Later-Onset Pompe Disease: Early Detection and Early Treatment Initiation Enabled by Newborn Screening

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Objective To determine whether newborn screening facilitates early detection and thereby early treatment initiation for later-onset Pompe disease.

Study design We have conducted a newborn screening program since 2005. Newborns with deficient skin fibroblast acid α-glucosidase activity and two acid α-glucosidase gene mutations but no cardiomyopathy were defined as having later-onset Pompe disease, and their motor development and serum creatine kinase levels were monitored every 3 to 6 months.

Results Among 344 056 newborns, 13 (1 in 26 466) were found to have later-onset Pompe disease. During a follow-up period of up to 4 years, four patients were treated because of hypotonia, muscle weakness, delayed developmental milestones/motor skills, or elevated creatine kinase levels starting at the ages of 1.5, 14, 34, and 36 months, respectively. Muscle biopsy specimens obtained from the treated patients revealed increased storage of glycogen and lipids.

Conclusion Newborn screening was found to facilitate the early detection of later-onset Pompe disease. A subsequent symptomatic approach then identifies patients who need early treatment initiation. *(J Pediatr 2011;158:1023-7)*.

Pompe disease (also known as glycogen storage disease type II, glycogenosis II, or acid maltase deficiency) is a lysosomal storage disorder in which a deficiency in acid α-glucosidase (GAA) causes intralysosomal accumulation of glycogen in all tissues, most notably skeletal muscles.[1] Clinically, Pompe disease presents a wide spectrum of phenotypes, ranging from the severe and rapidly progressive infantile-onset form characterized by the presence of hypertrophic cardiomyopathy to the heterogeneous and more slowly progressive later-onset form, which typically presents no cardiac manifestations.[1] In patients with the later-onset form of Pompe disease, the age at diagnosis can be from <1 to 78 years, and the age at symptom onset can be from <1 to 52 years.[2] Moreover, 50% of adult patients report the presence of mild muscular symptoms during childhood.[3] Earlier manifestation of the disease leads to earlier wheelchair or ventilator dependency.[4,5]

When an infant is diagnosed through newborn screening (NBS) and treated very early in life, normal motor function can be maintained.[6] Enzyme replacement therapy (ERT) for later-onset Pompe disease has been associated with improved motor capability and stabilized pulmonary function, but the best outcomes were in the youngest, non-ventilated patients.[7-10] Therefore, early diagnosis and early treatment are both important for later-onset Pompe disease, but the mean delay from onset of symptoms to diagnosis averages 10 years.[11] Screening of patients at high risk, such as those with limb-girdle muscular dystrophy, can identify undiagnosed later-onset Pompe disease,[12] but it might still be too late for treatment.

We conducted a large-scale NBS program for the early detection and treatment of Pompe disease.[6,13] In addition to patients with infantile-onset Pompe disease, newborns suspected of having the later-onset form were also identified. Here, we present our follow-up of 13 such newborns over a 4-year period.

Methods

The Newborn Screening Center at National Taiwan University Hospital initiated an NBS program for Pompe disease in 2005. GAA activity was measured in dried bloodspots via a fluorescence assay.[13] The screening algorithm was as follows:

CK	Creatine kinase
ERT	Enzyme replacement therapy
GMQ	Gross motor quotient
GAA	Acid α-glucosidase
NBS	Newborn screening
PDMS-II	Peabody Developmental Motor Scale, Second Edition

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first, newborns with dried bloodspot GAA activity <8% of the normal mean, a neutral α-glucosidase/GAA ratio >60, and GAA inhibition by acarbose >80% were first referred for confirmation; second, after the confirmatory testing, newborns with lymphocyte GAA activity <5% of the normal mean but with no cardiomyopathy underwent skin biopsy; finally, newborns with fibroblast GAA activity <5% of the normal mean and two identified GAA gene mutations were defined as having later-onset Pompe disease, and they were followed up every 3 to 6 months for motor development and serum CK. The criteria for ERT were a significant elevation of CK or motor developmental delay with or without the elevation of CK. A muscle biopsy specimen on the quadriceps muscle was obtained before the first alglucosidase alfa infusion.

The upper limits of CK for infants 1 week to 3 months of age were obtained from 32 male and 30 female infants who had a false-positive screening result (<245 IU/L for males and <200 IU/L for females). The upper limits for infants 3 to 12 months of age were according to the reference ranges published by Soldin et al[14] (<170 IU/L for males and <240 IU/L for females) The upper limits of CK for older children were from the hospital laboratory (<190 IU/L for males and <167 IU/L for females). The screening program and all subsequent analyses were conducted under institutional review board approval with appropriate informed consent.

GAA activity in lymphocytes and fibroblasts was measured by use of both the artificial substrate 4-methylumbelliferyl-α-D-glucopyranoside (Fluka Chemical Corp, Ronkonkoma, New York) and the natural substrate glycogen.[15] GAA gene sequencing was performed and analyzed as described.[16,17] Biochemical data were also obtained from 15 patients with later-onset Pompe disease identified through the emergence of clinical symptoms. Their median age of symptom onset was 13 (range 2 to 35 years), and their median age of any respirator use was 22 (range 14 to 45 years).

Follow-Up Clinical and Ancillary Tests

Muscle tone, muscle power, developmental milestones, and serum CK were assessed every 3 to 6 months. The Peabody Developmental Motor Scale, Second Edition (PDMS-II) was evaluated every 12 months. The PDMS-II is a skill-based measure of gross and fine motor development for infants and children from 6 months to 6 years of age consisting of four gross motor and two fine motor subtests.[18] The scores were normalized and presented as quotients (Normal mean = 100, 1 SD = 15).

Results

After screening 344 056 newborns from 2005 to 2009, 19 were confirmed to have Pompe disease (Table I). Six newborns showed hypertrophic cardiomyopathy during the neonatal period and were therefore classified as having infantile-onset Pompe disease, and five of them (NBS2-6) have been described.[6] The other 13 newborns (Cases 1 to 13, who did not have hypertrophic cardiomyopathy) were classified as having later-onset Pompe disease. The prevalence at live birth of all types of Pompe disease found by newborn screening in Taiwan was approximately 1 in 18 108; the prevalence of infantile-onset Pompe disease was 1 in 57 343; the prevalence of later-onset Pompe disease was 1 in 26 466.

All 13 newborns with later-onset Pompe disease had severe GAA deficiencies in their lymphocytes and skin fibroblasts (Table I). Their mutations were all identified, and most of the mutations were in association with the c.1726G>A (p.G576S) pseudodeficiency mutation (Table II; available at www.jpeds.com).[19,20] Both the severe Chinese founder mutation p.[D645E; G576S][17] and the later-onset Pompe disease mutation p.[W746C; G576S][21] were found in this group of patients.[21]

Electrocardiography, chest radiography, and echocardiography revealed no cardiomegaly in the 13 newborns. Four newborns had initial CK levels that exceeded the upper limits of their age- and sex-matched normal ranges (Figure 1, A, Cases 3, 4, 7, and 9), and most of the newborns showed fluctuating CK levels during follow-up (Figure 1, B). At least six children identified by NBS (46%) were reported by their parents as prone to falling, and four (31%) had poor endurance. Motor development tests were performed on 11 of the children (Cases 5 and 8 refused the tests); 10 (91%) were noted to have mild-to-moderate hypotonia, and seven (64%) had weakness in the trunk, gluteus, or abdomen muscle. Their PDMS-II gross scores (except for Case 13 who was too young) were often low or declining (Figure 1, C). Their fine motor skills seemed to be preserved, although the scores fluctuated because of poor cooperation of some of the children (Figure 1, D).

Case 1 had a significant delay in motor development at 7 months of age.[6] Her gross motor quotient (GMQ) was 72

Table I. Mutations and GAA activities in newborns with GAA deficiencies

No.	Sex	Current age (mo)	Age treated (mo)	GAA activity	
				Lymphocytes	Fibroblasts
NBS2	Female	53	0.9	0.68	0.06
NBS3	Male	52	0.97	1.58	-
NBS4	Male	45	0.6	0.45	0.11
NBS5	Female	39	1.17	3.82	0.14
NBS6	Male	34	0.4	0.83	0.06
NBS8	Female	17	0.47	1.41	0.19
1*	Female	59	14	1.65	0.65
2	Male	45	-	0.75	0.24
3	Male	44	36	0.8	0.14
4	Female	43	-	1.27	0.53
5	Female	41	-	1.45	0.27
6	Male	35	34	1.49	0.3
7	Male	33	-	1.93	0.51
8	Male	33	-	6.36	1.08
9	Male	29	1.5	0.46	0.11
10	Male	24	-	0.35	0.6
11	Male	30	-	0.58	0.49
12	Female	19	-	0.84	0.39
13	Male	12	-	0.69	0.22

*The patient was named as NBS1 in previous publications. Normal range of GAA activity in lymphocytes: 66.70 ± 33.70 nmol/mg Prot/hr; in fibroblasts: 106.29 ± 60.46 nmol/mg Prot/hr.

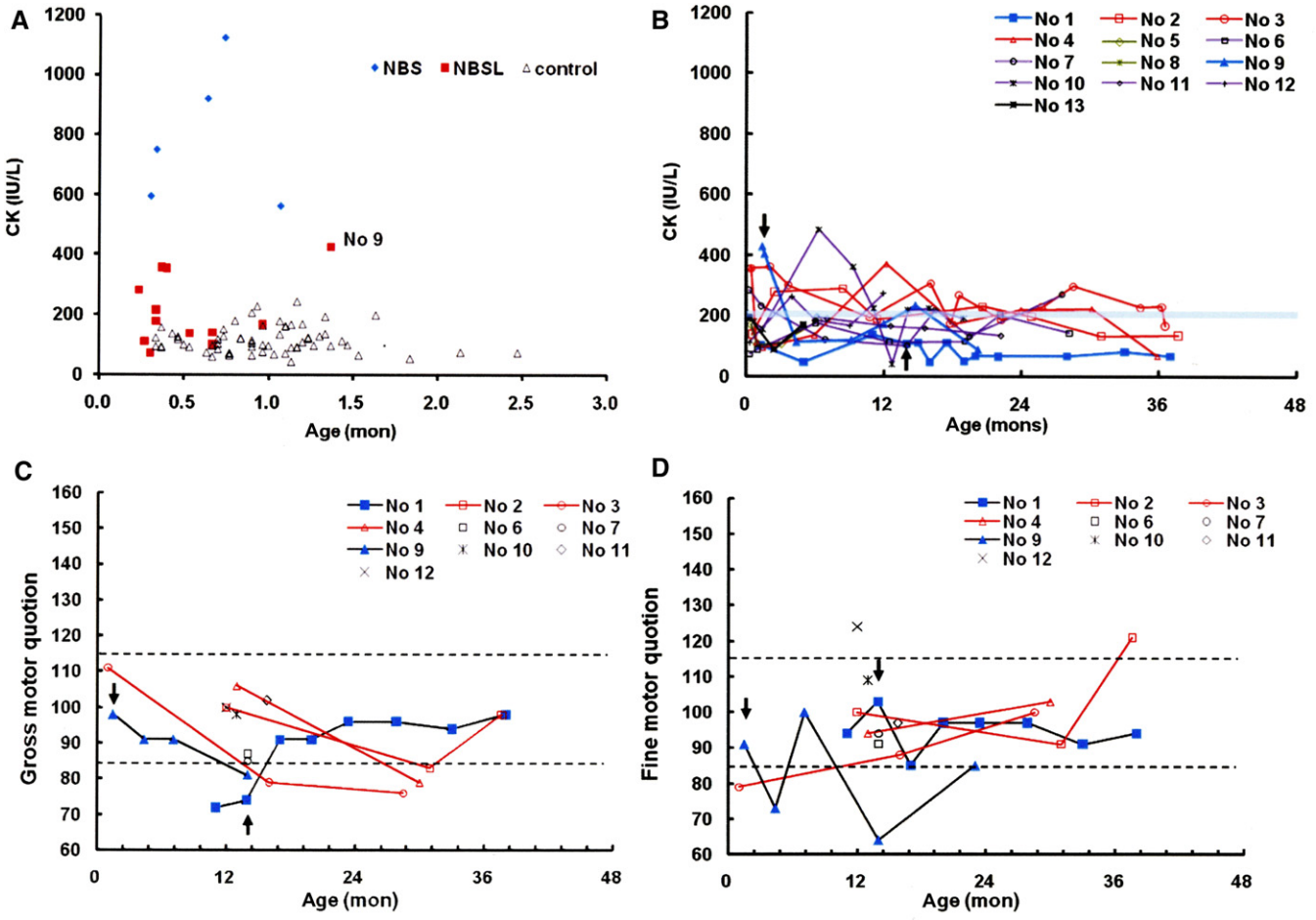

Figure 1. Laboratory and clinical manifestations of newborns with later-onset Pompe disease. **A,** Initial creatine kinase (CK) levels. The control group is newborns with a false-positive test result. **B,** Follow-up CK levels. The arrows indicate the time alglucosidase alfa treatment was started. **C,** Gross motor quotient of the newborns (Mean = 100, 1 SD = 15). The *dashed line* indicates the normal range (within ±1 SD). The *arrows* indicate the time alglucosidase alfa treatment was started. **D,** Fine motor quotient of the newborns (Mean = 100, 1 SD = 15). The *dashed line* indicates the normal range (within ±1 SD). The *arrows* indicate the time alglucosidase alfa treatment was started.

(3rd percentile) at 11 months. She began treatment at 14 months (Figure 1, C, *upward arrow*), and her motor development caught up quickly. She had a normal serum CK level.[6] Case 3 had an initial CK level of 356 IU/L (Figure 1, A), and his levels remained elevated during follow-up (Figure 1, B). His motor development was delayed such that he had complete head control only after 6 months of age, he walked at 13 months but fell frequently, and he did not jump well at 30 months. His GMQ was 79 (8th percentile) at 16 months and 76 (5th percentile) at 28.5 months. We started his treatment when he was 36 months old. Case 6 had a GMQ of 87 (19th percentile) at age 14 months. He was not cooperative at the second test, but he fell frequently and had prominent hypotonia and choking while drinking. His treatment started at 34 months of age. He had normal CK levels. Case 9 was found to have a CK level of 428 and 403 IU/L at 1.4 and 1.5 months of age, respectively. He was treated immediately, and his CK levels returned to normal rapidly. He had a borderline GMQ of 81 (10th percentile) at 14 months. His 7-year-old elder sister was later found to have Pompe disease, and she was also treated. Muscle biopsy specimens obtained from these treated patients revealed increased storage of glycogen, lipids, or both (data not shown).

Cases 4 and 7 both had an elevated initial CK level (359 and 284 IU/L, respectively), and their follow-up CK levels also fluctuated. Case 4 had a GMQ of 79 (8th percentile) at age 30 months, and case 7 had a GMQ of 85 (16th percentile) at 14 months. They are now being closely monitored.

Because lymphocyte GAA activity could not differentiate the patients with infantile and later-onset Pompe disease (Figure 2, A), fibroblast GAA activity was assayed with 4MU substrate. It was similar between the screened patients and those clinically identified with later-onset Pompe disease, but their GAA activities were significantly higher

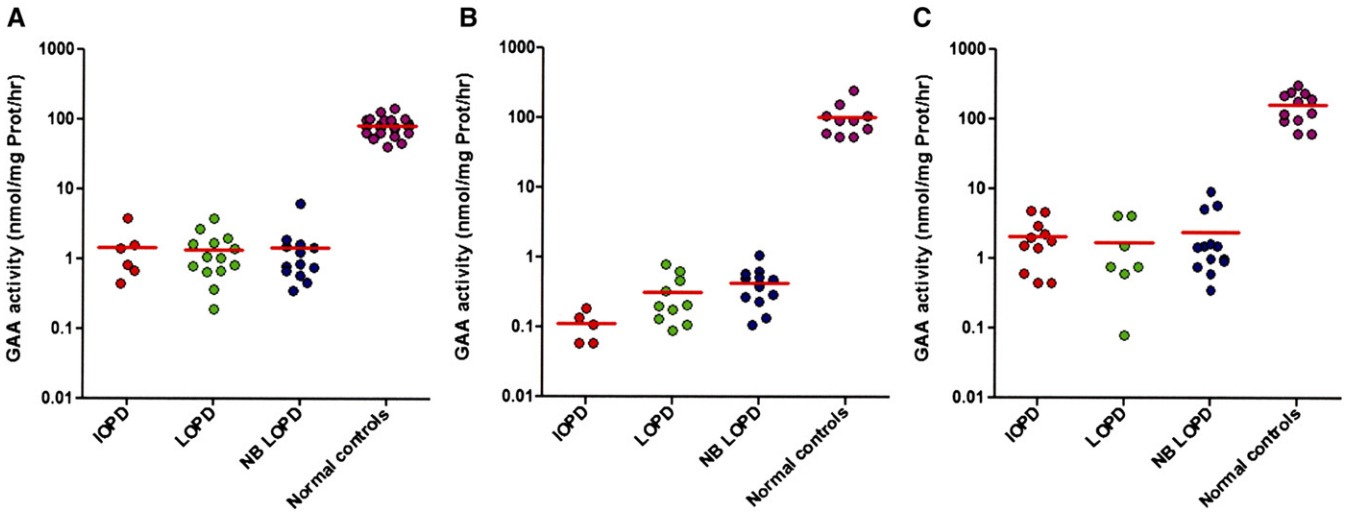

Figure 2. GAA activity of newborns with infantile-onset Pompe disease *(IOPD)*, patients with later-onset Pompe disease *(LOPD)*, newborns with later-onset Pompe disease *(NB LOPD)*, and control subjects. **A,** Lymphocyte GAA activity assayed with 4-methylumbelliferyl-α-D-glucopyranoside *(4MU)*; **B,** fibroblast GAA activity assayed with 4MU; **C,** fibroblast GAA activity assayed with glycogen.

than those of the screened patients with infantile-onset Pompe disease ($P < .005$) (Figure 2, B). A GAA assay with the natural substrate glycogen could not distinguish between the patients with infantile and later-onset Pompe disease (Figure 2, C).

Discussion

In this study, we detected 13 newborns with later-onset Pompe disease by newborn screening. Signs/symptoms such as hypotonia, muscle weakness, delayed developmental milestones or motor skills, or elevated CK levels were observed in most of them during follow-up, and four patients were treated starting from ages 1.5 months to 3 years. Because they need treatment from such a young age but they differ from patients with infantile-onset Pompe disease by the lack of hypertrophic cardiomyopathy, we prefer the term "later-onset" over the term "late-onset" to describe these patients. The 13 newborns described in this study could not be differentiated from the patients with clinically-recognized later-onset Pompe disease by GAA activity. In Taiwan, many of the patients in the latter group have juvenile-onset diseases and become wheelchair- or respirator-dependent in their twenties. We have seen one 11-year old patient who luckily was diagnosed because of an elevated CK level found in a routine health check. More than 50% of his muscle fibers in a quadriceps biopsy specimen had been replaced by either glycogen or fat. We suspect that the four babies who were screened and treated would have had a poor clinical course if they had not been diagnosed and treated early.

The pseudodeficiency mutation was found to be associated with the severe mutation p.[D645E; G576S], the later-onset Pompe disease mutation p.[W746C; G576S], and several other mutations identified by this study. The pseudodeficiency mutation may decrease the residual GAA activity of other mutations.[19,20] This may be the reason that Chinese patients with later-onset Pompe disease have earlier symptom onset than white patients, as with the four newborns who were treated in this study.

The best time to treat patients with Pompe disease is before irreversible damage to the muscles occurs. Unfortunately, no single measurement reliably detects early manifestations of later-onset Pompe disease. CK levels can be normal in adult patients with Pompe disease.[22] Muscle biopsy specimens may fail because patients usually demonstrate a mixture of affected and unaffected muscles.[4] Muscle strength is often measured in the extremities, but weakness of trunk muscles, an early sign of Pompe disease, can be underestimated. Therefore, for the maximum benefit to patients, treatment decisions should depend on a careful evaluation of multiple criteria. In this study, the criteria for initiating ERT included significantly elevated CK levels and the appearance of symptoms. However, it was not clear before the start of this study how much (if at all) the CK level would be elevated in infants and young children with later-onset Pompe disease. Individual variation in CK levels and motor development is also large. Therefore decisions to initiate ERT are taken on an individual basis at the present time, but we will be able to generate definitions for these criteria soon.

Currently, all nine untreated babies are being monitored every 3 to 6 months. At each visit, motor development is evaluated, and blood tests are performed. No invasive procedures are conducted until ERT is considered. It may be possible to omit the later-onset subtypes during newborn screening by excluding those newborns who do not have cardiomyopathy. However, many patients who may benefit from early

treatment at a later time will then be missed. Therefore our study reveals very important information regarding the earliest manifestations of later-onset Pompe disease. Only through these efforts can patients with later-onset Pompe disease be treated in a timely manner and obtain maximal benefit with minimal harm. ■

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Table II. Mutations and GAA activities in newborns with GAA deficiencies

No.	Mutation-paternal		Mutation-maternal	
NBS2	c.1411_1414del	p.E471PfsX5	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
NBS3	c.[1935C>A; 1726G>A]	p.[D645E; G576S]	**c.2842insT**	**p.L948SfsX70**
NBS4	c.784G>A	p.E262K	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
NBS5	c.[1935C>A; 1726G>A]	p.[D645E; G576S]	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
NBS6	c.1062C>G	p. Y354X	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
NBS8	c.1197_1208del	**p.V400_N403del**	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
1*	**c.[811A>G; 1726G>A]**	**p.[T271A; G576S]**	**c.424_440del**	**p.S142LfsX29**
2	**c.[752 C>T; 761 C>T]**	**p.[S251L; S254L]**	**c.[752 C>T; 761 C>T]**	**p.[S251L; S254L]**
3	c.[2238G>C; 1726G>A]	p.[W746C; G576S]	c.2662G>T	p.E888X
4	c.2662G>T	p.E888X	**c.1574T>A**	**p.F525Y**
5	**c.424_440del**	**p.S142LfsX29**	**c.[533 G>A; c.1726G>A]**	**p.[R178H; G576S]**
6	c.[1935C>A; 1726G>A]	p.[D645E; G576S]	**c.[752 C>T; 761 C>T]**	**p.[S251L; S254L]**
7	c.1080C>G	p.Y360X	**c.546+5G>T**	**splicing**
8	**c.546+5G>T**	**splicing**	**c.546+5G>T**	**splicing**
9	c.[2238G>C; 1726G>A]	p.[W746C; G576S]	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
10	**c.[1324G>A; 1726G>A]**	**p.[V442M; G576S]**	c.1843G>A	p.G615R
11	**c.[752 C>T; 761 C>T]**	**p.[S251L; S254L]**	**c.1958C>A**	**p.T653N**
12	**c.[752 C>T; 761 C>T]**	**p.[S251L; S254L]**	c.[1935C>A; 1726G>A]	p.[D645E; G576S]
13	c.[1935C>A; 1726G>A]	p.[D645E; G576S]	**c.[752 C>T; 761 C>T]**	**p.[S251L; S254L]**

*The patient was named as NBS1 in previous publications. Normal range of GAA activity in lymphocytes: 66.70 ± 33.70 nmol/mg Prot/hr; in fibroblasts: 106.29 ± 60.46 nmol/mg Prot/hr. Bold indicates the first reported mutation.