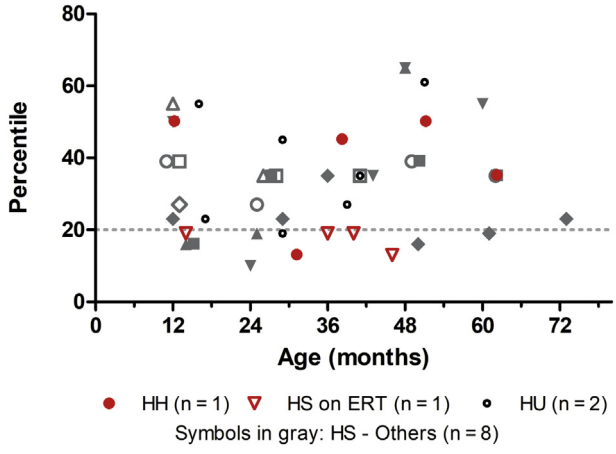


**Figure 5.** Gross motor development in subjects with the c.[752C>T;761C>T] variant. Individual data points from subjects harboring c.[752C>T;761C>T] are depicted according to age and percentile of the gross motor quotient of the PDMS-2. *HH*, homozygote, *HS*, compound heterozygotes with 1 severe (pathogenic/likely pathogenic) variant; *HU*, compound heterozygotes with 1 *GAA* gene variant of unknown significance.

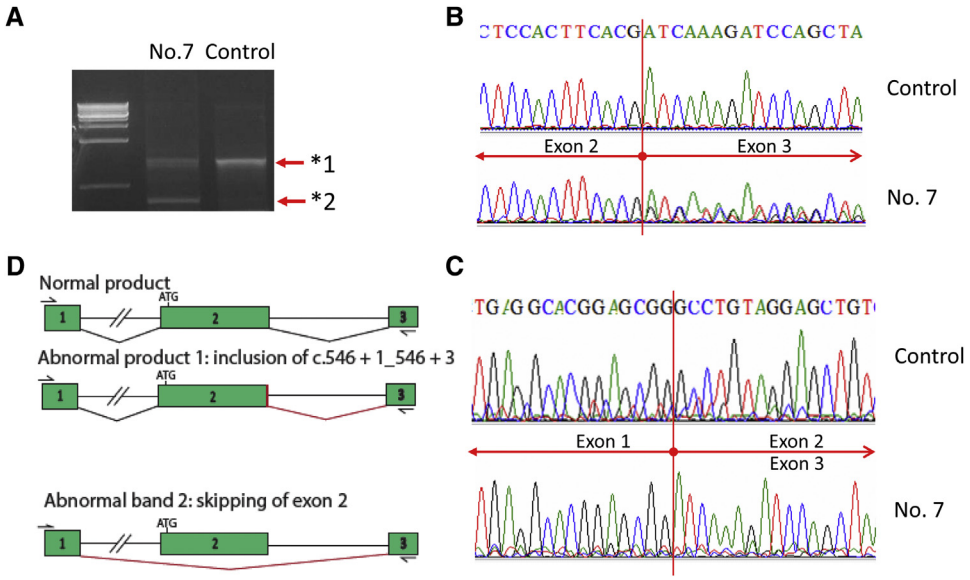


**Figure 6.** Effect of the splicing variant c.[546+5G>T; 1726G>A]. **A,** RT-PCR of RNA extracted from subject 7, heterozygous for c.[546+5G>T; 1726G>A], revealed 2 fragments. **B,** Sanger sequencing (from a primer on exon 2) of the normal-sized fragment (*1) showed aberrant sequences after the splice junction, marked by a *vertical line*. **C,** Sanger sequencing (from a primer on exon 1) of the aberrantly short fragment (*2) showed the connection of exon 3 to exon 1. The junction point is marked by a *vertical line*. **D,** Graphic view of aberrant splicing in subject 7. The normal-sized RT–PCR fragment contained 2 sequences, 1 normal and 1 with a 3-bp insertion; the aberrantly short fragment was the result of exon 2 deletion.