Krabbe disease: New hope for an old disease

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Abstract

Krabbe disease (globoid cell leukodystrophy) is a lysosomal storage disease (LSD) characterized by progressive and profound demyelination. Infantile, juvenile and adult-onset forms of Krabbe disease have been described, with infantile being the most common. Children with an infantile-onset generally appear normal at birth but begin to miss developmental milestones by six months of age and die by two to four years of age. Krabbe disease is caused by a deficiency of the acid hydrolase galactosylceramidase (GALC) which is responsible for the degradation of galactosyleramides and sphingolipids, which are abundant in myelin membranes. The absence of GALC leads to the toxic accumulation of galactosylsphingosine (psychosine), a lysoderivative of galactosyleramides, in oligodendrocytes and Schwann cells resulting in demyelination of the central and peripheral nervous systems, respectively. Treatment strategies such as enzyme replacement, substrate reduction, enzyme chaperones, and gene therapy have shown promise in LSDs. Unfortunately, Krabbe disease has been relatively refractory to most single-therapy interventions. Although hematopoietic stem cell transplantation can alter the course of Krabbe disease and is the current standard-of-care, it simply slows the progression, even when initiated in presymptomatic children. However, the recent success of combinatorial therapeutic approaches in small animal models of Krabbe disease and the identification of new pathogenic mechanisms provide hope for the development of effective treatments for this devastating disease. This review provides a brief history of Krabbe disease and the evolution of single and combination therapeutic approaches and discusses new pathogenic mechanisms and how they might impact the development of more effective treatment strategies.

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Declaration of Competing Interest
The authors report no declarations of interest.
Keywords
Krabbe disease; Globoid cell leukodystrophy; Lysosomal storage disease; Gene therapy

1. Introduction
Globoid cell leukodystrophy, commonly referred to as Krabbe disease, is a profoundly demyelinating inborn error of metabolism. Krabbe disease was first described over 100 years ago and has been extensively characterized [1]. Authentic small and large animal models of Krabbe disease have been available for over 40 years and countless pre-clinical therapeutic interventions have been attempted. Despite this enormous effort, there is no cure for Krabbe disease and the current standard-of-care simply slows the progression of the disease. However, the success of combinatorial therapeutic approaches, the identification of new pathogenic mechanisms, and the recent development of more efficient gene transfer vectors and precise substrate reduction drugs provide hope for the development of effective treatments for Krabbe disease. This review is not meant to be exhaustive, rather it provides a brief history of Krabbe disease and follows the evolution of single and combination therapeutic approaches (see Fig. 1 and accompanying citations 1–20). Finally, this review provides a discussion of new pathogenic mechanisms and how they might impact the development of even more effective treatment strategies.

2. Critical milestones in krabbe disease
Over 100 years ago, Dr Knud H. Krabbe reported the clinical and histological findings in five cases of what he referred to as a “familial, infantile form of diffuse brain sclerosis” [1]. Children with infantile Krabbe disease are typically pre-symptomatic at birth with clinical signs appearing around six months of age. The disease progresses rapidly with children initially becoming irritable, missing developmental milestones, and progressing to death between two and four years of age. He showed that the white matter of the brain was the primary site of pathology and contained “gigantic polynuclear glia-cells”. These are now commonly referred to as ‘globoid cells’, which is the derivation of the formal name for Krabbe disease, globoid cell leukodystrophy (GLD). The gray matter appeared grossly normal, although signs of axonal and neuronal degeneration were pointed out [1]. It is now understood that Krabbe disease is an inborn error of metabolism that is inherited as an autosomal recessive disease. The clinical signs of Krabbe disease can vary greatly with infantile-, juvenile- and adult-onset forms [21]. Although hundreds of genetic variants have been identified in the GALC gene, definitive genotype-phenotype correlations have been elusive [22]. Two exceptions are a 30 kilobase pair deletion encompassing much of the GALC gene and a single point mutation (T513M), both of which are associated with the infantile form of Krabbe disease [22]. More than 50 years after the initial report, a deficiency of the acid hydrolase, galactosylceramidase (GALC), was identified as the underlying enzymatic defect [3]. This identified Krabbe disease as a member of the much larger class of lysosomal storage diseases (LSDs). Shortly thereafter, Miyatake and Suzuki (1972) showed that one of the substrates of GALC, galactosylphosphatidylcholine (GLC), was identified as psychosine, accumulated in the brains of patients with Krabbe disease [4]. This led to...
the advancement of the ‘psychosine hypothesis’ which states that the clinical presentation of Krabbe disease was due to the accumulation of psychosine in the central (CNS) and peripheral nervous systems (PNS) ultimately leading to demyelination [4]. It was recently shown that the deacylation of galactosylceramide by acid ceramidase (ACD) is the main synthetic pathway for psychosine [23]. Genetic inhibition of ACD essentially eliminated both psychosine accumulation and the clinical/behavioral signs of disease in Twitcher mice, thus providing confirmation of the psychosine hypothesis. Although the psychosine hypothesis was only recently confirmed, it was one of the fundamental insights that provided a mechanistic basis for the disease and a biochemical surrogate for experimental therapeutic endpoints.

3. Tractable animal models of Krabbe disease

Krabbe disease research has been bolstered by the fact that spontaneously arising animal models of Krabbe disease were identified well before the advent of knockout or CRISPR-Cas9 technologies (Fig. 1). By 1990 it was known that Krabbe disease was naturally occurring in five mammalian species including the mouse, cat, dog, sheep, and rhesus monkey. By 1997 the disease-causing mutations had been identified in the mouse, dog [24], and rhesus monkey [25]. The spontaneously arising murine model of Krabbe disease (Twitcher) was first reported in 1980 [5]. A nonsense mutation creates a premature stop codon in the murine GALC gene leading to nonsense-mediated mRNA decay and a complete lack of GALC activity [26]. The Twitcher mouse appears essentially normal at birth but fails to thrive and only reaches a maximum body weight of 8–10 g. At ~25 days of age the Twitcher mouse develops a tremor with progressively worsening hind limb atrophy and death by ~40 days of age. Histologically, the disease in the Twitcher mouse appears very similar to human Krabbe disease with disorganized myelin, axonal degeneration and the presence of ‘globoid cells’ throughout the white matter tracts. Due to the rapid progression, the Twitcher mouse most closely mimics the infantile form of Krabbe disease. The Twitcher mouse has been the most widely used model of Krabbe disease due to its biochemical, histological, and phenotypical similarity to the human disease and the ability to generate large numbers of genetically uniform animals. In fact, the first pre-clinical experiment that demonstrated any meaningful increase in life span in Krabbe disease was performed in the Twitcher mouse [6].

Although the Twitcher mouse has served as the primary preclinical animal model for identifying disease mechanisms and evaluating therapeutic interventions for Krabbe disease, there are inherent limitations to murine model systems. Perhaps the biggest limitation is the size and complexity of the murine brain which is ~2500 fold smaller than the human brain. The rhesus monkey model of Krabbe disease is the most analogous to the human situation with respect to the complexity and size of brain, the amino acid sequence of GALC, immune system intricacies, and lifespan. However, this model also comes with the greatest constraints due to ethical considerations, the difficulty in generating meaningful numbers of animals, and the costs associated with maintaining these animals. Although closest to humans, the primate model of Krabbe disease has seen limited use in pre-clinical experiments due largely to these practical constraints.
The most tractable large animal model of Krabbe disease is the canine. Unlike the rhesus, canines can have multiple litters per year and routinely have >5 pups per litter allowing for greater numbers of affected animals. Affected dogs appear normal at birth but by six weeks of age have an obvious tremor and pelvic limb weakness. Disease progresses to include hearing loss, pelvic limb ataxia, thoracic limb dysmetria, urinary incontinence, and finally pelvic limb paralysis at ~16 weeks of age which defines the humane endpoint [27]. The size of the dog affords clinically relevant evaluations, such as nerve conduction velocity measurements and MRI, longitudinal sampling including CSF, and use of clinically relevant approaches, including various routes of administration (intrathecal, intracerebroventricular, intravenous, etc.). The canine GALC gene was cloned and the genetic deficiency revealed in 1996 [24]. In recent years a number of natural history studies have been conducted in the Krabbe dog including electrophysiological, imaging, and biochemical markers of disease progression [24,28–30]. These benchmark studies greatly increased the utility of this model and paved the way for meaningful therapeutic evaluations in a more relevant species.

4. The evolution and current status of therapies for Krabbe disease

It was demonstrated by Neufeld and Fratantoni (1970) that lysosomal enzymes, although localized within intracellular membrane-bound organelles, can be secreted by a cell and taken up by adjacent cells [31]. This process was originally referred to as ‘cross-correction’ and now forms the basis of many therapeutic approaches for LSDs. Based largely on the principle of cross-correction, it was hypothesized that donor-derived cells of hematopoietic origin can gain access to most organ systems, including the CNS, following bone marrow transplantation (BMT) and supply therapeutic levels of lysosomal enzymes to host cells. The enzyme-positive donor-derived cells essentially serving as enzyme delivery vehicles. In 1984, Yeager and colleagues performed syngeneic BMT in young (10-day-old) Twitcher mice and were able to double the life span from ~40 days to ~80 days [6]. This established the proof-of-principle and it was subsequently shown that allogeneic BMT could slow the progression of disease in children with Krabbe disease [9]. In fact, BMT is currently the standard-of-care for children with infantile Krabbe disease. However, BMT simply slows the progression of the disease and only when initiated in pre-symptomatic children [32]. There are additional limitations to BMT, some of which are life threatening. These include the difficulty in identifying matched donors, graft rejection, graft vs host disease, and identifying pre-symptomatic children with infantile Krabbe disease. Finally, clinical data from children receiving BMT suggest that BMT does not effectively treat the PNS disease. Clearly, there is a need to develop safer and more effective therapies for Krabbe disease.

Towards that goal, there have been many other therapeutic experiments performed in the Twitcher mouse. These include, but are not limited to: ex vivo gene therapy, direct gene therapy, enzyme replacement therapy (ERT), substrate reduction therapy (SRT), antinflammatories, anti-oxidants, etc. [reviewed in [33]]. Up until very recently, and with the notable exception of ex vivo lentiviral-mediated hematopoietic-directed gene therapy, none of those therapeutic interventions matched the increase in life span achieved with BMT. Even ex vivo lentiviral-mediated, hematopoietic-directed gene therapy performed in Twitcher mice only matched BMT with an increase in life span to ~80 days [16]. This is striking given that the level of GALC expression from the lentiviral vector exceeded that...
observed in normal hematopoietic-derived cells. Collectively, these single-therapy studies were disappointing and in stark contrast to the robust response of other LSDs to some of the identical therapeutic approaches.

A breakthrough came in 2002 when Biswas and Levine combined BMT and the small molecule SRT compound, L-cycloserine, in the Twitcher mouse [11]. The hypothesis was that BMT would provide a low but persistent source of GALC activity to the CNS through cross-correction while L-cycloserine would reduce the production of psychosine. This combination increased the life span of the treated Twitcher mice to ~120 days (~40 days longer than BMT). It was also shown that the combination of CNS-directed, AAV-mediated gene therapy and BMT resulted in a similar increase in life span [15]. Similarly, the combination of systemic lentiviral-mediated gene therapy and non-ablative BMT increased the life span of Twitcher mice to ~125 days [34]. Although it was widely believed that most of the therapeutic efficacy mediated by BMT was due to cross-correction by hematopoietic-derived cells, a separate combination therapy study revealed that gene therapy provided a persistent source of GALC while BMT provided a significant anti-inflammatory effect [35]. This basic concept of combining therapies has now been replicated and improved upon by numerous groups targeting different pathogenic mechanisms, using newer generation gene transfer vectors (eg. AAVrh10 and AAV9), and delivering vectors to various compartments through different routes of administration [25,36–38]. One of the more complicated therapeutic schemes combined AAV-mediated, CNS-directed gene therapy, BMT, and SRT using L-cycloserine [18]. This triple combination not only increased the median life span of Twitcher mice to ~300 days, it also resulted in significant and persistent behavioral improvements. The synergistic effects of various combination therapy approaches are perhaps best seen when directly comparing data from a single lab using identical reagents and techniques (Fig. 2). The life spans of Twitcher animals receiving single modality therapies all cluster between 40 and 80 days. However, the addition of AAV5-mediated gene therapy to BMT increased the life span by 81 days whereas AAV5 alone only increased the life span by 31 days. Even more strikingly, the addition of L-cycloserine to the combination of BMT +AAV5 increased the life span by ~180 days. In contrast, L-cycloserine by itself increased the life span by only ~18 days.

Clearly, SRT can synergize with other therapies to greatly increase therapeutic efficacy in the Twitcher mouse. The SRT compound that has been most extensively used in pre-clinical studies is L-cycloserine. Although L-cycloserine decreases psychosine levels, it inhibits the enzyme, serum palmitoyltransferase which is four enzymatic steps upstream of psychosine synthesis [11]. Therefore, it interferes with the normal physiological levels of a number of critical lipids, in particular, ceramide. This would likely preclude its use in humans. It was recently shown that acid ceramidase (ACD) is the enzyme directly responsible for the production of psychosine [23]. Pharmacological inhibition of ACD with the anti-cancer drug, carmofur, reduced psychosine levels and increased the life span of Twitcher mice, thus validating ACD as an SRT target. Interestingly, a recent report by Martino et al., (2020) describes the creation of a new class of compounds with drug-like properties that efficiently inhibit ACD and decreases psychosine levels in the brains of Twitcher mice [39]. Another potential SRT target for Krabbe disease is ceramide galactosyltransferase (CGT). CGT is the enzyme responsible for the addition of galactose to ceramide and is only one enzymatic
step removed from psychosine synthesis [40]. A new class of brain-penetrable compounds has been described that efficiently inhibit CGT activity [40]. Although not directly tested in GALC-deficient cells or in the Twitcher mouse, these compounds efficiently decrease the levels of galactosylceramide and, therefore, would also decrease psychosine. Drugs that inhibit an enzyme that is directly responsible for (ACD), or is in closer proximity to (CGT), psychosine production should be safer and possibly more effective than targeting an enzyme that acts further upstream. Although it is unlikely that SRT will be an effective stand-alone treatment for infantile Krabbe disease, these drugs might be sufficient to halt the progression of the juvenile- or adult-onset forms of the disease. In addition, these new inhibitors might play an important role as adjunct therapies for BMT and gene therapy approaches.

Although the median life spans reported in the combination therapy studies cited above were still less than the median life span of a normal laboratory mouse (~800 900d), these findings provided a conceptual framework for the rational development of other combination therapies. However, the pre-clinical studies cited above were all performed in the Twitcher mouse and, as stated above, there are significant limitations associated with mouse models. It will be important to determine whether newer generation gene transfer vectors, and rational combination therapy approaches with safer and potentially more effective SRT drugs can be translated to a larger animal model and eventually to children with Krabbe disease.

To date, two therapeutic studies have been conducted in the Krabbe rhesus monkey. The first study utilized direct intracranial targeting of lentiviral gene therapy [41]. Specifically, one wild type and one Krabbe affected rhesus were injected with a lentiviral vector encoding human GALC into the internal capsule and thalamus. Predictably, three months post treatment inflammation was noted near the injection sites. In addition, only 3% of the injected hemisphere contained integrated lentiviral genome, which closely correlated with the mRNA levels. These data suggest that the vector had negligible spread beyond the injection site. In fact, integrated lentiviral genomes and mRNA were not detected in the spinal cord, sciatic nerve, or peripheral organs. However, cross-correction resulted in detectable GALC activity in the contralateral brain hemisphere and the spinal cord. The motor performance of the treated Krabbe rhesus was comparable to an untreated animal for 2 months post-treatment. Interestingly, 3 months post-treatment the motor score rose to within normal range. Although promising, the short 3-month duration of the study with a single animal tempers the enthusiasm of this therapeutic evaluation. With diffuse disease pathology present throughout the CNS and PNS, it seems unlikely that direct targeting of limited brain structures will be a clinically relevant approach for Krabbe disease.

In a second study, a singular 4-week-old Krabbe rhesus received 4 injections of allogeneic mesenchymal stem cells targeted to the caudate nucleus [42]. There was a transient post-treatment improvement in nerve conduction and motor scores. In addition, cognitive scores had a delayed improvement at 5 months of age, which may correlate with the improved myelination seen on MRI at 4.5 months of age. However, clinical disease rapidly progressed at 5 months of age and warranted humane euthanasia at 7 months. Histological analysis showed the characteristic and age-appropriate signs of Krabbe disease. Transient improvements in motor function and nerve conduction velocity and improved cognition...
suggest a temporary benefit from the presence of mesenchymal stem cells. This effect was likely due to the anti-inflammatory effects of MSCs and highlights the importance of focusing on secondary disease mechanisms such as inflammation for complete resolution of disease [43].

Pre-clinical experiments in the canine model of Krabbe disease have been performed with larger numbers of animals and different approaches. The combination of intravenous (3 days of age) and intracerebroventricular (ICV) (6 weeks of age) injections of AAVrh10 to target the peripheral and central nervous systems, respectively, had a variable effect on the clinical outcomes and survival at the highest dose evaluated in a limited number of dogs [44]. More recently, a robust study of intrathecal (IT) delivery of AAV9 showed a clear dose- and time-dependent effect. Varying doses of AAV encoding canine GALC were administered through the cisterna magna in pre-symptomatic and symptomatic dogs. The higher doses delivered prior to the onset of symptoms delayed disease progression, normalized clinical and biochemical readouts, and extended survival beyond 3 years of age with the study still ongoing, the longest observed in this model [45].

These results exceed those observed with a similar approach in the Twitcher mouse [36]. The authors speculate that the differences could be due to several factors. First, the dose evaluated in the Twitcher mouse was equivalent to the low dose assessed in the dogs if scaled by brain weight or CSF volume. There was a clear dose response in the dogs with the low dose only doubling life span. Thus, potentially higher doses in the Twitcher mouse would have resulted in greater survival. Second, the Twitcher mouse results from a nonsense mutation and subsequently makes no functional GALC enzyme. The mice did not receive any immunosuppression as part of the gene therapy protocol. In contrast, the Krabbe dog results from a missense mutation in which low levels of endogenous GALC activity are detectable in untreated dogs. Additionally, immunosuppression was administered to the dogs prior to gene transfer and 4 months after. Taken together, it is likely that the Twitcher mouse had less GALC activity, and the naïve immune status and lack of immunosuppression could have resulted in immune related transgene loss. Lastly, Krabbe dogs that were treated prior to symptom onset (2 weeks of age) fared better than those treated after symptom onset (6 weeks). In contrast, the Twitcher mice were treated after signs of disease were present and it has been shown by several groups that dramatic therapeutic efficacy can be achieved in the Twitcher mouse when treatment is initiated during the neonatal period. Consequently, earlier intervention could have resulted in the greater therapeutic outcomes observed in the dog compared to the mouse.

Although promising, limitations of findings in the canine model include poor biodistribution of both vector and enzyme to deep white matter structures, including the internal capsule, permitting the continued accumulation of psychosine. Additionally, while cell-specific molecular analyses were not conducted, histology demonstrates primarily neuronal transduction as opposed to the cell population of interest, oligodendrocytes. Lastly, the most effective dose used in the dogs (1E14 vg) would likely translate to >1E15 vg in pediatric patients, which is considered a very high dose. Adaptations to the current gene therapy vectors could include alternate routes of administration to enhance distribution to deep...
structures, targeting of the necessary cell population, and/or increasing the potency of the vector in order to effectively reduce the necessary dose.

Despite the rapidly improving technology in the AAV gene therapy field, the aggressive nature of Krabbe disease, in which psychosine is often present at birth, will likely require multiple tactics to halt disease progression in a timely manner and provide sustained, lifelong therapeutic benefit. This might be best accomplished with a rational and effective combination approach. However, combination therapies have yet to be reported in a large animal model of Krabbe disease. Based on studies in the murine model, it would be of interest to determine if BMT provides more complete correction of disease in the canine model, particularly in combination with lower doses of AAV. Additionally, SRT using ACD inhibitors was recently shown to reduce psychosine in the brain of Twitcher mice [39]. With once-a-day intraperitoneal injection, Twitcher mice showed a strong dose response between 30 and 90 mg/kg of the inhibitor. The canine model could have great utility in evaluating dose, biodistribution, and alternative routes of administration to better predict the pharmacodynamics of SRT drugs, alone and in combination, in a larger animal.

While the utility of the murine model for early discovery and higher throughput experiments remains clear, the use of a large animal model to provide bridging studies from mouse to human is invaluable. This is effectively illustrated by data from the canine model that is being used to advance a gene therapy approach previously reported as nominally efficacious in the Twitcher mouse. Complementary studies in an animal that more closely models the human condition such as evaluating immune responses, dose scaling, longitudinal biomarkers (serum and CSF), and meaningful outcome measures (nerve conduction and MRI) will greatly increase confidence that an investigational new drug will be safe and efficacious. In fact, both the murine and canine models of Krabbe disease are being utilized in key IND-enabling studies in preparation for translation into the clinic.

5. Looking to the future

5.1 Mechanisms of psychosine pathogenesis

Perhaps one of the greatest puzzles associated with Krabbe disease is how relatively low levels of a single molecule, psychosine, can have such pleiotropic effects. Some of the wildly disparate pathways and cellular functions that are affected include, but are not limited to, mitochondrial potential, caspases, cytochrome C, AMPK, AKT, phospholipase A2, connexin 43, PKC, calcium, damage to myelin membranes, inflammation, axolemmal swelling, dephosphorylation of neurofilaments, and dying back neuropathy [46–67].

Two major hypotheses have been put forth to explain the toxicity associated with the accumulation of psychosine. One is that psychosine exerts a non-specific “detergent effect in cell membranes”. Another is that psychosine interacts directly with various proteins independent of their association with membranes to cause its effects. Although the hypothesis that psychosine binds to the G-protein coupled T cell death-associated gene 8 receptor has been disproven [68], more recent studies have presented new evidence that psychosine may exert at least some of its toxicity in membrane-free conditions. Cantuti et al. demonstrated that increasing the levels of psychosine in the axoplasm compartment significantly, and dose-dependently reduced anterograde and retrograde fast axonal vesicular
transport [67]. The mechanism involved a psychosine-triggered phosphorylation of motor proteins in axolemma-free axoplasm preparations, which promoted the dissociation of motor proteins and membranous cargoes following the activation of GSK3β activities [69]. Importantly, this study demonstrated that psychosine’s effect did not require myelin or axonal membranes and was fully prevented by inhibition of GSK3β [67], highlighting the potential of using appropriate drugs as co-adjuvants in combined therapies. Perhaps the best example that psychosine is capable of exerting its toxicity independently of membranes came from the work by Smith et al., who were the first to identify psychosine’s capacity to decrease the solubility of α-synuclein, thus promoting the aberrant formation of α-synuclein aggregates in neurons in the Krabbe brain [70]. In fact, nuclear magnetic resonance experiments later demonstrated that psychosine was sufficient to promote fibrillization of pure monomeric α-synuclein in vitro in the absence of any membranous matrix. Under these conditions, psychosine bound to negatively charged amino acids within the carboxy terminus of α-synuclein, leading to a change in protein conformation and decreased solubility [71]. Although these results clearly show the ability of psychosine to promote toxicity via direct interaction with some protein partners, they represent only a fraction of the aberrant functions observed in Krabbe disease. The absence of other identifiable psychosine interacting molecules associated with alterations in numerous pathways strengthens the hypothesis that membrane-based events are at the core of psychosine’s pathogenic mechanism. However, how membrane-bound psychosine triggers these responses remains largely unclear.

5.2 A unifying theory on the pleiotropic effects of psychosine

A growing body of evidence has contributed to the concept that, depending on their chemical structures, lipids may partition in more (raft) or less (non-raft) rigid micro-domains within most cell membranes. Under this theory, certain lipid species such as cholesterol and various sphingolipids rearrange and coalesce in small 10—250 nm rigid domains within the planar field of the phospholipidic bilayer [72–75]. It is hypothesized that these rafts serve as platforms for various receptors and scaffolding proteins that facilitate improved signalling across the membrane and are also important for membrane curvature [76–83]. This selective partitioning provides a conceptual framework that could explain how a single lipid such as psychosine can elicit a myriad of disparate effects by altering one common target: lipid rafts.

The architecture and global behavior of a membrane is greatly influenced by its chemical composition, particularly that of its lipids. This is primarily due to how variations in the chemical structures (aliphatic tails, aromatic rings, polar and non-polar head groups, etc.) impact the molecular shape, volume, mobility, and fluidity of membranes. The shape of a lipid species modifies membrane bending, which is fundamental for cell shape, endocytosis and exocytosis and is highly influenced by the molecular volume of lipid structure. Plasma membrane lipids can largely be grouped in three categories: 1) sterols (i.e. cholesterol), which are largely non-polar planar molecules inserted within the membrane bilayer, exposing a single hydroxyl group above the membrane surface; 2) glycerophospholipids (i.e. phosphatidylcholine), composed of saturated/unsaturated fatty acyl chains and a polar head-group, and 3) glycosphingolipids, composed by a hydrophobic sphingoid base (sphingosine), in most cases acylated with fatty acyl chains to form ceramides,
and linked to head-groups such as choline (sphingomyelin), sugars (galactosylceramide, glucosylceramide) or even larger more complex head-groups (i.e. sulfatides; gangliosides). Importantly, many glycosphingolipids have lysosphingolipid species, formed by sphingosine and the corresponding sugar such as the case of galactosyl-sphingosine or psychosine in Krabbe’s disease. The spaceoccupying volume of a given lipid is highly dependent on its chemical structure. For example, while most glycerophospholipids have cylindrical shapes, and cholesterol is a planar molecule, most glycosphingolipids and lysosphingolipids are inverted cones, with a membrane-inserted ceramide/sphingoid chain capped with an outward-facing large sugar head group. The shape and volume of the lipid influences its melting temperature, and consequently, the capacity to form rigid or less-rigid raft- and non-raft micro-domains, respectively. For example, glycerophospholipids have low-melting temperatures while glycosphingolipids and cholesterol have higher-melting temperatures [84]. At physiological temperature, cholesterol and sphingolipids tend to coalesce in more rigid rafts encircled by more fluid, less-rigid non-raft areas [85]. Therefore, it stands to reason that the composition of the different lipid domains can exert global control over membrane fluidity, thus affecting the rotational and lateral mobility of individual molecules embedded in the membrane [86]. It then follows that changes in lipid composition, particularly those driven by sphingolipids such as psychosine, will significantly alter the integrity of rafts. This can then lead to perturbations in cell membrane stability [87], membrane bending [88], surface tension and fluidity [89] and raft-mediated signaling [90,91] (Fig. 3).

The concept of upstream lipid membrane raft alterations/dysfunction rather than multiple direct downstream psychosine-mediated interactions provides a unifying model to understand the pleiotropic actions of psychosine [51,55,56,65,67,92–95]. The consequence of this has the potential to affect a myriad of cellular functions and responses such as myelination, remyelination, inflammation, neurodegeneration, synaptic activity, etc. One example that supports a unified model of psychosine’s pathogenicity is the disruption of IGF signaling by psychosine. Transduction of the IGF signal involves binding of IGF to the IGF-receptor. This extracellular binding then activates a complex cascade of events involving multiple steps occurring at the membrane. These include, production of PIP3 via PI3K, which is required for the phosphorylation and subsequent translocation of AKT to the cytosol for downstream signaling [92]. Sural-Fehr et al., recently demonstrated that psychosine interferes with this signaling through a dose-dependent raft-mediated uncoupling of IGF-1 receptor phosphorylation from downstream AKT activation. Decoupling is achieved by reduced recruitment of PI3K and mTORC2 to lipid rafts [92]. It follows that other key raft-dependent pathways such as those mediated by the PDGFrα-receptor [96], EGF receptor [97], Notch receptor [98], AMPA receptor [99], complement [100] or NMDA receptor [101] may also become dysfunctional with rising levels of raft-bound psychosine. In further support of psychosine exerting its effects through a membrane-mediated mechanism is the observation that the enantiomer of psychosine disrupts artificial lipid membranes, disrupts the translocation of protein kinase C to the plasma membrane, and has equal or greater toxicity compared to native psychosine [51]. Most proteins interact with other molecules in a stereo-specific manner. In contrast, membrane interactions are typically stereo-insensitive. Therefore, the fact that the stereoisomer of psychosine

Neurosci Lett. Author manuscript; available in PMC 2022 January 31.
acts similarly to the native molecule strongly suggests that stereo-insensitive, membrane-mediated mechanisms are crucial in psychosine’s pathogenicity. In contrast, stereo-sensitive interactions might be more relevant for psychosine-protein interactions such as those with α-synuclein. In conclusion, a lipid raft model provides a single yet powerful unified theory to understand how psychosine elicits several disparate pathogenic mechanisms in Krabbe disease.

6. Conclusions and the future of therapy for Krabbe disease

The first cases of Krabbe disease were reported over 100 years ago. Despite the wealth of knowledge about the histological and clinical characteristics of the disease and availability of both small and large animal models for at least 40 years, progress towards an effective therapy for Krabbe disease has lagged behind a number of other LSDs. This is largely due to the extreme toxicity elicited by one of the undegraded substrates, psychosine, and a poor understanding of the fundamental pathogenic mechanisms. The discovery of new pathogenic mechanisms driven by membrane-bound psychosine provides the impetus to engage in high throughput screening of drugs that may promote the removal or redistribution of psychosine from membranes, much like cyclodextrin eliminates excess cholesterol in Niemann-Pick type C disease [102]. Such compounds could serve as powerful co-adjuvants for combination therapies with newer generation SRT drugs, and gene transfer vectors and improved BMT methodologies. We are experiencing an exhilarating period of rapid discovery that will certainly result in the development of more effective therapies in the very near future. Indeed, it does appear that there is new hope for an old disease.

Acknowledgements

This work was supported by the following grants: R00HD096115 to A.M.B, R01NS065808 and Legacy of Angels Foundation to E.R.B., and R01NS100779 to M.S.S. The authors would like to thank Lauren Rohde for artistic assistance with Figure 1.

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Fig. 1. Critical Milestones in our Understanding and Treatment of Krabbe disease.

Although not exhaustive, this is a timeline showing a brief history of Krabbe disease, critical milestones in our understanding of the disease, the identification of animal models, and the evolution of single and multimodal therapies. The superscript numbers associated with each milestone identifies a reference/s associated with the first example of each finding.
Fig. 2. Synergistic Effects of Combination Therapies for Krabbe disease.
Kaplan-Meier curves of treated and untreated Twitcher and wild type mice. These data are compiled from several published studies from a single laboratory using identical reagents. Therefore, these life span curves can be directly compared. Twitcher mice treated with a combination of therapies survived significantly longer than those treated with any single therapy. The median life spans of untreated, BMT-treated, AAV5-treated, and L-cycloserine-treated (SRT) Twitcher mice are 39.5, 40.5, 71, and 58 days, respectively. If the combination of BMT and AAV5 (Twi AAV2/5 + BMT) were additive, the predicted median life span would be 70–75 days. However, the median life span for Twi AAV2/5 + BMT mice is ~120 days. If the effects of combining SRT (L-Cyc) with AAV5 + BMT (Twi AAV2/5 + BMT + L-Cyc) were additive, the predicted median life span would be 135–140 days. In actuality, the median life span of Twi AAV2/5 + BMT + L-Cyc mice is ~300 days. Alone, L-Cycloserine increases the life span of Twitcher mice by ~18 days. When added to AAV5 and BMT, L-Cycloserine adds an additional ~175 days to the median life span; clearly synergistic.
Fig. 3. A Unifying Theory to Understand Psychosine Pathogenic Mechanism: Disruption of Lipid Raft Architecture and Function.

The lipid raft microdomain theory provides a powerful platform to start understanding how psychosine triggers a broad spectrum of downstream pathogenic responses. Under physiological conditions (Panel A), sterols such as cholesterol and glycosphingolipids such as sphingomyelin, gangliosides, sulfatides and galactosylceramides tend to coalesce in more rigid lipid microdomains (also known as rafts). These domains provide platforms where multiple other components such as scaffolding proteins, receptors, etc participate in cell signalling, can interact with optimal efficiency. When GALC activity is present, psychosine homeostasis is maintained. In contrast, psychosine remains undegraded in the absence of sufficient GALC activity as observed in Krabbe disease (Panel B). Consequently, psychosine accumulates to toxic levels in lipid rafts, modifying fluidity and lateral mobility of raft-associated components. The figure illustrates the example of how psychosine interferes with the IGF-PIP3-AKT pathway in neurons [92]. Although the components of the IGF pathway remain essentially unaltered in Krabbe disease, psychosine accumulation in rafts deforms and alters the chemical composition of these microdomains, impeding the association of raft components and transduction of the AKT signal to the cytosol. In panel C, the application of this unifying raft theory facilitates our understanding of how psychosine may alter other unrelated pathways (e.g. Notch, EGF, PDGF, complement, and neurotransmitter receptors) impacting on distinct cellular aspects from membrane shedding [103] to myelin stability [104] to neuronal/synaptic function [94].