

# Randomized trial of L-serine in patients with hereditary sensory and autonomic neuropathy type 1

Vera Fridman, MD, Saranya Suriyanarayanan, PhD, Peter Novak, MD, PhD, William David, MD, PhD, Eric A. Macklin, PhD, Diane McKenna-Yasek, BSN, Kailey Walsh, BS, Razina Aziz-Bose, BA, Anne Louise Oaklander, MD, PhD, Robert Brown, MD, DPhil,\* Thorsten Hornemann, PhD,\* and Florian Eichler, MD\*

## Correspondence

Dr. Eichler  
feichler@partners.org

Neurology® 2019;92:e359-e370. doi:10.1212/WNL.0000000000006811

## Abstract

### Objective

To evaluate the safety and efficacy of L-serine in humans with hereditary sensory autonomic neuropathy type I (HSAN1).

### Methods

In this randomized, placebo-controlled, parallel-group trial with open-label extension, patients aged 18–70 years with symptomatic HSAN1 were randomized to L-serine (400 mg/kg/day) or placebo for 1 year. All participants received L-serine during the second year. The primary outcome measure was the Charcot-Marie-Tooth Neuropathy Score version 2 (CMTNS). Secondary outcomes included plasma sphingolipid levels, epidermal nerve fiber density, electrophysiologic measurements, patient-reported measures, and adverse events.

### Results

Between August 2013 and April 2014, we enrolled and randomized 18 participants, 16 of whom completed the study. After 1 year, the L-serine group experienced improvement in CMTNS relative to the placebo group (–1.5 units, 95% CI –2.8 to –0.1,  $p = 0.03$ ), with evidence of continued improvement in the second year of treatment (–0.77, 95% CI –1.67 to 0.13,  $p = 0.09$ ). Concomitantly, deoxysphinganine levels dropped in L-serine-treated but not placebo-treated participants (59% decrease vs 11% increase;  $p < 0.001$ ). There were no serious adverse effects related to L-serine.

### Conclusion

High-dose oral L-serine supplementation appears safe in patients with HSAN1 and is potentially effective at slowing disease progression.

### Clinicaltrials.gov identifier

NCT01733407.

### Classification of evidence

This study provides Class I evidence that high-dose oral L-serine supplementation significantly slows disease progression in patients with HSAN1.

## MORE ONLINE

### → Class of Evidence

Criteria for rating therapeutic and diagnostic studies

[NPub.org/coe](http://NPub.org/coe)

\*These authors contributed equally to this work.

From the Department of Neurology (V.F., W.D., K.W., R.A.-B., A.L.O., F.E.), Biostatistics Center, Department of Medicine (E.A.M.), and Department of Pathology (Neuropathology) (A.L.O.), Massachusetts General Hospital, Harvard Medical School, Boston; Clinical Chemistry (S.S., T.H.), University Hospital Zurich, Switzerland; and University of Massachusetts Medical School (P.N., D.M.-Y., R.B.), Worcester.

Go to [Neurology.org/N](http://Neurology.org/N) for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

## Glossary

**1-deoxySL** = 1-deoxysphingolipid; **AFT** = autonomic function testing; **CI** = confidence interval; **CIPN** = chemotherapy-induced peripheral neuropathy; **CMT** = Charcot-Marie-Tooth; **CMT1A** = Charcot-Marie-Tooth type 1A; **CMTES** = Charcot-Marie-Tooth Examination Score; **CMTNS** = Charcot-Marie-Tooth Neuropathy Score; **HSAN1** = hereditary sensory autonomic neuropathy type I; **MGH** = Massachusetts General Hospital; **NPS** = Neuropathy Pain Scale; **SF-36** = 36-item Short Form health questionnaire; **SPT** = serine palmitoyltransferase.

Hereditary sensory autonomic neuropathy type I (HSAN1) is a debilitating, progressive disorder of peripheral nerve that results in sensory loss, neuropathic pain, varying degrees of limb weakness, and mutilating skin ulceration severe enough to cause infection and warrant limb amputation.<sup>1</sup> The disease most commonly results from missense mutations in the genes *SPTLC1* and *SPTLC2*, which encode for 2 of the 3 subunits of serine palmitoyltransferase (SPT). SPT catalyzes the first step in the de novo synthesis of sphingolipids by conjugating palmitoyl-CoA and L-serine.<sup>2,3</sup> HSAN1 mutations reduce the affinity of the enzyme for L-serine and increase its affinity for alanine and glycine, thereby leading to formation of an atypical class of neurotoxic 1-deoxysphingolipids (1-deoxySLs).<sup>4,5</sup> To date, no effective disease-modifying therapy has been identified for HSAN1 or for any of the varied forms of Charcot-Marie-Tooth (CMT) disease.

The discovery that HSAN1 is driven by the formation of toxic lipid metabolites has provided a unique opportunity to devise a biologically rational and potentially disease-modifying therapy. Two important findings have implicated 1-deoxySL in HSAN1 pathogenesis. First, 1-deoxySLs accumulate preferentially in the peripheral nerves of transgenic HSAN1 mice while sparing the CNS. Second, 1-deoxySLs are neurotoxic and affect neurite outgrowth in cultures of dorsal root ganglia.<sup>4-6</sup> Elevated 1-deoxySLs have also been demonstrated in type 2 diabetes mellitus, where they might contribute to beta cell failure, and in diabetic neuropathy, which shares many phenotypic features with HSAN1.<sup>7-9</sup>

Importantly, it has been demonstrated that 1-deoxySL formation in humans and mice can be reduced with high doses of L-serine, the normal substrate of SPT.<sup>10</sup> Dietary supplementation in transgenic mice resulted in significant reductions of plasma 1-deoxySL levels paralleled by clinical improvement, including improved motor performance and increased unmyelinated sciatic nerve fibers.<sup>10</sup> A pilot study of L-serine in 14 patients with HSAN1 similarly demonstrated reductions in plasma 1-deoxySL levels.<sup>10</sup> Here, we report a randomized, placebo-controlled trial evaluating the safety and efficacy of L-serine as a treatment for HSAN1.

## Methods

The primary research question of this study was whether L-serine is safe and clinically efficacious in humans with HSAN1. The study provides Class I evidence that high-dose

oral L-serine supplementation is safe and significantly slows disease progression in HSAN1.

## Standard protocol approvals, registrations, and patient consents

Patients were enrolled at the Massachusetts General and University of Massachusetts Hospitals (ClinicalTrials.gov Identifier: NCT01733407). The protocol was approved by the institutional review boards at both sites and all participants provided written informed consent. The US Food and Drug Administration accepted the Investigational New Drug application (110,957).

## Patients

Eligibility required being at least 18 years of age and having confirmed HSAN1 based on *SPTLC1* mutations and clinical symptoms of a peripheral neuropathy. Exclusion criteria comprised other risk factors for polyneuropathy, history of nephrolithiasis, presence of other serious medical conditions, need for anticoagulation, psychiatric disease or cognitive impairment limiting ability to give consent, and exposure to L-serine within 30 days prior to enrollment. Women who were pregnant, breastfeeding, or planning to become pregnant during the study were also excluded. Women of childbearing age were advised to use contraception for the duration of the study and were counseled to stop L-serine immediately and notify the treating physician if they became pregnant.

## Randomization and masking

After enrollment, patients were referred to the Massachusetts General Hospital (MGH) pharmacy, where the research pharmacist independently provided study medications based on the allocation sequence. Patients were randomly assigned to receive either L-serine (administered in powder form to be dissolved in water) or matching placebo (dextrose hydrous powder) in a 1:1 ratio. L-Serine and placebo powder were identical in appearance, taste, and smell, and were centrally packed and labeled before being distributed from MGH. The randomization schedule used a permuted-block structure with blocks of 4. The schedule and allocation sequence was generated using *mrandomization.com* by CLIPA Clinical Packaging, an independent contract research organization. Only the study biostatistician and MGH research pharmacist were unblinded to the individual drug assignments in this study. The principal investigator and project management, data management, and site staff were blinded to treatment group assignments. Levels of alanine, citrulline, glycine, and serine were scrubbed by the study biostatistician from amino acid

panel results prior to distribution to the study team to maintain the blind. Unblinded laboratory assays and results of sphingolipid analyses were not shared with the study team until after the database was locked.

## Procedures

L-Serine 400 mg/kg/d was administered in 3 divided doses. This dose was chosen based on the HSAN1 pilot study and on previous observations that higher doses can cause reversible side effects including nausea, vomiting, nystagmus, and myoclonus.<sup>10,11</sup> The active drug was manufactured at Ajinomoto North America pharmacy and placebo was manufactured at University of Iowa. Participants were instructed to return all empty and unused study medication containers at each visit. Drug compliance was assessed by tracking the number of unused study medication bottles and analyzing plasma L-serine and 1-deoxySL levels.

After providing informed consent, patients underwent physical examinations and laboratory testing to determine eligibility. Eligible patients returned for baseline assessments and were randomly assigned to treatment groups. Participants were treated with either L-serine or placebo for 1 year, followed by crossover to open-label L-serine by all participants for 1 additional year. Follow-up visits were conducted at 24, 48, 72, and 96 weeks from baseline. Physical examination, Charcot-Marie-Tooth Neuropathy Score version 2 (CMTNS), laboratory studies including sphingolipid and amino acid levels, and skin biopsy were performed at every visit and adverse events were reviewed and coded to MedDRA terms (version 16.1). Nerve conduction studies, autonomic function tests, the 36-item Short Form health questionnaire (SF-36), and the Neuropathy Pain Scale (NPS) were performed yearly. Safety assessments comprised physical examination, adverse events, serious adverse events, and safety laboratory tests. All clinical assessments were performed by one of 2 examiners who were blinded to treatment group assignments.

The primary endpoint was the CMTNS, a reliable and valid composite score based on patient symptoms, signs, and electrophysiology that has been validated to measure progression in CMT type 1A (CMT1A). The score covers 9 items, each scored on a 0–4 scale for a total of 0–36 points, with higher scores indicating increased severity of neuropathy. Scores are classified as reflecting mild (CMTNS  $\leq 10$ ), moderate (CMTNS 11–20), or severe (CMTNS  $>20$ ) neuropathy. Secondary endpoints included nerve conduction studies, autonomic function testing (AFT), density of epidermal nerve fibers at the distal leg and proximal thigh, the SF-36, the NPS, patients' dietary journals with focus on amino acid consumption, plasma sphingolipid levels, plasma amino acid levels, and adverse events.

Nerve conduction studies (peroneal–extensor digitorum brevis, peroneal–tibialis anterior, tibial–abductor hallucis, median–abductor pollicis brevis, and ulnar–abductor digiti

minimi motor responses, and sural, superficial peroneal, radial, median, and lateral antebrachial sensory responses) were performed by one of 2 electrophysiologists at MGH using the same equipment and techniques. Limb temperature was maintained greater than 32°C.

Comprehensive AFT evaluated cholinergic, adrenergic, and sudomotor domains. Heart rate and blood pressure were monitored during tilt-table testing, deep breathing, and Valsalva maneuver. Deep breathing was performed at the rate of 6 breaths/minute for 1 minute. The Valsalva maneuver was performed with an expiratory pressure of 40 mm Hg for 15 seconds. Participants were tilted for 10 minutes. Quantitative sudomotor axonal reflex testing was performed at a simulation current of 2 mA for 5 minutes. Blood pressure was monitored continuously (Finometer; Finapres, Amsterdam, the Netherlands; and Dinamap ProCare 10; GE, Fairfield, CT). The validated Composite Autonomic Scoring Scale was used to quantitate and assess AFT results.<sup>12</sup>

For neuropathologic assessments, 3-mm-diameter skin punches were removed from the standard distal leg site (10 cm above the lateral malleolus) and in the upper thigh by experienced neurologists using local anesthesia and standard practices.<sup>13</sup> Biopsies were fixed in Zamboni fixative, then processed and analyzed by the clinical diagnostic skin biopsy laboratory at MGH according to consensus standards. Free-floating 50-mm vertical sections were labeled immunohistochemically against PGP9.5, a pan-neuronal marker (Chemicon, Temecula, CA), to reveal epidermal nerve fibers that were counted using standard rules by a skilled morphometrist blinded to group allocation.

For sphingolipid analyses, discarded venous blood was sampled in EDTA tubes and immediately frozen at 4°C until all clinical laboratory testing was complete. Plasma was obtained after spinning the samples at 3,400 rpm for 15 minutes at room temperature. Samples were aliquoted, anonymized, and immediately frozen to –80°C. For sphingoid base analysis, the samples were shipped on dry ice to the University of Zurich. The sphingoid base profile was analyzed by liquid chromatography/mass spectrometry after hydrolyzing the N-acyl and O-linked headgroups as described earlier.<sup>14</sup> Analyzed sphingoid bases included C16SO, C16SA, C17SO, C17SA, C18SO, C18SA, C19SO, C19SA, C20SO, C20SA, C18-Sadiene, 1-deoxysphinganine, and 1-deoxysphingosine. Other serum measures included amino acids, complete blood count, hepatic function, and renal function.

## Statistical analysis

The proportion of participants advancing by more than 1 point in the CMTNS from baseline to the 48-week visit was compared between the placebo and L-serine arms by Fisher exact test. As a more sensitive assessment, longitudinal changes in CMTNS were analyzed in a shared-baseline, random-slopes linear mixed model with fixed effects of visit (categorical) and treatment  $\times$  postbaseline visit interaction

and random participant-specific intercepts and slopes with unstructured covariance. Subgroup analyses of sex, age, baseline CMTNS, and time since symptom onset (median split for all continuous measures) were examined by adding fixed effects of subgroup, subgroup  $\times$  visit, and subgroup  $\times$  treatment  $\times$  postbaseline visit. Forty-eight-week CMTNS change scores were also compared by exact Wilcoxon rank sum test. Changes in L-serine, log-transformed 1-deoxySL levels, NPS scores, and SF-36 domain scores were all analyzed in equivalent shared-baseline, random-slope linear mixed models. Because of their coarse and strongly skewed distribution, AFT and density change scores were analyzed by exact Wilcoxon rank-sum test. Spearman correlation coefficients were used to estimate associations between CMTNS and 1-deoxySL levels at baseline and as change scores. All analyses were performed in SAS (version 9.4; SAS Institute, Cary, NC). Two-tailed *p* values are reported without adjustment for multiple comparisons given the limited power of the trial.

### Role of funding source and trial oversight

The study funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author and all coauthors had full access to all the data in the study and had final responsibility for the decision to submit for publication. The protocol was approved by the institutional review board at every site, and all participants provided written informed consent. The trial was performed under a Food and Drug Administration Investigational New Drug approval (110957) and registered on ClinicalTrials.gov (NCT01896102). An independent Data and Safety Monitoring Board reviewed the safety data annually. The study was conducted in accordance with the provisions of the Declaration of Helsinki and the authors vouch for the conduct of the trial, adherence to the trial protocol, and the accuracy of the data and analysis.

### Data availability

Trial design, eligibility criteria, and primary and secondary outcome measures have been published on [clinicaltrials.gov](http://clinicaltrials.gov). Any data not published within this article are available in the public repository of [clinicaltrials.gov](http://clinicaltrials.gov). Following publication, anonymized data will be shared by request from any qualified investigator.

## Results

Eighteen participants were deemed eligible and enrolled into the study between August 2013 and April 2014. There were no screen failures. Nine were randomized to L-serine and 9 to placebo (figure 1). Baseline characteristics were well-balanced between the 2 study groups, except for higher epidermal nerve fiber densities at the thigh biopsy site in the L-serine group (table 1). Participants' mean age ( $\pm$ SD) was  $47.8 \pm 14.0$  years (range 29.1–80.4 years), and 67% were female. Mean disease duration since symptom onset was  $24.7 \pm 14$  years (range 4–57 years). The average CMTNS at baseline was  $22.6 \pm 8.6$

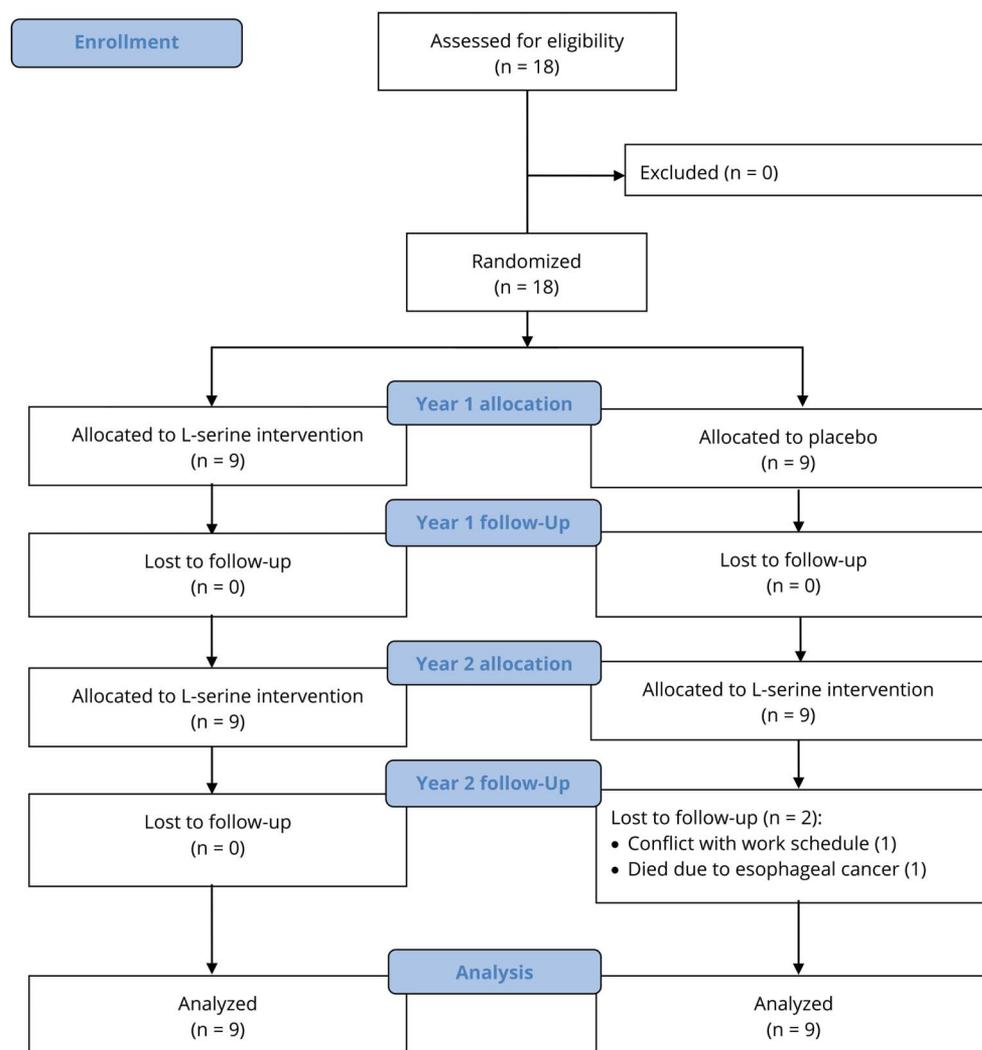
(range 5–33). All participants reported reduced sensation or painful paresthesias in their feet as the presenting symptom and all had experienced limb ulcers. One hundred percent of participants in the L-serine group and 78% in the placebo group completed the study. Of the 2 participants lost to follow-up, one had a malignancy prior to initiating the study and died of esophageal cancer before completing the study, and another left the study in the second year due to disease progression and increased work demands.

The primary outcome, defined as a  $>1$  point increase in CMTNS over 1 year, consistent with progression of neuropathy, was achieved by 1 participant in the L-serine group (11%) and 2 participants in the placebo group (22%,  $p = 1.0$ ). Participants randomized to L-serine had steady reductions in CMTNS over the 2 years of treatment (mean  $\pm$  SE change of  $-1.14$  points  $\pm 0.76$  between baseline and 2 years,  $p = 0.15$ ) (figure 2). In contrast, participants taking placebo experienced a mean  $\pm$  SE increase of 1.1 point  $\pm 0.47$  in CMTNS during the first year of the study ( $p = 0.02$ ). At 1 year, L-serine participants experienced a decline in CMTNS relative to those taking placebo ( $-1.5$  units, 95% confidence interval [CI]  $-2.8$  to  $-0.1$ ,  $p = 0.03$ ). Both groups improved on L-serine treatment during the second year, with the relative difference in the CMTNS unchanged ( $-1.4$  units, 95% CI  $-3.6$  to  $0.76$ ,  $p = 0.19$ ). Combining data from the first year of L-serine treatment for all participants (0–48 weeks for the L-serine group and 48–96 weeks for the placebo group) suggested a consistent treatment effect ( $-0.6$  points on the CMTNS,  $p = 0.08$ , 95% CI  $-1.3$  to  $0.08$ ), with no difference depending on the timing of treatment initiation ( $p = 0.52$ ). In addition to the CMTNS, we also examined the Charcot-Marie-Tooth Examination Score (CMTES), a subscore of the CMTNS, calculated as the sum of the symptoms and signs with exclusion of the electrophysiologic measures. The CMTES showed similar results to CMTNS (relative change of  $-1.2$  points, 95% CI  $-2.4$  to  $0.0$ ,  $p = 0.05$  at 1 year, and  $0.83$  points, 95% CI  $-2.7$  to  $1.0$ ,  $p = 0.37$  at 2 years), suggesting that electrophysiologic findings did not contribute importantly to treatment response.

The CMTNS items that contributed most to the benefit from L-serine supplementation vs placebo included sensory symptoms, strength of legs on examination, and strength of arms on examination (relative change of  $-0.48$ , 95% CI  $0.92$  to  $-0.04$ ,  $p = 0.03$ ;  $-0.44$ , 95% CI  $-0.78$  to  $-0.11$ ,  $p = 0.01$ ; and  $-0.40$ , 95% CI  $-0.77$  to  $-0.03$ ,  $p = 0.03$ , respectively). The distribution of change scores in participants taking L-serine varied across the component items. We performed subgroup analyses evaluating the effect of age (greater or less than 46 years), sex, time from diagnosis (greater or less than 20 years), and baseline CMTNS (greater or less than a score of 26 points) on treatment response and found no significant interaction between the listed variables and treatment effect.

1-deoxySL levels declined significantly in participants taking L-serine vs those on placebo following 1 year of treatment (41% decrease in 1-deoxysphingosine vs 9.2% increase on

**Figure 1** Trial profile



placebo,  $p = 0.001$ ; 60% decrease in 1-deoxysphinganine vs 9% increase on placebo,  $p < 0.001$ ) (figure 3). Most of the decline in 1-deoxySL levels occurred during the first 24 weeks of treatment. Placebo participants experienced similar declines in 1-deoxySL levels after crossing over to L-serine treatment (39% decrease in 1-deoxysphingosine,  $p = 0.001$ ; and 66% decrease in 1-deoxysphinganine,  $p = 0.001$ ). While a positive correlation was observed between baseline 1-deoxySL levels and baseline CMTNS ( $r = 0.50$ , CI  $-0.01$  to  $0.81$ ,  $p = 0.05$ ), we observed limited evidence of association between the change in 1-deoxySL levels and change in CMTNS that was largely attributable to a favorable response in one participant ( $r = 0.24$ ,  $-0.33$  to  $0.69$ ,  $p = 0.39$ ) (figure 3). No significant change was seen in the canonical sphingolipid levels following 1 year of treatment (6% decrease in sphingosine,  $p = 0.16$ , and 15% decrease in sphinganine,  $p = 0.08$ ).

At baseline, most of the distal leg skin biopsies were devoid of epidermal nerve fibers, whereas densities from the thigh site varied widely (figure 4). At 1 year, only distal biopsies from

L-serine-treated participants showed evidence of reinnervation (median change = 8 vs 0 fibers/mm<sup>2</sup> skin surface area,  $p = 0.014$ ). This pattern was not present at the thigh site, where there were no significant differences between the groups (median change = 2 vs 26 fibers/mm<sup>2</sup>,  $p = 0.099$ ) (figure 4). Changes in epidermal nerve fiber density at the distal site over 1 year were negatively correlated with changes in scores on question 1 on the CMTNS, which measured patient-reported sensory symptoms ( $r = -0.719$ ,  $-0.888$  to  $-0.380$ ,  $p < 0.001$ ); however, the opposite relationship was found at the upper thigh site ( $r = 0.62$ ,  $0.22$ – $0.84$ ,  $p = 0.004$ ). A negative correlation was also seen between changes in distal leg innervation at 1 year and 1-deoxySL levels (for 1-deoxysphingosine  $r = -0.73$ ,  $-0.91$  to  $-0.33$ ,  $p = 0.001$ ) (figure 4).

No treatment effects were detected in nerve conduction studies or AFT (table 2). In addition, none of the patient-reported outcomes revealed significant differences between treatment groups (table 2). No significant effect of treatment on NPS scores was observed, but mean levels of “unpleasant

**Table 1** Demographics and clinical characteristics at baseline

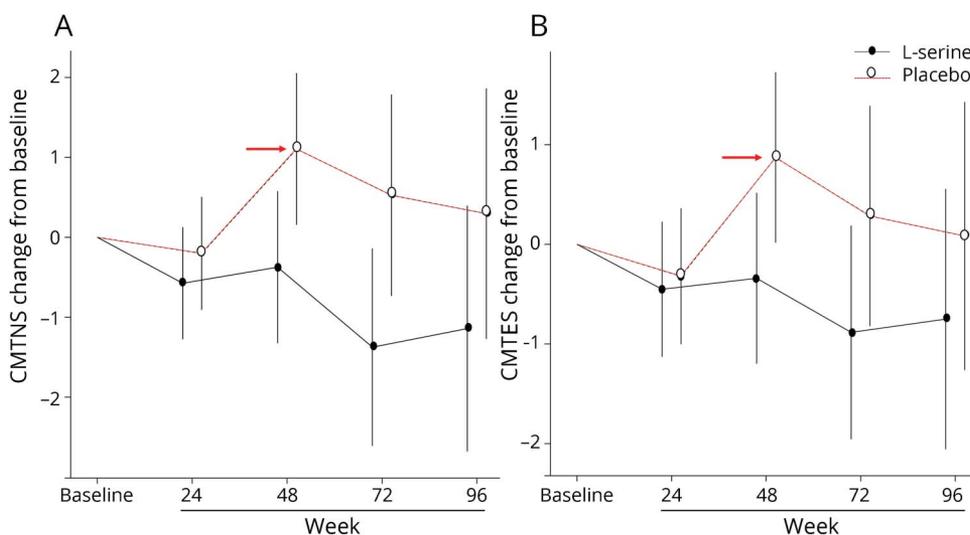
Variable	Overall, mean ± SD	Treatment assignment, mean ± SD		
		L-Serine	Placebo	p Value
Age, y	47.8 ± 14.0	45.8 ± 11.0	49.9 ± 16.9	0.55
Age at onset, y	22.7 ± 7.5	23.8 ± 9.6	21.7 ± 5.1	0.57
Right ulnar motor amplitude-wrist, mV	3.29 ± 3.92	4.16 ± 4.59	2.42 ± 3.16	0.36
Right ulnar CV forearm, m/s	45.0 ± 14.7	45.7 ± 14.8	43.9 ± 16.6	0.86
Radial sensory nerve action potential, $\mu$ V	7.32 ± 14.1	8.98 ± 17.2	5.46 ± 10.4	0.62
CMTNS	22.6 ± 8.6	20.6 ± 10.0	24.6 ± 7.0	0.34
CMTES	17.2 ± 5.8	15.8 ± 6.8	18.6 ± 4.5	0.33
ENFD distal leg, $\text{mm}^2$	0.61 ± 2.15	0.00 ± 0.00	1.22 ± 2.99	0.24
ENFD upper thigh, $\text{mm}^2$	35.8 ± 54.4	64.3 ± 66.4	7.3 ± 7.8	0.021 <sup>a</sup>
Pain score (how sharp 0–9)	7.19 ± 2.88	7.25 ± 3.20	7.13 ± 2.75	0.93
Plasma serine level, $\mu\text{mol/L}$	117 ± 22	120 ± 30	114 ± 9	0.60
1-Deoxysphingosine, $\mu\text{mol/L}$	0.58 ± 0.23	0.49 ± 0.12	0.64 ± 0.27	0.22
1-Deoxysphinganine, $\mu\text{mol/L}$	0.28 ± 0.13	0.23 ± 0.08	0.31 ± 0.16	0.27
Total CASS score	2.89 ± 1.37	2.33 ± 1.22	3.44 ± 1.33	0.084

Abbreviations: CASS = Composite Autonomic Scoring Scale; CMTES = Charcot-Marie-Tooth Examination Score; CMTNS = Charcot-Marie-Tooth Neuropathy Score version 2; CV = conduction velocity; ENFD = epidermal nerve fiber density.

<sup>a</sup>There was no significant difference between groups except for a higher baseline ENFD at the thigh in participants randomized to L-serine.

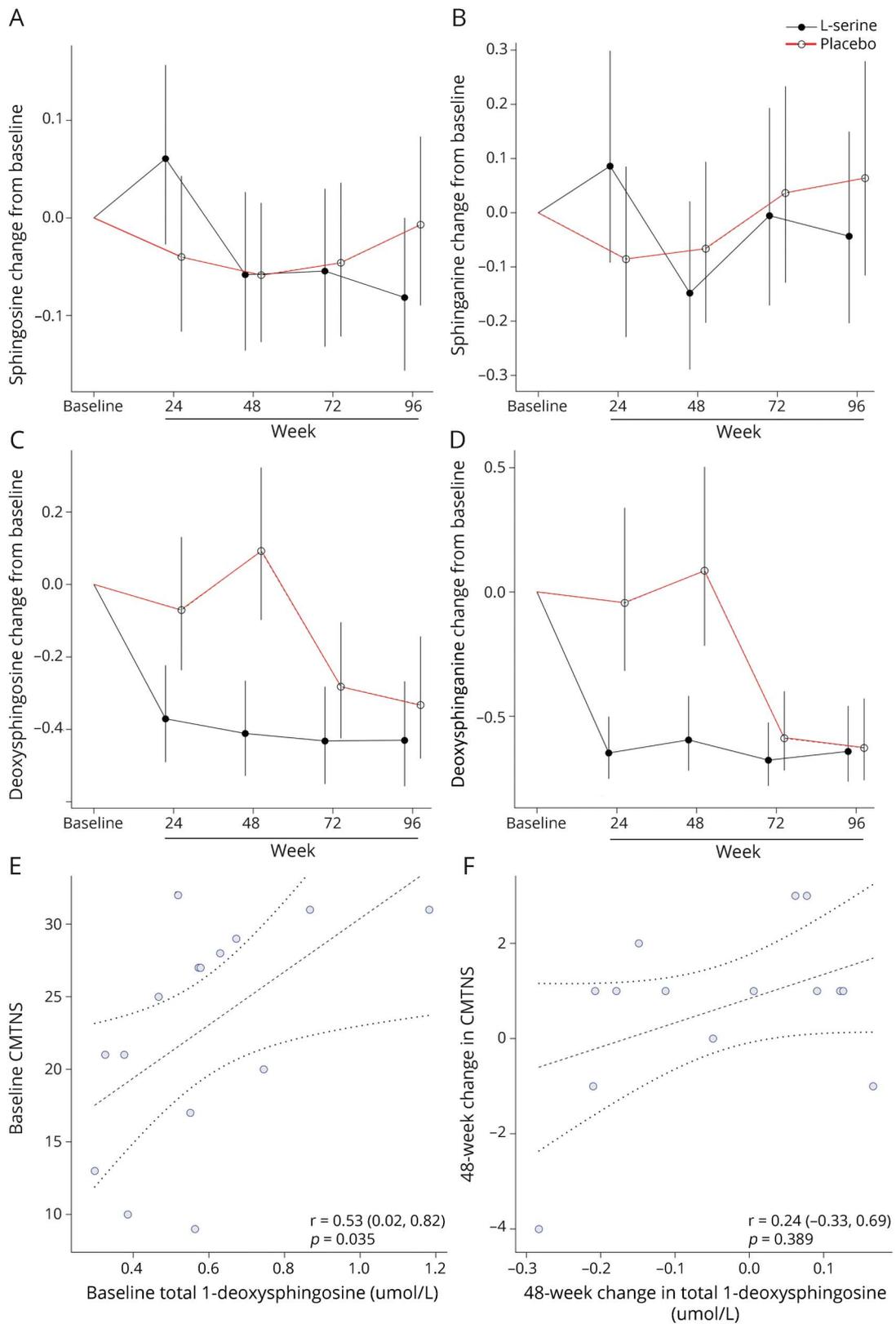
pain” were numerically lower among participants on L-serine vs those on placebo at 1 year, with further relative reduction in pain at 2 years (relative change of  $-0.18 \pm 1.2$  points,  $p = 0.88$ ; and  $-0.85 \pm 1.4$  points,  $p = 0.56$ , respectively). No difference in “sharp pain” was seen between the 2 groups following 1 year of treatment (L-serine participants had relative change

of  $0.14 \pm 1.4$  points,  $p = 0.92$ ). No significant effect of treatment on SF-36 scores was observed, but mean physical functioning component scores were numerically lower in the treatment group in the first year and in both groups following crossover to open-label L-serine ( $+4.2$  points at 1 year in L-serine group, 95% CI  $-4.2$  to  $1.5$ ,  $p = 0.29$ ) (table 2).

**Figure 2** Change in primary endpoints

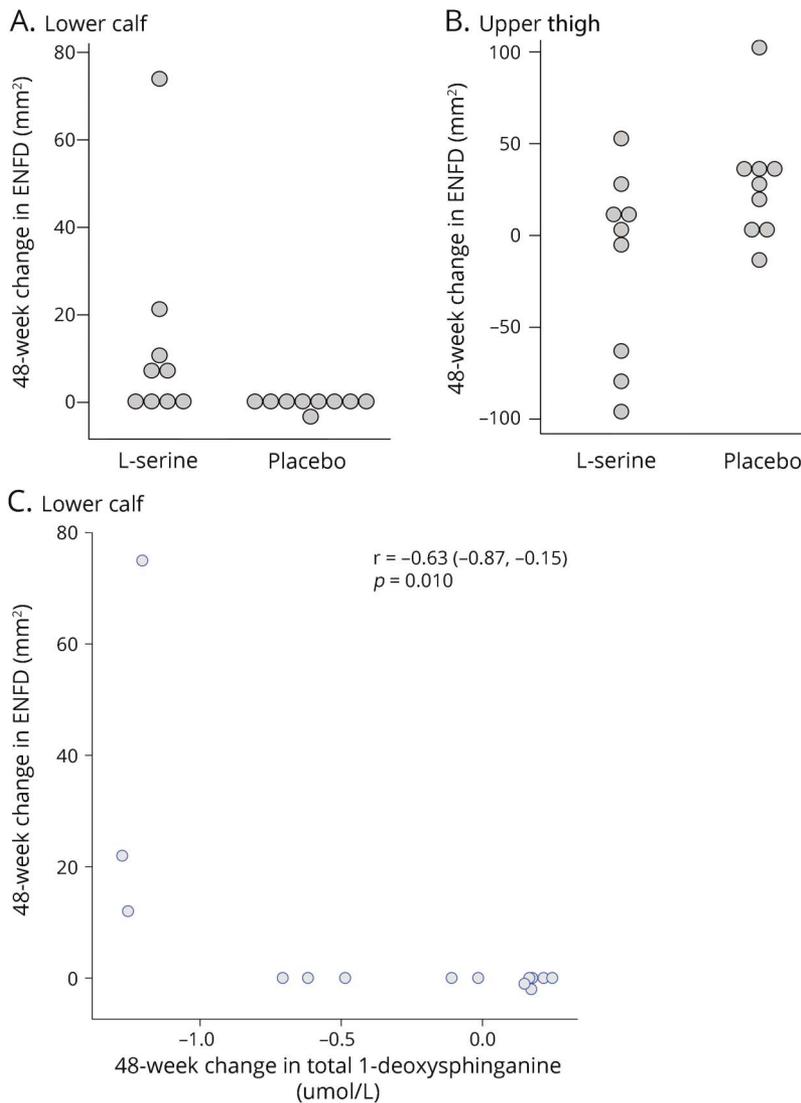
Charcot-Marie-Tooth Neuropathy Score (CMTNS) and Charcot-Marie-Tooth Examination Score (CMTES) in patients taking L-serine vs placebo during the trial period. Baseline values were normalized to zero, and data represent the mean change from baseline. At week 48, patients on placebo crossed over to L-serine (red arrows). I bars indicate 95% confidence bounds.

**Figure 3** Change in plasma sphingolipid levels



Change in plasma levels of canonical sphingolipids (A, B) and neurotoxic 1-deoxysphingolipids (C, D) over the course of the study. Baseline values were normalized to zero. Data represent the mean change from baseline. The correlation between the Charcot-Marie-Tooth Neuropathy Score (CMTNS) and deoxysphingosine levels is plotted for baseline (E) and change at 48-week follow-up (F). I bars indicate 95% confidence bounds.

**Figure 4** Change in epidermal nerve fiber density (ENFD)



Skin biopsies from the standard distal leg site (lower calf) were largely devoid of epidermal nerve fibers and demonstrated an increase in ENFD at 1 year ( $p = 0.014$ ) (A). Skin biopsies from the upper thigh revealed no significant change in ENFD in participants taking placebo vs serine (B). Correlation between changes in ENFD in the lower calf and changes in plasma deoxyseringanine levels is shown in (C).

Eighty-nine percent of the L-serine group and 100% of the placebo group reported adverse effects during the first year (table 3). Neither group had any serious adverse events, and most adverse events were of mild to moderate severity. During the second year, one placebo arm participant with a preexisting malignancy died of esophageal cancer, which was judged to be unrelated to L-serine. Fifty-six percent of participants in the treatment group reported adverse events vs 89% among participants following cross-over to open-label L-serine. Two participants (both of whom had crossed over from placebo) temporarily suspended treatment in year 2 in the setting of infection (bladder infection and a gastrointestinal virus). Adverse events that were more common in participants taking L-serine vs placebo included localized infection (6 of 9 participants [67%] vs 4 of 9 participants [44%]), MRSA infection (1 of 9 participants [11%] vs 0 participants), osteomyelitis requiring amputation (2 of 9 participants [22%] vs 0 participants), abdominal pain, and

dyspepsia (2 of 9 participants [22%] vs 0 participants). No participants reported vomiting, nystagmus, acoustic startles, or myoclonus, as in prior L-serine studies.<sup>11</sup> Analysis of vital signs, physical examination findings, and clinical laboratory measurements did not reveal adverse effects of L-serine. Plasma L-serine levels were highly variable in both the treatment and placebo group throughout the first year of treatment, with a nonsignificant relative increase seen among participants randomized to L-serine ( $95.6 \pm 48.3 \mu\text{mol/L}$ ,  $p = 0.06$ ), despite 1-deoxySL changes demonstrating good compliance in the treatment group.

## Discussion

In this randomized, double-blinded, placebo-controlled, 2-year trial in adults with HSAN1, L-serine treatment was well-tolerated and produced biochemical improvement with significant reductions in 1-deoxySL levels. While the primary

**Table 2** Change in secondary endpoints from baseline to 1 year

	L-serine, mean ± SD or mean (95% CI)	Placebo, mean ± SD or mean (95% CI)	<i>p</i> Value <sup>a</sup>
<b>Nerve conduction studies</b>			
Ulnar motor amplitude–wrist, mV	1.26 ± 1.42	−0.07 ± 1.12	0.026
Ulnar CV forearm, m/s	0.55 ± 4.58	−3.08 ± 4.72	0.26
Radial sensory amplitude, μV	0.74 ± 2.47	−1.69 ± 4.68	0.42
<b>Autonomic function testing</b>			
Cardiovagal	0.11 ± 0.33	0.00 ± 0.50	1.0
Sudomotor	−0.33 ± 1.12	−0.11 ± 0.60	0.84
Adrenergic	0.11 ± 0.60	0.22 ± 0.44	1.0
Total CASS score <sup>12</sup>	−0.11 ± 1.54	0.11 ± 0.93	0.97
<b>Neuropathy pain scale</b>			
Unpleasant pain	−0.02 (−2.39 to 2.35)	0.16 (−2.19 to 2.51)	0.88
Sharp pain	0.37 (−2.29 to 3.03)	0.23 (−2.41 to 2.88)	0.92
<b>SF-36</b>			
Physical functioning component score	1.21 (−5.14 to 7.56)	−2.97 (−9.33 to 3.40)	0.29
Social functioning component score	0.89 (−4.87 to 6.66)	1.25 (−4.54 to 7.03)	0.91

Abbreviations: CASS = Composite Autonomic Scoring Scale; CI = confidence interval; CV = conduction velocity; SF-36 = 36-item Short Form health questionnaire.

<sup>a</sup> *p* Values from exact Wilcoxon rank-sum tests for nerve conduction studies and autonomic function testing. *p* Values from shared-baseline random-slope models for Neuropathy Pain Scale and SF-36.

outcome of proportion of patients progressing more than 1 point on the CMTNS at 1 year did not differ between the L-serine and placebo groups (11% vs 22%, *p* = 1.0), the discriminatory power of this endpoint was limited by the small sample size and low event rate in both arms. Participants taking L-serine did demonstrate a significant quantitative improvement in CMTNS as compared to those taking placebo during the first year of the study. Furthermore, placebo-treated participants showed similar rates of improvement in CMTNS after crossover to L-serine treatment, further suggesting that L-serine may offer clinical benefit in HSAN1.

We found no evidence that the effect of L-serine on CMTNS differed according to age, sex, disease duration, or baseline CMTNS. The individual CMTNS items that contributed most to the L-serine-associated benefit vs placebo were patient-reported sensory symptoms and upper and lower extremity strength on examination; however, areas of improvement varied among participants. While no significant treatment effect was seen on quality of life measures, the observed numeric improvements seen on all the physical well-being domains of the SF-36 were consistent with benefit from L-serine treatment. Although we previously reported that some patients with HSAN1 experience worsening paresthesias on L-serine, potentially as a consequence of sensory nerve regeneration, we did not observe any treatment-

associated worsening in sharp pain in this study.<sup>10</sup> Treated participants did not have fewer skin ulcers, one of the more debilitating manifestations of HSAN1, and both skin infections and osteomyelitis occurred with higher frequency in the treatment group. To our knowledge, there are no prior reports of either alteration in immune function or heightened propensity towards infection resulting from L-serine supplementation. Many participants in our study had severe and longstanding neuropathy and were therefore susceptible to recurrent limb ulceration.<sup>1,15</sup> Treatment earlier in the disease course may therefore elucidate whether L-serine can prevent or reduce infections in HSAN1.

L-Serine supplementation was associated with significant reductions in levels of 1-deoxySL, with near-normal levels achieved within 24 weeks after starting on treatment, and continued 1-deoxySL suppression throughout the 2-year study period. In contrast to prior findings, supplementation was not associated with changes in the canonical, serine-based sphingolipids.<sup>10</sup> There are conflicting data regarding the role of endogenous lipid derangements in HSAN1, with initial reports indicating abnormal levels of select species, and subsequent studies showing no change in canonical lipid profiles.<sup>3,5,16,17</sup> Our findings further support the hypothesis that the potential treatment effect of L-serine is a consequence of 1-deoxySL suppression rather than shifts in endogenous

**Table 3** Adverse events, irrespective of cause, in the randomized study

MedDRA system organ class/ preferred term	Placebo period 1 (n = 9)	Placebo crossover to L-serine (n = 9)	L-Serine period 1 (n = 9)	Combined first-year exposure to L-serine (n = 18)	Second-year exposure to L-serine (n = 9)
L-Serine exposure, y	0	0–1	0–1	0–1	1–2
Any adverse event, n (%)	9 (100)	8 (89)	8 (89)	16 (89)	5 (56)
Vertigo	0	11	0	6	0
Gastrointestinal symptoms	11	0	22	11	0
Local swelling	0	11	0	6	0
Any infections or infestations	67	56	56	56	56
Any injury, poisoning, or procedural complication	22	0	44	22	0
Electrolyte or CBC abnormality	11	11	0	6	11
Vitamin D deficiency	0	0	11	6	0
Musculoskeletal or connective tissue disorder	33	0	22	11	0
Esophageal carcinoma	0	11	0	6	0
Oropharyngeal pain	0	11	0	6	0
Skin or subcutaneous tissue abnormality	11	11	22	17	11
Finger amputation	0	0	11	6	11
Hypertension	11	0	0	0	0

Abbreviation: CBC = complete blood count.  
Values are percentages.

sphingoid base formation.<sup>18</sup> Post hoc analysis indicated a positive correlation between baseline 1-deoxySL levels and CMTNS; however, we did not observe a clear relationship between the change in 1-deoxySL and neuropathy severity.

Regarding the 3 biomarkers evaluated (nerve conduction studies, autonomic function, and epidermal innervation), standard electrophysiologic measures did not prove helpful in defining treatment response in HSAN1. This is consistent with prior studies in CMT.<sup>19</sup> Only minimal autonomic abnormalities were seen at baseline, making treatment effect difficult to detect. In contrast, interpretation of nerve conduction studies was limited by the paucity of recordable sensory and motor responses prior to the start of treatment. We had previously found density of epidermal innervation promising in pilot studies, and studies in other small fiber predominant neuropathies have demonstrated excellent test–retest reliability.<sup>15,20</sup> However, in the current study we found high variability, particularly at the proximal site.<sup>20</sup> The main finding of a minor increase in the epidermal nerve fiber density at the distal site in L-serine-treated participants (median change of 8 vs 0 fibers/mm<sup>2</sup>, range 0–75,  $p = 0.014$ ) is surprising given the absence of treatment effects at the

proximal site. In length-dependent neuropathies, re-innervation would be expected to occur proximally, rather than in the more severely affected distal calf. The limitations of all the major electrophysiologic and pathologic measures underscore the importance of developing and evaluating novel biomarkers for HSAN1, as well as other severe, small fiber predominant polyneuropathies.

Our study had limitations, the most important being its small sample size, which limited its statistical power. HSAN1 is very rare, with an estimated prevalence of only a few hundred patients worldwide, and large clinical trials in the disease are therefore not feasible. Another limitation was the paucity of validated outcome measures and longitudinal natural history data specific to HSAN1. The CMTNS has been extensively studied in CMT1A but not been validated in HSAN1. Both neuropathies cause progressive, distally pronounced sensory loss and muscle weakness; however, HSAN1 is also distinct in its preferential involvement of the small nerve fibers with a propensity towards skin ulceration and neuropathic pain, features that cannot be adequately assessed using the CMTNS. Importantly, prior studies of CMT1A have shown a CMTNS change of only 0.23–0.68 points a year, and

a recent natural history study of HSAN1 demonstrated no change in CMTNS over 1 year.<sup>21,22</sup> In the current study, we did observe an increase in CMTNS scores in the placebo group (1.1 points  $\pm$  0.53,  $p = 0.04$ ); however, this must be interpreted with caution given the small sample size.<sup>23</sup>

Importantly, many participants in our study had advanced neuropathy at enrollment (mean CMTNS  $22.6 \pm 8.6$ ), and it is possible that earlier treatment would be more effective. Given that nerve injury in HSAN1 may begin in infancy or even in utero, long before symptoms are noted, treatment may be most beneficial in childhood with study endpoints focusing on delaying symptom onset and slowing disease progression. Furthermore, the optimal treatment dose of L-serine may require further examination. We selected a dose of 400 mg/kg/d on the basis of prior reports of reversible side effects at higher doses; however, these studies were performed in young children with disorders of serine metabolism rather than in adults with normal serine synthesis but dysfunctional use.<sup>11</sup> The absence of significant adverse events in our study suggests that even higher doses could be considered, although it is not clear if this would add benefit since 1-deoxySL formation was adequately suppressed in our study. In addition, we found that plasma L-serine levels do not reliably reflect treatment status. It is likely that these levels do not correlate with L-serine uptake into neurons, as plasma amino acid levels are heavily influenced by their metabolism in both liver and muscle. A follow-up study evaluating the clinical effect of L-serine in a larger number of patients using more sensitive biomarkers such as neuromuscular MRI is needed to help confirm the clinical efficacy of L-serine in HSAN1 and define the optimal timing and dosing of treatment.<sup>24</sup>

Our finding that L-serine reduces levels of 1-deoxySL in humans, and thereby may improve neuropathy, may have implications beyond HSAN1. Elevated plasma 1-deoxySL levels have been observed in patients with metabolic syndrome and type 2 diabetes mellitus and have been implicated as possible contributors to diabetic sensory neuropathy.<sup>7,8,25–27</sup> Elevated 1-deoxySL levels have also been observed in paclitaxel-induced peripheral neuropathy (chemotherapy-induced peripheral neuropathy [CIPN]).<sup>28,29</sup> L-serine supplementation has been shown to lower 1-deoxySLs and improve sensory function in a rat model of diabetic neuropathy as well as paclitaxel-induced CIPN.<sup>25,30</sup> 1-DeoxySLs may therefore also be important biomarkers and therapeutic targets in other small-fiber-predominant neuropathies.

We have shown that supplementation with high doses of L-serine is well-tolerated in patients with HSAN1 and results in reduced levels of neurotoxic 1-deoxySL. Our findings also suggest that treatment may slow clinical progression of the disease although further research will be needed to confirm this and optimize timing and dosage of L-serine supplementation. L-Serine may be the first rational therapy for HSAN1 as it addresses the metabolic effect of the underlying gene defect.

## Author contributions

The study was designed by Drs. Florian Eichler, Thorsten Hornemann, Eric Macklin, and Robert Brown. The authors had access to the full data from the study. All laboratory data were generated by the authors. Clinical data were collected by the authors, who treated participants and were responsible for all follow-up and clinical decisions. The analysis plan was developed and data analyses were performed by Dr. Eric A. Macklin. Dr. Vera Fridman performed clinical and electrophysiologic assessments, interpreted study results, wrote the first draft of the manuscript, which was revised by Dr. Eichler and edited and approved by all authors, and incorporated changes from all coauthors. Saranya Suriyanarayanan performed sphingolipid analysis throughout the study and revised the manuscript. Peter Novak performed autonomic function testing and revised the manuscript. William David performed electrophysiologic assessments and revised the manuscript. Eric Macklin prepared the randomization schedule, performed all statistical analysis, including those involved in study planning, wrote statistical methods for the manuscript, prepared all graphs for the manuscript, and revised the manuscript. Diane McKenna-Yasek served as the study nurse at UMass Medical and oversaw all assessments that were conducted at UMass Medical. Kailey Walsh served as the study coordinator throughout the duration of the trial and scheduled and oversaw all assessments. Razina Aziz-Bose performed study coordination. Anne Louise Oaklander performed skin biopsy analysis and interpreted the findings and revised the manuscript. Robert Brown performed autonomic function testing and revised the manuscript. Thorsten Hornemann performed all sphingolipid analyses and revised the manuscript. Florian Eichler served as principal investigator for the study, oversaw study design and implementation, conducted all regulatory affairs related to the study, oversaw data interpretation and manuscript preparation, and revised the manuscript.

## Acknowledgment

The investigators thank all the patients who participated in this study. Jessica Pan and Elizabeth Haxton contributed to the regulatory approval process and study coordination. Heather Downs assisted in skin biopsy analysis and John Vetrano contributed as research pharmacist. The investigators also thank Sarrah Knause for assisting with manuscript preparation.

## Study funding

This work was supported by the Deater Foundation, the FDA Orphan Disease Group (R01 FD004127), the National Institute of Health (R01 NS072446, R01 NS082331, R01 NS093653), the European Commission (7th Framework Program, “RESOLVE,” project number 305707), the Swiss National Foundation SNF (project 31003A\_153390/1), the Hurka Foundation, and the Rare Disease Initiative Zurich (“radiz,” Clinical Research Priority Program for Rare Diseases, University of Zurich).

## Disclosure

V. Fridman, S. Suriyanarayanan, P. Novak, and W. David report no disclosures relevant to the manuscript. E. Macklin receives research support from Acorda Therapeutics, serves on Data and Safety Monitoring Boards for Acorda Therapeutics and Shire Human Genetic Therapies, serves on a trial Steering Committee for Biogen, and consults for MyoLex Inc. and Lavin Consulting. D. McKenna-Yasek, K. Walsh, R. Aziz-Bose, A. Oaklander, R. Brown, and T. Hornemann report no disclosures relevant to the manuscript. F. Eichler receives research support from FDA Orphan Disease Group (R01 FD004127), NINDS (R01 NS072446, R01 NS082331), Retrophin, Neurovia, bluebird bio, and AGTC. Go to [Neurology.org/N](http://Neurology.org/N) for full disclosures.

## Publication history

Received by *Neurology* March 15, 2018. Accepted in final form September 28, 2018.

## References

1. Houlden H, King R, Blake J, et al. Clinical, pathological and genetic characterization of hereditary sensory and autonomic neuropathy type 1 (HSAN I). *Brain* 2006;129:411–425.
2. Bejaoui K, Wu C, Scheffler MD, et al. SPTLC1 is mutated in hereditary sensory neuropathy, type 1. *Nat Genet* 2001;27:261–262.
3. Dawkins JL, Hulme DJ, Brahmabhatt SB, Auer-Grumbach M, Nicholson GA. Mutations in SPTLC1, encoding serine palmitoyltransferase, long chain base subunit-1, cause hereditary sensory neuropathy type I. *Nat Genet* 2001;27:309–312.
4. Eichler FS, Hornemann T, McCampbell A, et al. Overexpression of the wild-type SPT1 subunit lowers deoxy-sphingolipid levels and rescues the phenotype of HSAN1. *J Neurosci* 2009;29:14646–14651.
5. Penno A, Reilly MM, Houlden H, et al. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. *J Biol Chem* 2010;285:11178–11187.
6. Jun BK, Chandra A, Kuljis D, Schmidt BP, Eichler FS. Substrate availability of mutant SPT alters neuronal branching and growth cone dynamics in dorsal root ganglia. *J Neurosci* 2015;35:13713–13719.
7. Dohrn MF, Othman A, Hirshman SK, et al. Elevation of plasma 1-deoxy-sphingolipids in type 2 diabetes mellitus: a susceptibility to neuropathy? *Eur J Neurol* 2015;22:806–814, e855.
8. Hammad SM, Baker NL, El Abiad JM, et al. Increased plasma levels of select deoxy-ceramide and ceramide species are associated with increased odds of diabetic neuropathy in type 1 diabetes: a pilot study. *Neuromolecular Med* 2016;19:46–56.
9. Zuellig RA, Hornemann T, Othman A, et al. Deoxy-sphingolipids, novel biomarkers for type 2 diabetes, are cytotoxic for insulin-producing cells. *Diabetes* 2014;63:1326–1339.
10. Garofalo K, Penno A, Schmidt BP, et al. Oral L-serine supplementation reduces production of neurotoxic deoxy-sphingolipids in mice and humans with hereditary sensory autonomic neuropathy type 1. *J Clin Invest* 2011;121:4735–4745.
11. de Koning TJ. Treatment with amino acids in serine deficiency disorders. *J Inher Metab Dis* 2006;29:347–351.
12. Low PA. Composite autonomic scoring scale for laboratory quantification of generalized autonomic failure. *Mayo Clin Proc* 1993;68:748–752.
13. England JD, Gronseth GS, Franklin G, et al. Practice parameter: evaluation of distal symmetric polyneuropathy: role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review): report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation. *Neurology* 2009;72:177–184.
14. Zitomer NC, Mitchell T, Voss KA, et al. Ceramide synthase inhibition by fumonisins B1 causes accumulation of 1-deoxysphinganine: a novel category of bioactive 1-deoxysphingoid bases and 1-deoxydihydroceramides biosynthesized by mammalian cell lines and animals. *J Biol Chem* 2009;284:4786–4795.
15. Fridman V, Oaklander AL, David WS, et al. Natural history and biomarkers in hereditary sensory neuropathy type 1. *Muscle Nerve* 2015;51:489–495.
16. Dedov VN, Dedova IV, Merrill AH Jr, Nicholson GA. Activity of partially inhibited serine palmitoyltransferase is sufficient for normal sphingolipid metabolism and viability of HSN1 patient cells. *Biochim Biophys Acta* 2004;1688:168–175.
17. McCampbell A, Truong D, Broom DC, et al. Mutant SPTLC1 dominantly inhibits serine palmitoyltransferase activity in vivo and confers an age-dependent neuropathy. *Hum Mol Genet* 2005;14:3507–3521.
18. Bode H, Bourquin F, Suriyanarayanan S, et al. HSN1 mutations in serine palmitoyltransferase reveal a close structure-function-phenotype relationship. *Hum Mol Genet* 2016;25:853–865.
19. Verhamme C, van Schaik IN, Koelman JH, de Haan RJ, de Visser M. The natural history of Charcot-Marie-Tooth type 1A in adults: a 5-year follow-up study. *Brain* 2009;132:3252–3262.
20. Smith AG, Howard JR, Kroll R, et al. The reliability of skin biopsy with measurement of intraepidermal nerve fiber density. *J Neurol Sci* 2005;228:65–69.
21. Shy ME, Chen L, Swan ER, et al. Neuropathy progression in Charcot-Marie-Tooth disease type 1A. *Neurology* 2008;70:378–383.
22. Lewis RA, McDermott MP, Herrmann DN, et al. High-dosage ascorbic acid treatment in Charcot-Marie-Tooth disease type 1A: results of a randomized, double-masked, controlled trial. *JAMA Neurol* 2013;70:981–987.
23. Kugathasan UEM, Laurá M, Sinclair C, et al. Natural history study in hereditary sensory neuropathy type 1 (HSN1): improving the responsiveness of outcome measures. *Peripheral Nerve Society annual meeting*; 2017: 81.
24. Morrow JM, Sinclair CD, Fischmann A, et al. MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. *Lancet Neurol* 2016;15:65–77.
25. Othman A, Bianchi R, Alecu I, et al. Lowering plasma 1-deoxysphingolipids improves neuropathy in diabetic rats. *Diabetes* 2015;64:1035–1045.
26. Othman A, Saely CH, Muendlein A, et al. Plasma 1-deoxysphingolipids are predictive biomarkers for type 2 diabetes mellitus. *BMJ Open Diabetes Res Care* 2015;3:e000073.
27. Mwinyi J, Bostrom A, Fehrer I, et al. Correction: plasma 1-deoxysphingolipids are early predictors of incident type 2 diabetes mellitus. *PLoS One* 2017;12:e0179313.
28. Othman A, Rutti MF, Ernst D, et al. Plasma deoxysphingolipids: a novel class of biomarkers for the metabolic syndrome? *Diabetologia* 2012;55:421–431.
29. Kramer R, Bielawski J, Kistner-Griffin E, et al. Neurotoxic 1-deoxysphingolipids and paclitaxel-induced peripheral neuropathy. *FASEB J* 2015;29:4461–4472.
30. Kiya T, Kawamata T, Namiki A, Yamakage M. Role of satellite cell-derived L-serine in the dorsal root ganglion in paclitaxel-induced painful peripheral neuropathy. *Neuroscience* 2011;174:190–199.

# Neurology®

## Randomized trial of l-serine in patients with hereditary sensory and autonomic neuropathy type 1

Vera Fridman, Saranya Suriyanarayanan, Peter Novak, et al.  
*Neurology* 2019;92:e359-e370 Published Online before print January 9, 2019  
DOI 10.1212/WNL.0000000000006811

**This information is current as of January 9, 2019**

<b>Updated Information &amp; Services</b>	including high resolution figures, can be found at: <a href="http://n.neurology.org/content/92/4/e359.full">http://n.neurology.org/content/92/4/e359.full</a>
<b>References</b>	This article cites 29 articles, 8 of which you can access for free at: <a href="http://n.neurology.org/content/92/4/e359.full#ref-list-1">http://n.neurology.org/content/92/4/e359.full#ref-list-1</a>
<b>Subspecialty Collections</b>	This article, along with others on similar topics, appears in the following collection(s): <b>Class I</b> <a href="http://n.neurology.org/cgi/collection/class_1">http://n.neurology.org/cgi/collection/class_1</a>
<b>Permissions &amp; Licensing</b>	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://www.neurology.org/about/about_the_journal#permissions">http://www.neurology.org/about/about_the_journal#permissions</a>
<b>Reprints</b>	Information about ordering reprints can be found online: <a href="http://n.neurology.org/subscribers/advertise">http://n.neurology.org/subscribers/advertise</a>

*Neurology*® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

