Journal of Dermatological Science 100 (2020) 156–159

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Letter to the Editor

Analysis of the mechanism underlying a mild phenotype of hereditary coproporphyria due to a homozygous missense mutation in the transcription initiation codon of the coproporphyrinogen III oxidase gene

Dear editor

Hereditary porphyrias are disorders caused by abnormalities in eight enzymes in the heme biosynthetic pathway. Hereditary coproporphyria (HCP) is a rare autosomal dominant disorder caused by deficiency of coproporphyrinogen III oxidase (CPOX) [1,2]. CPOX, the sixth enzyme in the heme biosynthetic pathway, converts coproporphyrinogen III to protoporphyrinogen IX through two sequential oxidative decarboxylation steps [3]. CPOX activity is located in the mitochondrial membrane. CPOX is expressed as a precursor and contains an amino-terminal mitochondrial targeting signal. After proteolytic processing, the protein is present as a homodimer in its mature form [4]. Clinical crises are characterized by an acute polysymptomatic syndrome with abdominal, cardiovascular, neurological, and psychiatric symptoms. Nearly 20 % of patients may experience photosensitivity [5]. We report a case of HCP with a mild clinical phenotype in which we investigated the mechanisms associated with lower severity.

The proband, a 56-year-old Japanese man with a 3-month history of solar hypersensitivity was referred to our department. Acute hepatitis had been found 2 months before. He had never experienced visceral or neuropsychiatric symptoms. Family history was unremarkable. No particular drug history was noted.

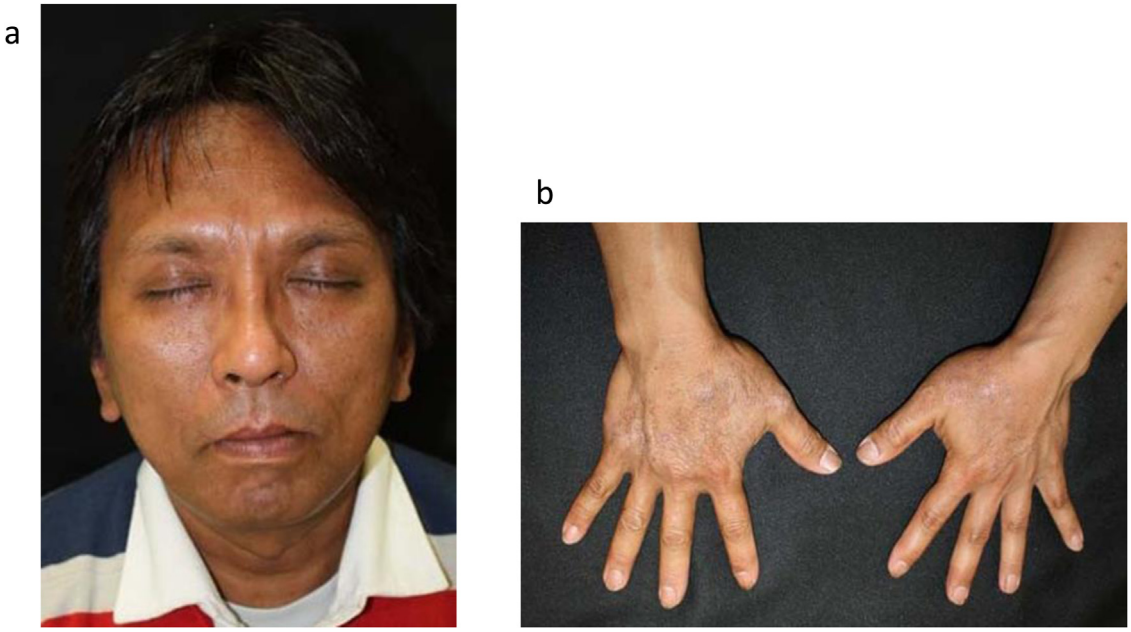

Fig. 1. a, b. Clinical findings. Physical examination revealed localized erythema, papules, and diffuse pigmentation predominantly on sun-exposed areas including the face (a) and the back of the hands (b).

https://doi.org/10.1016/j.jdermsci.2020.06.006

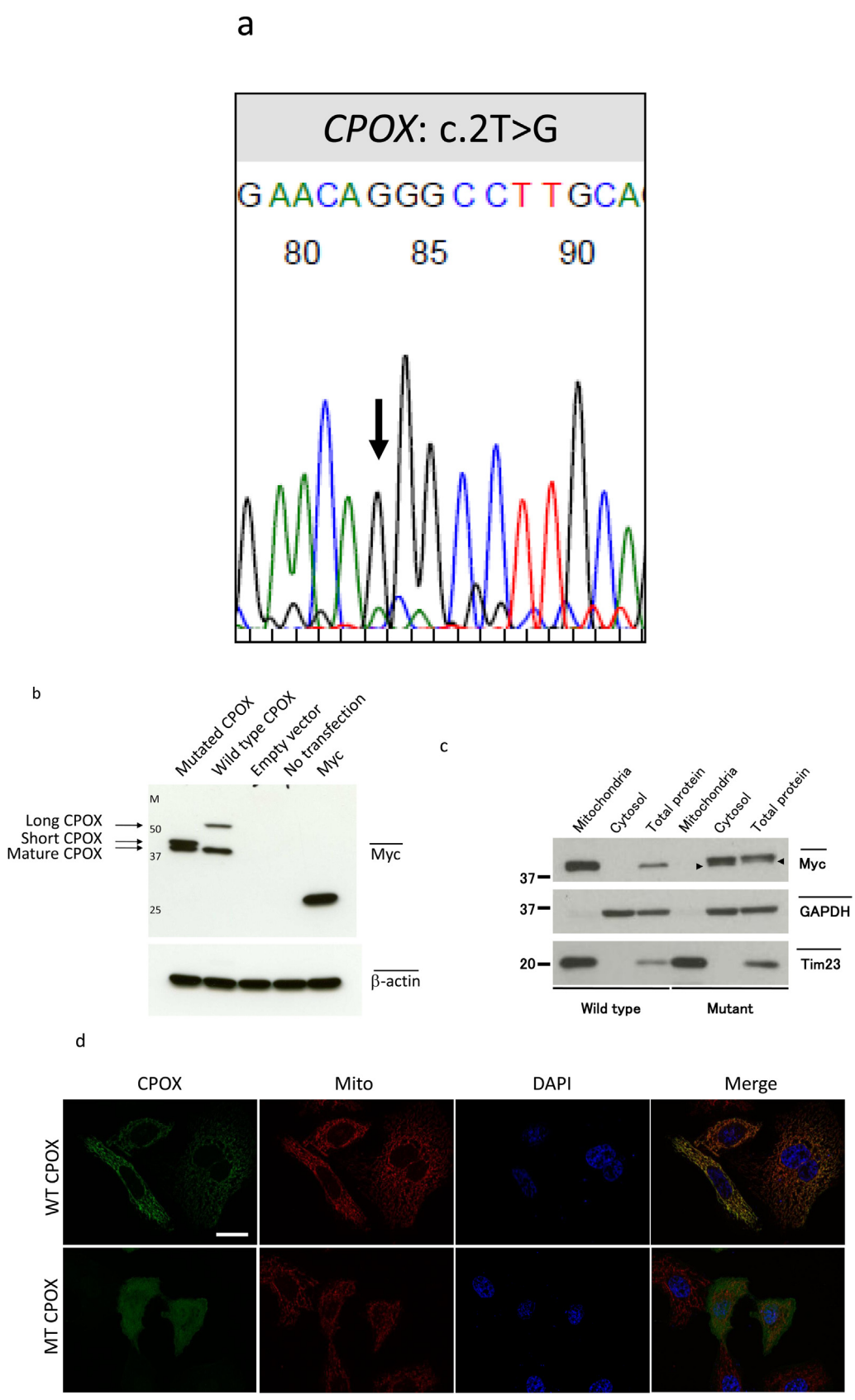

Fig. 2. a. Detection of the mutation. Nucleotide sequencing of the ***CPOX*** from the patient demonstrated novel homozygous missense mutations in the translation initiation codon: c.2 T > G (p.M1R) (arrow). *In silico* search of the publicly accessible Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/index.html) found that none of the 605 persons with HCP possessed the mutation. **b. Transient transfection experiments.** We generated full-length cDNA for mutated CPOX and constructed the pcDNA4-mut-myc expression vector. We also constructed the pcDNA4-Wt-myc expression vector with wild-type CPOX. We transfected these vectors into COS-7 cells and performed western blot analysis using an anti-myc antibody (Cell Signaling Technology). β-actin expression was used as an internal control. **c. Inducible expression system.** We utilized the tetracycline-regulated expression system and T-REx™-HeLa Cell Line (Invitrogen). Briefly, T-REx™-HeLa Cells were co-transfected with

Physical examination revealed localized erythema, papules, and diffuse pigmentation predominantly on sun-exposed areas including the face and hands (Fig. 1a, b). Minimal erythema dose (MED) for UVB was within normal range (MED for UVB, 120 mJ/cm^2), while minimal response dose (MRD) for UVA was decreased (MRD for UVA, 10 J/cm^2). Visible light phototest was negative (a slide projector halogen lamp, 30 cm distance, 15 min irradiation). Blood examination revealed elevations in ALT (54 IU/L), γGTP (112 IU/L), total bilirubin (2.8 mg/dL), direct bilirubin (1.0 mg/dL), triglycerides (350 mg/dL), total cholesterol (305 mg/dL), ferritin (587.2 mg/dL), and protoporphyrin (198 μg/dL RBC; reference interval: 30–86 μg/dL RBC), but coproporphyrin levels were in the normal range (<1 μg/dL RBC). Urine examination showed highly elevated levels of coproporphyrin (1200 μg/g CRE; reference level: <170 μg/g CRE), slightly elevated levels of porphobilinogen (2.9 mg/day; reference level: <2.0 mg/day), and normal levels of uroporphyrin and delta-aminolevulinic acid. Two months later, the elevated ALT was spontaneously normalized, indicating the associated acute hepatitis was transient.

We made a tentative diagnosis of HCP based on these findings, and examined the patient's *CPOX*. Sequence analysis was performed using previously reported primers [6]. We detected a novel missense mutation in the translation initiation codon (TIC): c.2 T > G (p.M1R) (Fig. 2a). This mutation was not detected in healthy control individuals. MLPA demonstrated that the copy number of *CPOX* of the patient was 2n, indicating that the patient is homozygote for the mutation (data not shown). Homozygous *CPOX* mutations have been reported in association with a more severe and phenotypically distinct condition called harderoporphyria that is characterized by neonatal hemolytic anemia with mild residual anemia during childhood and adulthood [7,8]. To learn more about the effect of the *CPOX* mutation, we performed the following experiments.

We generated the full-length cDNA of the mutated (mut) *CPOX* and constructed the pcDNA4/TO/myc-HisA expression vector (pcDNA4-mut-myc; Thermo Fisher Scientific). The pcDNA4-Wt-myc vector was used to serve as a normal control. We transfected these vectors into COS-7 cells and performed western blot analysis using an anti-myc antibody (Fig. 2b). The lane for wild-type *CPOX* (pcDNA4-Wt-myc) showed two bands, at 53 kDa and 39 kDa. Two bands were seen at 42 kDa and 39 kDa for mutated *CPOX* (pcDNA4-mut-myc). The *CPOX* has two possible TICs [9]. The first TIC (TIC-1) is located 300 bp upstream from the second TIC (TIC-2). Thus, there are three forms of CPOX: long CPOX transcribed from TIC-1, short CPOX transcribed from TIC2, and mature CPOX after proteolytic processing of the N-terminal presequence [4]. The three bands at 53 kDa, 42 kDa, and 39 kDa correspond to long CPOX, short CPOX, and mature CPOX, respectively (Fig. 2b). Long CPOX was not expressed in the COS-7 cells transfected with mutated cDNA because of the mutation in TIC-1. The absence of bands in the lane with the empty vector indicated that intrinsic enzyme could not be detected in this system.

Long CPOX can be imported into the intermembrane space of mitochondria via recognition of mitochondrial targeting signals. Long CPOX converts coproporphyrinogen III into protoporphyrinogen IX in the intermembrane space of mitochondria [4]. However, it has been reported that short CPOX is not effectively transported into mitochondria [10]. We performed another set of transfection experiments using tetracycline-regulated expression system, the T-REx^{TM}-HeLa Cell Line (Invitrogen) with which we can examine precise subcellular localization of the target molecule. We selected representative stable clones with pcDNA4-Wt-myc and pcDNA4-mut-myc and induced CPOX expression by adding tetracycline. Interestingly, CPOX expression of the clone with pcDNA4-Wt-myc was found predominantly in the mitochondrial fraction but not detected in the cytoplasmic fraction (Fig. 2c). CPOX expression of the clone with pcDNA4-mut-myc was specifically detected in the cytoplasmic fractions but not in the mitochondrial fraction. Notably, a little amount of mature CPOX was recognized in the cytoplasm of the mutant CPOX clone (Fig. 2c, arrowhead).

We performed another experiment with immunofluorescent staining. Stable cells expressing CPOX with the addition of tetracycline were stained with a FITC-labeled anti-myc antibody and an anti-Tim23 antibody labeled with red Alexa 647 (Fig. 2d). Examination of immunofluorescence staining under a confocal microscope revealed a merged image with wild-type CPOX, indicating intracellular localization in the mitochondria. However, mutant CPOX was localized in the cytoplasm and no colocalization of mutant CPOX with mitochondrial Tim23 was evident, consistent with the western blotting results.

In conclusion, we reported a mild case of HCP with a novel homozygous missense mutation in TIC-1: c.2 T > G (p.M1R). Transfection and immunostaining experiments indicated that the TIC-1 mutated *CPOX* only synthesized short CPOX and that mature CPOX can be produced from short CPOX in the cytoplasm. Generally, the complete blockade of heme synthetic pathway must be lethal. Therefore, the heme synthetic pathway in our patient with mild HCP may not be totally defected. It should be clarified that the cytoplasmic mature CPOX can participate in the heme synthetic pathway.

Funding

This study was supported by the Japan Society for the Promotion of Science KAKENHI [grant number 18K08261].

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgement

We thank Ms. Yukiko Kanda for technical assistance.

References

[1] M.H. Delfau-Larue, P. Martasek, B. Grandchamp, Coproporphyrinogen oxidase: gene organization and description of a mutation leading to exon 6 skipping, Hum. Mol. Genet. 3 (1994) 1325–1330.

pcDNA6/TR^{TM} and the pcDNA4-Wt-myc or pcDNA4-mut-myc. The transfected cells were selected in the DMEM medium containing zeocin (200 μg/mL) (Invitrogen) and blasticidin (5 μg/mL) (Invitrogen). The antibiotic resistant clones were screened for expression of CPOX by Western blot analysis using the anti-CPOX antibody (Abcam). The clones expressing CPOX in the presence of tetracycline (T+), but not ethanol (T-), were maintained in the medium supplemented with 50 μg/mL zeocin and 5 μg/mL blasticidin. A representative clones with the pcDNA4-Wt-myc and pcDNA4-mut-myc were chosen for further studies. 24 h after tetracycline in 2 ug/mL concentration was added to the stable cells, the protein were extracted after mitochondria and cytoplasmic separation by using QproteomeTM Mitochondria Isolation kit (QIAGEN) following the manufacturer's instructions. Expression of Tim23, cytochrome C and β-actin were also examined for reliability of the separation using anti-Tim23 antibody (Abcam), anti-cytochrome C antibody (Abcam) and anti-β-actin antibody (Abcam).

d. Immunofluorescent staining. Transiently transfected COS-7 cells expressing CPOX similarly utilized in the experiments in Fig. 2b were treated with the following antibodies. For CPOX, the first antibody was a rabbit anti–Myc tag antibody (1:500) and the second antibody was an anti-rabbit antibody fluorescently labeled with FITC (1:200). For staining of mitochondria (Mito), the first antibody was an anti-Tim23 antibody (1:200) and the second antibody was an anti-mouse antibody fluorescently labeled with Alexa Fluor 647 (1:200). Nuclei were labeled with DAPI. Scale bar = 20 μm.

[2] S. Taketani, H. Kohno, T. Furukawa, et al., Molecular cloning, sequencing and expression of cDNA encoding human coproporphyrinogen oxidase, Biochim. Biophys. Acta 1183 (1994) 547–549.
[3] S. Sano, S. Granick, Mitochondrial coproporphyrinogen oxidase and protoporphyrin formation, J. Biol. Chem. 236 (1961) 1173–1180.
[4] G. Attardi, G. Schatz, Biogenesis of mitochondria, Annu. Rev. Cell Dev. Biol. 4 (1988) 289–333.
[5] B. Wang, D.M. Bissell, Hereditary coproporphyria Nov, GeneReviews at GeneTests: Medical Genetics Information Resource (Database Online). Copyright, University of Washington, Seattle, 2018, pp. 1993–2019 Available at http://www.genetests.org, 2016 (Accessed 13 Dec 2016).
[6] F.J. Fiménez-Jiménez, J.A. Agúndez, C. Martínez, F. Navacerrada, et al., Hereditary coproporphyria associated with the Q306X mutation in the coproporphyrin oxidase gene presenting with acute Ataxia, Tremor Other Hyperkinet. Mov. 25 (2013) 3.
[7] Y. Nordmann, B. Grandchamp, H. de Verneuil, et al., Harderoporphyria: a variant hereditary coproporphyria, J. Clin. Invest. 72 (1983) 1139–1149.
[8] J. Lamoril, P. Martasek, J.C. Deybach, et al., A molecular defect in coproporphyrinogen oxidase gene causing harderoporphyria, a variant form of hereditary coproporphyria, Hum. Mol. Genet. 4 (1995) 275–278.
[9] M. Daimon, E. Gojyou, M. Sugawara, K. Yamatani, et al., A novel missense mutation in exon 4 of the human coproporphyrinogen oxidase gene in two patients with hereditary coproporphyria, Hum. Genet. 99 (1997) 199–201.
[10] S. Susa, M. Daimon, H. Ono, et al., The long, but not the short, presequence of human coproporphyrinogen oxidase is essential for its import and sorting to mitochondria, Tohoku J. Exp. Med. 200 (2003) 39–45.

Tomohisa Fukui[a], Eijiro Akasaka[a], Daiki Rokunohe[a], Yasushi Matsuzaki[a], Daisuke Sawamura[a], Kenji Kabashima[b], Hajime Nakano[a,*]
[a]Department of Dermatology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan,
[b]Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

* Corresponding author.
E-mail address: hnakano@hirosaki-u.ac.jp (H. Nakano).

Received 2 June 2020
Received in revised form 9 June 2020
Accepted 11 June 2020