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SHORT COMMUNICATION

An atypical case of *SPG56/CYP2U1*-related spastic paraplegia presenting with delayed myelination

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Hereditary spastic paraplegia (HSP) is a neurological disorder characterized by a progressive spasticity and muscle weakness of the lower limbs. It is divided into two subtypes, uncomplicated and complicated forms. Biallelic mutations in the cytochrome P450 2U1 gene (*CYP2U1*) are associated with spastic paraplegia type 56 (SPG56), manifesting both uncomplicated and complicated HSP. Accompanying clinical features include intellectual disability, dystonia, cerebellar ataxia, subclinical peripheral neuropathy, visual impairment, as well as abnormalities in brain magnetic resonance imaging. As a rare clinical feature, delayed myelination has previously been reported in only two patients with *CYP2U1* mutations. Here, we report a patient with SPG56 with novel compound heterozygous mutations in *CYP2U1* which were identified by whole exome sequencing. Our patient exhibited complex features together with delayed myelination, broadening the phenotypic spectrum of SPG56, and implying that *CYP2U1* should be screened in HSP with delayed myelination.
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INTRODUCTION

Hereditary spastic paraplegia (HSP) is a neurological disease characterized by a progressive spasticity and lower-limb muscle weakness.[1] Modes of HSP inheritance include autosomal dominant, autosomal recessive and X-linked recessive, as well as *de novo* occurrence. HSP is divided into two subtypes: uncomplicated and complicated.[1–3] Uncomplicated HSP presents with progressive bilateral leg spasticity and weakness, while complicated forms show additional neurological or extra-neurological features.

To date, 69 genes and/or loci are registered in OMIM (https://www.omim.org/phenotypicSeries/PS303350) for HSP. Among them, the cytochrome P450 2U1 gene (*CYP2U1*), encoding a member of the cytochrome P450 superfamily of enzymes, is associated with autosomal recessive spastic paraplegia type 56 (SPG56) (MIM 615030), also known as SPG49 in HUGO Nomenclature Committee. Patients with *CYP2U1* mutations mainly have complicated HSP, although some cases are uncomplicated. Previously reported additional features include intellectual disability, cerebellar ataxia, dystonia, subclinical peripheral neuropathy and optic atrophy.[4–8] Rarely, a thin corpus callosum, basal ganglia calcification and periventricular white matter lesions or delayed myelination have been found by brain magnetic resonance imaging (MRI).[4,6–8] Here, we report a Japanese patient with SPG56 having novel compound heterozygous mutations in *CYP2U1*. This is the first report of a Japanese patient with SPG56 including delayed myelination, extending our understanding of the clinical features of SPG56/*CYP2U1*-related HSP.

CASE REPORT

The patient was a 4-year-old boy at the time of the study. He is the third child of healthy, non-consanguineous Japanese parents (Figure 1a), and has a healthy elder brother and a sister. There are no other affected family members. His birth was uneventful. He attained head control at 4 months of age, rolled over at 6 months, crawled at 9 months of age without alternate movement of the legs, and spoke several words at 3 years of age. However, he was not able to stand, walk or use a pincer grasp at 4 years.

At the age of 1, he visited a hospital for developmental delay. On neurological examination, he showed muscle weakness and spasticity of both lower and upper limbs. Contracture of the ankles was also observed. Deep tendon reflexes in the lower and right upper limbs were increased with a positive forced grasp, and the palmomental reflex and Babinski sign were noted. No involuntary movements or cerebellar ataxia were observed. There is no foot deformity such as a cavovarus foot. Blood chemistries including activities of the lysosomal enzymes β-galactosidase, β-hexosaminidase, arylsulfatase and galactocerebrosidase were all normal. The plasma concentration of very long chain fatty acids was not elevated. A nerve conduction study in tibial nerves revealed that motor conduction velocities and compound

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muscle action potentials were within normal ranges (Supplementary Figure 1).

At 13 months and 26 months of age, brain MRI revealed delayed myelination in the frontal, temporal, parietal and occipital lobes, and insula, and minimal progress of myelination during this interval without demyelination (Figures 2a and b). Thinning of the corpus callosum was not observed (Figure 2c). Developmental evaluation at 3 years and 10 months of age (using the Kyoto Scale of Psychological Development), indicated a total developmental quotient of 26 (equivalent to that of 12 months) with subcategorized quotients as follows: Postural-Motor 11 (equivalent to 5 months), Cognitive-Adaptive 30 (equivalent to 14 months) and Language-Social 29 (equivalent to 13 months).

GENETIC ANALYSIS

The institutional review board of Yokohama City University School of Medicine approved this study. After obtaining informed consent from the patient's family, we performed whole exome sequencing as previously described.[9] The mean depth of the sequenced region was 108×, with 92.6% of total coding sequences covered by ⩾20 reads. Two novel heterozygous missense variants in *CYP2U1* (NM_183075.2) were detected: c.1055C>T (p.Ala352Val) and c.1616T>G (p.Ile539Arg). Sanger sequencing using DNA from the proband and his parents confirmed that these variants were compound heterozygous (Figure 1b). They were absent in dbSNP138, our 575 normal Japanese in-house exomes, the Exome Aggregation Consortium database, the NHLBI Exome Variant Server (ESP6500) (http://evs.gs.

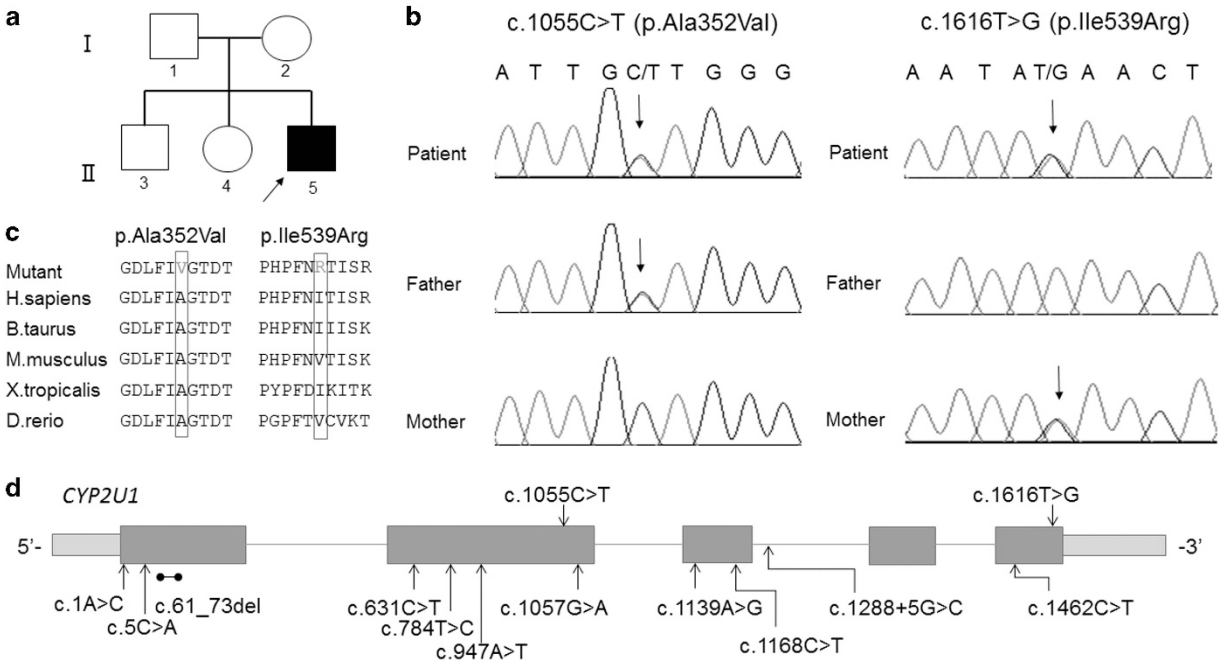

Figure 1 Familial pedigree and *CYP2U1* mutations. (**a**) Familial pedigree. (**b**) Electropherograms of identified *CYP2U1* mutations. c.1055C>T was inherited from the father, while c.1616T>G was inherited from the mother. (**c**) The evolutionary conservation of mutated amino acids (Ala352 and Ile539) from *Danio rerio* to *Homo sapiens*. (**d**) Schematic of *CYP2U1* and its mutations. Upper, novel mutations identified in our study; lower: previously described mutations. Blue boxes indicate coding exons, and light blue boxes indicate untranslated regions. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

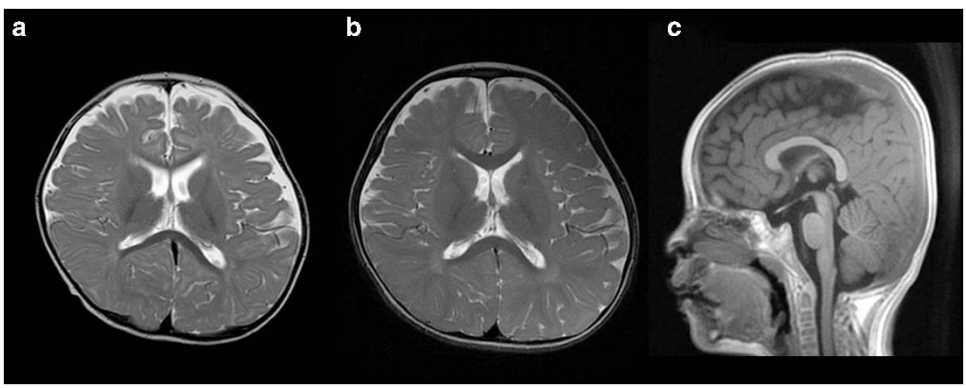

Figure 2 Brain magnetic resonance imaging (MRI) of the patient. Axial T2 weighted images of the brain MRI at 12 months (**a**) and 26 months (**b**). Both showed delayed myelination in the frontal, temporal, parietal and occipital lobes, and insula, and minimal progress of myelination during this interval without demyelination. Thinning of the corpus callosum was not observed in a sagittal T1 weighted image at 26 months (**c**).

Table 1 Clinical features in patients with *CYP2U1* mutations

Reference	Family1 Present case	Family2 Tesson et al.[4]						Family3 Tesson et al.[4]		Family4 Tesson et al.[4]	Family5 Tesson et al.[4]		Family6 Citterio et al.[5]	Family7 Leonardi et al.[6]			Family8 Masciullo et al.[7]	Family9 Karimine-jad et al.[8]	Family10 Karimi-nejad et al.[8]
Case number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Gender	male	male	female	female	female	female	male	male	male	male	female	male	male	male	female	male	female	female	female
Age when reported	4 years	33 years	21 years	18 years	11 years	25 years	21 years	12 years	5 years	31 years	29 years	27 years	4 years	50 years	46 years	42 years	16 years	16 years	15 years
Age at onset	8 months	3 years	5 years	1.5 years	8 months	Birth	5 years	1.5 years	Birth	8 years	13 months	16 months	18 months	25 years	30 years	30 years	15 months	11 months	11 months
Symptoms at onset	spasticity, developmental delay	delayed walking, spastic gait	unsteadiness, spastic gait	delayed walking, unsteadiness	delayed walking, spasticity	delayed walking, toe walking	toe walking	unsteady gait, tip-toe walking	spasticity, since birth	cannot run, frequent falls	delayed walking, spastic gait	spastic gait	developmental delay	visual impairment	visual impairment	visual impairement	delayed motor development	regression loss of acquired skills	regression, loss of acquired skills
Age at walking	not yet	3 years	1.5 years	1.5 years	never walked	6 years	1 year	2 years	never walked	2 years	delayed	normal	NA	normal	normal	normal	2 years	5 years	not yet
Cognitive delay	+	–	–	–	+	–	–	–	no speech	–	+	+	normal except for expressive language	–	–	–	mild	–	+
Lower-limb																			
Deep tendon reflexes	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased	increased
Babinski sign	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Weakness	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	–	–	–	–	+	–
Atrophy	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	NA	NA	NA	NA	+	–
Upper-limb																			
Deep tendon reflexes	increased	not increased	not increased	not increased	increased	increased	increased	increased	not increased	increased	increased	increased	slightly increased	not increased	not increased	not increased	not increased	increased	increased
Pathological reflexes	+	–	–	–	+	+	+	–	+	+	+	+	+ mild	–	NA	–	NA	NA	NA
Weakness	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	–	–	–	–	+	–
Atrophy	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	NA	NA	NA	NA	NA	–
Cerebellar ataxia	–	–	–	–	–	–	–	–	–	–	–	–	–	+	NA	–	–	NA	–
Dystonia	–	–	–	–	+	–	–	–	+	–	–	–	NA	–	NA	–	–	+	+
Visual impairement	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	–	–
Dysarthria	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–	+, speech delay	no speech
Brain MRI	delayed myelination at 2 years	ND	normal	normal at 17 years	normal at 10 years	ND	ND	normal at 5 years	TCC, WML, mild cortical changes at 2.5 years	normal at 28 years	normal at 14 years, thenglobus pallidus hypointensities emerged at 26 years	WML and globus pallidus hypointensities at 26 years	normal	mild brainstem and cerebellar atrophy, thin corpus callosum	NA	NA	normal	delayed myelination at 4 years	delayed myelination at 3 years
Spinal MRI	normal	ND	normal	normal at 17 years	normal at 10 years	ND	normal	normal at 4 years	ND	normal at 28 years	normal	normal	NA	NA	NA	NA	dorsal hydromyelia	normal at 4 years	ND
NCS	subclinical axonal neuropathy at 12 months	ND	subclinical axonal neuropathy at 21 years	subclinical axonal neuropathy at 18 years	subclinical axonal neuropathy at 10 years	subclinical axonal neuropathy at 25 years	subclinical axonal neuropathy at 21 years	normal	normal	normal at 28 years	ND	ND	normal	sensory-motor neurophathy	NA	NA	delayed conduction in the legs	sensory-motor neuropathy	ND

Abbreviations: MRI, magnetic resonance imaging; NA, information not available; NCS, nerve conduction study; ND, not done; TCC, thin corpus callosum; WML, white matter lesion; +, present; –, absent.

washington.edu/EVS/), and the Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/).

Ala352 is conserved in the eukaryotic CYP2U1 protein, and Ile539 is conserved among several species including *Homo sapiens*, *Bos taurus* and *Xenopus tropicalis* (Figure 1c). Possible pathogenic effects of these two variants on protein function were evaluated by web-based tools SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/). The c.1055C>T variant was predicted to be pathogenic by all three tools, while the c.1616T>G variant was predicted to be pathogenic by SIFT.

DISCUSSION

To date, a total of 11 *CYP2U1* mutations have been reported in 18 patients from nine families with SPG56/*CYP2U1*-related HSP (Figure 1d). Their clinical presentations are summarized in Table 1. Clinically, they often presented with complex forms of HSP, accompanied by various clinical features. Our patient showed delayed myelination, which is a rare feature of HSP as seen in spastic paraplegia 2 (MIM 312920) by proteolipid mutations and spastic paraplegia 44 by GJC2 (MIM 613206), and the third known case out of ten pedigrees with SPG56/*CYP2U1*-mutated HSP. Therefore, delayed myelination can be recognized as a possible clinical feature for SPG56, and *CYP2U1* screening should be considered in patients with HSP accompanying delayed myelination.

It is thought that the patho-mechanism of HSP includes defects in axon transportation, cytoskeleton control, mitochondrial function and/or axon development.[10] Regarding SPG56/*CYP2U1*-related HSP, mitochondrial dysfunction is suspected as a disease-causing mechanism. CYP2U1 is one of the most conserved cytochrome P450 proteins that catalyze the hydroxylation of arachidonic acid and related fatty acids. Its metabolites are local mediators of signal transduction,[4] and might indirectly mediate mitochondrial function via receptors in the brain that regulate mitochondrial respiration and energy production.[11] CYP2U1 was also shown to be partially localized in mitochondria, and mutations in *CYP2U1* might cause structural abnormalities in mitochondrial membranes, leading to the dysregulation of mitochondrial dynamics and respiratory impairment.[4,11] Indeed, CYP2U1-regulated mitochondrial function was preciously shown to be important for neuronal function in the corticospinal tract.[4] Our report, together with two other previous cases of delayed myelination in cerebral white matter,[8] further supports a possible association between normal myelination and mitochondrial function.

CONFLICT OF INTEREST

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. Authors declare noconflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)