MAYO CLINIC

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

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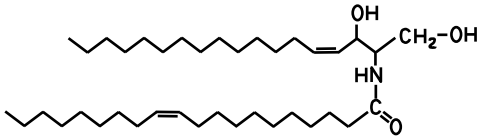
Outline

- 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

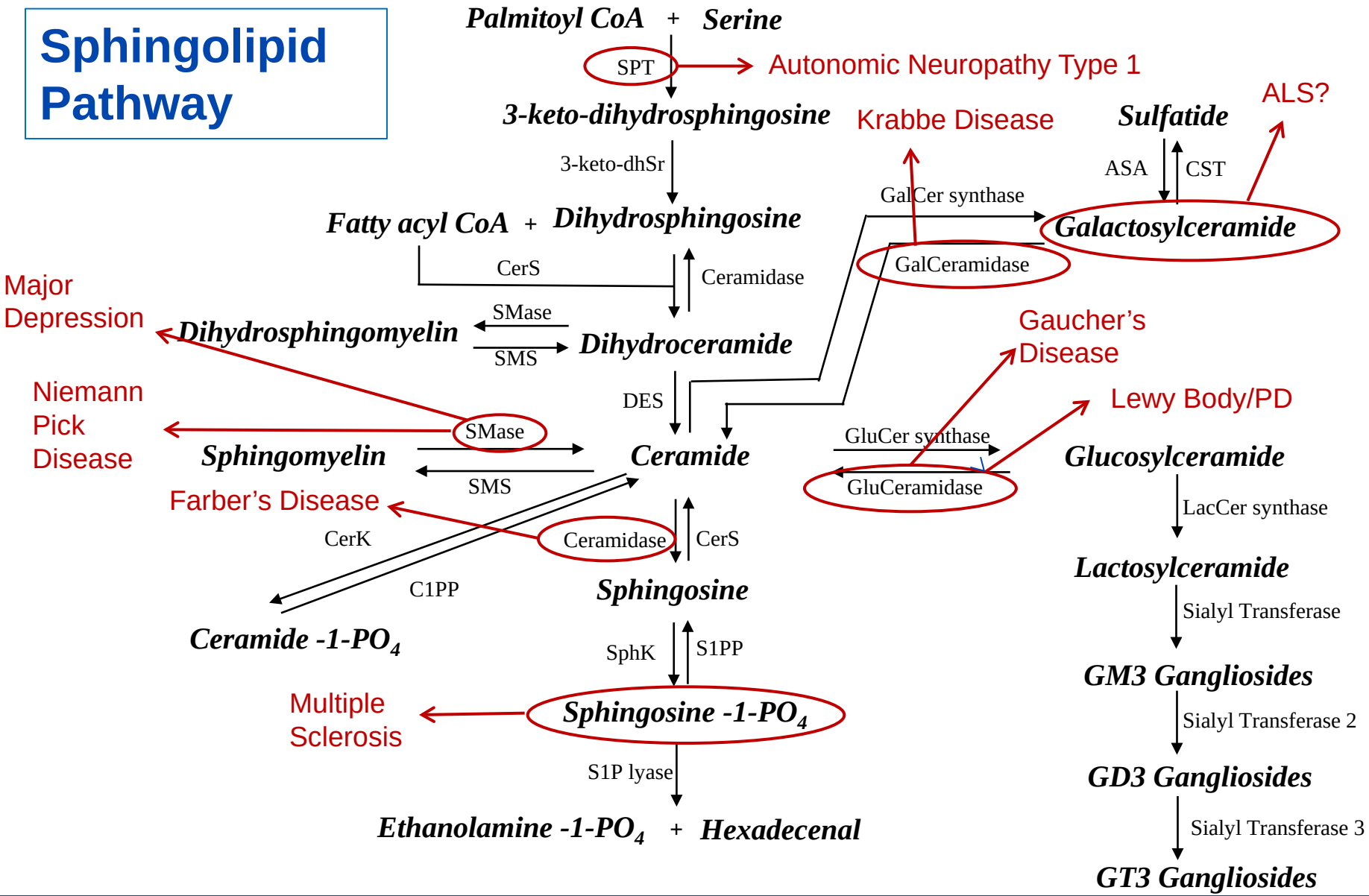
- Structure of neuronal cell membranes, directly affecting the solubility and fluidity (Needham & Nunn, 1990)
- Greatly affected by oxidative stress
  - Important in brain; low anti-oxidants
- Homeostasis in neuron and myelin is a key component in preventing loss of synaptic plasticity, cell death, and 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



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)

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)

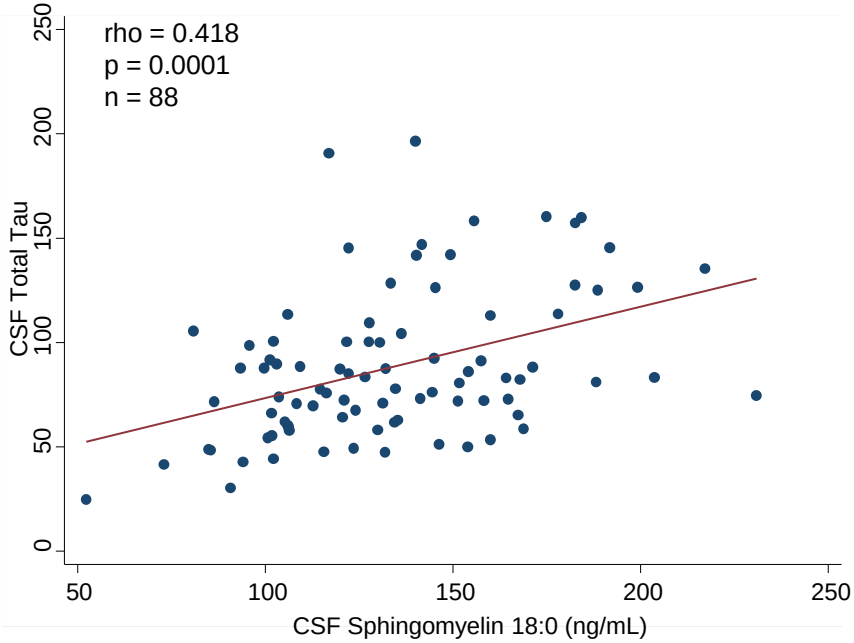
How do these markers relate to AD pathology?

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).

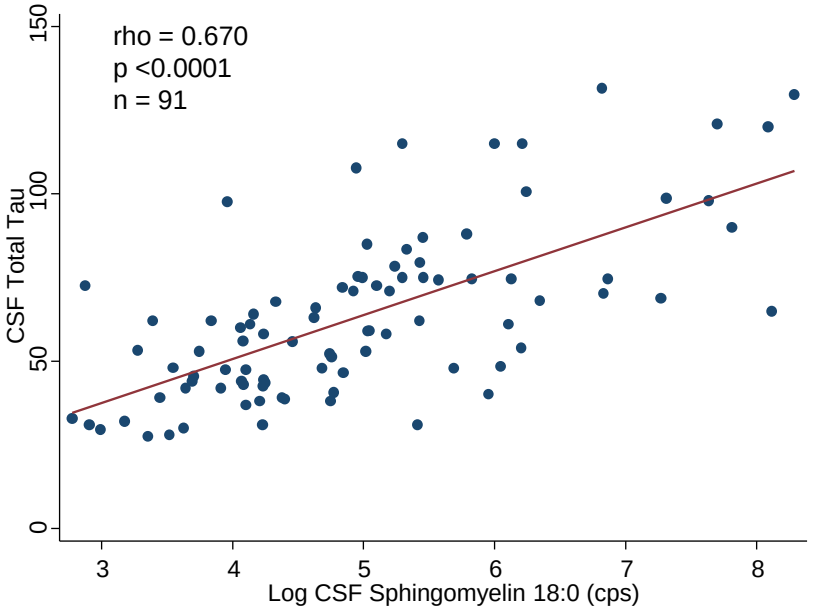
CSF Sphingomyelins and Tau

MCSA 70+ cognitively normal



Mielke MM, et al. unpublished

Wisconsin 36-69 cognitively normal



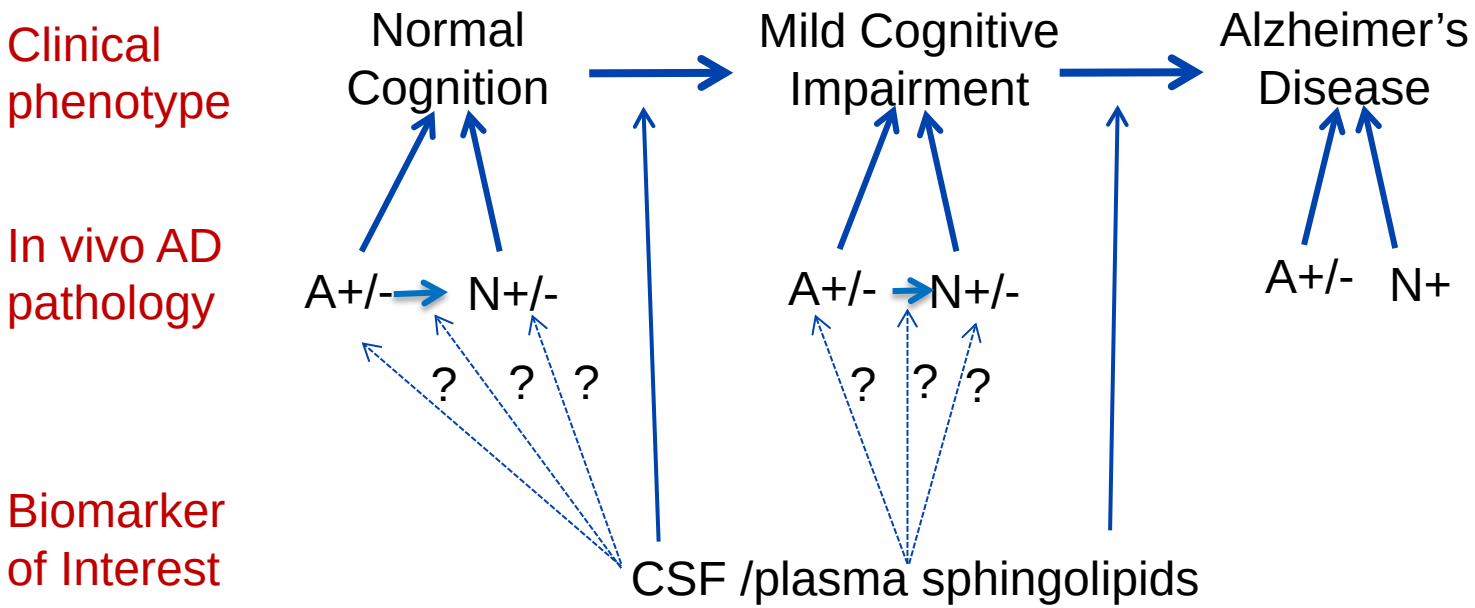
Mielke MM, et al. 2014

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



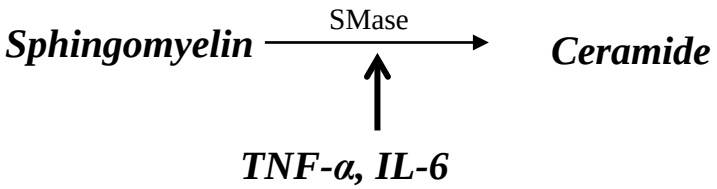
Interactions with PiB Amyloid

Baseline Log Plasma Ceramide	Change in Outcome	Baseline Ceramide * Time		Baseline Aβ * Time		Baseline Ceramide* Baseline Aβ* Time	
		b (se)	*p-value*	*b (se)*	*p-value*	*b (se)*	*p-value*
Ceramide carbon chain length or total ceramides							
C14:0	FDG-PET	0.027 (0.02)	0.092	0.044 (0.02)	0.012	**-0.06 (0.02)**	**0.004**
C16:0	Hippocampal volume	0.073 (0.10)	0.468	0.552 (0.25)	0.030	**-0.290 (0.13)**	**0.026**
	FDG-PET	0.035 (0.02)	0.179	0.158 (0.06)	0.014	**-0.084 (0.03)**	**0.011**
C18:0	Hippocampal volume	0.194 (0.08)	0.015	0.455 (0.18)	0.013	**-0.248 (0.10)**	**0.010**
C20:0	Hippocampal volume	0.080 (0.08)	0.320	0.490 (0.24)	0.038	**-0.223 (0.10)**	**0.032**
	FDG-PET	0.02 (0.02)	0.384	0.119 (0.06)	0.043	**-0.055 (0.03)**	**0.033**
C24:0	Hippocampal volume	0.100 (0.08)	0.215	0.833 (0.31)	0.008	**-0.286 (0.10)**	**0.006**
	FDG-PET	0.044 (0.02)	0.032	0.160 (0.08)	0.039	**-0.056 (0.03)**	**0.032**
C24:1	Hippocampal volume	0.838 (0.30)	0.005	0.258 (0.10)	0.008	**-0.355 (0.12)**	**0.004**
Total	Hippocampal volume	0.104 (0.09)	0.240	1.078 (0.42)	0.011	**-0.318 (0.12)**	**0.010**

*Linear mixed models adjust for age, sex, educ, APOE E4

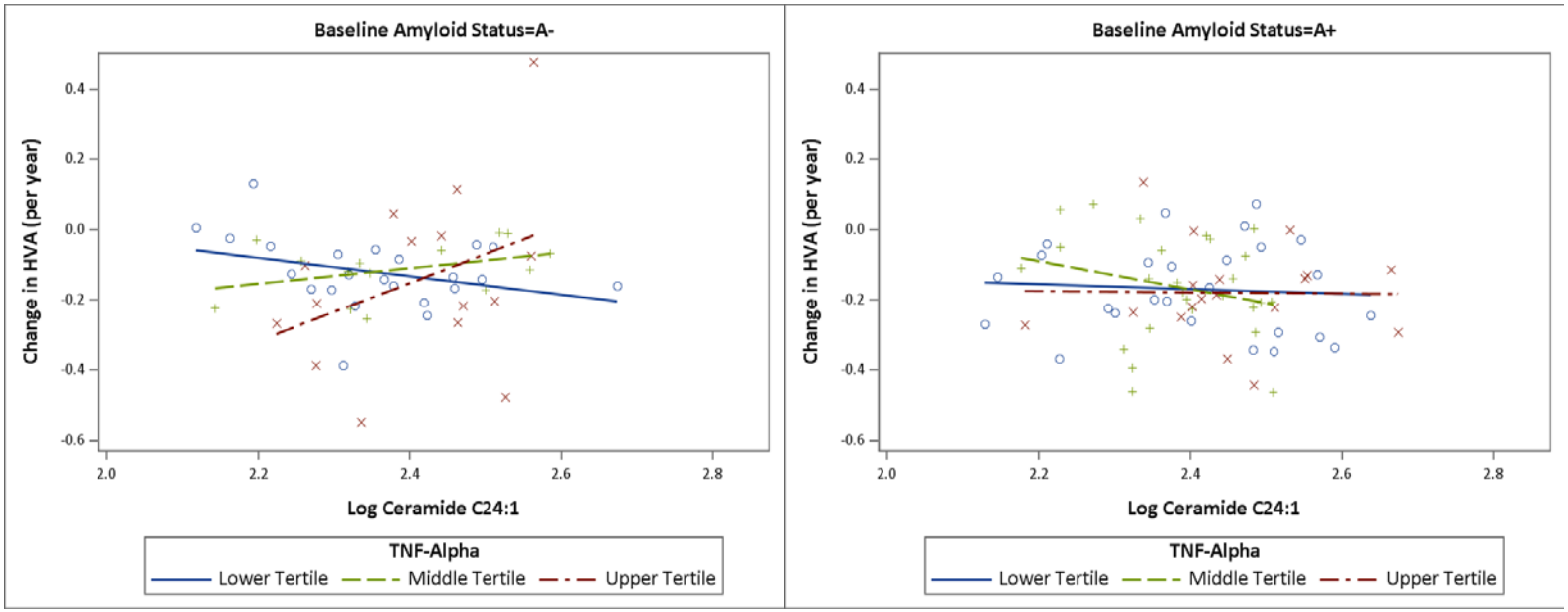
Ceramides-AD Neuropathology Link

Sphingolipids, Inflammation, and AD Pathology



		Log SM/Cer Ratio	
Log inflammatory marker	*N*	*b (95% CI)*	*p-value*
TNF-a	576	-0.06 (-0.09, -0.02)	0.002
IL-6	474	-0.04 (-0.06, -0.01)	0.005

*Models adjust for age and sex



AD – Funded R01

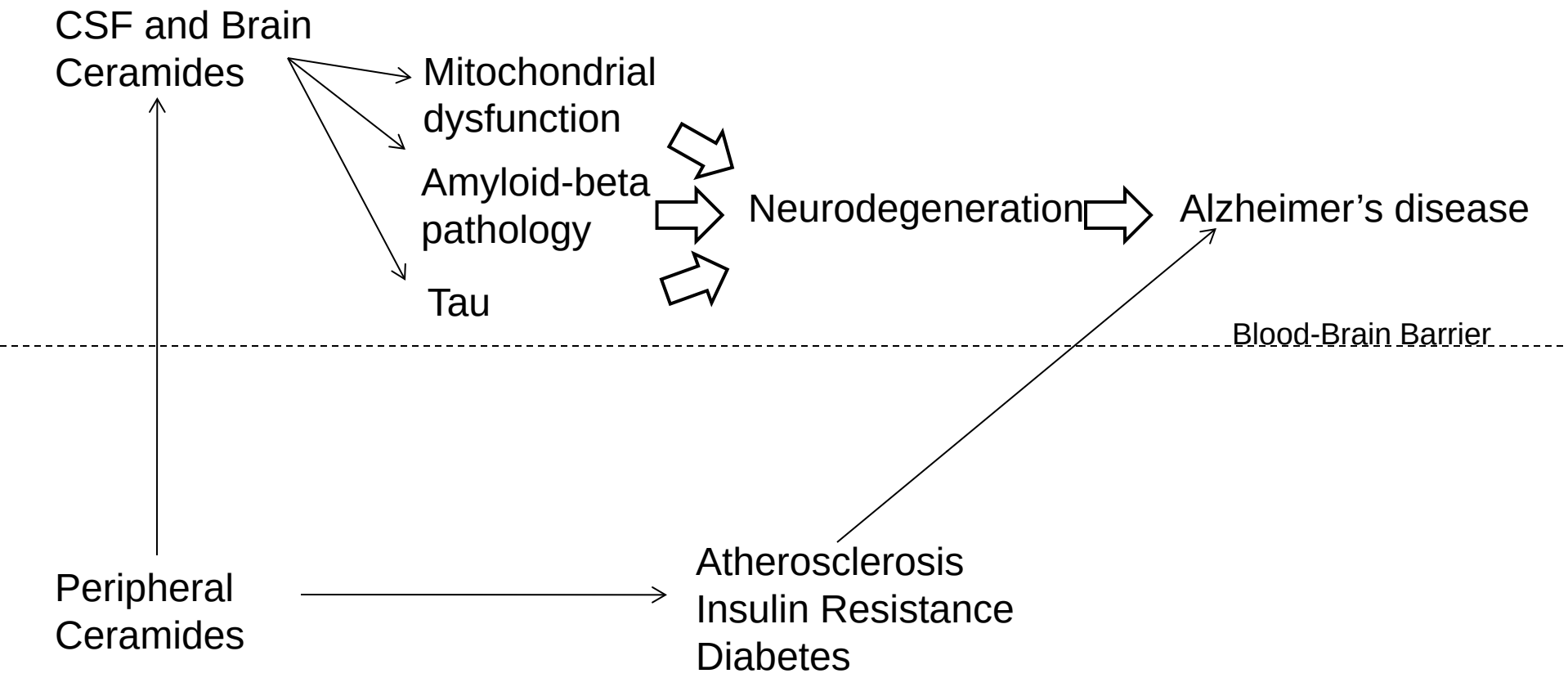
- ~2,400 plasma samples w/corresponding imaging (PiB, FDG-PET, MRI) and/or CSF
- ~1,000 CSF

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?

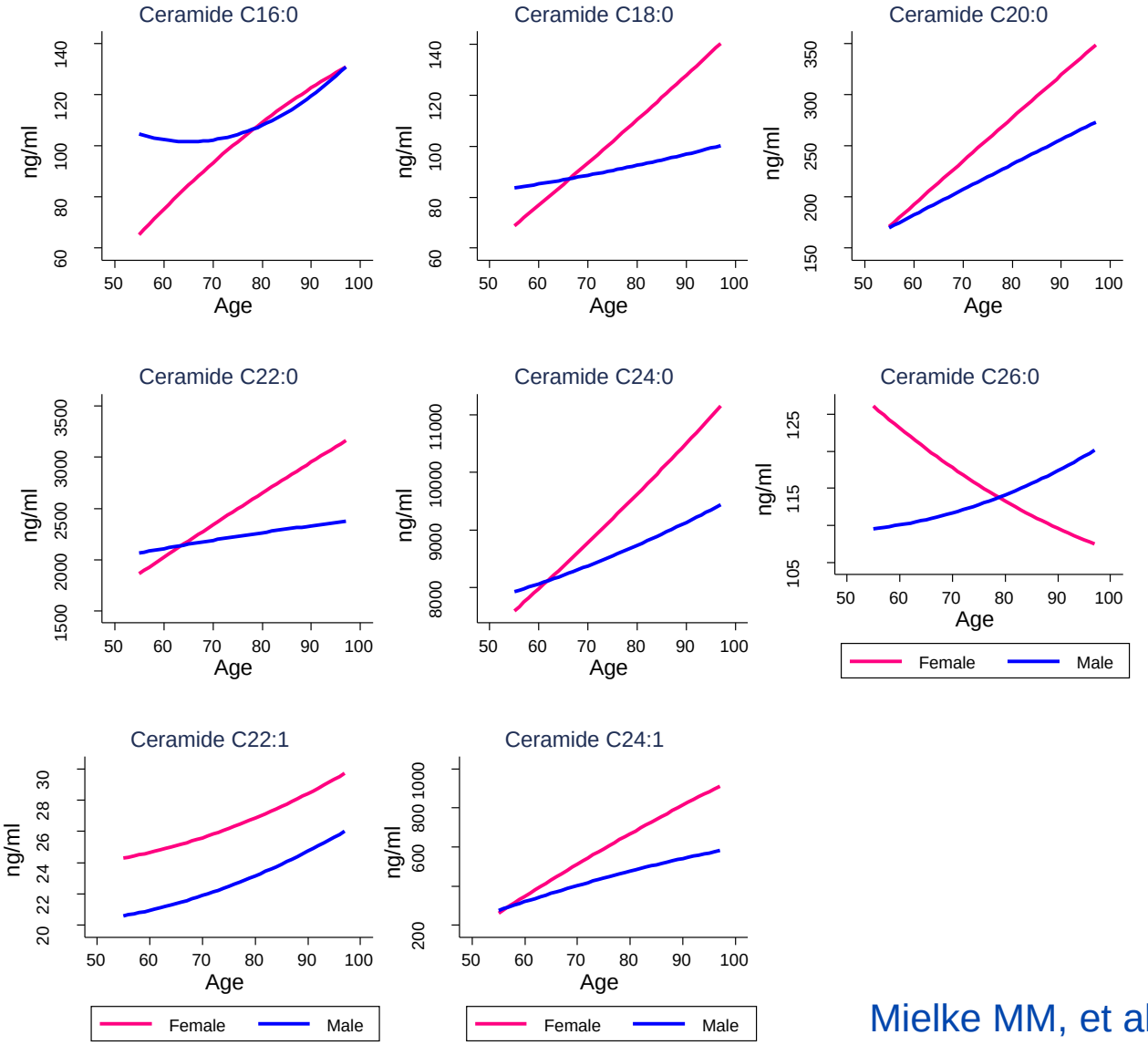


Mielke & Lyketsos, 2010

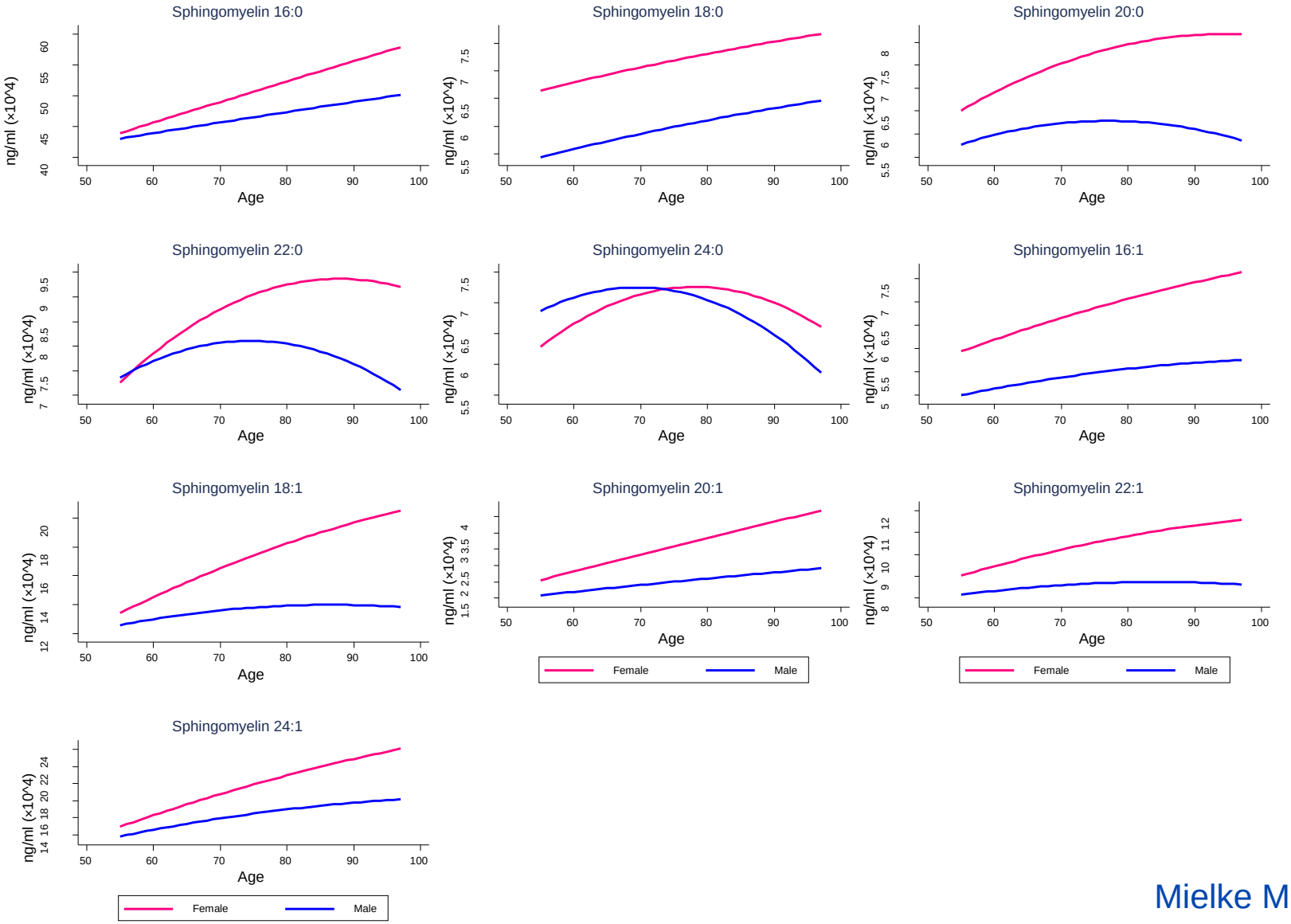
Sphingolipid Epidemiology

Baltimore Longitudinal Study of Aging (BLSA)

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



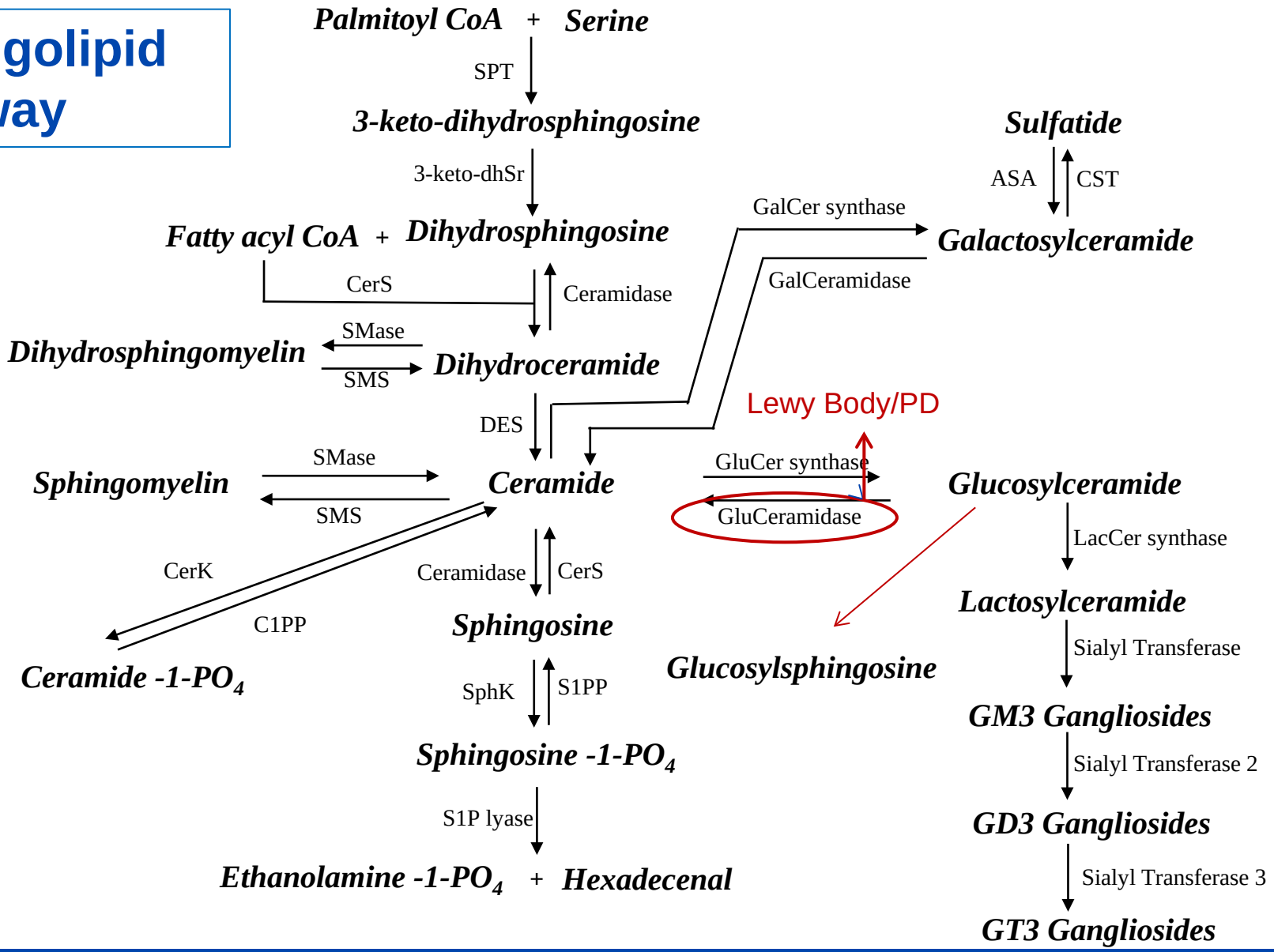
Mielke MM, et al., 2015b

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



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)

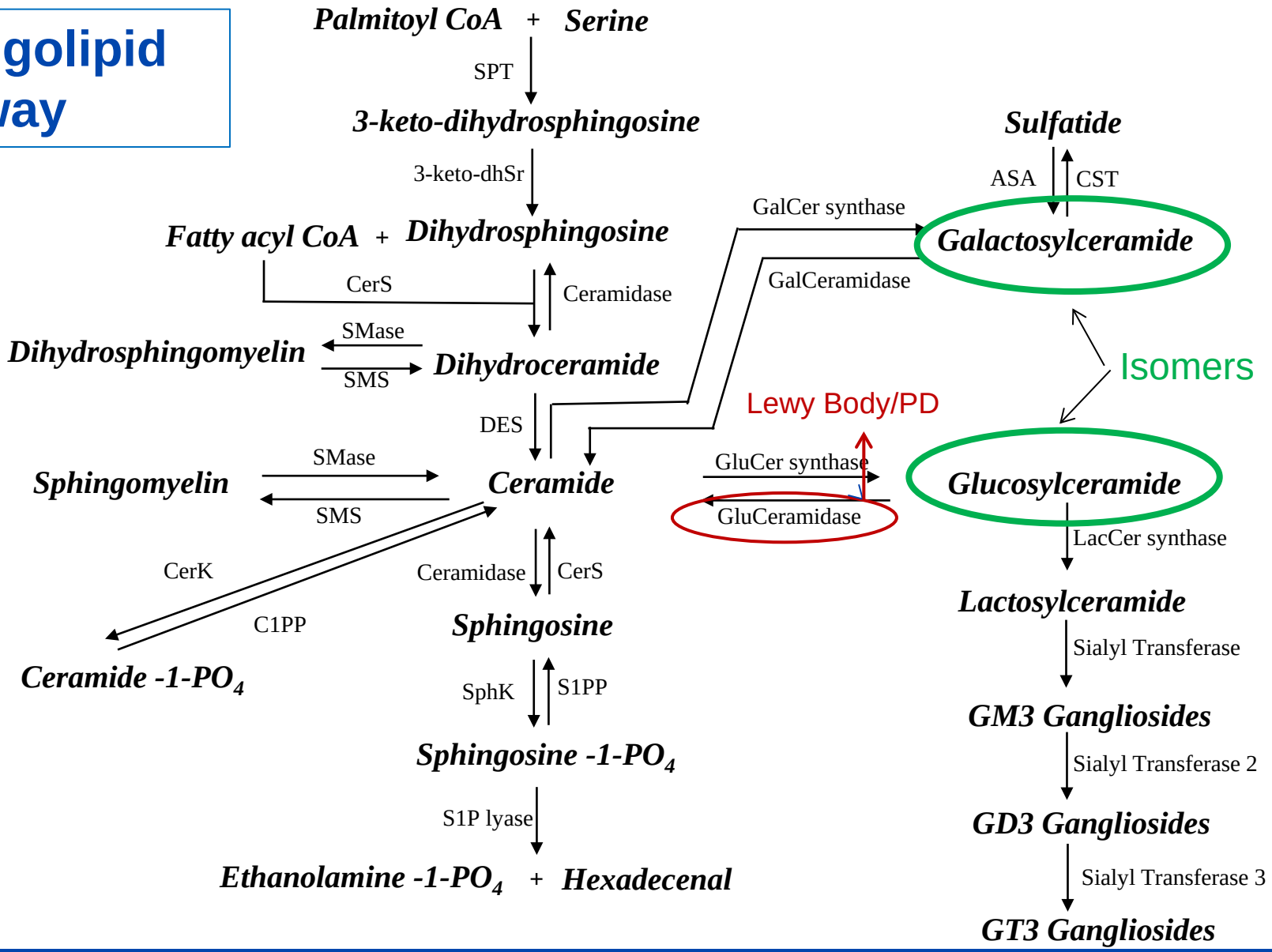
PD and LBD, cont.

- 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)

Plasma Log Lipid	**PD-NC (N=26)** *Median (range)*	**PD-MCI/PDD (N=26)** *Median (range)*	*p-value*
Ceramide			
C16:0	**11.48 (10.93, 12.26)**	**11.67 (11.27, 12.79)**	**0.035**
C18:0	**10.98 (10.24, 12.24)**	**11.18 (10.44, 12.54)**	**0.016**
C20:0	**12.21 (11.32, 13.58)**	**12.54 (11.80, 14.10)**	**0.037**
C22:0	**13.63 (12.83, 14.77)**	**13.94 (12.67, 16.14)**	**0.037**
C24:0	15.73 (14.37, 16.85)	16.01 (14.17, 18.00)	0.621
C26:0	11.94 (9.68, 12.84)	12.15 (9.70, 14.07)	0.510
C22:1	9.86 (8.83, 10.60)	9.95 (9.43, 11.92)	0.442
C24:1	**13.23 (12.34, 14.83)**	**13.44 (12.67, 15.32)**	**0.048**
C26:1	9.70 (8.46, 11.05)	9.90 (8.87, 11.77)	0.380
Monohexosylceramides (Glucosyl- & Galactosylceramides)*			
C16:0	**11.92 (11.03, 12.79)**	**12.09 (11.35, 14.29)**	**0.046**
C18:0	9.11 (8.40, 10.15)	9.15 (8.33, 10.80)	0.242
C20:0	**10.48 (9.79, 11.31)**	**10.72 (10.07, 12.11)**	**0.039**
C22:0	13.75 (12.97, 14.61)	13.95 (13.18, 15.52)	0.148
C24:0	**14.17 (13.15, 15.49)**	**14.56 (13.26, 16.31)**	**0.040**
C26:0	10.20 (8.82, 11.16)	10.23 (8.17, 12.28)	0.840
C16:1	9.50 (8.67, 9.99)	9.58 (9,12, 11.72)	0.089
C22:1	9.94 (9.02, 10.59)	10.00 (9.00, 11.88)	0.370
C24:1	10.02 (8.48, 11.06)	10.09 (9.26, 12.48)	0.272

Sphingolipid Pathway



Ceramides, Glucosylceramides and PD

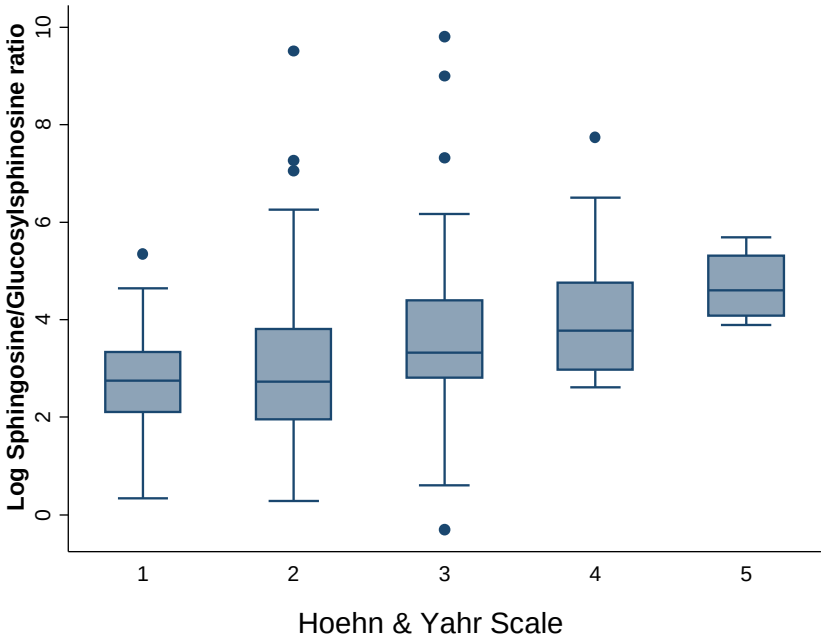
- Aims:
  - To replicate the previous cross-sectional study
  - To determine whether the lipids predict cognitive decline
  - To further explore GBA mutations and cognitive impairment in PD

Baseline Characteristics (N=412)

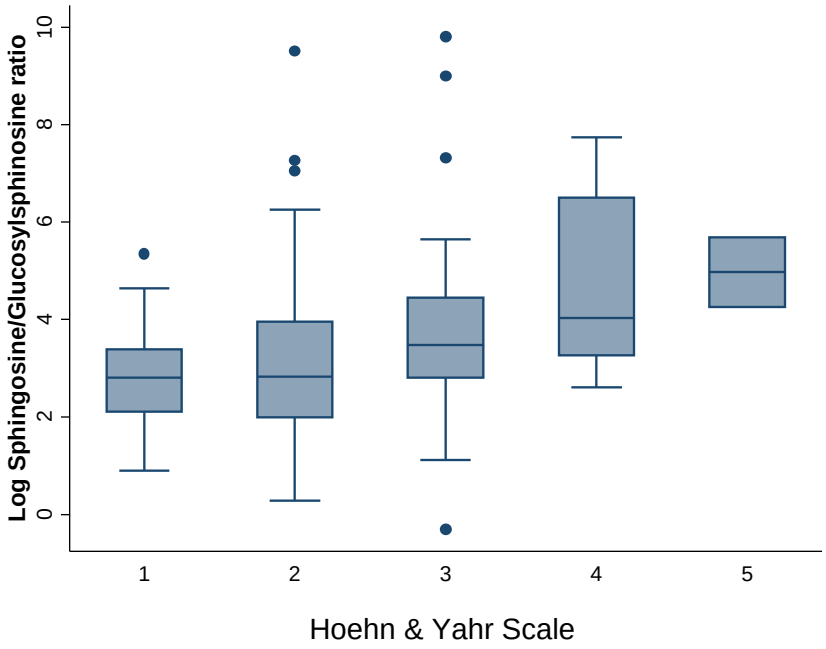
		PD-NC (N=272)		PD-MCI (N=85)		PDD (N=55)	
Characteristics	N	mean (SD)/N(%)	N	mean (SD)/N(%)	N	mean (SD)/N(%)	p-value
Baseline age	272	66.2 (8.2)	85	67.8 (7.0)	55	72.4 (4.9)	<0.001
Disease duration, years	262	5.9 (4.9)	84	7.6 (5.7)	55	9.3 (6.2)	<0.001
Male	272	188 (69.1%)	85	55 (64.7%)	55	38 (69.1%)	0.750
Education: University	268	188 (70.2%)	84	59 (70.2%)	55	41 (74.6%)	0.802
BMI	269	26.7 (4.2)	84	26.7 (4.1)	53	26.5 (4.9)	0.895
UPDRS I	270	1.7 (1.7)	85	2.4 (1.7)	55	5.2 (2.9)	<0.001
UPDRS III	264	20.8 (11.0)	84	24.9 (11.3)	53	30.6 (12.3)	<0.001
GDS	241	3.0 (2.6)	76	2.9 (2.7)	47	6.4 (3.4)	<0.001
AES	244	28.6 (7.9)	77	29.3 (2.7)	44	39.5 (10.6)	<0.001
PANDA	271	21.0 (5.2)	85	16.0 (5.1)	55	9.3 (4.7)	<0.001
PDQ-39	244	22.0 (13.6)	76	24.9 (12.5)	47	39.3 (14.8)	<0.001
MMSE	272	28.6 (1.5)	85	27.9 (1.9)	55	24.4 (3.1)	<0.001
Any GBA mutation	268	41 (15.3%)	84	19 (22.6%)	54	20 (37.0%)	0.001
Pathogenic GBA mutation	268	6 (2.2%)	84	2 (2.4%)	54	6 (11.1%)	0.011

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



A higher ceramide to glc-Cer ratio is associated with greater cognitive decline among cognitively normal patients with Parkinson's disease

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

*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

	DLB vs. control (N=40/group)	
lipid	*mean*	*p-value*
s1p	-0.018	0.557
cer C14:0	-0.0001	0.916
cer C16:0	**0.056**	**0.029**
cer C18:1	0.001	0.476
cer C18:0	0.016	0.265
cer C20:0	0.014	0.313
cer C22:0	0.040	0.142
cer C24:1	**0.135**	**0.046**
cer C24:0	0.047	0.590

	iRBD vs. control (n=17/group)	
lipid	*mean*	*p-value*
s1p	-0.022	0.672
cer C14:0	-0.002	0.178
cer C16:0	0.039	0.183
cer C18:1	**0.002**	**0.081**
cer C18:0	**0.025**	**0.070**
cer C20:0	0.005	0.727
cer C22:0	0.033	0.440
cer C24:1	0.114	0.221
cer C24:0	0.039	0.799

Higher plasma ceramides associated with lower grey matter mesopontine volume among iRBD

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

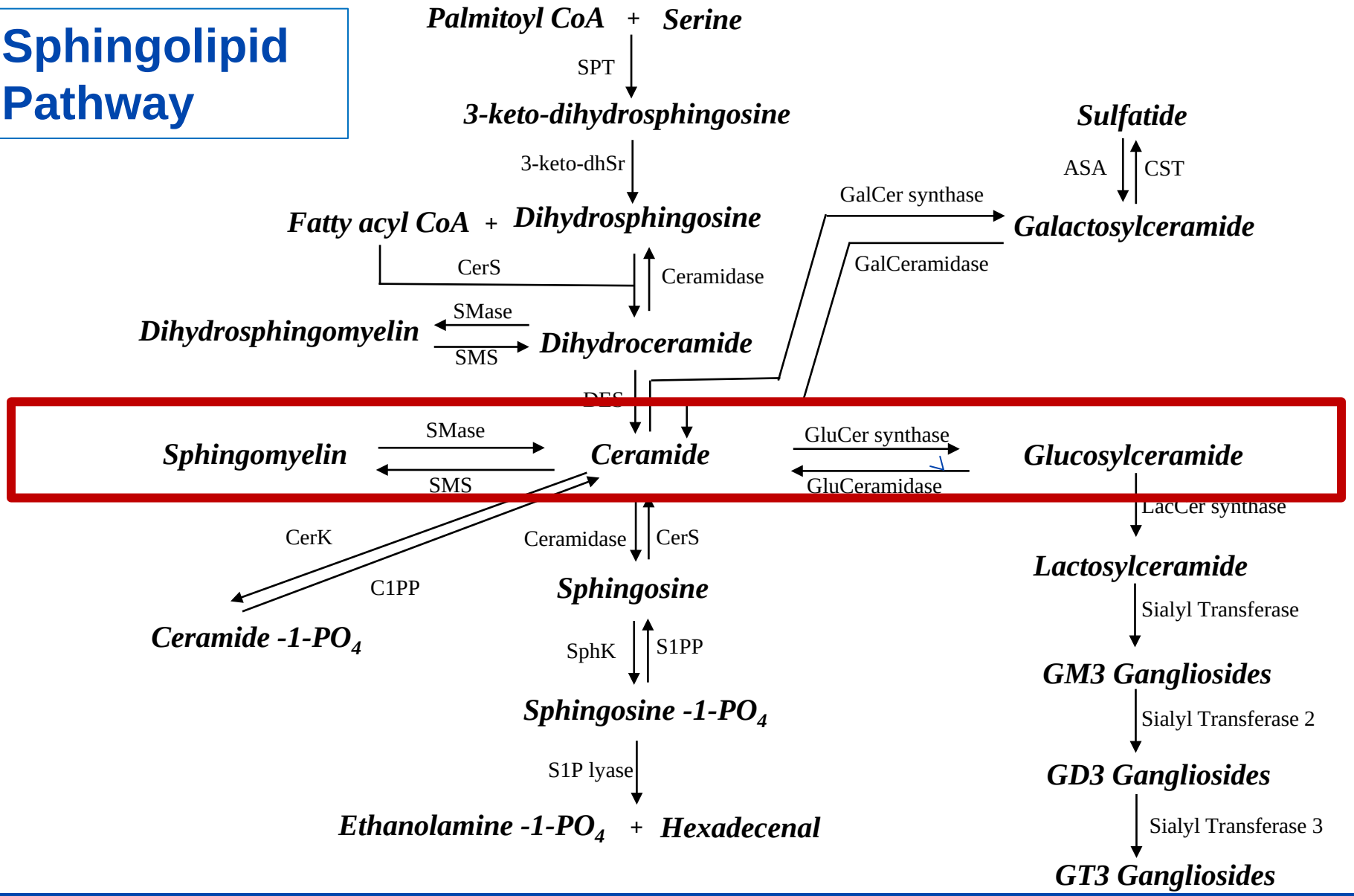
PD Ongoing Research

- 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



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- Cynthia Carlsson, MD

Baylor

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Questions & Discussion