Cells other than oligodendrocytes and Schwan cells may be involved in the rapid progression of Krabbe Disease

Karen J. Fernandez[1], Nadav I. Weinstock[1], Daesung Shin[1,3], Nicholas Silvestri[2], L. Wrabetz[1,2,3] and M. Laura Feltri[1,2,3]

(1) Hunter James Kelly Research Institute, (2) UB Department of Neurology, (3) UB Department of Biochemistry

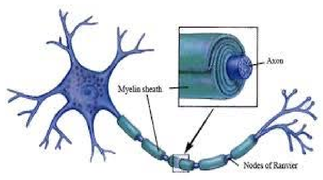
HJKRI - Hunter James Kelly Research Institute

University at Buffalo The State University of New York

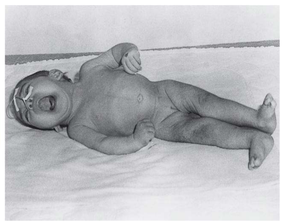
Abstract

Globoid Cell Leukodystrophy, or Krabbe disease (KD), is an autosomal recessive disease of the nervous system characterized by the deficiency of the enzyme galactocerebrosidase (Galc). KD occurs in infants and leads to death within two years of life. In KD, apoptosis of oligodendrocytes and Schwan cells (SC) leads to demyelination and neurodegeneration of the brain, spinal cord and nerves. Besides oligodendrocytes and SC, Galc is also present in other cells, like neurons and macrophages, which suggests that they may play an autonomous role in the disease. To further explore this hypothesis, mouse models were developed that can precisely remove Galc function in all cells, or only in Schwan cells, neurons or macrophages. These mice were compared based on morphology, and on behavior and clinical progression. By doing so, a better understanding of the existing role and interaction among these cells will be defined. This work will help us to better understand the pathophysiology of KD in the PNS, which has implications for designing better therapies to treat KD.

Background

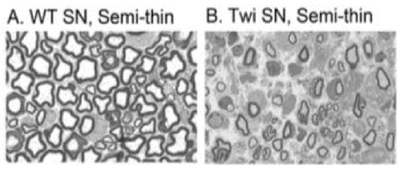


Representation of myelin around axon [1].



9-month-old patient with Krabbe Disease[2].

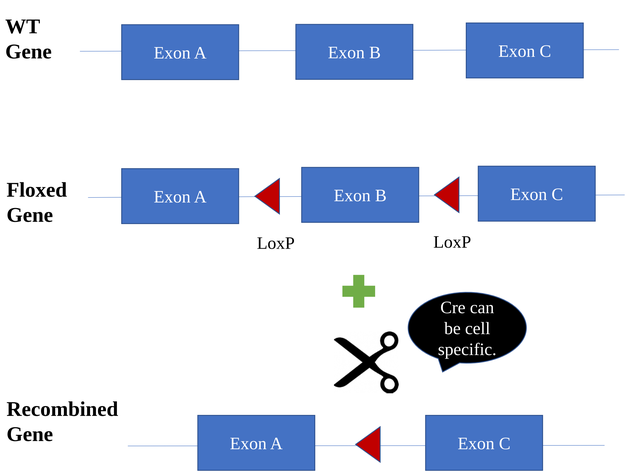
P35 *Twitcher* mice [3].



WT/ *Twitcher* SN semithins.

P35 *Twitcher* mice. The *twitcher* mouse is a well studied model of Krabbe Disease. Kyphosis and muscular atrophy are seen in this mice. **SN semithin of P35 WT and *twitcher* mice. A.** Normal myelinated fibers are present in the WT. **B.** *Twitcher* Sciatic nerve has fewer fibers and thinner myelin.

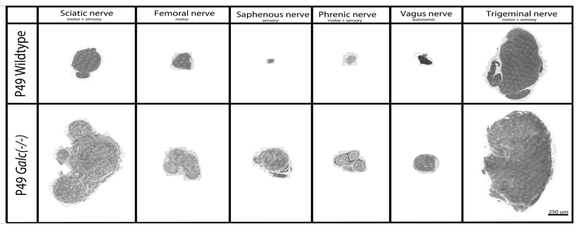
Experimental Approach



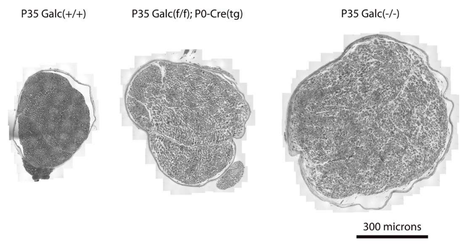
Cre-loxP strategy used to create the Schwan cell *Galc* cKO mice and the *Galc* null mice. In order to remove Galc function specifically in Schwan cells, P0- Cre was used to recombine the Galc allele, resulting in mice with *Galc(fl/fl);P0-Cre(tg)* genotype. CMW-Cre was used to create the Null (everywhere/ global Cre) mice, where Galc function was removed in all cells. These mice are referred to as *Galc* (-/-) mice.

Results

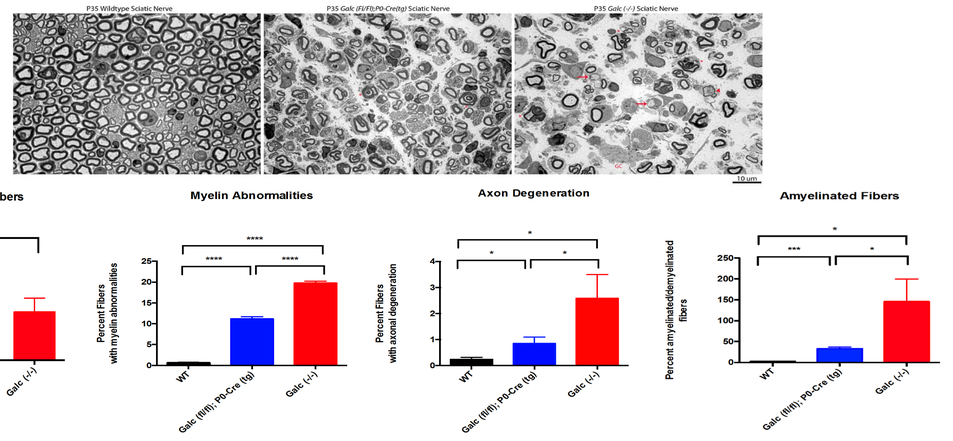
Morphology



Comparison of P49 WT and P49 global null semi-thin nerve reconstructions. Different types of nerves (motor, sensory, motor and sensory, and autonomic) are affected by Galc deficiency. Also, nerves in the *Galc* (-/-) mice appear to be four times bigger than the WT.

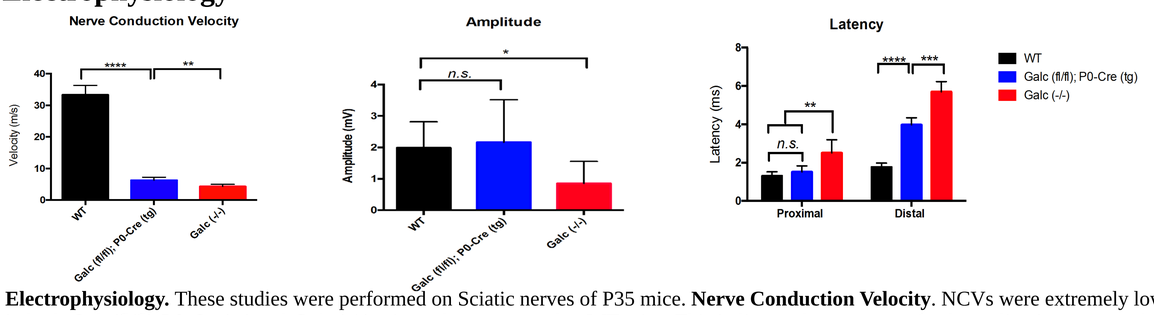


Comparison of P35 semi- thin, sciatic nerve reconstructions in WT, *Galc(fl/fl);P0-Cre(tg)* mice, and *Galc* (-/-) mice. The *Galc(fl/fl);P0-Cre(tg)* phenotype seems to be an intermediate of the WT and *Galc* (-/-) phenotypes.



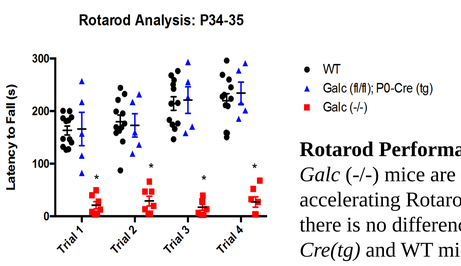
Morphology. The top three images are close-ups of the semithin reconstructions of P35 Sciatic nerves in the WT, *Galc(fl/fl);P0-Cre(tg)*, and *Galc* (-/-) mice. Clearly, in the *Galc* (-/-) mice, the myelinated axons are less densely packed and have more debris (probably due to nerve swelling and degeneration) than the *Galc(fl/fl);P0-Cre(tg)* and WT mice. **Myelinated Fibers.** There isn't a big difference in the number of myelinated fibers between the WT and the *Galc(fl/fl);P0-Cre(tg)* mice, but, in the *Galc* (-/-) mice there is a large decrease in the number of myelinated fibers. **Myelin Abnormalities.** When compared to the *Galc(fl/fl);P0-Cre(tg)* mice, the *Galc* (-/-) mice have twice as many myelin abnormalities (infoldings and outfoldings). There are no myelin abnormalities in the WT. **Axon degeneration.** The percent of axonal degeneration is higher in the *Galc* (-/-) mice than in the *Galc(fl/fl);P0-Cre(tg)* and WT mice. **Amyelinated Fibers.** Almost no amyelinated fibers are present in the WT. There are approximately six times more amyelinated fibers in the *Galc* (-/-) than in *Galc(fl/fl);P0-Cre(tg)* mice.

Electrophysiology



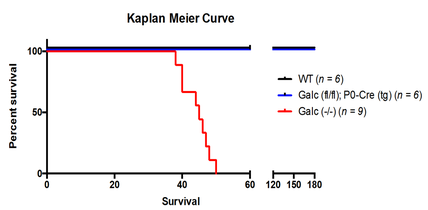
Electrophysiology. These studies were performed on Sciatic nerves of P35 mice. **Nerve Conduction Velocity**. NCVs were extremely lower in both *Galc(fl/fl);P0-Cre(tg)* and *Galc* (-/-) mice, when compared to WT mice. This indicates the presence of a demyelinating neuropathy. A small, but statistically significant, decrease in NCV was seen in *Galc* (-/-) mice compared to *Galc(fl/fl);P0-Cre(tg)* mice. **Amplitude**. The amplitude of the *Galc* (-/-) Sciatic nerves were decreased from WT, but *Galc(fl/fl);P0-Cre(tg)* mice were not. **Latency**. *Galc* (-/-) nerves had an increased latency for electrical signal to spread along the nerve along both short (proximal) and long (distal) measurements. *Galc(fl/fl);P0-Cre(tg)* nerves had increased latency at long (distal) distances but not when measured proximally. Tests were performed by Dr. Nick Silvestri.

Functional Analysis



Rotarod Performance Test. *Galc* (-/-) mice are not able to run on an accelerating Rotarod at P35. In contrast, there is no difference between *Galc(fl/fl);P0-Cre(tg)* and WT mice. These differences may be due to CNS abnormalities that are seen in *Galc* (-/-) mice but not *Galc(fl/fl);P0-Cre(tg)* mice.

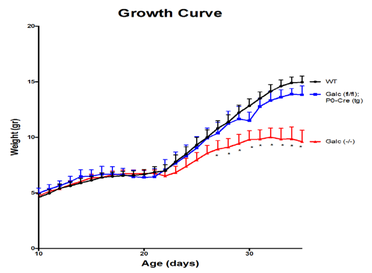
Survival



Galc mice survival. *Galc* (-/-) mice have an average lifespan of 45 days. In contrast, *Galc(fl/fl);P0-Cre(tg)* exhibit no change in survival compared to WT littermates up to 180 days.

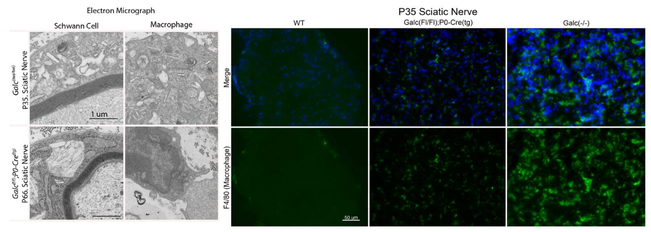
Results Cont.

Growth



Galc mice survival curve. All of the mice show normal growth from P10-P21. At P22, *Galc* (-/-) mice start to lose weight when compared to WT and *Galc(fl/fl);P0-Cre(tg)* mice. *Galc(fl/fl);P0-Cre(tg)* mice have similar growth patterns when compared to WT mice, but start to have a decreasing weight trend around P30 and onward.

Macrophages (Globoid Cells) in Sciatic Nerve



Analysis for cellular abnormalities in P35 SN of *twitcher* and *Galc(fl/fl);P0-Cre(tg)* mice by electron microscopy. *Twitcher* Schwann cells and macrophages both contain crystals of galactosylceramide, the substrate for Galc. In the *Galc(fl/fl);P0-Cre(tg)* mice, SC contain galactosylceramide crystals, while the macrophages seem normal and contain no crystals. **Immunofluorescence of macrophages (F4/80 positive) in P35 Sciatic nerves.** Endoneurial macrophages were almost absent in WT nerves. On the other hand, macrophages were present in both *Galc(fl/fl);P0-Cre(tg)* and *Galc* (-/-) nerves. However, there were more and larger macrophages present in the *Galc* (-/-) mice.

Conclusions

It was found that the *Galc(fl/fl);P0-Cre(tg)* mouse had a strong demyelinating neuropathic phenotype, despite endogenous Galc expression in the remaining cell types. Even though a strong demyelinating phenotype was seen, *Galc(fl/fl);P0-Cre(tg)* mice had a less severe phenotype when compared to the *Galc* (-/-) mice. Despite this demyelination, *Galc(fl/fl);P0-Cre(tg)* mice showed only moderate decreases in weight loss and motor function in comparison to the *Galc* (-/-) mice, and had no mortality in the 6-month survival curves. Sciatic nerves from *Galc(fl/fl);P0-Cre(tg)* mice were morphologically different from *Galc* (-/-) mice. They had more myelin and fewer myelin abnormalities. It was hypothesized that these differences were caused by Galc in cells other than SCs, specifically macrophages and neurons. The incomplete *Galc(fl/fl);P0-Cre(tg)* phenotype may therefore be caused by a small, but insufficient, amount of Galc delivered to SCs by Galc-producing macrophages or neurons.

Future Directions

To compare the morphology, behavior and clinical progression of the motor neuron and macrophage *Galc* cKO mice to the WT, *Galc(fl/fl);P0-Cre(tg)*, *Galc* (-/-), and *twitcher* mice, in order to have a more clear understanding of the specific cells that play a role in the rapid progression of Krabbe Disease.

References

1. http://multiple-sclerosis-research.blogspot.com
2. Atlas of Inherited Metabolic Diseases 3E. Dec 2011, 726- 732
3. De Gasperi, R, V L Friedrich, G M Perez, E Sentrurk, P H Wen, K Kelly, G A Elder, and M A Gama Sosa. "Transgenic Rescue of Krabbe Disease in the Twitcher Mouse." *Gene Therapy* 11, no. 15 (2004): doi:10.1038/sj.gt.3302282