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# 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 galactosylceramides and sphingolipids, which are abundant in myelin membranes. The absence of GALC leads to the toxic accumulation of galactosylsphingosine (psychosine), a lysoderivative of galactosylceramides, 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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**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, galactosylsphingosine, commonly referred to 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), antiinflammatories, 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, serine 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 lysophingolipid 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 PDGFα-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

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.

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## References

[1]. Krabbe K, A new familial, infantile form of diffuse brain-sclerosis, Brain 39 (1916) 74–114.

[2]. Fletcher TF, Kurtz HJ, Low DG, Globoid cell leukodystrophy (Krabbe type) in the dog, J. Am. Vet. Med. Assoc 149 (1966) 165–172. [PubMed: 5950438]

[3]. Suzuki K, Suzuki Y, Globoid cell leukodystrophy (Krabbe disease): deficiency of galactocerebroside β-galactosidase, Proc. Natl. Acad. Sci 66 (1970) 302–309. [PubMed: 5271165]

[4]. Miyatake T, Suzuki K, Globoid cell leukodystrophy: additional deficiency of psychosine galactosidase, Biochem. Biophys. Res. Commun 48 (1972) 538–543.

[5]. Duchen LW, Eicher EM, Jacobs JM, Scaravilli F, Teixeira F, Hereditary leucodystrophyin the mouse: the new mutant twitcher, Brain 103 (1980) 695–710. [PubMed: 7417782]

[6]. Yeager AM, Brennan S, Tiffany C, Moser HW, Santos GW, Prolonger survival and remyelination after hematopoietic cell transplantation in the twitcher mouse, Science 225 (1984) 1052–1054. [PubMed: 6382609]

[7]. Baskin G, Alroy J, Li YT, Dayal Y, Raghavan SS, Charer L, Galactosylceramide lipidosis in Rhesus monkeys, Lab. Invest 60 (1989) 7A.

[8]. Chen YQ, Rafi MA, de Gala G, Wenger DA, Cloning and expression of of cDNA encoding human galactocerebrosidase, the enzyme deficient in globoid cell leukodystrophy, Hum. Mol. Genet 2 (1993) 184.

[9]. Krivit W, Shapiro EG, Peters C, Wagner JE, Cornu G, Kurtzberg J, Wenger DA, Kolodny EH, Vanier MT, Loes DJ, Dusenbery K, Lockman LA, Hematopoietic stem cell transplantation in globoid cell leukodystrophy, N. Engl. J. Med 338 (1998) 1119–1126. [PubMed: 9545360]

[10]. LeVine SM, Pedechenko TV, Bronshteyn IG, Pinson DM, L-cycloserine slows the clinical and pathological course in mice with globoid cell leukodystrophy (Twitcher mice), J. Neurosci. Res 60 (2000) 231–236. [PubMed: 10740228]

[11]. Biswas S, LeVine SM, Substrate reduction therapy enhances the benefits of bone marrow transplantation in young mice with globoid cell leukodystrophy, Pedagog 51 (2002) 40–47.

[12]. Rafi MA, Rao HZ, Passini MA, Curtis M, Vanier MT, Zaka M, Luzi P, Wolfe JH, Wenger DA, AAV-mediated expression of galactocerebrosidase in brain results in attenuated symptoms and extended life span in murine models of globoid cell leukodusytrophy, Mol. Ther 11 (2005) 734–744. [PubMed: 15851012]

[13]. Lin DS, Fantz CR, Levy B, Rafi MA, Vogler C, Wenger DA, Sands MS, AAV2/5 vector expressing galactocerebrosidase ameliorates CNS disease in the murine model of globoid cell leukodystrophy more efficiently then AAV2, Mol. Ther 12 (2005) 422–430. [PubMed: 15996520]

[14]. Lee WC, Tsoi YK, Troendle FJ, DeLucia MW, Ahmed Z, Dicky CA, Dickson DW, Eckman CB, Single-dose intracerebroventricular administration of galactocerebrosidase improves survival in a mouse model of globoid cell leukodystrophy, FASEB J 21 (2007) 2520–2527. [PubMed: 17403939]

[15]. Lin DS, Donsante A, Macauley S, Levy B, Vogler C, Sands MS, Central nervous system-directed AAV2/5-mediated gene therapy synergizes with bone marrow transplant in the murine model of globoid cell leukodystrophy, Mol. Ther 15 (2007) 44–52. [PubMed: 17164774]

[16]. Gentner B, Visigalli I, Hiramatsu H, Lechman E, Ungari S, Giustacchini A, Schira G, Amandola M, Quattrini A, Martino S, Orlacchio A, Dick JE, Biffi A, Naldini L, Identification of hematopoietic stem cell-specific miRNAs enables gene therapy of globoid cell leukodystrophy, Sci. Transl. Med 2 (2010), 58ra84.

[17]. Rafi MA, Rao HZ, Luzi P, Curtis MT, Wenger DA, Extended normal life after AAVrh10-mediated gene therapy in the mouse model of Krabbe disease, Mol. Ther 20 (2012) 2031–2042. [PubMed: 22850681]

[18]. Hawkins-Salsbury JA, Shea L, Xiang X, Hunter DA, Guzman AM, Reddy AS, Qin EY, Li Y, Gray SJ, Ory DS, Sands MS, Mechanism-based combination treatment dramatically increases therapeutic efficacy in murine globoid cell leukodystrophy, J. Neurosci 35 (2015) 6495–6505. [PubMed: 25904800]

[19]. Meneghini V, Lattanzi A, Tiradani L, Bravo G, Moreno F, Sanvito F, Calabria A, Bringas J, Fisher-Perkins JM, Dufour JP, Baker KC, Doglioni C, Montini E, Bunnell BA, Bankiewicz K, Martino S, Naldini L, Gritti A, Pervasive supply of therapeutic lysosomal enzymes in the CNS of normal and Krabbe-affected non-human primates by intracerebral lentiviral gene therapy, EMBO Mol, Med 8 (2016) 489–510. [PubMed: 27025653]

[20]. Bradbury AM, Bagel JH, Nguyen D, Lykken EA, Salvador JP, Jiang X, Swain GP, Assenmacher CA, Hendricks IJ, Miyadera K, Hess RS, Ostrager A, ODonnell P, Sands MS, Ory DS, Shelton GD, Bongarzone ER, Gray SJ, Vite CH, Krabbe disease successfully treated via monotherapy of intrathecal gene therapy, J. Clin. Invest 130 (2020) 4906–4920. [PubMed: 32773406]

[21]. Wenger DA, Suzuki K, Suzuki Y, Suzuki K, Galactosylceramide lipidosis: globoid cell leukodystrophy (krabbe disease), in: Scriver CR, Beaudet A, Sly WS, Valle D (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001, pp. 3669–3694.

[22]. Kleijer WJ, Keulemans JLM, van der Kraan M, Geilen GG, van der Helm RM, Rafi MA, Luzi P, Wenger DA, Halley DJJ, van Diggelen OP, Prevalent mutations in the GALC gene of patients with Krabbe disease of Dutch and other European origin, J Inher Metab Dis 20 (1997) 587–594. [PubMed: 9266397]

[23]. Li Y, Xu Y, Benitez BA, Nagree MS, Dearborn JT, Jiang X, Guzman MA, Woloszynek JC, Giaramita A, Yip BK, Elsbernd J, Babcock MC, Lo M, Fowler SC, Wozniak DF, Vogler CA, Medin JA, Crawford BE, Sands MS, Genetic ablation of acid ceramidase in Krabbe disease

confirms the psychosine hypothesis and identifies a new therapeutic target, Proc. Natl. Acad. Sci 116 (2019) 20097–20103. [PubMed: 31527255]

[24]. Victoria T, Rafi MA, Wenger DA, Cloning of the canine GALC cDNA and identification of the mutation causing globoid cell leukodystrophy in West Highland White and Cairn terriers, Genomics 33 (1996) 457–462. [PubMed: 8661004]

[25]. Luzi P, Rafi MA, Victoria T, Baskin GB, Wenger DA, Characterization of the rhesus monkey galactocerebrosidase (GALC) cDNA and gene and identification of the mutation causing globoid cell leukodystrophy (Krabbe disease) in this primate, Genomics 42 (1997) 319–324. [PubMed: 9192853]

[26]. Sakai N, Inui K, Tatsumi N, Fukushima H, Nishigaki T, Taniike M, Nishimoto J, Tsukamoto H, Yanagihara I, Ozono K, Okada S, Molecular cloning and expression of cDNA for murine galactocerebrosidase and mutation analysis of the Twitcher mouse, model of Krabbe's disease, J. Neurochem 66 (1996) 1118–1124. [PubMed: 8769874]

[27]. Bradbury AM, Bagel JH, Jiang X, Swain GP, Prociuk ML, Fitzgerald CA, O'Donnell PA, Braund KG, Ory DS, Vite CH, Clinical, electrophysiological, and biochemical markers of peripheral and central nervous system disease in canine globoid cell leukodystrophy (Krabbe's disease), J. Neurosci. Res 94 (2016) 1007–1017. [PubMed: 27638585]

[28]. Bradbury A, Peterson D, Vite C, Chen S, Ellinwood NM, Provenzale J, Diffusion tensor imaging analysis of the brian in the canine model of Krabbe disease, Neuroradiol. J 29 (2016) 417–424. [PubMed: 27677296]

[29]. Li JY, Middleton DM, Chen S, White L, Corado CR, Vite C, Bradbury A, Provenzale JM, Quantitative DTI metrics in a canine model of Krabbe disease: comparisons versus age-matched controls across multiple ages, Neuroradiol. J 31 (2018) 168–176. [PubMed: 29350082]

[30]. Corado CR, Pinkstaff J, Xiang X, Galban EM, Fisher SJ, Scholler O, Russell C, Bagel JH, O'Donnell PA, Ory DS, Vite CH, Bradbury AM, Cerebrospinal fluid and serum glycosphingolipid biomarkers in canine globoid cell leukodystrophy (Krabbe disease), Mol. Cell. Neurosci 102 (2020), 103451. [PubMed: 31794880]

[31]. Neufeld EF, Fratantoni JC, Inborn errors in mucopolysaccharide metabolism, Science 169 (1970) 141–146. [PubMed: 4246678]

[32]. Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S, Wenger DA, Pietryga D, Wall D, Champagne M, Morse R, Krivit W, Kurtzberg J, Transplantation of umbilical-cord blood in babies with infantile Krabbe disease, N. Engl. J. Med 352 (2005) 2069–2081. [PubMed: 15901860]

[33]. Mikulka CR, Sands MS, Treatment for Krabbe's disease: finding the combination, J. Neurosci. Res 94 (2016) 1126–1137. [PubMed: 27638598]

[34]. Galbiati F, Givorgi MI, Cantuti L, Rosas AL, Cao H, van Breemen R, Bongarzone ER, Combined hematopoietic and lentiviral gene transfer therapies in newborn Twitcher mice reveal contemporaneous neurodegeneration and demyelination in Krabbe disease, J. Neurosci. Res 87 (2009) 1748–1759. [PubMed: 19185028]

[35]. Reddy AS, Kim JH, Hawkins-Salsbury JA, Macauley SL, Tracy ET, Vogler CA, Han X, Song SK, Wozniak DF, Fowler SC, Klein RS, Sands MS, Bone marrow transplantation augments the effects of brain- and spinal corddirected adeno-associated virus 2/5 gene therapy by altering inflammation in the murine model of globoid cell leukodystrophy, J. Neurosci 31 (2011) 9945–9957. [PubMed: 21734286]

[36]. Karumuthil-Melethil S, Marshall MS, Heindel C, Jakubauskas B, Bongarzone ER, Gray SJ, Intrathecal administration of AAV/GALC vectors in 10–11 day old Twitcher mice improves survival and is enhanced by bone marrow transplant, J. Neurosci. Res 94 (2016) 1138–1151. [PubMed: 27638599]

[37]. Marshall MS, Issa Y, Jakubauskas B, Stoskute M, Elackattu V, Marshal JN, Bogue W, Ngyuen D, Hauck Z, Rue E, Karumuthil-Melethil S, Zaric V, Bosland M, van Breemen RB, Civorgi MI, Gray SJ, Crocker SJ, Bongarzone ER, Long-term improvement of neurological signs and metabolic dysfunction in a mouse model of Krabbe's disease after global gene therapy, Mol. Ther 26 (2018) 874–889. [PubMed: 29433937]

[38]. Rafi MA, Luzi P, Wenger DA, Conditions for combining gene therapy with bone marrow transplantation in murine Krabbe disease, BioImpacts 10 (2020) 105–115. [PubMed: 32363154]

[39]. Martino SD, Tardia P, Cilibrasi V, Caputo S, Mazzonna M, Russo D, Penna I, Realini N, Margaroli N, Migliore M, Pizzirani D, Ottonello G, Bertozzi SM, Armirotti A, Ngyuen D, Sun Y, Bongarzone ER, Lansbury P, Liu M, Skerlj R, Scarpelli R, Lead optimization of benzoxazolone carboxamides as orally bioavailable and CNS penetrant acid ceramidase inhibitors, J. Med. Chem 63 (2020) 3634–3664. [PubMed: 32176488]

[40]. Thurairatnam S, Lim S, Baker RH, Choi-Sledeski YM, Hirth BH, Jiang J, Macor JE, Makino E, Maniar S, Musick K, Pribish JR, Munson M, Brain penetrable inhibitors of ceramide galactosyltransferase for the treatment of lysosomal storage disorders, ACS Med. Chem. Lett 11 (2020) 2010–2016. [PubMed: 33062186]

[41]. Meneghini V, Lattanzi A, Tiradani L, Bravo G, Morena F, Sanvito F, Calabria A, Bringas J, Fisher-Perkins JM, Dufour JP, Baker KC, Doglioni C, Montini E, Bunnell BA, Bankiewicz K, Martino S, Naldini L, Gritti A, Pervasive supply of therapeutic lysosomal enzymes in the CNS of normal and Krabbe-affected non-human primates by intracerebral lentiviral gene therapy, EMBO Mol. Med 8 (2016) 489–510. [PubMed: 27025653]

[42]. Isakova IA, Baker KC, Dufour J, Phinney DG, Mesenchymal stem cells yield transient improvements in motor function in an infant rhesus macaque with severe early-onset Krabbe disease, Stem Cells Transl. Med 6 (2017) 99–109. [PubMed: 28170189]

[43]. Scruggs BA, Zhang X, Bowles AC, Gold PA, Semon JE, Fisher-Perkins JM, Zhang S, Bonvillain RW, Myers L, Li SC, Kalueff AV, Bunnell BA, Multipotent stromal cells alleviate inflammation, neuropathy, and symptoms associated with globoid cell leukodystrophy in the Twitcher mouse, Stem Cells 31 (2013) 1523–1534. [PubMed: 23606584]

[44]. Bradbury AM, Rafi MA, Bagel JH, Brisson BK, Marshal MS, Pesayco-Salvador J, Jiang X, Swain GP, Prociuk ML, O'Donnell PA, Fitzgerald C, Ory DS, Bongarzone ER, Shelton GD, Wenger DA, Vite CH, Hum. Gene Ther 29 (2018) 785–801.

[45]. Bradbury AM, Bagel JH, Nguyen D, Lykken EA, Pesayco-Salvador J, Jiang X, Swain GP, Assenmacher CA, Hendricks IJ, Miyadera K, Hess RS, Ostrager A, O'Donnell P, Sands MS, Ory DS, Shelton GD, Bongarzone ER, Gray SJ, Vite CH, J. Clin. Invest 130 (2020) 4906–4920. [PubMed: 32773406]

[46]. Cho KH, Kim MW, Kim SU, Tissue culture model of Krabbe's disease: psychosine cytotoxicity in rat oligodendrocyte culture, Dev. Neurosci 19 (1997) 321–327. [PubMed: 9215877]

[47]. Giri S, Khan M, Nath N, Singh I, Singh AK, The role of AMPK in psychosine mediated effects on oligodendrocytes and astrocytes: implication for Krabbe disease, J. Neurochem 105 (2008) 1820–1833. [PubMed: 18248608]

[48]. Giri S, Khan M, Rattan R, Singh I, Singh AK, Krabbe disease: psychosine-mediated activation of phospholipase A2 in oligodendrocyte cell death, J. Lipid Res 47 (2006) 1478–1492. [PubMed: 16645197]

[49]. Graziano AC, Parenti R, Avola R, Cardile V, Krabbe disease: involvement of connexin43 in the apoptotic effects of sphingolipid psychosine on mouse oligodendrocyte precursors, Apoptosis 21 (2016) 25–35. [PubMed: 26459425]

[50]. Haq E, Giri S, Singh I, Singh AK, Molecular mechanism of psychosine-induced cell death in human oligodendrocyte cell line, J. Neurochem 86 (2003) 1428–1440. [PubMed: 12950451]

[51]. Hawkins-Salsbury JA, Parameswar AR, Jiang X, Schlesinger PH, Bongarzone E, Ory DS, Demchenko AV, Sands MS, Psychosine, the cytotoxic sphingolipid that accumulates in globoid cell leukodystrophy, alters membrane architecture, J. Lipid Res 54 (2013) 3303–3311. [PubMed: 24006512]

[52]. Inamura N, Kito M, Go S, Kishi S, Hosokawa M, Asai K, Takakura N, Takebayashi H, Matsuda J, Enokido Y, Developmental defects and aberrant accumulation of endogenous psychosine in oligodendrocytes in a murine model of Krabbe disease, Neurobiol. Dis 120 (2018) 51–62. [PubMed: 30176352]

[53]. Smith B, Galbiati F, Castelvetri LC, Givogri MI, Lopez-Rosas A, Bongarzone ER, Peripheral neuropathy in the Twitcher mouse involves the activation of axonal caspase 3, ASN Neuro 3 (2011).

[54]. Voccoli V, Tonazzini I, Signore G, Caleo M, Cecchini M, Role of extracellular calcium and mitochondrial oxygen species in psychosine-induced oligodendrocyte cell death, Cell Death Dis 5 (2014) e1529. [PubMed: 25412308]

[55]. White AB, Galbiati F, Givogri MI, Lopez Rosas A, Qiu X, van Breemen R, Bongarzone ER, Persistence of psychosine in brain lipid rafts is a limiting factor in the therapeutic recovery of a mouse model for Krabbe disease, J. Neurosci. Res 89 (2011) 352–364. [PubMed: 21259322]

[56]. White AB, Givogri MI, Lopez-Rosas A, Cao H, van Breemen R, Thinakaran G, Bongarzone ER, Psychosine accumulates in membrane microdomains in the brain of krabbe patients, disrupting the raft architecture, J. Neurosci 29 (2009) 6068–6077. [PubMed: 19439584]

[57]. Won JS, Kim J, Paintlia MK, Singh I, Singh AK, Role of endogenous psychosine accumulation in oligodendrocyte differentiation and survival: implication for Krabbe disease, Brain Res 1508 (2013) 44–52. [PubMed: 23438514]

[58]. Zaka M, Wenger DA, Psychosine-induced apoptosis in a mouse oligodendrocyte progenitor cell line is mediated by caspase activation, Neurosci. Lett 358 (2004) 205–209. [PubMed: 15039117]

[59]. Claycomb KI, Winokur PN, Johnson KM, Nicaise AM, Giampetruzzi AW, Sacino AV, Snyder EY, Barbarese E, Bongarzone ER, Crocker SJ, Aberrant production of tenascin-C in globoid cell leukodystrophy alters psychosine-induced microglial functions, J. Neuropathol. Exp. Neurol. 73 (2014) 964–974. [PubMed: 25192051]

[60]. Ijichi, Brown GD, Moore CS, Lee JP, Winokur PN, Pagarigan R, Snyder EY, Bongarzone ER, Crocker SJ, MMP-3 mediates psychosine-induced globoid cell formation: implications for leukodystrophy pathology, Glia 61 (2013) 765–777. [PubMed: 23404611]

[61]. LeVine SM, Brown DC, IL-6 and TNFalpha expression in brains of twitcher, quaking and normal mice, J. Neuroimmunol 73 (1997) 47–56. [PubMed: 9058758]

[62]. Ohno, Komiyama A, Martin PM, Suzuki K, Proliferation of microglia/ macrophages in the demyelinating CNS and PNS of twitcher mouse, Brain Res 602 (1993) 268–274. [PubMed: 8448672]

[63]. Snook ER, Fisher-Perkins JM, Sansing HA, Lee KM, Alvarez X, MacLean AG, Peterson KE, Lackner AA, Bunnell BA, Innate immune activation in the pathogenesis of a murine model of globoid cell leukodystrophy, Am. J. Pathol 184 (2014) 382–396. [PubMed: 24316110]

[64]. Kondo Y, Adams JM, Vanier MT, Duncan ID, Macrophages counteract demyelination in a mouse model of globoid cell leukodystrophy, J. Neurosci 31 (2011) 3610–3624. [PubMed: 21389217]

[65]. Castelvetri LC, Givogri MI, Zhu H, Smith B, Lopez-Rosas A, Qiu X, van Breemen R, Bongarzone ER, Axonopathy is a compounding factor in the pathogenesis of Krabbe disease, Acta Neuropathol 122 (2011) 35–48. [PubMed: 21373782]

[66]. Cantuti-Castelvetri L, Zhu H, Givogri MI, Chidavaenzi RL, Lopez-Rosas A, Bongarzone ER, Psychosine induces the dephosphorylation of neurofilaments by deregulation of PP1 and PP2A phosphatases, Neurobiol. Dis 46 (2012) 325–335. [PubMed: 22326830]

[67]. Cantuti Castelvetri L, Givogri MI, Hebert A, Smith B, Song Y, Kaminska A, Lopez-Rosas A, Morfini G, Pigino G, Sands M, Brady ST, Bongarzone ER, The sphingolipid psychosine inhibits fast axonal transport in Krabbe disease by activation of GSK3beta and deregulation of molecular motors, J. Neurosci 33 (2013) 10048–10056. [PubMed: 23761900]

[68]. Im DS, Heise CE, Nguyen T, O'Dowd BF, Lynch KR, Identification of a molecular target of psychosine and its role in globoid cell formation, J. Cell Biol 153 (2001) 429–434. [PubMed: 11309421]

[69]. Morfini G, Pigino G, Beffert U, Busciglio J, Brady ST, Fast axonal transport misregulation and Alzheimer's disease, Neuromolecular Med 2 (2002) 89–99. [PubMed: 12428805]

[70]. Smith BR, Santos MB, Marshall MS, Cantuti-Castelvetri L, Lopez-Rosas A, Li G, van Breemen R, Claycomb KI, Gallea JI, Celej MS, Crocker SJ, Givogri MI, Bongarzone ER, Neuronal inclusions of alpha-synuclein contribute to the pathogenesis of Krabbe disease, J. Pathol 232 (2014) 509–521. [PubMed: 24415155]

[71]. Abdelkarim H, Marshall MS, Scesa G, Smith RA, Rue E, Marshall J, Elackattu V, Stoskute M, Issa Y, Santos M, Nguyen D, Hauck Z, van Breemen R, Celej MS, Gaponenko V, Bongarzone ER, Alpha-Synuclein interacts directly but reversibly with psychosine: implications for alpha-synucleinopathies, Sci. Rep 8 (2018) 12462. [PubMed: 30127535]

[72]. Singer SJ, Nicolson GL, The fluid mosaic model of the structure of cell membranes, Science 175 (1972) 720–731. [PubMed: 4333397]

[73]. Simons K, Ikonen E, Functional rafts in cell membranes, Nature 387 (1997) 569–572. [PubMed: 9177342]

[74]. Lingwood D, Simons K, Lipid rafts as a membrane-organizing principle, Science 327 (2010) 46–50. [PubMed: 20044567]

[75]. Carquin M, D'Auria L, Pollet H, Bongarzone ER, Tyteca D, Recent progress on lipid lateral heterogeneity in plasma membranes: from rafts to submicrometric domains, Prog. Lipid Res 62 (2016) 1–24. [PubMed: 26738447]

[76]. Ramstedt B, Slotte JP, Membrane properties of sphingomyelins, FEBS Lett 531 (2002) 33–37. [PubMed: 12401199]

[77]. Fidorra M, Duelund L, Leidy C, Simonsen AC, Bagatolli LA, Absence of fluid-ordered/fluid-disordered phase coexistence in ceramide/POPC mixtures containing cholesterol, Biophys. J 90 (2006) 4437–4451. [PubMed: 16565051]

[78]. Dietrich C, Bagatolli LA, Volovyk ZN, Thompson NL, Levi M, Jacobson K, Gratton E, Lipid rafts reconstituted in model membranes, Biophys. J 80 (2001) 1417–1428. [PubMed: 11222302]

[79]. Kahya N, Scherfeld D, Bacia K, Poolman B, Schwille P, Probing lipid mobility of raft-exhibiting model membranes by fluorescence correlation spectroscopy, J. Biol. Chem 278 (2003) 28109–28115. [PubMed: 12736276]

[80]. Pinto SN, Silva LC, de Almeida RF, Prieto M, Membrane domain formation, interdigitation, and morphological alterations induced by the very long chain asymmetric C24:1 ceramide, Biophys. J 95 (2008) 2867–2879. [PubMed: 18586849]

[81]. Bernardino de la Serna J, Perez-Gil J, Simonsen AC, Bagatolli LA, Cholesterol rules: direct observation of the coexistence of two fluid phases in native pulmonary surfactant membranes at physiological temperatures, J. Biol. Chem 279 (2004) 40715–40722. [PubMed: 15231828]

[82]. Baumgart T, Hammond AT, Sengupta P, Hess ST, Holowka DA, Baird BA, Webb WW, Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles, Proc Natl Acad Sci U S A 104 (2007) 3165–3170. [PubMed: 17360623]

[83]. Plasencia I, Norlen L, Bagatolli LA, Direct visualization of lipid domains in human skin stratum corneum's lipid membranes: effect of pH and temperature, Biophys. J 93 (2007) 3142–3155. [PubMed: 17631535]

[84]. de Almeida RF, Fedorov A, Prieto M, Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts, Biophys. J 85 (2003) 2406–2416. [PubMed: 14507704]

[85]. Bagatolli LA, Ipsen JH, Simonsen AC, Mouritsen OG, An outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes, Prog. Lipid Res 49 (2010) 378–389. [PubMed: 20478336]

[86]. Lenaz G, Lipid fluidity and membrane protein dynamics, Biosci. Rep 7 (1987) 823–837. [PubMed: 3329533]

[87]. McMahon HT, Boucrot E, Membrane curvature at a glance, J. Cell. Sci 128 (2015) 1065–1070. [PubMed: 25774051]

[88]. Cooke IR, Deserno M, Coupling between lipid shape and membrane curvature, Biophys. J 91 (2006) 487–495. [PubMed: 16807230]

[89]. Mollinedo F, Gajate C, Lipid rafts as major platforms for signaling regulation in cancer, Adv. Biol. Regul 57 (2015) 130–146. [PubMed: 25465296]

[90]. Gomez-Mouton C, Lacalle RA, Mira E, Jimenez-Baranda S, Barber DF, Carrera AC, Martinez AC, Manes S, Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis, J. Cell Biol 164 (2004) 759–768. [PubMed: 14981096]

[91]. Iwabuchi K, Nakayama H, Iwahara C, Takamori K, Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction, FEBS Lett 584 (2010) 1642–1652. [PubMed: 19852959]

[92]. Sural-Fehr T, Singh H, Cantuti-Catelvetri L, Zhu H, Marshall MS, Rebiai R, Jastrzebski MJ, Givogri MI, Rasenick MM, Bongarzone ER, Inhibition of the IGF-1-PI3K-Akt-mTORC2 pathway in lipid rafts increases neuronal vulnerability in a genetic lysosomal glycosphingolipidosis, Dis. Model. Mech 12 (2019).

[93]. Cantuti-Castelvetri L, Zhu H, Givogri MI, Chidavaenzi RL, Lopez-Rosas A, Bongarzone ER, Psychosine induces the dephosphorylation of neurofilaments by deregulation of PP1 and PP2A phosphatases, Neurobiol. Dis 46 (2012) 325–335. [PubMed: 22326830]

[94]. Cantuti-Castelvetri L, Bongarzone ER, Synaptic failure: the achilles tendon of sphingolipidoses, J. Neurosci. Res 94 (2016) 1031–1036. [PubMed: 27638588]

[95]. Cantuti-Castelvetri L, Maravilla E, Marshall M, Tamayo T, D'Auria L, Monge J, Jeffries J, Sural-Fehr T, Lopez-Rosas A, Li G, Garcia K, van Breemen R, Vite C, Garcia J, Bongarzone ER, Mechanism of neuromuscular dysfunction in Krabbe disease, J. Neurosci 35 (2015) 1606–1616. [PubMed: 25632136]

[96]. Pituch KC, Moyano AL, Lopez-Rosas A, Marottoli FM, Li G, Hu C, van Breemen R, Mansson JE, Givogri MI, Dysfunction of platelet-derived growth factor receptor alpha (PDGFRalpha) represses the production of oligodendrocytes from arylsulfatase A-deficient multipotential neural precursor cells, J. Biol. Chem 290 (2015) 7040–7053. [PubMed: 25605750]

[97]. Liu YT, Song L, Templeton DM, Heparin suppresses lipid raft-mediated signaling and ligand-independent EGF receptor activation, J. Cell. Physiol 211 (2007) 205–212. [PubMed: 17226785]

[98]. Watanabe K, Nagaoka T, Lee JM, Bianco C, Gonzales M, Castro NP, Rangel MC, Sakamoto K, Sun Y, Callahan R, Salomon DS, Enhancement of Notch receptor maturation and signaling sensitivity by Cripto-1, J. Cell Biol 187 (2009) 343–353. [PubMed: 19948478]

[99]. Zhang L, Zhang P, Wang G, Zhang H, Zhang Y, Yu Y, Zhang M, Xiao J, Crespo P, Hell JW, Lin L, Huganir RL, Zhu JJ, Ras and rap signal bidirectional synaptic plasticity via distinct subcellular microdomains, Neuron 98 (783–800) (2018) e784. [PubMed: 29706584]

[100]. Presumey J, Bialas AR, Carroll MC, Complement system in neural synapse elimination in development and disease, Adv. Immunol 135 (2017) 53–79. [PubMed: 28826529]

[101]. Delint-Ramirez I, Fernandez E, Bayes A, Kicsi E, Komiyama NH, Grant SG, In vivo composition of NMDA receptor signaling complexes differs between membrane subdomains and is modulated by PSD-95 and PSD-93, J. Neurosci 30 (2010) 8162–8170. [PubMed: 20554866]

[102]. Davidson CD, Ali NF, Micsenyl MC, Stephny G, Renault S, Dobrenis K, Ory DS, Vanier MT, Walkley SU, Chronic cyclodextrin treatment of murine Niemann-Pick C disease ameliorates neuronal cholesterol and glycosphingolipid storage and disease progression, PLoS One 4 (2009) e6951. [PubMed: 19750228]

[103]. D'Auria L, Reiter C, Ward E, Moyano AL, Marshall MS, Nguyen D, Scesa G, Hauck Z, van Breemen R, Givogri MI, Bongarzone ER, Psychosine enhances the shedding of membrane microvesicles: implications in demyelination in Krabbe's disease, PLoS One 12 (2017), e0178103. [PubMed: 28531236]

[104]. Takahashi H, Suzuki K, Demyelination in the spinal cord of murine globoid cell leukodystrophy (the twitcher mouse), Acta Neuropathol 62 (1984) 298–308. [PubMed: 6730907]

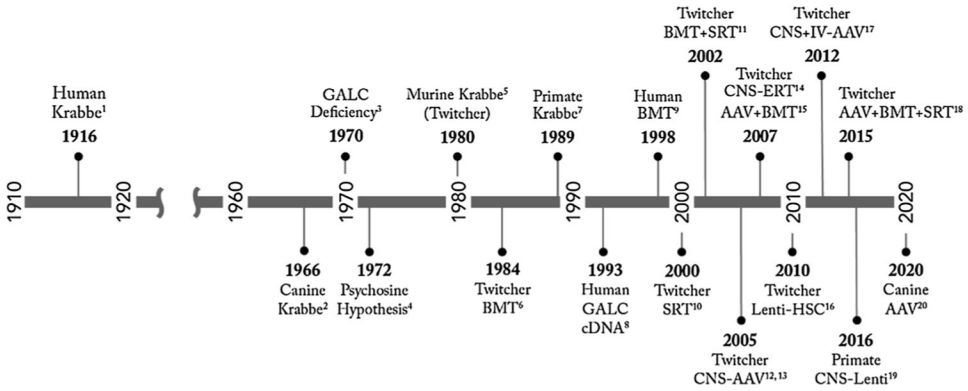


**Fig. 1. Critical Milestones in our Understanding and Treatment of Krabbe disease.**

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.

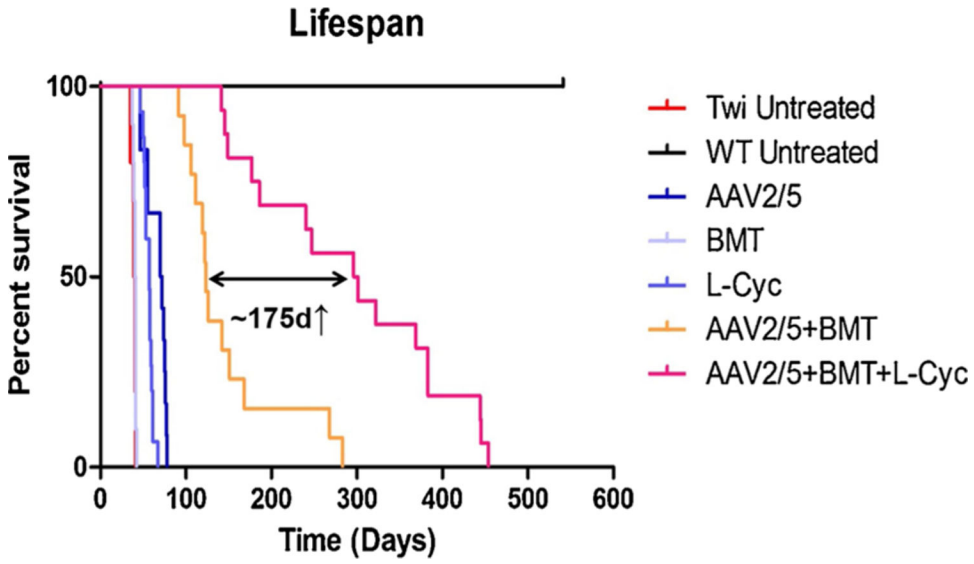


**Fig. 2. Synergistic Effects of Combination Therapies for Krabbe disease.**

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.

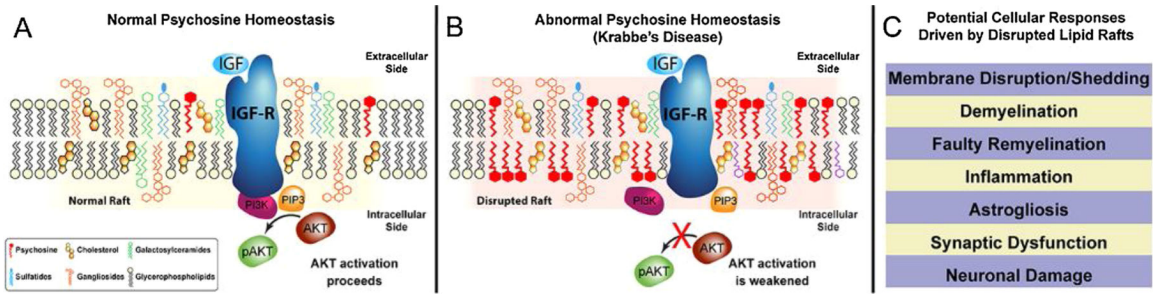


**Fig. 3. A Unifying Theory to Understand Psychosine Pathogenic Mechanism: Disruption of Lipid Raft Architecture and Function.**

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].