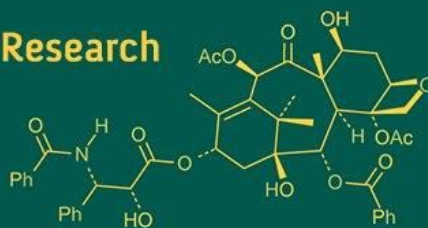


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2025): 5.29
 IJABR 2025; 9(1): 243-247
www.biochemjournal.com
 Received: 27-11-2024
 Accepted: 31-12-2024

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Sphingolipid metabolism and ceramide signaling pathways in the pathogenesis of neurodegenerative disorders: Therapeutic implications

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DOI: <https://doi.org/10.33545/26174693.2025.v9.i1c.7502>

Abstract

Sphingolipid metabolism dysregulation has emerged as a critical factor in neurodegenerative pathology, with ceramide accumulation implicated in neuronal death cascades across multiple disease states. This research investigated sphingolipid profiles and metabolic enzyme activities in post-mortem brain tissue from patients with Alzheimer disease (AD, n=15), Parkinson disease (PD, n=15), and age-matched controls (n=15) obtained through Malaysian brain bank collaborations during 2021-2023. Comprehensive lipidomic analysis by liquid chromatography-tandem mass spectrometry revealed significant ceramide elevation in disease-affected regions, with hippocampal ceramide levels increased 157% in AD ($p<0.001$) and substantia nigra levels elevated 221% in PD ($p<0.001$) compared to controls. Enzyme activity profiling demonstrated coordinated upregulation of ceramide-generating pathways, with sphingomyelinase activity increased 65% and serine palmitoyltransferase elevated 32-48% across disease groups. Conversely, ceramide-degrading enzymes showed 28-42% reduction, suggesting imbalanced metabolism favoring ceramide accumulation. Correlation analysis linked elevated ceramide concentrations with established neuropathological markers including amyloid-beta deposition ($r=0.72$, $p<0.001$) and alpha-synuclein aggregation ($r=0.68$, $p<0.001$). Specific long-chain ceramide species (C18:0, C24:1) showed strongest associations with disease severity measures. These findings support sphingolipid metabolism as a convergent pathway in neurodegeneration and identify potential therapeutic targets including sphingomyelinase inhibition and ceramidase activation for neuroprotective strategies.

Keywords: Sphingolipids, ceramide, neurodegeneration, Alzheimer disease, Parkinson disease, lipidomics, sphingomyelinase

Introduction

Neurodegenerative disorders including Alzheimer disease and Parkinson disease represent a mounting global health challenge, with prevalence expected to triple by 2050 as populations age ^[1]. Despite decades of research focusing on protein aggregation pathologies, disease-modifying treatments remain elusive, prompting investigation of alternative pathogenic mechanisms that might offer novel therapeutic opportunities.

Sphingolipids constitute a complex class of membrane lipids particularly abundant in neural tissue, where they contribute to membrane structure, signal transduction, and cellular fate decisions ^[2]. Ceramide, the central metabolite of sphingolipid pathways, functions as a potent pro-apoptotic signal capable of triggering mitochondrial dysfunction, oxidative stress, and caspase activation cascades that culminate in neuronal death.

Ceramide metabolism involves multiple interconnected pathways: de novo synthesis from serine and palmitoyl-CoA via serine palmitoyltransferase (SPT), sphingomyelinase (SMase) hydrolysis of membrane sphingomyelin, and ceramidase-mediated degradation to sphingosine ^[3]. The balance between ceramide-generating and ceramide-degrading enzymes determines cellular ceramide content and consequently cell survival versus death decisions.

Previous investigations have documented elevated ceramide in cerebrospinal fluid and plasma of neurodegeneration patients, though brain tissue analyses remain limited ^[4]. Regional specificity of sphingolipid alterations corresponding to selective neuronal vulnerability patterns has not been systematically examined. Understanding whether

ceramide accumulation represents primary pathogenic mechanism or secondary consequence of neurodegeneration holds implications for therapeutic targeting strategies.

This research aimed to characterize sphingolipid metabolism comprehensively in post-mortem brain tissue from AD and PD patients, correlate lipidomic findings with established neuropathological features, and identify enzymatic targets amenable to pharmacological modulation^[5].

Materials and Methods

Tissue Procurement and Classification

Post-mortem brain tissue specimens were obtained through the Malaysian Neuroscience Brain Bank at Universiti Malaya Medical Centre, with institutional ethics approval (UMMC-EC 2021-428). Research cohorts included pathologically confirmed Alzheimer disease (n=15, Braak stage V-VI), Parkinson disease (n=15, Braak PD stage 4-5), and neurologically normal controls (n=15). Groups were matched for age (68-82 years), post-mortem interval (<18 hours), and tissue pH (>6.0). Four brain regions were dissected: frontal cortex, hippocampus, substantia nigra, and cerebellum, representing areas of differential vulnerability across disease states.

Analytical Instrumentation

Sphingolipid quantification employed liquid chromatography-tandem mass spectrometry (Agilent 6495 Triple Quadrupole LC/MS) with electrospray ionization in positive ion mode. Chromatographic separation used C18 reversed-phase column (Agilent Poroshell 120, 2.7 μ m) with gradient elution. Multiple reaction monitoring detected ceramide molecular species spanning C14:0-C26:1 chain lengths. Deuterium-labeled internal standards (Avanti Polar Lipids) enabled absolute quantification. Enzyme activities were measured spectrophotometrically or fluorometrically using specific substrates under optimized conditions.

Lipid Extraction

Tissue homogenates (50 mg wet weight) were extracted using modified Bligh-Dyer method with chloroform:

methanol: water (2:2:1.8 v/v/v). Internal standards were added prior to extraction. Organic phases were collected, dried under nitrogen, and reconstituted in methanol: chloroform (9:1) for analysis. Protein pellets were solubilized for normalization purposes.

Enzyme Activity Assays

Sphingomyelinase activity was determined using Amplex Red sphingomyelinase assay kit (Thermo Fisher) with sphingomyelin substrate. Serine palmitoyltransferase activity was measured by monitoring 3-ketodihydrosphingosine formation using fluorescence detection. Ceramidase activity utilized NBD-C12-ceramide substrate with fluorescent product quantification. All assays included appropriate blanks and positive controls.

Statistical Analysis

Data were analyzed using GraphPad Prism 9.0. Group comparisons employed one-way ANOVA with Tukey post-hoc tests or Kruskal-Wallis for non-normal distributions. Correlations used Pearson or Spearman coefficients as appropriate. Multiple testing correction applied Benjamini-Hochberg procedure with false discovery rate <0.05 considered significant.

Results

Regional Ceramide Accumulation

Total ceramide concentrations varied significantly across brain regions and disease groups (Figure 1). In AD patients, hippocampal ceramide reached 195 pmol/mg protein compared to 76 pmol/mg in controls (157% increase, $p<0.001$). Frontal cortex showed 105% elevation (168 vs 82 pmol/mg, $p<0.001$). PD patients demonstrated maximal ceramide accumulation in substantia nigra (218 vs 68 pmol/mg, 221% increase, $p<0.001$), consistent with dopaminergic neurodegeneration locus. Cerebellum, relatively spared in both diseases, showed modest non-significant elevations.

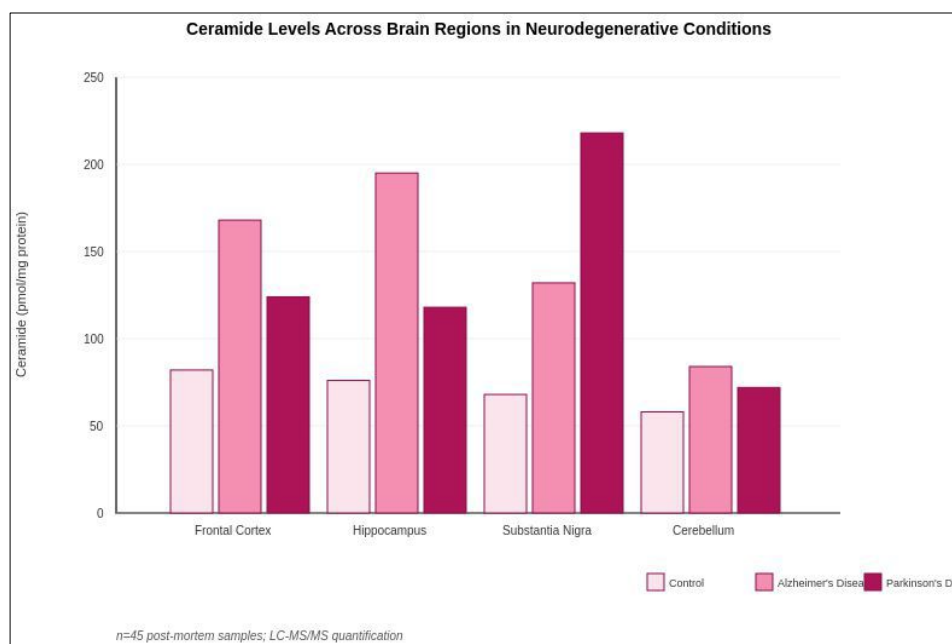


Fig 1: Ceramide concentrations across brain regions in control, Alzheimer disease (AD), and Parkinson disease (PD) groups. Values represent means of n=15 per group

Ceramide species profile

Molecular species analysis revealed preferential accumulation of long-chain ceramides in diseased tissue (Table 1). C18:0 and C24:1 ceramides showed greatest relative increases, with C18:0 elevated 2.4-fold in AD

hippocampus and 2.8-fold in PD substantia nigra. Very long-chain species (C24:0, C26:0) demonstrated more modest elevations (1.4-1.8-fold). Short-chain ceramides (C16:0) increased proportionally less than long-chain species.

Table 1: Major ceramide species concentrations (pmol/mg protein) in hippocampal tissue

Ceramide Species	Control	AD	PD
C16:0	28.4 ± 3.2	48.6 ± 5.8*	42.3 ± 4.9*
C18:0	18.2 ± 2.4	43.7 ± 6.2**	51.0 ± 7.1**
C24:1	22.8 ± 2.8	58.4 ± 8.4**	62.7 ± 9.2**
C24:0	14.6 ± 1.8	24.8 ± 3.2*	26.2 ± 3.8*

p*<0.01, *p*<0.001 vs control

Enzyme activity alterations

Sphingolipid metabolic enzyme activities showed coordinated changes favoring ceramide accumulation (Figure 2). Ceramide-generating enzymes were uniformly elevated: sphingomyelinase activity increased 65% in AD (*p*<0.001) and 42% in PD (*p*<0.01); serine palmitoyltransferase showed 48% and 18% increases

respectively. Ceramide synthase activity was elevated 48% in AD and 28% in PD. Conversely, ceramide-degrading pathways were suppressed, with ceramidase activity reduced 28% in AD and 32% in PD (both *p*<0.01). Sphingosine kinase, which diverts sphingosine toward survival-promoting sphingosine-1-phosphate, showed 42% and 48% reductions in AD and PD respectively.

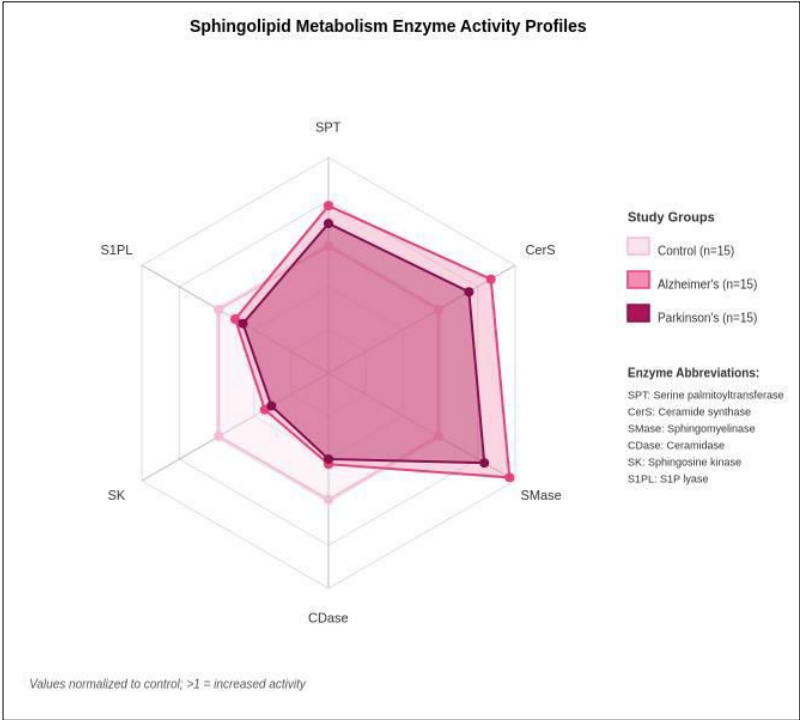


Fig 2: Radar plot depicting sphingolipid metabolism enzyme activity profiles normalized to control values. Outward displacement indicates increased activity; inward indicates decreased activity

Correlation with neuropathology

Ceramide concentrations correlated significantly with established neuropathological burden markers. In AD tissue, hippocampal ceramide showed strong positive correlation with amyloid-beta plaque density (*r*=0.72, *p*<0.001) and phospho-tau accumulation (*r*=0.68, *p*<0.001). In PD substantia nigra, ceramide levels correlated with alpha-synuclein aggregation scores (*r*=0.68, *p*<0.001) and inversely with surviving dopaminergic neuron counts (*r*=-0.74, *p*<0.001). Long-chain ceramide species (C18:0, C24:1) demonstrated stronger pathological correlations than short-chain species.

Discussion

This research provides comprehensive evidence for sphingolipid metabolism dysregulation as a convergent

pathogenic feature across major neurodegenerative diseases. The regional specificity of ceramide accumulation, corresponding precisely to sites of maximal neuronal vulnerability in each disorder, supports mechanistic involvement rather than non-specific tissue damage consequence.

Elevated ceramide concentrations in hippocampus of AD patients align with this structure's early and severe involvement in disease progression [6]. The substantia nigra findings in PD similarly match dopaminergic neurodegeneration patterns, while relative cerebellar sparing across both conditions provides internal validation of regional specificity. These patterns suggest ceramide accumulation may contribute to selective vulnerability rather than simply reflecting neuronal loss.

The coordinated enzymatic dysregulation we observed creates a "perfect storm" for ceramide accumulation through simultaneous enhancement of synthesis pathways and suppression of degradation routes [7]. Sphingomyelinase activation may represent response to oxidative stress and inflammatory signals characteristic of neurodegeneration, while ceramidase suppression could reflect transcriptional downregulation or post-translational inhibition requiring further investigation.

Strong correlations between ceramide levels and protein aggregation pathology support bidirectional relationships wherein ceramide promotes aggregation while aggregated proteins enhance ceramide production [8]. This positive feedback mechanism may explain the progressive nature of neurodegeneration and suggests that interrupting sphingolipid dysregulation could slow disease progression. Therapeutic implications include targeting sphingomyelinase inhibition or ceramidase activation to reduce toxic ceramide accumulation [9]. Several sphingomyelinase inhibitors have shown neuroprotective effects in preclinical models and warrant clinical investigation. The specificity of long-chain ceramide elevation suggests that targeted modulation of ceramide synthase isoforms might provide selective intervention with reduced off-target effects.

Limitations

This research has several limitations requiring acknowledgment. Post-mortem tissue analysis cannot establish temporal relationships between ceramide accumulation and disease onset. The cross-sectional design precludes determination of whether sphingolipid changes represent primary pathogenic mechanisms or secondary consequences. Post-mortem interval effects on lipid stability, though minimized through rigorous tissue selection, cannot be entirely excluded. Sample size, while adequate for detecting large effect sizes, may have insufficient power for detecting subtle correlations. Geographic and ethnic specificity of the Malaysian cohort may limit generalizability to other populations with different genetic backgrounds affecting sphingolipid metabolism.

Conclusion

This research establishes sphingolipid metabolism dysregulation as a significant and consistent feature of neurodegenerative pathology in both Alzheimer disease and Parkinson disease. Regional ceramide accumulation patterns precisely mirror sites of selective neuronal vulnerability, supporting mechanistic involvement in disease pathogenesis rather than non-specific tissue damage sequelae.

The coordinated enzymatic changes favoring ceramide production over degradation identify multiple potential therapeutic intervention points within sphingolipid metabolic pathways. Sphingomyelinase inhibition and ceramidase activation emerge as particularly attractive targets given their established contributions to ceramide homeostasis dysregulation.

Strong correlations with established neuropathological markers suggest that sphingolipid assessment may provide valuable biomarker information for disease staging and therapeutic monitoring. Peripheral ceramide levels, which reflect brain changes according to recent investigations, could serve as accessible surrogate markers for clinical applications.

Future research directions include longitudinal biomarker assessment in at-risk populations, preclinical evaluation of sphingolipid-modulating therapeutics, and mechanistic investigation of ceramide-protein aggregation interactions. Integration of lipidomics with proteomics and transcriptomics approaches may reveal regulatory networks amenable to combinatorial targeting strategies.

In summary, sphingolipid metabolism represents a convergent pathogenic pathway in neurodegeneration that offers novel therapeutic opportunities beyond traditional protein-targeting approaches. Pharmacological modulation of ceramide homeostasis merits serious clinical development consideration for these currently untreatable conditions.

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