Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.1839-18
Intern Med 58: 719-722, 2019
http://internmed.jp

【 CASE REPORT 】

Exome Sequencing Reveals a Novel Homozygous Frameshift Mutation in the *CYP7B1* Gene in a Japanese Patient with SPG5

Haitian Nan[1], Keisuke Shimozono[1], Yuta Ichinose[1], Mai Tsuchiya[1], Kishin Koh[1], Masaki Hiraide[2] and Yoshihisa Takiyama[1]

Abstract:
SPG5 is a rare subtype of autosomal recessive hereditary spastic paraplegia caused by a homozygous mutation in the *oxysterol 7α-hydroxylase* gene, *CYP7B1*. We describe the first Japanese patient with SPG5 with a novel mutation in the *CYP7B1* gene. On exome sequencing, we identified a homozygous frameshift mutation, c.741delA, p.K247fs, in exon 3 of the *CYP7B1* gene. The patient showed spastic paraparesis with white matter hyperintensities in the bilateral corona radiata and periventricular and subcortical regions on brain magnetic resonance imaging. The present study expands the mutation spectrum of *CYP7B1* and provides an opportunity to study the genotype-phenotype correlation in SPG5.

Key words: hereditary spastic paraplegia, SPG5, *CYP7B1*, frameshift mutation, white matter lesions

(Intern Med 58: 719-722, 2019)
(DOI: 10.2169/internalmedicine.1839-18)

Introduction

Hereditary spastic paraplegias (HSPs) are clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive weakness and spasticity in the lower limbs due to pyramidal tract dysfunction (1). Spastic paraplegia type 5 (SPG5) is an autosomal recessive (AR) HSP that may present with a pure or complicated phenotype (2). The gene responsible for SPG5, *CYP7B1*, encodes enzyme cytochrome P450 oxysterol 7α-hydroxylase, which is implicated in cholesterol metabolism (3). To date, over 50 different *CYP7B1* mutations associated with HSPs have been described, and white matter hyperintensity has been reported in several families with a pure form of HSP (4).

We herein report the first Japanese patient with SPG5 with a novel homozygous frameshift mutation of *CYP7B1* accompanied by white matter lesions.

Case Report

A 66-year-old woman was the second of 5 siblings born to healthy, unrelated parents. However, it should be noted her parents were born in the same village. Her only child and all of her brothers and sisters were unaffected. The patient complained of pain in the lower limbs at 10 years of age. At 20 years of age, she underwent arthroscopy surgery because of worsening pain in her knees and ankle joints. Nevertheless, there was no pain relief after surgery. After 40 years of age, she developed gait unsteadiness with slowly progressive aggravation. At 50 years of age, she was diagnosed with HSP because of the development of overt spasticity in the lower limbs. She used a walking stick from that time. From 60 years of age, she relied on a walking frame and was only able to walk for less than 100 m without resting.

On a neurological examination, she was intellectually normal, showed spastic paraparesis, and could not stand or

[1]Department of Neurology, Graduate School of Medical Sciences, University of Yamanashi, Japan and [2]Department of Neurology, Kyonan Hospital, Japan
Received: July 17, 2018; Accepted: August 5, 2018; Advance Publication by J-STAGE: October 17, 2018
Correspondence to Dr. Yoshihisa Takiyama, ytakiyama@yamanashi.ac.jp

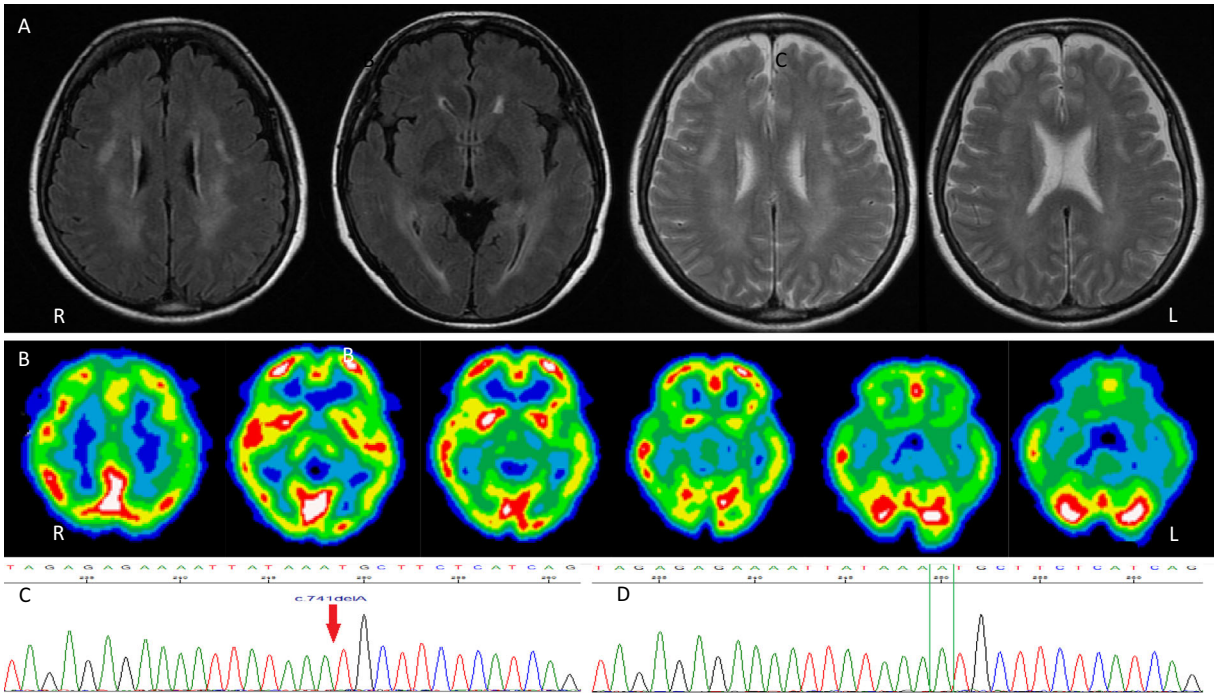

Figure. **A: Brain MRI showed areas of white matter hyperintensity in the bilateral corona radiata and periventricular and subcortical regions on T2WI and FLAIR. B: Brain SPECT revealed mild hypoperfusion in the left temporal lobe. C: Sequence analyses revealed the c.741delA mutation in exon 3 of *CYP7B1* in the patient. The red arrow indicates the position of the c.741 nucleotide. D: Sequence analyses revealed no mutation in exon 3 of *CYP7B1* in an unrelated negative control. The green frame indicates the position of the c.741 nucleotide.**

walk unaided. She had stiff legs, reduced muscle strength, loss of deep and superficial sense in the lower limbs, and positive Romberg's sign. Brisk tendon reflexes in the lower limbs and bilateral Babinski signs were detected. We did not observe dysarthria, nystagmus, or dysdiadochokinesia. In addition, cataract, optic atrophy, and urinary disturbance were absent. Metabolic and routine blood investigations were unremarkable. Serologic testing for the main autoimmune diseases was negative. Her low-density lipoprotein (LDL-Chol) level was 124 mg/dL, high-density lipoprotein (HDL-Chol) level 63 mg/dL, and triglyceride (TG) level 93 mg/dL, all of which were within the respective normal ranges. Brain magnetic resonance imaging (MRI) showed areas of white matter hyperintensity in the bilateral corona radiata and periventricular and subcortical regions on T2-weighted imaging (T2 WI) and fluid-attenuated inversion recovery (FLAIR). Mild cerebral cortical atrophy was also noted (Figure A). Single-photon emission computed tomography (SPECT) revealed mild hypoperfusion in the left temporal lobe (Figure B).

We carried out whole-exome sequencing of genomic DNA from the patient. Genomic DNA was extracted from the peripheral blood. Exome capture was performed with a SureSelect Human All Exon V6+UTR (89Mb) Kit (Agilent Technologies, Santa Clara, USA). Paired-end sequencing was carried out on a HiSeq2500 (Illumina, San Diego, USA) using the HiSeq SBS Kit V4 (Illumina), which generated 100-bp reads. The reference databases utilized included hg19 (GRCh37) (http://genome.ucsc.edu), ExAC (http://exac.broadinstitute.org), HGMD (https://portal.biobase-international.com), and dbSNP (5), and the 1000 Genomes Project (6). We examined variants of 85 genes known to be responsible for HSP (Table). Through this analysis, we identified a novel frameshift mutation (c.741delA, p.K247fs) in exon 3 of the *CYP7B1* gene and ruled out the possibility of other causative genes. We then examined exon 3 of the *CYP7B1* gene in the patient and an unrelated negative control via polymerase chain reaction (PCR). On Sanger sequencing, we reconfirmed p.K247fs in exon 3 of the *CYP7B1* gene, which was in a homozygous state in the patient (Figure C). This mutation causes a frameshift that results in a premature stop codon. At the protein level, this mononucleotide deletion results in the disruption of the amino acid reading frame that causes lysine to be replaced by asparagine at position 247, with a resulting premature stop codon at position 254, p.Lys 247Asnfs*8. As the *CYP7B1* gene encodes the 506-amino acid protein hydroxycholesterol 7-α-hydroxylase, half of the coding sequence of *CYP7B1* was not translated in our patient. This mutation was not present in dbSNP, HGMD, or ExAC, thus representing a novel etiology in a Japanese AR HSP patient. This mutation was not detected in an unrelated negative control who had no symptoms (Figure D).

Discussion

SPG5 is a rare subtype of ARHSP caused by a homozygous mutation in the *oxysterol 7α-hydroxylase* gene,

Table. **Genes Known to Be Responsible for HSP.**

ATL1	SLC16A2	WDR48	GJA12	CYP7B1	KIF1A	IBA57	c12orf65
SPAST	Xp25	ARL6IP1	NT5C2	SPG7	FAM134B	MAG	CYP2U1
NIPA1	HACE1	ERLIN1	GBA2	ALDH18A1	ALS2	MTCO3	TFG
KIAA0196	LYST	AMPD2	AP4B1	SPG11	EXOSC3	MTTI	KIF1C
ALDH18A1	ALS2	ENTPD1	KIAA0415	ZFYVE26	SPOAN	MTND4	USP8
KIF5A	SACS	ARSI	TECPR2	ERLIN2	GAD1	MTATP6	FA2H
RTN2	SPPRS	PGAP1	AP4M1	SPG20	ARSACS	L1CAM	PNPLA6
HSPD1	BICD2	FLRT1	AP4E1	ACP33	MAG	PLP	c9orf12
BSCL2	CHS	RAB3GAP2	AP4S1	B4GALNT1	ARSPG75	Xq11	ZFYVE27
ATSV	IFIH1	MARS	VPS37A	DDHD1	REEP2	CPT1C	SLC33A1
REEP1	CCT5	ZFR	DDHD2	ATSV			

CYP7B1 (7). This enzyme is involved in the degradation of cholesterol into primary bile acids. Cholesterol is initially side-chain oxidized, and the resulting oxysterols 25-hydroxycholesterol (25-OHC) and 27-hydroxycholesterol (27-OHC) are 7α-hydroxylated by *CYP7B1* (8). In patients with SPG5, there are dramatic increases in oxysterol substrates in the plasma and cerebrospinal fluid (CSF) (9). Both the serum and CSF levels of 25-OHC and 27-OHC are therefore potentially useful diagnostic biomarkers in SPG5 and are recommended for guiding genetic analyses (10). In addition, two randomized placebo-controlled clinical trials had already shown decreased total cholesterol, 27-OHC, and 25-OHC levels in serum with atorvastatin treatment. However, the clinical effect of statin treatment remains to be determined (8, 10). Although the cholesterol levels in our patient were all within the normal range, comprehensive measurement of the related oxysterol levels is required. The long-term follow-up of our patient with statin treatment is expected.

The overall *CYP7B1* mutation frequency among HSPs was shown to be 7.3% in familial and 1.25% in sporadic cases (4, 11). The median age at onset of SPG5 is 13 years, and truncating mutations are associated with an earlier age at onset (8). It is generally accepted that generated truncated proteins are often non-functional or exert dominant-negative effects. In our case, half of the coding sequence of *CYP7B1* was not translated, which is primarily deleterious. Consequently, our patient had an early onset at 10 years of age.

By whole-exome sequencing of genomic DNA from the patient, we discovered multiple contiguous loci adjacent to *CYP7B1*, which displays identical alleles reported as a single nucleotide polymorphism (SNP). These genes include *ASPH, TTPA, YTHDF3, BHLHE22, CYP7B1*, and *ADHFE1*, which appear in the homozygous state in stretches of chromosome 8. The distance from the first SNP to the last SNP is about 5,000,000 bp. The disease-causing mutation in *CYP7B1* of the present patient was surrounded by a long homozygous haplotype, which may have originated from the same common ancestor. We therefore suspect that the patient's parents might have been ancestrally consanguineous.

To date, according to the genetic classification, a total of 78 types of HSP have been described, among which SPG2, SPG5, SPG7, SPG11, SPG13, SPG21, SPG35, SPG44, SPG47, SPG50, SPG51, SPG54, SPG56, and SPG63 are reported to include white matter abnormalities (12, 13). Patchy periventricular and subcortical hyperintense T2-weighted lesions were observed in our case. The origin of these lesions remains uncertain; vascular, inflammatory, metabolic, or degenerative processes, or a combination thereof, might be involved. The present case also showed mild cerebral cortical atrophy and hypoperfusion in the left temporal lobe, which had not been reported in the literature. This might expand the phenotypic spectrum of SPG5. Further studies are needed to ascertain whether or not abnormal SPECT findings are also common in SPG5/*CYP7B1*.

In conclusion, we herein report the first Japanese SPG5 patient with a novel frameshift mutation, p.K247fs, in *CYP7B1* with white matter lesions. Our results expand the mutation spectrum of *CYP7B1* and provide an opportunity to study the genotype-phenotype correlation of SPG5.

The present clinical and genetic study was approved by the institutional review board of Yamanashi University, and written informed consent was obtained from all participating individuals.

The authors state that they have no Conflict of Interest (COI).

Financial Support

This work was supported by Grants-in-Aid from the Research Committee for Ataxic Disease (Y.T.), the Ministry of Health, Labor and Welfare, Japan, JSPS KAKENHI Grant Numbers JP17K 17772 (K.K.) and JP18K07495 (Y.T.) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and grants for AMED under Grant Number JP17ek0109078 (Y.T.).

Acknowledgement

We thank the patient for participating in this study.

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