EVIDENCE REVIEW: Krabbe Disease

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MATERNAL AND CHILD HEALTH BUREAU

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*****Appendices C, D and E available upon request.

i. Abbreviations used

ACHDNC	Advisory Committee on Heritable Disorders in Newborns and Children
BAEP	Brain-stem auditory evoked potential (also known as **BAER**, brain-stem auditory evoked response)
BMT	Bone marrow transplant
CBT	Cord blood transplant
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebral spinal fluid
CT	Computed tomography
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
EIKD	Early infantile Krabbe disease
ERG	Evidence review group
FDA	Federal Drug Administration
GALC	Galactosylceramidase
GLD	Globoid Cell Leukodystrophy
GVHD	Graft versus host disease
HSCT	Hematopoietic stem cell transplant
KD	Krabbe disease
LIKD	Late infantile Krabbe disease
LOKD	Late onset Krabbe disease
LSD	Lysosomal storage disease
MeSH	National Library of Medicine medical subject heading
MRI	Magnetic Resonance Imaging
MS/MS	Tandem mass spectrometry
NCS	Nerve conduction studies
NCV	Nerve conduction velocity
VEP	Visual evoked potential

I. Introduction

Krabbe disease (OMIM #245200) is an inborn error of lipid metabolism associated with mutations in the galactosylceramidase (GALC) gene, which is located on the long arm of chromosome 14 (14q31). Krabbe disease is a lysosomal storage disease (LSD) inherited in a classic autosomal recessive manner. Mutations in the GALC gene can cause a deficiency of the hydrolytic enzyme galactocerebrosidase (Wenger et al. 2000), which is responsible for the degradation of certain galactolipids, including galactosylceramide (gal-cer), psychosine (galactosylsphingosine), and monogalactosyldiglyceride (MGD) (Wenger et al. 2000). The inability to degrade these galactolipids, which are found almost exclusively in the myelin sheath, causes abnormal accumulation of galactosylceramide and psychosine, resulting in the death of myelin-producing oligodendrocytes, myelin breakdown, and the presence of Krabbe disease's histologic hallmark: globoid cells (Suzuki 2003). Globoid cells are macrophages, often multi-nucleated, accumulating myelin fragments and undigested galactosylceramide, and are frequently found clustered around blood vessels and abundant in the region of active demyelination (Wenger et al. 2000, Suzuki 2003). Nearly all progressive damage occurs in the white matter of the peripheral and central nervous systems, leading to a rapidly fatal course for untreated infants. Krabbe disease is also referred to as globoid cell leukodystrophy (GLD) for its distinguishing attribute.

In 1916, Danish neurologist Knud Krabbe first described infantile Krabbe disease in two siblings with spasticity who died in infancy and were found to have "diffuse sclerosis" of the brain (Krabbe 1916). A broad range of ages at symptom onset have been documented since the original description of Krabbe disease, leading to four main clinical sub-types distinguished by age of symptom onset: early infantile, late infantile, juvenile and adult (Suzuki 2003, Escolar et al. 2005). Over 60 disease-causing mutations have been identified in the GALC gene (Wenger et al. 2001). Only homozygosity for one specific mutation (a 30-kb deletion that eliminates the entire coding region for one of the enzyme subunits and 15% of the coding region for the other subunit) has been found to be strongly predictive of infantile Krabbe disease (Wenger et al. 2000).

Across all types of Krabbe disease, the average incidence based on data collected prior to the implementation of newborn screening is approximately 1/100,000 in the United States and Europe (Wenger 2008, Wenger et al. 2001). Data from these studies suggest that most (85-90%) of those with Krabbe disease have the infantile form presenting with extreme irritability, spasticity, and developmental delay before age six months (Wenger et al. 2000, Wenger 2008).

Patients with early infantile Krabbe disease (EIKD) present with extreme irritability, spasticity, and developmental delay before six months of age. These children will enter a decerebrate state in early infancy with most dying before two years of age (Wenger et al. 2000). Hematopoietic stem cell transplant (HSCT), often through cord blood transplant (CBT) is the only currently available treatment. HSCT prior to the development of symptoms is believed to be important to maximize treatment outcomes

(Suzuki 2003, Escolar et al. 2005). Although family history leads to early detection of some infants, most infants with Krabbe disease are not detected until they develop clinical symptoms and irreversible neurologic damage (Escolar et al. 2005, Weinberg 2005). The potential value of presymptomatic HSCT has led to a search for methods for population-based newborn screening.

II. Case definition

In order to identify asymptomatic infants with Krabbe disease through newborn screening it is necessary to have a reliable approach to rapid diagnosis in children one month of age or younger. At this age, globoid cells, the pathologic hallmark of Krabbe disease, may not yet be present. Interpretation of genetic testing results is problematic because of the heterogeneity of mutations associated with Krabbe disease and the lack of clear genotype-phenotype correlations.

For this report, we based our case definition on the one used by the New York State screening program. This case definition was based on a consensus of expert opinion. The New York State screening program (Duffner et al. 2009) considers an individual with galactocerebrosidase activity < 0.5 nmol/h/mg protein in peripheral blood leukocytes to be potentially affected. Individuals are then further subdivided by galactocerebrosidase level into high risk (≤ 0.15 nmol/h/mg protein), moderate risk (0.16- < 0.30 nmol/h/mg protein), and low risk (0.3-0.5 nmol/h/mg protein). This risk stratification is used to determine the frequency of evaluation. To be referred for HSCT, children have to receive a certain score based on a combination of neurologic exam findings, magnetic resonance imaging (MRI), cerebral spinal fluid (CSF) protein levels, nerve conduction velocity (NCV), brainstem auditory evoked potentials (BAEP), and visual evoked potentials (VEP). Points are given for abnormal findings as follows: neurological exam (2 points), MRI (2), increased CSF protein (2), NCS (1), BAEP (1), VER (1) and genotyping results of homozygous 30-kb deletion (4). A total score of greater than or equal to 4 indicates the patient may be considered for transplant. Alternatively, individuals who are found to have the 30-kb deletion mutation in both alleles of the GALC gene are referred directly for HSCT. We expect that this case definition will evolve as more data are collected from the New York State screening program.

III. Rationale for review

The Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) has directed the Evidence Review Group (ERG) to produce this report for the nominated condition of Krabbe disease. Krabbe disease has been nominated for the following reasons:

1. Without treatment, individuals with early or late infantile Krabbe disease have significant morbidity and die by six years of age (Wenger et al. 2001, Escolar et al. 2005).

2. HSCT before the onset of symptoms may decrease the morbidity and mortality associated with infantile Krabbe disease (Escolar et al. 2005).
3. Methods to screen infants for Krabbe disease have been developed. New York State began newborn screening for Krabbe disease in August 2006.

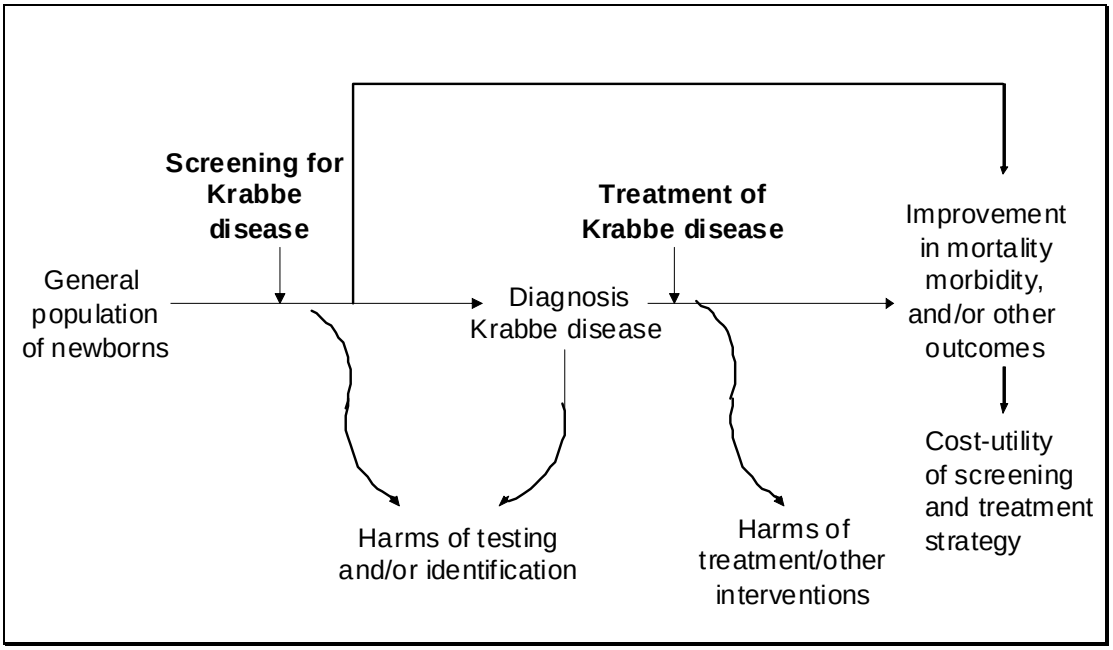
IV. Objectives

The objective of this review is to provide information to the ACHDNC to guide recommendations regarding screening newborns for Krabbe disease. Specifically, the ERG's goal was to summarize the evidence available from published studies, and the critical unpublished data available from key investigators and experts in the field.

V. Conceptual framework

The conceptual framework below (Figure 1) illustrates our approach to evaluating the evidence regarding the potential benefits and harms of newborn screening for infantile onset Krabbe disease. Our goals are (1) to assess the effectiveness of screening and (2) to assess the impact of treatment for those identified through newborn screening versus those identified later through clinical diagnosis.

Figure 1 - Conceptual framework



VI. Statement of main questions

We sought to answer the following questions, with a particular emphasis on the questions related to screening and the potential benefits of early treatment.

A. Natural History and Diagnosis:
   i. Is the condition well-defined?
   ii. What are the prevalence and incidence of the condition and its variations?
   iii. What is the natural history, including the spectrum of severity, of the condition and are there clinically important phenotypic or genotypic variations?

B. Screening Test:
   i. What methods exist to screen newborns for the condition? How accurate are those methods? Do they distinguish between infantile/juvenile and late onset? What are their sensitivity, specificity and analytic validity?
   ii. Do current screening tests effectively and efficiently identify cases of the condition that may benefit from early identification?
   iii. What are the potential harms or risks associated with screening?
   iv. What pilot testing has there been—population-based or other? Which populations have been screened? What have the results shown regarding the sensitivity and specificity of the screening test?

C. Diagnostic Test:
   i. What methods exist to diagnose individuals with positive screens?

D. Treatment:
   i. What treatment options and interventions exist for affected children? Is treatment for affected children standardized, widely available and/or FDA approved?
   ii. Does presymptomatic or early symptomatic intervention in newborns or infants with the condition improve health outcomes? What benefit does treatment, particularly presymptomatic, confer? What is the relationship between treatment outcomes and the timing of treatment intervention? In other words, does identification prior to clinical detection allow for better outcomes?
   iii. What are the potential harms or risks associated with treatment?

E. Economic Evaluation:
   i. What are the incremental costs associated with the screening test for newborn screening programs? What is the cost-effectiveness of newborn screening for the condition?
   ii. What are the costs associated with diagnosis, and the failure to diagnose in the presymptomatic period?
   iii. What is the availability of treatment and what are the costs associated with treatment?

VII. Literature review methods

For this report, we conducted a systematic evidence review. We searched MEDLINE for all relevant studies published over the 20 year period from January 1988 to November 2008. We completed searches combining the National Library of Medicine Medical Subject Heading (MeSH) “Leukodystrophy, Globoid Cell” and the keywords “Krabbe’s Disease” and “Krabbe disease” in an effort to capture all articles written about the disease over this time period. In order to capture articles that have not yet been assigned MeSH terms, we also searched the following keywords within the OVID In-Process and Other Non-Indexed Citations database: Leukodystrophy, Globoid Cell, Krabbe disease and Krabbe’s disease. The search was limited to human studies and

English language publications. This search strategy yielded 316 articles, and captured all references included on the nomination form submitted to the ACHDNC.

Two investigators (authors ARK and AAK) reviewed all abstracts to select articles for inclusion in the review. Articles were eliminated if they were: not human studies; did not focus on infantile onset Krabbe disease; did not address at least one key question; reviews or editorials that did not include new data; case reports or case-series of four or fewer subjects unless there were novel data not available in other larger studies (Appendix B). After abstract review, 72 articles were reviewed in full. After this process, 24 articles met all inclusion criteria and were included in this evidence review. The process was repeated in late March 2009 and again in July 2009 to capture articles published since the initial November 2008 search. The March 2009 search yielded six abstracts, from which three articles were included in this review. The July 2009 search yielded eight abstracts, of which two were included in this review.

The 29 selected manuscripts were divided in half for independent data abstraction by the two investigators. A 20% subset was selected for data abstraction by each investigator to validate the process. Each article was evaluated, using standardized tools, for the quality of the study design (NHS Center for Reviews and Dissemination March 2001, Accessed: October 17, 2008) and the quality of the evidence, as it relates to the category of evidence (Pandor et al. 2004, Pollitt et al. 1997). A given article received only one rating per reader for study design, but may have received multiple quality evaluations for the type of evidence. For example, a study that discusses prevalence and natural history would be evaluated for the quality of the evidence in each of those domains. There were no significant differences in the data extracted by the reviewers.

Table 1 - Study design for abstracted articles

Study Design	Number of Articles
Experimental intervention	0
Cohort study	1
Case-control study	4
Case series	15
Sample size ≤ 10	5
Sample size 11 to 50	7
Sample size ≥ 51	3
Economic Evaluation (from Drummond)	0
Cross-Sectional study	9
Total studies	29

To assure completeness and clarity of the report, a draft of the report was sent to an independent external review panel (see Appendix C for sample conflict of interest form). The report was revised based on their suggestions.

VIII. Methods for interviews with experts

The ERG and the ACHDNC recognize that in a rapidly developing field such as newborn screening for Krabbe disease there may be important but unpublished data. We identified experts, including researchers and Krabbe disease newborn screening advocates, to help us identify this information. These individuals were identified as authors of key papers included in the literature review, through discussions with content experts, and through recommendations from the ERG. These individuals are listed in Table 2.

Experts were sent a letter via e-mail (Appendix D for researchers, Appendix E for advocates) explaining the purpose of the review, a conflict of interest form (Appendix C) and an open-ended survey. Experts had one week to respond. The project coordinator sent a reminder e-mail to those who did not reply. In cases where clarifications were needed regarding the responses, individuals were either sent a follow-up e-mail or contacted via telephone by a member of the ERG (ARK, AAK, or JP). Information from survey responses is provided in this report when the experts and advocates provide information regarding the key questions that are not otherwise available from the selected articles.

Table 2 - List of experts contacted and degree of participation

Name		Title	Replied	Completed written survey	Telephone interview
Georgianne	Arnold, MD	Director of Inherited Metabolic Disorders Clinic, Department of Pediatrics and Genetics, Associate Professor, University of Rochester School of Medicine and Dentistry, Rochester, New York		✓	
Scott	Baker, MD, MS	Director, Fred Hutchinson Cancer Research Center Survivorship Program, Seattle Cancer Care Alliance, Seattle, WA, Professor of Pediatrics, University of Washington, Seattle, WA	✓^		
Susan	Berry, MD	Professor & Director, Division of Genetics & Metabolism, Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota	✓^		
Paula	Brazeal	President, United Leukodystrophy Foundation	✓#		
Barbara	Burton, MD	Member of the Division of Genetics, Birth Defects and Metabolism at Children's Memorial Hospital in Chicago, Director of PKU Program and Professor of Pediatrics, Northwestern University's Feinberg School of Medicine, Chicago, Illinois		✓	
Michele	Caggana, ScD	Director, Newborn Screening Program, New York State Department of Health, New York		✓	✓
Victor	De Jesus, PhD	Newborn Screening and Molecular Biology Branch Centers for Disease Control and Prevention Atlanta, Georgia		✓	

Name		Title	Replied	Completed written survey	Telephone interview
Patricia	Duffner, MD	Clinical Director, Hunter James Kelly Research Institute, Professor of Neurology and Pediatrics, Children's Hospital, Buffalo, New York		✓	✓
Florian	Eichler, MD	Assistant Professor of Neurology, Harvard Medical School, Director of the Leukodystrophy Clinic at the Massachusetts General Hospital (MGH), Boston, Massachusetts		✓	
Maria	Escolar, MD	Director, Neurodevelopmental Function in Rare Disorders (NFRD), Carolina Institute for Developmental Disabilities, University of North Carolina, Chapel Hill, North Carolina	✓		✓
Bob & Sonja	Evanosky	Evanosky Foundation	✓#		
Michael	Gelb, PhD	Harry and Catherine Jaynne Boand Professor of Chemistry, Departments of Chemistry and Biochemistry, University of Washington, Seattle, Washington	✓^		
George	Hoganson, MD	Associate Professor of Pediatrics, Head, Division of Genetics, Director, Biochemical Genetics Laboratory University of Illinois at Chicago Medical School, Chicago, Illinois			
Rhona	Jack, PhD	Division Head, Clinical Chemistry, Associate director of biochemical genetics laboratory, Clinical Associate Professor of Laboratory Medicine, Children's Hospital and Regional Medical Center, Seattle, Washington	✓^		
David	Jinks, PhD	Newborn Screening Laboratory Director Division of Laboratories Illinois Dept. of Public Health, Chicago, Illinois	✓*		
Joan	Keutzer, PhD	Vice-President of Scientific Affairs Genzyme Corporation, Cambridge, Massachusetts	✓#		
Edwin	Kolodny, MD	Bernard A. and Charlotte Marden Professor of Neurology and Chairman, New York University, New York, New York	✓#		
Kim	Kubilus	Director of member services, National Tay-Sachs and Allied Diseases Association	✓^		
Joanne	Kurtzberg, MD	Director, Duke Pediatric Blood & Marrow Transplant Program, Chapel Hill, North Carolina		✓	✓
Jennifer	Kwon, MD	Pediatric Neurologist and Associate Professor of Neurology and Pediatrics, University of Rochester Medical Center, Rochester, New York		✓	
Joe	Orsini, PhD	New York Department of Health, Wadsworth Center, Albany, New York		✓	✓
Lawrence	Shapiro, MD	Director of Regional Medical Genetics Center, Professor of Pediatrics and Pathology, New York Medical College, Valhalla, New York	✓^		
Jakub	Tolar, MD	Assistant Professor of Pediatrics, Division of Hematology-Oncology and Blood and Marrow Transplantation, University of Minnesota, Minneapolis, MN		✓	✓
Jacque	Waggoner	CEO of Hunter's Hope Foundation		✓	

Name		Title	Replied	Completed written survey	Telephone interview
Melissa	Wasserstein, MD	Director of the Program for Inherited Metabolic Diseases (PIMD), Associate Professor of Genetics, Genomic Sciences and Pediatrics, Mount Sinai Medical Center, New York, New York			
Kenneth	Weinberg, MD	Anne T. and Robert M. Bass Professor in Pediatric Cancer and Blood Diseases, Division Chief, Division of Stem Cell Transplantation, Stanford Institute for Cancer/Stem Cell Biology and Medicine, Stanford School of Medicine, Stanford, CA			
David	Wenger, PhD	Director, Lysosomal Diseases Testing Laboratory, Neurology Department, Thomas Jefferson University Hospital, Philadelphia, Pennsylvania	✓		✓

* Unable to contribute due to internal policy reasons
# Unable to contribute due to time constraints
^ Deferred to other experts

IX. Results: evidence findings to address the main questions

This section presents the evidence from the included articles organized by key question. Each subsection includes a summary of findings from the literature review, an assessment of the quality of the evidence from each included article, and additional information from the Krabbe disease experts.

A. **Natural history and diagnosis:**

Table 3 - Quality assessment of abstracted literature pertaining to condition

Type of evidence	Number of articles
Incidence (cases per 100,000), average within the U.S.	**4**
Data obtained from whole-population screening or comprehensive national surveys of clinically detected cases.	1
Ia. As in I but more limited in geographical coverage or methodology.	3
Extrapolated from class I data for non-U.S. populations.	0
Estimated from number of cases clinically diagnosed in U.S.	0
Genotype-Phenotype correlation	**8**
Data from retrospective screening studies in U.S. or similar population.	0
Data from systematic studies other than whole population screening.	2
Estimated from the known clinical features of the condition as described for individual cases or short series.	6
Other natural history of disease	**8**

Adapted from Pandor et al. 2004, Pollitt et al. 1997

We sought to answer the following questions on the natural history and diagnosis of Krabbe disease through a literature review and information provided by experts:

i. Is the condition well-defined?
ii. What are the prevalence and incidence of the condition and its variations?

iii. What is the natural history, including the spectrum of severity, of the condition and are there clinically important phenotypic or genotypic variations?

Literature review:
Estimates of the birth incidence of all types of Krabbe disease based on large population case ascertainments in Europe and Japan ranges from about 0.6 – 2 per 100,000 (Heim et al. 1997, Poorthuis et al. 1999). However, the rate has been reported to be higher in some populations (e.g., a specific Arab population) (Korn-Lubetzki, Nevo 2003).

Of the approximately 550,000 newborns screened as of June 30, 2008 (Duffner et al. 2009) in New York, four high-risk children have been identified (0.73 per 100,000), of whom two met criteria for infantile Krabbe disease (0.36 per 100,000) (Duffner et al. 2009). The other two children have not met the case definition criteria by 16 and 8 months of age and are developmentally normal. Six moderate-risk (1.09 per 100,000) and 15 low-risk newborns (2.72 per 100,000) have been identified, none of whom met their case definition criteria.

EIKD is associated with profound neurological impact leading to death. The neurologic effects can be seen as white matter changes on MRI and prolonged or absent BAEP and VEP. There is poor genotype-phenotype correlation (Tatsumi et al. 1995) other than homozygosity of the 30-kb deletion, which is strongly predictive of EIKD. Low levels of galactocerebrosidase activity (Duffner et al. 2009) do not entirely predict the age of symptom onset or severity of white matter changes. There are ongoing efforts being made to develop methods for determining the degree of neurologic involvement prior to symptom development. In EIKD patients, Husain et al. (2004) found that abnormal nerve conduction studies (NCS) were seen first, followed by BAEP, electroencephalogram (EEG) and eventually VEP abnormalities. Their findings illustrated peripheral nervous system involvement very early in EIKD disease process, even before central nervous system (CNS) involvement and onset of symptoms. Additionally, Aldosari et al. (2004) studied BAEP and VEP, and found abnormal BAEP is among the first indications of EIKD onset, and may precede clinical symptoms. Barone et al. 1996 used computed tomography (CT) scans and found high density areas as an early and specific finding in EIKD patients. They also found through imaging studies that EIKD patients displayed cerebellar atrophy, appearing during the first year of life.

Most recently, Escolar et al. (2009) performed diffusion tensor imaging (DTI) studies to identify early markers of Krabbe disease onset in the motor tracts of neonates with EIKD. DTI with quantitative tractography is a method for assessing myelination patterns of the motor tracts. Fractional anisotropy (FA) allows for a quantitative and reproducible analysis of the motor tract images (McGraw et al. 2005). Six neonates with Krabbe disease, diagnosed because of family history or through the New York State screening program, were first evaluated and imaged between one and three weeks of age. Five of the neonates had relatively normal neurological findings and one of the neonates exhibited mild neurological symptoms (clonus in one lower extremity). Compared to 45 unaffected neonates, the images from the neonates with Krabbe showed a more

immature pattern of myelination in the first month of life. All six neonates with EIKD underwent HSCT. Two of the neonates died from complications of the HSCT procedure. The four remaining infants were evaluated again at six, nine, 12 and 24 months of age. At the time of publication, they were all within the age-appropriate ranges for cognitive, receptive language and fine motor development; however, three of the four had gross motor delays at the last follow-up visit. The findings show that DTI can detect differences in corticospinal tracts of neonates with Krabbe disease presymptomatically. Additionally, among the affected children in follow-up, those with higher FA values performed better in standardized assessments.

The most comprehensive description of the natural history of Krabbe disease comes from a description of 334 families abstracted from the Hunter's Hope Krabbe Family Database (Duffner, Jalal & Carter 2009). This database began collecting data on children with Krabbe disease in 1997 through family self-report. Subjects who underwent HSCT were excluded. Information regarding age of symptom onset was available for 114 children in the database. Among them, 71% presented with symptoms at or before six months, 19% between seven and 12 months, 6% between 13 and 24 months, 3% between 25 and 36 months, and 1% at five years. The most common presenting symptom was crying or irritability. The average time to diagnosis was approximately five months (n=103). Eleven children were not diagnosed until after death or the diagnosis of an affected sibling. The average survival was associated with the age of onset of symptoms, ranging from 24 months for those with symptoms that developed at or before 6 months compared to 89 months for those with symptoms that developed between 7 and 12 months.

Expert information:
Experts responding to our survey corroborated the literature findings. They agreed that prior to the New York State pilot screening experience, the incidence of Krabbe disease was estimated to be 1 per 100,000 births in the United States and nearly all (90%) were expected to have infantile onset disease.

Dr. Caggana and Dr. Orsini provided updated data regarding the New York State Department of Health Krabbe disease screening program. New York State has screened 769,853 newborns as of June 30, 2009; seven high-risk children have been identified (0.91 per 100,000), of whom two met criteria for EIKD (0.26 per 100,000). Thirteen moderate-risk newborns have been identified (1.69 per 100,000), and 36 low-risk (4.68 per 100,000). None of the screen positive newborns who have not been diagnosed with Krabbe disease have been diagnosed with another lysosomal storage disease. These data demonstrate a lower incidence (0.26 per 100,000) than initially estimated (approximately 0.9 per 100,000) for EIKD.

New York State created consensus-based criteria to determine which infants should be referred for transplant, and created "risk" categories (high, moderate, low). Dr. Caggana and Dr. Orsini state the risk categories are based primarily on the enzyme activity level measured at the diagnostic testing laboratory of Dr. Wenger, in conjunction with their genotyping results. Because there is a wide normal GALC activity range, the New York

screening program assesses any polymorphisms and sequences variants by full gene DNA sequence analysis of the coding and promoter regions and the 5' and 3' intronic sequences along with GALC activity to predict risk level. Dr. Wenger shared that there may be many more mutations that have never been previously identified, the impact of which are unknown. In individuals identified without a family history of Krabbe disease, Dr. Wenger's lab makes predictions of disease onset based upon experience with previously diagnosed individuals having severe and mild mutations and combinations of mutations. Dr. Wenger reports that approximately 45% of patients diagnosed with Krabbe disease have at least one copy of the 30-kb deletion.

Dr. De Jesus agrees that neither enzyme level nor genotype reliably predicts disease course, and Dr. Burton stated that the type of Krabbe disease is usually determined by clinical presentation and especially the age of symptom onset. Experts agreed that unless the child is homozygous for the 30-kb deletion, it is usually unclear what the significance of a given genotype will be since much of the literature consists of single case reports. Expert consensus maintains that genotypes homozygous for the 30-kb deletion are associated with the early infantile phenotype. Dr. Duffner reported more than 75 mutations associated with Krabbe disease exist, with numbers too small at this time to predict clinical course of a child's genotype with certainty.

Dr. Escolar believes a very detailed clinical exam of the baby is the most predictive of developing Krabbe disease; however, exams are not easily reproducible. Dr. Escolar is working to develop better tools for clinicians using MRI and nerve conduction studies, in the hope of developing consistent and standardized ways to detect the disease before children become symptomatic. Dr. Escolar states that her work with MRI to date has shown that even at birth, the corticospinal tracts of children with Krabbe disease look much more affected than age-matched control newborns. She reports that in general, MRIs of newborns with Krabbe disease compared to MRIs of unaffected newborns revealed demyelination of the cortical spinal tracts in the affected newborns. Due to the uncertainty of timing of symptom onset, Dr. Kurtzberg reports that infants diagnosed in-utero (because of a sibling's diagnosis) have been delivered earlier than term to try to start the transplant process as quickly as possible. No evidence was provided on outcomes of babies delivered early.

B. **Screening test:**

Table 4 - Quality assessment of abstracted literature pertaining to screening test

Type of evidence	Number of articles
Overall sensitivity and specificity of screening	**3**
Data obtained from screening programs in U.S. population or similar.	1
Data from systematic studies other than from whole population screening.	0
Estimated from the known biochemistry of the condition.	2
False positive rate	**2**
Data obtained from screening programs in U.S. population or similar.	1
Data from systematic studies other than from whole population screening.	0
Estimated from the known biochemistry of the condition.	1
Repeat specimen rate	**1**
Data obtained from screening programs in U.S. population or similar.	1
Data from systematic studies other than whole population screening.	0
Estimated from the known biochemistry of the condition.	0
Second-tier testing	**1**
Data obtained from screening programs in US population or similar.	1
Data from systematic studies other than whole population screening.	0
Estimated from the known biochemistry of the condition.	0
Other screening test characteristics	**6**

Adapted from Pandor et al. 2004, Pollitt et al. 1997

We sought to answer the following questions on the screening test for Krabbe disease through a literature review and information provided by experts:

i. What methods exist to screen newborns for the condition? How accurate are those methods? Do they distinguish between infantile/juvenile and late onset? What are their sensitivity, specificity and analytic validity?
ii. Do current screening tests effectively and efficiently identify cases of the condition that may benefit from early identification?
iii. What are the potential harms or risks associated with screening?
iv. What pilot testing has there been—population-based or other? Which populations have been screened? What have the results shown regarding the sensitivity and specificity of the screening test?

Literature review:

Newborn screening for Krabbe disease, which is based on determining GALC enzyme activity in dried blood spots (DBS), is possible with tandem mass spectrometry to detect enzyme products (Li et al. 2004a, Li et al. 2004b, Zhang et al. 2008). The New York State screening program, which has screened more than 700,000 newborns, retests the same blood spot if GALC activity is < 20% of the daily mean activity and considers the

result positive if the level is ≤ 8% of the daily mean activity (Duffner et al. 2009). The rationale for these cutoff values was published recently in Orsini et al. (2009). Data have not been reported in the literature regarding the overall number of positive test results or the false positive rate. There is a published report describing the number of infants who have tested positive. We updated those data below with the most recent data provided by the New York screening program.

The New York State screening algorithm cutoff values were developed as a result of a study that measured the GALC activity of 139,000 anonymous newborn DBS in comparison to unaffected and affected controls by MS/MS methodology (Orsini et al. 2009). GALC activities were converted to a percent of the daily mean activity (%DMA) over a seven month testing period. The cutoffs of the New York screening method were established by considering the highest %DMA of the Krabbe positive specimens. Because cross-contamination was thought to contribute to elevated %DMA, the screening method includes retesting samples with GALC activity less than or equal to 20% to minimize false negative results. The authors report that conservative cutoff values were set to minimize false negative results. Krabbe positive samples were compared to known Krabbe carrier samples (from parents and unaffected siblings of Krabbe disease patients) and the ranges of %DMA were found to overlap between the two groups. For this reason, the screening method includes sequence analysis so that only those individuals with a GALC activity in the intermediate range with one or more known or novel mutations are considered a screen positive referral (Orsini et al. 2009) – i.e., those without any mutations are considered screen negative.

Figure 2 - New York State pilot screening program cutoffs, testing algorithm and number of newborns screened (in parentheses) at each stage as of June 2009

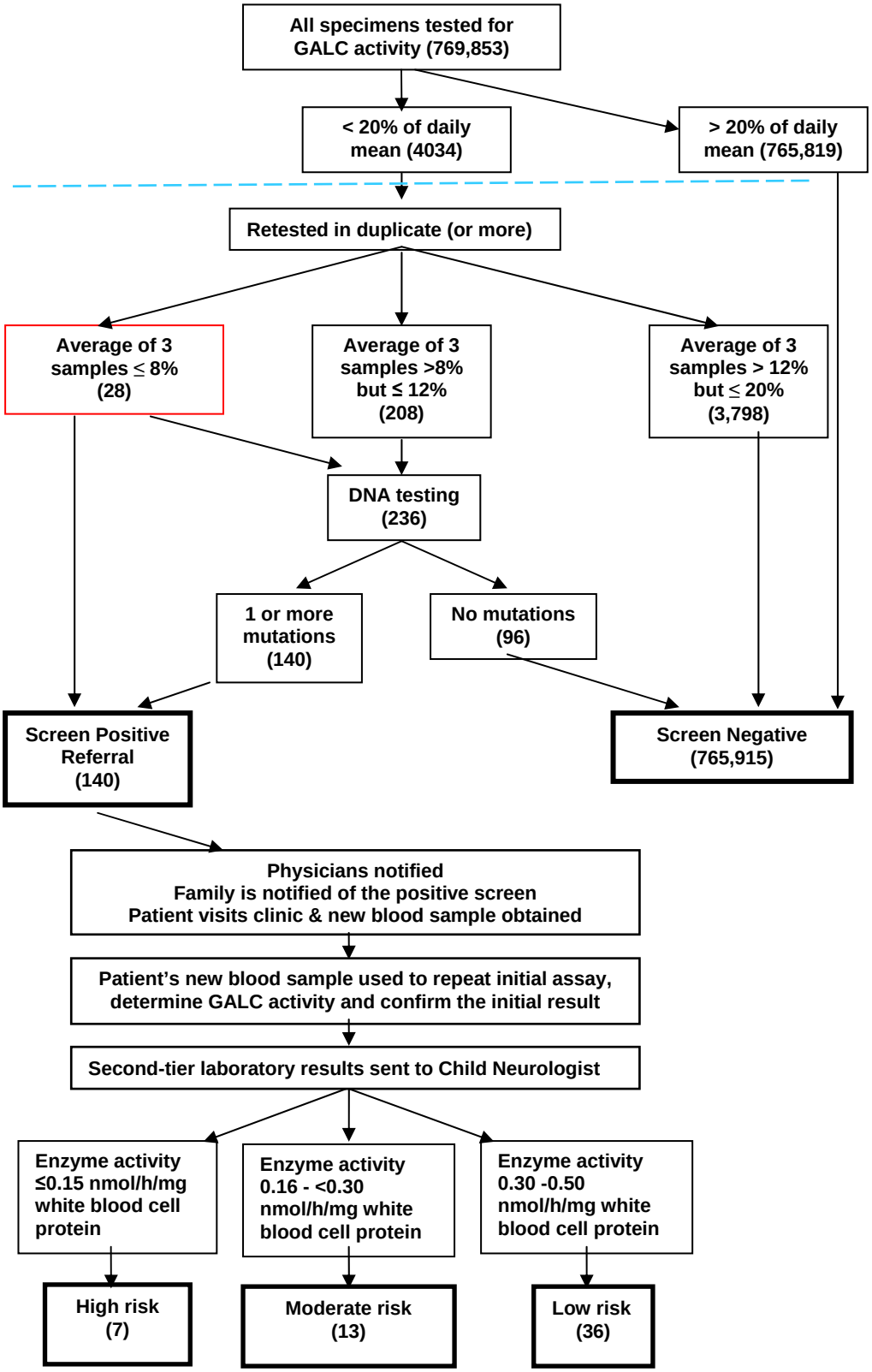


Figure adapted from the Management Guidelines: Krabbe disease published by the Wadsworth Center, New York State Department of Health (http://www.wadsworth.org/newborn/krabbe.htm), Duffner et al. 2009 and data from interviews with Dr. Caggana and Orsini

Expert information:

The most recent New York state pilot screening program information has been provided by Dr. Caggana and Dr. Orsini from the New York State Department of Health. They have tested 769,853 specimens for GALC activity as of June 2009. Those with less than 20% GALC activity are retested in duplicate using at least two new dried blood spot punches from the original sample. To that date, 4,034 specimens had been retested at this stage. Any specimen with less than 8% GALC activity is referred. All specimens with less than or equal to 12% GALC activity detected undergo DNA testing to determine genetic mutations. DNA results are usually available the afternoon after the low GALC activity is determined.

Full gene DNA sequence analysis is completed for all specimens with activities less than or equal to 12% GALC activity to determine the complete genotype. New York State does not refer the newborn for either diagnosis or treatment, if DNA testing indicates only polymorphisms, i.e., benign sequence variations known to not be associated with Krabbe disease. Any specimen with less than 8% GALC activity is considered a screen positive, even if no mutations are detected upon DNA testing. However, Dr. Caggana and Dr. Orsini believe that as more data become available, this protocol will likely be modified to refer any specimen with GALC activity less than or equal to 12% and 1 or 2 mutations identified, in order to minimize false positives. They report this second-tier test reduces their screen positive rate by approximately 40%. With GALC activity level in conjunction with DNA sequencing results, newborns are placed into either the high- or moderate-risk category. Both the newborn screening GALC activity and DNA results are made available to the newborn's physician when they are notified of the screen positive result; typically five days after the lab receives the initial sample. New York State and the Krabbe Consortium have no evidence of any missed cases, no evidence of screen positive newborns being diagnosed definitively with later onset Krabbe disease, and none of the screen positive newborns have been diagnosed with another leukodystrophy.

Table 5 - New York State pilot screening program August 2006 - June 2009*

Activities of pilot screening program	June 2009 data
Total newborns screened	769,853
Screen positive newborns (referred for and completed diagnostic evaluations)	140
High-risk newborns (enzyme activity ≤ 0.15 nmol/h/mg white blood cell protein)	7
Referred for HSCT (Genotype:1 newborn homozygous for 30-kb deletion, 1 newborn compound heterozygous for 30-kb deletion and novel mutation)	2/7
Moderate-risk newborns (enzyme activity 0.16 - <0.30 nmol/h/mg white blood cell protein)	13
Low-risk newborns (enzyme activity 0.30 - 0.50 nmol/h/mg white blood cell protein)	36

*Most recent data provided by Dr. Orsini and Dr. Caggana

Of the seven high-risk cases detected in New York (Table 6), two were considered EIKD and referred for HSCT because of their GALC genotypes and the early signs of neurologic disease. One of these patients was homozygous for the 30-kb deletion mutation, while the other patient was heterozygous for the 30-kb deletion and a novel mutation. Dr. Wenger reports that the five remaining children who screened high risk had genotypes considered to put them at a low risk for early onset of disease. Dr. Caggana and Dr. Orsini state that two of these children were lost to follow-up and three are being followed on a quarterly basis by a neurologist. One of these children is known to be asymptomatic and the other two are assumed to be asymptomatic as Dr. Caggana and Dr. Orsini have not heard otherwise. If the goal of screening is to detect those at moderate or high risk for Krabbe disease, the positive predictive value is 14.3%. If the goal is only to detect those at high risk, the positive predictive value is 5.0%.

Regarding the two high-risk screen positive infants who are not followed up clinically, Drs. Duffner, Caggana and Orsini shared that one is a child whose family returned to their country of origin and the other child's family refused follow-up.

Table 6 - Follow-up of the seven high-risk infants from New York State*

Infant	Birth month	Outcome
1	March 2007	Following up, assumed asymptomatic
2	March 2007	Confirmed EIKD, underwent HSCT
3	July 2007	No follow-up, returned to country of origin
4	August 2008	No follow-up, family refused
5	August 2008	Confirmed EIKD, underwent HSCT, died approximately 11 days posttransplant **
6	November 2008	Following up, asymptomatic
7	December 2008	Following up, assumed asymptomatic

*As described by Dr. Caggana and Orsini
** As described by Dr. Kurtzberg

Dr. Duffner currently follows patients who have screened in the low and moderate risk categories. She says that when these children are asymptomatic, families agree to BAEP, NCS, and other testing that does not require anesthesia. She reports that no one performs tests such as MRI that require anesthesia on low- and moderate-risk children unless there is a particular concern, since it is difficult for families of otherwise healthy appearing children to agree to participate in exams needing anesthesia.

Hunter's Hope Foundation has compiled a database with over 400 Krabbe-affected families sharing their personal experiences with their child's clinical course. When parents with an affected child were asked whether they think newborns should be screened for Krabbe disease and why, many stated their frustrations with initial wrong diagnoses or too late diagnosis, thereby missing the treatment window prior to their child's onset of severe symptoms. Parents state that learning of the diagnosis earlier, through newborn screening, would have decreased the lengthy search for a diagnosis, which families report was a painful experience. They believe that learning of the diagnosis through newborn screening would also provide the choice of a treatment option. One family that tested positive through the New York state pilot screening program contributed to the database. This newborn and mother were both found to be carriers through the newborn screening process.

Dr. Caggana and Dr. Orsini share that New York State uses full gene sequencing to distinguish affected newborns from carrier newborns. They explained the difficulty in cases where a novel mutation is detected, and it is difficult to determine whether it is a "disease-causing" mutation or a common polymorphism. They also test both parents (if possible) and examine their genotypes to determine mutations present only in the newborn. New York State has sequenced over 200 specimens with normal activity to acquire data on common polymorphisms, giving them more information than what is currently reported in the literature. DNA results from New York State show all newborns in the high, and all newborns but one in the moderate risk categories have two variants and multiple polymorphisms in the GALC gene. Some of the mutations found are known to be "disease-causing," while others are novel mutations. Predictions of phenotype,

which can be difficult, are based on the presence of a frameshift, type of amino acid change, and location in the gene.

Dr. Kwon adds that she cannot predict which, if any, of the moderate- and low-risk patients will become symptomatic. She suspects some of these patients are carriers, but the program will continue to follow them. Dr. Arnold agrees and adds that they cannot predict which, if any, of the asymptomatic infants will develop Krabbe disease in their lifetime, particularly among the moderate- and low-risk infants having two variants. She appreciates the dilemma created by the identification of novel variants. She notes that the number of infants having two variants (known or novel) is many times higher than the predicted incidence of Krabbe disease and thus late onset Krabbe disease (LOKD) is either underdiagnosed or these patients will not become symptomatic in their lifetime. She adds that because of this uncertainty, physicians are unable to provide prognostic information to parents of asymptomatic patients at the present time.

Dr. Duffner has created a registry to track the outcomes of each child who screens positive for Krabbe disease in New York State, in addition to an international Krabbe disease registry. Dr. Duffner reports that as of July 2009, of the 60 New York children who have screened low to high risk, 22 are followed by her registry. Participation in the Krabbe disease registry and outcome study varies between follow-up centers. Follow-up data is not consistently captured from all of the screen positive children, even among those in the low and moderate risk ranges that are being followed clinically. Dr. Duffner also described a proposed follow-up study of Krabbe disease screen positive children based on a model for studying quality of life in infants with medulloblastomas. This model uses telephone-based interviews with parents and has the potential to be independent of the child's clinical follow-up visits.

Dr. De Jesus stated the Centers for Disease Control and Prevention (CDC) has established a quality assurance program for LSD newborn screening, including Krabbe disease (De Jesus et al. 2009). This effort was created in response to New York's Krabbe disease newborn screening activities. Dr. De Jesus also informed us that the State of Illinois has mandated newborn screening for five LSDs, including Krabbe disease, for which the CDC provides reagents. Dr. Burton states the newborn screening program has not yet been initiated in Illinois, and Dr. De Jesus estimated that the pilot testing program in Illinois will commence in late 2010. In addition, Missouri has pending legislation to mandate newborn screening for five LSDs in its newborn screening panel.

C. **Diagnostic test**

We sought to answer the following question on a diagnostic test for Krabbe disease through a literature review and information provided by experts:

i. What methods exist to diagnose individuals with positive screens?

Literature review:

Diagnosis is based on GALC activity with either supportive genetic analysis (i.e., homozygous for 30-kb deletion) or clinical findings (Duffner et al. 2009). Approximately

nine laboratories in the country offer GALC enzyme testing (University of Washington, 2009). Close follow-up is necessary because of the variable phenotypic expression of Krabbe disease associated with low GALC activity. Clinical evaluation and follow-up schedule for children who are screen positive for Krabbe disease in New York State depend on the risk category of the positively screened child (Duffner et al. 2009). All risk categories maintain specific intervals of follow-up and children in all risk categories undergo a neurologic examination at each visit. If this examination is abnormal, all patients proceed to have neurodiagnostic studies completed which include MRI, CSF protein and cells, BAEP, VEP, and NCS (Duffner et al. 2009). Children in the high-risk category undergo these exams at the baseline visit and every three months; those in the moderate-risk category only annually or if other concerns arise; and those in the low-risk category only when the neurologic examination is abnormal (Duffner et al. 2009). All patients annually undergo neuro-psychologic testing.

Expert information:
Experts responding to our survey corroborated the literature findings.

Dr. Caggana and Dr. Orsini report that measurement of GALC activity in lymphocytes is most commonly used to diagnose patients. This diagnostic test cannot accurately phenotype Krabbe patients, thus if the activity is low, a combination of diagnostic tests described by Duffner et al. (2009) (in literature section above) is performed to phenotype the patients. They shared that one issue concerning the diagnosis is that there is a limited, but growing, body of reference data for newborns for the panel of tests used (NCS, BAEP, VEP, MRI).

Dr. Kwon shares that when there is referral to her metabolic center, they send a confirmatory enzyme level to Dr. David Wenger's lab as a part of the New York State pilot screening program. They also send blood for HLA typing at the same time to expedite tissue matching in the event that the child should need a rapid transplant. In addition, they repeat the heel stick on a fresh screening card. At times, they have had to use arterial puncture or scalp veins to obtain the blood. Samples are also obtained from the parents to confirm the DNA findings. Dr. Kwon adds that it is challenging to diagnose any young infant with only subtle neurologic abnormalities.

Dr. Wenger believes that his laboratory could manage nationwide confirmatory GALC testing for Krabbe disease screen positive samples, based on the volume he receives from New York State (approximately one sample per week). He says he is unsure of the potential number of samples to expect with a nationwide screening program; he is not sure if the burden on his laboratory would be too great if other state programs had higher false positive rates, thereby more samples, than New York. Dr. Wenger has spoken with Illinois concerning their potential screening program.

D. **Treatment:**

Table 7 - Quality assessment of abstracted literature pertaining to treatment

Type of evidence	Number of articles
Effectiveness of treatment	**5**
I. Well-designed RCTs.	0
II-1. Well-designed controlled trials with pseudo randomization or no randomization.	0
II-2. Well-designed cohort studies:	1
A. prospective with concurrent controls	0
B. prospective with historical control	1
C. retrospective with concurrent controls.	0
II-3. Well-designed case-control (retrospective) studies.	1
III. Large differences from comparisons between times and/or places with and without intervention	0
IV. Opinions of respected authorities based on clinical experience, descriptive studies and reports of expert committees.	2
Other treatment characteristics	1

Adapted from Pandor et al. 2004, Pollitt et al. 1997

We sought to answer the following questions on the treatment of Krabbe disease through a literature review and information provided by experts:

i. What treatment options and interventions exist for affected children? Is treatment for affected children standardized, widely available and/or FDA approved?
ii. Does presymptomatic or early symptomatic intervention in newborns or infants with the condition improve health outcomes? What benefit does treatment, particularly presymptomatic, confer? What is the relationship between treatment outcomes and the timing of treatment intervention? In other words, does identification prior to clinical detection allow for better outcomes?
iii. What are the potential harms or risks associated with treatment?

Literature review:

The only currently accepted treatment for Krabbe disease is HSCT, usually accomplished through CBT (Escolar et al. 2005, Siddiqi, Sanders & Massey 2006, Escolar et al. 2006, Gaipa et al. 2003, McGraw et al. 2005). As described, the New York screening program has referred two infants for HSCT. Both received CBT before 28 days of life. One of the infants died during transplantation (Duffner et al. 2009).

Enzyme replacement therapy (ERT) for EIKD has not yet been developed. Shire Human Genetic Therapies has announced a clinical trial for Krabbe disease ERT that is currently in the preclinical phase with a projected launch between 2012 and 2015.

Characteristics used for determining which children receive transplants

A retrospective case series (Escolar et al. 2006) was conducted to develop a staging system to predict outcome after CBT. This study included 26 patients with EIKD and 11 patients with late infantile Krabbe disease (LIKD), of whom 29 were treated (26 with CBT, three with matched related donor bone marrow). The authors created a four-stage system based on groupings of neurologic problems: stage 1 patients appeared to be developing normally but may have had inconclusive neurological findings, stage 2 patients had obvious neurological symptoms, stage 3 patients had signs of moderate to severe neurological involvement and stage 4 patients had advanced disease. Individuals in stages 1 and 2 had 100% survival through HSCT versus those in stages 3 (61.5% survival) and 4 (0% survival). The five stage 3 individuals who died lived 7.5–50 months posttransplant. Follow-up data are available for ten of the stage 3 individuals; 6/10 made no developmental gains over time.

Infants with an older sibling with EIKD may be tested and diagnosed prenatally or at birth based on this family history. Duffner et al. (2009) state that with EIKD there is much less clinical variability within families than in LOKD and that there is certain knowledge to assume that without transplant, an infant diagnosed with EIKD will experience neurological destruction and eventually death (Duffner et al. 2009).

Mortality and morbidity for those transplanted presymptomatically and postsymptomatically (see also: Table 8 & 9 and Expert information)

Data about the effectiveness of HSCT for Krabbe disease are available from outcomes of children who were treated after the development of symptoms compared to outcomes of asymptomatic newborns diagnosed prenatally or soon after birth. Because of the difficulty in establishing the diagnosis of EIKD in asymptomatic newborns and the genotype-phenotype variability, it is possible that the asymptomatic neonates may have had a different disease course than their affected siblings.

One case series (Escolar et al. 2005) identified 11 asymptomatic newborns and 14 symptomatic infants, all of whom underwent CBT. This report compared survival for these two groups and an untreated control group. The untreated control group was made up of 190 individuals from the Hunter's Hope registry. This report did not provide the ages of symptom onset for the control group.

Among the 11 asymptomatic children, six were diagnosed with Krabbe disease prenatally and five shortly after birth because of an affected sibling. This report did not provide the case definition used to diagnose Krabbe disease, the age of symptom onset for the affected sibling and did not provide the children's' genotypes and pre-transplant GALC levels for the asymptomatic newborns. All untreated affected siblings died between approximately 10 and 50 months of age. Nine of the 11 asymptomatic newborns had abnormal NCS before transplantation. Two of the 11 asymptomatic newborns had an abnormal EEG before transplantation. Seven of the 11 asymptomatic newborns had abnormal MRI findings before transplantation. Three of the eight

asymptomatic newborns tested had abnormal VEP before transplantation. Four of the eight asymptomatic newborns tested had abnormal BAEP before transplantation. The median age at CBT was 28 days for the asymptomatic neonates. The 14 symptomatic children were diagnosed between four and nine months of age. The median age at CBT was 236 days for the symptomatic children.

All 11 of the asymptomatic newborns survived for 36 months, the entire period for which data were available for this group at the time of writing. In contrast, only six of the 14 symptomatic infants survived for a median follow-up of 41 months. Death was due to progressive disease (n=4), graft-versus-host disease (GVHD) (n=1), procedural complication (n=1), and infection (n=2). The survival rate among these asymptomatic newborns was greater than both the control group of untreated children (P=0.001) and the symptomatic infants (P=0.01). The survival rate of the symptomatic infants was not statistically different from the control group (P=0.28),

A group of experts convened in July 2008 to address the long-tem outcomes of presymptomatic infants transplanted for EIKD (Duffner et al. 2009). Transplant centers from around the United States and Canada presented 25 cases of presymptomatic transplant for Krabbe disease. The mortality rate from transplant in this cohort was 15%. Of the children who survived, none have died of progressive Krabbe disease (with the oldest among them at 13 years of age) as compared to the average lifespan of children with untreated EIKD, which is 23 months (however children living beyond eight years has been reported). HSCT appears to attenuate the disease, but over time most children have developed slowly progressive spasticity, leading to eventual inability to walk without assistive devices, somatic growth failure, expressive language deficits and poor brain growth (Duffner et al. 2009).

Table 8 - Abstracted literature regarding early transplant morbidity

Study	Population	Patient Survival Outcomes
Escolar et al. 2005 & 2006* (USA)	Asymptomatic children: 2005:11 patients diagnosed prenatally or at birth because of an affected sibling 2006: 11 Stage 1 patients (appear to be developing normally but may have inconclusive neurological findings) Symptomatic children: 2005:14 patients diagnosed between 4 and 9 months of age 2006: 4 Stage 2 patients 13 Stage 3 patients 1 Stage 4 patient	2005: • Donor cell engraftment was 100% for both infants and newborns • Transplants prior to symptom onset maintained progressive central myelination, maintained normal vision and hearing and normal cognitive development except for areas influenced by gross motor development, some continued to gain gross motor skills compared to untreated controls • Transplants post symptom onset did not result in substantive neurologic improvement from transplant • GVHD developed in 8/11 newborns and 5/14 infants 2006: • All stage 1 and 2, and some stage 3 children achieved normal enzyme levels (1–7 nmol/hour/mL) posttransplant • Two children in stage 3 and the child in stage 4 failed to meet normal enzyme levels posttransplant • All eleven Stage 1 children continued to show an adequate rate of development in all of the domains except for gross motor development, the greatest variation among the group was in gross motor development, most of these children seem to gain skills normally for a period of time after transplant followed by no further development of skill • Stage 2 late infantile patients posttransplant continued to gain skills in all areas except gross motor, where there was no further development of skills • Stage 2 early infantile patients showed gains in most developmental domains except gross motor function • Stage 3 late infantile patients showed very minimal gains in most developmental areas and had no gains in motor function posttransplant • Stages 3 and 4 children had no developmental gains posttransplant •
Gaipa et al. 2003^ (Italy)	Symptom status not stated: 3 patients	• All 3 patients achieved 100% donor chimerism, required only one HSCT each • One patient's GALC activity was equal to that of donor's post-HSCT • No patients developed GVHD
McGraw et al. 2005*,^ (USA)	Asymptomatic children: 3 patients identified because of an affected sibling and very low or absent levels of GALC, received transplant in first month of life Symptomatic children: 4 patients received clinical diagnosis based on neurologic symptoms and signs of Krabbe disease and very low or absent levels of GALC, received transplant in first year of life	• Neurodevelopmental evaluations were performed and compared to age-matched controls, a standard score of 5 in each domain represented a score equal to or above the age-adjusted general population score • Early transplant group mean scores - expressive language, 3.33 (range, 3– 4), receptive language, 3.67 (range, 3–4), gross motor skills, 2.67 (range, 1–5), fine motor skills, 3.67 (range, 3–5), cognitive ability, 3.33 (range, 3– 4) • All patients in the late transplantation group had developmental scores of 1 in every category

Study	Population	Patient Survival Outcomes
Siddiqi et al. 2006*,^ (USA)	Early treatment: 3 patients with EIKD – transplant within first month of life	• At baseline, the average peroneal motor conduction velocity was comparable in the early and late treatment groups • One year after HSCT the average peroneal motor CV and F-wave latency improved in both groups though significantly more in the early group
	Late treatment: 3 patients with EIKD – transplant between four and six months	

Table 9 - Abstracted literature regarding symptoms at transplant and survival rates

Study	Population	Age at HSCT	Survival	Death
Escolar et al. 2005 & 2006* (USA)	Asymptomatic children: 2005:11 patients diagnosed prenatally or at birth because of an affected sibling 2006: 11 Stage 1 patients (appear to be developing normally but may have inconclusive neurological findings)	2005: 12-44 days 2006: Stated stage at transplant, but not age	2005: 100% survival at median of 36 months posttransplant (last data provided) 2006: 100% survival rate (follow-up between 24-108 months old)	2005: None 2006: None
	Symptomatic children: 2005:14 patients diagnosed between 4 and 9 months of age 2006: 4 Stage 2 patients 13 Stage 3 patients 1 Stage 4 patient	2005: 142–352 days 2006: Stated stage at transplant, but not age	2005: 6/14 at median of 41 months posttransplant (last data provided) 2006: Stage 2: 100% survival rate (follow-up between 24-108 months old) Stage 3: 61.5% survival rate;	2005: 8/14 patients died, due to: progressive disease (n=4), graft-versus-host disease (n=1), procedural complication (n=1), infection (n=2) 2006: Stage 3: 5/13 patients died, mean survival time was 21.4 months posttransplant (range 7.5-50 months) Stage 4: 1/1 patient died, a few weeks after the procedure
Gaipa et al. 2003^ (Italy)	Symptom status not stated: 3 patients	74, 79 and 109 months of age	100% survival at 68, 708, 384 days post-HSCT (last follow-up for each patient prior to publication), 100% donor chimerism, one patients' GALC activity was equal to that of donor's	None

Study	Population	Age at HSCT	Survival	Death
McGraw et al. 2005*^ (USA)	Asymptomatic children: 3 patients	Prior to 1 month of age	100% survival at 24, 36 and 48 months after transplant (last follow-up for each patient prior to publication)	None
	Symptomatic children: 4 patients	During first year of life (mean 6.5 months; range 5-8 months)	3/4 patients are alive approximately two years posttransplant (last follow-up for each patient prior to publication)	One patient died at 34 months of age, and 28 months posttransplant of unexplained causes
Siddiqi et al. 2006*^ (USA)	Asymptomatic children: 3 patients	Prior to 1 month of age	Average follow-up was 18 months (6 months to 3 years); did not state survival rates	Not stated
	Symptomatic children: 3 patients	During first year of life (average of 5 months; range 4-6 months)	Average follow-up was 18 months (6 months to 3 years); did not state survival rates	Not stated

*Potential patient overlap between Escolar et al. 2005, Escolar et al. 2006, McGraw et al. 2005 and Siddiqi et al. 2006
^Article included data on more subjects; however, patients with a different leukodystrophy than GLD, or later onset Krabbe disease were not included in table

Neurodevelopmental outcomes for those transplanted presymptomatically and postsymptomatically

Escolar et al. (2005) evaluated brain imaging and other neurologic studies to determine the differences in impact of treatment between the groups of asymptomatic and symptomatic children described above. All 11 of the asymptomatic newborns who underwent CBT had normal myelination changes by MRI. In contrast, disease progression was found by MRI among the symptomatic children (n=12 of 13 with available data). Among those asymptomatic neonates with VEP available before and after transplant (n=8), three were abnormal (considered abnormal if the P100 wave was missing) before HSCT but were normal by four months after transplant. Among symptomatic children with VEP available before and after transplant (n=12), eight were abnormal both before and after HSCT and four subjects developed abnormal VEP after CBT. CBT was associated with improvements in NCS among the asymptomatic neonates (7 out of 9 improved). However, two children in this group initially had improvement in NCS but worsened after one year. In the symptomatic group, thirteen had abnormal NCS (considered abnormal if they showed prolongation of the distal latency, low amplitude, no evoked response, or prolonged latency of the F wave) prior to treatment and seven had abnormal NCS after CBT. All symptomatic patients had abnormal EEGs (considered abnormal if focal or generalized slowing or if spikes or sharp waves were present) before and after CBT, and all survivors had clinical seizure activity. In contrast, eight of the 11 asymptomatic children had normal results both prior to CBT and 4 months to 6 years posttransplant. One asymptomatic child had a normal EEG prior to CBT but an abnormal result at six and a half months. However, subsequent EEGs were normal. Two of the asymptomatic children had abnormal EEGs prior to CBT (one newborn showed temporal sharp waves and the other showed sharp waves and asymmetric delta activity). Follow-up EEGs were not performed for either child.

Complete neurodevelopmental assessment was described for 10 of the 11 asymptomatic neonates and eight of the 14 symptomatic children who underwent CBT. One of the challenges of neurodevelopmental assessment is that motor delays can impact the assessment of cognitive function and language. Overall, all asymptomatic newborns developed cognitive skills at a normal rate, two were below average for adaptive behavior skills, one was below average for receptive language, two were below average for expressive language, four had mild-to-severe gross motor delay, two had subtle motor abnormalities, and two had severe delays in fine motor function. Over time, gross motor development could change. For example, two of six children with previously normal gross motor development developed leg spasticity and truncal weakness that interfered with standing or walking. In contrast to the asymptomatic newborns, the symptomatic children had poor neurodevelopment.

Expert information:
The consensus from experts is that HSCT is the only treatment option besides palliative care. Dr. Burton states that palliative care includes supportive care measures such as nutrition with gastric tube feedings if needed, sedation and/or pain medication. There is no standard protocol for transplantation.

Characteristics used for determining which children receive transplants

New York State follows a clinical and neurodiagnostic evaluation rating scale to determine which of the high-risk screen positive infants are candidates for HSCT (Duffner et al. 2009). Points are given for abnormal findings as follows: neurological exam (2 points), MRI (2), increased CSF protein (2), NCS (1), BAEP (1), VER (1) and genotyping results of homozygous 30-kb deletion (4). A total score of greater than or equal to 4 indicates the patient may be considered for transplant. Dr Kurtzberg reports she determines if a newborn with low GALC activity is a candidate for transplant by relying on family history, genotype and nine clinical parameters. These nine clinical parameters were the basis of the scoring system used by New York State (Duffner et al. 2009).

Mortality and morbidity for those transplanted presymptomatically and postsymptomatically

Dr. Escolar has managed the care of children with Krabbe disease for approximately ten years. She currently follows 17 patients post-HSCT, ranging from two to 12 years post-HSCT. Pre-transplant GALC values and genotype data for this cohort were unavailable. Regarding outcomes, onset of symptoms post-HSCT has varied among her patients, some before they were able to walk and others after. She has noted no further progress in development of their motor skills however, she has not observed regression. Of her 17 patients, two or three can ambulate completely independently (one can run, jump and has normal gross motor development); most of the others need walkers or other support for ambulation, and a few use wheelchairs. She has noted that peripheral neuropathy worsens over time.

Dr. Kurtzberg reported on her experience with children with Krabbe disease. She described the same cohort of newborns as Dr. Escolar who were transplanted for EIKD, not including the two children who screened positive in New York. Of the18 transplanted, she noted they all had a family history of Krabbe, at least one 30-kb deletion and in most of these children, six of the nine clinical parameters were abnormal upon examination. Of the18 newborns transplanted for EIKD in her cohort, one died from sepsis posttransplant. Referring to the same population as Dr. Escolar, she reports that a third of these individuals have had normal motor function through the first decade of life, another third are ambulatory but need devices to help them walk and the final third have severe spasticity and use wheelchairs. The oldest transplanted patient Dr. Kurtzberg follows is 13 years of age. This child was transplanted at three weeks old. She reports that this child is 95% normal. The child runs and plays normally but has some stiffness in one ankle that developed at nine years of age. Dr. Kurtzberg has also been involved in the transplants of the two children who were identified as high-risk through the New York State screening program. She reports that one of these children died approximately 11 days posttransplant.

Dr. Tolar reported on his experience in Minnesota. He shared that 17 children with symptomatic Krabbe disease have been transplanted in Minnesota since 1986. Nine are alive today, all of whom are quite delayed. He has experience with one child who was diagnosed with Krabbe very early in life due to a family history. This child had a transplant last April, at three and half months of age. He says that at the age of one year and three months, the child is able to sit, but not walk.

Dr. Burton reports that her team has performed HSCT on two EIKD patients not reported in the literature. Both infants were transplanted at under one month of age, and a second transplant was completed between two and three months of age on one patient due to failure to engraft. The patient who received two transplants is developmentally delayed, but is otherwise doing well at three years of age; this patient's older affected sibling died at 18 months of age. The other patient had symptoms at three weeks of age at the time of transplant and is ventilator dependent at five months of age and is doing poorly; the patient's affected sibling died at nine months of age.

Neurodevelopmental outcomes for those transplanted presymptomatically and postsymptomatically

Several experts reported on the neurodevelopmental outcomes of the children described above. Dr. Escolar reported that of the 17 post-HSCT patients she follows, the less involved patients have normal cognitive abilities. The more involved patients have difficultly with speed of processing. They are able to answer questions (for example, on an IQ test) but it takes them longer than control children. However, if these tests are adapted for their degree of motor impairment, the speed of processing appears more normal. Dr. Kurtzberg says of the same group of patients that they all have normal intelligence and all communicate well. Dr. Tolar reports on the one child he follows who was transplanted early at three and half months of age. He says that at

the age of one year and three months, the child can vocalize but lacks understandable words.

Dr. Eichler reported his experience that neurodevelopmental outcome is closely tied to the age of the child at HSCT. Furthermore, damage related to the EIKD continues until there is full engraftment and new glial cells develop. Dr. Eichler believes that the cortico-spinal tract is most sensitive to Krabbe disease, thus explaining the greater impact on motor function. He has not noted regression in neurodevelopment after HSCT, but at least ten years of follow-up would be helpful to ensure that this does not occur.

Experts concur about the lack of substantial data regarding the potential harms of HSCT. Dr. Duffner shared that the chemotherapy used to suppress the infant's immune system prior to HSCT is a potential harm of the treatment, including the potential damaging effects of chemotherapy on oligodendrites and myelin in the brain. She notes that late deterioration occurring in children who have had transplants could reflect a combination of chemotherapy toxicity, pre-existing disease and progressive Krabbe disease.

Treatment locations and Krabbe disease transplant registry

Duke University in North Carolina and the University of Minnesota in Minnesota are the main sites currently treating Krabbe disease with HSCT. Mt. Sinai Hospital in New York has begun to perform transplants in metabolic patients, and Dr. Kurtzberg states there are approximately eight centers total in the United States currently experienced in transplantation of infants with Krabbe disease. Dr. Duffner shares that there have been transplants performed for both EIKD and LOKD at sites besides Duke University and University of Minnesota, which include sites in: Chicago, Illinois, Columbus, Ohio, St. Louis, Missouri, Grand Rapids, Michigan in the United States and Canada (Montreal, Quebec and Vancouver, British Columbia). Additionally, Dr. Burton reports two centers in Illinois. Dr. Kurtzberg states that the protocol for transplant for Krabbe disease is the same as for other childhood diseases except that radiation is not used. She believes other centers familiar with this protocol can be trained to transplant for Krabbe disease.

The registry of the Pediatric Bone Marrow Transplantation Consortium (PBMTC) continues to compile data on patients transplanted for Krabbe disease. Dr. Duffner shared that a multidisciplinary workshop is planned for late summer 2009 with the goal of developing a standardized protocol to assess long-term outcomes.

E. **Economic evaluation**

Table 10 - Quality assessment of abstracted literature pertaining to economic evidence

Type of evidence	Number of articles
Economic	**0**
I. Evaluation of important alternative interventions comparing all clinically relevant outcomes against appropriate cost measurement and including a clinically sensible sensitivity analysis.	0
II. Evaluation of important alternative interventions comparing a limited number of outcomes against appropriate cost measurement, but including a clinically sensible sensitivity analysis.	0
III. Evaluation of important alternative interventions comparing all clinically relevant outcomes against inappropriate cost measurement, but including a clinically sensible sensitivity analysis.	0
IV. Evaluation without a clinically sensible sensitivity analysis	0
V. Expert opinion with no explicit critical appraisal, based on economic theory	0

Adapted from NHS Centre for Reviews and Dissemination Report 4, March 2001

We sought to answer the following questions on the economic evaluation of newborn screening for Krabbe disease through a literature review and information provided by experts:

i. What are the incremental costs associated with the screening test for newborn screening programs? What is the cost-effectiveness of newborn screening for the condition?
ii. What are the costs associated with diagnosis, and the failure to diagnose in the presymptomatic period?
iii. What is the availability of treatment and what are the costs associated with treatment?

Literature review:
We found no economic evaluations of screening for Krabbe disease.

Expert information:
Dr. Caggana and Dr. Orsini report the startup cost of the New York State laboratory was approximately $1,000,000, which included three tandem mass spectrometers, two liquid handlers, evaporators, and two DNA fragment analyzers. The actual reagent screening costs for tandem mass spectrometry testing, reagents and consumables are $0.39 per baby, and $283,530 for the 727,000 screens completed. For diagnostic enzyme analysis, the cost is $12,500 per year, which has been derived from approximately 50 referrals annually at $250 per newborn. In terms of DNA costs, after screening 769,853 babies, 236 have had full DNA sequence analysis completed. The DNA analysis costs about $650 per newborn, and per infant screened it is approximately $0.20. There is no estimate available regarding medical work-up costs.

X. Key findings and summary

Children with EIKD develop profound neurologic deficits and typically die by two years of age. Advances in technology have made it possible to detect those with Krabbe disease in newborn screening blood spots. Direct enzyme replacement therapy is not available; however, galactocerebrosidase can be provided to affected individuals via HSCT.

The most comprehensive information about the birth incidence of Krabbe disease comes from the New York screening data, which could identify those with all forms of Krabbe disease. No cases of infantile Krabbe disease have been reported to be missed by that program (i.e., sensitivity = 100%). Population-based screening often uncovers a different spectrum of disease than epidemiology based on case-finding. For example, screening can detect those cases that would go undiagnosed, including those with severe disease leading to rapid death prior to diagnosis, those with typical disease that was never properly diagnosed, and those with minor disease for which diagnosis was never sought. In New York, the observed prevalence of infantile-onset Krabbe disease is less than that predicted based on other epidemiologic evaluations (0.26 cases per 100,000 vs. 0.9 cases per 100,000) but the overall prevalence of Krabbe disease, regardless of type, has been higher (5 per 100,000 vs. 1 per 100,000). The greatest challenge to understanding the epidemiology is the classification into type based on risk categorization. Because of the lack of genotype-phenotype correlation except for one specific mutation, complex criteria based on genotype, exam, and other neurologic tests have formed the basis for the case definition with regards to determining when HSCT should be offered.

The overall specificity of the New York screening program is >99.9% if a positive screen is considered the point of family and physician notification and a positive result is the identification of a high risk newborn (765,915 screened negative, 140 families and physicians notified leading to seven high risk newborns). The specificity is still >99.9% if a positive result is considered to be referral to bone marrow transplantation. The subsequent risk stratification leads to uncertainty about those who are high risk but not referred for bone marrow transplantation (which is five out of the seven identified in New York), those who are moderate risk (13 in New York), and low risk (36 in New York). Refining the process of risk stratification and subsequent follow-up, which is an active area of research, may decrease the uncertainty and decrease the amount of testing required. As described by the experts, this follow-up can be challenging (e.g., one high-risk child moved out of the country, one high-risk patient's family refused follow-up) which emphasizes the importance of the ongoing research to improve the process of diagnosis.

Currently only one laboratory provides diagnostic confirmation of GALC levels. The director of the laboratory believes that there is sufficient capacity to serve as a national confirmatory laboratory center as long as the false positive rate remains low. Experts believe that there are sufficient numbers of bone marrow transplantation programs for those with early-onset Krabbe disease, although families may have to travel far for

treatment. These different sites seem to use generally similar protocols (e.g., no radiation). However, efforts to standardize approaches are ongoing. If such standardized protocols are developed, other sites may be able to offer transplantation.

Evaluating the outcomes of treatment is challenging. Although bone marrow transplantation involves risk of morbidity and mortality, treatment of those with infantile Krabbe disease presymptomatically or at the first development of symptoms appears to decrease the risk of mortality.

Assessing the impact of transplantation on neurodevelopment is challenging. There are several challenges to evaluating this evidence:

1. Heterogeneity in how the disorder was diagnosed (e.g., newborn screening, sibling of affected individual)
2. Differences in the age at the time of HSCT
3. Variability in follow-up with few data extending into the second decade of life
4. Incomplete data with some loss to follow-up
5. Lack of standardized measures at specific time intervals

The evidence suggests that HSCT in presymptomatic or early symptomatic children with EIKD improves neurodevelopmental outcome. Motor function appears to be more affected after HSCT than cognitive development. At least one-third of children would need some ambulatory assistance. Insufficient long-term data are available to evaluate whether there is a plateau or regression in neurodevelopment.

We identified several questions that we were unable to answer from the available evidence. Most of these are active areas of research.

- What are the appropriate ways to identify asymptomatic infants with low galactocerebrosidase levels who would benefit from bone marrow transplantation?
- What are the harms associated with screening, especially in the identification of asymptomatic infants with low galactocerebrosidase levels?
- What are the long-term neurodevelopmental outcomes for children who have received transplant?
- What is the cost-effectiveness of screening for Krabbe disease?

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XII. Appendix A - Krabbe disease evidence tables

Natural History

Authors/ Researcher Title of Paper Year	Study Population Description	Significant Findings
Aldosari,M.;Altuwaijri,M.;Husain,A. M. Brain-stem auditory and visual evoked potentials in children with Krabbe disease. 2004*	26 patients total 20 with EIKD, median age at diagnosis 6 months (range 2-12 months); 16 "symptomatic" and 4 "presymptomatic" - unclear how this is defined 6 with LOKD, median age at diagnosis 14 months (range 10-87 months) Ages at time of BAEP and/or VEP unclear.	• Prolonged or absent Bilateral Auditory Evoked Potentials (BAEP) were present in 88% (15/17) of those with EIKD • 40% of LOKD patients had abnormal BAEP • Abnormal BAEP is among first indication of disease onset in EIKD (may precede clinical symptoms) • Visual evoked potential (VEP) abnormalities were present in 53% (8/15) with EIKD • 0% of LOKD patients had abnormal VEP • VEP abnormality occurs later in illness progression
Barone,R.;Bruhl,K.;Stoeter,P.;Fiumara,A.;Pavone,L.;Beck,M. Clinical and neuroradiological findings in classic infantile and late-onset globoid-cell leukodystrophy (Krabbe disease). 1996	11 patients total 2 with EIKD 9 with LOKD age range 2 months - 5 years	• Variability of KD refers to clinical manifestations, CT and MRI findings • High density areas on CT exam is early and specific finding of EIKD • EIKD patients displayed cerebellar atrophy appearing during the first year of life • 2 patients with LOKD had follow-up MRI 18 months and 8 years after initial MRI • Follow-up MRIs displayed clear progression of white matter changes and clinically both had tetraplegic spasticity
Duffner,P. K.;Jalal,K.;Carter,R. L. The Hunter's Hope Krabbe family database 2009	334 families with children affected by Krabbe disease completed questionnaires as of June 2006	• Most common initial symptoms for age 0 to 12 months were crying and irritability, stiffness, and seizures • 71% developed symptoms at 0-6 months of age, 19% between 7-12 months of age, 10% at 13 months or more (13 months to 5.5 years of age) • Survival differed according to age of symptom onset: 0-6 months of age had worse survival than onset between 7-12 and 13 months or more • Three symptoms predictive of poor survival: stiffness, loss of vision, and poor feeding • Median survivals in the early infantile group: 17 months of age • Mean survival: 24.1 months of age • Neither GALC nor mutation analysis reliably predict disease severity

Escolar,M. L.;Poe,M. D.;Martin,H. R.;Kurtzberg,J. A staging system for infantile Krabbe disease to predict outcome after unrelated umbilical cord blood transplantation. 2006*	42 patients total 37/42 had sufficient data in medical chart (26 EIKD, 11 LIKD) 29/37 patients received a transplant 26 had unrelated CBT and 3 had BMT from sibling with conditioning	• Clinical staging system developed found to be predictive of neurodevelopmental outcome after CBT based on pretransplant progression of disease • Clinical interpretations of brain MRI, NCV, EEG, VEP, BAEP studies and CSF protein levels at initial evaluation were compared with age-equivalent normal controls, and all failed to correlate with disease stage • Clinical signs and symptoms alone sufficient in staging; undetectable GALC levels confirmed diagnosis in asymptomatic cases • Stage 1 and stage 2 patients had 100% survival rate (follow-up between 24-108 months old) • Stage 3 patients had 61.5% survival rate; mean survival time for 5 patients who died posttransplant was 21.4 months (range 7.5-50 months posttransplant) • Only 1 stage 4 patient received transplant and died weeks after; 8 patients at stage 4 were not transplanted and died
Escolar ML, Poe MD, Smith JK, Gilmore JH, Kurtzberg J, Lin W, Styner M. Diffusion tensor imaging detects abnormalities in the corticospinal tracts of neonates with infantile Krabbe disease. 2009	51 patients total 6 patients with KD: 4 infants with infantile Krabbe disease identified by family history with MRI in the first 4 weeks of life and 2 by NBS 45 controls	• After adjusting for gestational age, gestational age at birth, birth weight, sex, and race, those with Krabbe disease had significantly lower FA ratios than controls • In those with Krabbe disease, neurodevelopment in the motor area is associated with FA ratios
Guzzetta,F.;Rodriguez,J.;Deodato,M.;Guzzetta,A.;Ferriere,G. Demyelinating hereditary neuropathies in children: a morphometric and ultrastructural study. 1995	28 patients total 4 patients with KD: 12 months, 12 months, 18 months, 35 months of age at biopsy 5 normal age-matched controls (range 28 months - 17 years of age)	• Myelinated and unmyelinated number of fibers in KD patients were comparable to controls, but density in both fibers were comparatively low to controls • KD patients had: no nerve hypertrophy, relative loss of larger myelinated fibers, demyelination with significant low slope of the regression line of the ratio of myelin thickness to axon diameter, small onion bulb were evident • Typical inclusions in Schwann cells and macrophages found
Heim,P.;Claussen,M.;Hoffmann,B.;Conzelmann,E.;Gartner,J.;Harzer,K.;Hunneman,D.H.;Kohler,W.;Kurlemann,G.;Kohlschutter,A. Leukodystrophy incidence in Germany. 1997	All 439 pediatric, 463 neurology, and 41 neuropathology departments of Germany 353/439 (80%) pediatric centers replied 300/463 (65%) neurology centers replied 24/41 (58%) neuropathology centers replied	• A total of 617 leukodystrophy cases found • 80 patients with KD found, representing 14.4% of all leukodystrophies • In-depth national survey yielded an incidence of 0.6/100,000 for KD in Germany • Sweden reported 1/53,000 in 1970 (Hagberg et al. 1970) • Japan reported 1/100,000 in 1989 (Suzuki and Suzuki 1989)

Husain,A. M.;Altuwaijri,M.;Aldosari,M. Krabbe disease: neurophysiologic studies and MRI correlations 2004*	26 total patients 20/26 (77%) EIKD 16/20 EIKD (80%) symptomatic, 2 months at symptom onset (range 0.5-6 months) and 6 months at diagnosis (range 2-12 months) 4/20 EIKD presymptomatically diagnosed at birth; family history 6/20 LOKD 5/6 LOKD symptomatic, 12 months at symptom onset (range 10-60 months) and 14 months at diagnosis (range 10-87 months) 1/6 LOKD presymptomatically diagnosed at 48 months	• EIKD patients: all NCS abnormal, 88% of BAEPs abnormal, 65% of EEGs abnormal, 53% flash VEPs abnormal • Symptomatic EIKD patients more likely to have abnormal BAEPs, EEGs, and flash VEPs • In EIKD patients: NCS abnormality seen first, followed by BAEP, EEG and eventually flash VEP abnormalities • Findings show peripheral nervous system involved very early in EIKD disease process, even before CNS involvement and onset of symptoms • In LOKD patients, BAEPs most often abnormal (40%), followed by EEG abnormality (33%), NCS only abnormal in 20%, and all had normal flash VEPs
Kaye,E. M.;Ullman,M. D.;Kolodny,E. H.;Krivit,W.;Rischert,J. C. Possible use of CSF glycosphingolipids for the diagnosis and therapeutic monitoring of lysosomal storage diseases. 1992	59 total patients 2 patients with EIKD 2 patients with LOKD 23 patients: other LSDs 32 controls: 9 controls for ganglioside content, 12 for galactosylceramide, 11 for galactosylceramide sulfate	• All KD patients did not demonstrate higher levels of NFA- (non-hydroxy fatty acid) or HFA- (alpha-hydroxy fatty acid) galactosylceramide compared to control CSF • Trace amounts of lactosylceramide and globotriaosylceramide detected in KD compared to controls potentially due to myelin breakdown • CSF is not a pathway for galactosylceramide excretion in KD
Kleijer,W. J.;Keulemans,J. L.;van der Kraan,M.;Geilen,G. G.;van der Helm,R. M.;Rafi,M. A.;Luzi,P.;Wenger,D. A.;Halley,D. J.;van Diggelen,O. P. Prevalent mutations in the GALC gene of patients with Krabbe disease of Dutch and other European origin. 1997	111 total patients 41 Dutch with EIKD 23 of other European origin with EIKD 47 Dutch controls	• Of the 23 European EIKD patients, allele frequency of 30-kb deletion was 16/46 (35%), 502T polymorphism was 18/46 (39%) and the T513M base substitution was 0/46 (0%) • Of the 41 Dutch EIKD patients, allele frequency of 30-kb deletion was 43/82 (52%), 502T polymorphism was 55/82 (67%%) and the T513M base substitution was 7/82 (8.5%) • Of the 41 Dutch EIKD patients, coinheritance of the 30-kb deletion and 502T polymorphism was 41/43 30-kb deletion alleles and 41/55 of the 502T polymorphism alleles • Of the 47 controls, the allele frequency of the 502T polymorphism was 5/94 (5.3%)Together, the 502T polymorphism and 30-kb deletion are responsible for half of the GLD alleles in Caucasians in general, and 60% in Dutch patients

Korn-Lubetzki,I.;Dor-Wollman,T.;Soffer,D.;Raas-Rothschild,A.;Hurvitz,H.;Nevo,Y. Early peripheral nervous system manifestations of infantile Krabbe disease. 2003	8 patients total 2.5 - 5 months at symptom presentation, 2 weeks- 10 months until confirmed diagnosis of EIKD	• EIKD relatively frequent in Muslim-Arab population in Israel • 8/8 patients homozygous for same 1582 G-to-A mutation • 6/8 demonstrated CNS symptoms and signs of which seizures, poor focusing and irritability most common • 4/8 patients presented with hyperreflexia at first examination (4-5 months of age) • 2/8 patients detected with areflexia at first visit (3-5 months of age) • All 6/8 with CNS symptoms were blind and cognitively deteriorated 6-7 months after first visit • 2/8 patients had no CNS involvement for 9-10 months after initial symptom of peripheral neuropathy
Lissens,W.;Arena,A.;Seneca,S.;Rafi,M.;Sorge,G.;Liebaers,I.;Wenger,D.;Fiumara,A. A single mutation in the GALC gene is responsible for the majority of late onset Krabbe disease patients in the Catania (Sicily, Italy) region. 2007	8 families with a child affected by KD Ages not stated; LIKD, juvenile KD, and LOKD	• Identification of founder mutation in Italy of pGly41Ser (c.121 G>A) mutation in patients with LIKD, juvenile KD and LOKD
Loes,D. J.;Peters,C.;Krivit,W. Globoid cell leukodystrophy: distinguishing early-onset from late-onset disease using a brain MR imaging scoring method 1999	22 patients total 3 asymptomatic KD 10 EIKD over 2 years of age 9 LOKD under 2 years of age Age range: 1 month -18.5 years at exam; 3 months - 18 years old at onset of symptoms	• Identification of brain involvement among those with Krabbe is possible by MRI • Cerebellar white matter and deep gray matter involvement are present only in early onset Krabbe disease. • Pyramidal tract involvement is present in both early and late onset disease • All MRIs had abnormalities; KD has a characteristic pattern dependent on age of onset • The authors have developed a scoring method • Mean MRI severity scores of 8.1 for EIKD (range 3-18), 5.6 for LOKD (range 4-10) and 3.2 for asymptomatic (range 1.5-5)
Poorthuis,B. J.;Wevers,R. A.;Kleijer,W. J.;Groener,J. E.;de Jong,J. G.;van Weely,S.;Niezen-Koning,K.;E. van Diggelen,O. P. The frequency of lysosomal storage diseases in The Netherlands. 1999	963 enzymatically confirmed LSD cases between 1970-1996 in the Netherlands (assuming complete ascertainment) 70 with confirmed KD between 1971-1995	• The birth prevalence of Krabbe disease in the Netherlands is 1.35 per 100,000 between 1970-1996 • Combined birth prevalence in the Netherlands is 14 per 100,000 • KD diagnosed in 17% (70/424) of cases of lipidosis from 1970-1996

Provenzale,J. M.;Escolar,M.;Kurtzberg,J. Quantitative analysis of diffusion tensor imaging data in serial assessment of Krabbe disease 2005*	9 patients total All 9 infants with EIKD Ages at time of transplantation ranged from 3 weeks to 9 months of age, Pre and posttransplantation MR Images	• Very good correlation between clinical testing and Loes scores for entire brain • Moderately good correlation between clinical testing and Loes scores for specific brain regions • Loes scoring system from MRI likely provides reasonable assessment of prognosis and therapeutic response
Siddiqi,Z. A.;Sanders,D. B.;Massey,J. M. Peripheral neuropathy in Krabbe disease: electrodiagnostic findings 2006*	24 patients total All 24 with EIKD (others excluded) diagnosed between 1990 and 2002 seen at Duke for possible transplant	• Peripheral neuropathy occurs early in the disease • Nerve conduction tests provide a sensitive tool to "screen" this patient population - may reflect the degree of CNS involvement
Tatsumi,N.;Inui,K.;Sakai,N.;Fukushima,H.;Nishimoto,J.;Yanagihara,I.;Nishigaki,T.;Tsukamoto,H.;Fu,L.;Taniike,M. Molecular defects in Krabbe disease. 1995	11 patients total 7 Japanese, 4 non-Japanese patients Variable age of onset - difficult to tell from the paper; unclear how cases were assembled	• Mutations in infantile and late infantile patients are relatively heterogeneous
Tullu,M. S.;Muranjan,M. N.;Kondurkar,P. P.;Bharucha,B. A. Krabbe disease--clinical profile. 2000	9 patients total 5 "classical infantile", 3 "late infantile", and 1 juvenile KD Mean age of presentation 9.4 months (range 2.5-21 months); 1 case identified at 8 years of age	• Optic atrophy is uncommon • Most have elevated CSF protein • Most have peripheral neuropathy • Most have characteristic findings on MRI
Zlotogora,J.;Levy-Lahad,E.;Legum,C.;Iancu,T. C.;Zeigler,M.;Bach,G. Krabbe disease in Israel. 1991	26 patients total 18 patients with Krabbe disease diagnosed between 1975-1989, and 8 affected siblings who died without enzymatic confirmation 23 patients presented symptoms before age of 5 months; 3 patients presented symptoms between 6-11months of age	• Presenting symptoms are usually motor regression or irritability • All died before age 2 years

*Potential patient overlap between Aldosari et al. 2004, Escolar et al. 2006, Husain et al. 2004, Provenzale et al. 2005 and Siddiqi et al. 2006

Screening

Authors/Researcher Title of Paper Year	Study Population Description	Significant Findings
Duffner,P. K.; Caggana,M.; Orsini,J. J.;Wenger,D. A.; Patterson,M. C.;Crosley,C. J.;Kurtzberg,J.;Arnold,G. L.;Escolar,M. L.;Adams,D. J.;Andriola,M. R.;Aron,A. M.; Ciafaloni,E.;Djukic,A.;Erbe,R. W.;Galvin-Parton,P.; Helton,L. E.;Kolodny,E. H.;Kosofsky,B. E.;Kronn,D. F.;Kwon,J. M.;Levy,P. A.; Miller-Horn,J.;Naidich,T. P.;Pellegrino,J. E.; Provenzale,J. M.; Rothman,S. Newborn screening for Krabbe disease: the New York State model 2009	550,000 newborn babies screened for Krabbe disease as of June 30, 2008 Newborn screening program in New York state began in August 2006	• Formed the Krabbe Consortium for New York State to address the need for clinical evaluation and follow-up for screen positive babies • Developed a rapid and accurate technique for assessing GALC activity and performing DNA mutation analysis • Designed a standardized clinical evaluation protocol based on available literature • Formulated criteria for transplantation for EIKD phenotype • Developed a clinical database and registry • Instituted a study of developmental and functional outcomes • As of June 30, 2008, 550,000 babies have been screened: 4 high-risk, 6 moderate-risk, and 15 low-risk children have been identified to date
Kaye,E. M.;Ullman,M. D.;Kolodny,E. H.;Krivit,W.;Rischert,J. C. Possible use of CSF glycosphingolipids for the diagnosis and therapeutic monitoring of lysosomal storage diseases. 1992	59 total patients 2 patients with EIKD 2 patients with LOKD 23 patients: other LSDs 32 controls: 9 controls for ganglioside content, 12 for galactosylceramide, 11 for galactosylceramide sulfate	• All KD patients did not demonstrate higher levels of NFA- or HFA galactosylceramide compared to control CSF • Trace amounts of lactosylceramide and globotriaosylceramide detected in KD compared to controls potentially due to myelin breakdown • CSF is not a pathway for galactosylceramide excretion in KD
Kleijer,W. J.;Keulemans,J. L.;van der Kraan,M.;Geilen,G. G.;van der Helm,R. M.;Rafi,M. A.;Luzi,P.;Wenger,D. A.;Halley,D. J.;van Diggelen,O. P. Prevalent mutations in the GALC gene of patients with Krabbe disease of Dutch and other European origin. 1997	111 total patients 41 Dutch with EIKD 23 of other European origin with EIKD 47 Dutch controls	• Of the 23 European EIKD patients, allele frequency of 30-kb deletion was 16/46 (35%), 502T polymorphism was 18/46 (39%) and the T513M base substitution was 0/46 (0%) • Of the 41 Dutch EIKD patients, allele frequency of 30-kb deletion was 43/82 (52%), 502T polymorphism was 55/82 (67%%) and the T513M base substitution was 7/82 (8.5%) • Of the 41 Dutch EIKD patients, coinheritance of the 30-kb deletion and 502T polymorphism was 41/43 30-kb deletion alleles and 41/55 of the 502T polymorphism alleles • Of the 47 controls, the allele frequency of the 502T polymorphism was 5/94 (5.3%)Together, the 502T polymorphism and 30-kb deletion are responsible for half of the GLD alleles in Caucasians in general, and 60% in Dutch patients

Li,Y.;Brockmann,K.;Turecek, F.;Scott,C. R.;Gelb,M. H. Tandem mass spectrometry for the direct assay of enzymes in dried blood spots: application to newborn screening for Krabbe disease. 2004	28 total patient samples 4 KD, 4 Fabry, 3 Gaucher all diagnosed prior to use in assay 16 control samples	• Utilized dried blood 2mm punches and tandem mass spectrometry for direct enzyme assay • Activity in KD samples much lower with a range of 0.05-0.23 umol h-1 (L blood)-1 when compared to: controls (1.4-3.7), Fabry (range 1.28-5.34), and Gaucher (1.35-7.49) • The 5 actual KD results were 0.08, 0.05, 0.23, 0.07, 0.08 umol h-1 (L blood)-1 • C8-Cer (GALC) in MS/MS allows for high-sensitivity detection and quantification
Li,Y.;Scott,C. R.;Chamoles,N. A.;Ghavami,A.;Pinto,B. M.;Turecek,F.;Gelb,M. H. Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening 2004	70 total patient samples 38 different lysosomal storage diseases 12/38 diagnosed with KD: 1 infant, 2 juvenile, 1 adult, 5 of unknown age Samples compared to 17 healthy adults and 15 healthy infant controls	• Used rehydrated DBS from NBS cards and tandem mass spectrometry for a multiplex of 5 lysosomal enzymes and corresponding diseases • GALC shows decrease in activity over a 4 year period; sufficient for a retrospective analysis only over a 4 year period • DBS from patients with KD had lower GALC levels than DBS from healthy patients collected in the same year • No data from KD heterozygotes available (only homozygous NBS cards) • GALC best identified using 2mm DBS punch alone instead of 5mm DBS punch and multiplex • Detection rate for affected patients in study was 100%
Meikle,P. J.;Ranieri,E.;Simonsen,H.;R ozaklis,T.;Ramsay,S. L.;Whitfield,P. D.;Fuller,M.;Christensen,E.; Skovby,F.;Hopwood,J. J. Newborn screening for lysosomal storage disorders: clinical evaluation of a two-tier strategy. 2004	547 total patient samples 47 Guthrie cards from newborns in Denmark collected from 1982-1997 who were diagnosed with a LSD (12 disorders represented) 227 control Guthrie cards from newborns in Denmark collected from 1982-1997 273 additional control cards from Australia	• The first tier of this two tiered strategy will not identify cases of Krabbe disease
Orsini JJ, Morrissey MA, Slavin LN, Wojcik M, Biski C, Martin M, Keutzer J, Zhang XK, Chuang WL, Elbin C, Caggana M. Implementation of newborn screening for Krabbe disease: Population study and cutoff determination. 2009	139,146 total patient samples Evaluate the % Daily Mean Activity (DMA) to set reasonable thresholds for the NY screening algorithm	• %DMA is approach to normalize for variability in reagents or other day-to-day changes • The main goal was to set %DMA for the first point to figure out who screens negative and who should be retested (from the same blood spot), and who (on the average of 3 samples in subsequent testing) should screen negative or go on to be considered to be a positive. • The range of activities was 0.17-335 micromol/L/H • Overall average DMA for Krabbe disease is 4.6% • The highest DMA% for Krabbe positive controls was 10.9%, therefore set to 20% to avoid missing any cases • "Immediate action" was set to 8% which overlapped with some controls • The 8-12% DMA threshold for DNA sequence analysis was set to assure no missed cases

Zhang,X. K.;Elbin,C. S.;Chuang,W. L.;Cooper,S. K.;Marashio,C. A.;Beauregard,C.;Keutzer,J. M. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry 2008	309 total patient samples 149 DBS from healthy adults 100 DBS newborn screening cards 60 DBS from 60 patients with LSDs 10/60 LSD DBS samples previously diagnosed with KD	• Modified Li et al. assay: eluted GALC directly from NBS card punch directly into assay cocktail • Showed unambiguous distinction between samples from healthy individuals and corresponding samples from patients with LSDs • GALC assay separation between normal and disease samples became more pronounced than prior assay • Limits of detection observed were 2-fold below maximum observed disease activity, indicating higher precision • Assay was validated using CLSI standard protocol • Method works and could be implemented in high-throughput laboratory setting

Treatment

Authors/Researcher Title of Paper Year	Study Population Description	Significant Findings
Escolar,M. L.;Poe,M. D.;Martin,H. R.;Kurtzberg,J. A staging system for infantile Krabbe disease to predict outcome after unrelated umbilical cord blood transplantation. 2006*	42 patients total 37/42 had sufficient data in medical chart (26 EIKD, 11 LIKD) 29/37 patients received a transplant 26 had unrelated CBT and 3 had BMT from sibling with conditioning	• Clinical staging system developed found to be predictive of neurodevelopmental outcome after CBT based on pretransplant progression of disease • Clinical interpretations of brain MRI, NCV, EEG, VEP, BAEP studies and CSF protein levels at initial evaluation were compared with age-equivalent normal controls, and all failed to correlate with disease stage • Clinical signs and symptoms alone sufficient in staging; undetectable GALC levels confirmed diagnosis in asymptomatic cases • Stage 1 and stage 2 patients had 100% survival rate (follow-up between 24-108 months old) • Stage 3 patients had 61.5% survival rate; mean survival time for 5 patients who died posttransplant was 21.4 months (range 7.5-50 months posttransplant) • Only 1 stage 4 patient received transplant and died weeks after; 8 patients at stage 4 were not transplanted and died
Escolar,M. L.;Poe,M. D.;Provenzale,J. M.;Richards,K. C.;Allison,J.;Wood,S.;Wenger,D. A.;Pietryga,D.;Wall,D.;Champagne,M.;Morse,R.;Krivit,W.;Kurtzberg,J. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease 2005*	215 patients total 190 untreated patient data from registry 25 infantile KD: 11/25 asymptomatic newborns (age range 12 to 44 days) 14/25 symptomatic infants (age range 142 to 352 days)	• Initiation of myeloablative chemotherapy for newborns was at a median of 18.5 days of age, transplant median of 28 days old • Transplanted umbilical cord blood from unrelated donors with partial HLA mismatches (4 to 6 /6 HLA loci matched) • Donor cell engraftment was 100% for both infants and newborns • Follow-up 4 months to 6 years posttransplantation (median 3 years) • At follow-up, survival rate was 100% for asymptomatic newborns, and 43% among symptomatic infants • Transplants prior to symptom onset maintained progressive central myelination, continued gains in developmental skills not present in controls • Transplants post symptom onset did not result in substantive neurologic improvement from transplant • GVHD developed in 8/11 newborns and 5/14 infants • Compared to affected siblings, 6/12 outlived untransplanted siblings by 8 to 48 months • The other 6/12 are still alive, but have yet to surpass the age of their deceased sibling

Gaipa,G.;Dassi,M.;Perseghin,P.;Venturi,N.;Corti,P.;Bonanomi,S.;Balduzzi,A.;Longoni,D.;Uderzo,C.;Biondi,A.;Masera,G.;Parini,R.;Bertagnolio,B.;Uziel,G.;Peters,C.;Rovelli,A. Allogeneic bone marrow stem cell transplantation following CD34+ immunomagnetic enrichment in patients with inherited metabolic storage diseases 2003	9 patients total 11 HSCTs completed with conditioning but no irradiation 3 GLD (KD) patients each received 1 haploidentical HSCT from unrelated donors GLD patients were 74, 79 and 109 months of age at HSCT 6 other metabolic storage diseases	• All 3 GLD (KD) patients achieved 100% donor chimerism post one HSCT each • All 3 alive at 68, 708, 384 days post-HSCT (last follow-up for each patient prior to publication) • One KD patients' GALC activity was equal to that of donor's post-HSCT • No patients developed acute or chronic GVHD
McGraw,P.;Liang,L.;Escolar,M.;Mukundan,S.;Kurtzberg,J.;Provenzale,J. M. Krabbe disease treated with hematopoietic stem cell transplantation: serial assessment of anisotropy measurements--initial experience 2005*	12 patients total 7 patients diagnosed with EIKD: 3 received HSCT by 1 month of life, and 4 who received HSCT in first year of life (range 5-8 months of age at HSCT) 5 age-matched retrospective controls for MR Imaging comparison	• Pretransplantation fractional anisotropy ratios (marker of myelination) for early transplanted were normal • Pretransplantation fractional anisotropy ratios for the later treated were decreased • After 1 year, there was increases in the fractional anisotropy ratio among the early treated • In the late treated group, the change in fractional anisotropy ratio was variable
Siddiqi,Z. A.;Sanders,D. B.;Massey,J. M. Peripheral neuropathy in Krabbe disease: effect of hematopoietic stem cell transplantation 2006*	12 patients total All 12 patients diagnosed with KD and treated with HSCT: 9 with EIKD, 2 juvenile KD, 1 LOKD	• Average follow-up was 18 months (6 months to 3 years) after HSCT • Results indicate that HSCT may have a beneficial effect on the neuropathy in that most nerve conduction abnormalities improve after transplantation and some previously absent responses become obtainable • Sural sensory responses most robust indication of improvement • However, nerve conduction can worsen after an initial improvement • One year after transplant the average peroneal motor conduction velocity and FWL improved in both early and late onset disease though significantly more in the early than the late onset cases

*Potential patient overlap between Escolar et al. 2005, Escolar et al. 2006, McGraw et al. 2005 and Siddiqi et al. 2006

XIII. Appendix B - Articles excluded due to ≤4 Krabbe disease subjects

Authors	Title	Journal	Year
Bambach,B. J.;Moser,H. W.;Blakemore,K.;Corson,V. L.;Griffin,C. A.;Noga,S. J.;Perlman,E. J.;Zuckerman,R.;Wenger,D. A.;Jones,R. J.	Engraftment following in utero bone marrow transplantation for globoid cell leukodystrophy.	Bone marrow transplantation	1997
Bernal,O. G.;Lenn,N.	Multiple cranial nerve enhancement in early infantile Krabbe's disease.	Neurology	2000
Beslow,L. A.;Schwartz,E. S.;Bonnemann,C. G.	Thickening and enhancement of multiple cranial nerves in conjunction with cystic white matter lesions in early infantile Krabbe disease.	Pediatric radiology	2008
Breningstall,G. N.;Patterson,R. J.	Acquired obstructive hydrocephalus in globoid-cell leukodystrophy	Pediatric neurology	2008
Caniglia,M.;Rana,I.;Pinto,R. M.;Fariello,G.;Caruso,R.;Angioni,A .;Dionisi Vici,C.;Sabetta,G.;De Rossi,G.	Allogeneic bone marrow transplantation for infantile globoid-cell leukodystrophy (Krabbe's disease).	Pediatric transplantation	2002
De Meirleir,L. J.;Taylor,M. J.;Logan,W. J.	Multimodal evoked potential studies in leukodystrophies of children.	Canadian Journal of Neurological Sciences	1988
Del Bigio,M. R.;Chudley,A. E.;Booth,F. A.;Pacin,S.	Late infantile onset Krabbe disease in siblings with cortical degeneration and absence of cerebral globoid cells.	Neuropediatrics	2004
Finelli,D. A.;Tarr,R. W.;Sawyer,R. N.;Horwitz,S. J.	Deceptively normal MR in early infantile Krabbe disease.	Ajnr: American Journal of Neuroradiology	1994
Percy,A. K.;Odrezin,G. T.;Knowles,P. D.;Rouah,E.;Armstrong,D. D.	Globoid cell leukodystrophy: comparison of neuropathology with magnetic resonance imaging.	Acta Neuropathologica	1994
Randell,E.;Connolly-Wilson,M.;Duff,A.;Skomorowski,M. A.;Callahan,J.	Evaluation of the accuracy of enzymatically determined carrier status for Krabbe disease by DNA-based testing.	Clinical biochemistry	2000
Sasaki,M.;Sakuragawa,N.;Takashi ma,S.;Hanaoka,S.;Arima,M.	MRI and CT findings in Krabbe disease.	Pediatric neurology	1991
Vanhanen,S. L.;Raininko,R.;Santavuori,P.	Early differential diagnosis of infantile neuronal ceroid lipofuscinosis, Rett syndrome, and Krabbe disease by CT and MR.	Ajnr: American Journal of Neuroradiology	1994
Wang,P. J.;Wang,T. Z.;Shen,Y. Z.	A study of genetic leukodystrophies in Chinese children.	Chung-Hua Min Kuo Hsiao Erh Ko i Hsueh Hui Tsa Chih	1992
Yamanouchi,H.;Kaga,M.;Iwasaki,Y .;Sakuragawa,N.;Arima,M.	Auditory evoked responses in Krabbe disease.	Pediatric neurology	1993
Zafeiriou,D. I.;Anastasiou,A. L.;Michelakaki,E. M.;Augoustidou-Savvopoulou,P. A.;Katzos,G. S.;Kontopoulos,E. E.	Early infantile Krabbe disease: deceptively normal magnetic resonance imaging and serial neurophysiological studies.	Brain & development	1997