Expanding the spectrum of *SPTLC1*-related disorders beyond hereditary sensory and autonomic neuropathies: a novel case of the distinct "S331 syndrome"

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Abstract

Background: Hereditary sensory and autonomic neuropathies (HSAN) encompass a group of peripheral nervous system disorders characterized by remarkable heterogeneity from a clinical and genetic point of view. Mutations in *SPTLC1* gene are responsible for HSAN type IA, which usually starts from the second to fourth decade with axonal neuropathy, sensory loss, painless distal ulcerations and mild autonomic features, while motor involvement usually occur later as disease progresses.

Aim and Methods: Beyond the classic presentation of HSAN type IA, an exceedingly rare distinct phenotype related to *SPTLC1* mutations at residue serine 331 (S331) has recently been reported, characterized by earlier onset, prominent muscular atrophy, growth retardation, oculo-skeletal abnormalities and possible respiratory complications. In this report, we describe clinical, instrumental and genetic aspects of a 13-year-old Sri Lankan male carrying the rare *de novo* p.S331Y heterozygous mutation in *SPTLC1* gene found by whole exome sequencing.

Results: Patient's phenotype partly overlaps with the first case previously reported, however with some additional features not described before. This work represent the second report about this rare mutation and our findings strongly reinforce the hypothesis of a clearly distinct "S331 syndrome", thus expanding the spectrum of *SPTLC1*-related disorders.

Keywords

- HSAN
- Polyneuropathy

- Genetic diseases

- Neuromuscular diseases

- SPTLC1

Background and Aims

Inherited peripheral neuropathies comprise a group of genetically and clinically heterogeneous disorders of the peripheral nervous system. Based on the predominant involvement of motor or sensory nerve fibers, three main subclasses have been defined: hereditary motor and sensory neuropathies (HMSN), hereditary motor neuropathies and hereditary sensory and autonomic neuropathies (HSAN) (1). HSAN are further classified into HSAN types I–VIII, according to clinical characteristics, electrophysiological features, inheritance pattern and genetic defect (1). In particular, HSAN type IA (HSAN-IA) is related to autosomal dominant mutations in *SPTLC1* gene, which is located on chromosome 9q22 and encodes for the serine palmitoyltransferase (SPT) long chain subunit 1, a key enzyme in sphingolipid synthesis (1). HSAN-IA usually starts from the second to fourth decade of life, and is mainly characterized by axonal sensory neuropathy without marked autonomic features. Prominent sensory loss can lead to painless ulcerations that, if unrecognized, result in mutilating distal arthropaties. Positive sensory phenomena, including dysesthesia and characteristic "lightning" or "shooting" pains also occur early, while motor involvement, sometimes responsible for wheelchair dependence, is a typical finding of more advanced stages (1). Beyond this classical presentation, it has recently been suggested that specific *SPTLC1* mutations at residue serine 331 (S331) may result in a more severe and complex phenotype (2). We here report the case of a young male patient with early-onset sensori-motor polyneuropathy and additional ocular and skeletal abnormalities, carrying a *de novo* heterozygous p.S331Y mutation in *SPTLC1* gene.

Case Report

1. Clinical Report

A 13-year-old boy native of Sri Lanka, first-born of non-consanguineous and healthy parents, was admitted to our Neurology Division. His medical history began at age 4 with gastrointestinal involvement, i.e. lack of appetite, early satiety, diarrhea, poor weight gain. Subsequently, progressive balance and “walking on toes” gait difficulties associated with frequent falls appeared. Wheelchair dependence occurred at age 8. Skeletal deformities became progressively evident

during growth, including severe scoliosis, pes cavus and Achilles tendon retraction, which required surgical tenotomy. At the time of our observation, a general physical examination revealed abdominal breathing, underweight body mass index (BMI 10 Kg/m^2), prominent growth retardation and delayed puberty. Metacarpo-phalangeal joints hypermobility and fingers "swan-neck" deformities were evident too, along with right clubfoot, a crossover toe in left foot, bilateral pes cavus and hammertoes (Fig. 1). Neurological examination disclosed intermittent exotropia, marked photophobia and tongue fasciculations/fibrillations. Brisk tendon reflexes without extensor plantar response were also observed, as well as steppage gait and severe diffuse muscular wasting, with a more prominent weakness in distal regions. A remarkable postural instability resulted in the inability to stand up for more than a few seconds without support. Moreover, stereotyped swinging movements of trunk and legs were detected. We did not observed clear sensory loss. Intellectual development was normal as demonstrated by psychometric and neuropsychological evaluations. The patient underwent a complete diagnostic protocol for neuromuscular disorders. Laboratory tests, including autoimmune panel, serum vitamins and hormone screening, disclosed a subclinical autoimmune thyroiditis with anti-thyroperoxidase antibodies (32.2 UI/mL; normal values, n.v.<16.0 UI/mL) and vitamin D deficiency (14.5 ng/mL; n.v. 30-100 ng/mL). Ophthalmological evaluation revealed bilateral cataracts with reduced visual acuity. Cardiologic assessment, including electrocardiogram and cardiac ultrasound, did not detected any abnormality. Nerve conduction velocities (NCVs) study was indicative of mixed demyelinating-axonal sensori-motor polyneuropathy. In particular, median nerve motor study showed a reduced conduction velocity (29.6 m/s) with prolonged F-wave minimal latency (37.25 ms with stimulation at wrist) and decreased compound muscle action potential (CMAP) amplitude (3.3 mV). Ulnar nerve motor study also disclosed a marked slowing in NCVs (27.0 m/s) with reduced CMAP amplitude (3.6 mV). Sensory conduction studies in upper limbs detected a moderate slowing of conduction velocities in median nerve (36.4 m/s) and radial nerve (39.0 m/s), along with severely diminished sensory nerve action potential amplitude in both nerves (3.7 μV and 2.7 μV, respectively). There was no response for motor or sensory nerves in the lower limbs. Unfortunately, electromyography (EMG) was not performed due to patient's refusal to cooperate. Brain Magnetic Resonance Imaging (MRI) did not detected any significant alteration. Considering the whole clinical and instrumental picture, we suspected a hereditary neuropathy and performed genetic molecular testing.

2. Genetic Analysis

After informed consent, whole blood (3 ml) was collected for genetic analysis. The first step included *PMP22* analysis performed by multiplex ligation-dependent probe amplification (MLPA) assay, which resulted in normal findings, subsequently a whole exome sequencing (WES) was carried out. Exome sequencing data and reads alignment analysis were checked for coverage depth and alignment quality employing Bedtools v.2.24.0 software package. Variant classification was performed in accordance with the guidelines from the American College of Medical Genetics and Genomics (ACMG). Phenotype driven analysis coupled with the employment of *in silico* multigene panels specific for hereditary motor and sensory neuropathies (HMSN), hereditary motor neuropathies (HMN) and hereditary sensory and autonomic neuropathies (HSAN) was used to filter, select and interpret genetic variants obtained following exome sequencing.

3. Genetic Results

NGS analysis and mutation interpretation highlighted the presence of the heterozygous c.992C>A mutation in the *SPTLC1* gene resulting in the amino acidic substitution p.S331Y, subsequently validated by *Sanger* sequencing. The mutation here found, although already reported in correlation with HSAN-IA, is not reported in the allele frequency databases (ExAC, EVS and GnomeAD). Bioinformatics predictors indicate that the Serine in position 331 is highly conserved through high vertebrates (PhyloP-Vertebrates=6,02/6,42; PhyloP-Primate=0,65/0,65) and the missense change Serine to Tyrosine is suspected to be deleterious (Polyphen2=0,454/1,00; SIFT=0,001/0,00; CADD-Phred=24). Moreover, segregation analysis highlighted the *de novo* occurrence of the mutation here found. According to the ACMG guidelines and considering the partial overlap of our patient's phenotype with those already described for mutations at residue S331, the concordance with the mode of inheritance together with the result of family segregation analysis, the variant p.S331Y (c.992C>A), here found in heterozygosis in the *SPTLC1* gene of the proband, may be considered pathogenic.

Interpretation

Serine palmitoyltransferase (SPT), located at the outer membrane of the endoplasmic reticulum, catalyzes the pyridoxal-5'-phosphate dependent condensation of L-serine with palmitoyl-CoA. This is the first and rate-limiting step in the *de novo* biosynthesis of sphingolipids (1). *SPTLC1* mutations typically result in a permanent shift in the SPT substrate preference, determining the use of L-alanine and glycine as substrate instead of L-serine, which leads to formation of atypical deoxysphingoid bases (DSB) (3). It has been demonstrated, using mouse model mutant for *SPTLC1* C133W and C133Y, that elevated levels of DSB are cytotoxic, but the mechanism of their toxicity is not fully clear (3). From a clinical point of view, a remarkable phenotypic variability occurs within *SPTLC1*-related HSAN cases. For example, mutations at position p.V144, p.C133, p.C144 and p.A352 result in the typical phenotype with predominantly sensory axonal neuropathy. Conversely, some other variants, namely p.S331F and p.S331Y, have been associated with a more severe early-onset phenotype, characterized by motor neurons involvement, prominent muscle atrophy, growth retardation, ocular abnormalities, and possible respiratory complications (2). Noteworthy, it has been reported that C_{20}-DSB, a specific type of sphingoid base, may be a peculiar biochemical hallmark of p.S331F/Y mutations, compared to other *SPTLC1* variants (3). Currently, four other cases of *SPTLC1* mutations at residue S331 have been reported (1,2,4) with only one case due to the p.S331Y variant (2). Comparing our proband with the already reported p.S331Y patient (Table I), they share most of clinical characteristics, including skeletal abnormalities, diffuse muscle weakness, joint hypermobility, brisk tendon reflexes and tongue fasciculations/fibrillations. This latter feature was not reported in the three p.S331F cases, thus suggesting that it could be a peculiar finding of patients carrying p.S331Y mutation. However, some differences between the two p.S331Y cases should be underlined. First, although in both reports NCVs revealed a polyneuropathy with significant sensory nerves involvement, our proband did not refer any loss of sensation, and no scars at the extremities or painless injuries were observed. Hand tremor was described in the first p.S331Y report, while our patient only showed stereotyped non-rhythmic movements of trunk and legs.

In conclusion, this is the second report of the *SPTLC1* p.S331Y mutation, that we detected in a young Sri Lankan boy showing an unusual phenotype, more similar to HMSN than classical HSAN-1A. Our case confirms the existence of a clearly distinct "S331 syndrome" related to pathogenic variants at residue S331 in *SPTLC1* gene, characterized by early motor involvement,

prominent muscle atrophy, growth retardation and oculo-skeletal abnormalities. However, we here provide some additional features, such as the relative preservation of distal sensory function, and consequent absence of foot ulcerations. Since oral L-serine supplementation has been identified as an effective therapy to slow disease progression (5), we believe it is important for clinicians to be aware of this extremely rare, but potentially treatable syndrome within the spectrum of *SPTLC1*-related disorders.

Acknowledgements

Not applicable.

Ethics approval

No experimental procedure was performed, but only the investigations required by clinical practice. For this reason it was not needed to submit the case to the approval of the Ethics Committee. Clinical Investigations were conducted according to the principles expressed in Declaration of Helsinki.

Patient consent for publication

Informed consent was acquired both for the diagnostic procedures required for diagnosis and for the use of data for scientific purposes in respect of privacy. Informed consent for publication was obtained from the parents of the patient.

Authors' contributions

GB and FR were the major contributors in writing the manuscript. DC analyzed the blood samples of the proband and their parents and found the proband mutation. MF and SC recruited the patient and performed clinical and instrumental assessment.GB, FR and MO analyzed the data, performed the bibliographic research and drafted manuscript, figure and table. GP and SS reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Competing interests

These data have not been published elsewhere nor are they under consideration by any other journal. The authors declare that they have no financial or competing interests.

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Table I. Clinical features of the proband compared with the first reported case of p. S331Y *SPTLC1* mutation

CLINICAL FEATURES	This study	Auer-Grumbach et al. (2)
Sex	Male	Female
Diffuse muscular atrophy	YES	YES
Growth retardation	YES	YES
Delayed puberty	YES	YES
Mental retardation	NO	NO
Nerve conduction study	mixed MSN	mixed MSN
Sensory disturbances	NO	YES
Foot ulcerations	NO	YES
Tremor	NO	YES
Juvenile cataract	YES	YES
Pes cavus/foot deformities	YES	YES
Joint hypermobility	YES	YES
Scoliosis	YES	YES
Motor system involvement	YES	YES
Wheel chair dependence	YES (8 years old)	YES (14 years old)
Tongue fasciculations	YES	YES
Respiratory problems	YES	YES
Brisk tendon reflexes	YES	YES
Babinski sign	NO	NO
Brain MRI abnormalities	NO	NO

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MSN: motor-sensory neuropathy. Main differences are in **bold**.

Figure Legend

Figure 1. Clinical features of the patient

Swan-neck deformities of the fingers during active extension with hyper-extended proximal interphalangeal joints and flexed distal interphalangeal joints [A]. Metacarpophalangeal joints hypermobility [B]. Right clubfoot; cross-over toe in left foot *(arrow);* bilateral pes cavus and hammer toes [C-D].

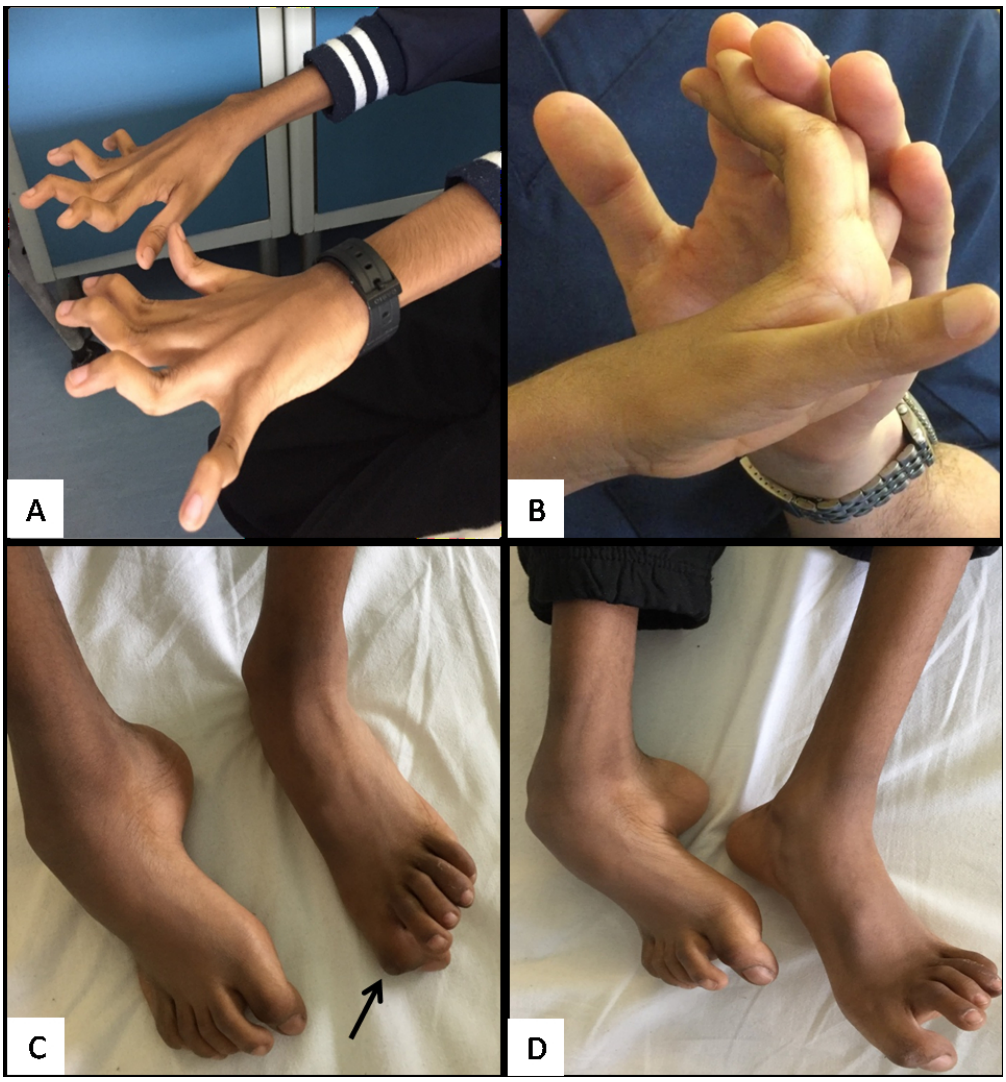

Figure 1. Clinical features of the patient"*Swan-neck* deformities of the fingers during active extension with hyper-extended proximal interphalangeal joints and flexed distal interphalangeal joints [A]. Metacarpophalangeal joints hypermobility [B]. Right clubfoot; cross-over toe in left foot (arrow); bilateral pes cavus and hammer toes [C-D].

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