Neonatal-Onset Hereditary Coproporphyria: A New Variant of Hereditary Coproporphyria

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Abstract Genetic mutation of the coproporphyrinogen oxidase (*CPOX*) gene causes either hereditary coproporphyria (HCP) or harderoporphyria. HCP, a rare hepatic porphyria, causes acute attacks after puberty and rarely accompanies cutaneous symptoms. In contrast, harderoporphyria is an erythropoietic porphyria that represents photosensitivity and hemolytic anemia from the neonatal period. In patients with harderoporphyria, the p.Lys404Glu mutation is found in the homozygous or compound heterozygous state with another mutation, and a marked increase in harderoporphyrin is observed. This report describes a neonate with symptoms of erythropoietic harderoporphyria (photosensitivity of the skin, hemolytic anemia, and jaundice). However, the pattern of porphyrin metabolites of feces was consistent with that of typical HCP, not of harderoporphyria. We found a heterozygous, novel, four-base pair deletion in exon 7 of the *CPOX* gene, although other mutations including the p.Lys404Glu mutation in *CPOX* were not found. By unknown etiology, our patient had accompanying adrenocortical insufficiency and 46, XY disorders of sex development. Based on genetic mutation of the *CPOX* gene and information from a previous similar

case report, we consider that neonatal-onset HCP is a variant of HCP.

Introduction

Hereditary porphyrias are disorders that are caused by abnormalities in eight enzymes of the heme biosynthesis pathway. Coproporphyrinogen oxidase (CPOX; EC 1.3.3.3) is the sixth enzyme of the heme biosynthesis pathway. CPOX catalyzes the two steps of oxidative decarboxylation from coproporphyrinogen III to protoporphyrinogen IX via an intermediate metabolite, harderoporphyrinogen (Elder et al. 1978). Genetic mutation of the *CPOX* gene causes either hereditary coproporphyria (HCP: MIM 121300) or harderoporphyria (Nordmann et al. 1983; Lamoril et al. 1995). HCP is a rare hepatic porphyria that causes acute attacks and rarely (5%) accompanies skin photosensitivity. The symptoms of HCP usually begin after puberty. Acute attacks are notable for various symptoms, and often begin with mental disturbance such as anxiety, restlessness, and so on (Kauppinen 2005; Puy et al. 2010). Gastrointestinal symptoms (abdominal pain, nausea, vomiting, and constipation) usually follow the mental disturbance. Tachycardia, excess sweating, and hypertension, which are signs of increased sympathetic activity, are frequently observed. Myopathy is a very common symptom and muscle pain in the arms and legs is also observed in early stages. Diffuse muscle weakness begins in the proximal muscles and can lead to respiratory and bulbar paralysis which can be life threatening. Sensory neuropathy such as sensory loss can develop. Hyponatremia due to inappropriate anti-diuretic hormone secretion may lead to convulsions. Convulsions

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are also observed as a central nervous feature of acute porphyric attacks. Daily hem infusions for 3–4 consecutive days are enough to abolish the porphyric symptoms associated with an acute attack. To prevent an acute attack, avoidance of precipitating factors such as certain drugs, alcohol intake, infection and so on, is needed.

Harderoporphyria is an erythropoietic porphyria that is characterized by hemolytic anemia, jaundice, and photosensitivity, which begin from the neonatal period (Nordmann et al. 1983). In feces of patients with harderoporphyria, a marked increase in harderoporphyrin is observed in contrast to patients with HCP in whom a marked increase in fecal coproporphyrin III is observed.

In patients with harderoporphyria, the p.Lys404Glu mutation in *CPOX* has been found in the homozygous or compound heterozygous state with another *CPOX* mutation, which results in exon 6 skipping at the mRNA level (Lamoril et al. 1995, 1998; Schmitt et al. 2005). Amino acids around p.Asp400-Lys404 in *CPOX* have been reported to be a putative hot spot for harderoporphyria (Schmitt et al. 2005). Kim et al. reported that p.Asp 400 is required for dimerization of *CPOX* and p.Arg401-Lys404 is necessary for catalysis of the second step in oxidative decarboxylation from harderoporphyrinogen to protoporphyrinogen (Kim et al. 2013).

We describe a neonate representing with clinical symptoms of erythropoietic porphyria (photosensitivity of the skin, hemolytic anemia, and jaundice) with congenital adrenocortical insufficiency and 46, XY disorders of sex development.

Case Report

At our affiliated hospital, a neonate was delivered by cesarean section because of reduced fetal movement at 40 weeks and 4 days of gestational age. The father and mother of the neonate were non-consanguineous. His birth weight was 3,318 g (0.75 SD) with Apgar scores of 9 at 1 and 5 min. He had hypospadias, a bifid scrotum, and bilateral cryptorchidism (Fig. 1a). The testes were palpable bilaterally in the inguinal regions. Chromosomal analysis showed that he had a 46, XY male karyotype. The neonate also showed marked jaundice and hepatosplenomegaly. Biochemical studies that were conducted 1 h after birth showed leukocytosis, hypoglycemia, and an elevated total bilirubin level (183.0 $\mu\text{mol/L}$). A continuous glucose infusion and phototherapy were begun. Hypoglycemia recovered spontaneously. The first urine, which was reddish brown, was excreted at 16 h after birth. Serum total and direct bilirubin levels increased to 218.9 and 100.9 $\mu\text{mol/L}$, respectively, in spite of continuous phototherapy. Therefore, exchange transfusion was performed twice on days 1 and 2. The

serum total bilirubin level reached its highest level on day 2 (270.2 $\mu\text{mol/L}$) and decreased daily thereafter. Phototherapy was discontinued on day 4. On day 5, blisters appeared on the front side of the patient's trunk, limbs, and face. On the other hand, no blisters were observed on his back, which had not been exposed to green light (Fig. 1b). On day 9, he became anemic but recovered spontaneously by day 12. An auto-immune basis for the anemia and congenital infections were excluded. He was referred to our hospital on day 17 to confirm the cause of blistering and ambiguous genitalia.

After admission to our hospital, the blisters spontaneously healed with no specific therapy. Skin biopsy of a blister was conducted on day 19, which showed a subepidermal blister without overt inflammation, and a church spire-like protrusion of the dermal papillae (so-called festooning phenomenon) (Fig. 2a–c). Clumpy deposits of IgG were observed along the basement membrane zones and vessels (Fig. 2d). Electron microscopy showed blister formation above the basal lamina, and cytolysis of some basal cells (Fig. 2e, f). These findings were consistent with those of porphyria, excluding autoimmune blistering disease or epidermolysis bullosa hereditaria.

We speculated that photosensitivity due to porphyria might have caused the patient's skin lesions. Therefore, porphyrin levels were analyzed in erythrocytes and urine. We observed elevated concentrations of urinary uroporphyrin and coproporphyrin, and elevated concentrations of protoporphyrin and coproporphyrin in erythrocytes (Table 1). These results suggested that a disordered porphyrin metabolism caused his skin lesions. After admission, skin pigmentation developed around his perineal and axillary regions. His serum potassium level rose slightly. Because adrenal insufficiency was suspected, we analyzed plasma adrenocorticotrophic hormone levels, plasma renin activity, and serum steroid metabolites to identify the cause of ambiguous genitalia (Table 2). Although a urinary steroid profile analysis conducted on day 21 showed ACTH resistance, no known steroidogenic enzyme deficiency was not suggested. Abdominal computed tomography on day 5 and ultrasonography on day 19 showed that his adrenal glands were of normal size. Oral hydrocortisone was started and serum adrenocorticotrophic hormone and potassium levels were reduced to the normal range. Skin hyperpigmentation improved.

At 1 year and 9 months old, we examined the urinary steroid profile again, which was again nondiagnostic. Fecal porphyrin metabolites were also analyzed. The coproporphyrin III level was extremely elevated (Table 1). He has had no acute attack as at 1 year 9 months of age.

Based on the clinical manifestations and laboratory testing, we considered that our patient might have HCP.

Fig. 1 Distinctive features of our patient. (a) External genitalia of the patient on day 17. Hypospadias, a bifid scrotum, and bilateral cryptorchidism are apparent. (b) Skin lesions of the patient. Blisters are apparent, restricted to his front on which green light was illuminated for phototherapy

Therefore, we conducted mutational analysis related to the *CPOX* gene.

Methods

Porphyrin metabolites in urine and erythrocytes were measured in a commercial laboratory, SRL (Tokyo, Japan). Fecal porphyrin metabolites were measured at Mayo Medical Laboratories (Rochester, MN) via LSI Medience Corporation (Tokyo, Japan).

To analyze the *CPOX* gene (reference sequence: Genomic, NG_012594.2; mRNA, NM_000097.5), genomic DNA from our patient and the mother was extracted using the QIAamp DNA Mini Kit (Qiagen Inc., Tokyo, Japan) after informed consent was obtained from his mother. Total

RNA was also extracted from peripheral blood of the patient using the RNeasy Mini Kit (Qiagen Inc.). A total of 1.0 µg of RNA was reverse-transcribed using the Omni-script RT Kit (Qiagen Inc.) to obtain cDNA. A PCR was conducted using standard PCR methods (primers are available on request). The PCR products were sequenced using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and Genetic Analyzer (ABI Prism 310; Applied Biosystems, Foster City, CA). The PCR products were then cloned into the pCR[®]4-TOPO vector using the TOPO TA Cloning[®] kit for sequencing (Thermo Fisher Scientific, Inc., Waltham, MA). The obtained clones were sequenced. The father was not analyzed because the mother had divorced the father soon after the birth of our patient.

Results

A heterozygous deletion of four base pairs was found in exon 7 of the *CPOX* gene (c.1286_1289 del ACAT; Fig. 3a, b). Other mutations, including the p.Lys404Glu mutation, were not identified in the *CPOX* gene. This four-base pair deletion was not found in single nucleotide polymorphism databases, the 1,000 Genomes and the ExAC databases. This mutation was not found in his mother and is presumed to be novel. The mutant allele with the four-base pair deletion was expressed as mRNA (Fig. 3c). This mutation is predicted to add an abnormal 64 amino acids instead of the normal 27 amino acids (p. Tyr429Cys fs64*). Enzymatic activity of CPOX was not analyzed in this patient. No other mutation was found in the respective coding regions of protoporphyrinogen oxidase, uroporphyrinogen decarboxylase, or ferrochelatase genes (data not shown).

Discussion

This report describes a case of a neonatal-onset HCP with adrenocortical insufficiency and a disorder of sex development associated with a normal male karyotype. Clinical features of our patient, such as neonatal-onset jaundice, hemolytic anemia, and photosensitivity, were not consistent with typical HCP, which causes acute attacks after puberty. However, these features are consistent with harderoporphyria. In spite of the similar clinical features to harderoporphyria, our patient showed a marked increase in the level of fecal coproporphyrin III, which is observed in patients with HCP (Table 3). Furthermore, our patient did not have previously reported harderoporphyria mutations, but had a novel, heterozygous, four-base pair deletion. These results are consistent with HCP, but not harderoporphyria.

Similar to our patient, another neonatal patient with HCP showed neonatal jaundice and hemolytic anemia with a

Fig. 2 Results of skin biopsy. (a) Subepidermal blister with church spire-like dermal papillae (festooning phenomenon). (b) Expanded image of (a). Note basal cell damage. (c) The blister can be seen above the periodic acid-Schiff-positive basement membrane. (d)

Clumpy deposits of IgG can be seen along the basement membrane zone and blood vessels. (e) Electron microscopic findings show blister formation above the basal lamina. (f) Expanded image of the white box in (e)

heterozygous *CPOX* gene mutation, which caused exon 6 skipping (Table 3) (Takeuchi et al. 2001). This case also did not have a mutation that is found in patients with harderoporphyria and the coproporphyrin III level in the feces was greatly increased.

Based on these clinical and biological results, we concluded that our patient was an atypical case of HCP, who showed symptoms of erythropoietic porphyria from the neonatal period, as in a previous case (Takeuchi et al. 2001), thus our case is only the second reported case of neonatal-onset HCP twice. We consider that neonatal-onset HCP is a variant of HCP.

Hypospadias and adrenocortical insufficiency were observed in our patient and in the previously reported neonatal patient with HCP (Table 3) (Takeuchi et al. 2001). In patients with harderoporphyria and compound heterozygous mutations of p.Lys404Glu and IVS6 + 3A>G (Lamoril et al. 1998), which result in exon 6 skipping at the mRNA level, only hypospadias was observed. Hypospadias is the result of a lack of androgenic steroid during the embryonic period. Synthesis of steroid hormones, such

as cortisol and androgen, depends on the heme-thiolate enzyme, cytochrome P450. Therefore, heme synthesis disorders might be linked to impairment of steroid hormone synthesis, although the mechanisms remain unknown. Further investigations, including the relationship between the genotype of *CPOX* and hypospadias and adrenocortical insufficiency, are needed because exon 6 skipping of *CPOX* also causes typical HCP (Delfau-Larue et al. 1994).

We conclude that the neonatal-onset HCP is a recognized variant of HCP caused by genetic mutations of *CPOX*. However, questions remain as to why some *CPOX* gene mutations cause photosensitivity, jaundice from the neonatal period, hypospadias, and adrenocortical insufficiency. Findings from our case have further expanded the clinical spectrum of diseases caused by *CPOX* gene mutations.

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Table 1 Porphyrin metabolite concentrations

Age	3 Months	5 Months	1 Year and 9 months	Reference range
Urine				
Uroporphyrin (μg/g Cre)	336	89	117	≤36
Coproporphyrin (μg/g Cre)	15,235	14,762	16,284	≤170
δ-Aminolevulinic acid (mg/L)		0.4	1.4	≤5
Erythrocytes				
Uroporphyrin (μg/dL RBC)		≤1		≤1
Coproporphyrin (μg/dL RBC)		3	≤1	≤1
Protoporphyrin (μg/dL RBC)		323.0	122.1	30–86
Feces				
Coproporphyrin I (μg/24 h)			452 (3.5%)	<500
Coproporphyrin III (μg/24 h)			10,541 (82%)	<400
Isocoproporphyrin (μg/24 h)			622 (4.8%)	<200
Protoporphyrin (μg/24 h)			54 (0.4%)	<1,500
Harderoporphylin (μg/24 h)			1,112 (8.7%)	<200
Coproporphyrin III/coproporphyrin I			23.3	<1.20

Cre creatinine, RBC erythrocyte

Abnormal findings are shown in bold font

Compliance with Ethics Guidelines

Conflict of Interest

Kosei Hasegawa, Hiroyuki Tanaka, Miho Yamashita, Yousuke Higuchi, Takayuki Miyai, Junko Yoshimoto, Ayumi Okada, Norihiro Suzuki, Keiji Iwatsuki, and Hirokazu Tsukahara declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. This study was approved by the Ethical Committee of Okayama University Hospital. Informed consent was obtained from patient's mother. Additional informed consent was also obtained from patient's mother for which identifying information is

Table 2 Analysis of steroid metabolites and stimulating hormones

	Day 19	Day 28	Day 36	Reference range ^a
Pregnenolone(nmol/L)		0.02		0.01–0.04
Progesterone (nmol/L)		14.9		0.22–1.7
17α-Hydroxypregnenolone (nmol/L)		0.34		0.01–0.23
17α-Hydroxyprogesterone (nmol/L)		0.08		0.00–0.03
Cortisol (nmol/L)		66.2		77.3–634.8
Androstenedione (nmol/L)		0.13		0.01–0.03
DHEA-S (μmol/L)		0.73		0.14–3.0
Testosterone (nmol/L)	12.0	4.9	2.2	2.1–13.9
Estradiol (pmol/L)		48.4		36.7–117.4
Aldosterone (nmol/L)		1.4		0.14–2.5
ACTH (pmol/L)	296.7	189.2	47.3	1.63–12.3
Plasma renin activity (μg/L/h)		17		3.6–24
Luteinizing hormone (IU/L)	4.8			4.85–10.02
Follicle-stimulating hormone (IU/L)	1.2			1.22–5.19

Abnormal findings are shown in bold font

DHEA-S dehydroepiandrosterone sulfate

^a Diamond and Bercu (2004)

included in this article. Proof that informed consent was obtained is available upon request.

Details of the Contributions of Individual Authors

Study design: Kosei Hasegawa and Hiroyuki Tanaka.

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Take-Home Message

Neonatal-onset hereditary coproporphyria is a variant of HCP.

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Fig. 3 Results of sequential analyses of the *CPOX* gene of our patient. **(a)** Sequence of PCR products of exon 7. A frameshift was found. **(b)** Sequence of TA cloning sample of PCR products of exon 7. A four-base pair (indicated by a red bold line) deletion (c.1286_1289

del ACAT) was found in the mutant allele. **(c)** Sequence of *CPOX* cDNA. The mutant allele including the four-base pair deletion (red bold line) was demonstrable in cDNA cloned into the sequencing vector

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Table 3 Comparison of genotype–phenotype correlation and porphyrin metabolites in diseases caused by *CPOX* gene mutation

Diseases (onset)	<i>CPOX</i> mutation	Enzymatic activity	Increased metabolite in feces (ratio to total fecal porphyrin)	Neurological attack	Cutaneous lesion due to sensitivity	Hemolytic anemia and jaundice	Adrenocortical insufficiency	Hypospadias
HCP (after puberty to adult)	Heterozygous or homozygous mutation of <i>CPOX</i>	~50% (hetero) ^{a,b,c} <10% (homo) ^{d,e,f}	COPRO III dominant	+	Rarely +	–	–	–
Harderoporphyria (neonate)	p.Lys404Glu homo ^{g,h}	18–25%	COPRO III (19–29%) HARDERO (65–72%)	–	+	+	–	–
	p.Lys404Glu and IVS6 + 3A>G (exon 6 skipping at mRNA level) ⁱ	18%	COPRO III (6.2%) HARDERO (90%)	–	+	+	–	+
Takeuchi et al. (neonate) ^j	c.1277G>A hetero (exon 6 skipping at mRNA level)	ND	COPRO III (63%) HARDERO (7%)	–	+	+	+	+
Present case (neonate)	c.1286_1289del ACAT hetero	ND	COPRO III (82%) HARDERO (8.7%)	–	+	+	+	+

HCP hereditary coproporphyria, *CPOX* coproporphyrinogen oxidase, COPRO coproporphyrin, HARDERO harderoporphyria, homo homozygous mutation, hetero heterozygous mutation, ND not determined

^a Elder et al. (1976)

^b Nordmann et al. (1977)

^c Grandchamp and Nordmann (1977)

^d Grandchamp et al. (1977)

^e Martasek et al. (1994)

^f Kuhnel et al. (2000)

^g Nordmann et al. (1983)

^h Schmitt et al. (2005)

ⁱ Lamoril et al. (1998)

^j Takeuchi et al. (2001)

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