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Review Article

From pathological mechanisms in Krabbe disease to cutting-edge therapy: A comprehensive review

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Since its initial documentation by Knud Krabbe in 1916, numerous studies have scrutinized the characteristics of Krabbe disease (KD) until the identification of the mutation in the GALC gene. In alignment with that, we investigated the natural history of KD spanning eight decades to gain a deeper understanding of the evolutionary trajectory of its mechanisms. Through our comprehensive analysis, we unearthed additional novel elements in molecular biology involving the micropathological mechanism of the disease. This review offers an updated perspective on the metabolic disorder that defines KD. Recently, extracellular vesicles (EVs), autophagy impairment, and α-synuclein have emerged as pivotal players in the neuropathological processes. EVs might serve as a cellular mechanism to avoid or alleviate the detrimental impacts of excessive toxic psychosine levels, and extracting EVs could contribute to synapse dysfunction. Autophagy impairment was found to be independent of psychosine and reliant on AKT and B-cell lymphoma 2. Additionally, α-synuclein has been recognized for inducing cellular death and dysfunction in common biological pathways. Our objective is to assess the effectiveness of advanced therapies in addressing this particular condition. While hematopoietic stem cells have been a primary treatment, its administration proves challenging, particularly in the presymptomatic phase. In this review, we have compiled information from over 10 therapy trials, comparing them based on their benefits and disadvantage.

Key words: autophagy impairment, extracellular vesicles, gene therapy, hematopoietic stem cell transplantation, Krabbe disease.

INTRODUCTION

Krabbe disease (KD) (Online Mendelian Inheritance in Man [OMIM] No. 245200) is an autosomal recessive and metabolic inherited disorder affecting both males and females.[1] It belongs to the heterogeneous group of genetic leukodystrophies characterized by a primary and selective impairment of the myelin sheath through a disturbance in cellular metabolism.[2,3] This condition is a rare lysosomal disease commonly known as globoid cell (GC) leukodystrophy (GLD) or galactosylceramide (GalCer) lipidosis.[3,4] Its incidence in the global population varies between 1 in 100 000 and 1 in 310 000 children per year.[5] KD arises from a mutation within the galactocerebrosidase (GALC) gene situated on chromosome 14.[6,7] This mutation results in a deficiency of the GALC enzyme, which is vital for the lysosomal breakdown of specific galactolipids, including GalCer, psychosine or galactosylsphingosine (GalSph), monogalactosyldiglyceride, and lactosylceramide.[6,7] GalCer and its sulfated form, sulfatide, play crucial roles as glycosphingolipids within the structure of myelin.[6,7] While these mechanisms were the main pathway of this rare disease, several studies have broadened our perspectives on various aspects of the novel biological elements involved in its metabolic intricacies.[7–10] Recent investigations highlight an important involvement of extracellular vesicles (EVs), autophagy impairment, saposin D, and other GalSph-independent mechanisms in the pathophysiological pathways of KD.[7–11] Meanwhile, the exact mechanism pathways remained unclear until now. KD manifests in four forms based on the age of onset: early infantile KD (0 to 6 months), late infantile KD (6 months to 3 years), juvenile KD (≤16 years) and adult KD (>16 years).[12] The most common form is the early infantile (85–90%) with unfavorable prognosis leading to death in nearly 100% of cases.[13] The diagnosis of the disease is confirmed through a genetic assay and/or measurement of the GALC enzyme.[6] Up to this point, hematopoietic stem cell transplantation (HSCT) has stood as the sole authorized remedy for presymptomatic GLD.[14] Meanwhile, investigation into KD treatment has

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expanded significantly, with various therapeutic approaches being explored.[15–18] Notably, gene therapy has emerged as a promising avenue for addressing the underlying genetic and metabolic disorders associated with KD.[15] These therapies aim to expand the lifespan of patients and improve their quality of life.

Within the vast field of inherited metabolic disease, KD has emerged as an interesting subject for its complexity and its poor prognosis. Until now, no review has been conducted to consolidate published papers on the mechanisms of KD into common pathways and explore its cutting-edge therapy strategies based on these novel mechanisms. Our comprehensive review is the first to summarize and illustrate the intricate mechanisms and metabolic disorders of KD that is up to date with a review of various therapies.

EXPLORING 80 YEARS OF DISCOVERY: FROM FIRST CASE REPORT OF KRABBE DISEASE TO REVEALING MUTATIONS

Exploring the natural history of a child disease from its initial case to contemporary developments holds significant importance in emphasizing our comprehension of this condition. After reviewing literature data, we yielded 19 papers resuming differently the course of KD.[2,19–35] It seems interesting to have gathered all these papers into a single natural history collection over eight decades. We illustrated the natural history of human KD in Figure 1.

First, in 1906, Bullard and South provided a description of patients exhibiting histopathological changes similar to those outlined in Knud Krabbe's study.[2,28,29] This proposal implies that these cases could potentially be the initial occurrences of the disease, although such a hypothesis lacks confirmation in the existing literature.[2,28,29] In 1908, Beneke *et al.* documented comparable cases and referred to them as diffuse sclerosis of the brain.[30] In 1916, Knud Krabbe described a child aged 1 year and five other infants within the same family exhibiting clinical features previously described in the Benke study.[31] At that time, the name of the disease was changed due to histopathological findings obtained during autopsy. In fact, Knud Krabbe was the first to describe the presence of glial cells, gigantic cells with a large irregular outlined protoplasm and several nuclei and fatty granule cells around the vessels of the white matter, accompanied by changes in the adventitia, while the media and intima remained normal. Consequently, the disease was labeled as perivascular necrosis of the medullary substance, or KD.[31]

In 1924, Collier and Greenfield affirmed the existence of these cells, identifying multinuclear macrophages in the white matter, which were subsequently named GC.[32] As a result, the condition was renamed GLD. GC has been the specific and distinctive histopathological alteration in white matter during KD.[32]

In 1954, Blackwood and Cumings observed an elevation in GalCer or cerebroside in the brain of a deceased KD case aged 3 years.[19,33] The cerebroside was likely present in the so-called GC. Thus, they suggested that these GC formed as phagocytes attempted to handle the periodic acid–Schiff-positive material, and these cells likely originated from microglia.[19,33] Moreover, the researchers proposed three hypotheses regarding the mechanism of KD: (i) a malfunction in the cells responsible for forming myelin, (ii) disruption in the synthesis, or (iii) degradation of a crucial component necessary for the production of stable and compact myelin.[19,33] In 1970, Malone and Suzuki documented significantly reduced activity of a lysosomal enzyme responsible for the decomposition of GalCer.[34,35] This enzyme was identified as a GALC enzyme.[35] This finding was interestingly used in screening KD in risky pregnancies by measuring GALC activity in chorionic villus samples.[20] Soon after, it was determined that another

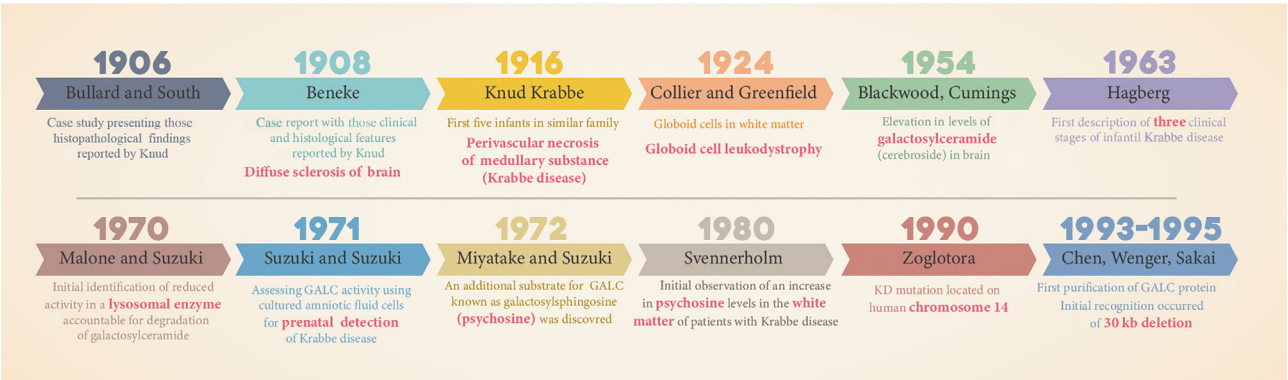


Fig 1 History of eight decades, Krabbe disease. The illustration depicts the chronological progression of Krabbe disease, beginning with the first instance of the early infantile form in 1916, leading up to the initial cloning of the GALC enzyme and the discovery of the 30 kb deletion in 1995. Notably, the cases reported in 1906 and 1908, while not acknowledged in the literature as the inaugural cases, exhibited clinical manifestations similar to the early infantile form.

galactolipid, GalSph or D-galactosyl-beta1-1′ sphingosine, served as a substrate for the GALC enzyme, as reported by Miyatake and Suzuki in 1972.[21] Several studies confirmed this result, as documented by Svennerholm *et al.* in 1980.[22] Zoglotora *et al.*, in 1990, demonstrated that the mutation responsible for KD was situated on chromosome 14 in humans.[26] In 1993, after numerous attempts and experiments over the years, Chen and Wenger successfully cloned the cDNA encoding the human ß-GALC enzyme and achieved the first purification of the protein from human urine.[23,36] Subsequently, isolating GALC from human lymphocytes provided an opportunity to leverage details about its N-terminal and internal amino acid sequences to clone a complete cDNA for the enzyme.[27] In 1995, the first mutation was described as a 30 kb deletion (*c.1161 + 6532_ polyA + 9kbdel*) among patients with KD.[37] All these findings changed the diagnostic methods and aided in the screening of the disease in the prenatal period. Examination of these studies yields evidence of the extended period required to uncover the origins of KD. This underscores the importance of raising awareness about rare diseases, necessitating further research to assist patients and enhance their quality of life.

EXPLORING INTRICATE PATHOPHYSIOLOGICAL MECHANISMS

The GALC gene is located on the long arm of chromosome 14 (14q31) and spans 58 kb and comprises 17 exons.[25,38] It encodes for the lysosomal hydrolytic enzyme (GALC enzyme) comprising 669 amino acids.[25,39] Its production involves its synthesis in the endoplasmic reticulum and subsequent glycosylation in the Golgi apparatus.[39] The glycans on GALC are modified with mannose-6-phosphate (M6P) groups, allowing recognition by the M6P receptor.[39] The M6P receptor–GALC complex is then transported to the early endosomal compartment. In the acidic environment of the late endosome, the complex dissociates.[39] The M6P receptor is recycled back to the Golgi apparatus, while GALC is directed to the lysosome.[39] Alternatively, the GALC enzyme can be transported via the constitutive secretory pathway and delivered to the lysosome through reuptake facilitated by the M6P receptor.[39]

According to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), more than 300 GALC mutations have been reported. The mutation can be homozygotes or compound heterozygotes.[40] Null mutations entirely eliminate normal GALC transcripts and functional GALC protein.[40] The common genetic aberration observed in KD entails a 30 kb deletion that stretches from intron 10 to intron 17 of the gene, existing in a homozygous state.[40,41] While a range of mutations, such as nonsense, missense, small-insertion, and small-deletion mutations, have been identified across the entire span of the GALC gene, the most prevalent among them is the missense mutation.[42]

To date, several studies have explored the pathophysiological mechanism of KD, which affects both the central nervous system (CNS) and the peripheral nervous system (PNS).[7,8] It is known that the mutation in the GALC gene results in a functional deficiency in the GALC enzyme.[6] Theoretically, the mutant protein may manifest various harmful characteristics: (i) misfolding or loss of stability; (ii) alteration of the active site of substrate binding; (iii) enzymatic activity alteration; and (iiii) protein structure modification, affecting regions beyond the active site impacting domains that engage with other cofactors essential for its correct functioning.[42]

The GALC enzyme plays a crucial role in neuron development and homeostasis by degrading specific galactolipids, such as GalCer, GalSph, and monogalactosyldiglyceride.[6,7] In particular, the GALC enzyme facilitates the hydrolysis of GalCer in the presence of saposin A, leading to the release of ceramide and galactose.[6,7] Additionally, supported by saposin A, it is responsible for the degradation of GalSph into sphingosine.[6,7] Indeed, the production of GalSph can occur through two distinct processes. The initial mechanism involves the catabolic pathway, wherein acid ceramidase, supported by saposin D, assumes a pivotal role in the deacylation of GalCer into GalSph and fatty acids.[6,7] The second pathway is the anabolic process, where sphingosine is transferred to GalSph by ceramide galactotransferase.[43] In recent studies, Watanabe *et al.* demonstrated that demyelination and neuroinflammation in a mice model may involve a GalSph-independent and saposin D-dependent mechanism.[11] In fact, in this study, investigators created a mouse model that exhibited genetic deficiencies in both GALC and saposin D. They found a minimal accumulation of GalSph in the CNS or PNS of these mice. As anticipated, the demyelination process, marked by the presence of GC, was less severe in these mice compared to mice that had had only GALC deficiency, both in the CNS and PNS, particularly during the early stages of the disease.[11] Meanwhile, in a later stage, the deficiency in saposin D led to demyelination, notably in PNS with GC formation. Thus, we propose that the transfer of GalCer into GalSph by acid ceramidase may not occur in the absence of saposin D.[11] However, the organism might resort to utilizing alternative coenzymes or alternative pathways to compensate for the deficiency of saposin D, leading to the accumulation of GalSph in later stages.

Overall, these various mechanisms collectively result in the accumulation of both GalCer and GalSph.[6] Their overload occurs not only within oligodendrocytes and Schwann cells but also within the myelin membrane itself.[9]

Unmetabolized GalCer and GalSph attract the influx of macrophages into the brain, and these macrophages undergo transformation into the characteristic multinucleated GC, wherein cytokinesis is arrested.[28] Additionally, the redistribution of GalCer from healthy myelin to irregular crystal inclusions might play a role in the pathology of the disease.[43] Thus, this crystal GalCer accumulation in cells and particularly innate immune cells can be toxic due to the formation of the inflammasome or lysosome membrane permeabilization.[43–45] Furthermore, two studies underscored the central role played by the extracellular matrix protease in GC formation.[46,47] Ijichi *et al.* observed elevated levels of the extracellular matrix protease, particularly matrix metalloproteinase-3, in GLD, emphasizing its involvement in regulating microglial activation and the formation of GC induced by GalSph.[46] In a separate investigation, Claycomb *et al.* showed that the formation of GC triggered by GalSph was promoted by tenascin-C, a protein that constitutes one of the components of the extracellular matrix.[47] While researchers have demonstrated that GalSph is the primary substance responsible for initiating KD pathogenesis, the available data indicate the involvement of multiple interconnected microbiological organisms in facilitating the role of GalSph.[7–9,46,47]

GalSph accumulation results in numerous metabolic and molecular alterations, as follows: release of oxidative stress, neuron membrane destabilization, decreased membrane fluidity, and alteration of neuron lipid rafts with structure disturbance.[4,7–9,48] Furthermore, it is associated with alterations in microtubule stability, leading to impaired axonal transport, synaptic structures, and neuronal defects.[49,50] The lipid raft disturbance leads to the alteration of the protein kinase (AKT) signaling pathway including those involving the Insulin-like Growth Factor-receptor.[50]

Recently, four significant research discoveries using a mouse model highlighted the participation of EVs, autophagy impairment, α-synuclein, and pentraxin-3 (PTX3) in the neuropathological processes associated with KD.[7–10] While the involvement of EVs has been demonstrated in numerous neurodegenerative diseases and other pathologies associated with endolysosomal dysfunction, such as multiple sclerosis, Alzheimer's disease, and Parkinson's disease, it has recently been identified in the pathophysiological mechanism of KD.[9,51] EVs (0.5–4 μm) are released by the accumulation of GalSph in a lipid raft.[52] According to Reiter *et al.*, EVs could potentially serve as a mechanism for cells to evade or reduce the harmful effects of an overload of toxic levels of GalSph.[9] EVs represent a diverse group of stable lipid membrane particles and play a crucial role in modulating various physiological and pathological processes.[53] They participate in cell-to-cell communication, the transmission of signals, and inflammation.[54] They achieve this by transporting RNA (including mRNA and long noncoding RNAs), lipids, and proteins, including lysosomal enzymes.[9,53] Considering that research has demonstrated the ability of EVs to traverse the blood–brain barrier (BBB),[9,50] we propose exploring their involvement in neurodegeneration within the PNS in KD. Additionally, given that in the initial phase of the disease, there was an elevated presence of EVs,[9,50] with a subsequent decline in the later stages, and we suggest that EVs had a crucial role in the early infantile KD form rather than other forms.

α-Synuclein is also abnormally expressed in KD.[10] It accumulates in the frontal lobe and spinal cord and in GC, especially in the brainstem and cerebral cortex.[10] This substance induces cellular death and dysfunction of common biological pathways.[10] Additionally, a relationship between the impairment of the autophagic-lysosomal pathway and the α-synuclein aggregation has been demonstrated.[55] Interestingly, Hatton *et al.* observed a rapid accumulation of prion-like α-synuclein in KD, occurring within the time frame of 4–10 months.[10] Considering α-synuclein's role as a presynaptic protein, it is plausible to suggest that synaptic impairment in KD may also contribute to α-synuclein accumulation. In alignment with our suggestion, it has been demonstrated that the disturbance of lipid raft architecture by GalSph in the Krabbe brain may impact the localization of α-synuclein to synapses, potentially leading to an increase in its aggregation within the neuronal cytoplasm.[56] Additionally, recent research has substantiated a significant role played by synaptic defects, including alterations in synaptic spines, proteins, and postsynaptic densities.[57] Several other hypotheses have been proposed in the literature to explain α-synuclein accumulation in fibrils. Abdelkarim *et al.* suggested a plausible underlying mechanism that involves the interaction of GalSph and α-synuclein accumulation.[58] GalSph can bind to the negatively charged carboxyl-terminus of α-synuclein, leading to the formation of galactosyl hydrophilic clusters. This interaction exposes the aggregation-prone, non-amyloid-β component domain of α-synuclein. Consequently, elevated GalSph levels lead to the aggregation of α-synuclein.[56,58] The aggregation of α-synuclein follows a nucleation-dependent first-order process characterized by distinct phases, including a lag phase, an elongation phase, and a steady-state phase.[56] Moreover, in the twitcher brain, the presence of GalSph was found to be adequate for inducing a shortening of the lag phase, consequently resulting in the generation of α-synuclein.[56] On the other hand, the abnormal accumulation of lipid products and the sphingolipid metabolism alteration in KD has emerged as a potential shared factor initiating α-synuclein accumulation.[59] Nevertheless, the underlying mechanism remains complicated, and a multitude of questions persist without conclusive answers, necessitating further research.

Recent discoveries have identified changes in autophagy in KD.[60] Despite the findings of earlier studies in which autophagy impairment was associated with GalSph overload,[61] current investigations using a fibroblast model demonstrated that this dysfunction is due to other pathways.[62,63] In fact, fibroblasts were chosen because they constitute a cellular model ideal for examining cell migration and interactions with the extracellular environment, without the accumulation of GalSph.[62,63] These studies indicate that autophagy impairment was linked to the overload of lactosylceramide and an increase in AKT and B-cell lymphoma 2 independently of GalSph.[63,64] Mezzena *et al*. demonstrated in a GALC enzyme-deficiency model mouse an impairment of mechanotransduction with reduced mobility of cells via dysfunction of the autophagic process.[62] Furthermore, they showed an increased count of focal adhesions per cell and reduction in N-cadherin junctions, suggesting other pathways in KD mechanisms.[62] Remarkably, GALC-deficient macrophages *in vivo* have decreased expression of CD206, a surface receptor that enables macrophage phagocytosis, suggesting a direct role of GalCer in impairing macrophage function.[65] The alteration of cell migration induced by all these mechanisms leads to a reduction in autophagosomes, to delays in the maturation of neurons, and to a severe clinical course of KD.[62,63] On the other hand, myelin debris induced by different mechanisms are phagocytosed by microglia, inducing microglial activation and astrocyte stimulation (astrogliosis), resulting in canonical and noncanonical inflammasome formation and release, then interleukine-1 α and interleukine-1 β through caspase-1 and caspase-11 (Fig. 2).[67]

PTX3, an acute phase protein known for its role in innate immunity, has been implicated in neurodegeneration.[7] It is stimulated by cytokines released due to the GALC enzymatic deficiency.[7] Coltrini *et al.* performed immunohistochemical analysis on brain specimens from individuals with KD, uncovering robust expression of PTX3 receptors and significant immunoreactivity for PTX3 in both macrophages and GC.[7] In GALC-deficient twitcher mice, PTX3 expression increased progressively in the cerebrum, cerebellum, and spinal cord throughout the disease course.[7] This elevation was accompanied by a reduction of proinflammatory genes in both the CNS and plasma of twitcher animals.[7] Thus, the PTX3 may have an anti-inflammatory property and help to decrease the neuroinflammatory response in KD.[7]

In general, it is evident that the mechanisms of KD are complex, involving interactions between various substances and cells. While EVs and PTX3 have emerged as novel avenues for comprehending the disease pathology, advancements in genetic and molecular research techniques may offer additional insights into the specific genetic mutations linked to KD and their role in contributing to the disease pathology. It is evident that further exploration of additional pathological mechanisms is necessary.

Another noteworthy observation is the potential role of the GALC gene in the development of cancer.[21] The overload of GalSph induces apoptosis in natural killer cells, which are involved in defending against tumor cells.[70] Reiter *et al*. showed that eliminating β-galactose from β-GalCer results in the generation of oncosuppressor metabolite ceramide, which could have implications for suppressing tumor growth and impacting the process of differentiation.[9] On the other hand, GALC gene mutation can lead to multiresistant treatment of cancer.[71]

Figure 2 illustrates the pathophysiological pathways of KD.

PHENOTOPIC SPECTRUM MANIFESTATIONS AND RADIOLOGICAL FEATURES

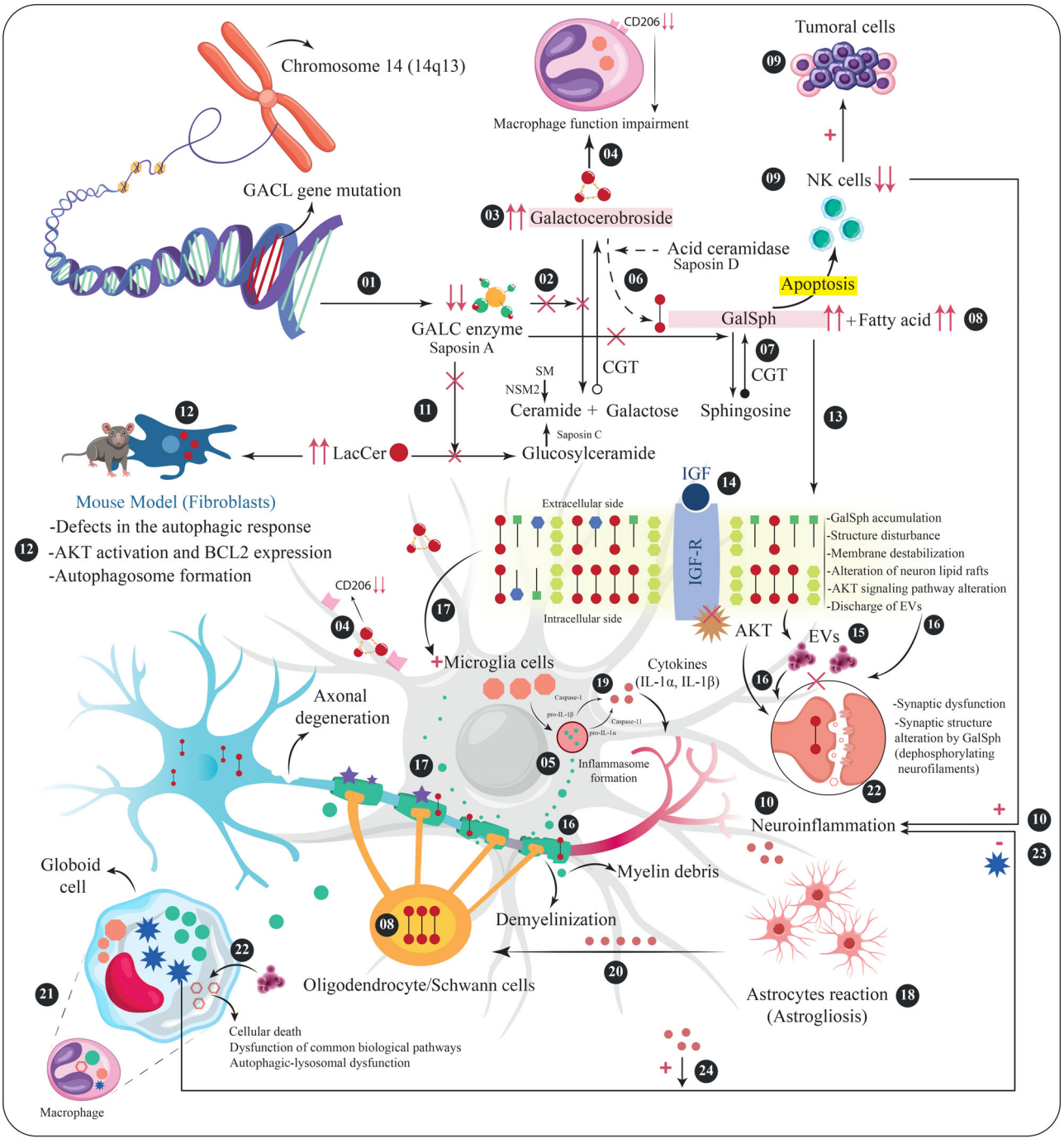
KD involves four forms: early infantile KD, late infantile KD, juvenile KD, and adult KD.[72,73] Each form exhibits distinct clinical and radiological characteristics. These various clinical signs are explained by the direct or indirect consequences of lysosomal dysfunction affecting different organs, resulting in ocular, auditory, visceral, and, notably, neurological signs.[72,73] Table 1 summarizes the various features of KD forms.

TREATMENT

KD is a devastating and dreadful neurodegenerative disorder by its evolution. In the face of this severe illness, researchers have consistently conducted studies to discover the most effective treatments. Nevertheless, to date, no curative therapy for KD has been developed.[17,87] Currently, HSCT represents the only approved and available treatment.[88] Hence, various treatment strategies have been assessed for KD. Our exploration of the literature identified a total of 15 therapies that were applicable either in humans or in animals. Table 2 summarizes the different therapies *in vivo* or *in vitro*. In addition, we delve more deeply into the most extensively described therapies.

Hematopoietic stem cells

The proposed mechanism of action for HSCT involves the secretion of GALC by monocytes or macrophages, followed by uptake by myelinating glia, a process known as cross-correction (increased expression of GALC can result in the secretion and absorption of GALC by adjacent cells).[44] Initially, it was primarily sourced from bone marrow due to their immediate availability for urgent



AKT, protein kinase
BCL2, B-cell lymphoma 2
CGT, ceramide galactosyltransferase
CD206, cluster of differentiation 206
IL-1α, interleukine-1α
IL-1β, interleukine-1β
GalCer, galactocerobroside
GalSph, psychosine
LacCer, lactosylceramide
NK, natural killer
NSM2, neutral sphingomyelinase-2
SM, sphingomyelin
PTX3, pentraxin-3
EVs, extracellular vesicles
IGF, insulin-like growth factor
IGF-R, insulin-like growth factor receptor

(Figure legend continues on next page.)

transplant procedures. However, in more contemporary approaches, there is a growing trend toward using HSCT obtained from umbilical cord blood.[14,89] This significantly enhances the likelihood of identifying a compatible human leukocyte antigen match for a specific patient.[89] Although this treatment has been demonstrated to extend the lifespan of infantile patients, nearly all individuals still encounter challenges with walking and experience expressive language difficulties by the conclusion of the first decade of life.[89] The challenges in walking seem to arise from a deficiency in the ability of HSCT to sufficiently address issues in the PNS.[89] Additionally, this is likely attributable to the rapid progression of KD, while the rate of transplanted cell infiltration into the nervous system is comparatively slow.[89] Earlier research indicated that individuals who undergo transplantation after the emergence of clinical symptoms experience unfavorable functional outcomes and have a lower likelihood of survival.[90] In contrast, favorable clinical outcomes are observed when HSCT is conducted before the initiation of symptoms.[14] In fact, HSCT has the potential to arrest cerebral demyelination if performed at an early stage, prior to the onset of neurological symptoms and before the progression of advanced brain disease.[91] HSCT not only contributes some level of GALC activity to tissues but also imparts certain anti-inflammatory effects to nerve tissue.[91] Hence, individuals who undergo HSCT for KD require comprehensive and continuous long-term monitoring. In addition to regular post-transplant evaluations, annual examinations should include magnetic resonance imaging, electroencephalogram, electromyogram/nerve conduction studies, brainstem auditory evoked response, visual evoked potentials, and measurement of GALC enzyme levels.[14] Furthermore, it is advisable to conduct a dental examination every 6 months.[14] Anomalies in tooth development were observed in all patients who survived beyond 5 years after HSCT, a phenomenon distinctively linked to the administration of chemotherapy in very young children.[14,130]

Novel synergistic therapeutics may hold the potential to accelerate the biomechanism and enhance efficacy of combined treatment. In fact, information from twitcher mice suggests that there is a span of several weeks after transplantation before there is an elevation in GALC activity within the brain, a decrease in GalSph levels, and an improvement in pathological features.[131,132] HSCT leads to a decrease in GC within nerves, indicating that the therapeutic effect is likely attributable to the phagocytic response of healthy macrophages, rather than cross-correction.[44] Another possible explanation for the lack of effectiveness of HSCT on peripheral neuropathy is that Schwann cells exhibit a restricted capacity to uptake the GALC enzyme from neighboring cells, and the process of cross-correction is not efficiently executed.[44] Individuals treated with HSCT demonstrate enhanced vision, cognitive abilities, and peripheral nerve conduction in comparison to untreated KD children. A majority of patients in the presymptomatic treatment group exhibit improved language and gross motor skills, and approximately 50% have normal hearing.[133]

Substrate reduction therapy

Substrate reduction therapy is a therapeutic approach aimed at reducing the accumulation of certain substances within cells, particularly in the context of lysosomal storage disorders.[95,96]

(Figure legend continued from previous page.)

Fig 2 Molecular mechanisms of pathophysiological pathway of Krabbe disease. The mutation in GALC gene results in GALC enzyme deficiency (1), leading to the absence of GalCer degradation into ceramide and galactose (2). Thus, GalCer crystals are accumulated in the central nervous system (3). Additionally, GalCer crystal accumulation in macrophage and innate immune cells results in macrophage function impairment by decreasing the CD206 receptors (4). Theses crystals contribute to the inflammasome formation and lysosome membrane permeabilization (5). On the other hand, there are two pathways of formation of GalSph. The first is catabolic pathway (6), including deacylation of GalCer by acid ceramidase supported by saposin D. The second pathway involves an anabolic process wherein sphingosine is converted into GalSph by the ceramide galactotransferase enzyme (7). Consequently, GalSph accumulates in oligodendrocytes and Schwann cells (8), which triggers apoptosis in NK cells and activates tumor cells (9). Further, this apoptosis activation results in enhanced neuroinflammation (10). Moreover, the GALC enzyme is responsible for converting LacCer into glucocylceramide (11). Thus, there is an accumulation of LacCer, resulting in a defect in autophagy response independent of GalSph. This phenomenon has been demonstrated in both fibroblast mice cells and fibroblasts derived from KD patients[62,63] (12). Indeed, these studies indicated that the impairment was linked to the overload of lactosylceramide and an increase in B-cell lymphoma 2 expression (12). On the other hand, the accumulation of GalSph induces lipid raft disturbance (13) and AKT pathway alteration (14) and EV release (15), resulting in impairment of synaptic plasticity and myelination and in neurodegeneration (16).[9,50,52,63,66] Furthermore, myelin debris, resulting from various mechanisms such as lipid raft alteration and dysfunction of oligodendrocytes and Schwann cells, are phagocytosed by microglia, inducing microglial activation (17) and astrogliosis (18).[67] This activation leads to the release of cytokine (IL-1 α and IL-1 β) (19) with increasing neuroinflammation (10) and oligodendrocytes and Schwann cell alteration (20).[67] Moreover, macrophages are transformed into globoid cells (characteristic multinucleated of KD) (21).[28] α-Synuclein may be generated due to synaptic dysfunction and EVs (22),[68,69] leading to its accumulation in globoid cells and neurological cells.[10] This accumulation results in alterations in autophagic-lysosomal pathway.[55] PTX3 is also accumulated in macrophage and globoid cells, resulting in a reduction of proinflammatory genes (23).[7] It is stimulated by cytokines released by astrocytes and microglia (24).[7]

Table 1 Characteristics of Krabbe disease in its various forms

	EIKD[6,8,12,15,41,74–77]	LIKD[6,8,73,78,79]	JKD[6,8,12,77]	AKD[8,12,77,80]
Age at onset	0–6 months	6 months–3 years	3–16 years	>16 years
Clinical features	3 stages after normal development[6,81]: - First stage: nonspecific generalized signs (irritability, axial hypotonia, stiffness, feeding difficulties, growth delay, intermittent thumb pinching, episodes of hyperthermia, delayed DM) -Second stage: intensification of symptoms observed in first phase (hypertonicity and opisthotonus, psychomotor and growth regression, peripheral neuropathy, apnea episodes, frequent epileptic seizures[†],[82–86] hand-grip difficulties, and visual impairments[‡]) - Third stage: Bedridden children[41] (deafness, blindness, loss of voluntary movement with progressive spastic paraparesis, pseudobulbar syndrome, decerebrate postures)	-Early normal development -Decreased muscle strength -Spasticity -Irritability -Ataxia -Peripheral neuropathy -Epileptic seizures[§] -Visual impairments[‡] -Loss of millstones development	-Early normal development -Loss of millstones development and psychomotor dysfunction -Irritability -Ataxia -Peripheral neuropathy -Epileptic seizures[§] -Cognitive disorder -Visual impairments[‡]	-Spastic paraparesis -Peripheral neuropathy -Ataxia
MRI features	Can be normal in early stage Lesions are vertically expanded Tigroid pattern (inconstant)	Lesions are vertically expanded Tigroid pattern (inconstant)	Lesions are vertically expanded	Lesions are vertically expanded
Periventricular	+++	+++	++	+
Parieto-occipital	+++	+++	++	++
SOC	+++	+++	++	+
CI	++	++	+	+
SCC	++	++	+	+
CST	+	+	++	+++
Dentate nuclei	+++	+++	+	+
Thalamus	++	++	+	+
NOH	+++	++	−	−
GCP	−	−	−	−
U fiber	−	−	−	−
Confirmation	Genetic assay and/or GALC enzyme dosage			
Survival	<2 years	< 10 years	Extended lifespan accompanied by mild symptoms	Prolonged and significantly extended lifespan

Abbreviations: AKD, adult Krabbe disease; DM, developmental milestone; EIKD, early infantile Krabbe disease; JKD, juvenile Krabbe disease; LIKD, late infantile Krabbe disease; MRI, magnetic resonance imaging. [†]Febrile seizures, myoclonic seizures, generalized tonic–clonic seizures, West syndrome. [‡]Disconjugate eye movements, abnormal pupillary responses, strabismus, nystagmus, optic atrophy or choroidosis or cherry-red macular in fundus examination; CI, internal capsule; SOC, semi-oval center; SCC, splenium corpus callosm; CST, corticospinal tract; GCP, genu corpus callosum; NOH, nerf optic hypertrophy; [§]Less frequent than the early infantile form.

Table 2 Current and novel therapies of Krabbe disease

	Studies	Advantage	Disadvantage	Remarks
HSCT	Allewelt *et al.*[14] Page *et al.*[88] Wright *et al.*[89] Prasad *et al.*[90] Rafi *et al.*[91] Weinstock *et al.*[44] Koffler *et al.*[92] Heller *et al.*[93] Krieg *et al.*[78]	-Arrested cerebral demyelination -Anti-inflammatory effects -Decreased globoid cells within nerves -Effective on CNS -Markedly improved myelin preservation -If performed in presymptomatic phase: *Delayed disease progression but poor motor performance in follow-up *Good outcome and extension of lifespan (median survival 11 years) *Improved quality of life	-Requires long-term follow-up -Rate of transplanted cell infiltration into nervous system is comparatively slow -Normal myelination levels not reached -Requires a long time to be effective -Not effective on peripheral demyelination in KD -If performed in symptomatic patient *Unfavorable functional and survival outcomes (average survival 30 months) -Transplant-related complications	-Should be performed before symptom onset -Age <1 month for optimal benefits -Umbilical cord blood is more beneficial than Bone marrow -Effectiveness on CNS > > PNS -Mechanism: cross-correction and immunomodulation (phagocytose)
Substrate reduction therapy				
L-cycloserine	Sundaram *et al.*[94] LeVine *et al.*[16] Biswas *et al.*[95] LeVine *et al.*[96] Hawkins-Salsbury *et al.*[97]	-Improved body weight -Increase in median life by 13 days (model mice) -Reduced psychosine, number of globoid cells and astrogliosis -Cross BBB	-Narrow therapeutic window (diminishing numerous other crucial lipids alongside psychosine) -Induced toxicity at elevated dose -Not tolerable -Dose and time dependent -Not approved for human use -Alteration of balance of other important lipids	-Mechanism: irreversible inhibitor of serine palmitoyltransferase -Subcutanous injection
D-cycloserine	LeVine *et al.*[96] Sundaram *et al.*[94] Kang *et al.*[98]	-Antibiotic properties -Anti-inflammatory properties -Prolonged lifespan -Increased body weight -Approved for human use -Cross BBB	-Safety in KD unknown -100-fold lower efficiency compared to L-cycloserine	-Mechanism: inhibitor of serine palmitoyltransferase
CGT inhibitor				
Compound S202	Babcock *et al.*[99]	-Improved body weight -Increase in median life by 20 days (model mice) -Reduced psychosine, brain GalCer -Decreased neuroinflammation -Normalized myelination -Selective inhibition for CGT -Selective inhibitor for non-hydroxy-GalCer (dose-dependent effect) -Tolerable	-Side effect: *Deteriorated nerve conduction speed *Formation of vacuoles in CNS	-Lipid inhibitors against CGT -Intraperitoneal injections

(Continues)

Table 2 (Continued)

	Studies	Advantage	Disadvantage	Remarks
Thienopyridine (Compound 19) (RA 5557)	Zaccariatto *et al.*[100] Thurairatnam *et al.*[101]	-Decreased psychosine metabolites in the brain -Absence of toxicity of myelin produced in cells -Decreased GalCer and sulfatide and inflammatory response	-Poor survival outcomes	-UGT8 inhibitor -Inhibit synthesis of GalCer and sulfatide in both brain and kidney
Acid ceramidase inhibitor				
Piperidine 22 m	Di Martino *et al.*[102]	-Redution in psychosine and glucopsychosine -Reduction of toxicity is correlated to 22 m dose -Oral route -Tolerable -Excellent brain penetration	Not available	-A class of benzoxazolone carboxamides -Inhibitor of acid ceramidase
Carmofur	Li *et al.*[103]	-Reduction in psychosine and GalCer -Increased lifespan -Reduction in globoid cells and demyelination -Preserved axonal structures	-No effect on body weight -Side effects (leukoencephalopathy, liver dysfunction, neuropathy, skin rash, and diarrhea)[96]	−5-fluorouracil-releasing chemotherapeutic agent -Inhibitor of acid ceramidase
Enzyme replacement therapy	Neufeld *et al.*[104] Lee *et al.*[105] Desnick *et al.*[106] Matthes *et al.*[107] Del Grosso *et al.*[108] Herzeg *et al.*[109] Del Moral *et al.*[110]	-Lifespan extended to 47 days -Administering through intracerebroventricular route decrease significantly psychosine levels -rhGALC penetrated BBB, targeting lysosomes in brain macrophages, astrocytes, and neurons with reduction in psychosine level -Innovative method of utilizing cross-linked enzyme aggregates (CLEAs) encapsulated in poly-(lactide-co-glycolide) (PLGA) nanoparticles, as described by Del Grosso *et al.*,[108] has demonstrated a notable impact on CNS when administered through intraperitoneal injection -In utero injection provides opportunity for enzyme GALC to reach CNS before establishment of BBB	-Incapacity to generate significant amounts of typical enzymes -Do not cross BBB if administrated by intravenous or intraperitoneal route -Requires installation of implantable pump necessary for repeated administration through intracerebroventricular route -Necessitates use of multiple dosing sites -Administering through intravenous route results in only minimal reduction in psychosine levels -Rapid enzyme turnover -rhGALC did not alleviate neuroinflammation, demyelination, or extend lifespan of mice	-Employs M6P receptor-mediated transport -Must be administrated both intravenously and through intracerebroventricular route -Mechanism: temporary augmentation of GALC function

(Continues)

Table 2 (Continued)

	Studies	Advantage	Disadvantage	Remarks
Pharmacological chaperon therapy	Lee *et al.*[111] Parenti *et al.*[112] Biela-Banaś *et al.*[113] Berardi *et al.*[114] Hossain *et al.*[115] Hill *et al.*[116] Graziano *et al.*[42] Spratley *et al.*[39]	-Favorable properties: low molecular weight, low toxicity, high bioavailability -Cross the BBB -Positive impact on patient quality of life	-Selective to certain missense mutation -Treatment depending on: *Identifying suitable candidates *Nature of protein misfolding *Certain type of mutation -Personalized therapy	-Targets misfolded proteins -Pharmacological chaperone therapy tested: *N-octyl-4-epi-β-valienamine binds specifically to the active site of the human GALC protein *α-lobeline can bind to GALC protein in nine distinct sites *Azasugars exhibit specific binding within active site pocket *Iminosugar, 4-epi-isofagomine emerged as most significant inhibition of both lysosomal β-galactosidase and GALC
Anti-inflammatory therapies†	Suzumura *et al.*[117] Kagitani-Shimono *et al.*[118] Luzi *et al.*[119]	-Decreased tumor necrosis factor-alpha -Less severe disease symptoms -Reduction in demyelination -Ibuprofen, indomethacin, minocycline: *Neuroprotective and anti-apoptotic properties *Increased lifespan (10–12 days)	-Absence of significant symptomatic improvement -No effect on lifespan (phosphodiesterase inhibitor ibudilast)	-Phosphodiesterase inhibitor ibudilast -Ibuprofen, indomethacin, minocycline
Antioxidant Therapy†	Paintlia *et al.*[120]	-Extended lifespan, with mean of 50 days -Reduce psychosine levels -Reduce demyelination	Not available	-Vitamin D -Mechanism: antioxidant
Autophagy activation†	Del Grosso *et al.*[60] Papini *et al.*[63] Mezzena *et al.*[62]	-Reducing autophagosome formation independently of GalSph	Not available	
Oligodendrocyte transplant†	Kuai *et al.*[121]	-	-No increase in level of myelin -No improvement in clinical symptoms or severity -No effect on lifespan	-Mechanism: enzyme expression
Neural stem-cell gene therapy†	Strazza *et al.*[122]	-Notable decrease in astrocyte gliosis and globoid cells -Minor increase in lifespan	Not available	-Mechanism: enzyme expression
Antipsychotic†	Sharma *et al.*[18]	-Typical and atypical antipsychotic: *Reduce psychosine level *Reduce psychosine-induced cell damage and morphological abnormalities -Haloperidol and clozapine *Protective effects on astrocytes and microglia	Not available	Mechanism: antipsychotics have conflicting effects on oligodendrocyte function and regulate glial cell dysfunction

(Continues)

Table 2 (Continued)

	Studies	Advantage	Disadvantage	Remarks
		*Restoring nonphosphorylated neurofilament levels *Improves mobility and significantly enhances survival		
Gene therapy				-Mechanism: long-term replacement of GALC activity
AAV1[†]	Herdt *et al.*[123]	-Improving body weight -Increased median life by 35 days -Normalizing psychosine levels	Not available	
AAVrh9	Bradbury *et al.*[124]	-Increase in GALC enzyme activity -Efficacy of presymptomatic monotherapy (administered to cisterna magna) -Improved myelination -Reduced inflammation in CNS and PNS -Lifespan up to 2.5 years		
AAVrh10	Bradbury *et al.*[125] Rafi *et al.*[91] Bradbury *et al.*[126]	-If administered alone or in combinaison with HSCT: *Normalized nerve conduction velocity in PNS *Normalized GALC enzyme activity and psychosine levels in PNS *Normalized GALC enzyme and psychosine if administered with HSCT *Typical myelination observed in both CNS and PNS *Average survival age 41.2 weeks	-If administered alone in CNS: *No effect on GALC enzyme or psychosine in CNS -Low dose of AAvrh10 had no effect on lifespan	-Significant synergistic effect with bone marrow transplantation
Gene therapy AAVrh68	Hordeaux *et al.*[127]	-Administration to lateral ventricle significantly prolonged median survival to 130 days. - Administration of same dose intravenously resulted in median survival of 49 days -Preserved peripheral nerve myelination -Reduced psychosine levels -Reduced brain neuroinflammation and demyelination	-Some regions remained abnormal and exhibited persistent demyelination	
rAAV2 [‡]	Tian *et al.*[17]	-Increased GALC enzyme activity -Reduce psychosine -Restore neuronal cell populations	-Time-related heterogeneity impacts infection efficiency -Difficult to cross BBB	

(Continues)

Table 2 (Continued)

	Studies	Advantage	Disadvantage	Remarks
α-tubulin deacetylase HDAC6 inhibitor (ACY-738) ‡	Braz *et al.*[49]	-Neuroprotective -Delays axonal degeneration in CNS -Improves overall disease presentation -Correction of neuronal defect -Stabilization of microtubule dynamic -Enhanced axonal transport of mitochondria	Not available	-Supplementary therapy when combined with approaches targeting metabolic correction
Sphingomyelinase 2 inhibitor ‡	Reiter *et al.*[9]	-Decline in total extracellular vesicle levels -Decrease in psychosine	-Increase in disease severity -Marked gliosis	-GW4869 -Mechanism: inhibition of Sphingomyelinase 2, responsible for catalysis of conversion of sphingomyelin to ceramide and phosphocholine
Immunoglobulin†	Ryosuke *et al.*[128] Debs *et al.*[80] Adachi *et al.*[129]	-Successfully treated with intravenous immunoglobulin in one case report (16-year-old)[128]	-Failed in two case reports (22-year-old and 54-year-old)[80,129]	-For juvenile and adult forms -Papers were restricted to case reports

Abbreviations: AAV, adeno-associated virus; BBB, blood–brain barrier; CGT, ceramide galactosyltransferase; CNS, central nervous system; GalcCer, galactosylceramide; HSCT, hematopoietic stem cell transplant; M6P, mannose 6-phosphate receptors; PNS, peripheral nervous system; rhGALC, recombinant human GALC; UGT8, uridine diphosphate-galactose glycosyltransferase 8. †Limited studies.

L-Cycloserine

L-Cycloserine is capable of penetrating the BBB, where it irreversibly inhibits serine palmitoyltransferase (SPT).[94,134] This enzyme represents the starting point in the synthetic pathway for various ceramides and sphingolipids.[94,134] It may reduce the GalSph.[97] Repeated subcutaneous administrations of L-cycloserine extended the median survival in mice by 13 days, resulted in increased body weight, lowered the GC count, and diminished astrogliosis.[16] While findings of this treatment are intriguing and remarkable, it is important to note that L-cycloserine is not approved for human use given its narrow window of therapy inducing toxicity at elevated doses (Table 1).[96] In fact, as sphingolipids play crucial roles in various tissues, complete inhibition of SPT cannot be tolerated.[135] Indeed, partial inhibition of certain levels may lead to adverse effects, and these effects are likely to be dependent on both the dosage and the duration of treatment.[135]

D-Cycloserine

D-Cycloserine represents an enantiomer of L-cycloserine and was approved in 1964 for human use.[96] Meanwhile, it exhibited a 100-fold lower efficiency compared to the L enantiomer.[94] Reduced efficiency for the D enantiomer allows for a wider range of drug concentrations to attain partial enzyme inhibition.[94] Indeed, unlike L-cycloserine, the inhibition of brain microsomal SPT did not occur after a single dose of D-cycloserine administered via intraperitoneal injection in mice.[94] In a recent study, D-cycloserine extended the lifespan of twitcher mice and increased the body weight.[96] Additionally, combining D-cycloserine with pyridoxine, also known as vitamin B6, may enhance the treatment outcome.[96] D-cycloserine also exhibits anti-inflammatory effects, particularly in macrophages. When stimulated with lipopolysaccharide, mice macrophages treated with D-cycloserine demontrated a reduction in the phosphorylation of extracellular signal-regulated kinase.[96,98]

Ceramide galactosyltransferase inhibitor

Two compounds have been recognized as inhibitors of the ceramide galactotransferase. The initial one is the compound S202 described by Babcock *et al.*[99] This compound represents a selective and potent small molecular inhibitor of the ceramide galactotransferase.[99] Research findings indicate that S202 resulted in a dose-dependent reduction of GalCer and GalSph in both the CNS and PNS. Additionally, the treatment significantly increased the lifespan of mice. Interestingly, S202 is able to inhibit selectively non-hydroxy-GalCer, and this could have an important impact on KD treatment.[99]

The second one is a thienopyridine that was described in two recent studies by Thurairatnam *et al.* (Compound 19) and Zaccariatto *et al.* (RA 5557).[100,101] It serves as an effective *in vivo* inhibitor of uridine diphosphate-galactose glycosyltransferase-8.[100,101] Uridine diphosphate-galactose-glycosyltransferase-8 is the enzyme responsible for the final step in the production of GalCer.[101] *In vivo* experiments conducted on healthy mice demonstrated that this compound effectively suppressed the synthesis of both GalCer and sulfatide in both the brain and kidney and led to a decrease in inflammatory response.[101] Treatment with RA 5557 markedly decreased elevated GalSph levels (72–86%) in the midbrain and cerebral cortex of twitcher mice.[100] The inhibitor also reduced GalCer levels by around 70% in these brain regions without detectable toxicity to myelin-producing cells.[100] Despite these favorable outcomes, the lifespan of twitcher mice was not extended.[100]

Acid ceramidase inhibitor

Acid ceramidase plays a pivotal role in controlling the metabolism of ceramides and glycosphingolipids.[102] Given that GalSph was identified as the main pathophysiological mechanism in KD and is produced through the catabolic pathway from GalCer, research has been concentrated on therapeutic approaches aimed at reducing this substrate.[103] Two inhibitors of acid ceramidase (carmofur and 22 m) have been assessed in various studies.[102,103,136,137] Li *et al.* demonstrated that carmofur, a 5-fluorouracil-releasing chemotherapeutic agent, significantly decreased GalSph accumulation in cells obtained from a Krabbe patient.[103] Additionally, this inhibition prolonged the lifespan of the twitcher mouse model of KD.[103] Furthermore, Di Martino *et al.* has demonstrated that piperidine 22 m induced a reduction in both GalSph levels in the brains of twitcher mice and glucosylsphingosine levels in a mouse model of Gaucher disease and KD.[102] Additional efforts are required to actively pursue the development of an acid ceramidase inhibitor with optimal properties.

Enzyme replacement therapy

KD is identified by a deficiency in the GALC enzyme, prompting various studies to explore the possibility of enzyme replacement.[106] Enzyme replacement therapy (ERT) involves the introduction of externally produced replacement enzymes to address the enzyme deficiency. It has been demonstrated to be effective and safe in treating various other diseases, such as Gaucher disease.[106,138,139] ERT employs M6P receptor-mediated transport, enabling enzyme-deficient cells with M6P receptors to uptake M6P-modified recombinant enzyme, offering a transient source of active GALC for deficient cells and tissues, as demonstrated in an evaluation using the twitcher model.[104]

Administering recombinant GALC successfully reduced GalSph levels and prolonged the lifespan of twitcher mice to 47 days.[105] Meanwhile, it is essential to administer ERT both intravenously and through the intracerebroventricular route to address the challenge posed by the BBB, ensuring effective treatment.[105] Treating KD involves frequent injections with prolonged durations, and although implantable CNS injection devices have been accessible for a substantial amount of time, their application in ERT has been limited due to concerns about adverse events, notably the risk of infection.[140] To address these challenges, three approaches were assessed. Matthes *et al.* demonstrated the effectiveness of intravenous injection of recombinant human GALC in a unique "humanized" mouse model of KD.[107] This method successfully penetrated the BBB, targeting lysosomes in brain macrophages, astrocytes, and neurons.[107] Despite this positive outcome, the treatment did not alleviate neuroinflammation or demyelination or extend the lifespan of the mice.[107] Del Grosso *et al.* presented an enzyme delivery system employing cross-linked enzyme aggregates (CLEAs) encapsulated in poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) that are functionalized with brain-targeting peptides. Successful testing in twitcher mice through intraperitoneal injections revealed restored enzymatic activity in the nervous system, suggesting a significant CNS involvement.[108] In this novel approach, the researchers utilized CLEAs of the enzyme GALC.[108] These CLEAs were encapsulated within PLGA NPs.[108,141] The purpose of this encapsulation is likely to protect the enzyme aggregates during transport and delivery, ensuring their stability and targeted release at the desired site, which is the CNS.[108,141] The addition of brain-targeting peptides (Ang2, g7, or Tf2) further enhances the specificity of the delivery system, ensuring that the encapsulated enzyme reaches the intended target within the CNS.[108] Del Moral *et al.* examined the PLGA NP technique, evaluating the efficacy of NPs based on their lactide and glycolide composition.[110] Their findings indicate a preference for the 60:40 NP formulation due to its superior encapsulation efficiency. Additionally, 50:50 NPs present a feasible alternative, while the increased stability of 75:25 NPs may pose a challenge to lysosomal function.[110] Herzeg *et al.* conducted an assessment of in utero ERT, demonstrating its potential capacity to prevent early pathology.[109] This approach provides an opportunity for the enzyme GALC to reach the CNS before the establishment of the BBB. Moreover, it takes advantage of the distinctive fetal immune system, which has the potential to alleviate immune responses and foster sustained tolerance to novel proteins.[109] Overall, increasing the GALC enzyme by ERT contributes several benefits, interestingly in the fetal period. Weinstock *et al.* showed an unexpected neuroprotective role of the prenatal GALC enzyme that was not associated with its usual role in myelination.[142] In fact, although the GALC enzyme is necessary in the myelination period, the researchers showed that certain important events related to GALC enzyme seemed to take place before the main phase of myelination in the CNS with different temporal expression of the GALC enzyme.[142] Augmenting the GALC level might also enhance other treatments like HSCT. It has been shown that the effectiveness of HSCT may stem from the necessity of GALC in early brain development.[142] This suggests an effectiveness from combining ERT and HSCT. The GALC enzyme is not only essential for myelinating cells but also expressed in various other types of brain cells.[142] Brainstem development is dependent on GALC expression by neurons. Specifically, brainstem neurons lacking GALC had perturbed differentiation and were in a more immature state.[142] On the other hand, the authors showed that the GALC enzyme plays a cell-autonomous role in the development and maturation of immature neurons in the brainstem.[142] When Galc was deleted, neurons in this region ultimately experienced increased axonal atrophy and degeneration, suggesting that the GALC enzyme might contribute to axonal growth.[142] Additionally, as GALC regulates T-box brain transcription factor 1 in the perinatal period and T-box brain transcription factor 1 contributes to cellular differentiation,[142] we suggest GALC enzyme therapy plays a role in enhancing cellular differentiation and neuronal maturation and migration. Weinstock *et al.* showed that early axonal pathology might occur independently of demyelination and was related to a neuron-autonomous effect of GALC enzyme deficiency.[142] Furthermore, Visigalli *et al.* showed that the GALC enzyme is vital for maintaining the function of hematopoietic stem/progenitor cells by regulating crucial sphingolipid levels.[143] Meanwhile, elevated levels of GalSph were shown to induce cell death through an apoptotic mechanism.[144] The findings suggest the potential involvement of caspases in severe demyelination. Recent evidence has suggested that GalSph exerts its cytotoxic effect by possibly initiating the apoptotic cell process.[144] Taken together, GALC enzyme therapy may contribute to neuronal maturation, stem cell proliferation, axonal development, and myelination and regulate the cell death program.

Pharmacological chaperone therapy

Pharmacological chaperone therapy (PCT) involves the use of small molecules specifically at low concentration designed to bind and facilitate the stabilization of the natural conformation of misfolded proteins.[39] With the available crystal structure of GALC, it is anticipated that almost 70% of missense mutations associated with

diseases may induce instability and misfolding in the GALC protein.[42] Consequently, these mutations are expected to become targets of PCT.[42] Certain small-molecule chaperones possess favorable properties, such as low molecular weight, low toxicity, and high bioavailability, making them well suited for addressing CNS pathology.[39,112] Their ability to efficiently traverse the BBB exceeds that of enzymes, rendering them promising candidates for therapeutic interventions in CNS-related conditions.[39] PCT offers advantages such as oral administration, broad biodistribution, and a positive impact on patient quality of life compared to other therapeutic methods.[112] These PCT molecules, resembling the glycan on the substrate, not only possess the capability to bind their respective hydrolases but also have the potential to interact with the enzyme responsible for glycan synthesis.[39] The initial report detailing the application of chaperones to reinstate GALC activity was authored by Lee *et al.*[111] Their findings indicated that PCT (α-lobeline) exhibited specificity for particular mutations, which impacts protein folding.[111] It was demonstrated that α-lobeline had the capability to bind to the GALC protein in nine distinct sites, as indicated through molecular docking analysis in the study conducted by Berardi *et al.*[114] Hossain *et al.* assessed another chaperone treatment involving N-octyl-4-epi-β-valienamine. Their study revealed that N-octyl-4-epi-β-valienamine binds specifically to the active site of the human GALC protein.[115] Furthermore, in various studies, azasugars and iminosugars were investigated for their dual roles as inhibitors and pharmacological chaperones for GALC.[111,113,116] Azasugars exhibit specific binding within the active site pocket, where the position of the ring nitrogen and its positive charge collectively contribute to an enhanced stabilization of GALC.[116] On the other hand, the iminosugar 4-epi-isofagomine emerged as the most promising, displaying significant inhibition of both lysosomal β-galactosidase and GALC.[113]

Indeed, a significant challenge in chaperone therapy lies in accurately identifying suitable candidates.[42] The success of chaperone therapy is contingent upon precise patient selection, considering factors such as the specific genetic mutations present, the nature of the protein misfolding, and the overall condition of the individual.[42] This personalized approach is crucial to optimizing the effectiveness of PCT and ensuring its potential benefits for individuals with various genetic and protein misfolding disorders.

Autophagy activation

Given that autophagy impairment has been linked to numerous neurodegenerative disorders, it has become a focal point in studies centered on KD. Lithium was discovered to boost autophagy, consequently improving cell tolerance to GalSph but not affecting GalSph levels.[63,145] Meanwhile, recent studies have demonstrated that autophagy impairment is not necessarily linked to the level of GalSph and can occur independently of it in KD.[63,145] Papini *et al.* triggered autophagy in fibroblasts obtained from KD patients through starvation.[63] Their research demonstrated that the inhibitory AKT-mediated phosphorylation of beclin-1 and the creation of the B-cell lymphoma 2-beclin-1 complex collaboratively contribute to reducing autophagosome formation independently of GalSph.[63] On the other hand, the administration of rapamycin, an autophagy activator that functions by inhibiting the mTORC1 pathway, was also capable of partially reinstating the levels of autophagy markers.[60–62] Nevertheless, studies in this field are limited so far. Understanding the intricate details of autophagy induction in KD is crucial for developing therapeutic strategies. The identification of pathways involved in autophagy induction may provide insights into potential targets for interventions aimed at mitigating the progression of KD and improving cellular health in affected individuals.

Gene therapy

Over the past two decades, gene therapy, whether used alone or in combination with transplantation, has been progressing in mouse models. Adeno-associated virus (AAV), a frequently utilized virus, has emerged as an attractive vector for gene therapy owing to its nonpathogenic nature and its capacity to target a diverse array of hosts and tissues.[145] Meanwhile, the primary challenge lies in the ability of AAV to cross the BBB.[15] Although AAV can cross the BBB in mice, it has been noted to do so in restricted amounts and with decreased efficiency.[15] To enhance the quantity in the CNS and address this issue, Rafi proposed the following solutions: increasing the viral load, implementing double injections, mobilizing innate bone marrow cells before viral injection, and developing a carrier-mediated method to cross the BBB.[15] Various AAV types studied in the literature are summarized in Table 2. Variations in AAV serotype and dosage are likely responsible for the differences observed in survivability.

Overall, various treatments are undergoing testing in experimental animal models of KD.[95] The only approved treatment is HSCT. Additionally, several studies have shown a more significant therapeutic effect with combined therapy compared to individual treatments with a synergetic and beneficial impact.[95] In contrast, studies exploring the role of a fetal therapy are rare in literature reviews. Weinstock *et al.* proved that treatments administered in utero at or before 32 weeks should yield superior results compared to standard postnatal treatment.[142]

Recently, some studies evaluated in utero a novel ERT approach to alleviate KD symptoms and reported positive outcomes.[109,146] Additionally, Shen *et al.* showed the role of in utero gene therapy by retrovirus vector in lysosomal disorder treatment.[147] In fact, they showed that injecting the retrovirus intrauterinely through cerebral ventricles proved to be a successful method for achieving efficient transfer and sustained expression of an exogenous gene throughout the entire brain.[147] In the meantime, additional research is necessary to evaluate and develop a novel fetal therapy.

Prospective future

This comprehensive review, with a specific emphasis on the metabolic disorder occurring in KD, elevates our understanding of the various biological elements involved in its pathophysiological pathways. This effort contributes to the exploration of novel approaches to diagnosis and therapy. Through ongoing research, collaboration, and a commitment to advancing medical knowledge, the possibility of revolutionary treatments is promising and should usher in a bright future for individuals affected by metabolic disorder. Since the emergence of artificial intelligence and machine learning in various metabolic and endocrine diseases,[148] we suggest that comprehension of the exact pathophysiological mechanisms of KD can help to establish a deep learning machine. Development of an algorithm focused on metabolic disorder to prompt diagnosis and screening of infantile forms can facilitate early diagnosis, making it possible to start treatment in the presymptomatic stage. Despite the rarity of this inherited disease, establishing regional registers worldwide is essential to accumulate a substantial patient pool, contributing to the refinement and effectiveness of the machine learning processes.

Despite the initial discovery of KD occurrence 117 years ago, advancement in the comprehensive understanding of this rare neurodegenerative disease has been slow. Our in-depth review presents an overview of KD's pathophysiological pathways, contributing to a better understanding of the disease. Recently, EVs and α-synuclein have shown their involvement in the metabolic disorder of KD. Autophagy impairment was observed to be unrelated to GalSph but dependent on AKT and BCL-2. Furthermore, our review comprehensively summarizes all therapy trials for KD, providing a detailed comparison between their benefits, administration mode, and disadvantages. It brings new hope for treating the disease, suggesting the possibility of discovering an effective therapy, particularly for the early infantile form, in the future.

DISCLOSURE

The authors declare no conflicts of interest.

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