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Beyond Krabbe's disease: the potential contribution of GALC deficiency to neuronal vulnerability in late onset synucleinopathies

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Abstract

New insights into the pathophysiological mechanisms behind late onset neurodegenerative diseases have come from unexpected sources in recent years. Specifically, the group of inherited metabolic disorders known as lysosomal storage diseases that most commonly affect infants has been found to have surprising similarities with adult neurodegenerative disorders. Most notable has been the identification of Gaucher's disease as a co-morbidity for Parkinson's disease. Prompted by the recent identification of neuronal aggregates of α -synuclein within another lysosomal storage disease, Krabbe's disease, we propose the idea that a similar connection exists between adult synucleinopathies and Krabbe. Similarities between the two diseases, including the pattern of α -synuclein aggregation in the brain of the Twitcher mouse (the authentic murine model of Krabbe's disease), changes to lipid membrane dynamics, and possible dysfunction in synaptic function and macroautophagy underline a link between Krabbe's disease and late onset synucleinopathies. Silent *GALC* mutations may even constitute a risk factor for the development of Parkinson's in certain patients. More research is required to definitively identify any link and the validity of this hypothesis, but such connection would prove invaluable for developing novel therapeutic targets for Parkinson's based on our current understanding of Krabbe's disease and establishing new biomarkers for the identification of at-risk patients.

Keywords

Parkinson's disease; Krabbe's disease; Alzheimer's disease; dementia; lysosomal deficiency; psychosine; α -synuclein; neuronal degeneration; synaptic failure

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CONFLICT OF INTEREST STATEMENT

ERB is a consultant for Lysosomal Therapeutics, Inc.

ROLE OF AUTHORS

MM and ERB contributed equally to the drafting and critical revision of the manuscript for important intellectual content

INTRODUCTION AND HYPOTHESIS

Disease indiscriminately affects people of all ages, from the earliest appearances of inborn genetic disorders in neonates, to the late-onset neurodegenerative conditions that plague our geriatric populations. Although these diseases affect widely disparate patients, it has only recently been appreciated that many of these disorders may share a commonality in their underlying pathological mechanisms. Recent attention has been given specifically to the group of inherited metabolic disorders known as lysosomal storage diseases (LSDs) and their increasingly noticeable connection to late-onset neurodegenerative disorders such as Alzheimer's and Parkinson's disease, among others.

The strongest connection between these diseases has been found between the LSD Gaucher's disease and Parkinson's, the two of which have been robustly linked via genetic, cellular, and biochemical connections (Shachar et al. 2011). Sprouting from these discoveries has been an interest to identify mechanisms inherent to LSDs that may be useful for the identification of novel pathophysiological processes in adult neurodegenerative disorders. One LSD that had not been associated with late-onset neurodegenerative disorders until recently was Krabbe's disease. With the recent identification of proteinaceous aggregates in the Krabbe murine and human brain (Smith et al. 2014), this connection can now be better appreciated. When recent studies on Krabbe are examined in this context, one can recognize the potential that Krabbe research has for broadening our understanding of neurodegenerative disorders, especially the synucleinopathies, and why future research into these connections is needed.

In this context, we hypothesize that pathophysiological mechanisms underlying Krabbe's disease share a connection to neuronal vulnerability in neurodegenerative synucleinopathies, suggesting that mutations in Krabbe's disease may even constitute risk factors for synucleinopathies such as Parkinson's disease.

KRABBE'S DISEASE

Krabbe's disease is a rare, inherited lysosomal storage disorder caused by mutations in the *GALC* gene for the lysosomal enzyme galactosylceramidase (GALC). Loss of function of this enzyme results in the accumulation of its undigested substrates, most toxically, the sphingolipid psychosine and a progressive demyelination of the central and peripheral nervous systems. Symptoms of Krabbe's disease include neurosensory deficits, bradykinesia, muscle rigidity/atrophy, and ultimately premature death by age two without treatment. Some of these symptoms have "Parkinsonian" characteristics, but due to the age of Krabbe patients no definitive diagnosis can truly be made. However, in addition to the loss of oligodendrocytes, a neuronal component to the pathophysiology of this disease has been elucidated in recent years, which appears to be driven, at least in part, by the toxicity of psychosine (Castelvetri et al. 2013; Castelvetri et al. 2011; White et al. 2009). These neuronal deficits bring attention to Krabbe's disease's potential importance for understanding neurodegenerative disorders.

EVIDENCE FOR HYPOTHESIS

Protein aggregates

One of the most common and defining characteristics of late-onset neurodegenerative disorders is their classification as proteopathies due to accumulations of misfolded proteins into amyloid structures. The most common proteopathic species have all been identified in multiple forms of LSDs, including Amyloid- β (Niemann Pick-Type C, Sandhoff), Tau (Sanfilippo syndrome type B), and α -synuclein (Gaucher, Metachromatic leukodystrophy, Krabbe) (Keilani et al. 2012; Mattsson et al. 2012; Mazzulli et al. 2011; Ohmi et al. 2009; Shachar et al. 2011; Smith et al. 2014). The physiological impact of these aggregates on the clinical course of these LSDs though is unclear. Still, investigating the mechanisms responsible for their accumulation could bring important insights into late-onset disorders, which may be influenced by similar cellular mechanisms.

The accumulation of α -synuclein within the brain of the Twitcher mouse (the authentic murine model for Krabbe's disease) is particularly interesting for the parallels it displays with the adult synucleinopathies. Normally, α -synuclein is a small disordered protein that localizes to the pre-synaptic terminal, and appears to have some impact on synaptic release, though its precise function is unknown (Cabin et al. 2002; Maroteaux et al. 1988). In pathological conditions, α -synuclein can undergo neuronal aggregation into Lewy Bodies, the pathological hallmark of Parkinson's and Lewy body dementia, or glial aggregation as seen in Multiple System Atrophy. Aggregates of α -synuclein are almost exclusively neuronal in Twitcher, originating in the hindbrain's medulla and pontine regions before the pathology spreads rostrally and dorsally into the midbrain structures, affecting the cerebral cortex in only the late stages of the disease (Smith et al. 2014). This pattern of accumulation is precisely the order of Lewy body accumulation that is described in Parkinson's brains via Braak staging (Braak et al. 2003). These aggregates also display regional specificity that follows the Braak staging, such as specificity for the CA2 region of the hippocampus and the A9 nuclei (Smith et al. 2014). Similar alpha-synuclein aggregations have been identified within the brains of human Krabbe patients, particularly the cortex (Smith et al. 2014), but their spatial and temporal distribution has yet to be established.

Given the similar spatial and temporal pattern of these aggregates within the Twitcher mouse, further investigation may reveal a common mechanism responsible for the manner in which these aggregates seem to spread through the brain. Some studies have suggested a "prionic" mechanism of action for these aggregates as seeds that induce aggregation in previously normal neurons (Visanji et al. 2013). There may also be a non-protein species that is transported between neurons to induce aggregation, such as exosomes. This pattern of α -synuclein accumulation could also be the result of intrinsic factors of certain neuronal populations that delay their susceptibility to protein aggregates relative to other populations.

Currently, α -synuclein is the only protein that has been identified to aggregate within the Krabbe and Twitcher brain. The specificity by which LSDs accumulate certain proteins could provide important information about the cellular and biochemical factors that are most instrumental for their aggregation. This is of particular importance given that many late-onset neurodegenerative disorders are compounded by multiple aggregate species, with

some evidence suggesting that α -synuclein and amyloid- β could mutually promote each other's accumulation (Marsh and Blurton-Jones 2012). While the presence of other protein aggregates within the Twitcher and Krabbe brain may still be identified, it could also be that Krabbe's disease provides a unique environment for α -synuclein aggregation. Accumulations in cellular aging, while largely absent in a primarily infantile disease such as Krabbe's disease, likely have a significant influence on the course of protein accumulation with the late-onset neurodegenerative diseases. However, identifying the underlying impact that GALC mutations and the subsequent accumulation of psychosine may play on protein aggregation could be invaluable for understanding mechanisms universal to their formation.

Lipid and membrane interaction

Aggregation of α -synuclein was shown *IN VITRO* to be accelerated in the presence of psychosine, suggesting that there may be a direct interaction between the two (Smith et al. 2014); however, biophysical experiments are required to characterize any potential intermolecular associations. An interaction may be possible given α -synuclein's association with lipid membranes, which has been shown to have a clear effect on the dynamics of α -synuclein aggregation. α -Synuclein also has been shown to bind specifically to gangliosides such as GM1 (Martinez et al. 2007). The mechanism of aggregation may not require direct interaction with psychosine, but rather a perturbation of membrane dynamics due to the imbalance of psychosine degradation. In fact, pathological levels of psychosine in Twitcher accumulate within lipid rafts of the membrane, disrupting their architecture (White et al. 2009). These rafts are locations where α -synuclein interacts with the membrane, actually mediating the localization of α -synuclein to the pre-synaptic terminal (Fortin et al. 2004). Mutations in the *GALC* gene leading to non-lethal enzyme deficiencies and small accumulations of psychosine in lipid rafts of aging neurons could be a contributing mechanism that triggers α -synuclein dysregulation in late onset conditions.

Lysosomal/autophagic function

The presence of proteinaceous deposits across nearly all of the LSDs suggests that this phenomenon could also result from or at least be compounded by a more basic and universal mechanism than substrate- α -synuclein interactions. By definition, these disorders have a deficiency in their clearance of waste material, usually via a hydrolase deficiency and less frequently through impaired fusion of the autophagosome with the lysosome. There is evidence that reduced metabolism of these waste products can increase toxicity due to upstream blockage of the macroautophagy system. This could be a key factor as these oligomeric and fibrillized species of α -synuclein may be formed under homeostatic conditions but are quickly metabolized and removed before any adverse interaction can take place. However, once the system is compromised, these proteopathic species are allowed to accumulate. This may be exacerbated through a feedback loop in which α -synuclein adversely interacts with the macroautophagy system. Such a feedback loop has been identified within Gaucher between α -synuclein and glucocerebrosidase (Mazzulli et al. 2011). More generally, mutated forms of α -synuclein can reduce the formation and transport of autophagic vacuoles, along with inhibiting the key chaperone mediated autophagy receptor Lamp2a, limiting a primary route of wild type (WT) α -synuclein clearance (Xilouri et al. 2016). Increased autophagy markers (LC3) have been observed in GALC deficient

cells (Ribbens et al. 2014), indicating that a similar process may be occurring in this disease state.

With nearly all the neuronal populations of the brain being post-mitotic, the possibility of diluting accumulated waste material that results from reduced autophagic clearance by cell division is highly unlikely. This leaves these neurons particularly vulnerable, increasing neuronal dysfunction, and eventually cell death. The significance that this autophagic dysfunction may have on late-onset neurodegenerative disorders is becoming clearer. Parkinson's brains have displayed a depletion of lysosomes and a corresponding elevation in autophagic vacuoles that suggests a lysosomal dysfunction could be inhibiting the clearance of autophagosomes (Shachar et al. 2011). The precipitating event that causes a dysfunction of autophagy in Parkinson's is unknown, but investigating known mutations of LSDs as potentially risk factors could provide new crucial information.

Synaptic function

α -Synuclein's localization to the pre-synaptic terminal has led to the proposal that it functions in synaptic release of vesicles. Studies utilizing transgenic knock-outs of α -synuclein and mutated isoforms of α -synuclein have demonstrated its role in the trafficking, clustering, and fusion of pre-synaptic vesicles (Cabin et al. 2002; Scott and Roy 2012), potentially through interactions with the SNARE complex (Burre et al. 2010). This may be a critical early step in the pathogenesis of Parkinson's, especially in the development of cognitive symptoms outside of the traditional motor deficits. There are interesting parallels within the Twitcher brain that suggest a potential synaptic dysfunction, which may also be physiologically relevant to neurodegenerative disorders.

Although psychosine has yet to be implicated, multiple sphingolipids have been shown to regulate synaptic vesicle release (Haughey 2010). Additionally, the disruption of lipid rafts, which psychosine is known to do, can inhibit NMDA receptor currents (Haughey 2010). Twitcher mice also display a deficit in fast axonal transport that suggests the possibility that trafficking of synaptic vesicles could be compromised as well (Castelvetri et al. 2013). This effect on axonal transport has been solely attributed to psychosine, and whether α -synuclein plays a mediating role is yet to be determined. Synaptic failure often leads to a dying back neuropathy that could be a potential mechanism for vulnerability in certain neuronal populations. The role of synaptic failure in synucleinopathies is gaining prominence, with some suggesting it as the primary event in the pathogenesis of these diseases (Calo et al. 2016).

Genetic predisposition

It is currently unknown if a genetic link between mutations in the *GALC* gene and a predisposition for late onset neurodegenerative disorders similar to the link found for Gaucher's and Parkinson's exists (Shachar et al. 2011). Gaucher's disease is the most common LSD, whose larger patient population allowed for a registry of Gaucher patients that originally identified Gaucher's as a co-morbidity for Parkinson's disease. Rare diseases such as Krabbe's are likely underreported, especially if mild LSD symptomatology is masked by more overt Parkinson's symptoms. Further, it is unlikely that mutations to the

GALC gene are a dominant risk factor for the development of a synucleinopathy. Rather, we speculate that a reduction in the efficiency of the *GALC* enzyme induces a susceptible condition in neurons that when combined with other genetic and environmental factors, leads to pathology (Figure 1).

This deficiency could be caused by the accumulation of mild mutations in the *GALC* gene, of which at least 130 are already identified. Many of these single nucleotide polymorphisms (SNPs) have been identified as silent mutations in the short time frame that these patients were studied; however, a longitudinal study into the later decades of their lives has not been performed. Effects from epigenetic regulation of the *GALC* gene are unknown and could also play a role to down regulate enzyme efficiency. A graphical representation is provided in figure 1, which depicts how these mutations may manifest in fulminant cases, late-onset Krabbe (adolescence), and a potentially very-late onset state that is influenced by additional risk factors. The most common mutation for the early infantile form is a homozygous 30kb deletion (C502T), eliminating all enzymatic activity. A mutational analysis of a cohort of adult onset Krabbe patients found that half of these cases were heterozygous for this mutation (De Gasperi et al. 1996), suggesting that reduced enzymatic activity can result in delayed pathology. SNPs that result in a milder reduction of *GALC* activity may remain silent for decades, only clinically relevant when combined with other genetic and environmental influences. Inter-patient variability may even mask these deficits, delaying the susceptibility for neurodegeneration during a patient's lifetime.

SIGNIFICANCE

Research into neurodegenerative disease has been a lengthy and costly endeavor. These diseases have come to be recognized as more heterogeneous than previously thought, suggesting that it's unlikely a solitary solution will be effective for all patients diagnosed under the same disease classification. Therefore, new and creative approaches need to be sought out through the identification of novel mechanisms. A range of novel therapeutics would potentially benefit any patients with a neurodegenerative disorder whose condition is influenced by *GALC* insufficiency. Researchers for Krabbe's disease are rapidly developing a wide variety of promising therapeutic strategies that include enzyme replacement therapy, substrate reduction therapy, chemical chaperone therapy, and gene therapy. Not to mention, the current best practice for the treatment of *GALC* deficiency, which is hematopoietic stem cell transplantation. Additionally, a connection between Krabbe's disease and late onset synucleinopathies would open the door for powerful new biomarkers to identify subsets of patients at risk for the development of Parkinson's or possibly other synucleinopathies.

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SIGNIFICANCE STATEMENT

Lysosomal storage disorders and late onset neurodegenerative diseases have displayed interesting connections in recent years. This commentary hypothesizes whether GALC deficiency as seen in the lysosomal storage disorder Krabbe's disease, can elucidate new mechanisms for neuronal vulnerability in late onset synucleinopathies. This idea is supported by evidence related to α -synuclein aggregation, lipid membrane dynamics, and possible deficits to synaptic function and macroautophagy displayed in Krabbe's disease. A connection between the two disorders would prove invaluable for developing novel therapeutic targets for Parkinson's based on our current understanding of Krabbe's disease and establishing new biomarkers for the identification of at-risk patients.

Figure 1.

Infantile Krabbe's disease follows a fulminant clinical course that is typically diagnosed around 6 months of age. The infantile disease is often the result of severe homozygous mutations that if not completely eliminating GALC function, severely limit it. The late onset form of Krabbe's disease has been known to commonly manifest in patients that are heterozygous for severe *GALC* mutations, suggesting delayed pathology can be due to reduced enzymatic function. Here, a new condition is proposed in which mild SNPs to the *GALC* gene result in slight deficiencies of GALC's activity that may lead to accumulation of low quantities of toxic material. Over decades of slow and progressive metabolic deficiency, this neuronal vulnerability is increased, leading to late onset synucleinopathies. This vulnerability could be influenced by other genetic and environmental factors that are either pathogenic or protective.