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BRIEF COMMUNICATION

Novel compound heterozygous *DPH1* mutations in a patient with the unique clinical features of airway obstruction and external genital abnormalities

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Abstract

The diphthamide biosynthesis 1 (*DPH1*) gene encodes one of the essential components of the enzyme catalyzing the first step of diphthamide formation on eukaryotic elongation factor 2 (EEF2). Diphthamide is the posttranslationally modified histidine residue on EEF2 that promotes protein chain elongation in the ribosome. DPH1 defects result in a failure of protein synthesis involving EEF2, leading to growth defects, embryonic lethality, and cell death. In humans, *DPH1* mutations cause developmental delay with a short stature, dysmorphic features, and sparse hair, and are inherited in an autosomal recessive manner (MIM#616901). To date, only two homozygous missense mutations in *DPH1* (c.17T>A, p.Met6Lys and c.701T>C, p.Leu234Pro) have been reported. We used WES to identify novel compound heterozygous mutations in *DPH1* (c.289delG, p.Glu97Lysfs*8 and c.491T>C, p.Leu164Pro) in a patient from a nonconsanguineous family presenting with intellectual disability, a short stature, craniofacial abnormalities, and external genital abnormalities. The clinical phenotype of all patients with *DPH1* mutations, including the current patient, revealed core features, although the external genital anomaly was newly recognized in our case.

Diphthamide biosynthesis 1 (DPH1), encoded by *DPH1* (MIM*603527, NM_001383.3), is an essential component of the enzyme complex that carries out the diphtamide modification on eukaryotic elongation factor 2 (EEF2) [1, 2]. Diphthamide is a post-translationally modified histidine residue essential for ribosomal protein synthesis by EEF2 [3, 4], and *Dph1*-null mice show cell death, embryonic lethality, and growth defects [5, 6]. To our knowledge, only two homozygous missense mutations in *DPH1* have been reported in nine individuals from three families, which are responsible for developmental delay, a short stature, dysmorphic features, and sparse hair, and demonstrate autosomal recessive inheritance (MIM#616901) [5, 7]. Here, we report the detailed clinical features of a patient with novel compound heterozygous mutations in *DPH*.

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The patient was born to nonconsanguineous healthy parents with no family history of disease. At 30 weeks of gestation, his mother was referred to us for sudden-onset polyhydramnios and posterior cranial fossa enlargement, hypoplasia of the cerebellar vermis, and intrauterine growth retardation of the fetus. Chromosomal analysis of amniotic fibroblasts revealed a normal karyotype (46,XY). The patient was born by vaginal delivery with vacuum extraction at 37 weeks of gestation with Apgar scores of 3 and 7 at 1 and 5 min, respectively. He required mechanical ventilation for 5 days immediately after birth. His birth weight, length, and head circumference were 2269 g (−1.13 SD), 43.2 cm (−1.75 SD), and 32.5 cm (−0.09 SD), respectively. Characteristic facial features (a prominent forehead, upslanted palpebral fissures, posteriorly rotated ears, and a depressed nasal bridge), a short stature, bilateral single transverse palmer creases, hypospadias, and cryptorchidism were noticed (Fig. 1a–c).

From 26 days after birth, nasal biphasic positive airway pressure was applied because of his laryngo/bronchomalacia. Irritability and myoclonus of the bilateral upper limbs began soon after birth and at 5 months of age, respectively. Brain magnetic resonance imaging (MRI) performed at the age of 2 weeks revealed hypoplasia of the gyrus, a thin corpus

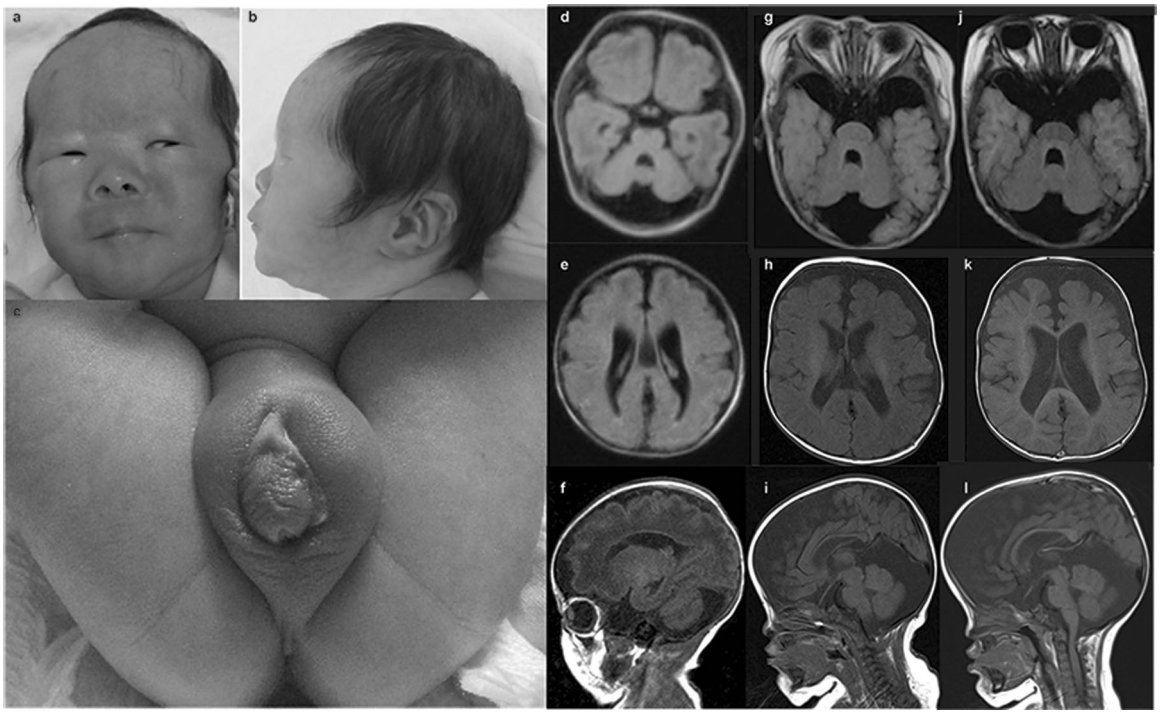

Fig. 1 **a**, **b** Photographs of the patient's face at the age of 2 weeks. A prominent forehead, upslanted palpebral fissures, posteriorly rotated ears, and a depressed nasal bridge were recognized. **c** The external genitals at 2 weeks. **d–l** Brain MRI of the patient at 2 weeks (**d–f**), 7 months (**g–i**), and 1 year (**j–l**). (Color figure online)

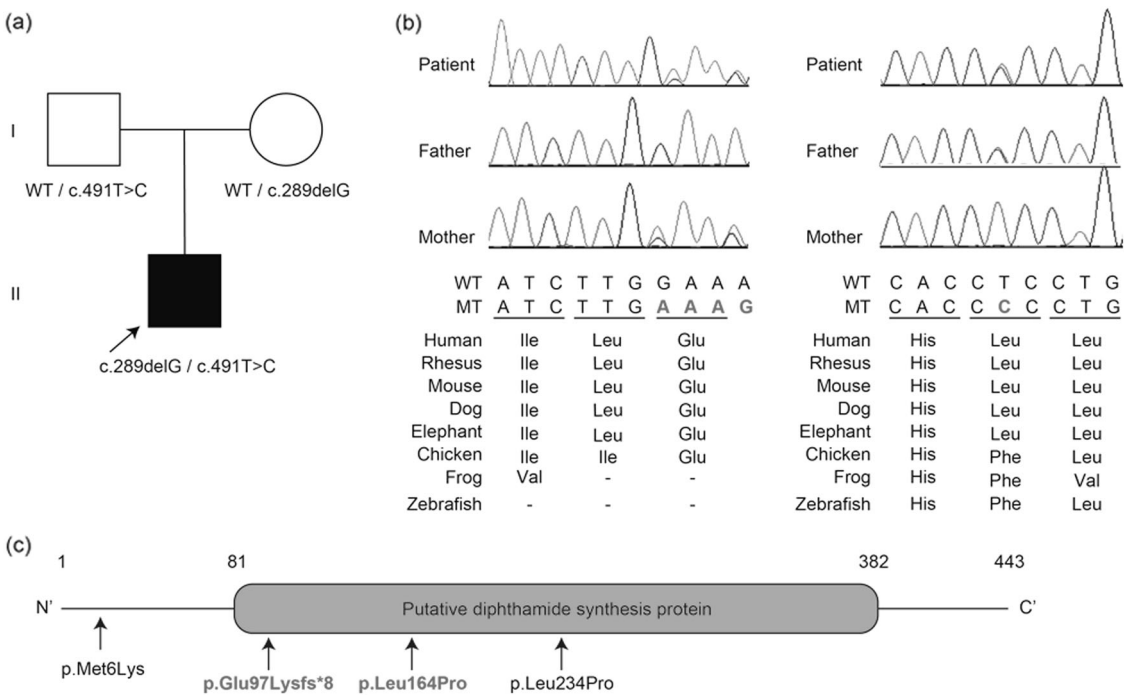

Fig. 2 The patient's family and *DPH1* mutations. **a** Familial pedigree and mutations. WT wild type allele. **b** Electropherograms of the site of the *DPH1* mutations. Evolutionary conservation of the altered amino residue is shown at the bottom. MT mutant allele. **c** Schema of DPH1 protein and the location of known *DPH1* mutations (arrows). The mutations reported in this study are indicated in red. (Color figure online)

callosum, and enlargement of the posterior cranial fossa, and lateral and third ventricles (Fig. 1d–f). Cerebellar vermis hypoplasia was suspected at the age of 2 weeks, but no abnormality was found at 7 months (Fig. 1g–i). Brain MRI at 1 year revealed persistent enlargement of the posterior cranial fossa, and hypoplasia of the cerebral hemisphere, especially the frontal and temporal lobes (Fig. 1j–l). At 1 year 11 months, he could crawl, sit alone for short periods, and stand up to hold onto something with a little help. Although babbling was recognized, he spoke no meaningful words. His hormone levels at baseline (cortisol, adrenocorticotropic hormone, thyroid-stimulating hormone, free triiodothyronine, free thyroxine, vasopressin, aldosterone, testosterone, plasma renin activity, angiotensin I, and somatomedin C) at 2 years 5 months was within normal ranges. As the patient was too young, pituitary provocation test was not performed.

To identify the genetic cause, we performed whole exome sequencing (WES) on the patient and his parents after obtaining their written informed consent. This study was approved by the institutional review board of Yokohama City University School of Medicine. Genomic DNA was extracted from peripheral leukocytes using QuickGene-610L (Fujifilm, Tokyo, Japan). WES was performed as previously described [8]. More than 95.6% of the coding sequences were covered by 10 or more reads in all analyzed samples. Synonymous variants and variants registered as common in dbSNP137, the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/), our in-house database (exome data of 575 Japanese individuals), ExAC (http://exac.broadinstitute.org), and the Exome Variant Server (http://evs.gs.washington.edu/EVS/) (variants with a minor allele frequency >0.005 under the autosomal recessive model) were excluded from the candidates. Candidate genes with de novo, homozygous, and compound heterozygous mutations were evaluated based on respective models, the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), and by three forms of prediction software: SIFT (http://sift.jcvi.org) [9], PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) [10], and MutationTaster (http://www.mutationtaster.org/) [11].

Compound heterozygous mutations (c.289delG, p.Glu97Lysfs*8 in exon 3 and c.491T>C, p.Leu164Pro in exon 5) in *DPH1* were highlighted because these variants were consistent with the patient's phenotype and were predicted to be pathogenic by the prediction software (SIFT, 0; Polyphen-2, 0.993; MutationTaster, 0.999). Sanger sequencing confirmed that c.289delG was inherited from the patient's mother, and c.491T>C was from his father (Fig. 2a, b). Leu164 is evolutionarily conserved in mammals, and is located within the diphthamide synthesis protein domain predicted by Pfam (http://pfam.xfam.org/family/PF01866). The frameshift mutation c.289delG, p.Glu97Lysfs*8 was assumed to result in nonsense-mediated decay because it was located >50 nucleotides upstream of the final splice junction at exon 3 [12]. No pathogenic mutation associated with genital anomalies has been detected in the WES data.

Table 1 Clinical comparison of patients with *DPH1* mutations

	Present case	Alazami et al.	Loucks et al.								Total
			1	2	3	4	5	6	7	8	
Sex	Male	Male	Male	Male	Male	Male	Female	Male	Female	Female	
Region	Japan	Saudi Arabia	Saudi Arabia				North America				
Mutation	c.289delG/ c.491T>C	c.701T>C/ c.701T>C	c.701T>C/ c.701T>C				c.17T>A/ c.17T>A				
Amino acid change	p.Glu97Lysfs*8 p. Leu164Pro	p.Leu234Pro	p.Leu234Pro				p.Met6Lys				
Intellectual disability	+	+	+	+	+	+	+	+	+	+	10/10
Abnormal skull shape	+	n. m.	+	+	+	+	+	+	+	+	9/9
Dysmorphic face	+	n. m.	+	+	+	+	+	+	+	+	9/9
Sparse hair on scalp/eye lash, eye blows	+	n.m.	+	+	+	+	+	+	+	+	9/9
Central nervous malformation	+	+	+	+	+	+	n. m.	n. m.	–	–	6/8
Short stature	+	n. m.	+	+	+	–	+	+	+	–	7/9
Ventricular septal defect	–	+	+	+	–	+	–	–	–	–	4/10
Renal anomaly	–	–	+	+	+	–	+	–	–	–	4/10
Cryptorchidism	+	–	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	1/2
Hypospadias	+	–	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	n. m.	1/2

n. m. not mentioned

By reviewing the clinical features of 10 individuals with biallelic *DPH1* mutations including the present case, we conclude that almost all show an intellectual disability, have an abnormal skull shape, characteristic facial features (a prominent forehead, depressed nasal bridge, low-set ears, and micrognathia), sparse hair on their scalp, eyelashes, and eyebrows, and a short stature (Table 1) [5, 7]. Approximately half of the patients show central nervous malformations (Dandy–Walker malformation, cerebellar vermis hypoplasia, posterior fossa cysts, and concomitant hydrocephalus), ventricular septal defects (VSD), and renal anomalies. Airway obstruction and external genital abnormalities were uniquely recognized in the present patient. Because the number of patients with *DPH1* mutations is limited and some symptoms such as VSD and renal anomaly were variable even in the same family sharing the same mutation, further patient accumulation is needed to precisely understand the genotype-phenotype correlation in biallelic *DPH1* mutations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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