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Consensus recommendations for the classification and long-term follow up of infants who screen positive for Krabbe Disease

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ABSTRACT

Objective: To provide updated evidence and consensus-based recommendations for the classification of individuals who screen positive for Krabbe Disease (KD) and recommendations for long-term follow-up for those who are at risk for late onset Krabbe Disease (LOKD).

Methods: KD experts (KD NBS Council) met between July 2017 and June 2020 to develop consensus-based classification and follow-up recommendations. The resulting newly proposed recommendations were assessed in a historical cohort of 47 newborns from New York State who were originally classified at moderate or high risk for LOKD.

Results: Infants identified by newborn screening with possible KD should enter one of three clinical follow-up pathways (Early infantile KD, at-risk for LOKD, or unaffected), based on galactocerebrosidase (GALC) activity, psychosine concentration, and GALC genotype. Patients considered at-risk for LOKD based on low GALC activity and an intermediate psychosine concentration are further split into a high-risk or low-risk follow-up pathway based on genotype. Review of the historical New York State cohort found that the updated follow-up recommendations would reduce follow up testing by 88%.

Conclusion: The KD NBS Council has presented updated consensus recommendations for efficient and effective classification and follow-up of NBS positive patients with a focus on long-term follow-up of those at-risk for LOKD.

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1. Introduction

Krabbe disease (KD) is an autosomal recessive neurodegenerative disorder that can affect individuals from early infancy to adulthood [1]. The most severe form of KD, herein referred to as early infantile

KD (EIKD, historically also referred to as infantile KD), is a rapidly progressive and quickly fatal disease presenting in the first few months of life. Mean life expectancy of infants with untreated EIKD is 2 years [2]. KD can also occur in older infants (sometimes referred to as late infantile KD), toddlers, children, and rarely adults, and all fall into the designation of late onset KD (LOKD).

KD is the result of deficient activity of the enzyme galactocerebrosidase (GALC) caused by variants in the GALC gene. Diagnosis of KD has traditionally relied on low enzyme activity but this method is not sensitive for distinguishing patients with EIKD from those with or at risk for LOKD as both groups of patients overlap in the measured ranges of GALC activity [3]. Additionally, infants may screen positive for KD based on the presence of non-pathogenic

Abbreviations: BAER, brainstem auditory evoked potentials; DBS, dried blood spot; CSF, cerebrospinal fluid; EEG, electroencephalogram; EIKD, early infantile Krabbe disease; GALC, galactocerebrosidase; HSCT, hematopoietic stem cell transplantation; KD, Krabbe disease; LOKD, late infantile Krabbe disease; MRI, magnetic resonance imaging; NBS, newborn screening; NCS, nerve conduction studies; Psy, psychosine.

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enzyme-activity-lowering polymorphisms involving the *GALC* gene, a situation often referred to as pseudodeficiency [4].

For individuals with KD, hematopoietic stem cell transplantation (HSCT) can favorably modify the course of the disease, particularly if performed in the first month of life and before the onset of neurologic symptoms [5–7]. Patients with KD who have minimal symptoms but are >12 months can also benefit from HSCT [8–10]. Given the benefit of pre-symptomatic treatment, New York State began newborn screening (NBS) for KD in 2006 and several states have since followed suit. In an attempt to identify patients with EIKD through NBS, babies at risk of developing LOKD have also been identified. Over the course of approximately 14 years of screening in New York State, 47 babies were identified who were considered at moderate or high risk for KD, but were not diagnosed with EIKD. We review screening methods for KD, the use of second tier tests, previously employed follow up algorithms and propose updated recommendations for the diagnosis and long term follow up of those individuals at risk for LOKD.

1.1. Utility of psychosine in newborn screening

Despite the challenges of measuring *GALC* activity from dried blood spot (DBS), NBS programs continue to utilize *GALC* activity as their first-tier screening test because it is a high throughput assay and can be multiplexed with other screening tests [11,12]. When implementing KD screening, New York State adopted *GALC* gene sequencing as a 2nd tier test to help reduce the false positive rate of screening based on *GALC* activity alone. Infants with a positive screen, as defined by low *GALC* activity and at least one known or novel variant in the *GALC* gene, were referred for subspecialty evaluation. Although the genotype information did help to identify those infants at high risk for EIKD, the protocol still led to many false positives. This was due to frequently encountered genotypes that included variants of uncertain significance, and referral of apparent heterozygotes owing to the possibility of sequencing missing deletions or duplications [13].

Psychosine (Psy), a toxic substrate that accumulates as a result of deficient *GALC* activity, has emerged as a second tier test with better specificity and sensitivity for KD than *GALC* genotype [14–20]. With the advent of a Psy assay with enhanced sensitivity, minor elevations in Psy seen in LOKD can be detected [21–23]. Guenzel and Escolar et al. have shown that Psy in most instances can differentiate infants with low *GALC* activity on NBS into three groups - those with EIKD, LOKD and unaffected individuals [21,22]. New York and Kentucky have pioneered the use of Psy as reflex second tier testing and most other states that screen for KD are moving toward incorporating Psy into their screening protocols [24,25]. Psy measurement is more efficient and less complicated compared to genotyping, thereby expediting identification of infants with EIKD [21,26].

1.2. Follow up diagnostic testing algorithms for children at risk for LOKD

The purpose of NBS and diagnostic testing is to identify patients at the earliest stages of disease. If a child is identified as having EIKD, they require immediate referral for assessment of eligibility for transplant or other disease modifying treatments that may be available in the future [25]. Children identified as at risk for LOKD require longitudinal monitoring to determine if and when disease develops, at which point they will require referral for treatment. Review of the literature for evidence-based recommendations on the timing and most sensitive modalities of diagnostic testing is confounded by the frequent grouping of EIKD with LOKD, non-standardized ages of assessment, non-standardized timing of testing after symptom onset and the fact that most neuro-diagnostic studies are undertaken after disease onset. Based on a thorough review of the literature, magnetic resonance imaging (MRI) and nerve conduction studies (NCS) are the most likely neurodiagnostic studies to be abnormal in early-symptomatic patients with LOKD. Electroencephalogram (EEG), auditory and visual evoked

potentials as well as cerebrospinal fluid (CSF) protein have also been used to determine the extent of disease progression in KD. Testing, particularly if invasive or requiring sedation, must be balanced with its acceptability to parents and its benefit to the patient [27–31].

The first follow up algorithm proposed in New York seemed to be too intensive. Of the first 2 million infants screened in NY, 5 were identified as EIKD, and 51 were thought to be at risk for LOKD and referred to a designated referral center for long term follow-up. In retrospect, the intensive follow up protocol for infants at risk for LOKD was burdensome to most families and the health care system, and too aggressive to be practical. This includes the high frequency of invasive testing (e.g. lumbar puncture, imaging with anesthesia, nerve conduction studies), and large number of tests recommended for children who in retrospect may not have been high risk. Infants who could theoretically benefit from ongoing monitoring may have been lost to follow up [3,13,32].

As more infants are identified as being at risk for LOKD through newborn screening, there is an urgent need for updated guidelines regarding classification of individuals at risk and an appropriate clinical follow up algorithm. Below we propose recommendations based on expert consensus and new evidence, which will improve the accuracy of KD NBS and significantly reduce the amount of diagnostic testing required to follow individuals at risk for LOKD.

2. Material and methods

These recommendations are the result of multiple meetings of the Krabbe Disease NBS Council organized by the Leukodystrophy Care Network that consists of representatives of the following specialties/groups: genetics (including laboratory specialists), child neurology, childhood neurodevelopment, pediatric hematopoietic stem cell transplant, state NBS programs, and patient advocates. Individuals for this council were recruited by the Leukodystrophy Care Network from all NBS labs and states that were screening for KD at the time, and any clinicians known to the group with experience in treating and managing KD. Overall, there were 21 group members who took part in some or all of the meetings. Council members met both in-person and by teleconference (audio and web-based video platforms) between July 2017 and June 2020 to review existing evidence, recent laboratory developments, examples from patient databases, and its members' extensive experiences following children identified through NBS. Members of the council performed a literature review of current NBS strategies and neuro-diagnostic testing in Krabbe disease. Available published evidence and unpublished data provided by council members were used for recommendation development. There were no specific outside groups that were consulted after consensus recommendations were determined. The guideline group meetings were organized by a moderator (A. Grantham) from the Leukodystrophy Care Network.

The key questions addressed in these recommendations include:

1. How does the inclusion of psychosine in KD testing algorithms help stratify babies into a diagnosis of EIKD vs at risk of LOKD?
2. What clinical follow-up and testing methods should be employed for children at risk for LOKD, including the associated urgency of follow-up?

As these key questions were discussed by the group, specific recommendations for action items, such as urgency of referral, and types of diagnostic tests required were brought up by group members based on clinical experience. However, the group did not set out with an agenda of specific sub-questions which could have introduced bias for or against pre-proposed algorithms. The consensus process was iterative. When there was significant disagreement on any point, one of the authors (RTS) revised the recommendations based on group feedback and subsequently brought it back for reconsideration. Eventually, over 90% agreement was reached on all points. After consensus was achieved, the resulting newly proposed recommendations were

assessed with a historical cohort of 47 newborns from New York. Patients in the cohort were originally classified to be at moderate or high risk for LOKD according to guidelines at the time of testing based on GALC activity and genotype [32]. Two authors (RTS, JO) independently reviewed de-identified patient data and reclassified risk based on the new recommendations. A meeting of the larger group was held to review the data and resolve discrepancies, and consensus was reached. Previous guidelines and our updated recommendations were then compared regarding risk classification and the subsequent clinical testing recommended over the first 2 years of life.

3. Results

3.1. Question 1: How does the inclusion of psychosine in KD testing algorithms help stratify babies into a diagnosis of EIKD vs at risk of LOKD?

Psy is increasingly used by NBS labs to confirm that a child has a positive KD screen, but it can also be used by clinicians to further classify patients as EIKD or being at risk for LOKD. In the case of low GALC activity, we consider DBS Psy concentrations ≥ 2 nmol/L to be abnormal [21,26]. Values ≥ 10 nmol/L are consistent with EIKD and should trigger immediate referral for evaluation and consideration of disease modifying treatment (e.g. HSCT) [5–7,26]. Infants with intermediate NBS Psy values (≥ 2 to < 10 nmol/L) should be referred for specialty evaluation within 2–4 weeks of birth. Psy ≥ 2 nmol/L in the setting of low GALC activity has 100% specificity for the diagnosis of KD [21], however given the small number set, it is difficult to make an exact and reliable assessment for LOKD cases. While the use of Psy in NBS protocols can help minimize false positives, not all NBS programs include Psy as part of their testing [25,26]. In order to evaluate their risk for EIKD or LOKD, the follow up of infants referred for KD-positive NBS should include: (1) measurement of GALC activity in leukocytes, (2) blood Psy level, and (3) GALC genotyping (including sequence analysis and targeted analysis for the 30 kb and 7 kb deletions) [21,25]. Parental genotyping should also be done, when possible, to help establish if identified variants are in *cis* or *trans*. Additionally, congruence has been achieved among 3 clinical laboratories performing Psy analysis, and it is important that any lab offering Psy analysis make every effort to achieve comparable results and participate in inter-laboratory assessments until a proficiency program is offered by the College of American Pathologists, the Centers for Disease Control and Prevention, or other relevant providers. As such, the authors suggest that the test be carried out by a laboratory that has achieved congruence to ensure valid meaning and comparability of the disease ranges referenced by Guenzel, Escolar, and Herbst et al. [21,22,26].

Infants identified by NBS with possible KD should enter one of three clinical follow-up pathways based on the above listed testing (Table 1, Fig. 1):

1. Early infantile Krabbe Disease (EIKD): requires immediate counseling of family and referral for disease modifying treatment (e.g. HSCT)
2. At-risk for Late Onset Krabbe Disease (LOKD): requires long term follow-up and monitoring, but not immediate referral as a neonate for treatment
3. Unaffected: either enzyme-lowering polymorphisms (pseudodeficiency) or heterozygote, where no follow-up is required

3.1.1. Early Infantile Krabbe Disease (EIKD)

The details of EIKD diagnosis and management are reviewed in detail elsewhere [25]. In summary, infants with NBS results including reduced GALC activity and high DBS Psy (≥ 10 nmol/L) must be placed in this category and referred immediately for disease modifying treatment if families consent to further evaluation and treatment [21,25]. The current standard of care indicates that HSCT should be initiated before 30 days of life to achieve the best outcomes [5–7]. NBS programs and relevant regional institutions should be prepared with processes already in

Table 1
Criteria for classification of newborn screen positive patients.

Early Infantile Krabbe Disease	
Requires the following criteria:	
1. Leukocyte GALC enzyme assay in Krabbe disease range [23,35]	
2. DBS or erythrocyte Psychosine in the high range ^a [21,27]	
Late Onset Krabbe Disease – criteria for risk stratification	
High Risk for onset of LOKD in early childhood	Low Risk for onset of LOKD in early childhood
Requires the following criteria:	
1. Leukocyte GALC enzyme assay in KD range	1. Leukocyte GALC enzyme assay in KD range
2. DBS Psychosine in intermediate range ^b	2. DBS Psychosine in intermediate range ^b
3. Genotype suspicious for KD with potential onset in early childhood	3. Genotype appears low risk for early onset KD and/or associated with adolescent or adult onset disease
Unaffected ^c	
Requires the following criteria:	
1. Leukocyte GALC enzyme assay above the range reported in affected patients	
2. Normal DBS psychosine level	

^a At the time of publication, we consider the high range of DBS Psy to be ≥ 10 nmol/L, but this value may change as more data become available or with future alterations in the assay.

^b At the time of publication, we consider the intermediate range of DBS Psy to be ≥ 2 nmol/L and < 10 nmol/L, but these values may change as more data become available or with future alterations in the assay.

^c If genotype was performed and is concerning for LOKD, assign risk group as “Low Risk for LOKD”.

place for rapid identification and referral so that referral can occur in parallel with confirmatory testing and not wait until genotyping is complete.

3.1.2. At risk for Late Onset Krabbe Disease (LOKD)

Patients in this category have low GALC activity and an intermediate DBS Psy concentration (≥ 2 to < 10 nmol/L). Infants determined to be at risk for LOKD are further split into a high-risk follow-up or low-risk follow-up pathway (Table 1). The high-risk pathway includes individuals with a severe genotype that could cause the onset of KD in early childhood (i.e. late infancy through the first decade of life). The low-risk pathway includes individuals with a genotype that could cause KD, but it is less likely to start in early childhood. Given the importance of early diagnosis of symptomatic LOKD for possible disease modifying therapy (e.g. HSCT), all patients at risk for LOKD should be seen within 2–4 weeks by a specialist or by their primary care provider in consultation with a Krabbe specialist for initial confirmatory testing and placement into the proper follow up pathway.

3.1.2.1. High-risk follow-up pathway. Infants at high risk for developing LOKD in early childhood have DBS Psy in the intermediate range and a severe GALC genotype that could be consistent with EIKD. A suspicious genotype would include bi-allelic GALC variants that had either been previously associated with EIKD or predicted to be pathogenic (e.g., frameshift variants, in-frame deletions, premature stop codons and splice-site variants). The most common pathogenic variant is a 30-kb deletion starting at intron 10 (of the 17-exon gene) and extending beyond the end of the gene [33].

3.1.2.2. Low-risk follow-up pathway. Infants should be placed in the low risk follow-up pathway if they have reduced leukocyte GALC enzyme activity with DBS Psy in the intermediate range, but the genotype appears unlikely to cause onset of KD in early childhood (e.g. variants most consistent with adolescent/adult onset) [4]. A low risk genotype

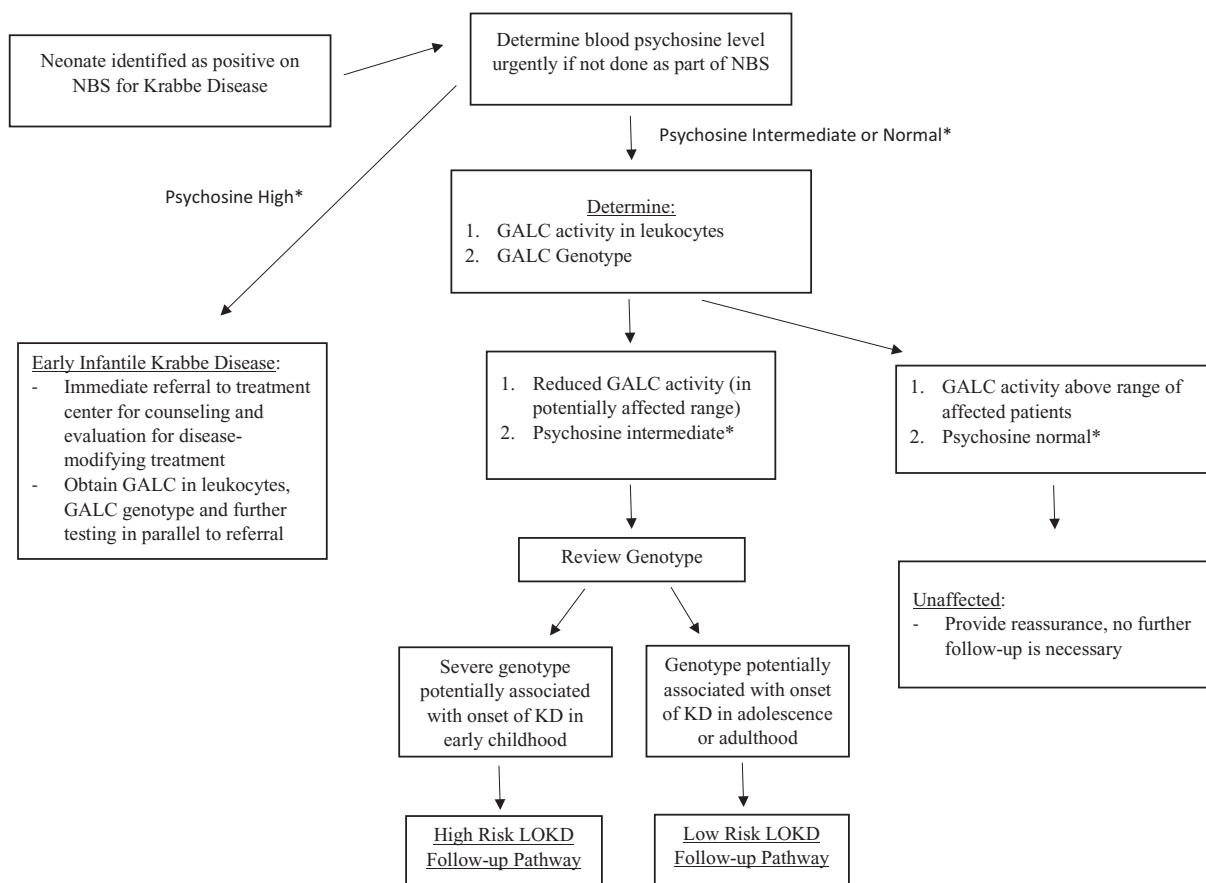


Fig. 1. Algorithm for classification and follow up of newborns who screen positive for Krabbe disease. *At the time of publication, we recommend the following interpretation of psychosine values on dried blood spot, but they may change as more data become available or with future alterations in the assay: High: ≥ 10 nmol/L, Intermediate: ≥ 2 nmol/L and < 10 nmol/L, Normal: < 2 nmol/L.

might include a variant known to be associated with adult onset disease or a variant classified as of uncertain significance, but seen in multiple other screen-positive patients who did not develop disease in childhood (e.g. NM_000153.3:c.956A>G). Consultation with the Krabbe Disease NBS Council could be considered for help in interpreting genetic results. Contact information will be available to all NBS labs testing for KD and through the Leukodystrophy Care Network to aid in web-based case submission for the group's review.

3.1.3. Unaffected

Some infants may screen positive when NBS relies on GALC activity without Psy as a second tier test [3]. These infants may have a positive screen due to (1) enzyme lowering polymorphisms (pseudodeficiency alleles), (2) being heterozygous for pathogenic variants, possibly in combination with enzyme lowering polymorphisms, or (3) inadequate DBS specimen processing. Such infants will have a normal DBS Psy (< 2 nmol/L), and typically a leukocyte GALC level above the range reported in affected patients. For those patients satisfying these two criteria, further testing is not necessary, and no follow-up is required. Reassurance and genetic counseling should be provided to the infant's family that the patient is not at risk for developing disease and the potential risk for future pregnancies. When a patient has a combination of normal Psy, but leukocyte GALC level in the affected range and concerning genotype results, the approach is variable. This situation would only arise in a state that incorporates genotype into the NBS and positively results cases with normal Psy. We recommend that follow-up testing be considered in this circumstance, and that the patient be seen by a provider with expertise in KD.

3.2. Question 2: What clinical follow-up and testing methods should be employed for children at risk for LOKD, including the associated urgency of follow-up?

Testing should aim to identify patients in the very earliest stages of disease in order to enable prompt referral for evaluation for disease modifying treatment, and balance this with the possibility that a given child may not develop disease until later in adulthood.

3.2.1. High-risk clinical follow-up pathway

Since speed of disease progression slows with advancing age of onset [2], early monitoring must be more rigorous and frequent in infants and young children than in older children. Infants identified to be at high-risk for LOKD must be monitored closely over the first 2 years as disease manifestations can arise quickly with a short window for potential treatment. The Krabbe Disease NBS Council recommends that follow-up occur with a physician with expertise in leukodystrophy care (typically a child neurologist, neurodevelopmental pediatrician or geneticist) in addition to routine primary care follow-up. Visits with the specialist should occur every 2–3 months for the first 24 months, every 6 months until age 3, then annually until 12 years of age. Following this, clinical follow-up should occur every 2–5 years until adulthood. At each visit, a comprehensive history and physical examination should be performed, and the family should be instructed on how to monitor for signs and symptoms (Table 2). For high-risk infants, symptoms such as colic or reflux, developmental delay, signs of spasticity, seizures or other unexplained neurologic signs could be indicators of progression to symptomatic disease and should be rapidly and thoroughly

Table 2
Signs and symptoms of Krabbe Disease in infants.

Irritability
Reflux
Feeding difficulties
Constipation
Loss of developmental milestones
Motor delay or regression
Muscle tone abnormalities
Seizures
Abnormal visual behavior (tracking or pupillary abnormalities)
Changes in gait (if ambulation achieved)

evaluated. The family should be counseled that symptoms can have an onset after an inciting event (e.g. febrile illness or head trauma) and, if possible, written educational materials should be provided. We do not advise any restrictions to the recommended vaccination schedule. If any early signs or symptoms occur, the patient should be urgently tested (including MRI of the brain, nerve conduction study, blood Psy level), and be considered for urgent referral for disease modifying treatment evaluation.

In the absence of clinical signs or symptoms, we recommend laboratory, neurophysiologic and imaging studies as outlined in Table 3. Within 2 months of diagnosis of high-risk status, the clinician should obtain a brain MRI (with diffusion tensor imaging if possible), nerve conduction studies and blood Psy level. If the results of initial screening tests are equivocal, the clinician could consider obtaining brainstem auditory evoked potentials (BAER) and/or a lumbar puncture with evaluation of CSF protein and CSF Psy. The core screening tests (MRI brain, nerve conduction studies, and blood Psy level) should be performed every 4 months for the first year, followed by every 6 months until 3 years of age. If no clinical symptoms or abnormalities are detected by that time, it is recommended that a brain MRI (with diffusion tensor imaging if possible), as well as blood Psy level be performed annually until 12 years of age. Following this, brain MRI should be performed every 2–5 years until adulthood.

Based on our literature review and experience in patients with EIKD, EEG is less likely to be abnormal in presymptomatic patients. Visual-evoked potentials (VEP) may be added to screening in presymptomatic patients at risk for LOKD, since visual loss can be the only presenting

symptom in a subgroup of patients [34]. BAER are often abnormal in EIKD, and could be considered based on the available evidence. A strong consensus regarding recommending for or against this test was not achieved among the experts in our workgroup.

3.2.2. Low-risk follow-up pathway

Children at lower risk for Krabbe disease are less likely to manifest symptoms in infancy, and thus are less likely to have rapid progression. As such, the Krabbe Disease NBS Council recommends follow-up occur with an individual with expertise in leukodystrophy care every 6 months for the first 24 months, then annually until 12 years of age. Following this, clinical follow-up should occur every 2–5 years until adulthood. At each specialty visit, a comprehensive history and physical examination should be performed and the family should be counseled about monitoring for signs and symptoms (Table 3). If any signs or symptoms occur, the patient should be urgently tested (including MRI head, nerve conduction study, blood Psy level), and considered for referral for disease modifying treatment evaluation.

In the absence of clinical signs or symptoms, we recommend diagnostic screening testing as outlined in Table 3. At 12–18 months of age, the clinician should obtain a baseline brain MRI (with diffusion tensor imaging if possible) along with nerve conduction studies and blood Psy level. Thereafter, brain MRI should be performed every 2–5 years until adulthood. Of note, if the *GALC* genotype is highly suggestive of adolescent or adult onset disease, follow-up testing could be deferred until the second decade of life.

3.3. Comparison between new and previous clinical follow-up recommendations

Initial guidelines for follow-up of at-risk infants published in 2009 recommended a schedule of frequent diagnostic testing in the first 2 years of life and were solely based on *GALC* activity level in leukocytes [32]. Given the difficulty in implementing those guidelines and concerns about the high frequency of invasive testing, we compared our current recommendations to these past guidelines. We reviewed 47 de-identified patients from the New York State NBS database from 2006 to 2019 who had been designated as moderate or high risk using the previous guidelines, but were not diagnosed with EIKD.

Table 3
Recommended clinical follow-up schedules for asymptomatic individuals at-risk for LOKD.

Follow-up pathway for individuals at high-risk for onset of LOKD in early childhood																
	By 2 mo	4 mo	6 mo	8 mo	10 mo	12 mo	14 mo	16 mo & 18 mo	20 mo	22 mo & 24 mo	26 mo	2.5 yrs	3 yrs	Annual until age 12	Every 2–5 yrs from 12–18 yrs	
History	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
PE	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
MRI brain	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
NCS	√	√	√	√	√	√	√	√	√	√	√	√	√	+/-	+/-	
Psy	√	√	√	√	√	√	√	√	√	√	√	√	√	√	+/-	
BAER	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	
LP	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	
VEP	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	
Follow-up pathway for individuals at low-risk for onset of LOKD in early childhood																
	By 6 mo	12 mo	18 mo	24 mo	Annual until age 12	Every 2–5 yrs until age 12	Every 2–5 yrs from 12–18 yrs									
History	√	√	√	√	√	N/A	√									
PE	√	√	√	√	√	N/A	√									
MRI brain				√	√	√	√									
Psychosine				√	√	+/-	+/-									
NCS				√	√	+/-	+/-									
BAER				+/-	+/-	+/-	+/-									
LP				+/-	+/-	+/-	+/-									
VEP				+/-	+/-	+/-	+/-									

PE = physical examination, MRI = magnetic resonance imaging, NCS = nerve conduction study, Psy = psychosine, BAER = brainstem auditory evoked potential, LP = lumbar puncture, VEP = visual evoked potentials.

Table 4

Case examples from New York State's newborn screening database.

Designation	Leukocyte GALC Activity (nmol/h/mg) [35]	Psychosine in DBS (nmol/L) [21,27]	Genotype
Early Infantile Krabbe Disease	0.05	32.2	Homozygous g.30 kb deletion (pathogenic)
High-risk LOKD follow-up pathway	0.05	8.9	Compound heterozygous for: NM_000153.3:c.195G>C (late onset splicing variant previously observed in one 16-year-old affected patient) and NM_000153.3:c.1045C>T (likely pathogenic, but not previously reported)
Low-risk LOKD follow-up pathway	0.15	4.6	Homozygous NM_000153.3:c.956A>G (most commonly associated with adolescent or adult onset)
Unaffected	0.13	0.72	Compound heterozygous for: g.30 kb deletion (pathogenic), homozygous for NM_000153.3:c.550C>T and hemizygous for NM_000153.3:c.1685T>C (both enzyme-lowering polymorphisms)

Leukocyte GALC enzyme levels obtained from the Lysosomal Diseases Testing Laboratory at Jefferson Medical College [35].

Using the 2009 criteria, 36 patients were designated as high risk and 11 as moderate risk. According to those recommendations, during the first 2 years of life, the entire cohort would require each of the following tests 238 times: lumbar puncture, MRI scan, nerve conduction study, and BAER, thus requiring a total of 952 diagnostic tests in the first 2 years [32]. Using our new criteria, 8 patients would have been designated at high-risk for LOKD, 15 patients at low-risk for LOKD, and 24 patients as unaffected. Given these designations, over the first 2 years of life, the cohort would need to undergo 63 MRI scans, 55 nerve conduction studies, and there would be no requirement for lumbar punctures, VEP, or BAER. The entire cohort would have required a total of 118 diagnostic tests, which represents an 88% reduction in invasive testing from the 2009 guideline. The reduction was due to both a decrease in the number of required tests per child, and fewer children undergoing follow-up testing. Table 4 details exemplar cases from the NYS database for each classification.

4. Discussion

These recommendations for risk assessment and recommended follow-up update the previous recommendations by the New York State Krabbe Consortium published in 2009 and 2016 [3,32]. A significant update was deemed necessary due to: 1) the large number of false positive cases identified utilizing existing guidelines, 2) lack of follow-up with current guidelines due to their invasiveness, and 3) improved laboratory techniques that have been developed, notably the addition of Psy, leading to an improved ability to discriminate risk level. Our recommendations strive to clearly identify those neonates at high risk for EIKD who require urgent workup for disease modifying treatment, and then classify the remaining positive screens into high-risk for LOKD, low-risk for LOKD, or unaffected based on GALC enzyme activity, Psy level, and genotype (Fig. 1).

Through expert consensus and literature review, we recommended a follow-up strategy for at risk patients, including frequency of clinical visits and diagnostic testing. Our review of previous patients from the New York State database indicated that the new recommendations would reduce overall testing by 88%, which should improve compliance without significantly increasing the risk of missing early symptomatic patients.

There are some limitations to these recommendations. The majority of the recommendations are derived from expert consensus of the Krabbe Disease NBS Council as there is a dearth of published data that could inform the best strategy for screening and clinically following patients using Psy as a prominent laboratory measure. It will be important to establish a collaborative longitudinal database to track NBS-positive patients and ensure the recommendations are effective, used as intended, and can be updated as new evidence emerges. With our analysis of de-identified patients from New York State, we were only able to report on how our new recommendations would change the early childhood testing strategy. The state NBS program does not formally

track patient outcomes, and thus we cannot report on a final diagnosis in these patients. The complexity of interpreting all NBS data (i.e. GALC level, blood Psy levels, and genotyping) is still high and will be challenging for clinicians less familiar with KD. There will also be some cases with ambiguity around interpretation of data, particularly with regards to whether a specific genotype confers a higher or lower risk for onset of KD in early childhood. There is no current evidence that longitudinal Psy measurements can determine need for transplant, however, we recommended a comprehensive approach of repeated Psy testing as values increasing over time may be an important component to clinical decision-making.

We achieved >90% consensus on all points with the main point of dissent being one author (MLE) who did not agree with the classification of LOKD for late-infantile onset or EIKD for early infantile onset, preferring the term IKD for all infantile onset. All authors agree that patients with psychosine values between 2 and 10 nmol/L are potentially at high risk, and that the number of these cases referred in the newborn period would exceed those who would ultimately need to be transplanted in the first 6 weeks of life. Therefore, authors recommended evaluation within the first 2 months of life, but one author (MLE) felt that all cases with psychosine ≥ 5 nmol/L should be referred to a transplant center.

KD NBS Council members continue to meet on a monthly basis to review and adjudicate results from NBS laboratories and provide input on follow-up of individuals at risk for LOKD. As such, we strongly recommend clinicians bring cases to the Council if further input is desired. The group is currently constructing a web-based submission process for cases with contact information available through NBS labs testing for KD and the Leukodystrophy Care Network.

In conclusion, updated recommendations for classification and follow-up of NBS positive patients have been developed. By following these recommendations, the specificity of NBS for KD can be improved thus minimizing the distress of patients and clinicians caused by ambiguity and unnecessary testing, and improving the success in identifying children with this devastating disease who may benefit from life-saving disease modifying treatment.

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Declaration of competing interest

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