

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

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Supplemental content

IMPORTANCE Juvenile amyotrophic lateral sclerosis (ALS) is a rare form of ALS characterized by age of symptom onset less than 25 years and a variable presentation.

OBJECTIVE To identify the genetic variants associated with juvenile ALS.

DESIGN, SETTING, AND PARTICIPANTS In this multicenter family-based genetic study, trio whole-exome sequencing was performed to identify the disease-associated gene in a case series of unrelated patients diagnosed with juvenile ALS and severe growth retardation. The patients and their family members were enrolled at academic hospitals and a government research facility between March 1, 2016, and March 13, 2020, and were observed until October 1, 2020. Whole-exome sequencing was also performed in a series of patients with juvenile ALS. A total of 66 patients with juvenile ALS and 6258 adult patients with ALS participated in the study. Patients were selected for the study based on their diagnosis, and all eligible participants were enrolled in the study. None of the participants had a family history of neurological disorders, suggesting de novo variants as the underlying genetic mechanism.

MAIN OUTCOMES AND MEASURES De novo variants present only in the index case and not in unaffected family members.

RESULTS Trio whole-exome sequencing was performed in 3 patients diagnosed with juvenile ALS and their parents. An additional 63 patients with juvenile ALS and 6258 adult patients with ALS were subsequently screened for variants in the *SPTLC1* gene. De novo variants in *SPTLC1* (p.Ala20Ser in 2 patients and p.Ser331Tyr in 1 patient) were identified in 3 unrelated patients diagnosed with juvenile ALS and failure to thrive. A fourth variant (p.Leu39del) was identified in a patient with juvenile ALS where parental DNA was unavailable. Variants in this gene have been previously shown to be associated with autosomal-dominant hereditary sensory autonomic neuropathy, type 1A, by disrupting an essential enzyme complex in the sphingolipid synthesis pathway.

CONCLUSIONS AND RELEVANCE These data broaden the phenotype associated with *SPTLC1* and suggest that patients presenting with juvenile ALS should be screened for variants in this gene.

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Amyotrophic lateral sclerosis (ALS) is a relatively common neurological disorder characterized by progressive paralysis and death from respiratory failure. The vast majority of cases occur in individuals older than 40 years.¹ In contrast, juvenile ALS (defined as an age of onset less than 25 years) is a rare form of motor neuron disease.² These early-onset cases are characterized by slow progression and a variable phenotype that often makes accurate diagnosis challenging.²

Considerable progress has been made in unravelling the genetic architecture underlying ALS, but much remains to be understood about this condition.³ Juvenile ALS is thought to be more frequently genetic in origin than the adult-onset forms, and the genetic analysis of these young-onset cases offers an opportunity to identify disease-causing genes.² By extension, any gene underlying juvenile ALS may also play a role in adult-onset ALS.

De novo genetic variants may underlie at least a portion of ALS cases. Such variants would not be detected by genome-wide association studies owing to their recent occurrence and corresponding low frequency within the community. Spontaneously occurring variants are a well-known cause of neurological conditions, such as neurofibromatosis type 1 and Duchenne muscular dystrophy.^{4,5} Indeed, de novo variants of the familial ALS genes *FUS*, *SOD1*, and *VCP* have been described in sporadic ALS cases.^{6,7} Such variants are more likely to present with early-onset disorders because of their impact on fitness.⁸

Here, we performed whole-exome sequencing of 3 patients diagnosed with juvenile ALS and their unaffected parents to identify the variants associated with their disease. None of these patients had a family history of neuromuscular disorders, suggesting de novo variations as the underlying genetic mechanism. After identifying variants in serine palmitoyltransferase, long-chain base subunit 1 (*SPTLC1*; OMIM, 605712) in all 3 cases, we also screened patients with juvenile ALS from Turkey for *SPTLC1* variants and identified a fourth patient carrying an *SPTLC1* variant.

Methods

Patients

Four unrelated patients with neuromuscular symptoms consistent with juvenile ALS participated in the study between March 2016 and January 2021. The **Table** summarizes the clinical features of the 4 patients. Detailed descriptions of each patient and their recruitment are available in the eMethods in the **Supplement**. All participants provided written informed consent for genetic analysis according to the Declaration of Helsinki, and the Institutional Review Board of the National Institutes of Health approved the study. Members of the FALS Sequencing Consortium, American Genome Center, International ALS Genomics Consortium, and ITALSGEN Consortium can be found in the eAppendix of the **Supplement**.

Patient 1 presented with gradually progressive spastic diplegia and growth retardation beginning at age 5 years. By age 20 years, she had quadriplegia with marked muscle atro-

Key Points

Question What genetic variants are associated with juvenile amyotrophic lateral sclerosis (ALS)?

Findings In this family-based genetic study, exome sequencing was performed in 3 patients diagnosed with juvenile ALS and failure to thrive; this identified de novo variants in *SPTLC1* (p.Ala20Ser in 2 patients and p.Ser331Tyr in 1 patient). Variants in *SPTLC1* are a known cause of hereditary sensory and autonomic neuropathy, type 1A, and these data extend the phenotype associated with this gene.

Meaning De novo variants in the *SPTLC1* gene are associated with juvenile ALS, a fatal neurological disorder.

phy and diminished weight, brisk lower limb reflexes, tongue fasciculations and weakness, dysarthria, mild cognitive dysfunction, and respiratory failure requiring tracheostomy and ventilation. Repeated neurophysiological testing did not show evidence of sensory or autonomic dysfunction. She was diagnosed with juvenile ALS based on the revised El Escorial criteria.⁹

Patient 2 was a teenaged girl of African American and White race who presented with a 6-year history of gradually progressive generalized limb and bulbar weakness. She had a long-standing history of progressive weight loss of unknown cause, and her school performance began to decline in her mid-teens. Her neurological examination at presentation revealed a body mass index less than the first percentile, exaggerated lumbar lordosis, tongue fasciculations and wasting, generalized muscle atrophy and weakness, brisk asymmetric ankle reflexes, a positive Gower sign, and normal sensation (**Figure 1A** and **B**). Neurophysiological testing revealed active and chronic denervation without evidence of sensory neuropathy. Decreased sustained attention and impaired executive functioning were evident in neuropsychological evaluation. She was diagnosed with juvenile ALS based on the revised El Escorial criteria.⁹

Patient 3 was an 11-year-old African American girl with a history of failure to gain weight and toe walking since age 4 years. She presented at age 10 years with a deteriorating gait, hand weakness, right foot paresthesia, dysphagia, and increased sweating. Examination revealed marked atrophy, postural tachycardia, bilateral cataracts, a wasted and fasciculating tongue with an exaggerated jaw jerk, generalized fasciculations and weakness associated with hyperreflexia, and decreased pinprick sensation in a glove-and-stocking distribution (**Figure 1C** and **D**). The patient walked abnormally owing to weakness and bilateral foot drop, and she had a positive Gower sign. Neurophysiological examination showed sensorimotor axonal neuropathy as well as polyphasic potentials on electromyography. She was diagnosed with juvenile ALS-Plus syndrome owing to her prominent motor symptoms and modest sensory-autonomic involvement. The pedigrees of patients 1, 2, and 3 are shown in **Figure 2A** to **C**.

Patient 4 was a 34-year-old Turkish woman with a history of arm and leg weakness and atrophy since age 15 years. There was no family history of neuromuscular disease, and

Table. Clinical Features of Patients Diagnosed With Juvenile Amyotrophic Lateral Sclerosis and Carrying Variants in *SPTLC1*

Clinical feature	Patient 1	Patient 2	Patient 3	Patient 4
Gene change	p.Ala20Ser	p.Ala20Ser ^a	p.Ser331Tyr	p.Leu39del
Age at onset	5 y	<10 y	4 y	15 y
Age at evaluation	20 y	10s	11 y	34 y
BMI (z score)	13th Percentile (-1.1)	<1st Percentile (-7.0)	<1st Percentile (-6.5)	Normal
Back deformities	Severe scoliosis	Lordosis	Normal posture	NA
Foot deformities	Pes cavus	NA	Pes cavus/varus	NA
Walking	Nonambulatory	Steppage	Steppage	Abnormal
Atrophy	Global, contractures	Global	Global	Global
Weakness	Generalized	Generalized	Generalized	Generalized
Reflexes	Hyporeflexia, Achilles tendon brisk	Hyporeflexia, Achilles tendon brisk	Hyperreflexia, Achilles tendon absent	Hyporeflexia
Tongue	Wasted, fasciculations	Wasted, fasciculations	Wasted, fasciculations	NA
Jaw jerk	Present	NA	Present	NA
Respiratory	Tracheostomy at 17 y	NA	Dyspnea on exercise	Normal
Cognition	Executive dysfunction	Executive dysfunction	Normal	NA
Sensory	Normal	Normal	Glove-stocking pain loss, foot ulceration	Normal
Neurophysiology				
Motor	Chronic denervation	Acute and chronic denervation	Axonal loss, polyphasia	Denerv
Sensory	Normal	Normal	Axonal loss	Normal
Additional features	NA	Scapular winging, Gower sign	Gower sign, vitamin D deficiency, hyperhidrosis	Uses a wheelchair

Abbreviations: BMI, body mass index; NA, not applicable.

^a Variant was detected in 1 of 49 and 0 of 149 next-generation sequencing reads from the father's saliva-derived and buccal-derived DNA, respectively.

none of her 5 siblings had symptoms (Figure 2D). She was diagnosed with juvenile ALS, and she has been taking riluzole since age 15 years. Her symptoms were slowly progressive, and there were no upper motor neuron signs on examination. During her last review at age 34 years, she used a wheelchair, although she could walk short distances with assistance. She had no dysphagia and did not require oxygen supplementation, and her weight was normal. Neurophysiological examination at that time revealed denervation activity in all muscles and no evidence of multifocal motor neuropathy.

For variant screening of *SPTLC1* in adult-onset ALS, we used 6258 DNA samples obtained from individuals diagnosed with adult-onset ALS (eTable 1 in the Supplement). Control data consisted of 5710 neurologically healthy US individuals who had undergone next-generation sequencing at the Laboratory of Neurogenetics of the National Institute on Aging, National Institutes of Health, Bethesda, Maryland, or the Alzheimer Disease Sequencing Project.

Next-Generation Sequencing in Juvenile ALS

Whole-exome sequencing was performed using 100 base-pair, paired-end sequencing on an Illumina sequencer (eg, HiSeq 2000) according to the manufacturer's protocol. DNA from patient 1 and her family was sequenced in the Laboratory of Neurogenetics using TruSeq library preparation version 1.0. DNA from patients 2 and 3 and their families was sequenced at GeneDx using IDT xGen Exome Research Panel version 1.0.¹⁰

Data were analyzed to identify de novo variants present in the affected child and not present in either parent. As the variants underlying a rare disease, such as ALS, are unlikely to be present in the general population, variants present in the Genome Aggregation Database (gnomAD; version 2.1) or the Kaviar Genomic Variant database (September 23, 2015, version) were excluded. Synonymous, intronic, and intergenic changes were excluded (ANNOVAR; August 11, 2016, version). Paternity and maternity were confirmed using identity-by-descent analysis, and exome data were reviewed to identify variants in known ALS genes.

SPTLC1 Sequencing in Adult-Onset ALS

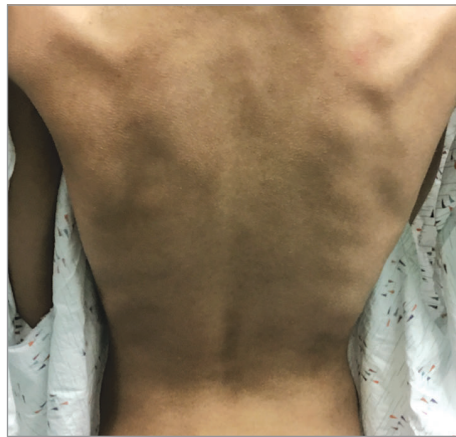
DNA from 6258 patients with adult-onset ALS were sequenced to identify variants in the *SPTLC1* gene (whole-exome sequencing, 3748 cases^{11,12}; whole-genome sequencing, 1860 cases; Sanger sequencing, 650 cases) (eTable 2 in the Supplement). Variants in *SPTLC1* were considered to be deleterious if they (1) were not present in the 4647 controls from the Alzheimer Disease Sequencing Project; (2) had a frequency less than 3.3×10^{-5} in human variant databases, including the 51 592 European and 8949 Finnish nonneurological individuals in gnomAD and the 77 301 samples in Kaviar database³; and (3) were designated as damaging according to 4 of 5 prediction algorithms,¹³ were identified as stop gain or frameshift, or were identified as splice-site variants with a dbcsSNV score higher than 0.6. Gene burden testing of *SPTLC1* was performed using publicly available control data (gno-

Figure 1. Clinical Features of Patients Diagnosed With Juvenile Amyotrophic Lateral Sclerosis

A Tongue wasting in patient 2



B Scapular winging in patient 2



C Tongue wasting in patient 3



D Leg muscle atrophy in patient 3



A and B, Tongue wasting and scapular winging in patient 2 carrying the p.Ala20Ser *SPTLC1* variant. C and D, Tongue wasting and muscle atrophy of the lower limbs in patient 3 carrying the p.Ser331Tyr *SPTLC1* variant. Note the hammertoe deformities of both feet.

mAD and Kaviar databases) as implemented in the Test Rare Variants With Public Data (TRAPD) software package version 1.0.¹⁴ The statistical significance threshold was set at a 1-tailed *P* value less than .05 for single-gene analysis and 2.5×10^{-6} for genome-wide significance (.05/20 000 genes).

Cellular Mitochondrial Assays

Variants were introduced into a plasmid containing the human *SPTLC1* open reading frame (Origene) using the QuikChange II XL kit (Agilent), followed by subcloning into pLenti-C-Myc-DDK-P2A-Puro lentiviral plasmid (Origene). Lentiviruses were produced with third-generation packaging plasmids (pMDLg/pRRE and pRSV-Rev; Addgene) and envelope plasmid (pMD2.G; Addgene).¹⁵ HEK293FT cells were transfected with wild-type or variant lentivirus transfer plasmid, and transduced cells stably expressing *SPTLC1* were selected by extended growth in 0.5- μ g/mL puromycin (Thermo Fisher Scientific). For the serine rescue experiment, a final concentration of 100-mM L-serine was added to the media for 48 hours. Mitochondria were imaged with MitoTracker Red CMXRos (Thermo Fisher Scientific) using a CellInsight imager (Thermo Fisher Scientific). A minimum of 6 wells were quantified for each condition, and all assays were performed at least twice.

Unpaired *t* test with Welch correction or analysis of variance were used to calculate statistical significance.

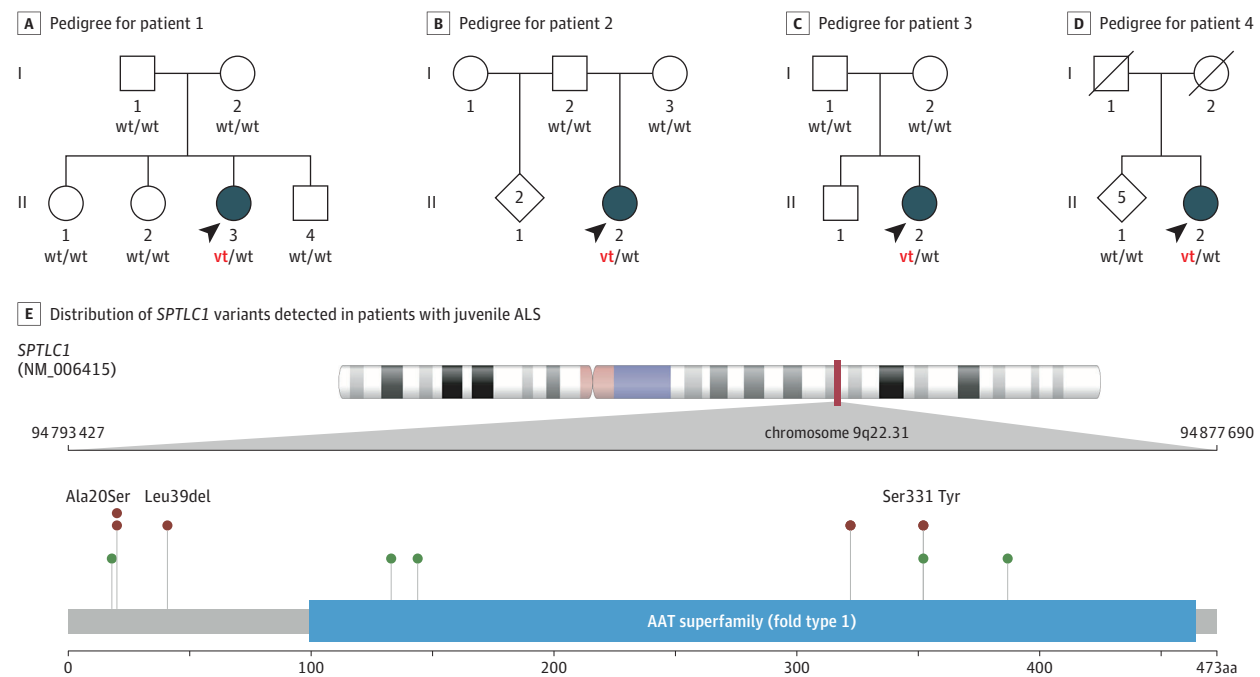
Sphingolipid Measurements

Plasma sphingolipid and glucosylceramide measurements were performed at the Biomedical Genetics Clinical Laboratory, Seattle Children's Hospital, Seattle, Washington, using high-performance liquid chromatography and tandem mass spectrometry.

Results

Identification of De Novo Variants in *SPTLC1* Associated With Juvenile ALS

We performed whole-exome sequencing in 3 unrelated patients who had been diagnosed with juvenile ALS and their healthy parents (Figure 1; Figure 2A to C; Table). The analysis of their genetic data identified de novo variants in the *SPTLC1* gene in each of the 3 patients that were absent in their parents (Figure 2E). Patients 1 and 2 carried the same heterozygous p.Ala20Ser variant in *SPTLC1* caused by variation in adjacent nucleotides (chr9:94874844C>A and chr9:

Figure 2. De Novo Variants of *SPTLC1* in Patients Diagnosed With Juvenile Amyotrophic Lateral Sclerosis (ALS)

A-D, Pedigrees of 4 patients diagnosed with juvenile ALS. The variant alleles in *SPTLC1* are indicated by vt, and wild-type alleles are indicated by wt. The arrowheads indicate the probands. E, Distribution of *SPTLC1* variants detected

in patients diagnosed with juvenile ALS. Variants identified in the 3 patients with juvenile ALS are noted in red, and variants previously described to cause hereditary sensory and autonomic neuropathy, type 1A, are shown in green.

94874843G>T; human genome build hg19). Patient 3 carried a p.Ser331Tyr (chr9:94809543G>T) heterozygous variation in *SPTLC1*. Screening of the *SPTLC1* gene in a cohort of 63 patients with juvenile ALS from Turkey who had undergone whole-exome sequencing identified a p.Leu39del (chr9:94874785_94874787del) heterozygous variant in patient 4 (Figure 2D). Parental DNA was not available, making it impossible to determine if the deletion had occurred spontaneously. These *SPTLC1* variants were not present in controls or online databases of human variants (142 489 individuals). The p.Ser331Tyr and p.Leu39del amino acid changes have been previously implicated in neurological disease.¹⁶

Serine and the Damaging Effects of the p.Ala20Ser Variant in Vitro

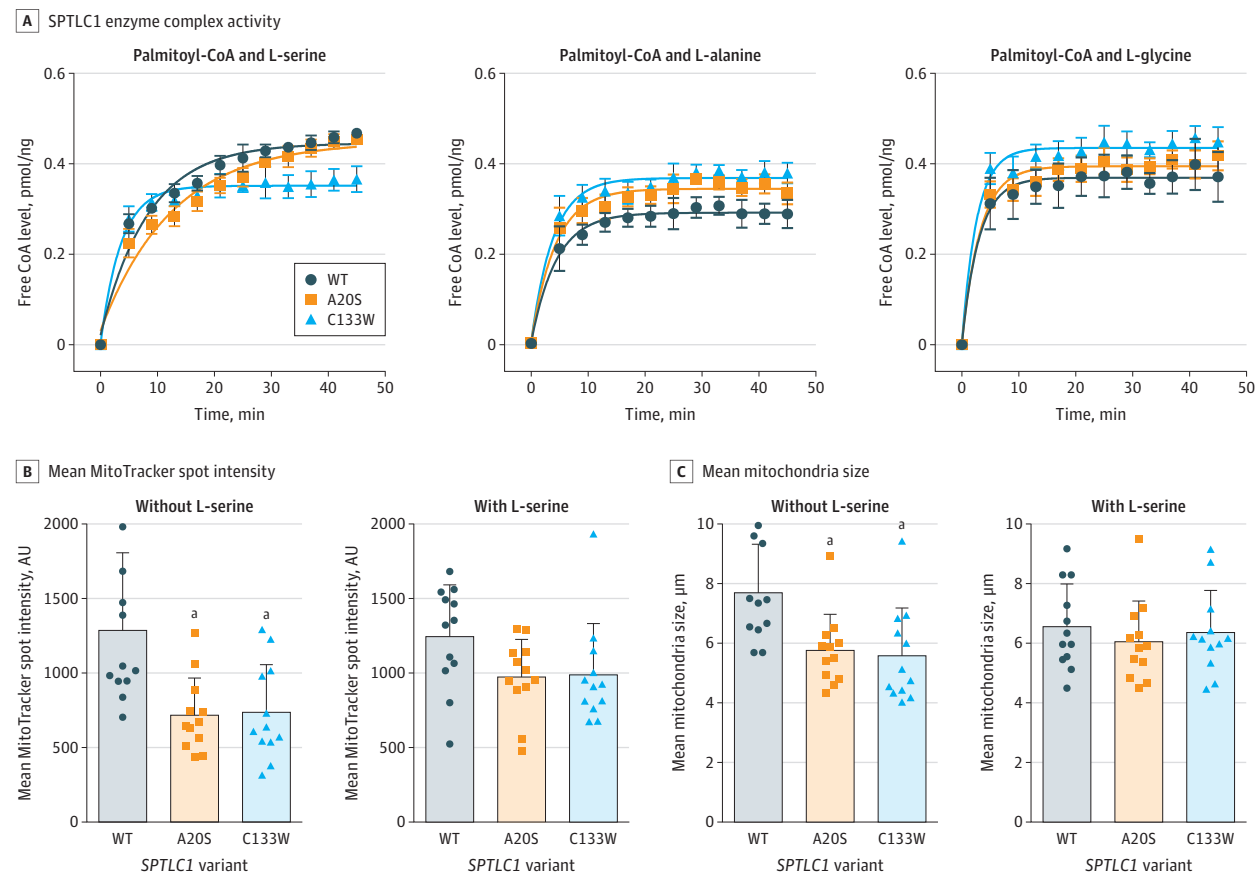
Variants in *SPTLC1* are a known cause of autosomal-dominant hereditary sensory and autonomic neuropathy, type 1A (HSAN1; OMIM, 162400).^{17,18} The protein encoded by *SPTLC1* is an essential subunit of serine palmitoyltransferase (SPT), the enzyme that catalyzes the first and rate-limiting step in the de novo synthesis of sphingolipids.¹⁹ A characteristic feature of *SPTLC1* variants associated with HSAN1 is a shift in substrate specificity of SPT to L-alanine and L-glycine, leading to the formation of an atypical class of deoxysphingolipids.²⁰ These neurotoxic metabolites accumulate within cells as they cannot be converted to complex sphingolipids nor degraded by the catabolic pathway.²⁰

Based on this information, we used a photometric assay of SPT enzyme activity to explore the association of the de novo

variants with protein function. We found that the p.Ala20Ser variant *SPTLC1* complex had an altered L-alanine and glycine preference over the canonical L-serine compared with the wild-type *SPTLC1* complex (Figure 3A; eFigure 1 in the Supplement). Differences were also observed using cell-based assays based on established HSAN1-mitochondrial phenotypes (Figure 3B and C; eFigure 1 in the Supplement).²¹ Mitochondrial size and intensity were defective to the same degree in cells expressing p.Ala20Ser and p.Cys133Trp. These defects were reversed to the wild-type phenotype on serine supplementation in the culture (Figure 3B and C; eFigure 1 in the Supplement).

SPTLC1 Variants in Patients With Adult-Onset ALS

Having established that variants in *SPTLC1* are associated with juvenile ALS, we explored the role of variation in this gene in the pathogenesis of adult-onset ALS by evaluating the occurrence of *SPTLC1* variants in a series of 6258 patients with adult-onset ALS. This screening identified 20 novel *SPTLC1* variants in 23 patients with ALS (0.4%) that were rare or absent in healthy controls and were predicted to be damaging (eTable 3 and eFigures 2 and 3 in the Supplement). The typical clinical features of ALS were observed among these patients with adult-onset disease, and none of the patients reported sensory or autonomic involvement (eTable 4 in the Supplement). The intensity and number of motor neurons staining with *SPTLC1* were diminished in autopsy tissue obtained from a patient with ALS carrying a p.Arg445Gln variant in *SPTLC1* (eFigure 4 in the Supplement). Gene burden testing was not sig-

Figure 3. Photometric and Cell-Based Assays in the Presence of Select *SPTLC1* Variants

A, The *SPTLC1* enzyme complex activity was determined using a photometric assay measuring the release of free coenzyme A (CoA) from the condensation reaction between palmitoyl-CoA and L-serine, L-alanine, and L-glycine. The variant p.Ala20Ser and p.Cys133Trp *SPTLC1* complex had increased preference for L-alanine and L-glycine over L-serine compared with the wild-type (WT) *SPTLC1* complex. B and C, Mitochondria in HEK293 cells expressing WT,

p.Ala20Ser, and p.Cys133Trp were assessed using MitoTracker on a high-content imager. Mitochondrial intensity and mitochondria size were smaller in cells expressing variant protein under standard culture conditions. Supplementation of 100 mM L-serine in the culture media for 48 hours rescued the mitochondrial abnormalities in the p.Ala20Ser and p.Cys133Trp lines.

nificant for *SPTLC1* variants as a cause of adult-onset ALS (87 variants in population samples; uncorrected 1-sided Fisher test *P* value using TRAPD software package = 1.9×10^{-4} ; not significant after correction for multiple testing of 20 000 genes).

Discussion

We provide genetic, biochemical, and cellular data that variations in *SPTLC1* are associated with juvenile ALS. First, we found 3 unrelated patients diagnosed with juvenile ALS who carried de novo variants in *SPTLC1* and identified a fourth patient with juvenile ALS carrying another *SPTLC1* variant for whom inheritance could not be determined. These variants were not present in our in-house control data set or in online databases of human variants, indicating they were rare variants in diverse populations. Two of the patients carried the same alanine to serine amino acid shift at position 20 of the protein, which arose from different nucleotide changes.

Second, cell-based assays of SPT activity confirmed that the p.Ala20Ser variation altered the encoded enzyme's function, leading to increased aberrant utilization of alanine and glycine as substrates. This biochemical pattern was consistent with a mechanism reported in patients with HSAN1 caused by *SPTLC1* variants.²⁰ Third, we used immunohistochemistry to demonstrate that *SPTLC1* is abundantly expressed within the motor neurons of healthy spinal cord tissue.

Though labeled as HSAN1, the phenotypes associated with variants in *SPTLC1* are varied, with patients manifesting various combinations of sensory loss, autonomic dysfunction, and motor weakness.²² Indeed, there is a previous report of a de novo p.Ser331Tyr variant in *SPTLC1* in a young French girl presenting with a similar phenotype to the patients in this article.¹⁶ Her clinical picture consisted of severe growth restriction, cognitive impairment, amyotrophy, hyperreflexia, vocal cord paralysis, and respiratory failure, although this patient was not diagnosed as having juvenile ALS. More recently, retinal disease has been reported in patients carrying *SPTLC1* variants.²³ This clinical heterogeneity has been linked to the differing ef-

fects of each variant on *SPTLC1* enzyme-substrate preference,²⁰ and we observed similar differences in substrate utilization across the variants that we had studied at the enzymatic level (Figure 3A). Alternatively, the phenotypes associated with variants in *HSAN1* may represent a continuum between sensory neuropathy and ALS. Future postmortem studies that determine the central nervous system pathology (eg, TAR DNA-binding protein 43, tau, β -amyloid deposition) underlying the motor neuron deficits and the cognitive impairment may resolve the nature of this overlap with other neurodegenerative diseases.

Perturbed sphingolipid metabolism underlies many neurological disorders, such as Niemann-Pick disease and Gaucher disease,²⁴ and may play a role in the pathogenesis of Alzheimer disease.²⁵ Sphingolipid metabolism has also been implicated in motor neuron degeneration. For example, patients with partial deficiency of hexosaminidase A enzyme activity (also known as GM2 gangliosidosis, a form of sphingolipidosis) may have clinical manifestations mimicking ALS.²⁶ The accumulation of ceramides and cholesterol esters also occurs within the spinal cords of patients with ALS and an *SOD1* transgenic mouse model of ALS.²⁷

Owing to the poor prognosis observed among patients with juvenile ALS and work published by other groups,^{20,28} patient 2 was commenced on high-dose (10 g per day) oral serine supplementation on a compassionate basis. Her body weight increased during this off-label treatment, which was the first time she had gained weight in several years. The patient's ceramide levels were within normal range and trending downwards, indicating that ceramide toxic effects, a theoretical possibility with serine treatment, were not present (eFigure 5 and eTable 5 in the Supplement). We did not observe evidence of neurological improvement, although prolonged therapy would be required to detect such an effect.²⁹

Serine is a nonessential amino acid that is available as a low-cost nutritional supplement. A 10% serine-enriched diet was associated with a reduction in neurotoxic deoxysphingo-

lipid plasma levels both in transgenic mice expressing the p.Cys133Trp *SPTLC1* variant and in human patients diagnosed with *HSAN1*.²⁸ Furthermore, a safety trial involving 20 patients with adult-onset ALS demonstrated that high doses of oral serine are well tolerated and that this polar amino acid is actively transported across the blood-brain barrier.³⁰ Nutritional supplementation has proven to be remarkably effective in other forms of ALS.³¹ Despite these supportive data, future clinical trials are needed to determine the effectiveness and safety profile of serine supplementation in patients with juvenile ALS owing to *SPTLC1* variants.

Limitations

Our study had limitations. DNA was not available from the parents of patient 4, so it was not possible to determine whether or not the variation arose spontaneously. Nevertheless, the lack of a family history supports the possibility that this variant was de novo in origin; there is only a 3.1% chance that none of her 5 siblings would have inherited an autosomal-dominant variant from a transmitting parent. Our evidence also demonstrates that variants in *SPTLC1* are not a common cause of adult-onset ALS. Overall, our data imply that the genetic causes of juvenile ALS and adult-onset ALS are distinct.

Conclusions

In conclusion, our data broaden the phenotype associated with variants in *SPTLC1* to include juvenile ALS and implicate sphingolipid metabolism as a pathway in motor neuron disease. Our findings are relevant in light of the fact that nutritional supplementation with serine has been postulated to ameliorate the toxic effect of abnormal sphingolipid metabolites if instituted at an early stage in the disease.²⁸ In such cases, abnormal plasma metabolites could be used as a marker of target engagement.³² This provides an early opportunity for future clinical trials to test the precision medicine approach in an otherwise fatal neurodegenerative disease.

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