The Role of Sphingolipids in Alzheimer’s Disease, Parkinson’s Disease and Lewy Body Dementia: A Common Pathway?

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Disclosures

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• Associate Editor: Alzheimer’s and Dementia – A Journal of the Alzheimer’s Association; Journal of Alzheimer’s Disease
Outline

• Importance of lipids in the CNS
• Sphingolipids in neurodegenerative diseases
• Link between sphingolipids, Alzheimer’s disease and pathology
• Link between sphingolipids, Parkinson’s disease and Lewy body dementia
• Ongoing research and future directions
Importance of Lipids in the CNS

- Structure of neuronal cell membranes, directly affecting the solubility and fluidity (Needham & Nunn, 1990)

- Greatly affected by oxidative stress
  - Important in brain; low anti-oxidants

- Homeostasis in neuron and myelin is a key component in preventing loss of synaptic plasticity, cell death, and ultimately, substantial neurodegeneration

- Act as second messengers
Ceramides

- Product of sphingomyelin metabolism or de novo synthesis
  - Precursor to more complex sphingolipids
- Structural role – lipid rafts
- 2nd messengers that regulate cellular differentiation, proliferation, and apoptosis
  - Low levels: promote cell division & play fundamental role in injury-induced cytokine production (TNF-a, IL-6)
  - High levels: activate signaling cascades, increase inflammation, promote free radical generation; sensitize neurons to oxidation
  - Autophagy
Sphingolipid Pathway

**Key Reactions and Pathways:**

- **Palmitoyl CoA + Serine** → Autonomic Neuropathy Type 1
- **3-keto-dihydrosphingosine** → Krabbe Disease
- **Fatty acyl CoA + Dihydrosphingosine**
  - **CerS** → Dihydroceramide
  - **SMase** → Sphingomyelin

**Metabolites:**

- **Ceramide**
  - **CerS Ceramidase**
  - **S1P lyase** → Sphingosine -1-PO₄
- **Sphingosine -1-PO₄**
  - **SphK** → S1P lyase
  - **S1PP** → Ethanolamine -1-PO₄ + Hexadecenal

**Diseases:**

- Niemann Pick Disease
- Farber's Disease
- Multiple Sclerosis
- Major Depression

**Sphingomyelin:**

- **SMase** → Dihydrosphingomyelin
- **SMS** → Dihydrosphingomyelin

**Lysosomal Enzymes:**

- **Ceramidase**
- **GalCer synthase**
- **GluCer synthase**
- **LacCer synthase**

**Sphingosine:**

- **S1P lyase** → Ethanolamine -1-PO₄ + Hexadecenal

**Gangliosides:**

- **GM3 Gangliosides**
- **GD3 Gangliosides**
- **GT3 Gangliosides**

**Clinically Relevant Conditions:**

- ALS
- Gaucher's Disease
- Lewy Body/PD
- Farber's Disease
- Multiple Sclerosis

**Genes:**

- **SPT**
- **ASA**
- **CST**
- **S1PP SphK**

**Note:** The diagram illustrates the Sphingolipid Pathway and its connection to various diseases and metabolites.
Ceramides-AD Neuropathology Link

- Exposure of cultured neurons to Aβ42 directly increases ceramide levels by activating neutral sphingomyelinase (nSMase) (Grimm, 2005; Jana & Pahan, 2004; Lee, 2004)
  - Inhibiting increase protects neuron from Aβ42-induced cell death (Cutler, 2004)

- Aβ42 can indirectly increase ceramides through an oxidative stress-mediated mechanism (Cutler, 2004; Matson, 2005; Gulbins & Kolesnick, 2003)

- Ceramides modulate BACE (but not gamma-secretase) activity (Kalvodova, 2005; Puglielli, 2003)

- Ceramides modulate PP2A activity, leading to tau phosphorylation (Mukhopadhyay et al., 2009)

Amyloid → PP2A → AKT dephosphorylation → Mitochondrial dysfx → Cell Death

Ceramide
Few Human Studies Conducted

- Mixed results for ceramides & SM in brain tissues of AD patients and controls (Cutler et al., 2004; Pettegrew et al., 2001)
  - SM and ceramide levels varied by disease severity (Satoi et al., 2005; Han et al., 2002; Cutler et al., 2004)
  - Altered gene expression patterns of enzymes in sphingolipid metabolism pathway varied by AD severity (Katsel et al., 2007)
  - Possible marker of AD progression??

- CSF ceramide and sphingomyelin levels also vary by disease severity (Satoi et al., 2005; Kosicek et al., 2010; Kosicek et al., 2012)
Blood-Based Predictor of Cognitive Progression?
Overview of Blood-Based Findings

• Cognitively Normal
  • High baseline ceramides associated with increased risk of:
    • Cognitive impairment (Mielke MM, et al., 2010a)
    • Alzheimer’s disease (Mielke MM, et al., 2012)

• Mild Cognitive Impairment (MCI)
  • High baseline ceramides associated with: (Mielke MM, et al., 2010b)
    • Memory decline
    • Hippocampal volume loss
    • White matter integrity, primarily the posterior cingulate

• Alzheimer’s Disease
  • High baseline ceramides and SM/Cer ratios associated with:
    • Faster cognitive progression on MMSE and ADAS-Cog
      (Mielke MM, et al., 2011)
How do these markers relate to AD pathology?
Relationship Between Sphingolipids, Amyloid, and Tau in Humans

• Brain
  • *He et al., 2010* – aSMase positive correlation with amyloid and tau
  • *Katsel et al., 2007* – LASS1 & LASS2 upregulated at Braak Stage VI, LASS6 downregulated; correlation between other enzymes and Braak stage

• CSF
  • Previous studies did not look at relation to amyloid and tau (e.g., Satoi et al., 2005; Kosicek et al., 2012).
CSF Sphingomyelins and Tau

MCSA 70+ cognitively normal

- rho = 0.418
- p = 0.0001
- n = 88

Wisconsin 36-69 cognitively normal

- rho = 0.670
- p <0.0001
- n = 91

Mielke MM, et al. unpublished

Next step: Mechanistic studies in Humans
Next Steps

• In vivo biomarker modeling opens up a whole new world

• Transgenic animal models primarily used for mechanistic studies, but not fully translatable

• Biomarker modeling allows for human mechanistic studies and identification of new biomarkers for given pathology and clinical phenotype
  • Clinic phenotype is heterogeneous
  • Allows for Individualized Medicine Approach
Diagram for the study of CSF and plasma sphingolipids with both clinical phenotypes and *in vivo* brain pathology

Clinical phenotype

In vivo AD pathology

Biomarker of Interest

CSF /plasma sphingolipids

Normal Cognition  \(\rightarrow\)  Mild Cognitive Impairment  \(\rightarrow\)  Alzheimer’s Disease

A+/\(-\)  \(\rightarrow\)  N+/\(-\)

\(\rightarrow\)  ?  \(\rightarrow\)  ?  \(\rightarrow\)  ?

\(\rightarrow\)  ?  \(\rightarrow\)  ?  \(\rightarrow\)  ?
## Interactions with PiB Amyloid

<table>
<thead>
<tr>
<th>Baseline Log Plasma Ceramide</th>
<th>Change in Outcome</th>
<th>Baseline Ceramide * Time</th>
<th>Baseline Aβ * Time</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>b (se)</td>
<td>p-value</td>
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<tr>
<td><strong>Ceramide carbon chain length or total ceramides</strong></td>
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<tr>
<td>C14:0</td>
<td>FDG-PET</td>
<td>0.027 (0.02)</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>Hippocampal volume</td>
<td>0.073 (0.10)</td>
<td>0.468</td>
</tr>
<tr>
<td>C16:0</td>
<td>FDG-PET</td>
<td>0.035 (0.02)</td>
<td>0.179</td>
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<tr>
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<td>Hippocampal volume</td>
<td>0.194 (0.08)</td>
<td>0.015</td>
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<tr>
<td>C18:0</td>
<td>FDG-PET</td>
<td>0.080 (0.08)</td>
<td>0.320</td>
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<tr>
<td></td>
<td>Hippocampal volume</td>
<td>0.02 (0.02)</td>
<td>0.384</td>
</tr>
<tr>
<td>C20:0</td>
<td>FDG-PET</td>
<td>0.100 (0.08)</td>
<td>0.215</td>
</tr>
<tr>
<td></td>
<td>Hippocampal volume</td>
<td>0.044 (0.02)</td>
<td>0.032</td>
</tr>
<tr>
<td>C24:0</td>
<td>FDG-PET</td>
<td>0.838 (0.30)</td>
<td>0.005</td>
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<tr>
<td></td>
<td>Hippocampal volume</td>
<td>0.104 (0.09)</td>
<td>0.240</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Linear mixed models adjust for age, sex, educ, APOE E4*
Ceramides-AD Neuropathology Link

Amyloid → PP2A → AKT dephosphorylation → Cell Death

Ceramide
Sphingolipids, Inflammation, and AD Pathology

Sphingomyelin $\xrightarrow{\text{SMase}}$ Ceramide

$\text{TNF-}\alpha$, IL-6

<table>
<thead>
<tr>
<th>Log inflammatory marker</th>
<th>$N$</th>
<th>$b$ (95% CI)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>576</td>
<td>-0.06 (-0.09, -0.02)</td>
<td>0.002</td>
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<tr>
<td>IL-6</td>
<td>474</td>
<td>-0.04 (-0.06, -0.01)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Models adjust for age and sex
AD – Funded R01

• ~2,400 plasma samples w/corresponding imaging (PiB, FDG-PET, MRI) and/or CSF
• ~1,000 CSF

Goals: Are sphingolipids predictive of and/or associated with neurodegeneration/cognition?
  - interaction with amyloid
  - assess inflammation as effect modifier
Are Peripheral Ceramides Related to AD?

Mielke & Lyketsos, 2010
Sphingolipid Epidemiology
Participants (n = 992; 3,960 total samples) were 55 years and older and had plasma at two or more study visits over a mean of 14.3 years (range: 2.0-38.9 years). 366 women 626 men.

Mielke MM, et al., 2015a
Sphingomyelin 16:0

Sphingomyelin 18:0

Sphingomyelin 20:0

Sphingomyelin 22:0

Sphingomyelin 24:0

Sphingomyelin 18:1

Sphingomyelin 20:1

Sphingomyelin 22:1

Sphingomyelin 24:1

Mielke MM, et al., 2015b
Sex Differences

- Estrogen may upregulate S1P
  - Most focus is on breast cancer
- Hormone therapy reduces ceramides in pilot study
- Future step:
  - To determine association between hormones (estrogen, testosterone), sphingolipids, and risk of AD
Parkinson’s Disease (PD) and Lewy Body Dementia (LBD)
**Sphingolipid Pathway**

**Palmitoyl CoA** + **Serine**

$\xrightarrow{SPT}$

3-keto-dihydrosphingosine

$\xrightarrow{3\text{-keto-dhSr}}$

**Fatty acyl CoA** + **Dihydrosphingosine**

$\xrightarrow{\text{CerS}}$

Ceramide

$\xrightarrow{\text{CerS Ceramidase}}$

**Sphingomyelin**

$\xrightarrow{\text{SPT}}$

Dihydrosphingomyelin

$\xrightarrow{\text{SMase SMS}}$

**Dihydroceramide**

$\xrightarrow{\text{CerS Ceramidase}}$

Ceramide

$\xrightarrow{\text{CerK}}$

Ceramide -1-PO$_4$

$\xrightarrow{\text{SphK S1P lyase}}$

Sphingosine -1-PO$_4$

$\xrightarrow{\text{S1P lyase}}$

Ethanalamine -1-PO$_4$ + Hexadecenal

$\xrightarrow{\text{S1P lyase}}$

**Sphingosine**

$\xrightarrow{\text{S1PP}}$

Glucosylsphingosine

$\xrightarrow{\text{SphK}}$

**Sphingosine -1-PO$_4$**

**Galactosylceramide**

$\xrightarrow{\text{GalCer synthase}}$

Glucosylceramide

$\xrightarrow{\text{LacCer synthase}}$

Lactosylceramide

$\xrightarrow{\text{Sialyl Transferase}}$

GM3 Gangliosides

$\xrightarrow{\text{Sialyl Transferase 2}}$

GD3 Gangliosides

$\xrightarrow{\text{Sialyl Transferase 3}}$

GT3 Gangliosides

$\xrightarrow{\text{CerS Ceramidase}}$

**Lewy Body/PD**

**Sulfatide**

$\xrightarrow{\text{ASA CST}}$

**Glucosylceramide**

$\xrightarrow{\text{GluCer synthase}}$

**Glucosylceramide**

$\xrightarrow{\text{GluCer synthase}}$

**Sphingolipid Pathway**

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Sphingolipids in PD and LBD

• Alpha-synuclein is a lipid-binding protein; involved in the regulation of membrane lipid composition (Jo E, et al., 2000)

Ceramides

• Dopaminergic neurons positively regulate neutral sphingomyelinase activity and can cause an increase in ceramide levels (Sofic et al., 2001)

• Post-mortem and in-vitro PD studies have demonstrated that the resultant increase in ceramide levels may mediate the apoptosis observed in the substantia nigra (Hunot et al., 1997)

• Reducing ceramide levels protect against MPTP neurotoxicity (Levenson et al., 2004)
Glucosylceramides

- **Glucocerebrosidase:**
  - Catalyzes breakdown of glucosylceramide to ceramide + glucose
  - Glucosylceramide levels increases alpha-synuclein
  - Alpha-synuclein inhibits glucocerebrosidase activation (Mazulli et al., 2012)

- **Mutations in the GBA gene coding for glucocerebrosidase:**
  - ~7% of sporadic PD patients (Sidransky E, et al., 2009)
  - Most prevalent genetic mutation

- **GBA mutations:**
  - Higher odds of LB pathology among PD patients (Clark LN, et al., 2009)
  - Greater cognitive impairment (Brockmann K, et al., 2011; Alcalay RN, et al., 2012)
PD and LBD, cont.

• Hypothesis:
  • Sphingolipid metabolism (ceramides and glucosylceramides) is also affected in non-GBA mutation carriers

• Clinical Study (Tübingen, Germany):
  • 26 PD cognitively normal; 14 PD-MCI; 12 PDD
  • 5 controls
  • All non-GBA mutation carriers (minor/major)
<table>
<thead>
<tr>
<th>Plasma Log Lipid</th>
<th>PD-NC (N=26)</th>
<th>PD-MCI/PDD (N=26)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Ceramide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>11.48 (10.93, 12.26)</td>
<td>11.67 (11.27, 12.79)</td>
<td>0.035</td>
</tr>
<tr>
<td>C18:0</td>
<td>10.98 (10.24, 12.24)</td>
<td>11.18 (10.44, 12.54)</td>
<td>0.016</td>
</tr>
<tr>
<td>C20:0</td>
<td>12.21 (11.32, 13.58)</td>
<td>12.54 (11.80, 14.10)</td>
<td>0.037</td>
</tr>
<tr>
<td>C22:0</td>
<td>13.63 (12.83, 14.77)</td>
<td>13.94 (12.67, 16.14)</td>
<td>0.037</td>
</tr>
<tr>
<td>C24:0</td>
<td>15.73 (14.37, 16.85)</td>
<td>16.01 (14.17, 18.00)</td>
<td>0.621</td>
</tr>
<tr>
<td>C26:0</td>
<td>11.94 (9.68, 12.84)</td>
<td>12.15 (9.70, 14.07)</td>
<td>0.510</td>
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<tr>
<td>C22:1</td>
<td>9.86 (8.83, 10.60)</td>
<td>9.95 (9.43, 11.92)</td>
<td>0.442</td>
</tr>
<tr>
<td>C24:1</td>
<td><strong>13.23 (12.34, 14.83)</strong></td>
<td>13.44 (12.67, 15.32)</td>
<td><strong>0.048</strong></td>
</tr>
<tr>
<td>C26:1</td>
<td>9.70 (8.46, 11.05)</td>
<td>9.90 (8.87, 11.77)</td>
<td>0.380</td>
</tr>
<tr>
<td><strong>Monohexosylceramides (Glucosyl- &amp; Galactosylceramides)</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>11.92 (11.03, 12.79)</td>
<td>12.09 (11.35, 14.29)</td>
<td>0.046</td>
</tr>
<tr>
<td>C18:0</td>
<td>9.11 (8.40, 10.15)</td>
<td>9.15 (8.33, 10.80)</td>
<td>0.242</td>
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<tr>
<td>C20:0</td>
<td><strong>10.48 (9.79, 11.31)</strong></td>
<td><strong>10.72 (10.07, 12.11)</strong></td>
<td><strong>0.039</strong></td>
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<tr>
<td>C22:0</td>
<td>13.75 (12.97, 14.61)</td>
<td>13.95 (13.18, 15.52)</td>
<td>0.148</td>
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<tr>
<td>C24:0</td>
<td><strong>14.17 (13.15, 15.49)</strong></td>
<td><strong>14.56 (13.26, 16.31)</strong></td>
<td><strong>0.040</strong></td>
</tr>
<tr>
<td>C26:0</td>
<td>10.20 (8.82, 11.16)</td>
<td>10.23 (8.17, 12.28)</td>
<td>0.840</td>
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<tr>
<td>C16:1</td>
<td>9.50 (8.67, 9.99)</td>
<td>9.58 (9.12, 11.72)</td>
<td>0.089</td>
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<tr>
<td>C22:1</td>
<td>9.94 (9.02, 10.59)</td>
<td>10.00 (9.00, 11.88)</td>
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<tr>
<td>C24:1</td>
<td>10.02 (8.48, 11.06)</td>
<td>10.09 (9.26, 12.48)</td>
<td>0.272</td>
</tr>
</tbody>
</table>
Sphingolipid Pathway

**Palmitoyl CoA** + **Serine**

- $3\text{-keto-dihydrosphingosine}$
  - $3\text{-keto-dhSr}$
  - **Fatty acyl CoA** + **Dihydrosphingosine**
    - **CerS**
    - **CerS Ceramidase**
  - **Dihydrosphingomyelin**
    - **SMase**
    - **SMS**

**Dihydrosphingomyelin** → **Dihydroceramide**

- **DES**
- **CerS**
- **Ceramidase**
- **CerK**
- **C1PP**

**Sphingomyelin**

- **SMase**
- **SMS**

**Sphingosine**

- **SphK**
- **S1PP**

**Sphingosine -1-PO$_4$**

- **S1P lyase**

**Ethanolamine -1-PO$_4$** + **Hexadecenal**

**Sulfatide**

- **ASA**
- **CST**

**Galactosylceramide**

- **GalCer synthase**
- **GalCeramidase**
- **Lewy Body/PD**

**Lactosylceramide**

- **GluCer synthase**
- **GluCeramidase**

**Glucosylceramide**

- **Ceramidase**
- **GluCer synthase**
- **LacCer synthase**

**Lactosylceramide**

- **Sialyl Transferase**

**GM3 Gangliosides**

- **Sialyl Transferase 2**

**GD3 Gangliosides**

- **Sialyl Transferase 3**

**GT3 Gangliosides**
Ceramides, Glucosylceramides and PD

• Aims:
  • To replicate the previous cross-sectional study
  • To determine whether the lipids predict cognitive decline
  • To further explore GBA mutations and cognitive impairment in PD
## Baseline Characteristics (N=412)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PD-NC (N=272)</th>
<th>PD-MCI (N=85)</th>
<th>PDD (N=55)</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>Baseline age</strong></td>
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<tr>
<td></td>
<td>272</td>
<td>85</td>
<td>55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>66.2 (8.2)</td>
<td>67.8 (7.0)</td>
<td>72.4 (4.9)</td>
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<tr>
<td><strong>Disease duration, years</strong></td>
<td>262</td>
<td>84</td>
<td>55</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>5.9 (4.9)</td>
<td>7.6 (5.7)</td>
<td>9.3 (6.2)</td>
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<tr>
<td><strong>Male</strong></td>
<td>272</td>
<td>85</td>
<td>55</td>
<td>0.750</td>
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<td></td>
<td>188 (69.1%)</td>
<td>55 (64.7%)</td>
<td>38 (69.1%)</td>
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<tr>
<td><strong>Education: University</strong></td>
<td>268</td>
<td>84</td>
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<td>188 (70.2%)</td>
<td>59 (70.2%)</td>
<td>41 (74.6%)</td>
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<td><strong>BMI</strong></td>
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<td>26.7 (4.2)</td>
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<td>55</td>
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<td>1.7 (1.7)</td>
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<td><strong>UPDRS III</strong></td>
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<td>20.8 (11.0)</td>
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<td><strong>GDS</strong></td>
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<td>3.0 (2.6)</td>
<td>2.9 (2.7)</td>
<td>6.4 (3.4)</td>
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<td><strong>AES</strong></td>
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<td>28.6 (7.9)</td>
<td>29.3 (2.7)</td>
<td>39.5 (10.6)</td>
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<td><strong>PANDA</strong></td>
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<td>55</td>
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<td>16.0 (5.1)</td>
<td>9.3 (4.7)</td>
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<td>47</td>
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<td>22.0 (13.6)</td>
<td>24.9 (12.5)</td>
<td>39.3 (14.8)</td>
<td></td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>272</td>
<td>85</td>
<td>55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>28.6 (1.5)</td>
<td>27.9 (1.9)</td>
<td>24.4 (3.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Any GBA mutation</strong></td>
<td>268</td>
<td>84</td>
<td>54</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>41 (15.3%)</td>
<td>19 (22.6%)</td>
<td>20 (37.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Pathogenic GBA mutation</strong></td>
<td>268</td>
<td>84</td>
<td>54</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>6 (2.2%)</td>
<td>2 (2.4%)</td>
<td>6 (11.1%)</td>
<td></td>
</tr>
</tbody>
</table>

BMI = body mass index; UPDRS = Unified Parkinson’s Disease Rating Scale; GDS = Geriatric Depression Scale; AES = Apathy Evaluation Scale; PANDA = Parkinson Neuropsychometric Dementia Assessment; PDQ = Parkinson’s Disease Questionnaire; MMSE = Mini-Mental Status Examination
All PD patients

Cognitively Normal PD patients

Log Sphingosine/Glucosylsphinosine ratio

Hoehn & Yahr Scale
A higher ceramide to glc-Cer ratio is associated with greater cognitive decline among cognitively normal patients with Parkinson’s disease.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Without GBA mutations/variants (N=194)</th>
<th>With GBA mutations/variants (N=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cross-sectional MMSE</td>
<td>cross-sectional MMSE</td>
</tr>
<tr>
<td></td>
<td>b          p-value          b          p-value</td>
<td>b          p-value          b          p-value</td>
</tr>
<tr>
<td>C16:0</td>
<td>0.94       0.151           -0.88       0.043</td>
<td>-2.82       0.159           1.80       0.150</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.86       0.099           -0.78       0.020</td>
<td>1.00       0.498           0.14       0.882</td>
</tr>
<tr>
<td>C18:1</td>
<td>0.04       0.906           -0.06       0.772</td>
<td>-0.61       0.366           0.05       0.897</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.48       0.336           -0.51       0.118</td>
<td>-1.62       0.226           0.90       0.281</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.87       0.102           -0.84       0.006</td>
<td>-1.92       0.176           1.17       0.211</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.58       0.296           -0.64       0.079</td>
<td>-2.07       0.113           1.17       0.171</td>
</tr>
<tr>
<td>C24:1</td>
<td>0.35       0.493           -0.49       0.144</td>
<td>-0.21       0.874           0.08       0.928</td>
</tr>
<tr>
<td>Sph</td>
<td>0.21       0.334           -0.16       0.237</td>
<td>0.18        0.676           0.01       0.966</td>
</tr>
</tbody>
</table>

*Association between each log-unit increase in baseline ceramide to glc-Cer and sphingosine to glc-Sph ratios and cross-sectional and one-year change in MMSE score.*
ADRC pilot project

- **Aim 1**: Comparison of 40 DLB, 12 MCI+RBD, 17 iRBD and 70 age- and sex-matched cognitively normal individuals

- **Aim 2**: Cross-sectional association between the lipids and neuroimaging
  - Dorsal mesopontine gray matter atrophy; occipital hypometabolism
  - Stratify by Amyloid status

- **Aim 3**: Determine whether the lipids predict disease progression
Ceramides elevated in DLB and iRBD versus matched controls

<table>
<thead>
<tr>
<th>lipid</th>
<th>DLB vs. control (N=40/group)</th>
<th>iRBD vs. control (n=17/group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>p-value</td>
</tr>
<tr>
<td>s1p</td>
<td>-0.018</td>
<td>0.557</td>
</tr>
<tr>
<td>cer C14:0</td>
<td>-0.0001</td>
<td>0.916</td>
</tr>
<tr>
<td><strong>cer C16:0</strong></td>
<td><strong>0.056</strong></td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td>cer C18:1</td>
<td>0.001</td>
<td>0.476</td>
</tr>
<tr>
<td>cer C18:0</td>
<td>0.016</td>
<td>0.265</td>
</tr>
<tr>
<td>cer C20:0</td>
<td>0.014</td>
<td>0.313</td>
</tr>
<tr>
<td>cer C22:0</td>
<td>0.040</td>
<td>0.142</td>
</tr>
<tr>
<td><strong>cer C24:1</strong></td>
<td><strong>0.135</strong></td>
<td><strong>0.046</strong></td>
</tr>
<tr>
<td>cer C24:0</td>
<td>0.047</td>
<td>0.590</td>
</tr>
</tbody>
</table>
Higher plasma ceramides associated with lower grey matter mesopontine volume among iRBD

<table>
<thead>
<tr>
<th>lipid</th>
<th>iRBD (N=16)</th>
<th></th>
<th>iRBD A- (N=12)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>p-value</td>
<td>b</td>
<td>p-value</td>
</tr>
<tr>
<td>sph</td>
<td>-54.26</td>
<td>0.492</td>
<td>60.65</td>
<td>0.560</td>
</tr>
<tr>
<td>spa</td>
<td>-223.91</td>
<td>0.354</td>
<td>-157.65</td>
<td>0.580</td>
</tr>
<tr>
<td>cer C14:0</td>
<td>-711.52</td>
<td>0.018</td>
<td>-697.79</td>
<td>0.012</td>
</tr>
<tr>
<td>cer C16:0</td>
<td>-16.26</td>
<td>0.069</td>
<td>-18.99</td>
<td>0.038</td>
</tr>
<tr>
<td>cer C18:1</td>
<td>-465.63</td>
<td>0.047</td>
<td>-577.93</td>
<td>0.006</td>
</tr>
<tr>
<td>cer C18:0</td>
<td>-44.88</td>
<td>0.003</td>
<td>-46.29</td>
<td>0.005</td>
</tr>
<tr>
<td>cer C20:0</td>
<td>-33.30</td>
<td>0.049</td>
<td>-34.78</td>
<td>0.059</td>
</tr>
<tr>
<td>cer C22:0</td>
<td>-9.43</td>
<td>0.321</td>
<td>-1.17</td>
<td>0.911</td>
</tr>
<tr>
<td>cer C24:1</td>
<td>-9.45</td>
<td>0.002</td>
<td>-8.97</td>
<td>0.022</td>
</tr>
<tr>
<td>cer C24:0</td>
<td>-0.37</td>
<td>0.900</td>
<td>2.22</td>
<td>0.451</td>
</tr>
</tbody>
</table>
PD Ongoing Research

• Predict PD progression
  • 500 PD patients (normal cognition, MCI, PDD).
  • Ceramides and glycosylceramides at baseline
  • Lipid levels associated with & predictive of cognitive decline (and other outcomes)

• Comparison of PD GBA mutation carriers, PD sporadic non-carriers, normal controls
  • CSF and plasma ceramides and glucosylceramides

• Comparison of DLB, MCI+RBD, iRBD and age- and sex-matched cognitively normal individuals
  • CSF and plasma ceramides and glucosylceramides
Conclusion

• Consistent evidence for role of ceramide metabolism in AD, LBD, and other neurodegenerative diseases
  • Animals and humans
  • CSF and plasma

• Common pathway; Different forks in the road?

• Identification in blood – blood-based biomarkers?

• Collaborative, translational effort needed
  • Better understanding of ceramides and AD/LBD pathology in animals
  • Roles of exosomes, ceramide transporters (CERT)
  • Better understanding and characterization of sphingolipid levels in the population and relation to disease pathology (direct vs. indirect)
**Sphingolipid Pathway**

- **Palmitoyl CoA + Serine**
  - SPT → 3-keto-dihydrosphingosine
  - 3-keto-dhSr → Fatty acyl CoA + Dihydrosphingosine
  - CerS → Dihydroceramide
  - SMase → Dihydrosphingomyelin

- **Serine Palmitoyl CoA**
  - SPT → 3-keto-dihydrosphingosine
  - 3-keto-dhSr → Fatty acyl CoA + Dihydrosphingosine
  - CerS → Dihydroceramide
  - SMase → Dihydrosphingomyelin

- **Ceramide Sphingomyelin**
  - CerS → Ceramide
  - SMase → Sphingomyelin

- **Ceramide -1-PO₄**
  - CerK → C1PP
  - SphK → S1P lyase → Ethanolamine -1-PO₄ + Hexadecenal

- **Sphingosine**
  - S1P lyase → SphK
  - S1PP → Cholinephosphoethanolamine

- **Sphingosine -1-PO₄**
  - S1P lyase → S1PP

- **Sulfatide**
  - ASA → CST

- **Galactosylceramide**
  - GalCer synthase → GalCeramidase

- **Glucosylceramide**
  - GluCer synthase → GluCeramidase

- **Lactosylceramide**
  - LacCer synthase → LacCeramidase

- **GM3 Gangliosides**
  - Sialyl Transferase 2

- **GD3 Gangliosides**
  - Sialyl Transferase 3

- **GT3 Gangliosides**
  - Sialyl Transferase 4
Collaborators & Funding

**Mayo Clinic**
- Bradley Boeve, MD
- Dennis Dickson, MD
- Clifford Jack, MD
- K. Sree Nair, MD
- Ronald Petersen, MD, PhD
- Walter Rocca, MD, MPH
- Rodolfo Savica, MD

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- Ratnam Bandaru, PhD
- Michelle Carlson, PhD
- Norman Haughey, PhD
- Constantine Lyketsos, MD

**Maastricht University**
- Pilar Martinez-Martinez

**University of Wisconsin**
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**Baylor**
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- Valory Pavlik, PhD

**NIA**
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- Susan Resnick, PhD

**Toronto**
- Krista Lanctôt, PhD

**Germany**
- Daniela Berg, MD
- Inga Liepelt-Scarfone, PhD
- Walter Maetzler, MD

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Call for Papers:

Special Issue – Sphingolipids in Neurodegeneration Diseases

Journal of Alzheimer’s Disease

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Thank You!

Questions & Discussion