


BRIEF COMMUNICATION

A novel *CPT1C* variant causes pure hereditary spastic paraplegia with benign clinical course

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Introduction

Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of disorders characterized by progressive spasticity and weakness of the lower limbs resulting from a length dependent degeneration of the corticospinal tract, ascending sensory fibers, spinocerebellar fibers, and neuronal cell bodies.¹ The phenotype of HSPs can be pure with spasticity of lower limbs alone or complicated with additional symptoms such as peripheral neuropathy, epilepsy, ataxia, optic neuropathy, retinopathy, deafness, dementia, mental retardation, ichthyosis, and bulbar dysfunction.^{2,3}

The inherited modes of HSPs include autosomal dominant (AD), autosomal recessive (AR), or X-linked recessive.⁴ At least 80 different genetic loci for HSPs (SPG1-80) have been identified, and 60 genes are currently cloned.⁵ Among pure and dominantly inherited HSPs, heterozygous variant in the neuronal isoform of carnitine

Abstract

Hereditary spastic paraplegia 73 (SPG73) was currently identified in only one family with variant in the neuronal isoform of carnitine palmitoyl-transferase 1C (*CPT1C*) gene. We described a new family, in which affected individuals exhibited pure hereditary spastic paraplegia with benign clinical course. Exome sequencing revealed a novel nonsense variant in the *CPT1C* gene. The level of *CPT1C* mutant transcript significantly decreased compared to that of wild-type transcript, and can be recovered after cycloheximide administration, which indicated that nonsense-mediated mRNA decay was a mechanism that might be responsible for the phenotype. Our findings expanded the clinical and genetic spectrum of SPG73.

palmitoyl-transferase (*CPT1C*) gene as the genetic cause of SPG73 is a new locus added to the genetic list of SPGs recently.⁶ However, definitive proof about the pathogenicity of *CPT1C* will require identification of more heterozygote variants in new HSP patients. Herein, we report that three patients present with pure SPG73 accompanied with a benign clinical course in an autosomal dominant family.

Methods

Information regarding age of onset, progression of disease, and clinical manifestations was collected. Multiple laboratory tests were examined. Cerebral and spinal Magnetic Resonance imaging (MRI) were conducted. All patients were examined by at least two neurologists after written informed consent. The research was approved by ethics committee of the Peking University People's Hospital.

Genetic test was initially conducted in the index patient through exome next generation sequencing (NGS).

Targeted exon enrichment was performed using SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, USA). This enriched library was sequenced on a HiSeq 2500 platform (Illumina, San Diego, CA) for paired-end reads of 100 bp. The reads were aligned against the human reference genome (hg19) using the SOAPaligner program. The single-nucleotide polymorphisms (SNPs) were identified using the SOAPsnp program. Subsequently, the reads were realigned to the reference genome by the BWA program, and insertions or deletions (InDels) were identified with the GATK program. Allele frequency and reported pathogenicity were assessed using the dbSNP138, Human Gene Mutation Database (HGMD), 1000 Genomes Project, and Exome Sequencing Project (ESP6500). DNA from all available family members was directly sequenced for cosegregation analysis. In addition, the dynamic (CAG)_n numbers for SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, and DRPLA were directly detected by PCR amplified fragments. The copy number variation in exons in the SPAST, REEP1, and ATL1 gene were examined by multiplex ligation-dependent probe amplification (MLPA).

Plasmids harboring wild-type GFP-CPT1C or mutant GFP-CPT1C were transfected into HEK 293T cells for over-expression. To explore the presence of nonsense mediated mRNA decay, cells were split into two subcultures. One subculture remained untreated, whereas the other was exposed to 150 mg/ml cycloheximide. The fluorescence of GFP was directly observed in these subcultures. Quantitative PCR (qPCR) for relative quantification of CPT1C transcript levels was performed on cDNA using primers specific for the CPT1C and GAPDH gene. Measurements were normalized against the GAPDH gene. The level of mutant transcript was relatively quantified to the level of wild-type transcript. The protein level of CPT1C was evaluated by a monoclonal antibody (H00126129-P01, Novus Biologicals, Centennial, CO, USA).

Results

The index patient (II1, Table 1) was a 13-year-old girl who came from a nonconsanguineous family (Fig. 1A). She had a milestone delay of motor ability characterized as crawling at age 1, learning to walk at age 2, and walking independently at age 3.5. Before the age of 9, she gradually developed difficulty of walking steadily, weakness of lower limbs, and frequent falling-down. Thereafter, she felt the aforementioned symptoms improved gradually, but still had muscle fatigue, stiffness, soreness, and clumsy gaits after walking with long-distance. Her muscle strength was grade 4/5 (Medical Research Council Scale) in the foot dorsiflexion, grade 5-/5 in the proximal lower limbs, and grade 5/5 in the upper limbs. The

muscle tone of lower limbs increased. Deep tendon reflexes were brisk in the lower extremities. Babinski signs were elicited bilaterally. Sensation of vibration in the feet slightly decreased, but the other sensations were normal. No oculomotor disturbance, slow saccades, dysarthria, and ataxia signs were identified. Bilateral pes cavus was obvious. Neuropsychological battery tests revealed a normal cognitive status. Cerebral and spinal MRI were normal. Somatosensory evoked potentials indicated a delay latency and poor waveform differentiation, but peripheral nerve conduction velocities (NCV) were normal.

The younger brother (II2) of index patient was 12 years old. He presented with clinical features similar to that of the index patient. The symptoms insidiously progressed before 10 years old, then gradually improved in the recent 2 years, despite the fact that spasticity of lower limbs still affected his gaits. Physical examination revealed spasticity and hyperreflexia in lower limbs, mild weakness of foot dorsi/plantar flexion, and bilateral pes cavus. Babinski signs were elicited bilaterally. No oculomotor disturbance, slow saccades, dysarthria, and ataxia signs were identified. Cerebral and spinal MRI were normal. Somatosensory evoked potentials indicated a delay latency and poor waveform differentiation, but NCV was normal. Before the index patient was diagnosed as SPG73, the 45-year-old mother (I2) felt no neurological symptoms. Physical examinations revealed high-brisk reflexes in both knees and a positive response of the right Oppenheimer sign, but no other extensor plantar responses, spasticity, gait abnormalities, and sensory loss were identified. The father and little younger brother (II3) were normal in

Table 1. Clinical characteristics of affected individuals of the family.

Clinical variables	Patient II1	Patient II2	Patient I2
Age at onset (years)	infancy	infancy	uncertainty
Age at walking (years)	3.5	3	1
Age at examinations (years)	13	12	45
Gender	female	male	female
Spasticity	+	+	–
Hyperreflexia	+	+	+
Plantar reflex	+	+	+
Muscle weakness	+	+	–
Muscle wasting	–	–	–
Vibration sensation	+	+	–
Urinary dysfunction	–	–	–
Pes cavus	+	+	–
Ataxia	–	–	–
Brain and spinal MRI	–	–	–
Neurophysiology	–	–	–
Progression	progression-reversible	progression-reversible	unnoticed

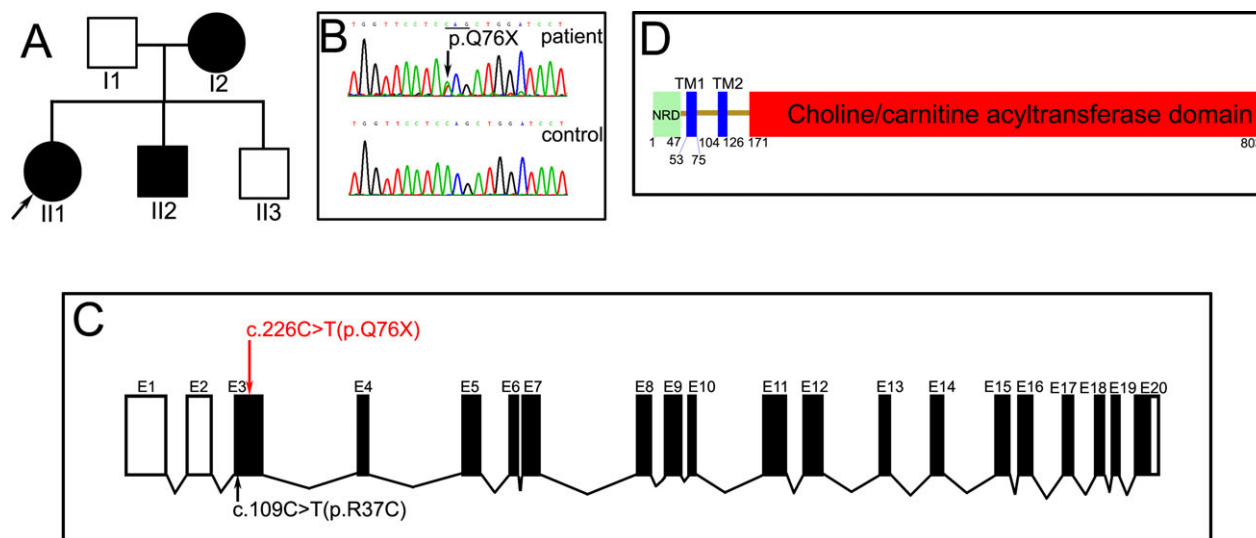


Figure 1. The clinical and genetic data in the SPG73 family. The family tree indicated an autosomal dominant inherited pattern (A, arrow indicates index patient). The chromatogram revealed a variant (c.226C>T) in the *CPT1C* gene (B). The novel variant (red font) caused a premature stop in exon 3 of the *CPT1C* gene, the reported variant (black font) was also located in the exon 3 (C). *CPT1C* protein is composed of a small N-terminal regulatory domain (NRD, 1-47) and a large catalytic-terminal domain (171-803), separated by two transmembrane (TM) domains and a short connecting loop (D).

clinical evaluations. No other family members were available to examine clinically.

A novel heterozygous variant (c.226C>T) resulting in a premature stop (p.Q76X, Fig. 1B) was detected in exon 3 of the *CPT1C* gene by exome NGS (Fig. 1C). The same variant was identified in her mother and younger brother, but not in her father and healthy little brother, which suggested the variant was closely segregated with the phenotype. The variant was not found in 200 healthy Chinese controls, 1000 genomes database, ExAC database, and gnomAD database. No other causative variants associated with other SPGs were found in the NGS data; no abnormal expansion of nucleotide repeats was identified in SCAs. No copy number variation in exons was found in the *SPAST*, *REEP1*, or *ATL1* gene.

The GFP fluorescence showed an increased expression of control (Fig. 2A) and wild-type *CPT1C* (Fig. 2B) in the 293T cells, but the expression of mutant *CPT1C* significantly decreased (Fig. 2C). After administration of cycloheximide, the expression of mutant *CPT1C* was recovered (Fig. 2D). Premature stop variant might induce mRNA degradation via nonsense-mediated mRNA decay. 293T cells carrying the p.Q76X variant indeed contained only about 5% *CPT1C* mRNA transcript compared to that of wild-type cells (Fig. 2E). Transcript levels could be increased after treatment with the translation inhibitor cycloheximide that blocks nonsense-mediated mRNA decay. No full-length or truncated *CPT1C* proteins were detected by immunoblot in the 293T cell homogenates (Fig. 2F).

Discussion

SPG73 has been considered a pure form of AD-HSP characterized by adult-onset progressive weakness and spasticity of the lower limbs, but the disease progression is relentless and will lead to loss of ambulation at 10 to 15 years after the onset of symptoms.⁶ Currently the clinical features of SPG73 were only described in an Italian family.⁶ Herein we reported the second SPG73 family with genetic confirmation. The affected patients presented with a typical phenotype of pure HSP characterized by spasticity of lower limbs and pes cavus, but they simultaneously showed some distinct characteristics. The index patient and her brother showed a motor milestone delay from infant period, and then exhibited a reversible course at the age of around 10. Conversely, the mother was only identified to have hyperreflexia and mild extensor plantar response without any other symptoms, which represented a putatively asymptomatic carrier. The phenotype of our patients indicated that the clinical course of SPG73 was relatively benign, despite the fact that the patients had congenital onset.

The list of genes responsible for HSPs is quickly expanding, mostly owing to the convenient availability of NGS method.⁷ The *CPT1C* gene responsible for SPG73 was reported in autosomal dominant HSP. Only a missense (p.R37C) in the *CPT1C* gene had been reported in an Italian family with pure HSP currently.⁶ In this study, we found a *CPT1C* variant in a Chinese family through

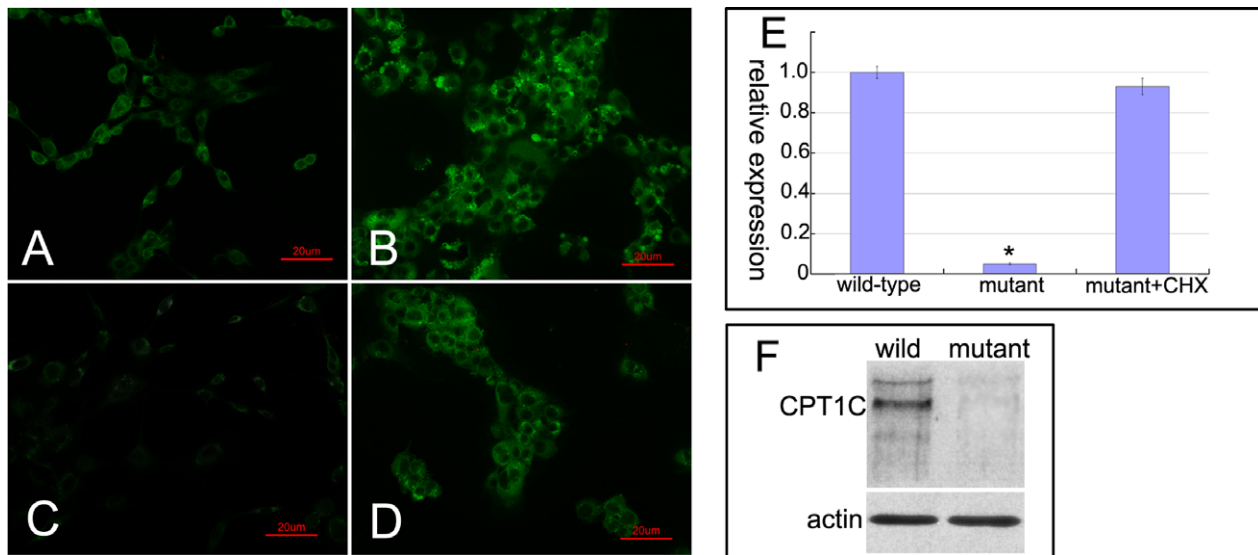


Figure 2. The pathogenic investigations of mutant *CPT1C*. The GFP fluorescence showed a normal expression in 293T cells transfected with pEGFP-N1 vectors (A), an increased expression in 293T cells transfected with wild-type pEGFP-N1-CPT1C vectors (B), and a significantly decreased expression in 293T cells transfected with c.226C>T mutant pEGFP-N1-CPT1C vectors (C). After administration of cycloheximide (CHX), the expression of mutant *CPT1C* was recovered (D). Quantitative PCR measurements showed a significant reduction in the mutant *CPT1C* transcript expression compared to the level of wild-type transcript. Repeated three times for every test. Data were analyzed by one-way ANOVA test; Error bars are SEM; * $P < 0.01$ (E). Immunoblot showed that no full-length or truncated *CPT1C* proteins were detected in the mutant cells (F).

NGS method. The nonsense mutation was a novel variant that was predicted to cause a premature stop, and closely segregated in the family. Therefore, the variant can be qualified as pathogenic according to the American College Medical Genetics and Genomics (ACMG) criteria.⁸

CPT1C encodes the neuronal isoform of the carnitine palmitoyl-transferase enzyme that is mainly expressed in the soma and dendritic projections of motor neurons, and localizes at the endoplasmic reticulum (ER).⁹ *CPT1C* protein is composed of a small N-terminal domain and a large catalytic-terminal domain, separated by two transmembrane domains and a short connecting loop (Fig. 1D).¹⁰ Rinaldi et al. found that the missense mutation (p.R37C) would impair the function of N-terminal regulatory domain, and markedly reduced the number and size of lipid droplets in the cortical neurons through a dominant negative mechanism.⁶ However, we found that the level of mutant *CPT1C* transcript significantly decreased compared to the level of wild-type transcript, and can be recovered after treatment with cycloheximide that blocks nonsense-mediated mRNA decay. The immunoblot study revealed that no full or truncated *CPT1C* proteins were detected. These findings indicated that the nonsense-mediated mRNA decay might be responsible for the pathogenesis of SPG73 in our patients.¹¹ The stop codon Pro76 is located at the last residue of the first transmembrane helix, and will lose the whole catalytic-terminal domain. The C-terminal choline/carnitine

acyltransferase domain of *CPT1C* can directly interact with atlastin-1 (SPG3A) in the network of ER, and assists in the formation and maintenance of long neuron processes, which suggest that abnormalities of ER morphogenesis may be a common pathogenesis of HSPs.^{12,13}

Carrasco et al. found that *CPT1C*-deficient mice developed early onset progressive motor disturbances, including impaired gait and coordination, severe muscle weakness, and reduced locomotor activity.¹⁴ During mouse development, *CPT1C* levels were low in the cerebellum, striatum, and motor cortex from birth to postnatal day 10. Levels then increased gradually and peaked on postnatal day 21. In adulthood, *CPT1C* expression was substantially reduced in the striatum and cerebellum, but remained high in the motor cortex. The experimental evidence suggested that the availability of *CPT1C* protein was important to the development of neurons in the young individual, but the insufficiency of *CPT1C* protein function might be tolerated in the adult. The pattern of dynamic expression might partially explain the phenotype of motor milestone delay and reversible course with age in our patients.¹⁵

In conclusion, our findings provided additional patients to confirm that autosomal dominant SPG73 could be caused by the *CPT1C* variant. The clinical features exhibited pure form of HSP with benign clinical course. Nonsense-mediated mRNA decay was a mechanism that might be responsible for the phenotype in our patients.

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Author Contribution

H.D. contributed to research execution and manuscript composition; C. L. contributed to genetic evaluation. Z.S. and L.L. contributed to cell and RNA experiments. X.Y. contributed to critical review and revision of manuscript; Z.J. contributed to design, conception, and manuscript composition.

Conflicts of Interest

None.

References

- Lo Giudice T, Lombardi F, Santorelli FM, et al. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. *Exp Neurol* 2014;261:518–539.
- Kara E, Tucci A, Manzoni C, et al. Genetic and phenotypic characterization of complex hereditary spastic paraplegia. *Brain* 2016;139:1904–1918.
- Blackstone C. Hereditary spastic paraplegia. *Handb Clin Neurol* 2018;148:633–652.
- Finsterer J, Löscher W, Quasthoff S, et al. Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance. *J Neurol Sci* 2012;318:1–18.
- Faber I, Pereira ER, Martinez ARM, et al. Hereditary spastic paraplegia from 1880 to 2017: an historical review. *Arq Neuropsiquiatr* 2017;75:813–818.
- Rinaldi C, Schmidt T, Situ AJ, et al. Mutation in CPT1C associated with pure autosomal dominant spastic paraplegia. *JAMA Neurol* 2015;72:561–570.
- Qiu Y, Zhong S, Cong L, et al. A novel KIF5A gene variant causes spastic paraplegia and cerebellar ataxia. *Ann Clin Transl Neurol* 2018; 5:1415–1420.
- Bush LW, Beck AE, Biesecker LG, et al. Professional responsibilities regarding the provision, publication, and dissemination of patient phenotypes in the context of clinical genetic and genomic testing: points to consider—a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2018;20:169–171.
- Price N, van der Leij F, Jackson V, et al. A novel brain-expressed protein related to carnitine palmitoyltransferase I. *Genomics* 2002;80:433–442.
- Gratacòs-Batlle E, Olivella M, Sánchez-Fernández N, et al. Mechanisms of CPT1C-dependent AMPAR trafficking enhancement. *Front Mol Neurosci* 2018;11:275.
- Baker KE, Parker R. Nonsense-mediated mRNA decay: terminating erroneous gene expression. *Curr Opin Cell Biol* 2004;16:293–299.
- Blackstone C, O’Kane CJ, Reid E. Hereditary spastic paraplegias: membrane traffic and the motor pathway. *Nat Rev Neurosci* 2011;12:31–42.
- Hu J, Shibata Y, Zhu PP, et al. A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell* 2009;138:549–561.
- Carrasco P, Jacas J, Sahún I, et al. Carnitine palmitoyltransferase 1C deficiency causes motor impairment and hypoactivity. *Behav Brain Res* 2013;256:291–297.
- Salinas S, Proukakis C, Crosby A, Warner TT. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. *Lancet Neurol* 2008;7:1127–1138.