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ORIGINAL PAPER

A Novel Variant (Asn177Asp) in SPTLC2 Causing Hereditary Sensory Autonomic Neuropathy Type 1C

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Abstract
Hereditary sensory and autonomic neuropathy type 1 (HSAN1) is a rare, autosomal dominantly inherited, slowly progressive and length-dependent axonal peripheral neuropathy. HSAN1 is associated with several mutations in serine-palmitoyltransferase (SPT), the first enzyme in the de novo sphingolipid biosynthetic pathway. HSAN1 mutations alter the substrate specificity of SPT, which leads to the formation of 1-deoxysphingolipids, an atypical and neurotoxic subclass of sphingolipids. This study describes the clinical and neurophysiological phenotype of a German family with a novel SPTCL2 mutation (c.529A > G; N177D) associated with HSAN1 and the biochemical characterization of this mutation.) The mutaion was identified in five family members that segregated with the diesease. Patients were characterized genetically and clinically for neurophysiological function. Their plasma sphingolipid profiles were analyzed by LC–MS. The biochemical properties of the mutation were characterized in a cell-based activity assay. Affected family members showed elevated 1-deoxysphingolipid plasma levels. HEK293 cells expressing the N177D SPTLC2 mutant showed increased de novo 1-deoxysphingolipid formation, but also displayed elevated canonical SPT activity and increased C20 sphingoid base production. This study identifies the SPTLC2 N177D variant as a novel disease-causing mutation with increased 1-deoxySL formation and its association with a typical HSAN1 phenotype.

Keywords HSAN1 · Serine-palmitoyltransferase · 1-deoxysphingolipids · Neuropathy · Mass spectrometry

Introduction

Hereditary sensory and autonomic neuropathies (HSAN) are rare inherited disorders of the peripheral nervous system, characterized by sensory and autonomic dysfunction (Houlden et al. 2004). Seven subtypes of HSAN (HSAN1–HSAN7) have been described (Dyck 1993; Edvardson et al. 2012; Leipold et al. 2013) of which HSAN type I (HSAN1) is the most frequent one. HSAN1 has an autosomal dominant (AD) trait and is primarily characterized by axonal atrophy and a progressive degeneration of sensory neurons (Houlden et al. 2006). Onset of the disease is variable and can occur between early childhood up to the sixth decade of life (Auer-Grumbach 2013; Suriyanarayanan et al. 2016). HSAN1 is associated with mutations in two subunits of serine-palmitoyltransferase (SPT) (Rotthier et al. 2011; Penno et al. 2010). SPT is a heteromeric enzyme, which catalyzes the condensation of palmitoyl-CoA and L-serine, the first and rate-limiting step in the de novo biosynthesis of sphingolipids (SLs). It is located at the outer membrane of the endoplasmic reticulum and composed of the three subunits SPTLC1, SPTLC2 and SPTLC3 (Hornemann et al. 2006). Further, regulatory components (ORMDL 1-3 and ssSPTa,b) were also reported (Breslow et al. 2010; Gupta et al. 2015; Davis et al. 2019). So far,

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seven missense mutations in SPTLC1 (p.C133W, p.C133Y, p.C133R, p.V144D, p.S331F, p.S331Y and p.A352V) (Houlden et al. 2006; Dawkins et al. 2001; Klein et al. 2005; Geraldes et al. 2004; Auer-Grumbach et al. 2013) and six missense mutations in SPTLC2 (p.V359M, p.G382V, p.I504F, p.A182P, p.S384F, and p.R183W) (Rotthier et al. 2010; Ernst et al. 2015; Murphy et al. 2013; Suriyanarayanan et al. 2016) have been associated with HSAN1.

HSAN1 mutations cause a change in the amino acid specificity of SPT, so that in addition to the canonical L-serine, also L-alanine is used as an alternative substrate. This leads to de novo synthesis of an atypical subclass of SLs termed 1-deoxysphingolipids (1-deoxySLs). 1-deoxySLs lack the C1 hydroxyl group of canonical sphingolipids and therefore, are neither converted to complex sphingolipids nor degraded by the canonical sphingolipid catabolic pathway. 1-deoxySLs are neurotoxic, affecting neurite formation and cytoskeleton integrity in cultured dorsal root ganglia (DRG) (Penno et al. 2010; Guntert et al. 2016). Moreover, pathologically elevated 1-deoxySL levels were found in plasma and lymphocytes of HSAN1 patients (Penno et al. 2010) and in plasma and tissues of transgenic HSAN1 mice (Eichler et al. 2009; Garofalo et al. 2011).

In addition to palmitoyl-CoA, SPT can also metabolize other acyl-CoAs in the range of C_{14}–C_{18}, which results in the formation of sphingoid bases with different carbon chain lengths (Hornemann et al. 2009). Besides increased 1-deoxySL formation, some HSAN1 mutations also affect canonical SPT activity and the affinity for alternative acyl-CoAs, therefore changing the spectrum of the sphingoid bases profiles (Hornemann et al. 2009). This is in particular true for the two SPTLC1 mutations (p.S331F, p.S331Y) and one SPTLC2 mutant (p.I504F). These mutants are associated with an exceptionally severe disease phenotype and show not only elevated 1-deoxySL formation, but also higher canonical activity and increased C20 sphingoid base formation (Auer-Grumbach et al. 2013; Suh et al. 2014).

Here, we report a novel HSAN1 causing mutation in SPTLC2 (c.529A > G; p.N177D). In addition to segregation studies in the family, we characterized the mutant biochemically in cell culture experiments to assess the pathogenicity of the mutation.

Materials and Methods

Patients

A family of five affected and three non-affected individuals participated in this study. All affected family members were clinically diagnosed for HSAN1. Patients carrying the mutations underwent a detailed diagnosis, based on clinical phenotype together with neurophysiological assessments. Plasma samples were obtained from all family members, with written informed consents. The manuscript is a retrospective case report that does not require ethics committee approval at our institution.

DNA Sequencing

Next generation sequencing (NGS) was performed after a gene enrichment procedure (Agilent in solution technology). The coding and the flanking intronic regions were sequenced (Illumina HiSeq 2500/4000). Only variants (SNVs/Small Indels) with a minor allele frequency of MAF < 1.5% in the coding and the flanking intronic regions (± 8 bp) were evaluated. Known disease-causing variants (according to HGMD) were verified in ± 30 bp of the flanking regions and up to a MAF of 5%. At least one causative or rare variant was re-sequenced using conventional Sanger sequencing, providing a second, independent, confirmation [for details refer to (Dohrn et al. 2017)].

Cloning

The N177D mutation was introduced into the SPTLC2 cDNA by site-directed mutagenesis as described previously (Penno et al. 2010), using the following primers:

hSPTLC2_N177D_FW 5′-GTTATAAACATGGGTTCCTACGACTATCTTGGATTTGCACGG-3′.

hSPTLC2_N177D_RV: 5′-CCGTGCAAATCCAAGATAGTCGTAGGAACCCATGTTTATAAC-3′. Constructs were verified by sequencing and by western blot for expression.

Sphingolipid Analysis in Serum

The Serum sphingoid base profile was analyzed in sera of blood samples collected from N177D carriers and unaffected healthy family members. 500 µl of methanol (Honeywell) including 200 pmol of deuterated internal standards (d7-sphinganine and d7-sphingosine, Avanti Polar Lipids, AL, USA) were added to the 100 µl of serum sample or frozen cell pellets resuspended in 100 µl PBS. Lipids were extracted at 37 °C for 1 h with constant agitation at 1400rcf. Protein precipitates were removed by centrifugation (16000 rcf, 5 min) and 500 µl of the supernatant was transferred to new tubes and 75 µl of HCl (32%, Sigma) was added. The extracted lipids were hydrolyzed for 16 h at 65 °C. Acid hydrolysis was stopped by addition of 100 µl KOH (10 M). The free sphingoid bases were extracted with 125 µl of chloroform followed by washing with 100 µl of ammonium hydroxide (2 N) and 500 µl of alkaline water. The extracted lipids were separated on C18 column (Uptispere 120 A°, 5 µm, 125 × 2 mm, Interchim) and analyzed on a TSQ Quantum Ultra MS analyzer (Thermo Scientific).

Cell based SPT Activity Assay

Stable HEK293 cell lines expressing either the vector control (VC), wildtype SPTLC2 (WT) or the SPTLC2 mutant (N177D) were generated. For the sphingolipid synthesis assay, cells were cultured in DMEM (Sigma) with 10% fetal bovine serum (Sigma), 400 µg/ml of Geneticin (Gibco) and penicillin/streptomycin (100 U/ml, and 0.1 mg/ml, respectively, Sigma) for 48 h. Followed by the exchange of medium with L-serine deficient DMEM (Genaxxon Bioscience, Ulm, Germany). After 2 h, isotopic labelled (d3, 15 N)-serine (1 mM) and (d4)-alanine (2 mM) were added. Cells were harvested after 24 h, SPT activity and 1-deoxySL formation was measured by the incorporation of isotope-labeled (d3, 15 N)-serine and (d4)-alanine into the newly formed sphingoid bases using mass spectrometry as described above.

Homology Modelling

The human SPTLC2 model was generated from the protein structure homology-modelling server Swiss Model (http://swissmodel.expasy.org) based on the SPT structure from *Sphingomonas paucimobilis* (PBD entry code 2JG2) (Yard et al. 2007). The resulting models showed QMEAN4 values of − 2.44 and GMQE values of 0.51. Structure of SPTLC2 with residue N177 was illustrated using VMD 1.9.2.

Results

Clinical Details

The pedigree revealed an AD inheritance of the clinical phenotype (Fig. 1a). The onset of symptoms varied from childhood to adults. Three (II-1; II-2; III-4) of five affected individuals had a late onset in the third decade, one patient (III-3) in the second decade presenting initially with reduced sensation in the feet and later with distal atrophy and paresis. All except the youngest affected family member (III-5; 15 years) had impaired wound healing and ulcerations. The youngest family member showed early onset symptoms in the first decade with slow motor development in childhood and the formation of a syrinx. Sensory loss occurred in gloves and stocking distribution; vibration perception was better than pinprick perception. Patient II-2 had restless legs; patients II-1 and III-3 had a history of diabetes mellitus. Electrophysiology was done in four of five patients. Due to the young age, patient III-5 was not analyzed. All patients had an axonal and demyelinating neuropathy (Table 1).

Sequencing

Sequencing of SPTLC2 revealed a missense mutation of A to G at nucleotide position 529, causing the substitution of Asparagine (N) to Aspartic acid (D) at amino acid 177. All five patients (II-1, II-2, III-3, III-4, and III-5) carried the same N177D mutation, while healthy individuals (Control 1-3) carried the normal allele. Interestingly, sequence analysis and alignment indicated that the N177 residue is highly conserved among species ranging from bacteria (*S. paucimobilis*) to *Arabidopsis* to humans (Fig. 1b).

1-Deoxysphingolipid Levels are Elevated in Patient Plasma

All N177D carriers showed significantly elevated plasma 1-deoxysphinganine (1-deoxySA) and 1-deoxysphingosine (1-deoxySO) levels compared to controls (Fig. 2a). Total C18 (Fig. 2b) and C20 sphingolipid levels were not different between patients and controls (Fig. 2c). Index patient (II-1) had borderline elevated C20 SLs.

Effect of the SPTLC2 N177D Mutation on SPT Activity

Expression levels of HEK293 cells transfected with SPTLC2wt were not different from SPTLC2 N177D cells (Fig. 3). At standard assay conditions [(d3, N15)-serine (1 mM), (d4)-alanine (2 mM)], N177D expressing HEK293 cells showed a 9-fold increase in isotope labeled 1-deoxySL compared to SPTLC2wt (Fig. 4a). 1-deoxySL formation was modulated in response to the availability of alanine or serine. In presence of increased (d4)-alanine (10 mM), 1-deoxySL formation was increased, whereas 1-deoxySL formation was considerably suppressed in presence of increased (d3, N15)-serine (10 mM) (Fig. 4a). For N177D expressing cells, we also observed an increased formation of isotope labelled C18 (Fig. 4b) and C20 sphingoid bases (Fig. 4c), compared to control cells.

Modelling

A homology model of human SPTLC2 was generated using the Swiss Model Server (http://swissmodel.expasy.org) based on the SPT structure from *S. paucimobilis* (PBD entry code 2JG2) (Yard et al. 2007). The structural analysis revealed that residue N177 is likely located in an internal loop of the protein and is not in the α-helix or the β-sheet (Fig. 5a, b). The exchange from a polar asparagine to the negatively charged aspartic acid could have an impact on salt bridges that might lead to changes in overall

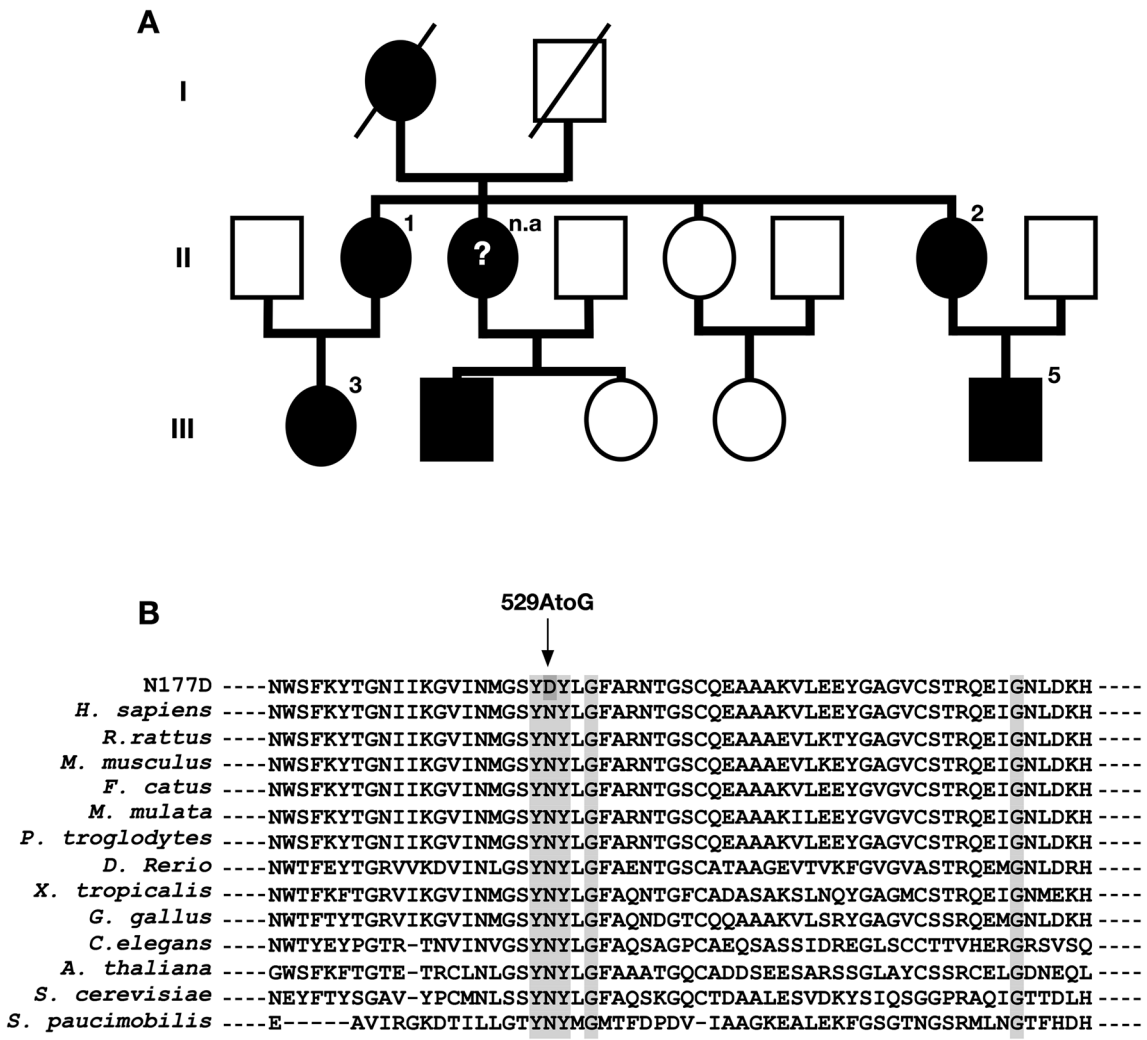

Fig. 1 **a** Pedigree of German family with SPTLC2 N177D mutation. Square = male; circle = female; diagonal line = deceased; filled symbol = affected; unfilled symbol = not affected; n.a = not available. **b** Sequence alignments showed that the N177 residue (arrow) is highly conserved between species

structure thereby affecting substrate binding affinity and activity.

Discussion

So far, thirteen mutations in either SPTLC1 or SPTLC2 have been functionally associated with HSAN1. In this study, we report a novel mutation (SPTLC2 p.N177D) in a German HSAN1 family. Clinically, the patients presented with typical features for HSAN1, including numbness, loss of sensation, tingling in hands and feet, distal atrophy and impaired wound healing. The age of onset in this family varied from childhood to adult. All N177D carriers were affected and had elevated plasma 1-deoxySL levels, which appeared to correlate with the severity of the disease (Laura et al. 2012). The youngest patient (III-5), showed slow motor development and a more severe neuropathy compared to other family members, although his plasma 1-deoxySL levels were not the highest within the family. The reason for this is not clear, but might be related to his younger age, duration of illness or due to the influence of environmental and dietary factors.

Compared to wild type cells, HEK293 cells overexpressing the SPTLC2 p.N177D mutant showed an increased activity in using alanine as the substrate and, as the consequence, increased 1-deoxySL formation. Similar to other HSAN1 mutants, 1-deoxySL formation was stimulated or suppressed in the presence high levels of L-alanine levels and L-serine, respectively. This suggests, that the patients might benefit from an oral L-serine supplementation that was recently tested as a therapy for HSAN1 (Garofalo et al. 2011; Auranen et al. 2017; Fridman et al. 2019).

Table 1 Clinical details of SPTLC2 N177D patients

Patien t	AAO	Age at exa m	Symptom at onset	Motor	Sensory	Reflexes	Ulzero-muti-lating compli-cations	Amputa-tion	Other	Nerve conduction studies						
										Ulnar DM	CVm (ms)	CMAP (mV)	CVs (ms)	SNAP (μV)	Radial CVs	NAP (μV)
II-1	25	56	Numb-ness of feet	Distal atrophy and paresis feet > hands	Vibration ancles 4/8, tin-gling, sensory loos	Absent at patellar and	Burns and ulcers	Yes left foot	DM[a]	3.4	34.4	2.5	23.7	2.2	16.8	8.4
II-2	25	51	Foot defor-mation, restless legs, delayed wound healing	Distal atrophy and paresis feet > hands	Vibration ancles 4/8, tingling	Absent at ancles	Burns and ulcers	No		2.83	62.5	4.2	33.5	10.7	10.2	60.3
III-3	14	32	Delayed wound healing	Distal atrophy and paresis feet > hands	Vibration ancles 4/8 pin above elbow and	Absent at patel-lar and ancles	Burns and ulcers	No	DM, multi-ple foot opera-tions	4.79	37.7	0.87	58.8	9	22	7.5
III-4	22	42		Distal atrophy and paresis feet	Ataxia, Vibra-tion ancles 4/8 pin above elbow		Burns and ulcers	No		3.62	29.8	3.8	19	12.4	10.1	2.8
III-5	3	15	Tingeling in hands and feets	Distal atrophy and paresis feet > hands	Vibration ancles 4/8	Absent at ancles	No	No	Syrinx	–	–	–	–	–	–	–

AAO age at onset, *DM* diabetes mellitus

[a]death

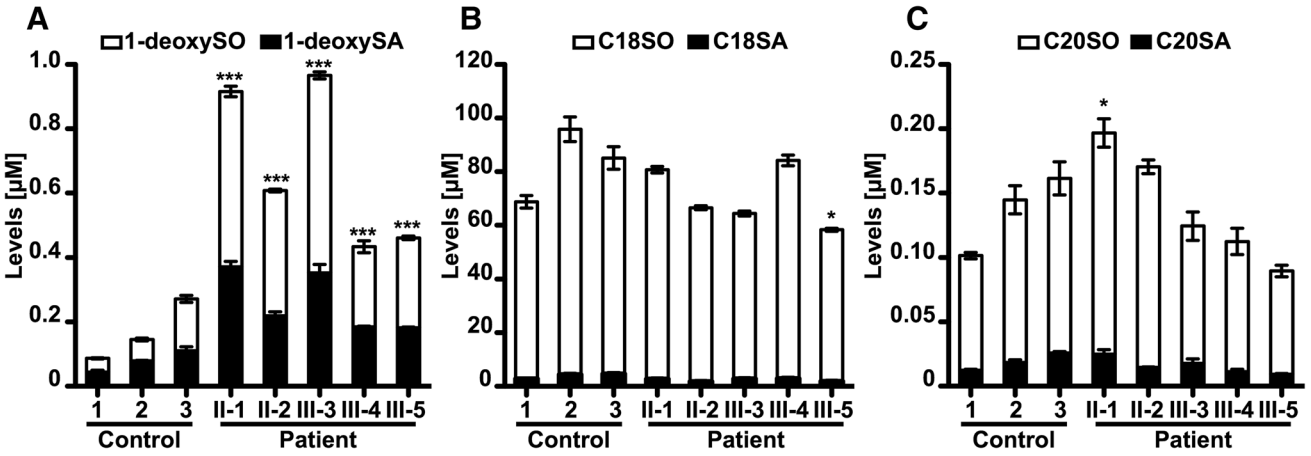

Fig. 2 **a** 1-deoxyphingolipid (1-deoxySA/1-deoxySO) levels in plasma of HSAN1 patients carrying N177D mutation and unaffected family members. 1-deoxySA and 1-deoxySO were significantly elevated in all the patients carrying the SPTLC2 p.N177D mutation **b** Canonical sphingolipids (C18SA/C18SO) were not different in any of the patients except III-5, who had slightly lower levels compared to controls **c** Total level of C20SLs were not elevated in patients compared to controls, except for index patient (II-1), who had borderline elevated levels. All data are shown as mean, with error bars representing SDs (unpaired, two tailed, *t* test; $*p<0.05$, $**p<0.01$, $***p<0.001$)

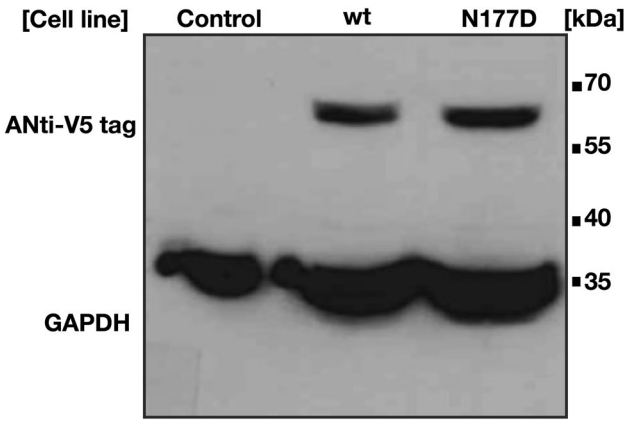

Fig. 3 Expression of N177D mutant in stably transfected HEK293 cells. Cells expressing vector control and SPTLC2 were used as control. SPTLC2 (63 kDa) was detected using antibody against the the C-terminal V5-tag. GAPDH (38 kDa) was used as a loading control

In cell culture, the N177D mutation also resulted in the increased formation of C18 and C20 sphingoid bases formation which was, however, not reflected by high concentrations of these metabolites in the serum of affected patients. Altered C18 and C20 sphingoid base formation was described previously for other HSAN1 mutants (SPTLC1 p.S331F, p.S331Y and SPTLC2 p.I504F) and was typically associated with a more severe disease phenotype. These severe variants are clinically associated with a congenital onset, development of cataracts and respiratory as well as growth complications and in atleast one case with mental retardation (Rotthier et al. 2011; Auer-Grumbach et al. 2013; Rotthier et al. 2010). However, in these severe cases, C18 and C20 sphingoid bases were also found to be elevated in plasma, which was not the case in the family studied here. (Table 2 shows an overview of all published SPTLC2 mutations). HSAN1 mutations that are associated with the mild phenotype typically cluster around the active site, whereas mutations associated with a severe phenotype are located on the surface of the SPT dimer (Bode et al. 2016). We used a protein structure homology modelling to model the N177D mutation into the homology structure for human SPTLC2 based on PBD 2JG2 (Yard et al. 2007). In these models, residue N177 appeared to be part of an internal loop which is neither in the vicinity of the active site nor exposed to the surface. However, a change from asparagine to aspartic acid could have an influence on salt bridges, which in turn affect the overall protein structure and thereby shifting the substrate-binding affinity towards alanine. We observed that the sequence motif is conserved among higher organisms and especially the N177 amino acid has been strictly retained in the evolution. The amino acid stretch falls within a region that corresponds to a loop structural motif, therefore, pointing to its essential role in selectivity and SPT function.

In summary, we identified the novel SPT variant SPTLC2p.N177D as a disease-causing HSAN1C mutation with combined sensory and motor involvement, associated with increased 1-deoxySL formation. In total, this expands the number of reported HSAN1 mutations to fourteen.

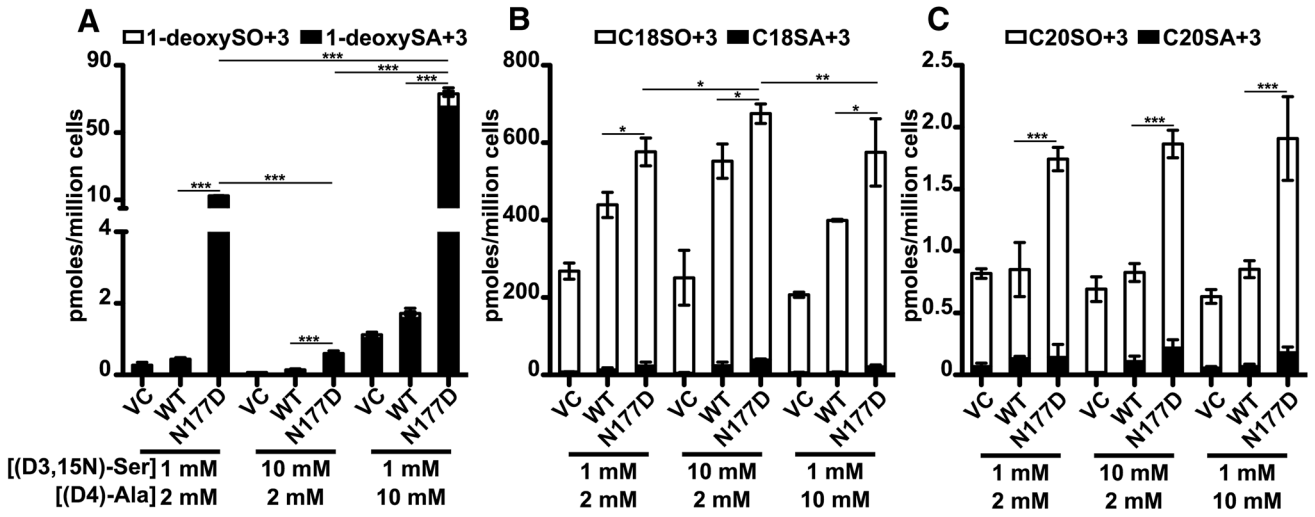

Fig. 4 Cells were treated with isotope-labelled (d3, 15N)-serine and (d4)-alanine. Levels of de novo formed isotope-labelled **a** (d3)1-deoxySA and (d3)1-deoxySO **b** (d3)-C18SA and (d3)-C18SO **c** (d3)-C20SA and (d3)-C20SO were significantly elevated in N177D cells compared to wt control. All data are shown as mean, with error bars representing SDs (unpaired, two tailed, *t* test; $*p<0.05$, $**p<0.01$, $***p<0.001$)

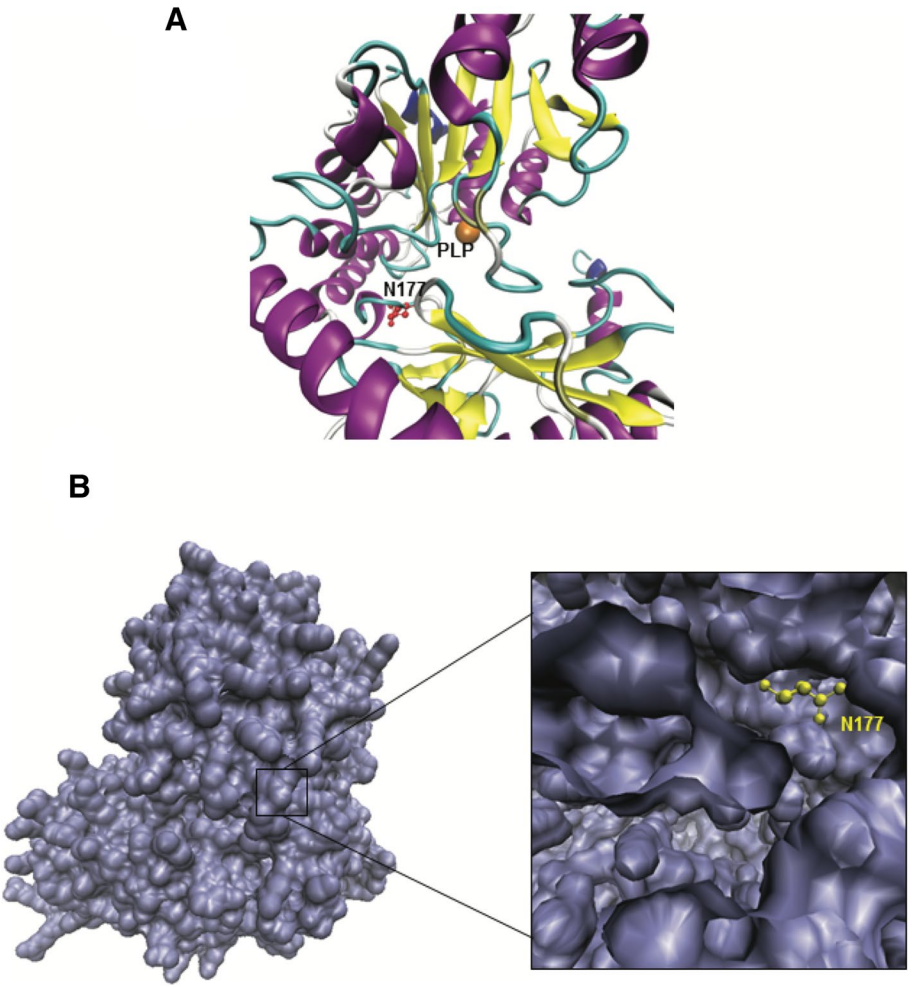

Fig. 5 Modelling of the human SPTLC2 subunit using the homology-modelling server Swiss Model based on the SPT structure from *S. paucimobilis* (PBD 2JG2). **a** Structure of SPTLC2 was illustrated using VMD 1.9.2. Residue N177 is located in the internal loop and is neither in α-helix, nor in β-sheets (N177 shown in red, PLP in orange) **b** (Left) Overall structure of SPTLC2 (illustrated using VMD 1.9.2). Space fill representation for SPTLC2 indicates that residue N177 is buried within the protein. (Right) Close-up view of N177 (yellow) in SPTLC2

Table 2 Overview of SPTLC2 mutations and their clinical and biochemical features

Mutation	DO.	Location in SPT	Clinical features	Biochemical characterization			Reference
				1-deoxySL	C18SL	C20SL	
c.1145G > T, p.G382 V	Adult	Active site	Axonal sensorimotor neuropathy with progressive distal sensory loss, distal muscle weakness in LL, dysesthesia in hands and feets, osteomyelitis	Elevated	Not elevated	Not elevated	Rotthier et al. 2010
c.1075G > A, p.V359 M	Late	Not-surface exposed	Axonal/intermediate sensorimotor neuropathy with distal sensory dysfunction, ulceration, amputation, osteomyelitis	Elevated	Not elevated	Not elevated	Rotthier et al. 2010
c.1510A > T, p.I504F	Early	Surface-exposed	Intermediate sensory neuropathy with distal LL weakness, ulceration, osteomyelitis, anhidrosis	Elevated	Elevated	Elevated	Rotthier et al. 2010
c.544G > C, p.A182P	Early	Around internal aldimine	Sensory neuropathy with progressive sensory loss, ulceration, prominent motor involvement	Elevated	Not elevated	Not elevated	Murphy et al. 2013
c.1151C > T, p.S384F	Late	Around internal aldimine	Sensory neuropathy with progressive distal sensory loss, ulcer mutilations, mild proximal and severe distal UL and LL weakness	Elevated	Not elevated	Not elevated	Ernst et al. 2015
c.547C > T, p.R183 W	Late	Not-surface exposed	Mild sensorimotor axonal neuropathy with UL and LL weakness	Elevated	Not elevated	Elevated	Suriyanarayanan et al. 2016
c.529A > G; p.N177D	2^{nd}–6^{th} decade	Internal loop	Axonal sensorimotor demyelinating neuropathy with ulcerations, impaired wound healing,	Elevated	Elevated	Elevated	–

DO disease onset, *LL* lower limb, *UL* upper limb

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to declare.

Ethical Approval The manuscript is a retrospective case report that does not require ethics committee approval at our institution.

Informed Consent Written informed consent was obtained from all participants involved in this study.

References

Auer-Grumbach, M. (2013). Hereditary sensory and autonomic neuropathies. *Handbook of Clinical Neurology, 115,* 893–906. https://doi.org/10.1016/b978-0-444-52902-2.00050-3.

Auer-Grumbach, M., Bode, H., Pieber, T. R., Schabhuttl, M., Fischer, D., Seidl, R., et al. (2013). Mutations at Ser331 in the HSN type I gene SPTLC1 are associated with a distinct syndromic phenotype. *European Journal of Medical Genetics, 56*(5), 266–269. https://doi.org/10.1016/j.ejmg.2013.02.002.

Auranen, M., Toppila, J., Suriyanarayanan, S., Lone, M. A., Paetau, A., Tyynismaa, H., et al. (2017). Clinical and metabolic consequences of L-serine supplementation in hereditary sensory and autonomic neuropathy type 1C. *Cold Spring Harbor Molecular Case Studies*. https://doi.org/10.1101/mcs.a002212.

Bode, H., Bourquin, F., Suriyanarayanan, S., Wei, Y., Alecu, I., Othman, A., et al. (2016). HSAN1 mutations in serine palmitoyltransferase reveal a close structure-function-phenotype relationship. *Human Molecular Genetics, 25*(5), 853–865. https://doi.org/10.1093/hmg/ddv611.

Breslow, D. K., Collins, S. R., Bodenmiller, B., Aebersold, R., Simons, K., Shevchenko, A., et al. (2010). Orm family proteins mediate sphingolipid homeostasis. *Nature, 463,* 1048–1053. https://doi.org/10.1038/nature08787.

Davis, D. L., Gable, K., Suemitsu, J., Dunn, T. M., & Wattenberg, B. W. (2019). The ORMDL/Orm-serine palmitoyltransferase (SPT) complex is directly regulated by ceramide: Reconstitution of SPT regulation in isolated membranes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.ra118.007291.

Dawkins, J. L., Hulme, D. J., Brahmbhatt, S. B., Auer-Grumbach, M., & Nicholson, G. A. (2001). Mutations in SPTLC1, encoding serine palmitoyltransferase, long chain base subunit-1, cause hereditary sensory neuropathy type I. *Nature Genetics, 27*(3), 309–312. https://doi.org/10.1038/85879.

Dohrn, M. F., Glockle, N., Mulahasanovic, L., Heller, C., Mohr, J., Bauer, C., et al. (2017). Frequent genes in rare diseases: panel-based next generation sequencing to disclose causal mutations in hereditary neuropathies. *Journal of Neurochemistry, 143*(5), 507–522. https://doi.org/10.1111/jnc.14217.

Dyck, P. J. (1993). Neuronal atrophy and degeneration predominantly affecting peripheral sensory and autonomic neurons. In P. J. Dyck, K. P. Thomas, J. W. Griffin, P. A. Low, & J. F. Poduslo (Eds.), *Peripheral Neuropathy* (3rd ed., pp. 1065–1093). Philladelphia: Saunders.

Edvardson, S., Cinnamon, Y., Jalas, C., Shaag, A., Maayan, C., Axelrod, F. B., et al. (2012). Hereditary sensory autonomic neuropathy caused by a mutation in dystonin. *Annals of Neurology, 71*(4), 569–572. https://doi.org/10.1002/ana.23524.

Eichler, F. S., Hornemann, T., McCampbell, A., Kuljis, D., Penno, A., Vardeh, D., et al. (2009). Overexpression of the wild-type SPT1 subunit lowers desoxysphingolipid levels and rescues the phenotype of HSAN1. *The Journal of Neuroscience, 29*(46), 14646–14651. https://doi.org/10.1523/jneurosci.2536-09.2009.

Ernst, D., Murphy, S. M., Sathiyanadan, K., Wei, Y., Othman, A., Laura, M., et al. (2015). Novel HSAN1 mutation in serine palmitoyltransferase resides at a putative phosphorylation site that is involved in regulating substrate specificity. *NeuroMolecular Medicine, 17*(1), 47–57. https://doi.org/10.1007/s12017-014-8339-1.

Fridman, V., Suriyanarayanan, S., Novak, P., David, W., Macklin, E. A., McKenna-Yasek, D., et al. (2019). Randomized trial of L-serine in patients with hereditary sensory and autonomic neuropathy type 1. *Neurology, 92*(4), e359–e370. https://doi.org/10.1212/wnl.0000000000006811.

Garofalo, K., Penno, A., Schmidt, B. P., Lee, H. J., Frosch, M. P., von Eckardstein, A., et al. (2011). Oral L-serine supplementation reduces production of neurotoxic deoxysphingolipids in mice and humans with hereditary sensory autonomic neuropathy type 1. *Journal of Clinical Investigation, 121*(12), 4735–4745. https://doi.org/10.1172/jci57549.

Geraldes, R., de Carvalho, M., Santos-Bento, M., & Nicholson, G. (2004). Hereditary sensory neuropathy type 1 in a Portuguese family-electrodiagnostic and autonomic nervous system studies. *Journal of the Neurological Sciences, 227,* 35–38. https://doi.org/10.1016/j.jns.2004.08.002.

Guntert, T., Hanggi, P., Othman, A., Suriyanarayanan, S., Sonda, S., Zuellig, R. A., et al. (2016). 1-Deoxysphingolipid-induced neurotoxicity involves N-methyl-d-aspartate receptor signaling. *Neuropharmacology, 110*(Pt A), 211–222. https://doi.org/10.1016/j.neuropharm.2016.03.033.

Gupta, S. D., Gable, K., Alexaki, A., Chandris, P., Proia, R. L., Dunn, T. M., et al. (2015). Expression of the ORMDLS, modulators of serine palmitoyltransferase, is regulated by sphingolipids in mammalian cells. *Journal of Biological Chemistry, 290*(1), 90–98. https://doi.org/10.1074/jbc.M114.588236.

Hornemann, T., Penno, A., Rütti, M. F., Ernst, D., Kivrak-Pfiffner, F., Rohrer, L., et al. (2009). The SPTLC3 subunit of serine palmitoyltransferase generates short chain sphingoid bases. *The Journal of Biological Chemistry, 284,* 26322–26330. https://doi.org/10.1074/jbc.M109.023192.

Hornemann, T., Richard, S., Rütti, M. F., Wei, Y., von Eckardstein, A., Serine-palmitoyltransferase, M., et al. (2006). Cloning and initial characterization of a new subunit for mammalian serine-palmitoyltransferase. *The Journal of Biological Chemistry, 281,* 37275–37281. https://doi.org/10.1074/jbc.M608066200.

Houlden, H., Blake, J., & Reily, M. M. (2004). Hereditary sensory neuropathies. *Current Opinion in Neurology, 17*(5), 569–577.

Houlden, H., King, R., Blake, J., Groves, M., Love, S., Woodward, C., et al. (2006). Clinical, pathological and genetic characterization of hereditary sensory and autonomic neuropathy type 1 (HSAN I). *Brain, 129,* 411–425. https://doi.org/10.1093/brain/awh712.

Klein, C. J., Wu, Y., Kruckeberg, K. E., Hebbring, S. J., Anderson, S. A., Cunningham, J. M., et al. (2005). SPTLC1 and RAB7 mutation analysis in dominantly inherited and idiopathic sensory neuropathies. *Journal of Neurology, Neurosurgery and Psychiatry, 76*(7), 1022–1024. https://doi.org/10.1136/jnnp.2004.050062.

Laura, M., Murphy, S. M., Hornemann, T., Bode, H., Polke, J., Blake, J., et al. (2012). P42 Hereditary sensory neuropathy type 1: correlation of severity and plasma atypical deoxy-sphyngoid bases. *Neuromuscular Disorders, 22,* S18. https://doi.org/10.1016/S0960-8966(12)70050-0.

Leipold, E., Liebmann, L., Korenke, G. C., Heinrich, T., Gieszelmann, S., Baets, J., et al. (2013). A de novo gain-of-function mutation in SCN11A causes loss of pain perception. *Nature Genetics, 45*(11), 1399–1404. https://doi.org/10.1038/ng.2767.

Murphy, S. M., Ernst, D., Wei, Y., Laurà, M., Liu, Y.-T., Polke, J., et al. (2013). Hereditary sensory and autonomic neuropathy type 1 (HSANI) caused by a novel mutation in SPTLC2. *Neurology, 80,* 2106–2111. https://doi.org/10.1212/WNL.0b013e318295d789.

Penno, A., Reilly, M. M., Houlden, H., Laurá, M., Rentsch, K., Niederkofler, V., et al. (2010). Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. *The Journal of Biological Chemistry, 285,* 11178–11187. https://doi.org/10.1074/jbc.M109.092973.

Rotthier, A., Auer-Grumbach, M., Janssens, K., Baets, J., Penno, A., Almeida-Souza, L., et al. (2010). Mutations in the SPTLC2 subunit of serine palmitoyltransferase cause hereditary sensory and autonomic neuropathy type I. *American Journal of Human Genetics, 87*(4), 513–522. https://doi.org/10.1016/j.ajhg.2010.09.010.

Rotthier, A., Penno, A., Rautenstrauss, B., Auer-Grumbach, M., Stettner, G. M., Asselbergh, B., et al. (2011). Characterization of two mutations in the SPTLC1 subunit of serine palmitoyltransferase associated with hereditary sensory and autonomic neuropathy type I. *Human Mutation, 32*(6), E2211–E2225. https://doi.org/10.1002/humu.21481.

Suh, B. C., Hong, Y. B., Nakhro, K., Nam, S. H., Chung, K. W., & Choi, B.-O. (2014). Early-onset severe hereditary sensory and autonomic neuropathy type 1 with S331F SPTLC1 mutation. *Molecular medicine Reports, 9,* 481–486. https://doi.org/10.3892/mmr.2013.1808.

Suriyanarayanan, S., Toppila, J., Auranen, M., Paetau, A., Shcherbii, M., Palin, E., et al. (2016). The variant p.(Arg183Trp) in SPTLC2 causes late-onset hereditary sensory neuropathy. *NeuroMolecular Medicine, 18*(1), 81–90. https://doi.org/10.1007/s12017-015-8379-1.

Yard, B. A., Carter, L. G., Johnson, K. A., Overton, I. M., Dorward, M., Liu, H., et al. (2007). The structure of serine palmitoyltransferase; gateway to sphingolipid biosynthesis. *Journal of Molecular Biology, 370*(5), 870–886. https://doi.org/10.1016/j.jmb.2007.04.086.