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Clinical outcomes of children with abnormal newborn screening results for Krabbe disease in New York State

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Background: Early infantile Krabbe disease is rapidly fatal, but hematopoietic stem cell transplantation (HSCT) may improve outcomes if performed soon after birth. New York State began screening all newborns for Krabbe disease in 2006.

Methods: Infants with abnormal newborn screen results for Krabbe disease were referred to specialty-care centers. Newborns found to be at high risk for Krabbe disease underwent a neurodiagnostic battery to determine the need for emergent HSCT.

Results: Almost 2 million infants were screened. Five infants were diagnosed with early infantile Krabbe disease. Three died, two from HSCT-related complications and one from untreated disease. Two children who received HSCT have moderate to severe developmental delays. Forty-six currently asymptomatic children are considered to be at moderate or high risk for development of later-onset Krabbe disease.

Conclusions: These results show significant HSCT-associated morbidity and mortality in early infantile Krabbe disease and raise questions about its efficacy when performed in newborns diagnosed through newborn screening. The unanticipated identification of "at risk" children introduces unique ethical and medicolegal issues. New York's experience raises questions about the risks, benefits, and practicality of screening newborns for Krabbe disease. It is imperative that objective assessments be made on an ongoing basis as additional states begin screening for this disorder.

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Key Words: globoid cell leukodystrophy; Krabbe disease; newborn screening

INTRODUCTION

Krabbe disease (globoid cell leukodystrophy, OMIM 245200) is a rare lysosomal storage disorder affecting the central and peripheral nervous systems.[1] It is caused by an inherited deficiency of galactocerebrosidase (GALC), an enzyme essential for the normal turnover of myelin.[2] Historically, Krabbe disease has been classified into three distinct phenotypes. Infants with the most severe form, early infantile Krabbe disease, present with irritability, cortical fisting, and stiffness by 6 months of age.[3] The disease progresses rapidly, with most dying by 2 years of age.[3,4] Individuals with late infantile Krabbe disease develop symptoms between 6 and 12 months and follow a progressive neurodegenerative course.[5] Those with later-onset phenotypes may present from childhood to adulthood with variable symptoms that may include ataxia, visual disturbances, and dementia.[6–8] Prior to screening, the reported incidence of all forms of Krabbe disease was 1 in 100,000, with 90% of diagnosed patients having infantile disease.[6]

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A 2005 report in the *New England Journal of Medicine* described the use of hematopoietic stem cell transplantation (HSCT) in 25 infants with Krabbe disease.[9] Although baseline GALC activity and genotype were not reported, all infants were presumed to have a form of infantile Krabbe disease. Eleven asymptomatic newborns underwent transplantation between 12 and 44 days of age and were followed for a median of 3.0 years. They had improved myelination and gained developmental skills, but delays in gross motor skills and expressive language were present. By contrast, infants who underwent transplantation after the onset of symptoms had minimal neurologic improvement. The investigators concluded that HSCT favorably alters the course of infantile Krabbe disease if performed presymptomatically. This served as the justification for newborn screening (NBS) for the disorder, a cause that was championed by advocacy groups. In 2006, New York State (NY) mandated the screening of all newborns for Krabbe disease.

Recognizing how complex the evaluation of asymptomatic newborns would be, the NY Krabbe Consortium was established, consisting of pediatric neurologists, biochemical geneticists, neuroradiologists, transplant physicians, and key members of NY's NBS Program.[10] Based on the findings in the *New England Journal of Medicine* paper,[9] early transplantation was crucial, so it was essential to identify those with early infantile Krabbe disease immediately. Conversely, newborn transplantation is not indicated for later-onset forms of Krabbe disease, so accurate differentiation between early-onset and later-onset phenotypes was necessary. Therefore, the Consortium developed a comprehensive protocol (**Table 1**) to help clinicians identify newborns with early infantile Krabbe disease in need of emergent HSCT and to provide guidance to evaluate children at risk for later-onset phenotypes.

Since 2006, almost 2 million infants have been screened for Krabbe disease in NY. The overall NBS and molecular results are detailed in a recently published companion article by Orsini et al.[11] This report describes the clinical results obtained thus far and focuses on the outcomes of those infants found to be at high risk for Krabbe disease.

MATERIALS AND METHODS

Patients

NBS data from 1,968,568 infants screened for Krabbe disease in NY between 7 August 2006 and 7 August 2014 are included in this report. Outcome data for 346 infants with abnormal NBS results for Krabbe disease who had confirmatory testing during that time period are also included. Institutional review board approval for chart review was obtained at Inherited Metabolic Disease Specialty Care Centers with data for children at high risk of developing Krabbe disease.

Specimen testing

GALC activity in newborn dried blood spots (DBS) was quantitated using a modified multiplex tandem mass spectrometry method as previously described.[12] The daily mean activity (DMA) was calculated from the results of all newborns tested on that particular day. DBS with ≤20% DMA were retested in duplicate.[10] Specimens with mean GALC activities ≤12% DMA underwent a rapid molecular analysis of the *GALC* gene, including PCR for the 30-kb and 7.4-kb deletions and bidirectional sequencing of all 17 exons and the promoter region.[10] Infants with mean GALC activity ≤12% DMA and at least one potentially disease-causing variant were referred for confirmatory testing. (**Figure 1**)

Confirmatory evaluation

At the confirmatory visit, detailed prenatal, medical, and family histories were obtained, and a physical examination was performed. DBS were obtained from the infant for identity confirmation and HLA typing. DBS were also obtained from both parents for *GALC* genotyping and phasing of the infant's mutations. Blood was collected from the infant to measure GALC activity in leukocytes, which was always performed[10] at the Thomas Jefferson University Lysosomal Diseases Testing Laboratory using a tritium-labeled galactosylceramide.[13] Because there were no validated methods to predict an infant's risk for development of Krabbe disease, leukocyte GALC activity was used to determine initial risk category (low, moderate, or high). Infants with the lowest enzyme activity were predicted to have the highest risk of developing Krabbe disease.

Neurodiagnostic evaluation schedule and scoring system

Table 1 shows the currently recommended neurodiagnostic procedures and schedule for infants in the high risk and moderate risk categories. Neurologic examinations were performed by experienced child neurologists. Neurodiagnostic batteries included lumbar puncture to measure cerebrospinal fluid protein, brain magnetic resonance imaging, brainstem auditory-evoked responses, and nerve conduction studies. The original algorithm, which is described elsewhere,[10] was further modified by Krabbe Consortium consensus in 2012 to remove visual-evoked responses and electroencephalograms and to reduce the number of neurodiagnostic evaluations. Abnormal results were scored as shown in **Table 2**, and infants who received scores of ≥4 were considered to be candidates for transplantation. The neurodiagnostic protocol and scoring systems were devised based on available evidence and the collective experience of the Krabbe Consortium, and they were not validated prior to implementation.

RESULTS

Overall screening results

As of 7 August 2014, 2,090,910 specimens from 1,968,568 infants had been screened, and more than 99.9% of screening results were negative;[11] 10,199 specimens had ≤20% DMA GALC activity and, within this group, 620 had ≤12% DMA on duplicate testing and underwent molecular analysis of the *GALC* gene. Of these, 272 carried only polymorphisms. A total of 348 infants had one or more known or potentially pathogenic *GALC* mutation and were referred to a designated specialty-care center in NY. Two infants were lost to follow-up prior to confirmatory testing[11] (**Figure 1**).

Table 1 Suggested evaluation schedule

	0–12 months	13–36 months	37–60 months	Years 6–10
High risk				
Neurological examinations	Every month	Every 3 months	Every 6 months	Annual
Neurodiagnostic evaluations	0, 4, 8, 12 months	As needed	As needed	As needed
Moderate risk				
Neurological examinations	Every 3 months	Every 3 months	Every 6 months	Annual
Neurodiagnostic evaluations	At 12 months	As needed	As needed	As needed

Infants in the high risk category had confirmatory GALC activity between 0.0 and 0.15 nmol/hour/mg protein. Infants in the moderate risk category had confirmatory GALC activities between 0.16 and 0.29 nmol/hour/mg protein. Neurodiagnostic evaluations included lumbar puncture to measure CSF protein, brain MRI, nerve conduction velocity, and brainstem auditory-evoked response.

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

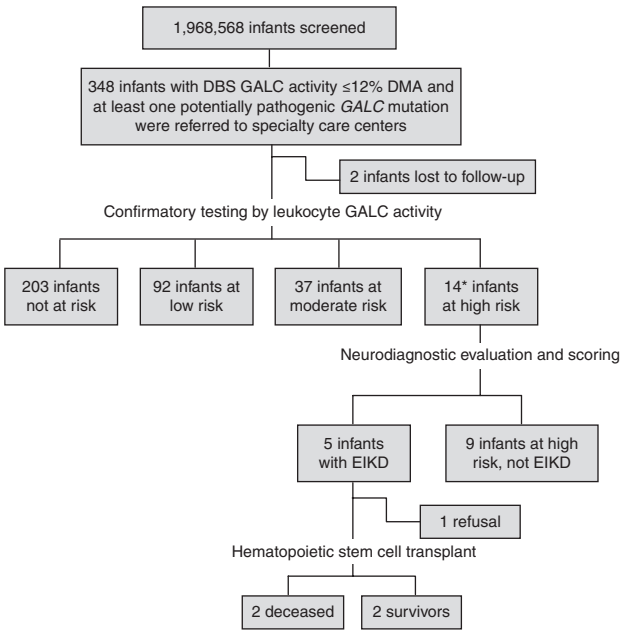

Figure 1 Numbers of infants who underwent screening, confirmatory testing, neurodiagnostic testing, and treatment. *Fifteen infants were originally determined to be at high risk, but repeat GALC testing for one infant changed his risk category to moderate.

Infants at low or no risk of developing Krabbe disease

From 2006 to 2012, infants with confirmatory residual GALC activities between 0.30 and 0.5 nmol/hour/mg protein were considered to be at low risk, and those with activities >0.5 nmol/hour/mg were considered to not be at risk. In 2012, the low-risk category was eliminated by consensus of the Krabbe Consortium, and infants with activities >0.3 nmol/hour/mg are now considered to not be at risk unless they carry two potentially pathogenic variants, which would classify them as moderate risk. During the 8-year period, 203 infants were determined not to be at risk and 92 were determined to be at low risk [11] (**Figure 1**).

Table 2 Scoring system for HSCT referral

Parameter	Points
Abnormal neurologic examination	2
Abnormal MRI	2
Elevated CSF protein	2
Abnormal nerve conduction velocity	2
Abnormal brainstem auditory-evoked response	1
30-kb homozygous deletion	4

Transplantation is considered for infants with scores ≥4.

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

Infants at moderate risk of developing Krabbe disease

Thirty-seven infants were found to be at moderate risk, 32 of whom had confirmatory GALC activity of 0.16–0.29 nmol/hour/mg protein and 5 of whom had GALC activity in the low-risk range but were classified as moderate because of two known or potentially pathogenic mutations, as mentioned previously[11] (**Figure 1**). Currently, no children in this group (who range in age from 13 months to 9 years) are known to be symptomatic.

Infants at high risk of developing Krabbe disease

Fourteen infants were considered to be at high risk for Krabbe disease based on very low confirmatory GALC activity of 0.0–0.15 nmol/hour/mg protein. However, a fifteenth infant who was initially classified as being at high risk (GALC = 0.09) was then reclassified as being at moderate risk after repeat testing at age 4 years revealed GALC activity of 0.21. All infants in this group underwent urgent neurodiagnostic evaluation, and their results and scores are shown in **Table 3**. Five were confirmed to have early infantile Krabbe disease. Of these, four underwent HSCT, and their outcomes are shown in **Table 4**. The first infant was transplanted on day 32 of life and subsequently developed autoimmune hemolytic anemia and steroid-related hypertrophic cardiomyopathy. The child, currently 8 years old, has receptive language skills that are appropriate for his age, but he has significant developmental delays and is unable to walk independently. His weight, height, and head circumference are all below the third percentile. Infant 2 was transplanted on day 31 of life but died 53 days later from transplant-related complications and multiorgan failure. Infant 3 was homozygous for the 30-kb deletion. His parents decided against transplant following extensive counseling about its risks, potential benefits, and limitations, and he died of early infantile Krabbe disease at approximately 18 months of age. Infant 4 underwent transplantation on day 41 of life. Her posttransplantation course was complicated by graft versus host disease. At 5 years of age, she is severely developmentally delayed and failing to thrive. Infant 5 was transplanted on day 24 of life. She died of respiratory failure on day 69 of life after experiencing progressive pulmonary hypertension. Posttransplantation neurodevelopmental assessments for infants 1 and 4 were performed by the transplantation centers; the results are shown in **Table 4**.

Nine additional infants were found to be at high risk for Krabbe disease. Two patients (13 and 14; **Table 3**) had initial

Table 3 Results of infants at high risk for Krabbe disease

	Enzyme and genotype				Neurodiagnostic evaluation							
Patient	NBS GALC DMA	Conf GALC	Allele 1	Allele 2	Age	Neurologic examination	CSF protein	Brain MRI	BAER	NCV	KC score	Age at last contact
Early infantile Krabbe disease												
1	9.9	0.01	Del30kb+p.R168C	c.-335G>A+ p.D94=+ p.I546T+p.*670Qext42	18–32 days	Cortical thumbs, mild jitteriness	523	Mild or moderately delayed myelination in the peri-Rolandic regions bilaterally	Abnl	Abnl	9	8 years
2	10.9	0.05	Del30kb+p.R168C	Del30kb+p.R168C	16–18 days	Cortical thumbs, inconsistent tracking	255	Normal myelination for patient age; multiple small foci of subdural hemorrhage	Abnl	Abnl	13	Deceased
3	7.6	0.02	Del30kb+p.R168C	Del30kb+p.R168C	10 days	Normal	547				6	Deceased
4	5.6	0.12	Del30kb+p.R168C	c.-335G>A + p.G360Dfs*2	16 days	Normal	441	Normal	Abnl	Abnl	5	5 years
5	4.3	0.05	Del30kb+p.R168C	Del30kb+p.R168C	6–8 days	Normal	483	Increased T2 signal in the bilateral periventricular white matter			8	Deceased
Not early infantile, but at high risk of developing Krabbe disease												
6	6.1	0.06	p.A5P + p.D232N+ p.Y303C	p.A5P + p.D232N+ p.Y303C	13 days	Normal	111, bloody	Normal			2	8 years
					3 months	Normal	39	Normal			0	
					6 months	Normal	24	Normal			0	
					12 months	Normal	14	Normal			0	
					21 months	Normal					0	
					28 months	Normal					0	
					3 years 5 months	Normal					0	
					4 years 2 months	Normal					0	
					4 years 9 months	Normal					0	
					6 years 1 months	Normal					0	
					7 years 3 months	Normal					0	
					8 years 2 months	Normal					0	

Pretransplantation results are shown for patients 1, 2, 4, and 5. NBS GALC is expressed in % daily mean activity, confirmatory GALC is reported in nmol/hour/mg protein, and CSF protein is expressed in mg/dl. Krabbe Consortium (KC) Score is defined in Table 2; score was calculated based on completed test results. No results indicate that the evaluation was not performed.

BAER, brainstem auditory evoked response; CSF, cerebrospinal fluid; DMA, daily mean activity; GALC, galactocerebrosidase; MRI, magnetic resonance imaging; NBS, newborn screening.

[a]NBS abnormality reported after fourth NBS at 4 months, first three NBS specimens were unsuitable.

Table 3 Continued on next page

Table 3 Continued

	Enzyme and genotype				Neurodiagnostic evaluation							
Patient	NBS GALC DMA	Conf GALC	Allele 1	Allele 2	Age	Neurologic examination	CSF protein	Brain MRI	BAER	NCV	KC score	Age at last contact
7	8.3	0.12	p.A5P + p.D232N+p.Y303C	p.I546T+p.D556fs*1	46 days	Normal	94	There is a suggestion of increased T2 signal within the white matter			2	7 years
					3 months	Normal					0	
					6 months	Normal	41	Normal			0	
					13 months	Normal					0	
					3 years 1 months	Normal					0	
					4 years 4 months	Normal					0	
					4 years 1 months	Normal					0	
					5 years 5 months	Normal					0	
					5 years 1 months	Normal					0	
					6 years 7 months	Normal					0	
8	9.6	0.07	p.H375Qfs*3+p.I546T	c.-348C>T+p.A5P+ p.D232N+p.Y303C	24 days	Normal	83	Normal	Normal		0	4 years
9	9.1	0.12	c.-128_-123delATCAGC+p.L618S	p.L618S	20 days	Normal	137	Normal			2	6 months
					2 months	Normal					0	
					3 months	Normal					0	
					6 months	Normal					0	
10	4.7	0.03	p.M101V+c.1786+5C>G+p.A625T	p.M309V+ p.I546T	27 days	Normal	35	Normal	Normal		0	5 years
11	6.2	0.05	c.147G>C/p.G49=+p.I546T	p.K83E+p.I546T	4 months[a]	Normal					0	4 years
					5 months	Normal	16	Normal			0	
					8 months	Normal					0	
					14 months	Normal					0	
12	4.9	0.05	p.T452I	p.A5P+ p.D232N+p.Y303C	13 days	Normal					0	6 months
					6 months	Normal					0	

Pretransplantation results are shown for patients 1, 2, 4, and 5. NBS GALC is expressed in % daily mean activity, confirmatory GALC is reported in nmol/hour/mg protein, and CSF protein is expressed in mg/dl. Krabbe Consortium (KC) Score is defined in Table 2; score was calculated based on completed test results. No results indicate that the evaluation was not performed.

BAER, brainstem auditory evoked response; CSF, cerebrospinal fluid; DMA, daily mean activity; GALC, galactocerebrosidase; MRI, magnetic resonance imaging; NBS, newborn screening.

[a]NBS abnormality reported after fourth NBS at 4 months, first three NBS specimens were unsuitable.

Table 3 Continued on next page

Table 3 Continued

	Enzyme and genotype				Neurodiagnostic evaluation							
Patient	NBS GALC DMA	Conf GALC	Allele 1	Allele 2	Age	Neurologic examination	CSF protein	Brain MRI	BAER	NCV	KC score	Age at last contact
13	10.8	0.09	p.R63C+p.I546T	p.R111*	29 days	Normal	193, bloody	Abnormal signal on the inferior aspect of the genu and the body of the corpus callosum; increased T2 signal within the hila of the dentate nuclei bilaterally and symmetrically	Normal	Normal	4	2 years
					3 months	Normal	32	Interval expected maturation of the myelination of the brain; the site of abnormality at the hila of the dentate nuclei appear to have matured			2	
					8 months	Normal					0	
					11 months	Normal					0	
					14 months	Normal					0	
					26 months	Normal					0	
14	8.0	0.07	c.-335G>A+ p.I546T+p.R380W	c.-128_-123delATCAGC+ p.L618S	18 days	Normal	484, bloody	Bilateral symmetrical increase in the T2 signal in the hilum of the dentate nucleus of cerebellum bilaterally and increased signal in the peridentate white matter		Abnl	5	13 months
					3 months	Normal	39	Since previous MRI, there is decreased dentate and peridentate signal; there is persistent greater-than-expected periventricular T2 flair hyperintensity bilaterally and symmetrically			2	
					6 months	Normal					0	
					9 months	Normal					0	
					13 months	Normal					0	

Pretransplantation results are shown for patients 1, 2, 4, and 5. NBS GALC is expressed in % daily mean activity, confirmatory GALC is reported in nmol/hour/mg protein, and CSF protein is expressed in mg/dl. Krabbe Consortium (KC) Score is defined in Table 2; score was calculated based on completed test results. No results indicate that the evaluation was not performed.

BAER, brainstem auditory evoked response; CSF, cerebrospinal fluid; DMA, daily mean activity; GALC, galactocerebrosidase; MRI, magnetic resonance imaging; NBS, newborn screening.

[a]NBS abnormality reported after fourth NBS at 4 months, first three NBS specimens were unsuitable.

Table 4 Outcomes of infants with early infantile Krabbe disease identified by newborn screening

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Overall status					
Age at death		84 days	18 months		69 days
Current age, if alive	8 years 5 months			5 years 4 months	
Transplant status					
Age at transplantation	32 days	31 days	Not transplanted	41 days	24 days
Complications from transplant	Autoimmune hemolytic anemia, steroid-related hypertrophic cardiomyopathy	Multiorgan failure	N/A	Graft-vs.-host disease	Seizures, pulmonary hypertension, progressive respiratory failure
Anthropometrics					
Last weight age	8 years 5 months			3 years 3 months	
Percentile (*Z* score)	0 (–6.65)			0 (–4.33)	
Last height age	8 years 3 months			3 years 3 months	
Percentile (Z score)	0 (–4.23)			2 (–2.06)	
Last head circumference age	5 years 8 months			2 years 6 months	
Percentile	2.7			60	
Developmental status					
Lansky performance score age	8 years 3 months			3 years 4 months	
Score	70			40	
Age at developmental assessment	7 years 3 months			3 years 4 months	
Interpretation	Significant delays in all domains; developmental profile-3 (ref. 27) score <50 (<0.1 percentile) across 5 scales: cognitive, physical, communication, adaptive behavior, social–emotional			Severe global developmental delays; unable to hold head up, communicate verbally, sit, or stand; able to see, hear, smile, babble, and has some receptive language skills	
Ambulation ability	Requires wheelchair/stander for ambulation			Nonambulatory	

Growth percentiles are according to Center for Disease Control Standards.[28,29] Patient 1 head circumference percentile is according to Nellhaus boys.[30]

scores of 4 and 5 and were considered candidates for HSCT. In both cases, the myelination pattern on initial magnetic resonance imaging was interpreted as abnormal by two independent neuroradiologists (**Supplementary Figure S1a,b** online). Both families refused HSCT but agreed to have the infants undergo another evaluation. In both cases, magnetic resonance imaging several weeks later showed improvements in myelination (**Supplementary Figure S1c,d**) and they were no longer considered candidates for HSCT. Four children at high risk had at least one additional neurodiagnostic evaluation, but the majority did not undergo all recommended assessments (**Table 1**). These nine children now range in age from 14 months to 8 years. Six children either have been examined by a child neurologist or were contacted within the past year, and all remain without overt symptoms of Krabbe disease. Of the remaining three children, two (patients 9 and 12) were last seen at 6 months of age but were later lost to follow-up, and another (patient 8) was well when last contacted at 4 years of age.

DISCUSSION

NBS has been an integral part of preventable health care for five decades. In 2006, NY became the first and only state to implement NBS for Krabbe disease, a decision that was lauded by many Krabbe disease families and support groups. However, 4 years later, the US Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children advised against NBS for Krabbe disease, citing insufficient knowledge about the accuracy of screening, diagnostic strategy, benefits and harms of treatment, and long-term prognosis.[14] The past 8 years of NBS for Krabbe disease have clarified some of these issues but have raised additional concerns.

After the screening of almost 2 million infants, only five were diagnosed with early infantile Krabbe disease. Of these, three have died—two from complications of HSCT and one from untreated Krabbe disease. One infant who underwent transplantation is severely developmentally delayed. Another is stable, albeit with failure to thrive, gross motor delays, and cardiomyopathy. These outcomes show significant HSCT-associated morbidity and mortality in newborns with early infantile Krabbe disease and raise questions about its efficacy when performed in infants diagnosed through NBS. In the interval between the NBS result and HSCT, it was necessary to perform confirmatory testing, complete the neurodiagnostic

battery, educate parents, obtain insurance approval for HSCT, and, in two cases, relocate families to a transplantation center. Chemotherapy induction added several more days. Although infants identified through NBS were transplanted between 24 and 41 days of age, it is possible that the disease progressed during the time between diagnostic confirmation and treatment, reducing the effectiveness of HSCT. An alternative explanation for the suboptimal outcomes is that babies with early infantile Krabbe disease may actually have had prenatal onset of disease, which is consistent with the presence of brain abnormalities in 22- to 24-week fetuses.[15–17] If true, this may limit the efficacy of HSCT for early infantile Krabbe disease regardless of timing. Nonetheless, it is possible that transplantation may be more effective for late infantile Krabbe disease; however, to date, no infants with this phenotype have been identified by NY's NBS, suggesting that its incidence is very low, i.e., less than one in 2 million.

In contrast to NY's experience, HSCT outcome in 16 children with a form of infantile Krabbe disease who were transplanted at Duke University and six more who were transplanted at other centers showed transplant-related mortality of only 10%.[18] Infants transplanted before 30 days of age, most of whom were diagnosed *in utero* because of family history, had better survival and functional outcome compared with those transplanted later.[19] Presymptomatic transplantation is reported to result in normal receptive language,[18] attenuation of symptom severity,[18] and longer survival compared with untreated infantile Krabbe disease.[20] However, most children have progressive gross motor delays ranging from mild spasticity to inability to walk independently, and a few have acquired microcephaly.[18]

Nine NY children have been identified to be at high risk for Krabbe disease, and 37 more are at moderate risk. These children are now past the ages for infantile forms of Krabbe disease, but they remain at risk for later-onset phenotypes, which have a variable age of onset and a broad phenotypic spectrum.[7] The only currently available treatment is HSCT, which has been effective in some later-onset Krabbe patients but has left residual neurologic deficits in others.[5] NY's "at risk" children will require follow-up for potentially decades, which presents unique logistical and medicolegal challenges for NBS teams, especially because there are no published guidelines for monitoring these patients and no validated markers that can be used to predict later disease onset. These uncertainties can be stressful for families,[21] and, consistent with NY's NBS experience, parents may be reluctant to subject their asymptomatic children to invasive assessments, thus increasing the likelihood of being lost to follow-up.

Interestingly, the incidence of Krabbe disease in NY is different than was anticipated, which may reflect the diversity of NY's population. Before screening, the estimated incidence of Krabbe disease was 1 in 100,000 (ref. 6), but the actual incidence of early infantile Krabbe disease in NY detected by NBS is only 1 in 394,000 (ref. 11). Based on the best evidence prior to screening, we anticipated that 90% of infants would have early infantile Krabbe disease and 10% would have later-onset forms. However, it appears that these percentages may be reversed, with only 5 infants having early infantile Krabbe disease and 46 children at moderate risk or high risk for later-onset phenotypes. It is possible that some individuals may never develop symptoms due to variable penetrance. It is also possible that later-onset Krabbe disease is more common than previously thought but is underdiagnosed owing to ambiguous clinical presentation. Importantly, these numbers suggest that we are screening infants for a predominantly later-onset disease. This challenges traditional NBS criteria, which generally recommend that newborns be screened for treatable childhood-onset disorders.[22,23]

Several polymorphisms in the *GALC* gene affect DBS GALC activity,[6,24] and NY's use of second-tier DNA testing was essential to reduce the number of false positives by only referring infants with at least one true or suspected pathogenic mutation.[11] Of 346 infants who underwent confirmatory testing, only 5 were diagnosed with early infantile Krabbe disease, which corresponds to a positive predictive value of 1.4% for this phenotype. In comparison, medium-chain acyl CoA dehydrogenase deficiency, another inherited metabolic disease that utilizes second-tier DNA testing as part of the NBS process, has a positive predictive value of 58.7% in NY. Because of the variability in symptom onset in later-onset Krabbe disease, it may be decades before it is possible to estimate the positive predictive value of NBS for this phenotype.

Importantly, the risk categories, neurodiagnostic protocol, and scoring system developed by the Krabbe Consortium correctly identified the infants with early infantile Krabbe disease. However, for one infant initially diagnosed as being at high risk a subsequent measurement of GALC activity changed the risk category to moderate. Two other infants had initial neurodiagnostic scores in the transplant referral range that improved with additional testing. Fortunately, none of these children underwent HSCT. False-positive results such as these, and the consequent risks of unnecessary invasive testing and inappropriate HSCT, may be reduced by adding biomarkers[25] to the Krabbe Consortium scoring system and including techniques that permit quantitative analysis of myelination, such as diffusion tensor imaging.[26] Compliance with the Krabbe Consortium protocol was low; of the high-risk infants, the majority did not complete all recommended neurodiagnostic studies, and none have undergone all recommended long-term follow-up assessments, suggesting that the testing schedule may be unrealistic and overly burdensome to families.

NY's current data do not support NBS for Krabbe disease because our experience has raised serious questions about its benefits, risks, and practicality. However, Missouri has recently started screening newborns for Krabbe disease, and other states are slated to do so; justification for continuing this screening requires identifying ways to improve diagnostic specificity and clinical outcome. It is imperative that objective assessments continue as Krabbe disease screening becomes more widespread.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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