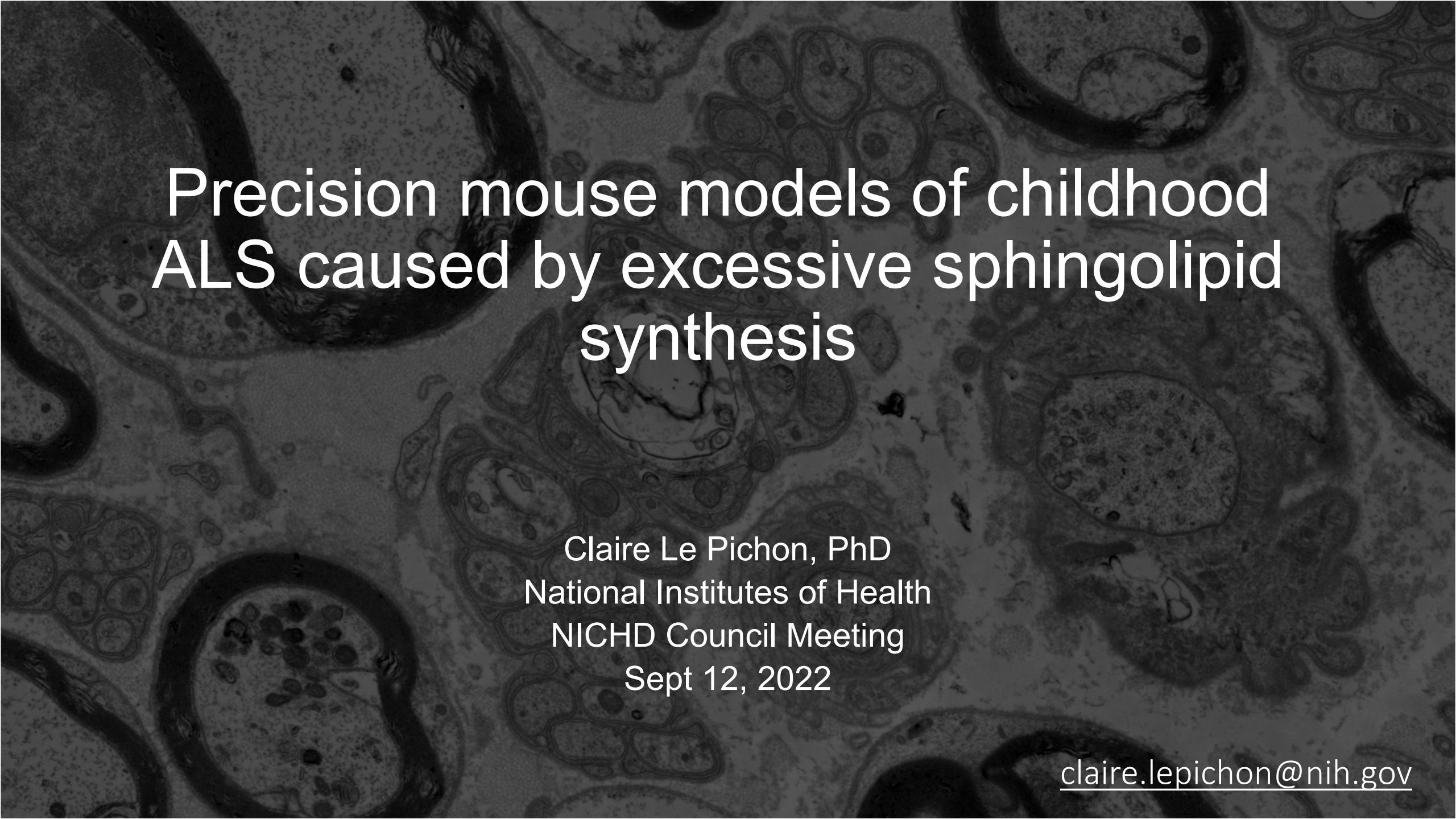
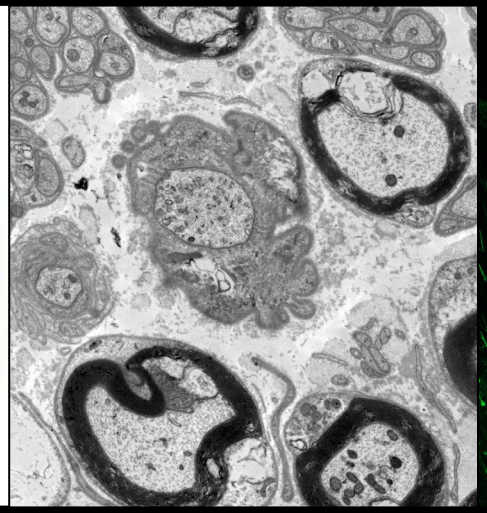


Precision mouse models of childhood ALS caused by excessive sphingolipid synthesis

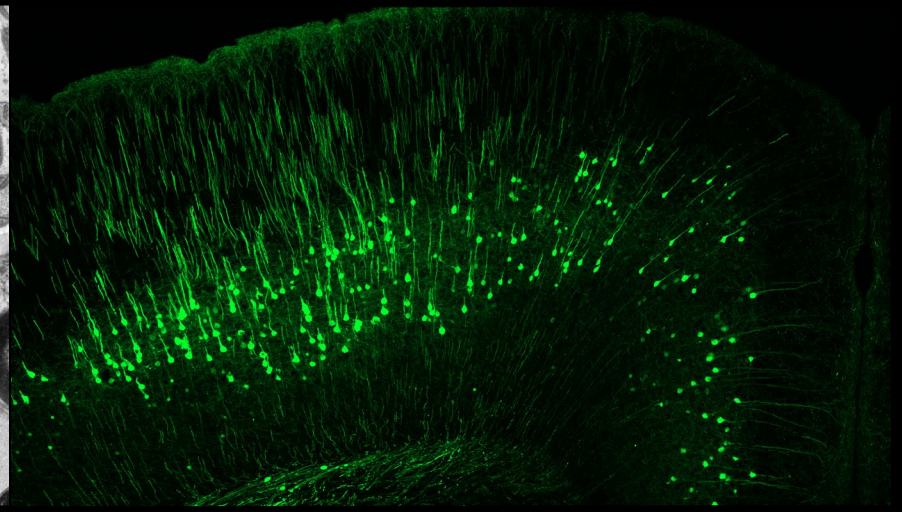
Claire Le Pichon, PhD
National Institutes of Health
NICHD Council Meeting
Sept 12, 2022

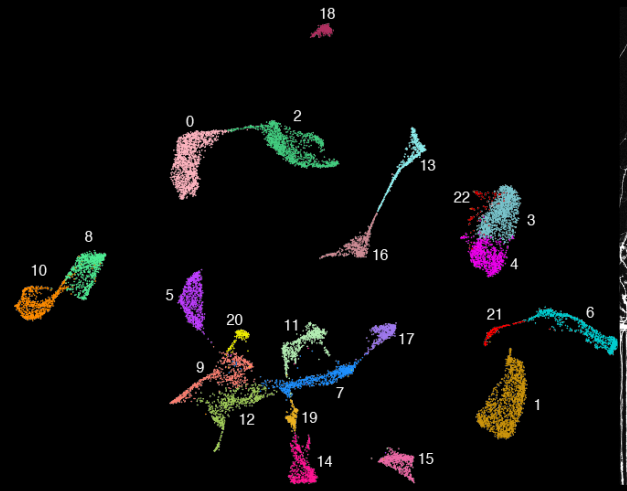
claire.lepichon@nih.gov

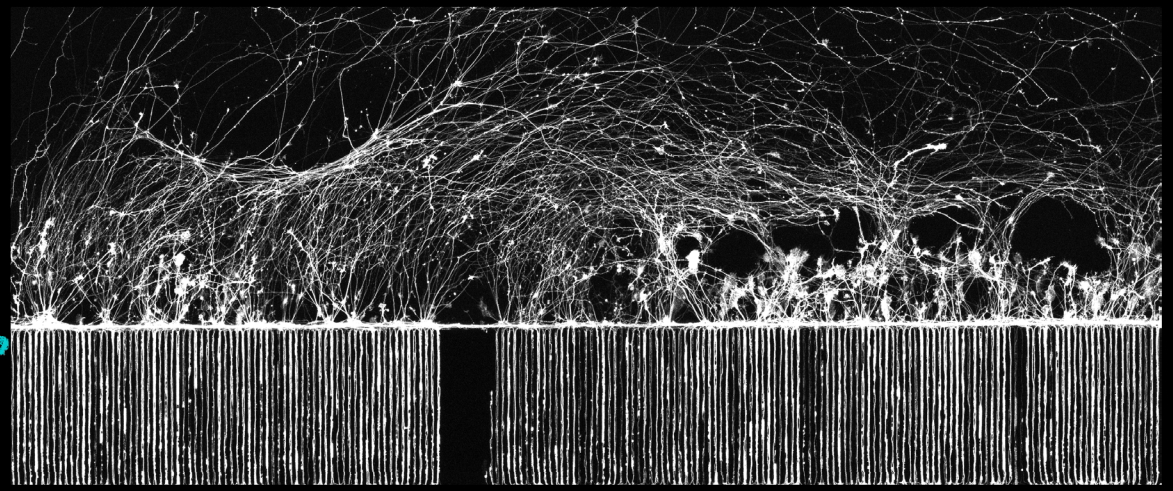
Precision mouse models of disease



Traumatic injury



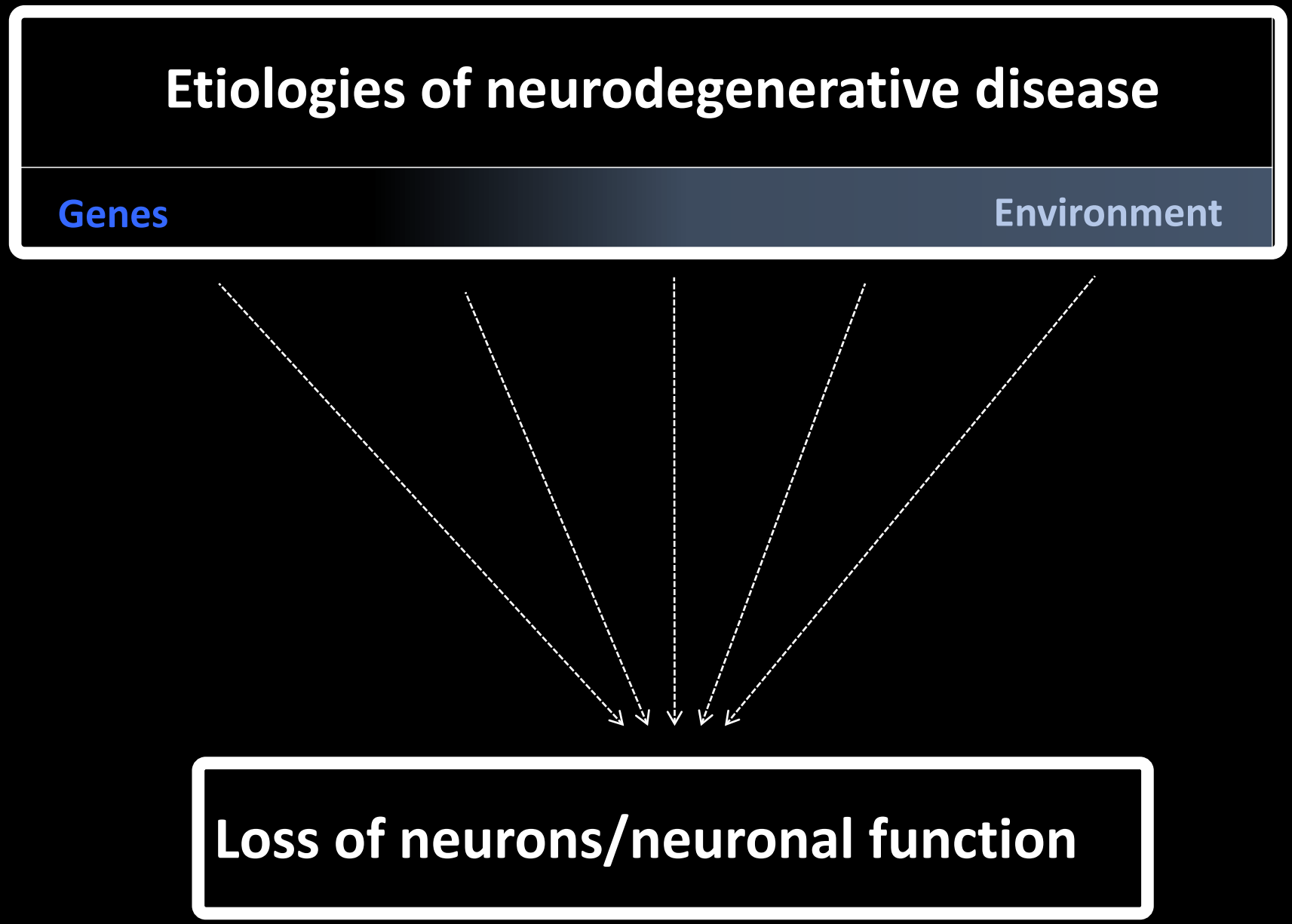




Cell type specificity

Axon injury responses in human neurons

Causes of neurodegeneration are still poorly understood



Amyotrophic lateral sclerosis

Placeholder for background on ALS

Mutations in SPTLC1 cause juvenile ALS

(serine palmitoyltransferase 1)

nature medicine

ARTICLES

https://doi.org/10.1038/s41591-021-01346-1

Check for updates

Childhood amyotrophic lateral sclerosis caused by excess sphingolipid synthesis

Payam Mohassel[1], Sandra Donkervoort[1,26], Museer A. Lone[2,26], Matthew Nalls[1,26], Kenneth Gable[3,26], Sita D. Gupta[3,26], A. Reghan Foley[1], Ying Hu[1], Jonas Alex Morales Saute[4], Ana Lucila Moreira[5], Fernando Kok[6], Alessandro Introna[7], Giancarlo Logroscino[7,8], Christopher Grunseich[9], Alec R. Nickolls[1], Naemeh Pourshafie[9], Sarah B. Neuhaus[1], Dimah Saade[1], Andrea Gangfuß[10], Heike Kölbel[10], Zoe Piccus[11], Claire E. Le Pichon[11], Chiara Fiorillo[12], Cindy V. Ly[13], Ana Töpf[14], Lauren Brady[15], Sabine Specht[14], Aliza Zidell[16], Helio Pedro[17], Eric Mittelmann[18], Florian P. Thomas[18], Katherine R. Chao[19], Chamindra G. Konersman[20], Megan T. Cho[21], Tracy Brandt[21], Volker Straub[14], Anne M. Connolly[22], Ulrike Schara[10], Andreas Roos[10], Mark Tarnopolsky[15], Ahmet Höke[23], Robert H. Brown[24], Chia-Hsueh Lee[25], Thorsten Hornemann[2], Teresa M. Dunn[3]✉ and Carsten G. Bönnemann[1]✉

NATURE MEDICINE | VOL 27 | JULY 2021 | 1197–1204 |

Research

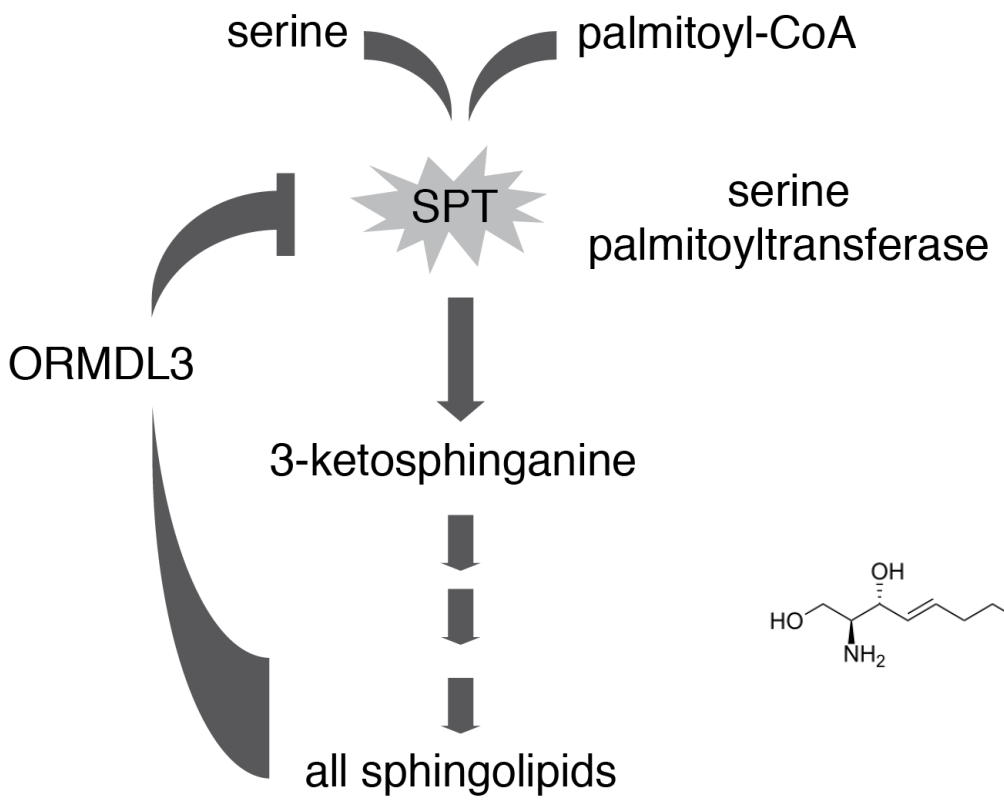
JAMA Neurology | Original Investigation

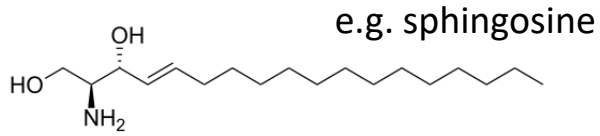
Association of Variants in the *SPTLC1* Gene With Juvenile Amyotrophic Lateral Sclerosis

Janel O. Johnson, PhD; Ruth Chia, PhD; Danny E. Miller, MD, PhD; Rachel Li, MD; Ravindran Kumaran, PhD; Yevgeniya Abramzon, BSc; Nada Alahmady, PhD; Alan E. Renton, PhD; Simon D. Topp, PhD; J. Raphael Gibbs, PhD; Mark R. Cookson, PhD; Marya S. Sabir, BSc; Clifton L. Dalgard, PhD; Claire Troakes, PhD; Ashley R. Jones, PhD; Aleksey Shatunov, PhD; Alfredo Iacoangeli, PhD; Ahmad Al Khleifat, PhD; Nicola Ticozzi, MD, PhD; Vincenzo Silani, MD; Cinzia Gellera, PhD; Ian P. Blair, PhD; Carol Dobson-Stone, PhD; John B. Kwok, PhD; Emily S. Bonkowski, ScM; Robin Palvadeau, MSc; Pentti J. Tienari, MD; Karen E. Morrison, MD; Pamela J. Shaw, MD; Ammar Al-Chalabi, PhD; Robert H. Brown Jr, MD, PhD; Andrea Calvo, PhD; Gabriele Mora, PhD; Hind Al-Saif, MD; Marc Gotkine, MBBS; Fawn Leigh, MD; Irene J. Chang, MD; Seth J. Perlman, MD; Ian Glass, MB ChB, MD; Anna I. Scott, PhD; Christopher E. Shaw, MD; A. Nazli Basak, PhD; John E. Landers, PhD; Adriano Chiò, PhD; Thomas O. Crawford, PhD; Bradley N. Smith, PhD; Bryan J. Traynor, MD, PhD; and the FALS Sequencing Consortium; American Genome Center; International ALS Genomics Consortium; and ITALSGEN Consortium

JAMA Neurol. 2021;78(10):1236-1248. doi:10.1001/jamaneurol.2021.2598
Published online August 30, 2021.

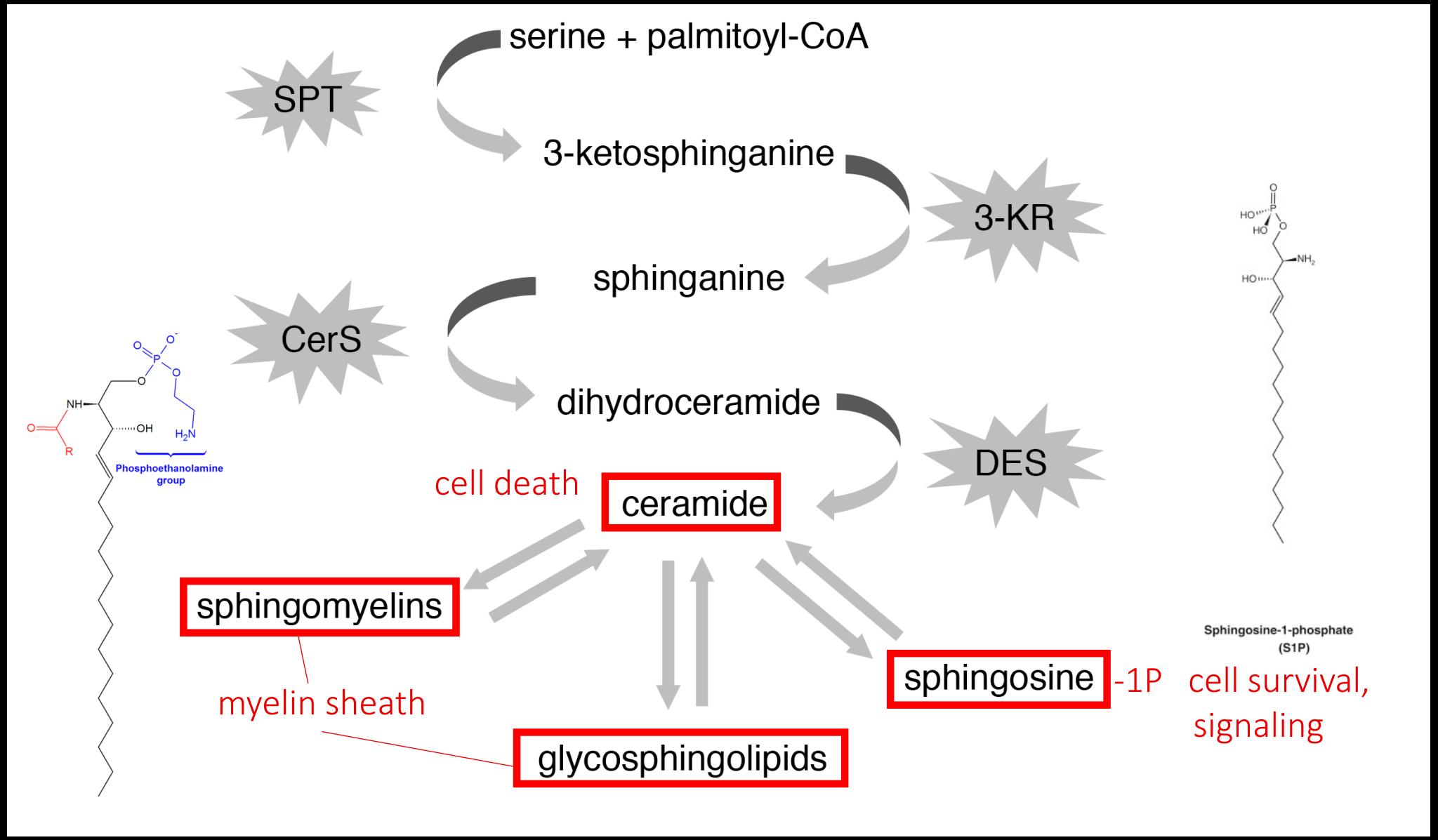
All sphingolipid biosynthesis is initiated by SPT





Essential lipids of membranes in mammalian cells
Wide spectrum of functions

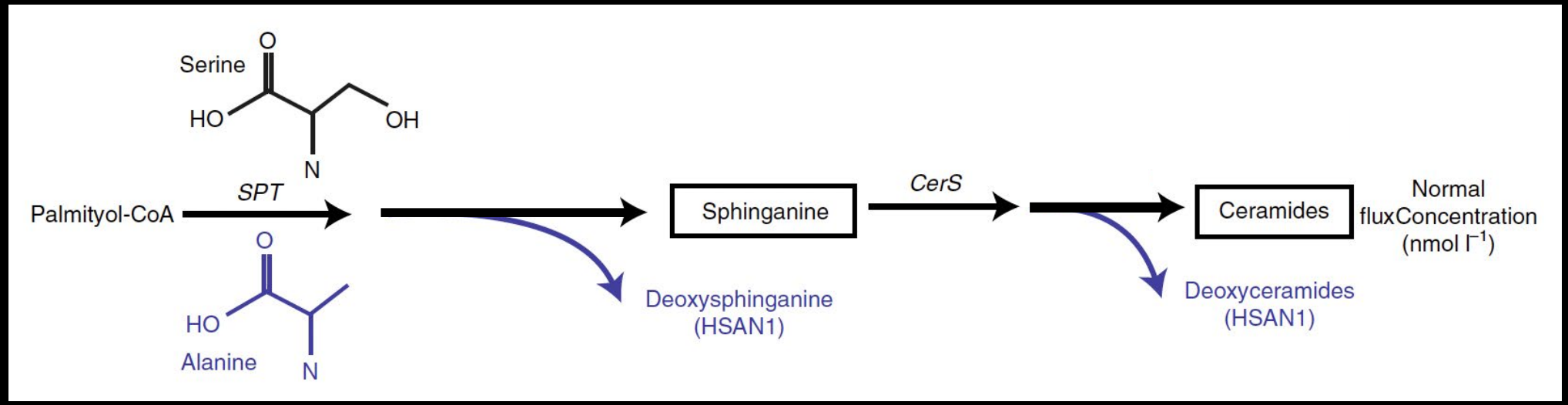
Sphingolipid biosynthesis pathway



Location of disease-causing mutations in SPTLC1

- Previously known SPTLC1 mutations cause HSAN1
  (Hereditary Sensory and Autonomic Neuropathy type 1)

Metabolomic alterations resulting from HSAN1 mutations in SPTLC1



Penno et al 2010
Rotthier et al 2011
Mohassel et al 2021

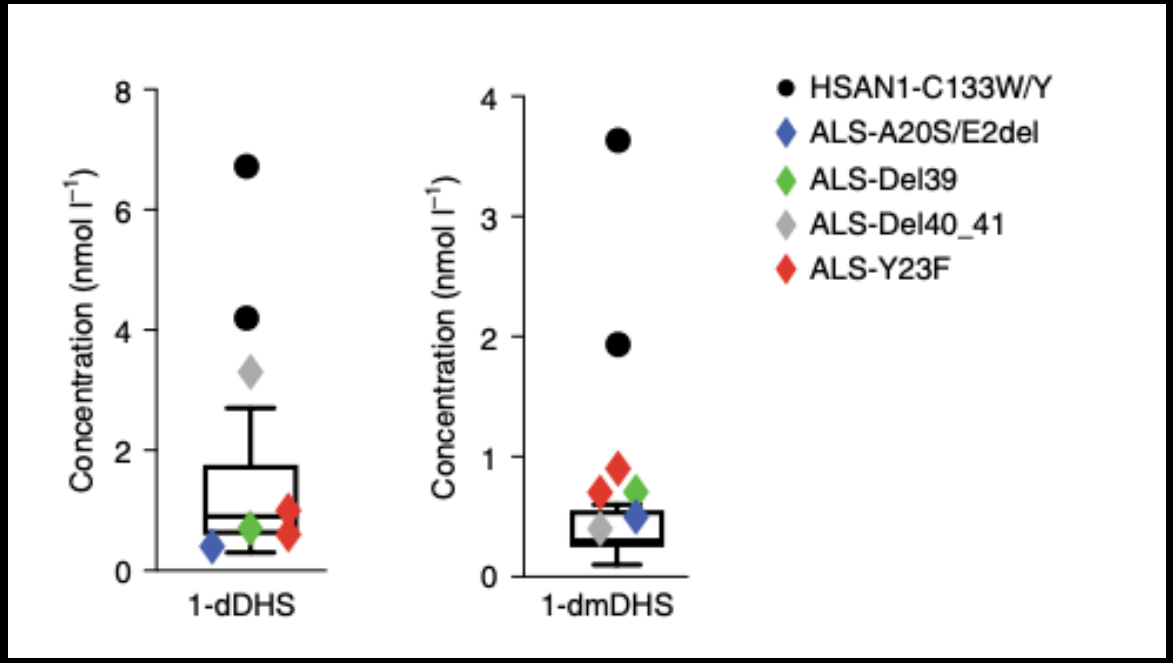
Location of disease-causing mutations in SPTLC1

- Previously known SPTLC1 mutations cause HSAN1

Location of disease-causing mutations in SPTLC1

- Previously known SPTLC1 mutations cause HSAN1
- The childhood ALS-linked mutations cluster in a different region: transmembrane domain 1 (TMD1)

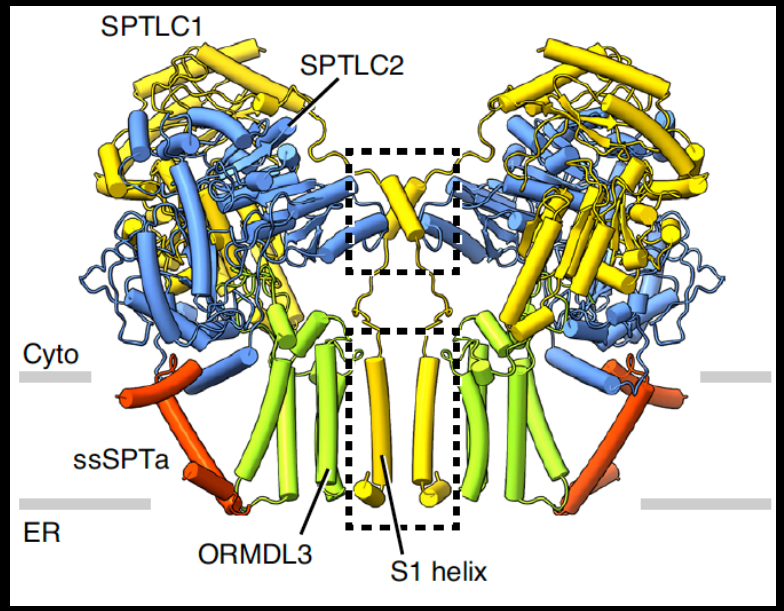
Metabolomic alterations resulting from ALS vs HSAN1 mutations in SPTLC1



Sphingolipidomic analysis of patient serum

Mohassel et al., Nat Med 2021

Location of disease-causing mutations in SPTLC1



A20S
Y23F
Δ39
Δ40-41

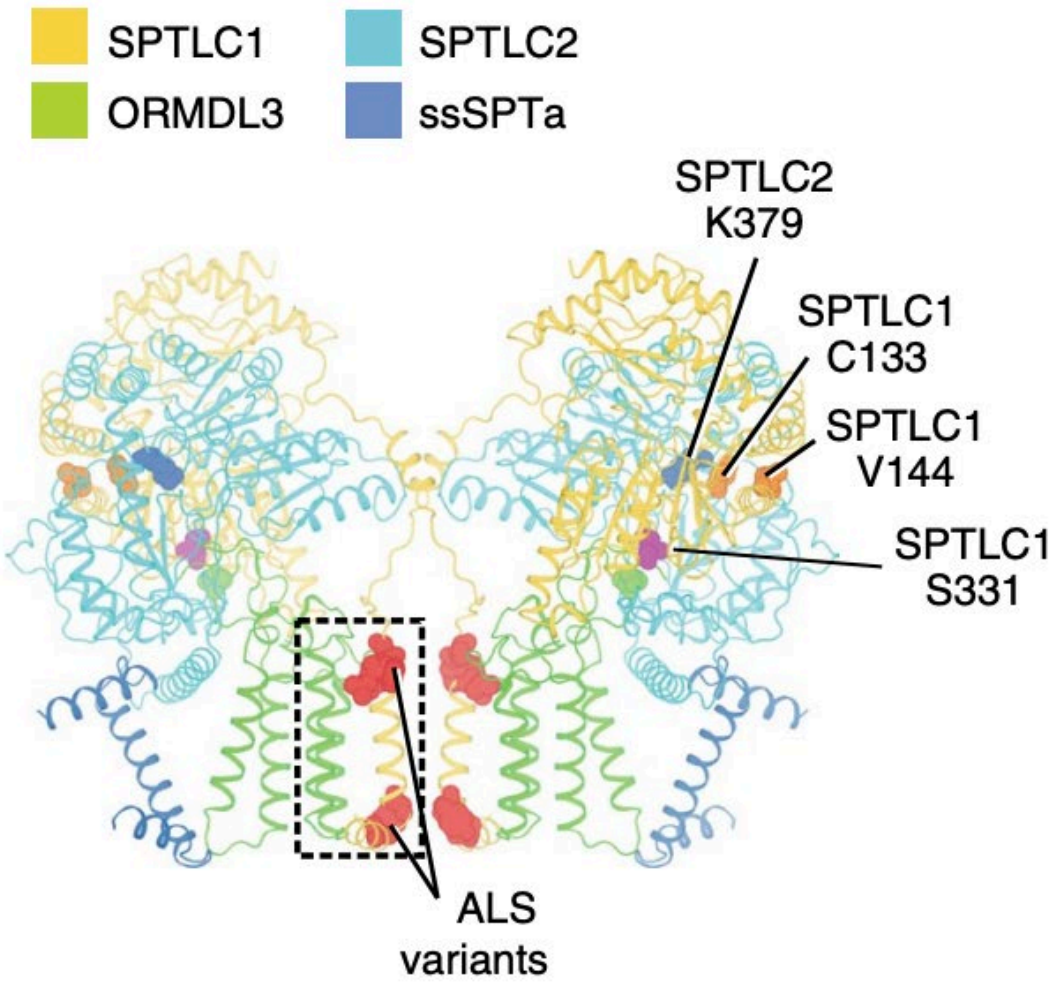
C133Y
C133W
V144D

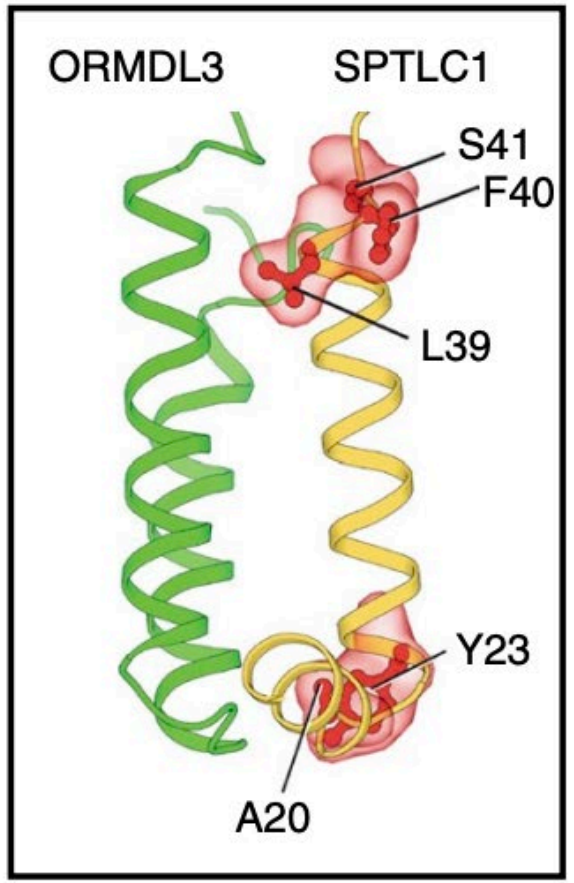
S331Y
S331F
A352V

HSAN1
Childhood ALS

TMD1 | Catalytic domain | 473 aa

Location of ALS mutations within SPTLC1 structure

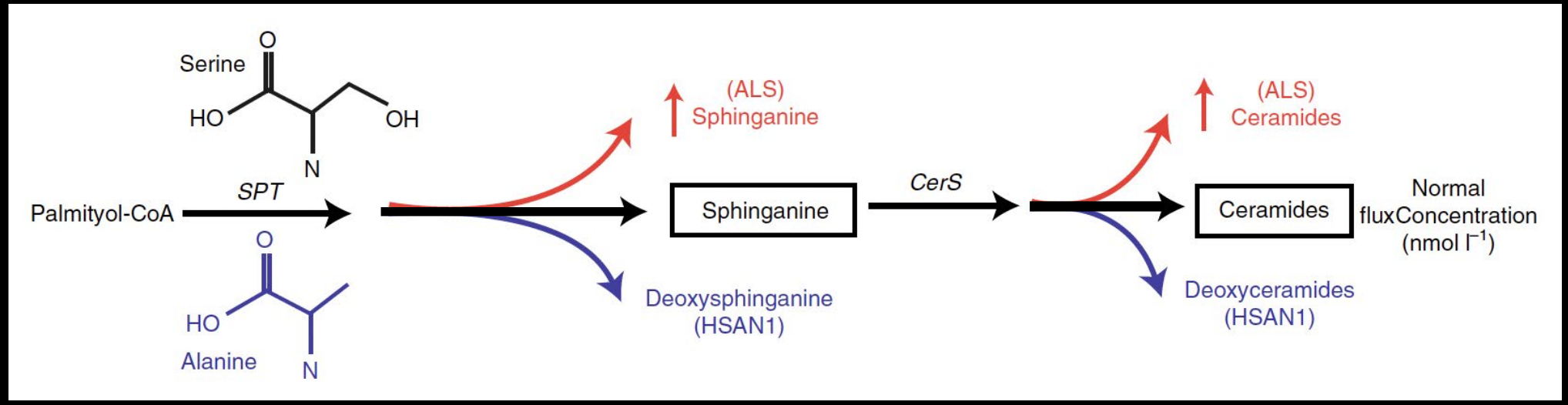




Wang et al., Nat Struc & Mol Bio 2021

Mohassel et al., Nat Med 2021

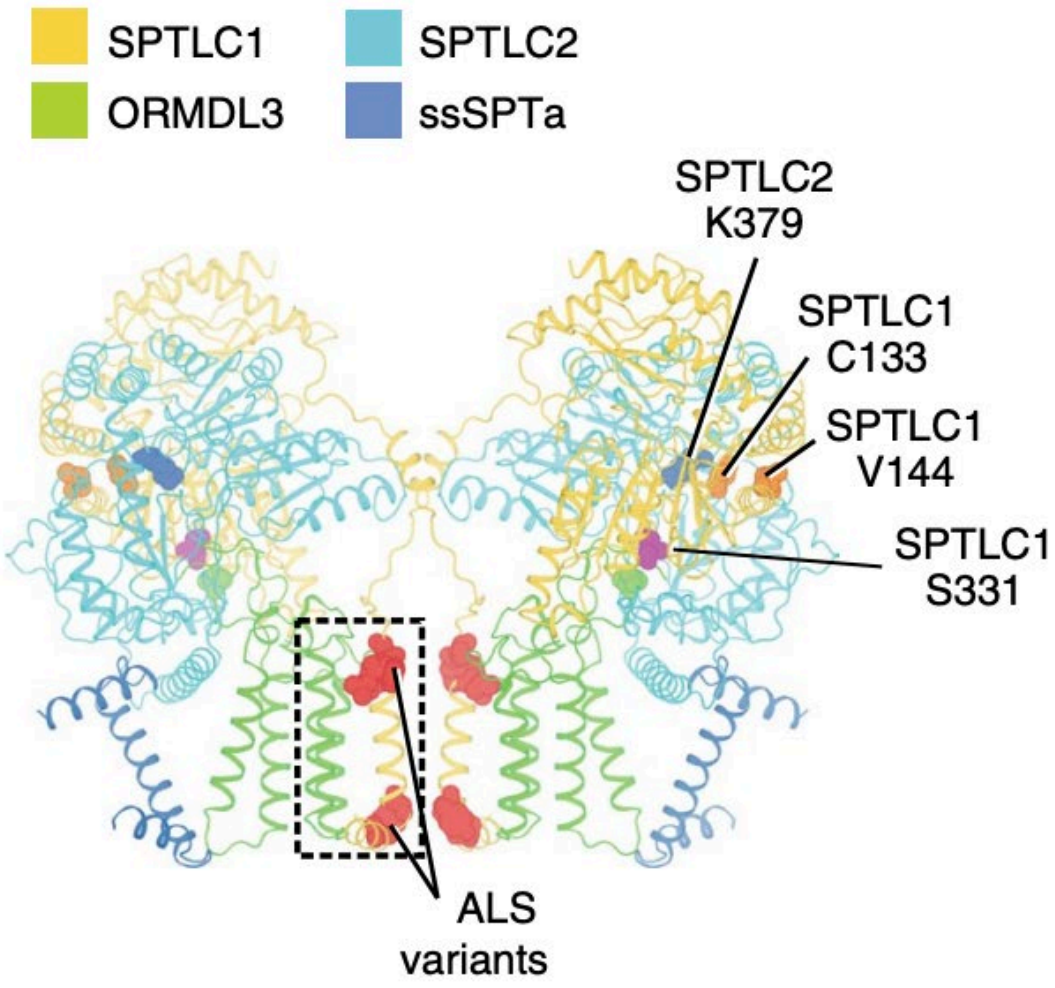
Metabolomic alterations resulting from ALS vs HSAN1 mutations in SPTLC1

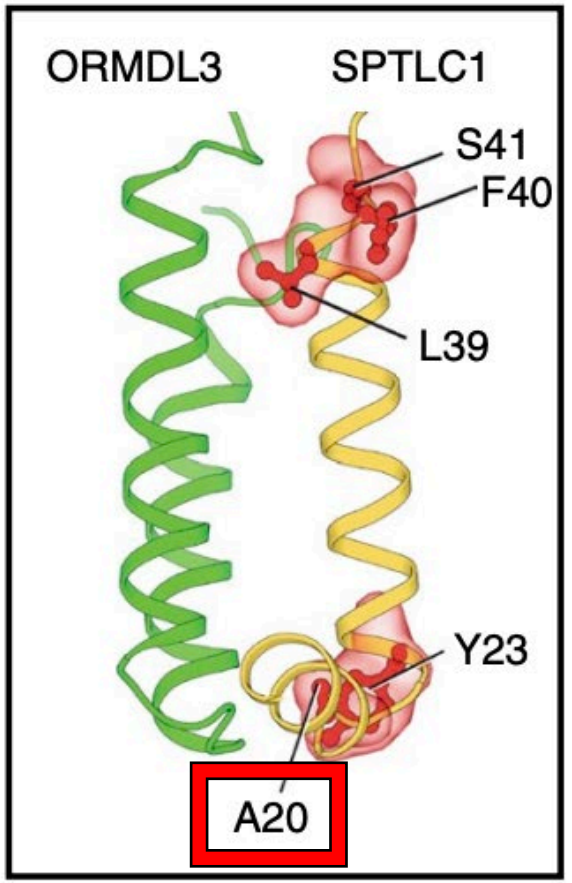


Hypotheses - mice engineered with these ALS mutations will exhibit:

1. elevated sphingolipid levels
2. ALS-like neurodegeneration

SPT-ORMDL3 interaction occurs at TMD1





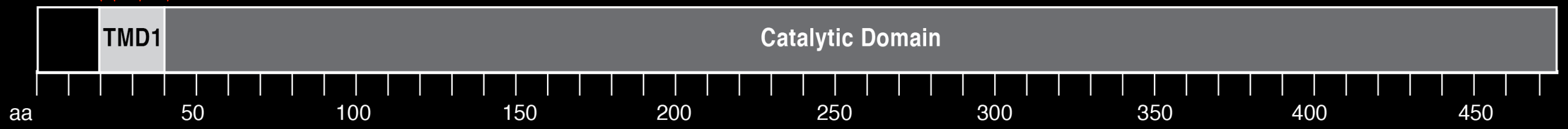
Wang et al., Nat Struc & Mol Bio 2021

Mohassel et al., Nat Med 2021

High conservation of TMD1 (encoded by exon 2)
Gene editing approach in mouse to knock in the mutation

AA number				20			23													38	39	40	41				
Homo sapiens	L	Y	E	**A**	P	A	**Y**	H	L	I	L	E	G	I	L	I	L	W	I	I	R	**L**	**L**	**F**	**S**	K	T	Y	K
Pan troglodytes	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Macaca mulatta	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Felis catus	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Mus musculus	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	V	F	S	K	T	Y	K
Gallus gallus	F	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	I	F	S	K	T	Y	K
Takifugu rubripes	F	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	F	R	L	L	F	S	K	T	Y	K
Danio rerio	F	Y	E	A	P	A	Y	H	L	I	L	E	G	F	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Xenopus tropicalis	F	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	I	F	S	K	T	Y	K

A20S
Y23F
Δ39
Δ40-41

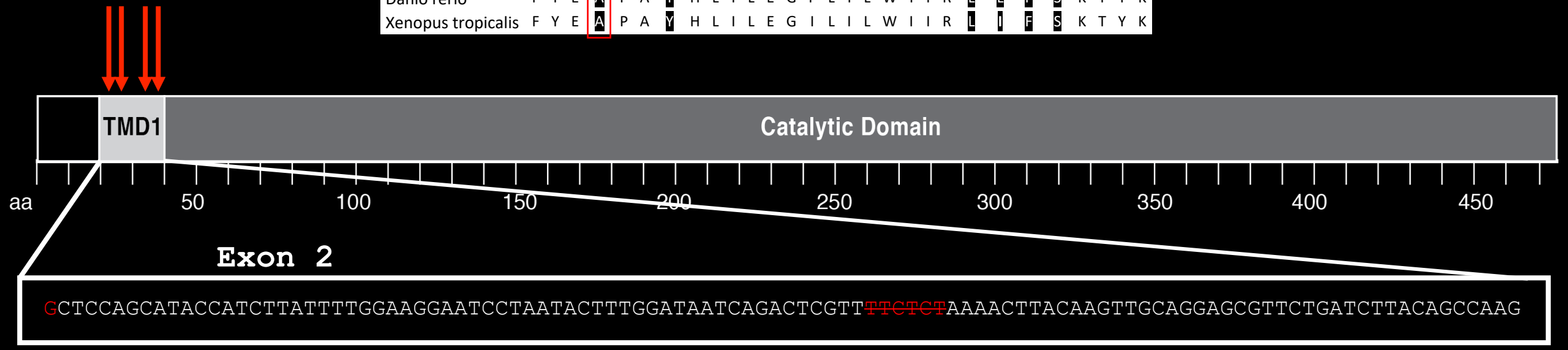


Zoe Piccus et al., unpublished

High conservation of TMD1 (encoded by exon 2)
Gene editing approach

AA number				20			23													38	39	40	41				
Homo sapiens	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Pan troglodytes	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Macaca mulatta	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Felis catus	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Mus musculus	L	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	V	F	S	K	T	Y	K
Gallus gallus	F	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	I	F	S	K	T	Y	K
Takifugu rubripes	F	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	F	R	L	L	F	S	K	T	Y	K
Danio rerio	F	Y	E	A	P	A	Y	H	L	I	L	E	G	F	L	I	L	W	I	I	R	L	L	F	S	K	T	Y	K
Xenopus tropicalis	F	Y	E	A	P	A	Y	H	L	I	L	E	G	I	L	I	L	W	I	I	R	L	I	F	S	K	T	Y	K

A20S



G->T
A20S

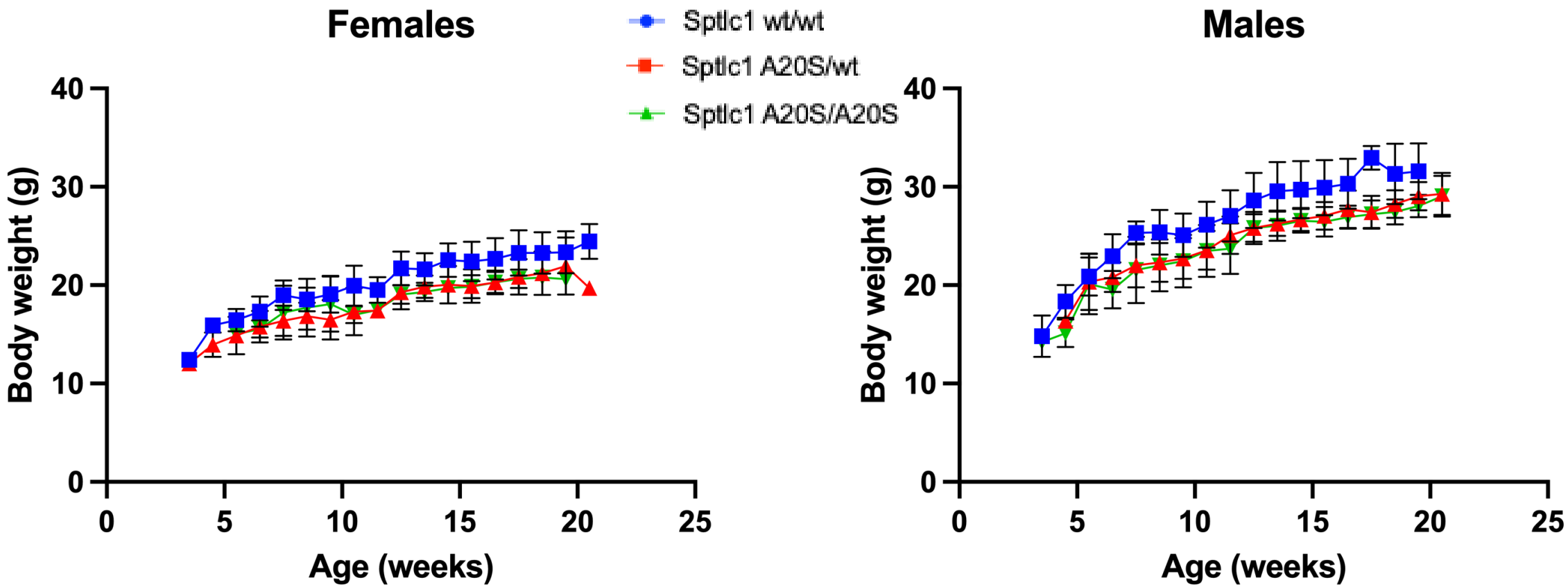
Zoe Piccus et al., unpublished

Characterization of Sptlc1^{A20S} knock-in mouse line

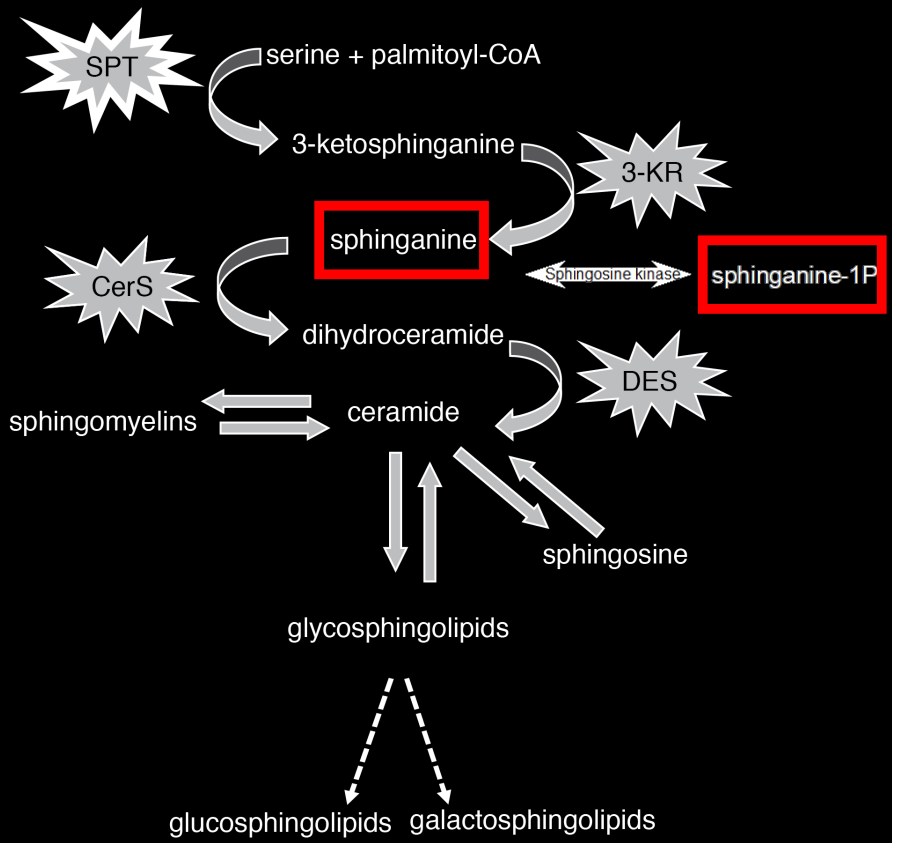
A20S	**del 40-41**
Normal lifespan	Homozygotes runted and die early (5-6 weeks old)
Mild motor symptoms	Tremors, muscle atrophy
Progressive motor neuron, nerve and muscle pathology (but no obvious motor neuron cell body loss)	Misdevelopment and neurodegeneration (more similar to a juvenile syndrome)
Homozygotes exhibit more severe pathology	Histological characterization ongoing

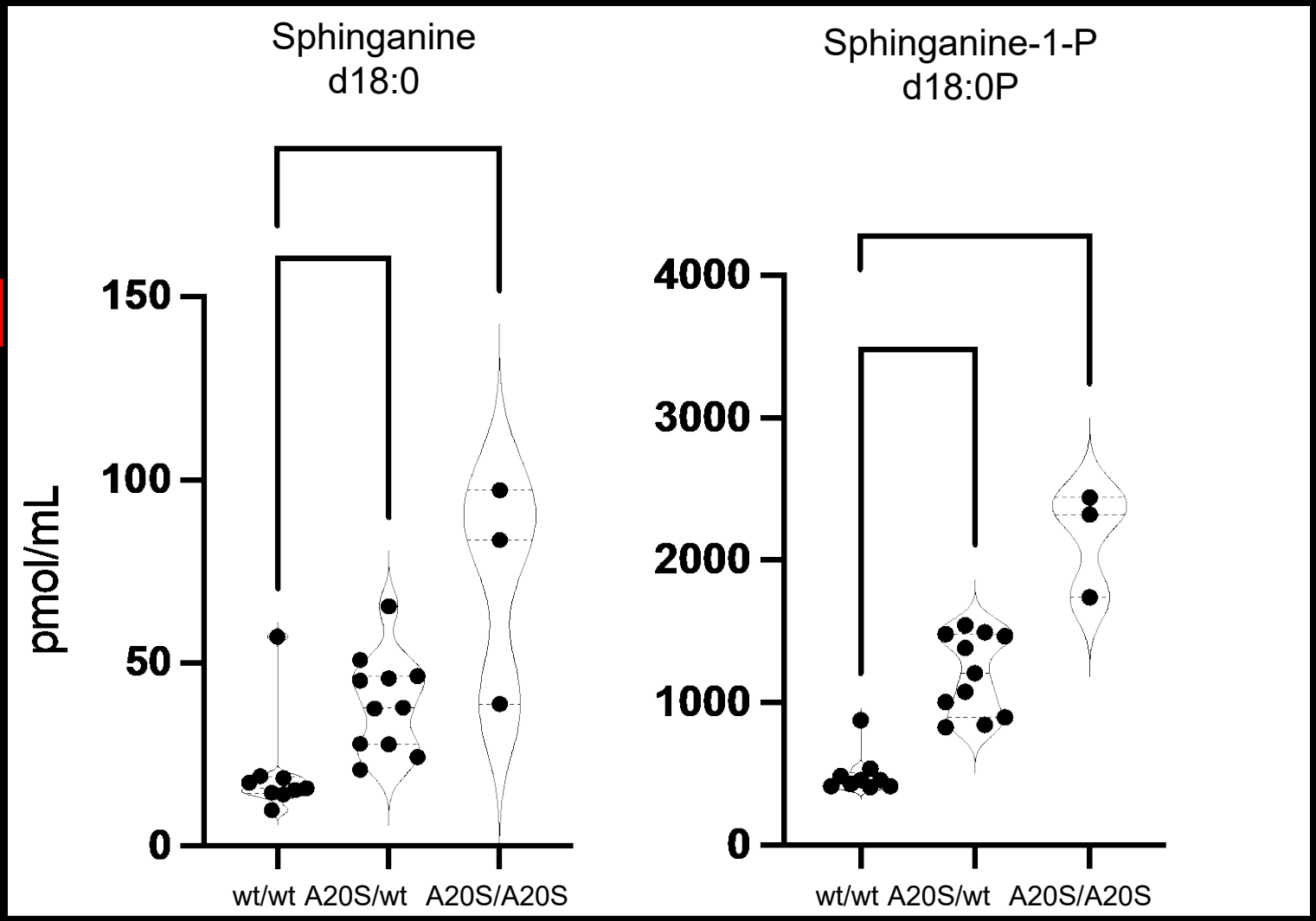
Zoe Piccus et al., unpublished

A20S carriers have slightly lower body weight



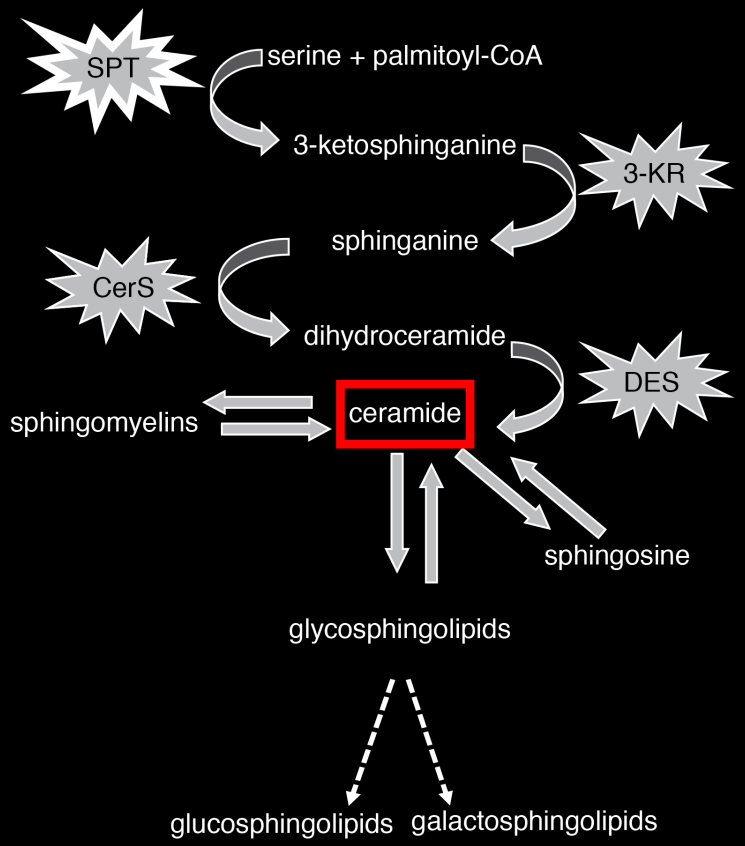
Sptlc1^{A20S} animals produce excess sphingolipids

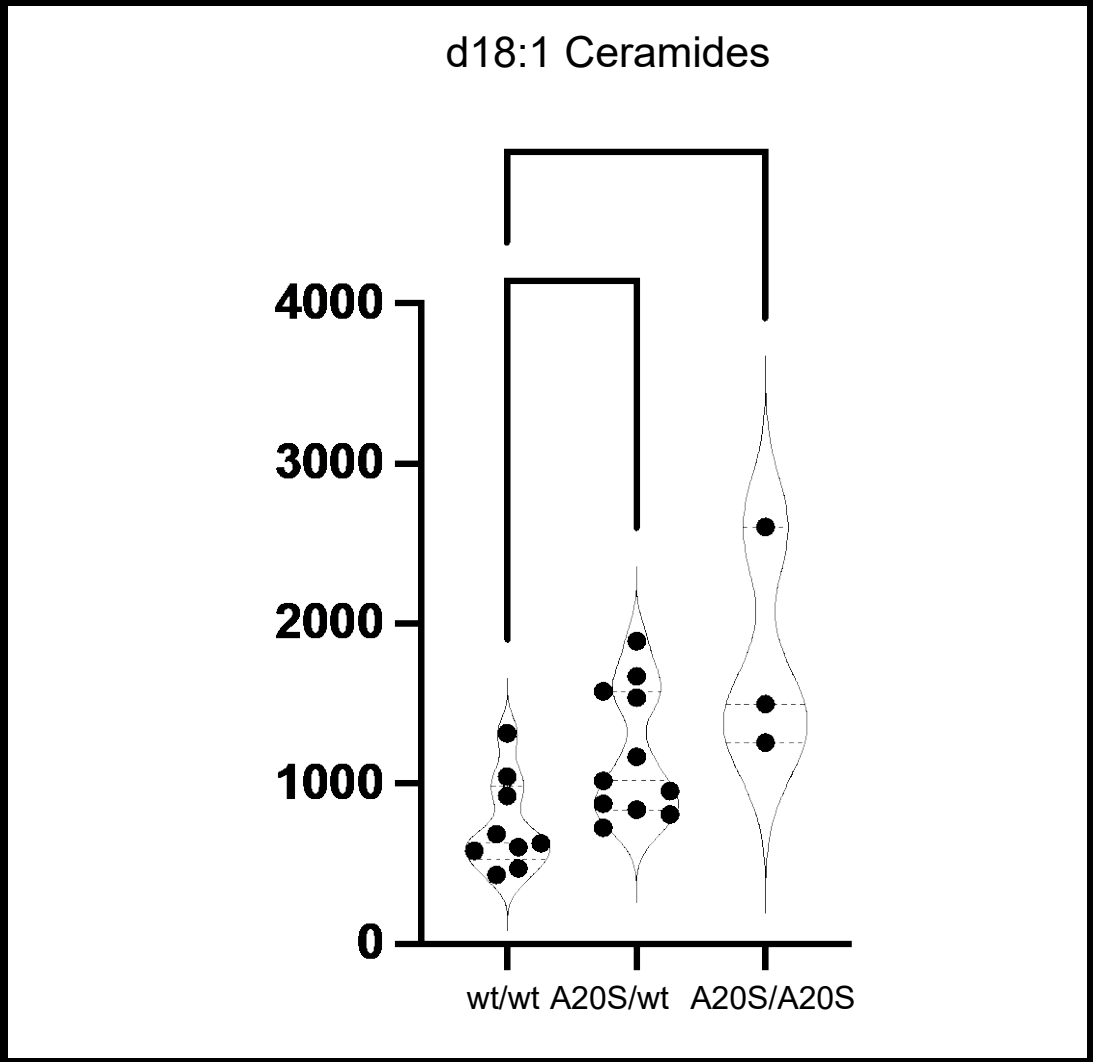




10 to 20-week-old serum

Sptlc1^{A20S} animals produce excess sphingolipids

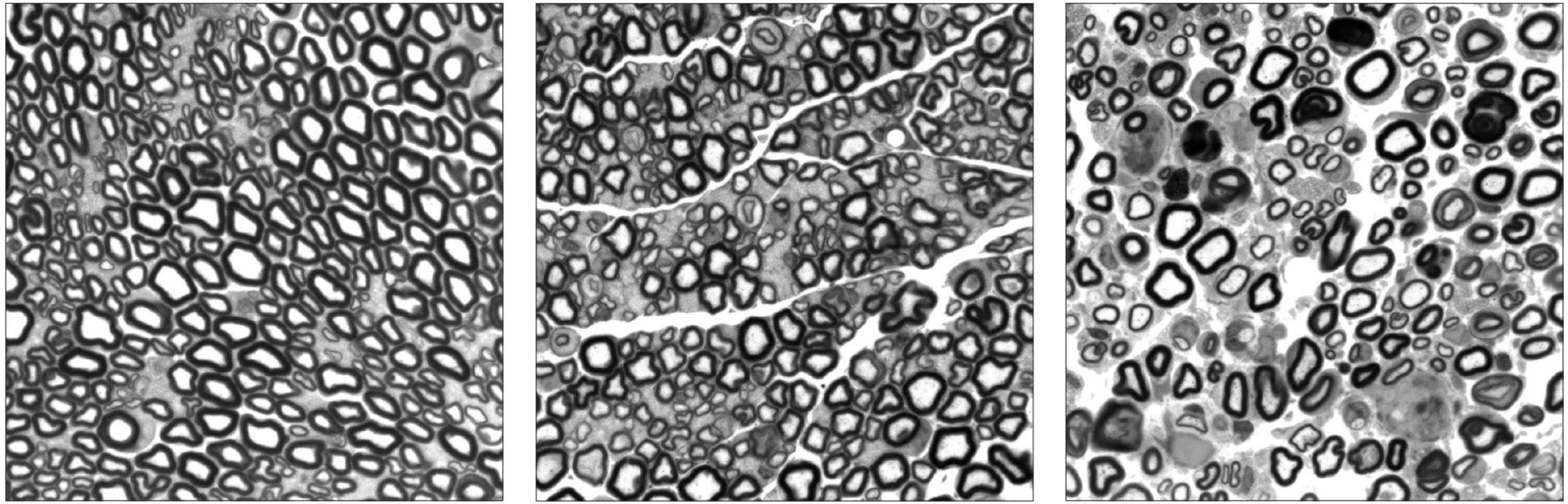




10 to 20-week-old serum

Sciatic nerve pathology in Sptlc1 A20S mutants

Sptlc1 wt/wt A20S/wt A20S/A20S



9-week old, myelin stain

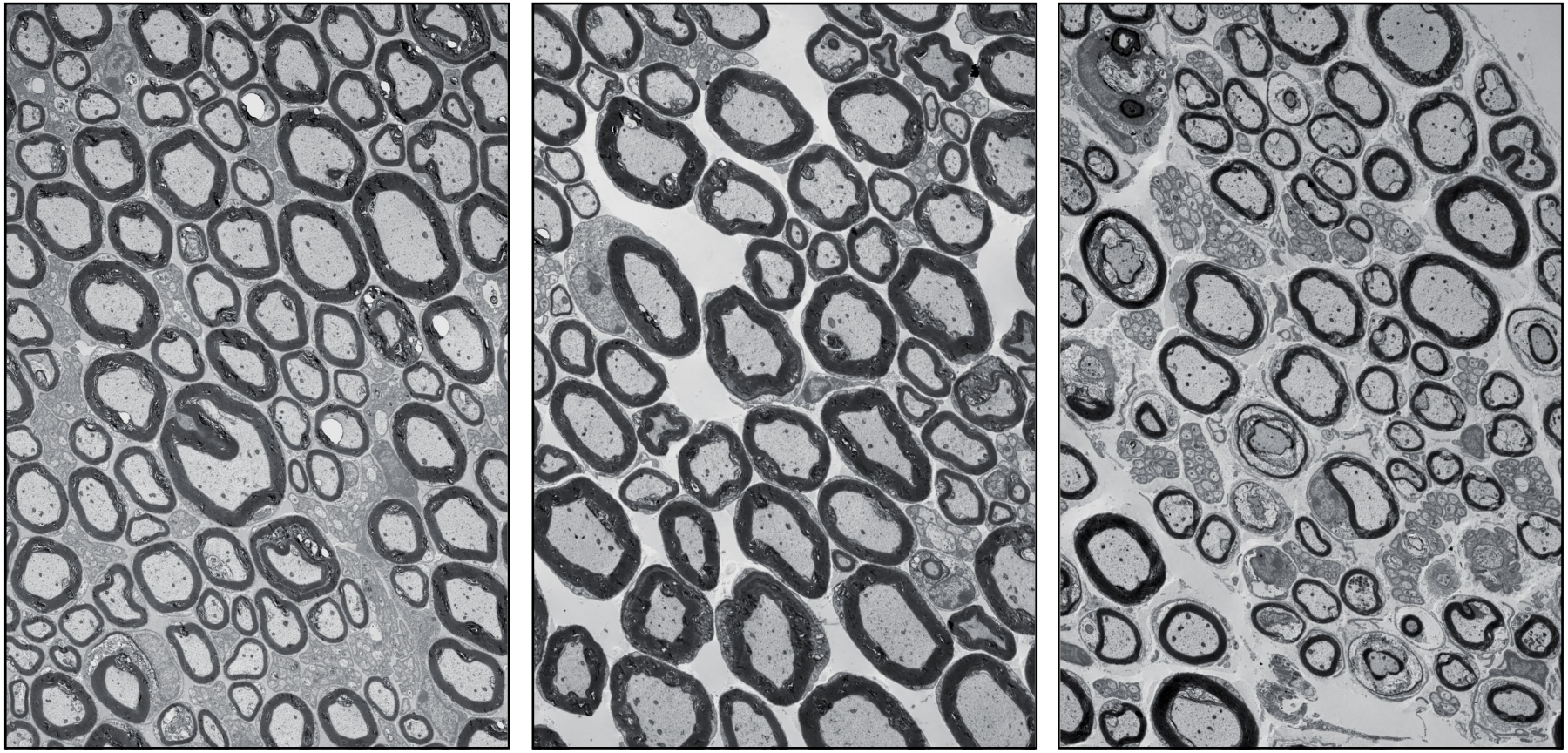
Sciatic nerve pathology in Sptlc1 A20S mutants

Sptlc1

wt/wt

A20S/wt

A20S/A20S



19-week old
Electron microscopy (EM)

Degenerative pathology in Sptlc1^{A20S} mutant nerve

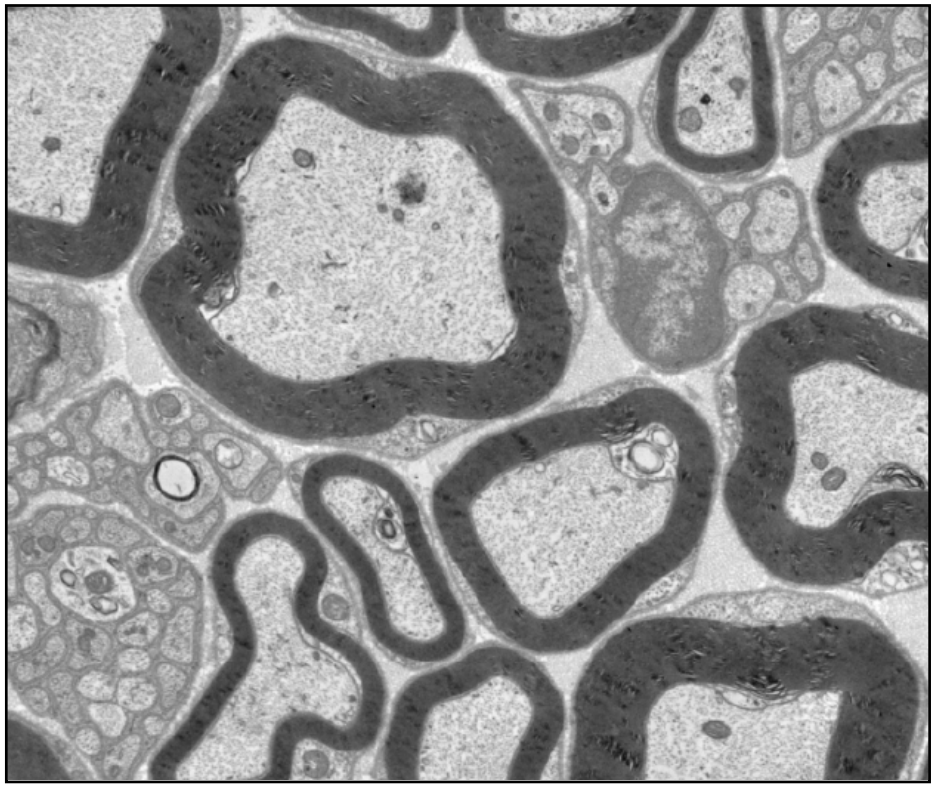
Sptlc1

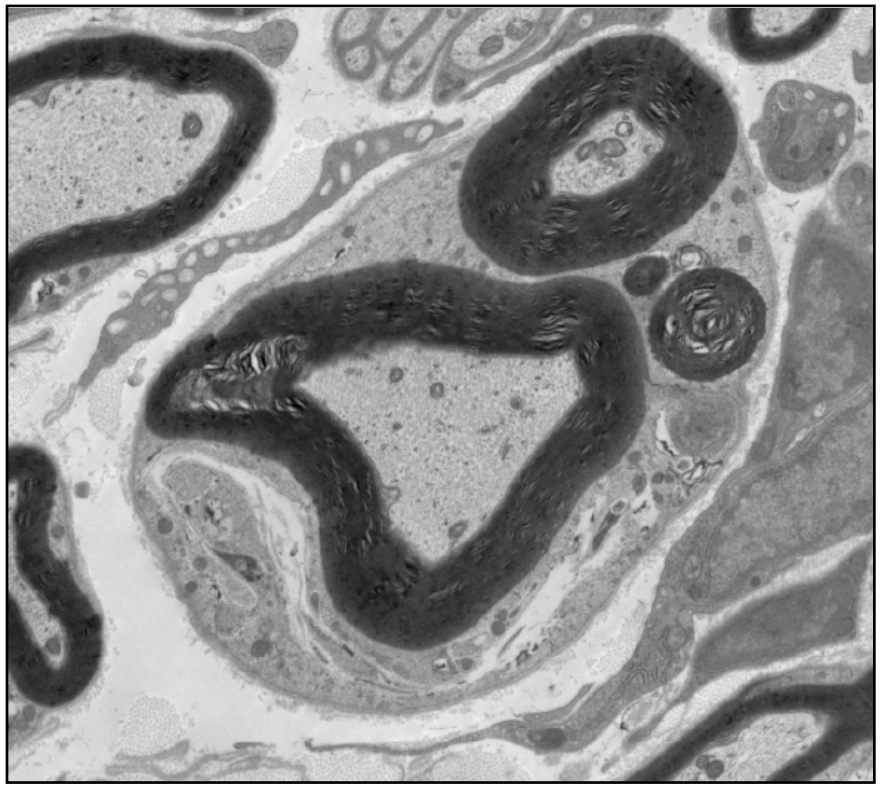
wt/wt

Healthy Schwann cells and axons

A20S/A20S

Schwann cell pathology /axonal sprouting





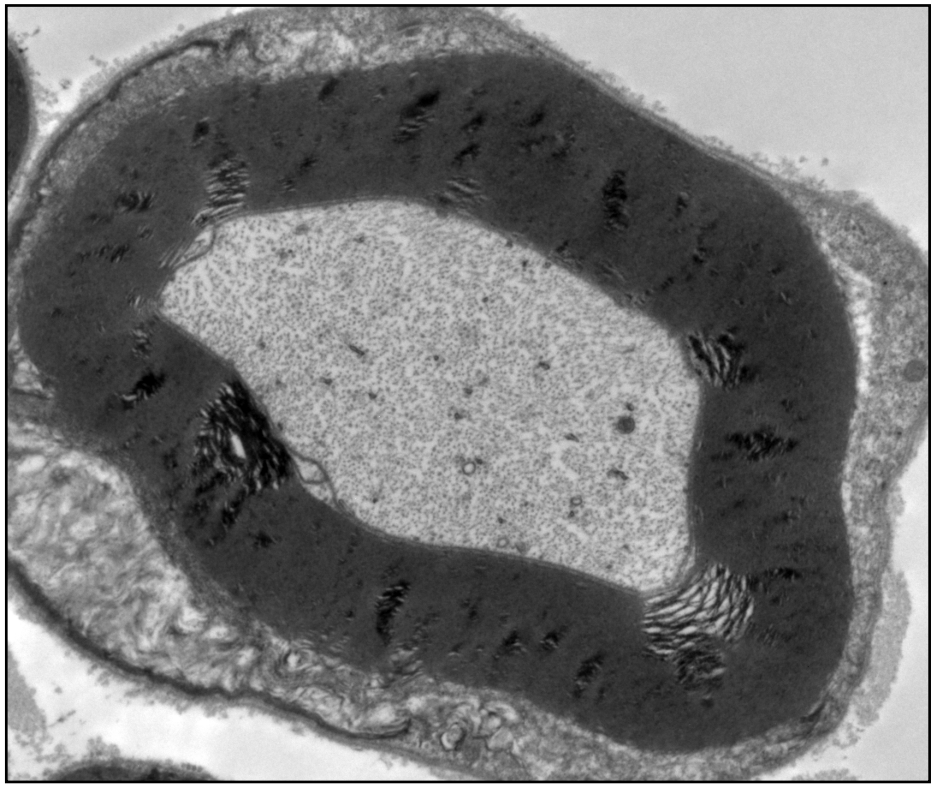
19-week old EM, littermates

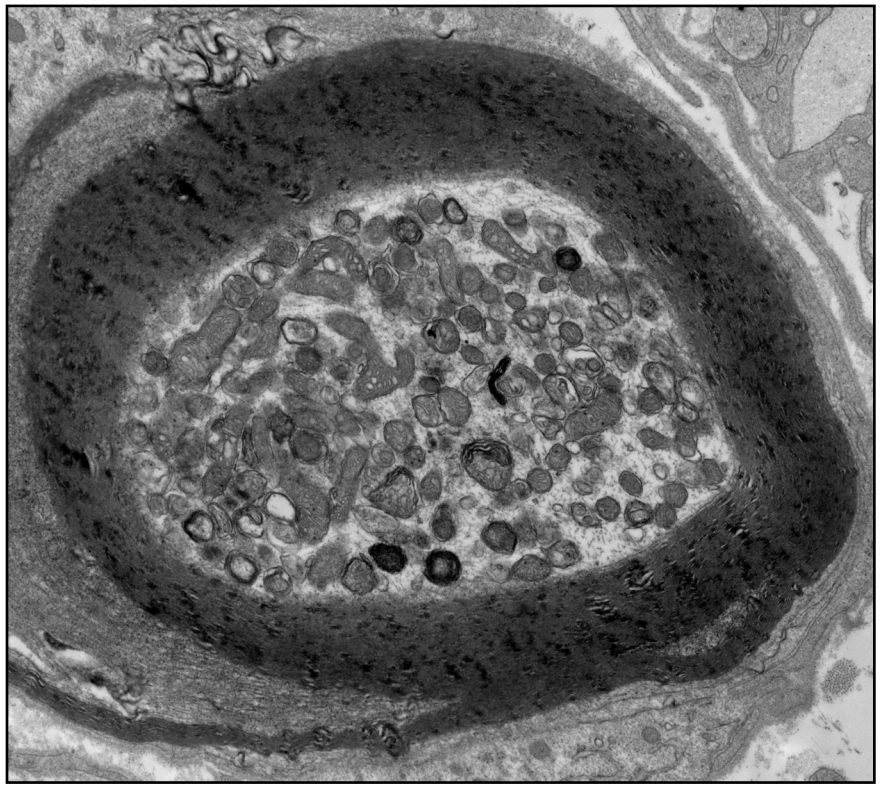
Degenerative pathology in Sptlc1^{A20S} mutant nerve

Sptlc1

wt/wt
Healthy axon

A20S/A20S
Axonal organelle accumulations





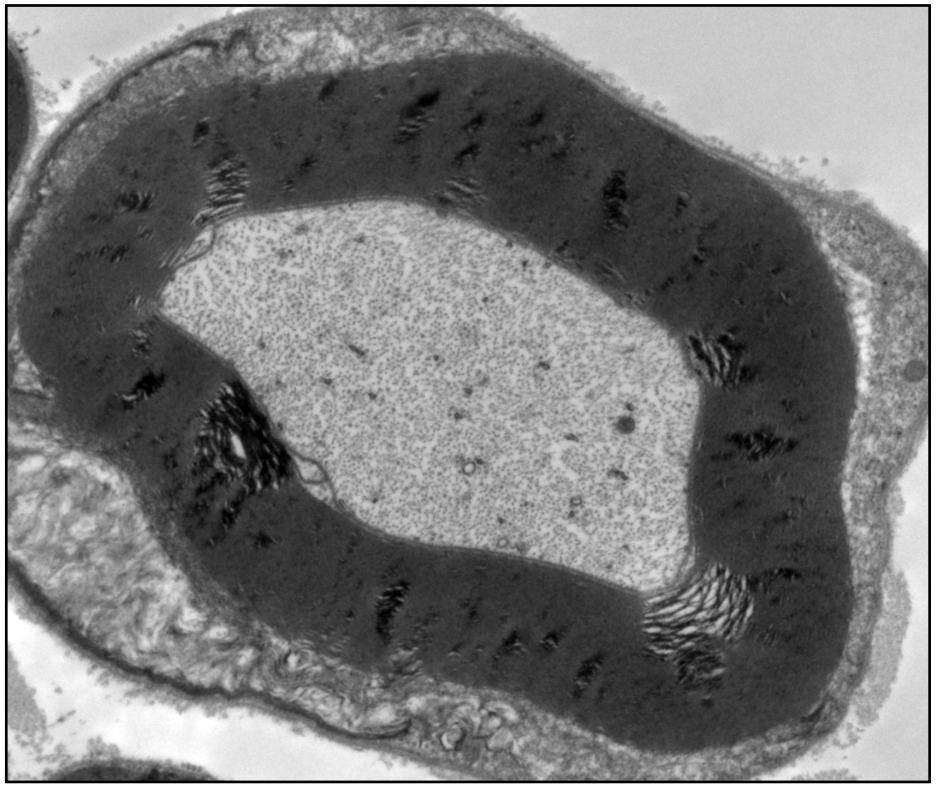
0.5 µm

19-week old EM, littermates

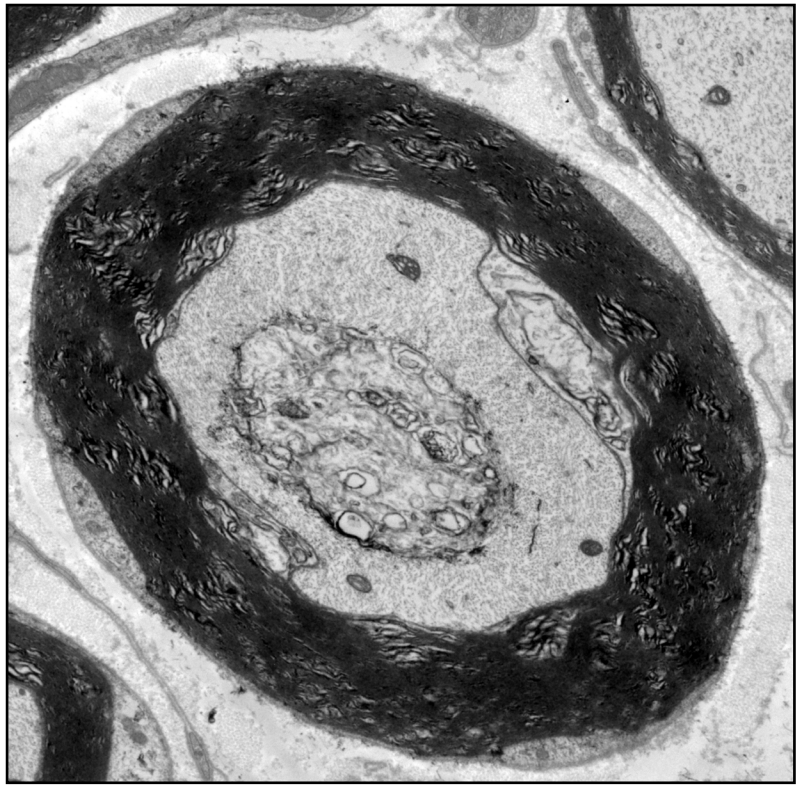
Degenerative pathology in Sptlc1^{A20S} mutant nerve

Sptlc1

wt/wt
Healthy axon



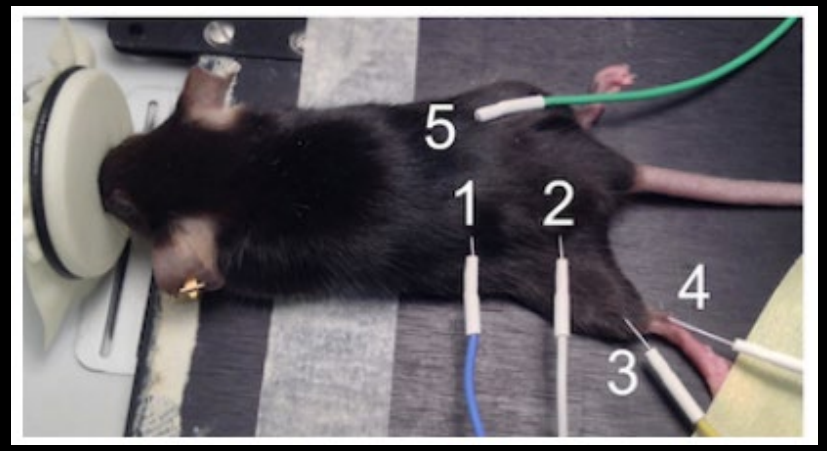
A20S/A20S
Degenerating axon



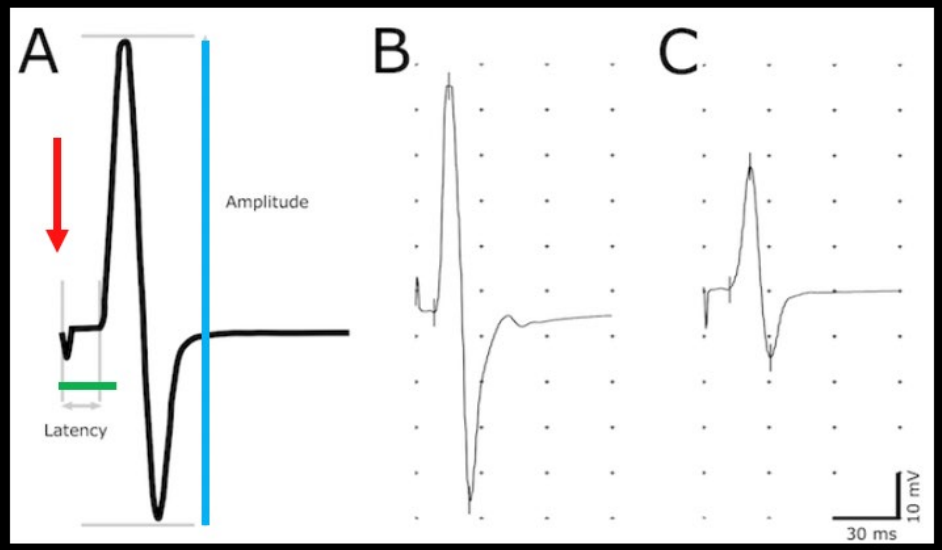
1 µm

19-week old EM, littermates

Compound Muscle Action Potential (CMAP) recordings reflect functional connectivity between nerve and muscle



1+2: stimulating electrodes
3: recording electrode
4: reference
5: ground

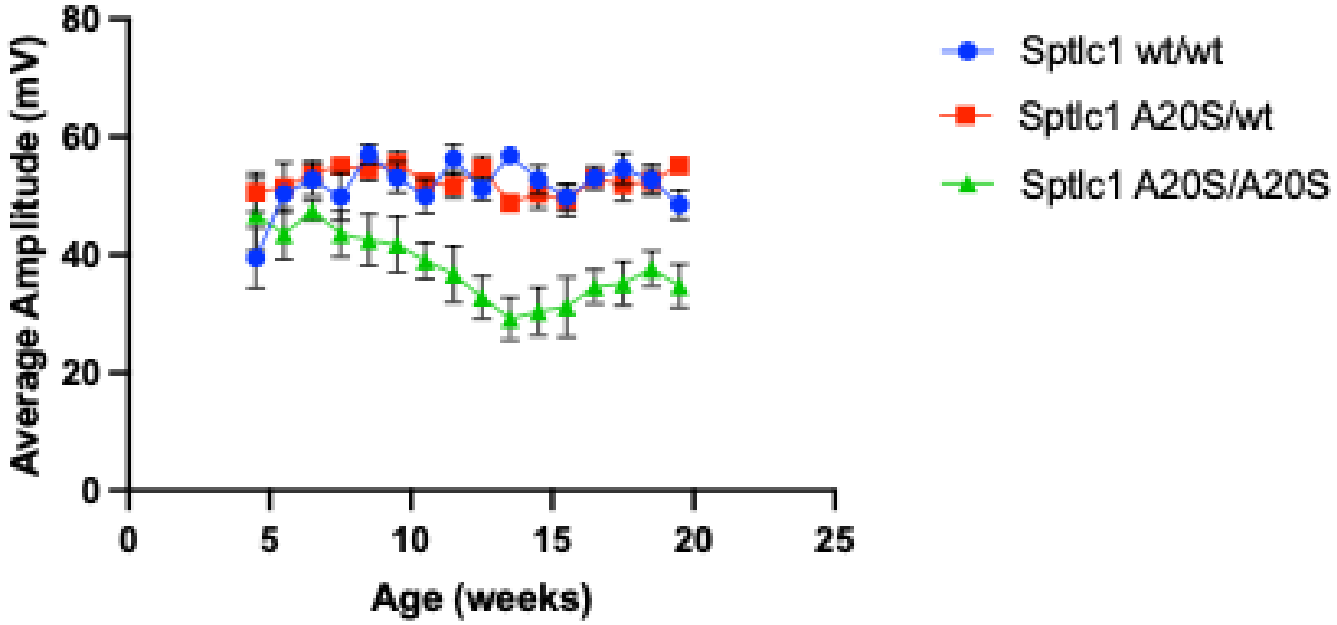


Stimulation (t=0)
Latency to peak
Max amplitude

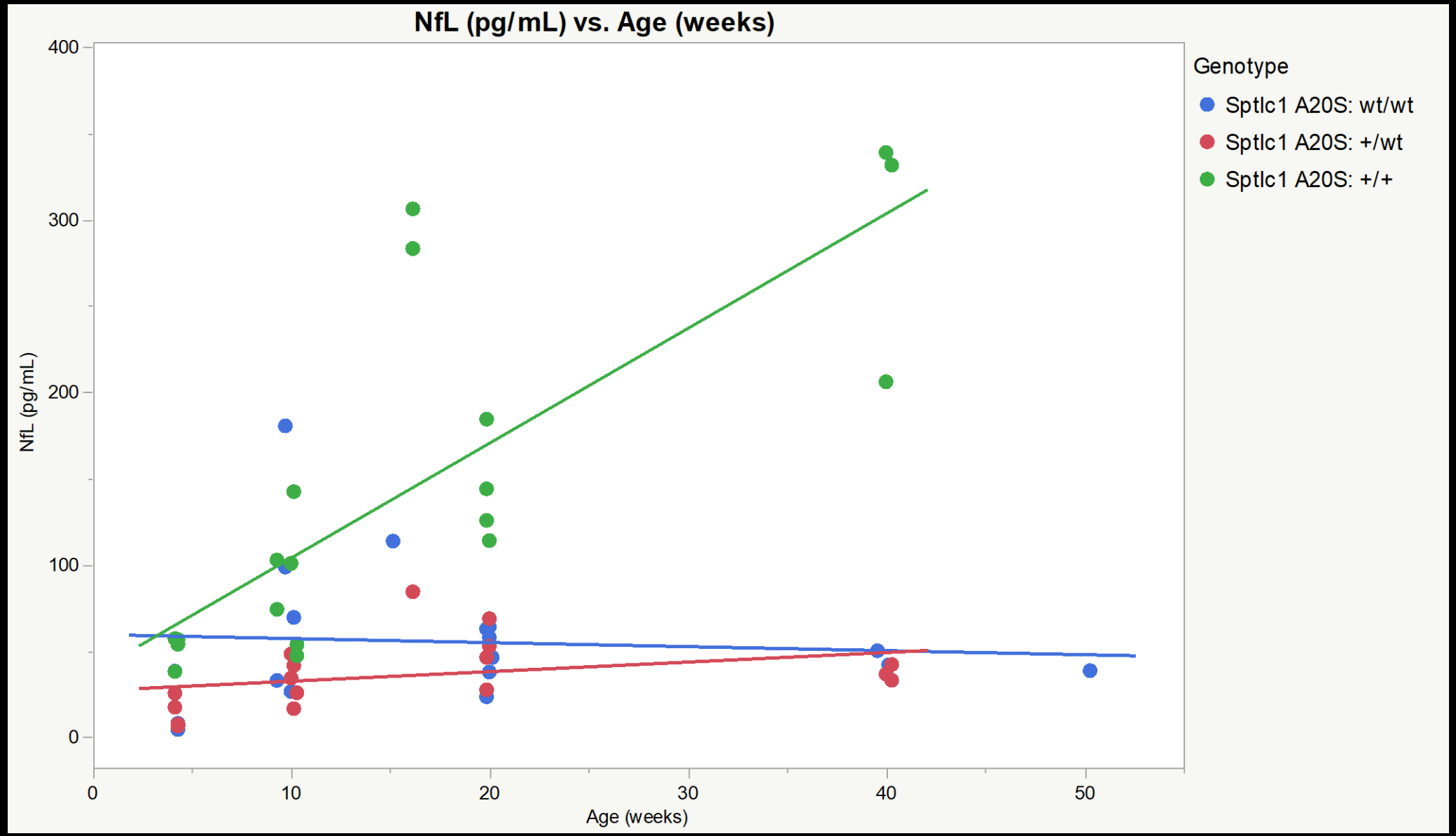
A+B: waveform in WT animals
C: diminished amplitude in SOD1 animal

Pollari, …, van den Bosch, JoVE 2018

Nerve to muscle communication is disrupted in homozygotes



A20S homozygotes have increased NfL in serum



The Sptlc1 A20S mouse model can be used for preclinical testing

Placeholder for prevention study
Placeholder for treatment study

Closing thoughts

- Mutations in SPTLC1 associated with ALS produce neurodevelopmental and/or neurodegenerative phenotypes in gene-edited mice
  - Ongoing characterization (motor neuron counts, electrophysiology, muscle histology)
  - Planning prevention study and treatment study using inhibitors of SPTLC1 in Sptlc1$^{A20S/A20S}$ mice
- Mutations in SPTLC1 have also been detected in sporadic ALS patients (Johnson et al., 2021)
- Possibility of a pathogenic role for disrupted sphingolipid metabolism in sporadic ALS
- Potential of sphingolipids as disease biomarkers for ALS

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