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Peripheral sphingolipids are associated with variation in white matter microstructure in older adults

Christopher E. Gonzalez [a], Vijay K. Venkatraman [a], Yang An [a], Bennett A. Landman [b], Christos Davatzikos [c], Veera Venkata Ratnam Bandaru [d], Norman J. Haughey [d], Luigi Ferrucci [a], Michelle M. Mielke [e,f,*,1], Susan M. Resnick [a,1]

[a] Laboratory of Behavioral Neuroscience, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA
[b] Institute of Imaging Science and Department of Electrical Engineering, Vanderbilt University, Nashville, TN, USA
[c] Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA
[d] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[e] Department of Health Science Research, Mayo Clinic, Rochester, MN, USA
[f] Department of Neurology, Mayo Clinic, Rochester, MN, USA

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ABSTRACT

Sphingolipids serve important structural and functional roles in cellular membranes and myelin sheaths. Plasma sphingolipids have been shown to predict cognitive decline and Alzheimer's disease. However, the association between plasma sphingolipid levels and brain white matter (WM) microstructure has not been examined. We investigated whether plasma sphingolipids (ceramides and sphingomyelins) were associated with magnetic resonance imaging-based diffusion measures, fractional anisotropy (FA), and mean diffusivity, 10.5 years later in 17 WM regions of 150 cognitively normal adults (mean age 67.2). Elevated ceramide species (C20:0, C22:0, C22:1, and C24:1) were associated with lower FA in multiple WM regions, including total cerebral WM, anterior corona radiata, and the cingulum of the cingulate gyrus. Higher sphingomyelins (C18:1 and C20:1) were associated with lower FA in regions such as the anterior corona radiata and body of the corpus callosum. Furthermore, lower sphingomyelin to ceramide ratios (C22:0, C24:0, and C24:1) were associated with lower FA or higher mean diffusivity in regions including the superior and posterior corona radiata. However, although these associations were significant at the a priori $p < 0.05$, only associations with some regional diffusion measures for ceramide C22:0 and sphingomyelin C18:1 survived correction for multiple comparisons. These findings suggest plasma sphingolipids are associated with variation in WM microstructure in cognitively normal aging.

1. Introduction

Sphingolipids, such as sphingomyelin and ceramide, contribute to the structural integrity and fluidity of cell membranes and are highly enriched in the central nervous system. Indeed, the specific concentrations of, and interaction between, sphingomyelin with cholesterol affect membrane permeability (Needham and Nunn, 1990) and are critical for the formation and structure of lipid rafts (Brown and London, 2000; Cremesti et al., 2002). Sphingomyelins are a type of phospholipid also found in myelin. Ceramide is both a precursor and a metabolite of sphingomyelin. Although ceramides do act as structural lipids, they are important bioactive lipids necessary for regulating signaling cascades involved in cellular senescence, apoptosis, and inflammation (Hannun and Obeid, 2008; Kolesnick, 1998). Both sphingomyelins and ceramides increase with age (Cutler et al., 2004; Mielke et al., 2015a, 2015b). Furthermore, these lipids, individually and the ratio of sphingomyelin to ceramide, have been implicated in the development and progression of neurodegenerative diseases (Ben-David and Futerman, 2010; Cutler et al., 2004; Haughey, 2010).

Several postmortem studies have found higher concentrations of ceramides and sphingomyelins in frontal and temporal brain regions in Alzheimer's disease (AD) patients compared with normal controls (Chan et al., 2012; Cutler et al., 2004; Han et al., 2002), and in persons with Lewy body pathology and frontotemporal lobar degeneration (Filippov et al., 2012). We have previously reported that high blood ceramide and sphingomyelin levels predicted cognitive impairment and AD among cognitively normal (CN)

* Corresponding author at: Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, 200 First Street South West, Rochester, MN 55905, USA. Tel.: +1 507 284 5545; fax: +1 507 284 1516.
E-mail address: mielke.michelle@mayo.edu (M.M. Mielke).
[1] Co-senior authors: Michelle M. Mielke and Susan M. Resnick.

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individuals (Mielke et al., 2010a, 2012) and memory decline and hippocampal volume loss among amnestic mild cognitive impairment patients (Mielke et al., 2010b). Notably, in AD patients, the ratio of sphingomyelin to ceramide was most predictive of rate of cognitive decline compared with sphingomyelin or ceramide levels alone (Mielke et al., 2011). Given the high concentration of sphingolipids in white matter (WM), it is possible that changes in circulating sphingolipids could be related to changes in microstructural diffusion measures such as fractional anisotropy (FA) and mean diffusivity (MD). However, the relationship of circulating sphingolipid concentrations on WM microstructure, as measured with diffusion tensor imaging (DTI), has not been reported.

A number of cross-sectional neuroimaging studies have shown lower FA or higher MD with increasing age in WM tracts (Abe et al., 2002; Bennett et al., 2010; Grieve et al., 2007; Head, 2004; Kennedy et al., 2009; Pfefferbaum et al., 2000; Salat et al., 2005), with the most significant associations often found in frontal regions. These decrease in FA with age remain significant even after correcting for WM volume loss (Hugenschmidt et al., 2008) suggesting these microstructural changes could precede WM atrophy. Identifying blood markers that predict deficits in WM integrity of CN individuals would improve our understanding of modifiers of healthy brain aging. The prospective design of the Baltimore Longitudinal Study of Aging (BLSA) allowed us to evaluate the association of peripheral sphingolipid levels with subsequent brain structure. Given the important structural and functional roles of sphingolipids in cellular membranes and within myelin sheaths, the goal of the present study was to examine whether plasma sphingolipids were associated with global and region-specific WM microstructure 10 years later.

2. Methods and materials

2.1. Participants

The present study included 150 CN BLSA participants who had (3T) DTI scans and available plasma ceramide and sphingomyelin levels an average of 10.5 years before the scan. We initially identified 168 BLSA participants with available plasma ceramide and sphingomyelin levels and a 3T DTI scan. The average time between the baseline plasma sphingolipid assays and the DTI scan was 17 years (standard deviation [SD] = 6 years) with a range of 8.5–32.0 years. To reduce the variability in time to DTI scan, we a priori decided to examine the sphingolipid measurement that was nearest to 10 years before each participant's DTI scan (mean = 10.2 years; SD = 2.5). Thus, we excluded 8 participants with a follow-up interval of less than 5 years and 3 participants over 15 years. Scans were also excluded from an additional 6 participants for whom WM hyperintensity volumes concurrent with the DTI scan were not available and another participant because the image processing pipeline failed to generate diffusion metrics for several brain regions. Compared with participants with a sphingolipid measurement but without a 3T DTI scan (N = 824), the 150 participants included in this study were slightly younger at their initial blood draw (one-way analysis of variance [ANOVA], $F(1,972) = 15.74$), were more likely to be female (Fishers' exact test, odds ratio = 2.37 [95%: 1.64–3.42]) and Caucasian (Fishers exact test, odds ratio = 5.7 [95%: 3.68–8.82]), had slightly lower systolic blood pressure (one-way ANOVA, $F(1,964) = 3.92$), had no prevalence of myocardial infarction compared with 3% in the larger group and lower total cholesterol (one-way ANOVA, $F(1,928) = 19.47$). However, the samples did not differ ($p > 0.05$) in the frequency of diabetes, hypertension, cancer, use of statins, body mass index (BMI), diastolic blood pressure, triglycerides, apolipoprotein E ε4 (APOE ε4) allele carrier status, or total sphingomyelin and ceramide levels.

All participants were CN at the time of both the blood and magnetic resonance imaging (MRI) assessments. Diagnoses of dementia and AD were determined by Diagnostic and Statistical Manual-III-R (1987) and the National Institute of Neurological and Communication Disorders—AD and Related Disorders Association criteria (McKhann et al., 1984), respectively. Mild cognitive impairment was based on the Petersen criteria (Petersen et al., 1999) and diagnosed when (1) cognitive impairment was evident for a single domain (typically memory) or (2) cognitive impairment in multiple domains occurred without significant functional loss in activities of daily living. Furthermore, all participants had no medical history of stroke at the time of blood sample or at the time of DTI. Blood samples were drawn at all visits from the antecubital vein between 7 and 8 AM after an overnight fast (Shock et al., 1984). Participants were not allowed to smoke, engage in physical activity, or take medications before the sample was collected. Plasma samples were immediately processed, cataloged, and stored at −80 °C. The protocol was approved by the local institutional review board, and all participants provided written informed consent.

2.2. Lipid extraction and liquid chromatography-electrospray ionization-tandem mass spectrometry analysis

The lipid extractions and methods for measuring plasma ceramide and sphingomyelin levels in the BLSA have previously been described in detail, including the inter- and intra-day coefficients of variation (Mielke et al., 2015a, 2015b). Briefly, a crude lipid extraction of plasma was conducted using a modified Bligh and Dyer procedure with ceramide or sphingomyelin C12:0 included as an internal standard (Avanti Polar Lipids, Alabaster, AL, USA; Bandaru et al., 2013; Haughey et al., 2004). Plasma extracts were dried in a nitrogen evaporator (Organomation Associates Inc, Berlin, MA, USA) and resuspended in pure methanol just before analysis. An autosampler (LEAP technologies Inc, Carrboro, NC, USA) injected extracts into an high performance liquid chromatography (PerkinElmer, MA, USA) equipped with a reverse phase C18 column (Phenomenex, Torrance, CA, USA). The eluted sample was then injected into an electrospray ion source coupled to a triple-quadrupole mass spectrometer (API3000, AB Sciex Inc, Thornhill, Ontario, Canada; Bandaru et al., 2007, 2011, 2013). Analyses were conducted by multiple reaction monitoring. Eight point calibration curves (0.1–1000 ng/mL) were constructed by plotting area under the curve, separately for ceramides and sphingomyelins, for each calibration standard d18:1/C16:0, d18:1/C18:0, d18:1/C20:0, d18:1/C22:0, d18:1/C24:0 (Avanti polar lipids, Alabaster, AL, USA) normalized to the internal standard. Correlation coefficients (R^2) obtained were >0.999. Ceramide concentrations were determined by fitting the identified ceramide species to these standard curves based on acyl chain length. Internal standards were run daily, and area under the curves plotted weekly, to track instrument efficiency. Plasma extracts were reanalyzed if the internal standard deviated more than 25% of the median value. Instrument control and quantitation of spectral data were performed using Analyst 1.4.2 and MultiQuant software (AB Sciex Inc, Thornhill, Ontario, Canada). All sphingolipids are expressed in μg/mL for statistical analyses, and descriptive statistics for sphingomyelins and ceramides for the current sample are summarized in Supplementary Table 1.

2.3. MRI protocol and image processing

The neuroimaging component of the BLSA incorporated diffusion-weighted imaging on 3T scanners in 2008. Data acquired at each BLSA visit included T1-weighted magnetization prepared rapid gradient recalled echo (MPRAGE) and DTI scans. This study