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Loss of ceramide synthase 2 activity, necessary for myelin biosynthesis, precedes tau pathology in the cortical pathogenesis of Alzheimer's disease

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ABSTRACT

The anatomical progression of neurofibrillary tangle pathology throughout Alzheimer's disease (AD) pathogenesis runs inverse to the pattern of developmental myelination, with the disease preferentially affecting thinly myelinated regions. Myelin is comprised 80% of lipids, and the prototypical myelin lipids, galactosylceramide, and sulfatide are critical for neurological function. We observed severe depletion of galactosylceramide and sulfatide in AD brain tissue, which can be traced metabolically to the loss of their biosynthetic precursor, very long chain ceramide. The synthesis of very long chain ceramides is catalyzed by ceramide synthase 2 (CERS2). We demonstrate a significant reduction in CERS2 activity as early as Braak stage I/II in temporal cortex, and Braak stage III/IV in hippocampus and frontal cortex, indicating that loss of myelin-specific ceramide synthase activity precedes neurofibrillary tangle pathology in cortical regions. These findings open a new vista on AD pathogenesis by demonstrating a defect in myelin lipid biosynthesis at the preclinical stages of the disease. We posit that, over time, this defect contributes significantly to myelin deterioration, synaptic dysfunction, and neurological decline.

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1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder associated with aging. It is estimated that around 30 million people worldwide are afflicted with AD, and this number is projected to triple by 2050 (Prince et al., 2013). The disease is characterized by loss of synapses and neurodegeneration in the basal forebrain and limbic system that initially produce short-term memory deficits, followed by more extensive cortical neurodegeneration that is associated with an array of neurological deficits. A fraction of AD cases are attributed directly to inheritance of mutations in the amyloid precursor protein (APP) or the proteases Presenilin-1 and -2 (PS1 and PS2), giving rise to accelerated proteolytic cleavage of APP and production of amyloid beta peptides. These peptides form insoluble extracellular aggregates (neuritic plaques) that are associated with dystrophic neurites and are one of the hallmark pathological features of the disease. Other pathological hallmarks of AD include astrogliosis and neuroinflammation, neurofibrillary tangles (NFTs) inside neuronal cell bodies, and lipid deposits within glia and endothelial cells (Braak and Braak, 1995; DeKosky and Scheff, 1990; Foley, 2010; Holtzman et al., 2011; Scheff et al., 2006).

Over 95% of AD cases are the late-onset form of the disease, for which the greatest overall risk factor is age. The greatest genetic modulator of risk for late-onset AD is the different alleles (ε2, ε3, and ε4) of the *APOE* gene. Homozygous carriers of the ε4 allele are at 12-fold increased risk of developing AD, compared with noncarriers (Corder et al., 1993). Conversely, the ε2 allele protects against AD (Corder et al., 1994). ApoE is the major apolipoprotein of the brain, responsible for transporting lipids in the extracellular space. Brain lipids that are directly regulated by ApoE include the prototypical myelin lipids galactosylceramide (GalCer) and its sulfated form, sulfatide (Cheng et al., 2007).

NFT pathology develops in a well-defined anatomical pattern during the course of AD pathogenesis (Braak and Braak, 1995). The tangles first appear in the entorhinal and perirhinal cortex (Braak stages I/II). This may be years to decades before clinical symptoms develop. NFT pathology then progresses into the limbic system,

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including the hippocampus, and associated temporal gyri (Braak stages III/IV), before spreading throughout the cerebral cortex (Braak stages V/VI). Braak stages V/VI are associated with widespread neuritic plaques and a clinical AD diagnosis, whereas Braak stages I-III represent preclinical AD (Hyman et al., 2012).

The pathologists Braak and Braak observed in 1996 that "The pattern of neurofibrillary changes which gradually develop in the course of Alzheimer's disease bears a striking resemblance to the inverse sequence of cortical myelination" (Braak and Braak, 1996). Brain regions that are myelinated later in life, and consequently have thinner myelin sheaths, are affected earlier by AD pathology. The same regions are also more heavily afflicted by normal myelin loss with aging (Bartzokis, 2011). These observations on myelin have received relatively scant attention in studies on the mechanisms of AD pathogenesis.

Myelin provides electrical insulation and is necessary for rapid impulse propagation along axons. Myelinating oligodendrocytes also provide direct trophic support to axons, illustrated by the fact that demyelinating diseases cause axon degeneration (Nave, 2010). Lipids comprise approximately 80% of the dry weight of myelin (O'Brien and Sampson, 1965), creating the hydrophobic insulation that is essential for neuronal conductivity. The major lipid constituents of myelin are cholesterol, glycerolipids, and sphingolipids. The sphingolipids sphingomyelin (SM), ceramide, GalCer, and sulfatide (Fig. 1) are all found in high proportions in myelin (O'Brien and Sampson, 1965). Of these, GalCer and sulfatide are specific markers of oligodendrocytes and myelin in the central nervous system (Schaeren-Wiemers et al., 1995). Mice lacking the GalCer synthase enzyme are unable to synthesize GalCer and sulfatide, resulting in myelin instability, conduction deficits, tremour, hindlimb paralysis, and premature death (Bosio et al., 1996; Coetzee et al., 1996).

Previous studies using classical thin layer chromatography separation of lipids reported significant loss of GalCer and sulfatide in late-onset AD brain tissue (Svennerholm and Gottfries, 1994; Wallin et al., 1989). Subsequent analyses with liquid chromatography-tandem mass spectrometry (LC-MS/MS) demonstrated the loss of sulfatide in cortical gray and white matter at the earliest clinically-detectible stage of AD-type dementia (CDR 0.5) (Han et al., 2002); and loss of GalCer in the CA1 region of the hippocampus of subjects with AD (Hejazi et al., 2011). These studies were performed on subjects with mild to severe dementia and do not address the possibility that myelin lipid defects are present in the pre-clinical AD state. This question was addressed in the present study, using tissue from subjects graded according to the Braak staging scheme for postmortem NFT pathology (Braak and Braak, 1995; Hyman et al., 2012). Although statistically significant depletion of myelin sphingolipids was only observed in Braak V/VI subjects, associated with clinical AD, we observed a significant reduction in ceramide synthase 2 (CERS2) activity, responsible for synthesis of very long chain (VLC) ceramides that are incorporated into GalCer and sulfatide, as early as Braak stages I/II in the inferior temporal lobe.

2. Materials and methods

2.1. Human brain tissues

Human brain tissue samples were obtained from the Sydney Brain Bank and NSW Tissue Resource Centre, under UNSW Human Research Ethics Advisory committee approval HC13120. Brains from 34 subjects were grouped according to NIH-Alzheimer's Association criteria (Hyman et al., 2012) into Braak stages I/II (n = 8), III/IV (n = 7), V/VI (n = 10), and age-matched controls exhibiting no Braak NFT pathology (n = 9). Information on the subjects is provided in Table 1. Samples were taken from the CA1 region of the hippocampus, inferior temporal and superior frontal gray and white matter, and cerebellum. Tissue samples were crushed over dry ice, then stored at −80 °C for lipid or protein extraction as described in the following. The same set of tissue samples have been used for a previously published study on sphingosine 1-phosphate (Couttas et al., 2014a).

2.2. Lipid quantification by LC-MS/MS

Lipids were extracted from approximately 20 mg crushed brain tissue according to a previously published 1-phase extraction protocol (Hejazi et al., 2011), with the addition of the following internal standards: 1250 pmoles C12:0 SM, 250 pmoles C12:0 sulfatide, 250 pmoles C12:0 GalCer, and 50 pmoles C17:0 ceramide (Avanti Polar Lipids). Samples were resolved on a 3 × 150 mm Agilent XDB-C8 HPLC column and quantified by LC-MS/MS, as described previously (Hejazi et al., 2011; Wong et al., 2012). Data processing was carried out using the MMSAT program (Wong et al., 2012), and the detected sphingomyelin, GalCer, ceramide, and sulfatide species were verified as conforming to a previously identified quadratic elution profile (Hejazi et al., 2011). Lipids, expressed as ratios to the relevant internal standard, were quantified using standard curves prepared with external standards for SM, sulfatide, GalCer, and ceramide (Avanti Polar Lipids).

2.3. Quantification of free fatty acids by gas chromatography-mass spectrometry (GC-MS)

Free fatty acids were extracted from 50 mg of brain tissue using iso-octane, as described (Quehenberger et al., 2011), in the presence of 25 ng deuterated eicosanoic acid as the internal standard (C/D/N Isotopes). Free fatty acids were converted to their tert-butyl dimethylsilyl derivatives by dissolving dried extracts in 60 μL pyridine and 30 μL N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (Sigma Aldrich). Samples were then incubated at 80 °C for 1 hour. Derivatized fatty acids were promptly analyzed by GC-MS using an HP-5ms capillary column (0.25 mm internal diameter × 30 m × 0.25 μm; Agilent) on an Agilent HP 6890-5973 GC-MS. Injection volume: 1 μL in splitless mode; inlet temperature 250 °C; helium flow 1.1 mL/min. The MS was operated in electron ionization mode at 70 eV. Source, quadrupole, and

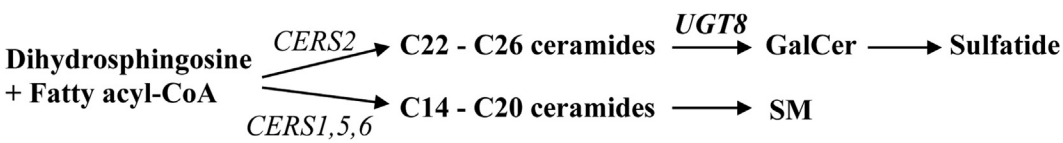

Fig. 1. The myelin sphingolipid pathway in oligodendrocytes. Very long chain (VLC; C22–C26) ceramides are synthesized from a long chain base (sphingosine or dihydrosphingosine) and a very long chain fatty acid-coenzyme A (CoA) conjugate, catalyzed by ceramide synthase 2 (CERS2). The prototypical myelin lipid galactosylceramide (GalCer) is formed by addition of a galactose headgroup to VLC ceramides. GalCer can then be sulfated, forming sulfatide. Alternatively, ceramide is used as a substrate to form sphingomyelin (SM), which is an abundant lipid present in all cell membranes, but enriched in myelin. SM is preferentially synthesized from C16 and C18 ceramides. The most abundant form of ceramide in the brain is C18, which is synthesized by CERS1.

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