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Myelin lipid deficiency: a new key driver of Alzheimer's disease

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Lipids play essential biological functions that include acting as components of biological membranes, energy storage, signaling, nutrients, transporters, enzyme activators, among others. Compared with the multiple research methods to assess DNA, RNA, and protein content, location, and function in cells, there are relatively fewer methods to study lipids. Therefore, lipid-oriented mechanistic studies remain rare and challenging. Lipidomics which allows large-scale analysis of cellular lipids, is a critical strategy for achieving this. One revolutionary advance in lipidomics pioneered by our group is the development of multidimensional mass spectrometry-based shotgun lipidomics, which has become a foundational analytical technology platform among current lipidomics practices due to its high efficiency, sensitivity, and reproducibility, as well as its broad coverage and minimal batch effects.

Alzheimer's disease (AD) is by far the most common form of dementia. The number of people living with AD is growing up fast as the world population ages and life expectancy increases. Unfortunately, effective disease-modifying therapies remain elusive (Cummings et al., 2018), highlighting the importance of better understanding the molecular mechanism(s) underlying disease etiology. The brain is the richest organ in terms of lipid content and diversity, largely due to the abundance of lipid-rich myelin. On the other hand, large-scale human genome-wide association studies have consistently linked lipid metabolism with AD (Wightman et al., 2021). However, there are relatively few studies focusing on the mechanisms regarding how specific lipids, other than cholesterol, affect AD pathogenesis. By exploiting multidimensional mass spectrometry-based shotgun lipidomics, our laboratory has revealed that a class of myelin-enriched lipids, i.e., sulfatide, is dramatically reduced at the earliest clinically recognizable stages of AD and in AD animal models and specifically lost compared to other myelin lipids and other neuropathological conditions, which showed a strong relationship with ApoE4 (Han et al., 2003), the strongest genetic risk factor for AD. In a recent study (Qiu et al., 2021), we reported that mild central nervous system (CNS) myelin sulfatide losses are sufficient to activate disease-associated microglia and astrocytes, and to increase the expression of AD risk genes (e.g., *Apoe*, *Trem2*, *Cd33*, *Mmp12*, and *Spi1*), as well as previously established causal regulators of the immune/microglia network in late-onset AD (e.g., *Tyrobp*, *Dock*, and *Fcerg1*), leading to chronic AD-like neuroinflammation and mild cognitive impairment. Furthermore, CNS sulfatide deficiency-induced astrogliosis and astrocytic ApoE upregulation were independent of microgliosis. Notably, all the phenotypes showed gender differences, being more pronounced in females than males.

To mimic and study the consequences and related molecular mechanisms of adult-onset sulfatide deficiency in very early AD, we generated a novel mouse model, i.e., myelinating cells specific cerebroside sulfotransferase (CST, a.k.a. Gal3st1) conditional knockout (cKO) mice. CST codes for the enzyme that catalyzes the last step of sulfatide biosynthesis. Myelin sulfatide is inducibly and conditionally depleted by tamoxifen in adult CST cKO mice. For the first time, multiple lines of evidence were provided at the lipid, RNA, and protein levels to demonstrate that inducible myelinating glia-specific CST depletion leads to specific losses of CNS sulfatide without major impact on overall oligodendrocyte/myelin homeostasis or cell death, but is sufficient to impair cognitive dysfunction, disrupting both spatial and non-spatial memory consolidation.

Accumulating evidence has implicated sustained glia-mediated inflammation as a major contributor to AD neurodegenerative processes and cognitive deficits (Newcombe et al., 2018). In the study of the related cell/molecular mechanisms of adult-onset myelin sulfatide deficiency, we found that most upregulated gene expression after sulfatide loss was enriched in activated microglia and astrocytes, which overlapped with the AD disease-associated microglia and astrocytes, leading to chronic AD-like neuroinflammation. The predictions from transcription factors scores based on the significantly changed genes in CST cKO/KO brain showed the top targets including interferon regulatory factor 8, signal transducer and activator of transcription 3 (STAT3), SPI1, and CCAAT/enhancer-binding protein beta (C/EBPbeta), and all of them have been reported to be involved in the activation of microglia or astrocytes. We confirmed that PU1/SPI1 and STAT3 were dramatically upregulated at the protein or phosphorylation levels. Meanwhile, interferon regulatory factor 8 and C/EBPβ were slightly upregulated. Strikingly, SPI1 is one of AD risk and microglia-enriched genes (Tansey et al., 2018), and C/EBPβ has been reported to play a role in neuroinflammation and is the mediator of ApoE4 expression in AD. Furthermore, the depletion of microglia in the brain of CST KO mice demonstrated that CNS sulfatide deficiency-induced astrogliosis was independent of microgliosis. The high STAT3 levels in CST KO brain after microglia depletion suggested the STAT3 upregulation was independent of microglia and might mainly come from activated astrocytes. Thus, our new study (Qiu et al., 2021) confirmed that adult-onset CNS myelin sulfatide deficiency is sufficient to cause AD-like neuroinflammation. To further understand the association between brain sulfatide loss and glia activation, we confirmed that CNS myelin sulfatide deficiency leads to activation of microglia/astrocytes within myelin-enriched brain regions, and the activated astrocytes were adjacent to myelin-rich regions as shown via immuno staining and electron microscopy. Sulfatide has been reported to interact directly with microglia enriched membrane protein TREM2, which can promote microglial activation in response to insults to the white matter (Poliani et al., 2015); and sulfatide can also interact directly with extracellular matrix proteins like laminin (Baron et al., 2014) and tenascin (Shao et al., 2007). So we hypothesized that sulfatide deficiency-induced microglia and/or astrocyte activation begins from the loss of interaction between myelin sulfatide and ligands on microglia or astrocyte processes.

ApoE ε4 allele is known as the strongest genetic risk factor for AD, and ApoE plays important roles that range from promoting amyloid-β pathology to inducing tau neurofibrillary degeneration, microglia and astrocyte responses, and blood-brain barrier disruption. Our group also confirmed that ApoE is necessary to bring down brain sulfatide levels, as ApoE transports brain sulfatide and modulates its turnover (Han et al., 2003). In this study (Qiu et al., 2021), we found that ApoE was upregulated in the CNS of CST cKO and KO mice, which suggested myelin sulfatide deficiency and ApoE upregulation formed a positive feedback loop. However, using ApoE and CST double KO mice, we confirmed that ApoE was not necessary for sulfatide deficiency-incluced neuroinflammation, which suggested that although ApoE drives sulfatide losses in AD, it is not required for activation of disease-associated microglia and astrocytes directly.

The depletion of microglia in the brain of CST KO mice demonstrated that CNS sulfatide deficiency-induced ApoE upregulation may have been primarily driven by activated astrocytes.

Our study strongly indicates that specific lipid abnormalities in the brain, such as the lack of myelin sulfatides, may also be an important driving and promoting factor of neuroinflammation and mild cognitive impairment in AD pathology (**Figure 1**). However, follow-up studies are needed to continue to clarify how the loss of myelin sulfatide regulates brain cell functions.

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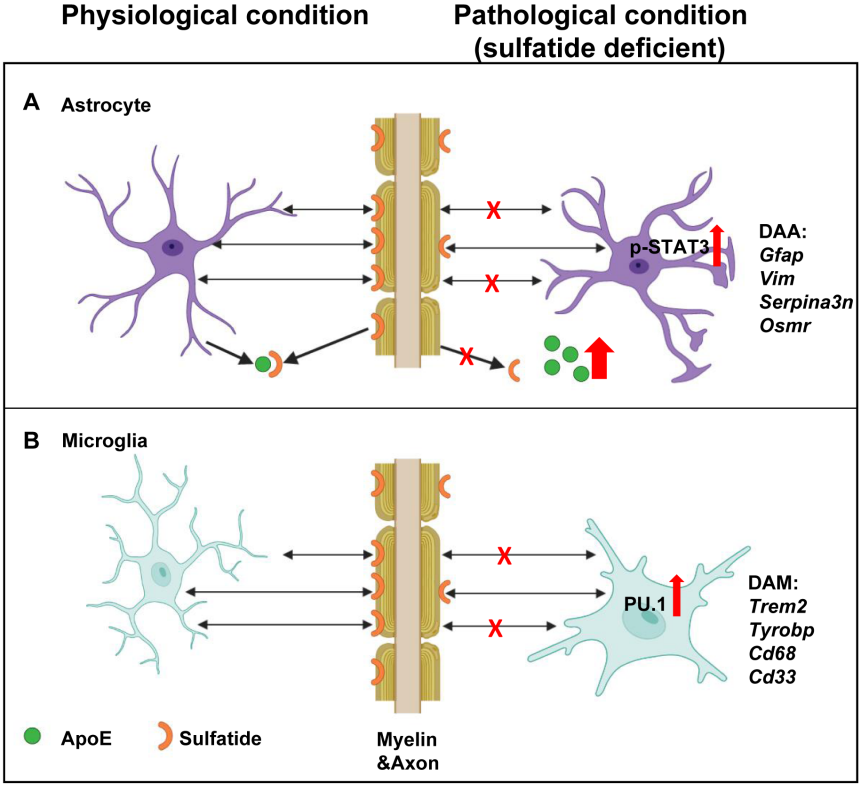

Figure 1 | **Schematic diagram summarizing how sulfatide losses cause Alzheimer's disease-like astrocyte (A) and microglia (B) activation through a loss of interaction and a positive feedback between sulfatide loss and astrocytic ApoE overexpression.**
DAA: Disease-associated astrocyte; DAM: disease-associated microglia; STAT3: signal transducer and activator of transcription 3.

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