Structural changes to nodes of Ranvier consequential of adult-onset sulfatide depletion

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Abstract

Multiple Sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system (CNS). MS affects ~750,000 people in the United States alone, and roughly 2 1/2 million worldwide presenting with a 3:1 disproportionate diagnosis for women. MS-related disabilities, which include motor, sensory and cognitive deficits, are highly varied depending on the region of the CNS that experiences myelin loss. To better understand the consequence of myelin loss, we use in vivo models that allow specific depletion of myelin components. Most recently, we have focused on the role that lipids play in the regulation of myelin stability and neuronal function. Specifically, we are studying a myelin glycosphingolipid known as sulfatide. Sulfatide, which is significantly depleted in the very earliest stages of MS development, constitutes 7% of the lipids in the myelin sheath in mice and 4% of myelin lipids in humans and has been implicated in regulating 1. the proliferation of oligodendrocytes, the cells that make myelin in the CNS, 2. membrane organization, and 3. protein trafficking within oligodendrocytes. Using a mouse incapable of synthesizing sulfatide prenatally, we have previously reported that the absence of sulfatide results in compromised structure of the nodes of Ranvier and the associated paranodes, which together constitute the axonal domains responsible for axon action potential conduction. Although the study of this mouse significantly furthered our understanding of the function of sulfatide, this mouse provides limited insight for adult-onset disease such as MS. Therefore, to more accurately model MS, we have created and analyzed a mouse that utilizes the cre/loxP system to specifically deplete sulfatide levels with age and cell-type specific control. Here, we have exploited this novel mouse to deplete sulfatide in adult mice and use electron microscopic analyses to quantify structural changes in the node of Ranvier and paranode. Our preliminary data indicate a progressive loss of domain integrity that is consistent with ultrastructural deterioration that has been reported in human disease.

Previous Work

Comparison of CST KO tissue to human MS tissue shows similar ultra-structural abnormalities

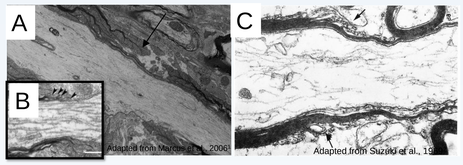


Figure 1: CST KO mouse shows loss of paranodal attachment to axon (A) and eversion of lateral loops[1] (B) Brain tissue from human MS patients[2] have pathology consistent with CST KO mouse including loop eversion (arrows) and loose contact between the myelin and axon.

Plp-cre^{ERT}/CST$^{fl/fl}$ Inducible model

Mouse Genotypes and Experimental Paradigm

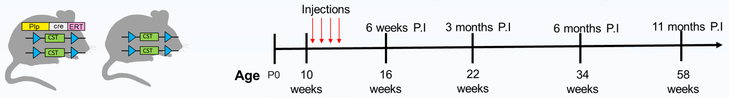
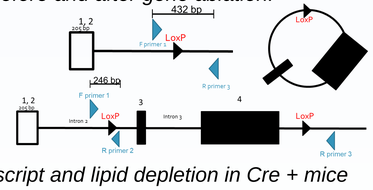


Figure 2: Generation of a Plp-cre^{ERT}/CST$^{fl/fl}$ mice. Cre+ mice are experimental and Cre- mice control for tamoxifen. After 4 consecutive days of i.p injections of tamoxifen, we age mice to above timepoints

Genomic map of CST construct before and after gene ablation.

Figure 3: Primers (blue arrowheads) were designed around LoxP sites to detect efficiency of ablation. After gene ablation, Cre+ samples reveal 432bp product. Lack of ablation reveals a 246bp product.



Detection of sulfatide gene ablation, transcript and lipid depletion in Cre + mice

Figure 4: Cre+ and Cre- cortex was removed and DNA and RNA were isolated. In the Cre+ mouse, the CST gene is ablated and transcript levels are significantly lowered.

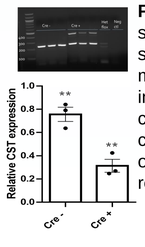
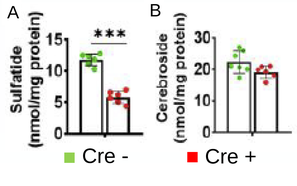


Figure 5: Based on mass spectrometry[3], sulfatide is significantly reduced by 9 months post tamoxifen injection in the cre+ vs cre- mice (A). Note that cerebroside, the precursor of sulfatide, was not reduced (B).



Materials and Methods

Animals: CST$^{fl/fl}$/Plp-creERT mice are aged to 10 weeks of age and given intraperitoneal injections of 60mg/kg of tamoxifen, dissolved in corn oil, for four consecutive days. Tissue was analyzed at 3 months, 6 months, and 11 months post injection (P.I). For each timepoint 5-7 mice will be used per genotype.

Gene Detection: Cre+ and Cre- were transcardially perfused with 0.9% saline and brain tissue was flash frozen in liquid nitrogen. DNA and RNA were isolated using Qiagen All Prep kit.

Electron Microscopy (EM) : Cre+ and Cre- mice were transcardially perfused with saline followed by a phosphate buffered solution containing 5% glutaraldehyde and 4% paraformaldehyde. Following post-fixation, cervical spinal cord, and brain were harvested and processed for standard electron microscopic analysis as previously described[1,4]. The ultrathin sections were examined with a JOEL 1400PLUS Transmission Electron Microscope.

Quantification: For g-ratio and axonal/myelin analysis, 10 images were collected per animal, containing 50-100 axons in cross section. Analysis of g-ratio quantification consisted of measuring the myelin and axonal widths. Axonal/myelin analysis including quantifying myelin and axonal pathologies such as; neurodegeneration, myelin splitting, vacuolar degeneration, and myelin uncompaction. For nodal length measurement, 10 images in longitudinal orientation were collected per animal. Nodes of Ranvier lengths were measured using Image-J

Electron Microscopy g-ratio

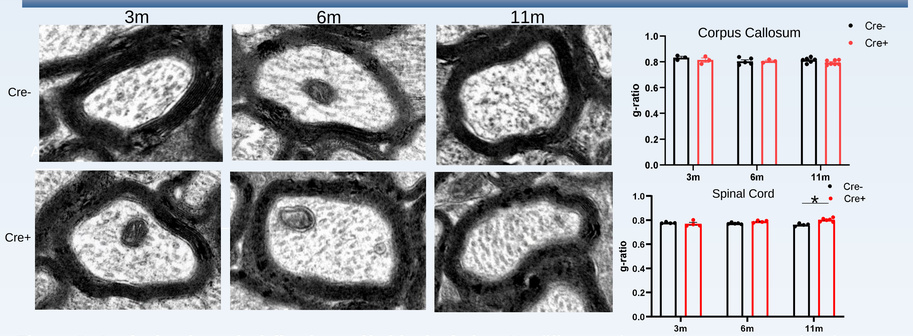
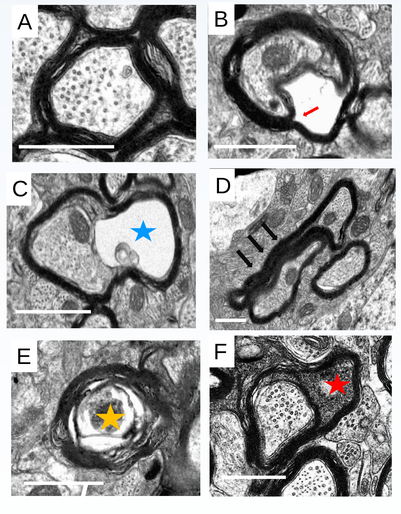


Figure 6: Analysis of g-ratio following sulfatide depletion. No difference in g ratio was observed in the corpus callosum at any of the time points. Similar observations were made for the spinal cord with the exception of a slight increase in g ratio (thinner myelin) at 11 months post injection**.**

Ultrastructural Analysis



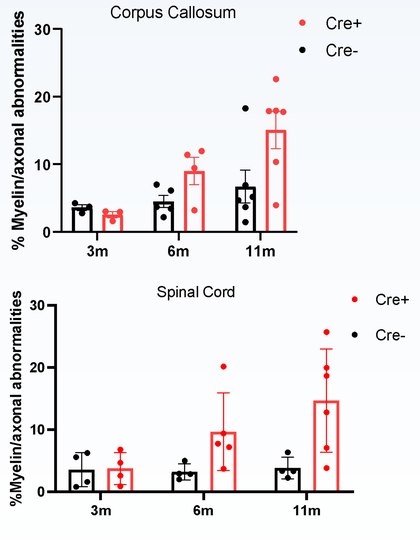
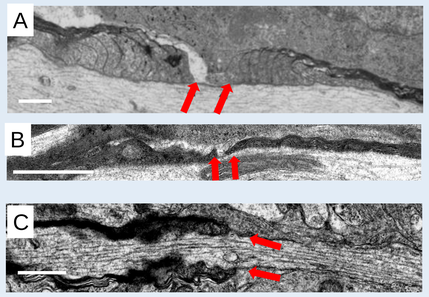


Figure 7: Myelin and axonal defects following sulfatide depletion in the adult CNS. The corpus callosum and spinal cord of Cre+ mice reveal; 1. presence of intralamellar splitting of the myelin consistent with unstable myelin (red arrow, B); 2. pulling away of the myelin sheath from the axon (blue star; C) 3. redundant myelin profiles (black arrows, D) 4. degenerated axonal profiles (orange star, E) and 5. myelin uncompaction (red star, F) Myelin sheaths from these mice in the spinal cord reveal. Magnification bars = 1um

Nodal length



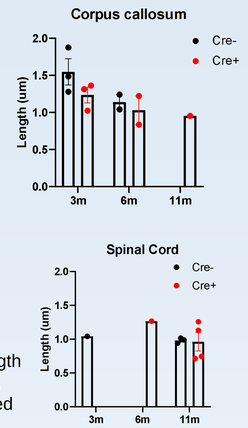


Figure 8: Variation in nodal lengths in sulfatide deficient mice. Preliminary data suggest sulfatide deficiency in adulthood increases variability in nodal length in the spinal cord by 11 months post tamoxifen injection. Panel B presents an extremely short node of Ranvier (red arrows) while panel C displays an extremely long node.

Transverse bands

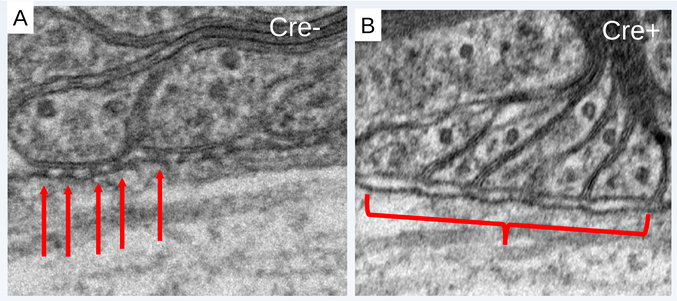


Figure 9: Loss of transverse bands in paranodes in sulfatide deficient mice (A) Cre- paranodes with preserved transverse bands (red arrows, B). Cre+ paranodes display a loss of transverse bands, indicated by red bracket**.**

Future Directions

We have evidence that adult onset sulfatide depletion is sufficient to cause myelin instability, moving forward we will:

- Have well powered quantification of structural and nodal protein distribution using EM and IHC, respectively
- Understand the ***mechanism*** by which lack of sulfatide causes myelin protein instability
  - Does sulfatide regulate lateral interactions at the myelin sheath, anchoring myelin proteins?
  - Does sulfatide aid in trafficking myelin proteins to their correct destination in microdomains?

References

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Acknowledgements

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