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Editorial

Sphingolipids in Alzheimer's Disease and Related Disorders

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Alzheimer's disease (AD) is a heterogeneous disorder and there is growing consensus that therapeutic targets other than amyloid-β or tau will also be necessary to reverse, or slow AD progression. Further elucidation of the dysregulated biological mechanisms that lead to the onset and progression of AD is critical to identify new treatment strategies. Lipids play an important role in the structure of neuronal cell membranes, directly affecting the solubility and fluidity of the membrane. The homeostasis of membrane lipids in neurons and myelin is a key component in preventing loss of synaptic plasticity, cell death, and ultimately, substantial neurodegeneration [1, 2]. Sphingolipids are a class of ubiquitous lipids derived from the aliphatic amino alcohol sphingosine. This class of lipids makes up approximately one third of the content in eukaryotic cell membranes and is highly enriched in the central nervous system in multiple cell types including neurons, glia, and the vascular compartment. In addition to important structural roles, sphingolipid metabolites function as second messengers that modulate critical signaling functions for inter- and intra-cellular signaling activities that affect cellular growth and differentiation, senescence, apoptosis, inflammation, immune cell trafficking, and the generation of reactive oxygen species [3]. In brain, the proper balance of sphingolipids is essential for optimal neuronal function [4]. As a consequence, alterations of the delicate balance in sphingolipid metabolism may contribute to the development of age-related neurological and neuro-inflammatory diseases. There is a growing literature suggesting the importance of sphingolipids and phospholipids in the development and progression of AD pathology. The focus of this mini-forum in the *Journal of Alzheimer's Disease* was to highlight this ongoing translational research from cellular studies to animal models, and finally to humans, and to emphasize the potential importance of this pathway in AD pathogenesis.

The first article, by Dinkins and colleagues [5], summarized the newest information regarding extracellular vesicles, particularly exosomes, and their potential role in the perturbation of sphingolipid metabolism and relation to AD pathogenesis. Exosomes and other extracellular vesicles are enriched with the sphingolipid ceramide and also other more complex glycosphingolipids such as gangliosides. As ceramide is often elevated in AD, exosome secretion may be potentially altered as well. The

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authors review the data regarding the role of exosomes in the aggregation and clearance of amyloid-β, extracellular tau propagation, and AD-related exosomal mRNA/miRNA cargo. The use of exosomes as biomarkers and gene therapy vehicles for diagnosis and potential treatment are also discussed.

The APOE E4 allele is the greatest genetic risk factor for sporadic AD. However, the relationship between sphingolipids and APOE in relation to the etiopathogenesis of AD remains unclear. The article by Den Hoedt and colleagues [6] aimed to determine the specific effects of human APOE4 (hE4) on cerebral ceramide and fatty acid content in mice on either a chow or a high fat/high cholesterol l (HFHC) diet. The authors first reported that the hE4 mice had lower cerebral ceramide levels compared to the E0 mice, and this difference was independent of diet. Interestingly, they also observed diet-specific differences in both fatty acid and the ceramide metabolism. In particular, the HFHC diet increased cerebral fatty levels in the hE4 mice. This increase, only among the hE4 mice, was subsequently associated with alterations in the expression of both ceramide and fatty acid transporters.

A gap in the current knowledge of the contribution of the sphingolipid pathway to AD is the relationship between sphingolipid transporters and the alterations observed in ceramide levels in AD. In order to better understand this relationship, there is a need to first demonstrate that current drugs targeting the transporters can cross the blood-brain barrier. Relevant to this gap, Crivelli and colleagues [7] investigated the ceramide transporter protein (CERT) because it is the only known protein able to mediate the non-vesicular transfer of ceramide between organelle membranes. Thus, the modulation of CERT function may impact on ceramide accumulation. The competitive CERT inhibitor N-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl) dodecanamide (HPA-12) is able to bind to a specific protein region and interferes with the ceramide trafficking. The authors utilized HPA-12 as a tool to modulate ceramide trafficking and also to study CERT dynamics. For the first time, they report the synthesis and *in vitro* properties of HPA-12 radiolabeled with fluorine-18, and present preliminary *in vitro* and *in vivo* positron emission tomography imaging and biodistribution data demonstrating that HPA-12 crosses the blood-brain barrier and is retained in the brain. These data open new opportunities to more comprehensively study the contribution of CERT to the progression and pathology of AD.

Cerebral amyloid angiopathy (CAA) involves the cerebrovascular deposition of amyloid-β beta and is highly prevalent in the brains of AD patients. Capillary CAA (capCAA) is a subtype of CAA that accumulates in the cortical capillaries and is found in over 50% of AD patients. To date, the pathways associated with capCAA are not well understood. The article by de Wit et al. [8] examined whether there were sphingolipid alterations in the brains of confirmed pathological AD with capCAA compared to without capCAA. Utilizing immunohistochemical analysis, AD brains with capCAA had altered expression of ceramide, acid sphingomyelinase, and sphingosine-1-phosphate receptors 1 and 3. The authors hypothesize that these results suggest that the sphingolipid pathway is involved in the neuroinflammatory response to capCAA.

A blood-based biomarker for AD would have advantage over cerebrospinal fluid (CSF) or neuroimaging markers with regards to cost, feasibility, and invasiveness. Previous studies have examined the association between plasma sphingolipids and odds of AD or risk of cognitive decline [9–12], but these studies have generally been limited by small sample sizes and have not adequately considered age, sex, and APOE E4 genotype as effect modifiers. The latter is important because plasma levels of both ceramides and sphingomyelins are known to be affected by these demographic factors [13, 14]. The study by Kim et al. [15], including 412 participants (205 AD and 207 cognitively normal individuals), reported that elevated plasma levels of ceramides 16 : 0, 18 : 0, and 20 : 0 were associated with hippocampal atrophy among participants aged <75 years. Among participants aged 75 and older, three phospholipids were most associated with hippocampal atrophy. In the study by Mielke and colleagues [16], the association between plasma ceramides or sphingomyelins and risk of AD differed by both sex and APOE E4 genotype. Among men, high levels of both ceramides and sphingomyelins were associated with an increased risk of AD; this relationship did not differ by APOE E4 genotype. In contrast, among women, low sphingomyelins were associated with the greatest risk of AD, with a strong association among APOE E4 carriers. Together, these studies suggest that future clinical and epidemiological studies examining sphingolipids and AD should consider age, sex, and APOE E4 genotype as potential effect modifiers.

The last article in this mini-forum, by Saleem and colleagues [17], examines plasma sphingolipids as

a biomarker of treatment response. Among a group of patients with coronary artery disease undertaking cardiac rehabilitation, the authors showed that low levels of ceramide C18 : 0 was associated with significant improvement across multiple cognitive domains. Thus, individuals undergoing cardiac rehabilitation with high ceramide levels were least likely to respond to an exercise intervention with regards to cognitive performance.

Based on the implication that impaired sphingolipid balance may be causative for AD pathogenesis, it is straightforward to propose that these lipids and their metabolites are of fundamental importance for the treatment or even prevention of AD. As a case in point, it is known that modulating this pathway is beneficial in models of neuroinflammation and neurodegeneration and that in postmortem material of patients with AD, levels of the lipid ceramide and the enzymes involved in its production are increasingly expressed.

Together, the articles included in this mini-forum provide further evidence for the important role of sphingolipid metabolism in the etiopathogenesis of AD. To further enhance our knowledge regarding perturbations of this pathway in AD, from the molecule to the whole individual, it will be essential to: 1) understand the molecular mechanisms contributing to the sphingolipid alterations observed in the AD brain relative to controls and other neurodegenerative diseases; 2) understand the cross-talk between sphingolipids, fatty acid metabolism, and APOE genotype; 3) quantify plasma and CSF sphingolipid levels, and the involved enzymes, serially across neurodegenerative diseases and in relation to specific neuropathologies and clinical systems; and 4) understand how plasma or CSF sphingolipid levels or enzymes can best be utilized as potential risk factors or biomarkers, and when across the spectrum of AD (i.e., pre-clinical to fully symptomatic phase) in both animal models and humans. Finally, the identification of additional therapeutics targets that can moderate the sphingolipid pathway, and that can cross the blood-brain barrier, enter the brain, and influence disease pathogenesis, are needed in the effort to develop future potential treatment strategies for AD and related disorders.

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