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

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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 schwann 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 Schwann 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.

Results
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.

Electrophysiology
Morphology
The top three images are close-ups of the seminum 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 (inclusions 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. Amylated Fibers. Almost no amylated fibers are present in the WT. There are approximately six times more amylated fibers in the Galc(-/-) than in Galc(fl/fl);P0-Cre(tg) mice.

Electrophysiology
The 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.

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.

Background

Comparison of P35 Global semi-thin sciatic 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.

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Analysis of 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) mice 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 macrophage 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