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Supplementary information

Childhood amyotrophic lateral sclerosis caused by excess sphingolipid synthesis

In the format provided by the authors and unedited

Table S1. Clinical features of patients with *SPTLC1* variants associated with hereditary amyotrophic lateral sclerosis

Family	1	2	3	4	5	6	7	7	7	7	7
Patient	1	2	3	4	5	6	I-1	II-1	II-2	II-3	II-4
SPTLC1[a] variant	p.(Y23F)	p.(Y23F)	p.(F40_S41del)	p.(L39del)	p.(L39del)	p.(A20S)[b]	p.(L39del)	p.(L39del)	p.(L39del)	p.(L39del)	p.(L39del)
Inheritance	De novo	De novo	De novo	De novo	De novo	De novo	AD	AD	AD	AD	AD
Sex/Age at last examination (years)	F/16	F/8	F/28	M/28	M/13	M/21	M/62	F/27	F/25	M/21	F/19
Symptom Onset (years)	LE spasticity (4)	LE spasticity (3)	LE spasticity (4)	Toe walking (8)	Falls (3)	Not able to run (3)	Ankle sprains (15) ; numb toes (30)	UE discoordination (16)	Abnl Gait (14)	Abnl Gait (10)	Abnl Gait (6)
Progressive Weakness	+	+	+	+	+	+	+	+	+	+	+
Loss of Ambulation (years)	13	A	13	17	10	10	A	19[c]	23	14[c]	13[c]
Respiratory Insufficiency[d]	++	-	++	+	-	++	-	-	-	-	-
Cognition	Nl	Nl	Nl	Nl	Nl	Nl	Nl	Nl	Nl	Nl	Nl
Exam: LMN signs	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L	C,L	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L
Exam: UMN signs	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L	Cr, C, L	L	L	L	L	-
Sensory Exam[e]	Nl	Nl	Nl	Nl	Nl	Nl	↓P, ↓T, ↓V, ↓Pr	Nl	Nl	Nl	Nl
Sensory NCS	Nl	Nl	Nl	Nl	Nl	Nl	↓amp/absent	Nl	Nl	Nl	Nl
Motor NCS: CMAP	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp/absent, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV	↓amp, Nl DL, Nl NCV
EMG: Fib/PSW	+	Not performed	+	+	-	Not performed	+	+	+	+	+
EMG: MUAP	↑amp, ↑duration	Not performed	↑amp, ↑duration	↑amp, ↑duration	↑amp, ↑duration	Not performed	↑amp, ↑duration	↑amp, ↑duration	↑amp, ↑duration	↑amp, ↑duration	↑amp, ↑duration

a Transcript ID: NM_006415.4
b This variant results in a splicing defect, predominantly causing exon 2 skipping.
c Uses a wheelchair outside of the home, but able to walk short distances.
d ++ =use of non-invasive or invasive (i.e. tracheostomy) ventilatory support. + = reduced forced vital capacity without non-invasive ventilation
e Bedside sensory testing assessing pinprick (P), temperature (T), vibration (V) and proprioception (Pr)

M=male, F=female, AD= autosomal dominant, LE= lower extremity, UE= upper extremity, Abnl=abnormal, A=ambulatory, Nl=normal, UMN= upper motor neuron signs, LMN= lower motor neuron signs/weakness, Cr=cranial, C=cervical, L=lumbar, NCS=nerve

conduction studies, EMG= electromyography, CMAP=compound muscle action potential, amp=amplitude, DL=distal latency, NCV=nerve conduction velocity, Fib=fibrillation potential, PSW= positive sharp wave, MUAP=motor unit action potential morphology.

Table S2. Four novel *SPTLC1* variants associated with amyotrophic lateral sclerosis

Genomic location[a]	Nucleotide Change[b]	Predicted Amino Acid Change	Exon	Polyphen-2	CADD	gnomAD AF	ACMG Variant Classification[1]
9:92112562	c.58G>T	p.A20S	2	0.973; Probably damaging	28	0	Likely Pathogenic (PS2, PM2, PP3)
9:92112552	c.68A>T	p.Y23F	2	0.973; Probably damaging	24	0	Likely Pathogenic (PS2, PM2, PP3)
9:92112503-505	c.115_117delCTT	p.L39del[c]	2	NA	19	0[d]	Likely Pathogenic (PS2, PM2, PM4, PP3)
9:92112497-502	c.118_123delTTCTCT	p.F40_S41del	2	NA	19	0	Likely Pathogenic (PS2, PM2, PM4, PP3)

a Genome reference GRCh38;
b Transcript ID: NM_006415.4
c Due to the repeat sequence (CTTCTT), the distinction of L39 vs L38 deleted residues based on genetic sequencing in humans is not possible; c.115_117delCTT, p.L39del is the current consensus Human Genome Variation Society format for reporting this variant.
d This variant is noted in a single allele (4e-6 allele frequency) in the gnomAD database (v2.1.1) in an individual from the neurologic/psychiatric cohort.

Polyphen-2 (http://genetics.bwh.harvard.edu/pph2) CADD= Combined Annotation Dependent Depletion score (https://cadd.gs.washington.edu) , gnomAD AF = Genome aggregate database allele frequency (v3.1) (https://gnomad.broadinstitute.org), ACMG=American College of Medical Genetics. NA= Not available for deletion variants.

1. Richards, S. *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17**, 405-24 (2015).

Table S3. Inhibition of serine-palmitoyltransferase activity by increasing ORMDL concentrations. IC_{50} and 95% confidence interval for each *SPTLC1* variant was calculated using a least squares, non-linear regression.

Genotype	IC_{50} (95% CI) ng
Wildtype	19.25 (14.7-24.46)
Y23F	70.04 (55.53-87.8)
Del39	236.7 (174.2-323.7)
E2del	376.5 (302.3-473.1)
Del40_41	361.4 (282.2-467.6)

Table S4. Details of clinical and research based genetic testing in each family with *SPTLC1*-associated amyotrophic lateral sclerosis

Family	Methodology	Library preparation	Sequencing platform	Alignment/Calling/ Annotation	Testing site
1	WES	SeqCap EZ Exome + UTR Library (Roche)	TruSeq V2:39 (Illumina)	Varsifter[50]	NIH
2	WES	GeneDx capture system	HiSeq (Illumina)	XomeAnalyzer (GeneDx)	GeneDx
3	Hereditary neuropathy panel		Multiplex Ligation-dependent Probe Amplification		ARUP Laboratories
4	WES	GeneDx capture system	HiSeq (Illumina)	XomeAnalyzer (GeneDx)	GeneDx
5	WES	Nextera Exome Capture	HiSeq (Illumina)	In-house software	Mendelics
6	WES	Nextera Rapid Capture Exome Kit 38Mb (Illumina)	HiSeq 4000 (Illumina)	BWA/ GATK Haplotype Caller/ Variant Effect Predictor (VEP)	Broad Institute of MIT and Harvard
7	WES	GeneDx capture system	HiSeq (Illumina)	XomeAnalyzer (GeneDx)	GeneDx

Table S5. Primers and probes used in this manuscript.

Primer or probe	Sequence	Figure/section
qPCR-del40_41-F	5'— CTCTGGATAATCAGACTTCTTAAG —3'	Fig 4a
qPCR-del40_41-R	5'— TTGGGACAGGAGGAACAA —3'	Fig 4a
Del40_41 probe	5'-/56-FAM/TGATTGAAG/ZEN/AGTGGCAACCAGAACCT/3IABKFQ/-3'	Fig 4a
qPCR-del39-F	5'— CCTCTGGATAATCAGACTTTTC —3'	Fig 4a
qPCR-del39-R	5'— GTAGTTGAGAGCAGGATGG—3'	Fig 4a
Del39 Probe	5'-/56-FAM/ACCTCTTGT /ZEN/TCCTCCTGTCCCAAA/3IABKFQ/-3'	Fig 4a
qPCR-del39/wt-F	5'— TCCTCTGGATAATCAGACTTCTT —3'	Fig 4a
qPCR-del39/wt-R	5'— CTGGTTGCCACTCTTCAATC—3'	Fig 4a
Del39/wt Probe	5'-/56-FAM/ACAAGAACG/ZEN/ATCTGATCTTACAGTCAAGGA/3IABkFQ/-3'	Fig 4a
qPCR-total SPTLC1-F	5'—TCTTGGATTGTTGGATAACCCTA—3'	Fig 4a, Extended Data Fig 5a
qPCR-total SPTLC1-R	5'—GTCCCCACGCCATACTTCT—3'	Fig 4a, Extended Data Fig 5a
qPCR-SPTLC1 probe	Probe #74-Roche Universal Probe Library	Fig 4a, Extended Data Fig 5a
qPCR-PGK1-F	5'— CAGCTGCTGGGTCTGTCAT —3'	Fig 4a, Extended Data Fig 5a
qPCR-PGK1-R	5'— GCTGGCTCGGCTTTAACC —3'	Fig 4a, Extended Data Fig 5a
PGK1 probe	Probe #67-Roche Universal Probe Library	Fig 4a, Extended Data Fig 5a
SPTLC1-cDNA -F	5'— TGCGGAGGTGATAACGACAC —3'	Extended Data Fig 2

SPTLC1-cDNA -R	5'— CAGGCGGTCTTCCAAATCCA —3'	Extended Data Fig 2
SPTLC1-gDNA-F	5'— AGGCTAAAAGTCACAGGAGGC —3'	iPSC-CRISPR
SPTLC1-gDNA-R	5'— GGTTGAGCCACCACAAATCC —3'	iPSC-CRISPR
SPTLC1-seq gDNA -F	5'— TGTGCAGGTGTTAGAAGTGTA —3'	iPSC-CRISPR
SPTLC1-seq gDNA -R	5'— TTTCTGGAAAGACATAGCAACT —3'	iPSC-CRISPR

Table S6. Sequence of siRNAs used in this manuscript

siRNA	**Sequence**
Del40_41 targeting	5’ ----AAUCAGACUUCUUAAGACAdTdT---- 3’ 3’---dTdTUUAGUCUGAAGAAUUCUGA---- 5’
Del39 targeting	5’----UGGAUAAUCAGACUUUUCUCAdTdT---- 3’ 3’---dTdTACCUAUUAGUCUGAAAAGAGA---- 5’
Scrambled	Dharmacon Inc., D-001810-01