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Case Report

A case of dihydropyrimidinase deficiency incidentally detected by urine metabolome analysis

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Abstract

Dihydropyrimidinase deficiency is a rare autosomal recessive disease affecting the second step of pyrimidine degradation. It is caused by mutations in the *DPYS* gene. Only approximately 30 cases have been reported to date, with a phenotypical variability ranging from asymptomatic to severe neurological illness. We report a case of dihydropyrimidinase deficiency incidentally detected by urine metabolome analysis. Gas chromatography-mass spectrometry-based urine metabolomics demonstrated significant elevations of dihydrouracil and dihydrothymine, which were subsequently confirmed by a quantitative analysis using liquid chromatography-tandem mass spectrometry. Genetic testing of the *DPYS* gene revealed two mutations: a novel mutation (c.175G > T) and a previously reported mutation (c.1469G > A). Dihydropyrimidinase deficiency is probably underdiagnosed, considering its wide phenotypical variability, nonspecific neurological presentations, and an estimated prevalence of 2/20,000. As severe 5-fluorouracil-associated toxicity has been reported in patients and carriers of congenital pyrimidine metabolic disorders, urinary pyrimidine analysis should be considered for those who will undergo 5-fluorouracil treatment.

Keywords: 5-Fluorouracil; *DPYS* gene; Pyrimidine metabolism; Screening

1. Introduction

Inborn errors of pyrimidine metabolism are a group of diseases with congenital defects affecting the degradation of the pyrimidine bases uracil and thymine, which are important constituents of nucleic acids. The symptoms are diverse, ranging from asymptomatic cases to epilepsy, intellectual disability, and autism [1–4]. Dihydropyrimidinase (DHP) deficiency (MIM #222748) is a rare autosomal recessive disease caused by mutations in the *DPYS* gene, and approximately 30 patients have been reported worldwide [1–3,5,6]. Elevation of dihydrouracil and dihydrothymine in body fluids has been

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demonstrated, and an analytical technique based on gas chromatography–mass spectrometry (GC-MS) has been developed to detect these abnormalities to screen patients [7]. Importantly, severe (sometimes fatal) 5-fluorouracil (5-FU) – associated toxicity has been reported in asymptomatic patients with DHP deficiency who underwent anticancer therapy [8,9]. Thus, an efficient screening method seems necessary to identify individuals at high risk for severe adverse effects of 5-FU. Here, we report a case of DHP deficiency incidentally detected by urine metabolome analysis.

2. Case report

The patient was a male born full-term (40 weeks of gestational age) to nonconsanguineous parents without perinatal complications. There was no family history of neurological diseases. The patient was enrolled in special needs classes during elementary school due to hyperactive behavior. An intelligence quotient (IQ) test (Wechsler Intelligence Scale for Children-IV) at 11 years and 7 months of age demonstrated a full-scale IQ of 80, a verbal comprehension index of 86, a perceptual reasoning index of 82, a working memory index of 79, and a processing speed index of 88. At 13 years of age, the patient started to experience painful muscle cramps involving the lower legs and thighs after vigorous exercise. There was no muscle weakness or hypoesthesia, and the cramps resolved spontaneously upon rest. However, symptoms recurred when the patient resumed exercising. He had no symptoms with daily activities. When he had a muscle cramp at 14 years of age, blood tests performed at a local clinic showed markedly elevated aspartate aminotransferase (AST, 74 U/L; reference range, 14–33 U/L), alanine aminotransferase (ALT, 37 U/L; reference range, 3–20 U/L), and creatine kinase (CK, 3600 U/L; reference range, 52–305 U/L). He was referred to Okayama University Hospital the next day. On examination, his height was 164.2 cm (+0.0 standard deviation [SD]), and his weight was 53.0 kg (−0.1 SD). The patient had no dysmorphism or malformation, and general and neurological examinations were unremarkable. Blood tests still showed markedly elevated AST (45 U/L), ALT (34 U/L), CK (1820 U/L), and aldolase (24.0 U/L; reference range, 2.7–7.5 U/L), although these values were lower than those on the previous day. Electrolytes, kidney function, thyroid function, lactate, and pyruvate were normal. The patient was admitted to Okayama University Hospital for investigation of muscle disorders at 14 years and 3 months of age.

On admission, the patient had no muscle cramps. Blood tests (when asymptomatic) showed normalization of muscle enzymes. Electrolytes, liver function, and kidney function were normal (Table 1). Intense exercise during admission induced a painful muscle cramp, and a blood test at that time showed a slight increase in CK (254 U/L), but normal levels of electrolytes, ammonium, lactate, and pyruvate. Acylcarnitine analysis for dried blood spot using tandem mass spectrometry was normal. An electrocardiogram, needle electromyogram, nerve conduction test, long exercise test, short exercise test, cooling test, and non-ischemic forearm exercise test were normal. Magnetic resonance imaging (MRI) of the lumbar spine and computed tomography (CT) of the muscles of the entire body including the thighs and lower legs were normal (Supplementary Figure).

Based on these test results, we concluded that channelopathies, muscular dystrophies, mitochondria diseases, glycogen storage diseases, and fatty acid metabolic disorders were unlikely. We instructed the patient to rest and consume appropriate amounts of water

Table 1
Laboratory findings (blood tests).

Test	Result	Reference range[†]
At the first visit (14 years and 1 month of age)		
AST (U/L)	45	10–40
ALT (U/L)	34	5–40
CK (U/L)	1820	57–197
ALD (U/L)	24.0	2.7–7.5
Free T_3 (pg/ml)	4.12	3.6–5.6
Free T_4 (ng/dL)	1.27	1.16–1.54
TSH (μU/ml)	3.47	0.34–3.5
Lactate (mg/dL)	14.7	3.0–17.0
Pyruvate (mg/dL)	1.4	0.3–0.9
On admission (14 years and 3 months of age)		
WBC (/μL)	4900	4500–13,000
RBC ($\times 10^4$ /μL)	525	505–575
Hemoglobin (g/dL)	15.8	15.0–17.0
Hematocrit (%)	47.1	44–50
PLT (/μL)	222,000	150,000–400,000
Total protein (g/dL)	7.3	6.5–8.1
Albumin (g/dL)	4.5	3.7–5.8
AST (U/L)	10	10–40
ALT (U/L)	15	5–40
γ-GT (U/L)	19	0–20
ALP (U/L)	448	240–1560
LDH (U/L)	229	230–460
CK (U/L)	100	57–197
Na (mmol/L)	138	138–145
K (mmol/L)	4.5	3.4–4.7
Cl (mmol/L)	104	98–106
Ca mg/dL	9.7	9.1–10.2
IP (mg/dL)	3.5	2.4–4.5
Mg (mg/dL)	2.0	1.8–2.2
UN (mg/dL)	17.2	6–20
Creatinine (mg/dL)	0.82	0.6–0.9
ANA	Negative	
Acylcarnitines	Normal	

ALD, aldolase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibodies; AST, aspartate aminotransferase; CK, creatine kinase; γ-GT, γ-glutamyltransferase; IP, inorganic phosphates; LDH, lactate dehydrogenase; PLT, platelets; RBC, red blood cells; TSH, thyroid stimulating hormone; UN, urea nitrogen; WBC, white blood cells

[†] Reference ranges at Okayama University Hospital.

and salt when he experienced muscle cramps. After discharge, the muscle cramps became less frequent and eventually stopped spontaneously.

Spot urine samples were collected and frozen at −80 °C during admission for metabolome analysis. Urine metabolomics using GC-MS performed at the Japan Clinical Metabolomics Institute [7] detected markedly elevated levels of dihydrouracil (+7.0 SD) and dihydrothymine (+7.0 SD), strongly suggesting a diagnosis of DHP deficiency. Quantitative analysis of pyrimidine compounds using liquid chromatography–mass spectrometry (LC-MS) performed at Fujita Health University School of Medicine [6] confirmed these abnormalities (Table 2). A genetic test was conducted after obtaining informed consent from the patient's parents. This study was approved by the ethics committee of Fujita Health University Hospital. Sequencing of the *DPYS* gene according to the Sanger method [6] demonstrated mutations of c.175G > T (p.Val59Phe) in exon 1 and c.1469G > A (p.Arg490His) in exon 9. Using computer prediction, c.175G > T was SIFT = 0 (damaging), PolyPhen-2 = 1.000 (probably damaging), and c.1469G > A was SIFT = 0 (damaging), PolyPhen-2 = 1.000 (probably damaging). The patient and his parents were informed about the possible risk for severe 5-FU toxicity should the patient need to undergo 5-FU treatment in the future. Genetic analysis of his parents was declined.

3. Discussion

We reported the case of a patient with DHP deficiency incidentally detected by urine metabolome analysis. Because the patient's muscle cramps improved spontaneously, we deemed the muscle symptoms coincidental. We were unable to determine whether the patient's behavioral problems and borderline IQ were directly caused by DHP deficiency, as these symptoms are nonspecific. Therefore, if urine metabolome analysis had not been conducted, the diagnosis could not have been made.

DHP deficiency is one of three congenital metabolic disorders affecting pyrimidine degradation (Fig. 1). In DHP deficiency, elevation of dihydrouracil and dihydrothymine in body fluids has been demonstrated, whereas uracil and thymine accumulate in dihydropyrimidine dehydrogenase (DPD) deficiency, and β-ureidopropionic acid and β-ureidoisobutyric acid accumulate in β-ureidopropionase (β-UP) deficiency. The symptoms of DHP deficiency are similar to those of DPD deficiency and β-UP deficiency and very diverse, ranging from asymptomatic cases to epilepsy, intellectual disability, microcephaly, and malformations such as spina bifida and aproctia [1–3]. Because these symptoms are nonspecific, it is difficult to consider DHP deficiency as a differential diagnosis from the beginning. Routine biochemical tests, amino acid analysis, organic acid analysis, and acylcarnitine analysis are unable to detect these diseases. Previously, we reported the case of a patient with β-UP deficiency detected by urine metabolome analysis [10]. Sumi et al. (1998) analyzed urine samples from 21,200 healthy Japanese infants and found two asymptomatic cases with dihydropyrimidinuria, which indicated that the prevalence of DHP deficiency was 1/10,000, and thus relatively frequent [1]. Although the carrier frequency is estimated to be one in several tens in the general population, DHP deficiency has been rarely reported, with only approximately 30 patients including our case reported worldwide [1–3,5,6], which suggests that this disease is probably underdiagnosed. Although most Japanese patients with DHP deficiency have been asymptomatic so far, it was recently reported that a patient with this disease developed neurological symptoms [2,6].

Methods for diagnosing DHP deficiency include urinary pyrimidine analysis, enzyme activity assay of DHP in liver tissue, and sequencing of the *DPYS* gene. Because urinary pyrimidine analysis can detect patients with DHP deficiency, urine metabolome analysis, which is noninvasive and can analyze many samples, may be useful for diagnostic screening [7]. In the present case, we detected two mutations, c.175G > T (p.Val59Phe) and c.1469G > A (p.Arg490His), in the *DPYS* gene. In a previous study, a DHP mutant carrying the p.Arg490His variant was reported to have a residual DHP activity of 0.3% [6]. Although c.175G > T (p.Val59Phe) is a novel mutation, it is likely pathogenic based on computer prediction. Although we could not

Table 2
Urinary concentrations of pyrimidine metabolities determined by liquid chromatography-mass spectrometry.

Compound	Result (μmol/mmol creatinine)	Reference range† (μmol/mmol creatinine)
Uracil	8.6	0–28.0
Thymine	0.9	0–2.5
Dihydrouracil	**122.6**	0–16
Dihydrothymine	**56.1**	0–3.4
β-Ureidopropionic acid	0.3	0–11.9
β-Ureidoisobutyric acid	0.0	0–5.5

Figures shown in bold font indicate abnormal values.
† Reference ranges at Fujita Health University Hospital.

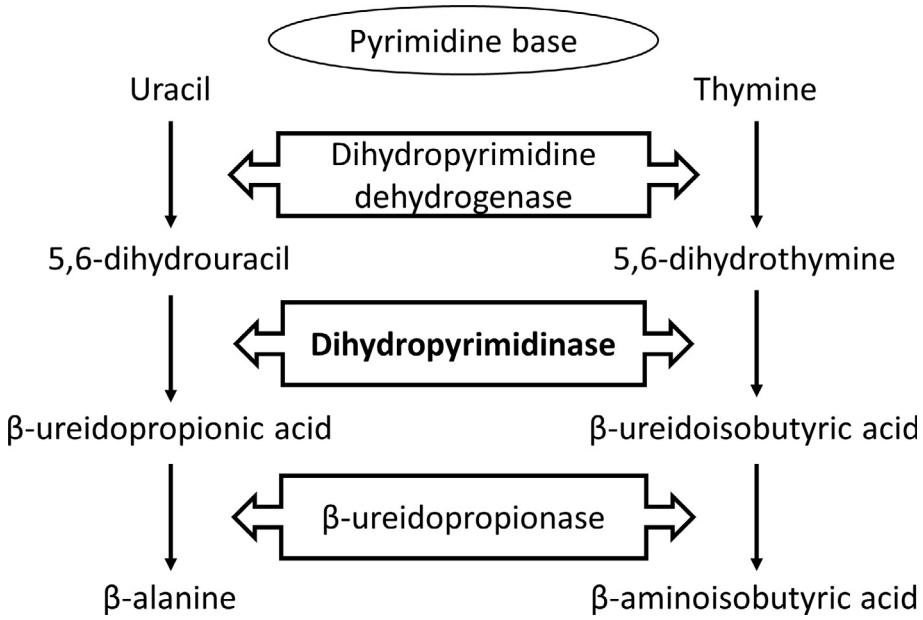

Fig. 1. Metabolic pathway of pyrimidine degradation. Dihydropyrimidinase catalyzes the second step of the three-step degradation of uracil and thymine.

confirm compound heterozygosity of these two mutations, this patient is likely a compound heterozygote based on the definite biochemical abnormalities we detected.

Severe 5-FU–associated toxicity has been reported in patients and carriers of DPD deficiency and in patients with DHP deficiency [8,9]. Because there is no radical therapy available for severe 5-FU – associated toxicity, a screening test for congenital disorders of pyrimidine degradation may be meaningful before 5-FU administration. Although it would be ideal to make a genetic diagnosis before 5-FU therapy, congenital disorders of pyrimidine degradation are rare, and genetic variants revealed by genetic analysis do not always prove pathological. Therefore, it is not realistic to screen all cases using genetic analysis considering cost-effectiveness and reliability. Although urinary pyrimidine analysis detects only patients but not carriers of pyrimidine degradation disorders [7,9], it should be considered before 5-FU treatment, because it can at least identify patients who are at high risk of developing severe adverse effects.

4. Conclusion

We reported the case of a patient with asymptomatic DHP deficiency incidentally detected by urine metabolome analysis. Because the symptoms of this disease are nonspecific, it is difficult to consider DHP deficiency as a differential diagnosis from the beginning, and routine biochemical tests, organic acid analysis, and acylcarnitine analysis are unable to detect this disease. Considering an estimated prevalence of 0.1% in Japan, this disease is probably underdiagnosed. As severe 5-FU – associated toxicity has been reported in congenital disorders of pyrimidine degradation including DHP deficiency, urinary pyrimidine analysis should be considered for those who will undergo 5-FU treatment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2018.10.005.

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