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Review

Krabbe Disease–To Add or Not to Newborn Screening?

Shibani Kanungo [1, †, *], Samantha A. Vergano [2, †], Thomas Clark [1, †], Rami Madani [1, †], Melissa Schott [1, †], Kira Couch [1, †], Rubie Villela [1, †], Natalie White [2, †]

1. Western Michigan University Homer Stryker MD School of Medicine; Kalamazoo, Michigan, USA; E-Mails: Shibani.Kanungo@wmed.edu; thomas.clark@wmed.edu; rami.madani@wmed.edu; Melissa.schott@wmed.edu; kira.couch@wmed.edu; rubie.villela@wmed.edu
2. Children's Hospital of The King's Daughters, Norfolk, Virginia, USA; E-Mail: samantha.vergano@chkd.org; natalie.white@wmed.edu

† These authors contributed equally to this work.

* **Correspondence:** Shibani Kanungo; E-Mail: Shibani.Kanungo@wmed.edu

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Abstract

Krabbe disease (KD), a severe neurodegenerative disorder, has been controversial in the space of newborn screening (NBS) in the United States. Families continue to advocate for the addition of KD to the Recommended Uniform Screening Panel (RUSP) after being declined for the second time in February 2023. Even with significant progress in KD screening tests, uncertainty about the phenotypic presentations, and effectiveness of hematopoietic stem cell transplant (HSCT) seems to have impeded the addition of this condition to the RUSP. Potential in-utero onset of symptoms in early infantile onset Krabbe disease (EIKD) raise questions on the 'pre-symptomatic' requirement of NBS. This paper reviews the current knowledge of KD, including accepted and investigational treatments to help further the discussion for adding KD on NBS panels.

Keywords
Krabbe disease (KD); early-infantile onset Krabbe disease (EIKD); newborn screening (NBS); GALC; psychosine (Psy); recommended uniform screening panel (RUSP); hematopoietic stem cell transplant (HSCT)

1. Introduction

Krabbe disease (KD) is a rare, neurodegenerative leukodystrophy caused by pathogenic variants in the *GALC* gene and the accumulation of galactolipids such as galactosylceramide and galactosylsphingosine in the brain, resulting in devastating neurologic consequences. Two main types of KD have been recognized: *the early infantile form* (EIKD or IKD) onset within the first year of life, which results in the loss of developmental milestones starting around 4-6 months of age and leading to spasticity, seizures, and ultimately death; and *late-onset KD*, with onset after a year of age and with variable outcomes. Newborn screening for KD has been implemented in 11 states (NY, MO, KY, OH, TN, IL, IN, NJ, PA, GA, and SC) but it has not yet been added to the Recommended Uniform Screening Panel (RUSP). Hematopoietic stem-cell transplant (HSCT) is currently the only treatment for KD, but for EIKD, it must be performed very early on (<30 days of age) and has variable outcomes [1].

2. Genetics & Epidemiology

KD is an autosomal recessive lysosomal storage disorder, with an estimated incidence of one in every 100,000 births [1]. This occurs due to a deficiency in galactosylceramide beta-galactosidase aka galactocerebrosidase (GALC) enzyme, which leads to galactosylceramide and toxic galactosylsphingosine aka psychosine accumulation resulting in decreased neural myelination [2]. The vast majority are EIKD. As late-onset KD can present with variable symptoms, the diagnosis may be delayed, and prevalence may be underestimated. The *GALC* gene, located on chromosome 14q31, is responsible for the GALC enzyme activity and breaks down galactolipids, particularly in those nervous system cells responsible for myelin production. Thus any *GALC* gene mutation leads to GALC enzyme deficiency with subsequent buildup of these galactolipids, which results in the neurologic symptoms. New York, the first state to implement KD newborn screening, screened nearly 2 million infants from 2006-2016. Of those, 346 were flagged with abnormal NBS for KD. Ultimately, 5 infants were diagnosed with EIKD and 9 were determined to be 'high-risk' but not EIKD [3].

The estimated incidence in 2006 was thought to be approximately 1 in 100,000 births [4]. While in 2017, the overall incidence of IKD in Illinois was found to be 1 in 250,000 [5], and 1 in 55,161 in Kentucky respectively [6]. However, a retrospective analysis identified 98 patients ages 0-3 between October 1, 2015, and December 31, 2020, and estimated a birth incidence of about 1 in 310,000 live births [7]. Pediatric Health Information System (PHIS) and Kids' Inpatient Database (KID) data sources indicated a disease burden of 736 hospital visits, with each patient averaging about 2.6 hospitalizations and a length of stay of 33 days [7]. These numbers may not be fully representative

of all racial and ethnic minorities, as milder cases may not have been hospitalized or picked up on screening, or symptom onset may have presented later.

The exact prevalence is difficult to estimate (See Table 1) due to lack of every state implementing KD screening [7]. Genomic allele frequency predictions estimate an incidence of 1 in 12,080 live births, which was approximately 8.3 times higher than the estimated incidence in 2006. This study using genomic allele frequency predictions examined all GALC alleles and determined that some variants showed milder effects [8]. On the other hand, newborn screening data in New York estimates an incidence of 1 in 394,000 [9], which is closer to the numbers reported by the retrospective analysis. Additionally, there have been differences reported among some ethnic groups. Two separate inbred communities in Israel reported 1 in 100-150 live births [10], whereas Japan only reported to 2 in 1,000,000 live births [11].

Table 1 Epidemiology of Krabbe Disease.

Country/ region/ethnic group	Incidence	Carrier Frequency	Reference
United States	1 in 310,679 (birth prevalence)	-	Ghabash, et al (2021) [7]
United States (New York and Missouri)	1 in 500,000 (infantile Krabbe)	-	Orsini, et al (2016) [1]
Jerusalem (Muslim Villages)	-	1 in 14 ~ 7.3%	Ezer, et al (2022) [12]
North Israel Druze community and Muslim villages	1 in 100 to 150 live births	1 in 6	Rafi, et al (1996) [10]
Czech Republic	1 in 249,935 (birth prevalence)	1 in 12,997	Poupetová, et al (2010) [13]
Sweden	1 in 39,000 live births	-	Hult, et al (2014) [14]
The Netherlands	1.35 per 100,000 (birth prevalence)	-	Poorthuis, et al (1999) [15]
Hong Kong	-	1 in 48	Chan, et al (2021) [16]
Europe	1 in 100,000 live births	-	Jain, et al (2023) [17]
Northern Finland	1.1 per 100,000 live births	-	Knuutinen, et al (2021) [18]

3. Clinical Signs of Krabbe

Clinically, infants with EIKD may present normally at birth, but begin to show neurologic symptoms after several months. They may begin to demonstrate severe irritability and crying and may develop signs of hypertonia, sometimes being diagnosed with cerebral palsy. Spasticity continues to worsen, and neurologic deterioration occurs, as does deafness, blindness, and eventual death. Death usually occurs around 2 years of age but may occur much earlier due to risk factors

such as aspiration pneumonia. With supportive care, some individuals may survive longer. Late-onset KD may appear after a year of age and symptoms are variable, including loss of milestones, seizures, gait changes, vision changes, or behavioral abnormalities. Individuals who have a late-onset form that manifests in adolescence or adulthood may demonstrate weakness, gait changes, paresthesia, and cognitive deterioration. Some individuals may survive into the 7th decade of life [1].

Infants who are picked up on NBS may be asymptomatic, or may have some subtle neurologic signs, such as clonus or difficulty feeding, and may already have changes on brain MRI making it challenging to determine disease onset and utility of newborn screening [19].

4. Krabbe Subtypes

4.1 Infantile

Among the different subtypes, Infantile Krabbe Disease (IKD) is the most common at approximately 85% of cases, and the majority of IKD is classified as Early-Infantile Krabbe Disease (EIKD). IKD is also considered the most severe subtype, with rapid neural decline accompanied by a life expectancy of around 2 years [19]. HSCT treatment has been shown to be effective in pre-symptomatic patients, while outcomes of post-symptomatic treatment have not shown significant clinical benefit [20]. The aggressive nature and emergent need for the treatment of IKD create a complex environment for successful treatment. An important development in the treatment of IKD came when New York State was the first to add IKD to the NBS conditions' list in 2006 [9]. Continued research provided advancements in the specificity of NBS for IKD over the century that followed and was centered mainly around the biomarker psychosine (also called galactosylsphingosine), with first reported longitudinal data showing that substantially elevated levels were a specific marker for IKD [21] and later on the value of second tier testing in differentiating high risk versus lower risk newborns [22, 23]. Despite such advancements, ethical concerns exists for NBS for IKD on all newborns with the difficulty in accurately predicting phenotype severity in lower risk newborn [24, 25].

Other studies have shown practical or theoretical benefits to patients from the NBS protocols. HSCT treatment by 30 days of life has been established as a potential benchmark for improving functional outcomes in EIKD, which is reliant upon the NBS [26]. A records review of 19 patients who underwent HSCT between 19-61 days of life, and those who received HSCT prior to 30 days of life showed a significant improvement in outcomes related to mobility, communication, and feeding [26]. In comparison of outcomes between asymptomatic and symptomatic infants at the time of umbilical cord blood (UCB) transplantation found that pre-symptomatic patients had 100 percent success in both engraftment and survival of UCB transplantation, while symptomatic patients had 100 percent engraftment but only 43 percent survival of UCB transplantation. All surviving patients experienced normalization of blood GALC enzyme levels. However, only asymptomatic patients showed improved central myelination and developmental gains, including age-appropriate cognitive function and receptive language, with some asymptomatic patients continuing to show varying degrees of developmental delays in expressive language and gross motor function [27]. A study of six cases of HSCT for patients with EIKD identified by NBS protocols found that all patients had successful HSCT at a median of 36 days old (range of 24-40 days). And, at a median of 4 years post-transplant, all were alive with normalized GALC enzyme levels and reduced but not normalized

psychosine levels. One patient was lost to follow-up, but the other five patients all continued to achieve developmental milestones, though delayed compared to age-matched unaffected peers. Gross motor skills showed the greatest developmental deficits, while cognitive and language skills showed better progression [20]. A post-mortem analysis of a 15-year-old patient who underwent USB transplant for IKD at 4 weeks of life showed notably improved myelination of the brain. The largest deficits were noted on the corticospinal tract, which correlated to the patient's clinical picture of gross motor skill deficits. In contrast to the maintained myelination in the central nervous system (CNS), the peripheral nervous system (PNS) showed loss of myelination and ultrastructural inclusions in the Schwann cells – this also matched the clinical presentation of progressive peripheral neuropathy [28].

4.2 Juvenile

Juvenile Krabbe Disease (JKD) is much rarer than IKD, and fewer cases are reported in the literature. A positive effect of treatment with HSCT and UCB transplantation has been described for two brothers. The older brother first showed symptoms of vision loss at 5 years old and was diagnosed at 9 years old. At the time of diagnosis, his symptoms had progressed to include walking difficulties. He underwent UCB transplantation at nine years old. The younger brother was diagnosed at 5 years of age and showed his first symptom of vision loss at 6 years old, at which point he underwent HSCT. Both cases showed a subsequent improvement of laboratory markers, although MRI showed continued worsening of disease until one-year post-transplant for both patients. Clinical outcomes showed correlative improvements as well. Cognitive function has been well-maintained for both patients. The older brother showed improvement in tremors and spastic walking post-transplant, though deficits remain. The younger brother never developed these symptoms, which suggests early treatment is key to improving long-term outcomes [29]. HSCT treatment for JKD has also been shown in two other patients to either slow or stop the progression of the disease [30].

Despite these successes, another case of a previously healthy 3-year-old girl with poor outcomes following HSCT has been described. She initially presented with ataxia but then experienced a rapid symptom progression over a 2-month period, including a loss of ability to walk independently, dysarthria, and facial asymmetry. The patient underwent 5/6 HLA-matched HSCT 2 months later. Despite a successful HSCT, symptoms of JKD worsened with continued loss of expressive language and new development of polyneuropathy and refractory seizures noted at 18 months post-HSCT. Nerve studies completed 7 months post-HSCT showed primarily a demyelinating peripheral polyneuropathy. The patient has maintained hearing and vision and continues to be closely monitored, so it is unknown if long-term outcomes may be improved [31].

While the rapid progression of symptomology is not expected with JKD, there are reports of patients with JKD who express an acute onset with rapid progression of symptoms. Meanwhile, sisters with the same genotype as the 3-year-old girl described above experienced a slow disease course [32].

This seemingly conflicting data highlights the difficulty of predicting phenotypes and outcomes for individual patients and contributes to the complexity of management and treatment, especially in the context of high-risk HSCT being the standard of care.

4.3 Adult

Adult-Onset Krabbe disease (AOKD) is incredibly rare, and therefore less is known about treatment and outcomes. Additionally, due to the less aggressive nature of AOKD and high rates of complications/morbidity associated with HSCT, patients may elect to pursue conservative/supportive treatment options. Timely diagnosis also presents a challenge yet is important for AOKD as it may potentially affect treatment decisions. In one case, diagnosis of AOKD took 13 years from initial symptom onset, and the patient ultimately elected supportive measures [33].

Despite the extensive challenges, HSCT has been reported to greatly improve outcomes for one patient with AOKD. The first AOKD-associated symptoms presented in the patient's 20's, and progressed to spasticity, ataxia, dysarthria, and pseudobulbar disorder. The patient received a lower-intensity bone marrow conditioning regimen, and subsequently underwent HSCT with a 10/10 HLA-matched sibling donor. At 4 years post-HSCT, the disease was stabilized with no evidence of new progression or demyelination. Additionally, at 7.5 years post-HSCT there were improvements seen in gait, dysarthria, and pseudobulbar affect [34].

5. Evaluation of Krabbe

With variable presentation and onset of KD, diagnosis typically requires high clinical suspicion from pediatric physicians [1], that is followed up with referral to biochemical geneticists for evaluation and management. Recently, however, eleven states have adopted a GALC enzyme screening test through tandem mass spectrometry in newborn dried blood spots (DBS) as part of the states' NBS program to evaluate for KD [35]. This method allows for effective follow-up of a positive screen with an algorithm that can help stratify a patient's risk for KD and identify a likely subtype requiring transplantation. At this time, further evidence can help determine best screening test approaches, given current prognostic odyssey in predicting phenotype severity in lower risk newborn or later onset KD accurately.

There are many biomarkers and identifiers that can be used to evaluate potential KD cases to provide both diagnostic and prognostic information. Tools such as cerebrospinal fluid (CSF) analysis, nerve conduction studies, and neuroimaging via computer topographic (CT) scan or magnetic resonance imaging (MRI) can be useful to evaluate disease progression and severity [36, 37]. Brain MRI in an affected infant typically identifies areas of inflammatory demyelination as contrast-enhancing regions [38]. Increased T2 signal intensity in deep cerebral white matter, dentate nuclei, and cerebellar white matter are typical areas of the brain affected in the infantile form [17]. In the later onset forms, juvenile and adult-onset, the dentate nuclei and cerebellar white matter are more frequently spared, and the parieto-occipital region and corticospinal tracts show more increased T2 signal intensity [22]. CT scans typically show affected areas as hyperdense due to demyelination. As the disease progresses, these areas become hypodense as the tissues atrophy [17]. Calcifications of the internal capsule and cortical white matter on CT have also been found in cases of KD [39]. Lumbar puncture and subsequent CSF analysis in affected patients usually demonstrate elevated protein levels [36]. A protein value of greater than 61.5mg/dl is associated with a significantly shorter survival period [36]. Additionally, CSF protein is inversely related to the age of onset of KD [36]. Further, nerve conduction studies are useful to look at delayed conduction velocity in the

peripheral motor and sensory nerves, which can be useful clinically to understand disease manifestations and symptom management [40].

Currently, GALC enzyme activity measurement by via Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS) has highest utility in supporting the diagnosis of KD [41], though additional high psychosine levels seems better at suggesting IKD [6] GALC enzyme activity assay can either be performed on DBS, as would be used in the NBS or can be run on a whole blood sample from an individual with suspected KD. Currently, multiple labs across the United States offer to test for KD, including the Lysosomal Diseases Testing Lab at Jefferson University or the Mayo Lab. GALC enzyme levels in white blood cells (lymphocytes) below 0.300 nmol/hour/mg protein is suspicious for KD, while levels below or equal to 0.10 nmol/hour/mg protein stratifies the patient into a much higher risk for having KD [42]. Both LC-MS/MS and fluorometry methods for detecting GALC activity have shown diagnostic success [43]. However, the GALC enzyme assay alone does not have acceptable specificity to suggest KD with possibility of pseudo-deficiencies as seen in DBS screening other lysosomal enzyme defects [5, 44, 45]. Following an abnormal result of GALC activity, a test for psychosine (also called galactosylsphingosine) levels, a toxic metabolite that becomes elevated in GALC-deficient cells, is warranted [46]. This metabolite accumulates within cells and results in the death of the oligodendrocytes, resulting in the clinical manifestations of KD [46]. The acceptable normal reference range for psychosine is <2 nmol/L and a level >10 nmol/L is indicative of IKD [6, 21, 23]. Abnormalities in both GALC activity and psychosine yield higher specificity for KD and will warrant genetic evaluation of the individual [5]. Evaluation of variants of the *GALC* gene via PCR and DNA sequencing of all 17 exons provides individuals with a confirmation of the diagnosis of KD. There is a common 30kb deletion in this gene and when an individual is homozygous for this deletion infantile onset of KD is likely [47]. Those who are compound heterozygotes for the 30kb deletion, and an additional alteration are also likely to have the infantile-onset type [47]. Individuals homozygous for the pGly286Asp variant are more likely to have the adult-onset phenotype [47].

Table 2 provides comparison of some evaluation tools for diagnostic and prognostic information in early-onset versus late-onset KD.

Table 2 Diagnostic and prognostic characteristics in early-onset versus late-onset Krabbe Disease.

Evaluation tool	GALC activity	PSY level	CSF protein	MRI findings
Infantile Krabbe Disease High-risk group	<0.150 nmol/hour/mg protein	>10 nmol/L	≥61.5 mg/dl	Increased T2 signal intensity in deep cerebral white matter, dentate nuclei and cerebellar white matter
Late-onset Krabbe Disease Low-risk group	<0.300 nmol/hour/mg protein	>2 - <10 nmol/L	<61.5 mg/dl	Increased T2 signal intensity in deep cerebral white matter, parieto-occipital region and corticospinal tracts

Considering the molecular advances in the diagnosis of typical and atypical KD, more costly and invasive procedures are often unnecessary diagnostically. Tools such as CSF protein quantification, while a nonspecific finding, can be used for prognosis and as an indicator of treatment success [48]. Additionally, nerve conduction studies and advanced imaging such as MRI or CT may help localize specific areas of demyelination and atrophy throughout the cortical white matter and peripherally to provide information about symptom manifestations and prognosis [37].

6. Metabolic Findings and Diagnosis

The diagnosis of both early and late-onset forms of KD is suspected in individuals who demonstrate deficiency levels of GALC enzyme activity. Psychosine levels are universally elevated (>10 nmol/L) in individuals with EIKD and are used in some states' newborn screening to increase the specificity of testing [5]. Psychosine may not be as elevated in late-onset KD and normal levels cannot be used to reliably exclude the diagnosis. Pseudodeficiencies for GALC exist, in which *in vitro* levels are low but do not affect *in vivo* levels and do not cause disease. Confirmation of the diagnosis is performed with molecular testing; most individuals with EIKD have a homozygous 30kb microdeletion in the *GALC* gene. Carriers may also have low enzymes but not in the range of homozygotes.

Rarely will a clinically affected individual with decreased GALC activity, and with or without increased psychosine levels, have genetic testing that does not show consistency with known pathogenic KD alleles. This situation prompts the evaluation of a similar disease, known commonly as atypical KD and more formally as Saposin A deficiency. Saposin A is a non-enzymatic glycoprotein encoded on the gene *PSAP* and is an activator of the GALC enzyme [49]. *PSAP* encodes multiple sphingolipid activator proteins, including Saposin A [49]. There have been only 3 published cases of this form of atypical Krabbe disease to date [50-52]. Genetic testing for this atypical manifestation of KD requires sequencing of the *PSAP* gene. Identification of a pathological allele on this test confirms the diagnosis of atypical KD and provides an explanation for symptom manifestation and atypical GALC activity and psychosine levels. Clinically, individuals may have features similar to that of early infantile Krabbe disease, with normal development for a period of time and then significant neurologic regression. [50, 51]. Algorithm for evaluating Typical and Atypical Krabbe Disease (Saposin A Deficiency) is shown in Figure 1.

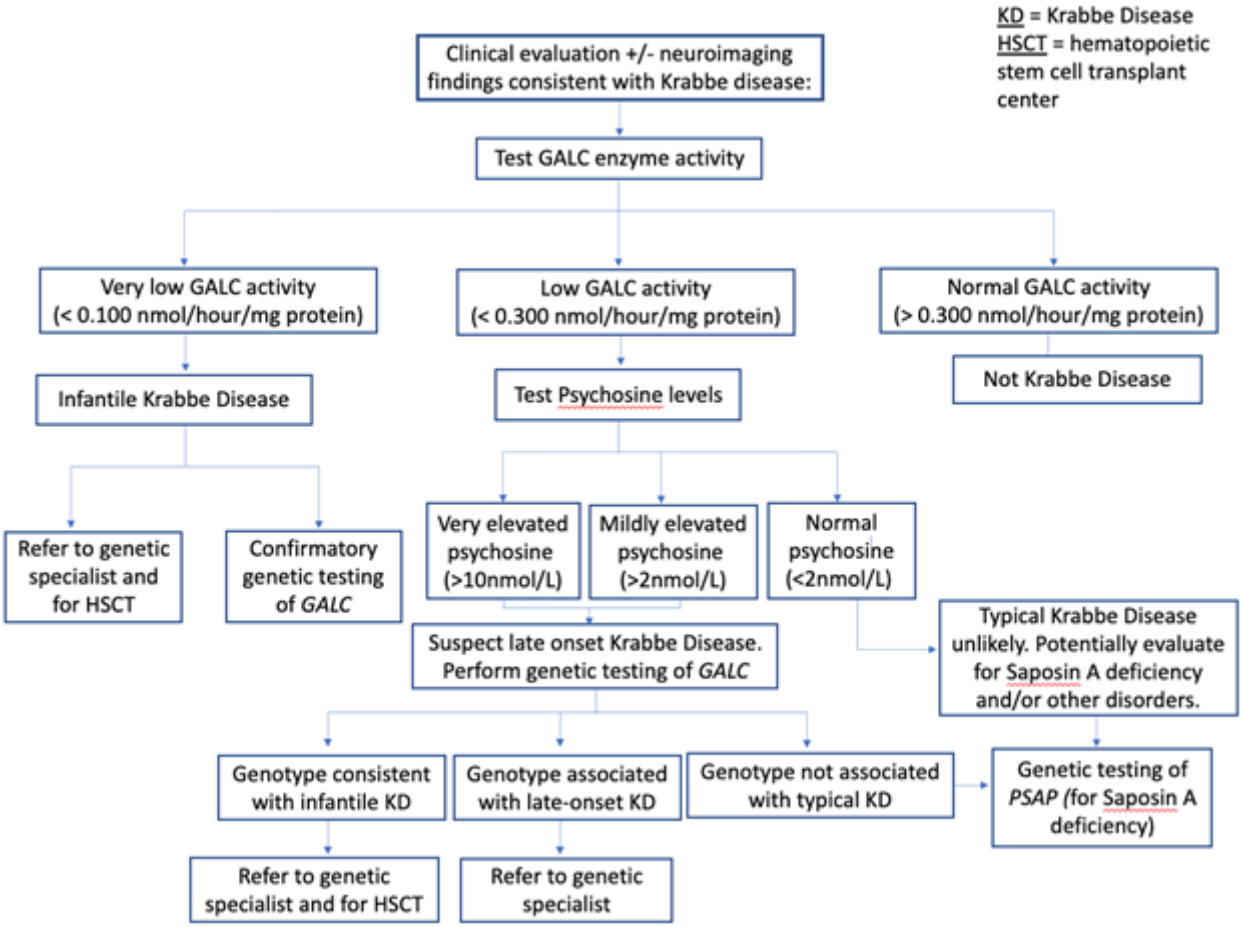

Figure 1 Algorithm for evaluating typical and atypical Krabbe Disease (Saposin A Deficiency).

7. Treatment

Treatment options for EIKD individuals who are already significantly symptomatic are limited and are primarily supportive. HSCT within 30 days of life for the most potential benefit is now recommended for newborns found to be KD positive on newborn screening or are diagnosed prenatally (due to an affected sibling). Several studies have looked at the outcomes of individuals who have received HSCT for EIKD. A review of six newborns with EIKD, detected by NBS, who underwent HSCT over a three-year period (2016-2019) demonstrated some degree of motor and speech delay, though cognitive skills were more developed than gross motor in most children, at a slower rate than their peers [20]. In late-onset KD who had onset between the ages of 6-36 months of age, children who were asymptomatic (5/19) at the time of HSCT had the most positive developmental outcomes, with cognitive development being normal. Gross motor skills were delayed but with gains. Individuals who developed symptoms at less than 12 months of age demonstrated outcomes similar to those who were untreated. At the time of publication, 14/19 individuals were living [19].

In 1972, the “Psychosine Hypothesis” was suggested as the underlying mechanism of disease for KD [53]. Since that time, considerable research efforts have gone into the development of treatment. A number of treatments that are effective for other lysosomal storage diseases seem to

not work for patients with KD. But the combination of multiple therapeutic approaches to address different aspects of KD pathogenesis, including inhibitors of sphingolipid synthesis, enzyme replacement therapy, gene therapy, anti-inflammatories, and antioxidants if administered together has shown promise in animal models [54-56]. This combination of therapies is hypothesized to be beneficial due to the complex biochemical pathways involved in the mechanism of KD, as the deleterious effects likely come from both the root cause as well as secondary pathogenic mechanisms [56].

These treatments largely remain in the preclinical phase due to various limitations. Inhibitors of sphingolipid synthesis reduce psychosine levels but show minimal clinical benefit, and there is a concern for toxicity due to the requirement of sphingolipids during normal brain development [57].

Non-steroidal anti-inflammatories (NSAIDs) have been utilized as a treatment in animal models because inflammation secondary to psychosine accumulation may theoretically lead to cytokine-mediated myelin damage, contributing to KD progression [58]. This approach has generally shown minimal clinical benefit in animal models [57].

Recent advancements in Adeno Associated Vectors (AAV) for gene therapy have shown the most promise in recent years. A positive treatment effect using a single dose of AAVhu68 with a codon-optimized GALC across murine, dog, and macaque trials was demonstrated with improved myelination in both the CNS and PNS, and additionally proved beneficial from a clinical standpoint as none of the test animals developed typical symptoms of KD. The study on Macaques helped confirm a safety profile suitable for humans, and a clinical trial is currently underway [59]. Potential limitations for this gene therapy may revolve around the well-established difficulty in the timeliness of disease diagnosis and the subsequent timing of treatment initiation, as the timing of treatment is a key factor in the current treatment of KD.

8. Krabbe and Newborn Screening

The purpose of newborn screening is to detect debilitating and potentially fatal diseases/ conditions in asymptomatic infants to allow for early, outcome-altering interventions, to prevent long-term morbidity or mortality. Many of these conditions are often as rare as they are devastating, especially the neurodegenerative conditions. Even with easily available accurate testing methodologies or molecular technological advances, screening for all conditions known to clinical medicine can be an attractive concept, though impractical to implement as a public health program at this time. A national experts panel of Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) are tasked with decisions to recommend any conditions to the Secretary Department of Health and Human Services (DHHS) to include on the RUSP. They conduct a systematic evaluation using three main criteria: 1) the net benefit of screening, 2) the readiness of state programs to implement expanded screening, and 3) the feasibility of implementing a comprehensive screening program [60]. While Krabbe disease was not included in RUSP in 2009 after initial review, once again in 2022 a formal evidence review was conducted by ACHDNC experts' panel for RUSP nomination, and declined once again in February 2023 with the DHHS Secretary's decision to not move KD forward for consideration to the RUSP [61, 62]. However, the KD community are advocating to have these review results re-interpreted. Here, we offer an independent evaluation of KD NBS based on the aforementioned standards.

There are currently eleven state programs that started to offer KD newborn screening: New York, Missouri, Kentucky, Ohio, Tennessee, Illinois, Indiana, New Jersey, Pennsylvania, Georgia and South Carolina. Most of these utilize tandem mass spectrometry (MS/MS) to measure GALC enzyme activity in dried blood spots (DBS). The natural substrate of GALC is a galactosylceramide, which is catabolized to galactose and a ceramide. The MS/MS assay involves incubating the DBS punch with a synthetic analog of a galactosylceramide with an eight-carbon fatty acyl chain and measuring the amount of ceramide generated in the sample compared to the internal control. In the initial study, five Krabbe patients were found to have significantly lower GALC activity than 16 healthy individuals and seven patients with other lysosomal storage disorders [63]. There have been multiple iterations of this method since its conception, and the latest is a 6-plex assay that will test for Krabbe, Gaucher, Niemann-Pick-A/B, Pompe, Fabry, and MPS-I [1]. The state of Missouri, also measures GALC activity but with fluorometry. By itself, though, reduced GALC activity is not specific to KD. An overlap of GALC activity levels has been found among those with KD, carriers of *GALC* pathogenic variants, and healthy subjects with GALC pseudo-deficiency, which is a genotype that causes in vitro GALC deficiency without manifesting in vivo. In the first years of newborn screening in New York, only 8% of infants with positive screens and confirmatory GALC activity testing developed early infantile KD [64]. And requires a second-tier testing, which was previously recommended to be *GALC* genotyping or specific testing for the most common pathogenic 30kb deletion. Though, recently DBS psychosine concentration has gained prominence as a specific biomarker for 'high-risk' IKD [4, 5, 21-23, 45]. When utilized in conjunction with genotyping, psychosine measurement allows for timely and accurate identification of EIKD those who would most benefit from prompt HSCT. However, psychosine testing is available in only four private lab and every state program lacks the in-state infrastructure and in-state readiness to test psychosine on DBS as a second-tier screening on all newborns. There is paucity of a clear genotype-phenotype correlation to help predict if and when an individual may develop clinically significant KD [4], creating prognostic odyssey. This incongruence has posed as an argument against newborn screening for KD altogether. Table 3 provides a comparison of current available screening mechanisms of Krabbe disease.

Table 3 Comparison of screening mechanisms of Krabbe Disease.

Screening Tool (Tier)	Advantages	Disadvantages
GALC activity assay (1)	-Simple test -Can be combined with other lysosomal storage diseases in multiplex assay	-Low GALC activity has low positive predictive value for KD -Causes many healthy children to undergo further unnecessary testing
Psychosine concentration (2)	-Simple test -Strong association between elevation and active KD	-Requires MS/MS with higher sensitivity than other screening tests -testing available in only 4 private labs

GALC genotyping (2)	-Differentiates those at highest risk from carriers and those with less concerning mutations -useful with psychosine levels	-Protocol beyond capability of many state laboratories -May detect pathogenic variants with uncertain clinical correlate, creating prognostic dilemma
30kbDel testing (2)	-Rapid test -Homozygosity indicates KD	-Rare variant -Strong possibility of both false positives (carrier status) and false negatives (other pathogenic variant)

While the first KD case was reported a century ago, there is still no definitive cure for this disease. HSCT only slows the progression at best and yields better outcomes when initiated in pre-symptomatic children [64]. Early onset of neurodevelopmental symptoms at 0-3 months of age in 14% (n = 16) of infants in a large prospective natural history study raises potential role of pediatricians in prompt KD diagnosis treatment management [65]. Thus, early and effective newborn screening and diagnosis can be crucial to improve survival in patients. In 2006, New York State initiated screening for all newborns. This proved to be a challenge because neither the enzyme level activity nor genotype was a reliable predictor of severity [64]. Nonetheless, they became the first to implement NBS via the GALC enzyme assay using DBS. Their outcomes, however, showed that even with identification by newborn screening, survival heavily depended on hematopoietic stem cell transplant occurring early enough. Recent collaborative study noting timely treatment interventions at four HSCT centers (major academic center hospitals in states offering KD NBS), evaluating 6 IKD cases identified through NBS, found that all neurodevelopmental milestone were >1SD below mean than unaffected peers and noted that HSCT itself is associated with developmental delays [20], raising concerns of effectiveness of currently available treatment. The hope is that the process of the NBS, referral, and the treatment can be done in a timely manner to improve survival and long term outcomes.

9. Conclusion: Future of Krabbe on NBS

The complexity of predicting affected from unaffected individuals for KD adds to the difficulty in implementing the disease into NBS. To date, prognostic odyssey on interventions on every KD positive screen exists. Role of psychosine in identifying a life threatening disease in asymptomatic newborns can be a very important initial part of the tenet or purpose of NBS process. But, we cannot overlook the subsequent equally important role of timely intervention and treatment implementation, to prevent long-term morbidity and mortality towards healthy productive quality of life of every newborn. There is potential for improvement in the future, but with our current knowledge, the literature seems still investigational [6]. Potential prenatal onset of KD affecting myelination/brain development in some newborns means some of infants born with EIKD were never asymptomatic [19]. And is contrary to the underlying tenet of NBS as a public health program in its entirety and burdens all newborns and public health infrastructure disproportionately. Without an effective prenatal screening option, for KD and other neurodegenerative diseases, it

becomes more difficult to justify these conditions as a population-based public health program [25]. Current limitations in federal and state funding raise questions about health care equity and ethical challenges for some of the existing RUSP conditions requiring HSCT (unpublished personal sources). Thus, beneficial pre-requisites for justification for any neurodegenerative conditions screening in future, should include a stable infrastructure for screening each condition in every state, short result turnaround time, availability of sensitive and specific laboratory tests, equitable and effective follow-up and treatment centers with availability of multi-disciplinary expertise in every state, and adequate federal and state funding for the NBS program [25]. Current contentions about asymptomatic status in all newborns with a positive KD NBS, along with prognostic odyssey in lower risk or late onset KD, and limited evidence that interventions prevent adverse long-term morbidity affecting quality of life may be moot over time with further research or evidence. These current concerns compounded by uncertainty of timely and equitable access to healthcare by all newborns, and potential for overt medicalization of a condition risks undue burden on families, healthcare systems and public health programs like NBS and need to be addressed prior to addition of any new condition to RUSP.

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Author Contributions

Shibani Kanungo (SK): Concept, design, method, writing of complete manuscript, critical review for important intellectual content. Samantha A. Vergano (SAV): Equal contribution of manuscript writing, critical review for important intellectual content. Thomas Clark (TC): Equal contribution of manuscript writing. Rami Madani (RM): Equal contribution of manuscript writing. Melissa Schott (MS): Equal contribution of manuscript writing. Kira Couch (KC): Equal contribution of manuscript writing. Rubie Villela (RV): Equal contribution of manuscript writing. Natalie White (NW): Equal contribution of manuscript writing.

Competing Interests

SAV is a member of the Commonwealth of Virginia Rare Disease Newborn Blood Spot Advisory Committee. SAV received a one-time honorarium as a non-voting member of ACHDNC technical workgroup. SK, TC, NW, RM, MS, KC, RV have declared that no competing interests exist.

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