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Letter to the editor

The first Japanese case of familial porphyria cutanea tarda diagnosed by a *UROD* mutation

Dear Editor,

Porphyria cutanea tarda (PCT) is a disorder of porphyrin metabolism associated with cutaneous photosensitivity that usually presents with blistering on the face and dorsal hands and signs of liver damage. The disease is caused by a deficiency in uroporphyrinogen decarboxylase (UROD), the fifth enzyme in the heme synthesis pathway. Three clinically similar forms of PCT can be distinguished: sporadic (type I or S-PCT), familial (type II or F-PCT), and type III [1]. F-PCT is an autosomal dominant disorder with low penetrance that is characterized by a heterozygous *UROD* mutation. Most PCT patients have the sporadic type, which is not associated with mutations in *UROD*. In this paper we report the first Japanese case of F-PCT with a previously unreported pathogenic *UROD* mutation.

A 26-year-old Japanese fisherman presented with a 4-year-history of exanthema after sun exposure. He was diagnosed with liver damage 2 months previously. His alcohol intake was more than 28 g daily for more than 9 years. His family history of PCT was negative. Physical examination revealed multiple fine pitted scars across the face (Fig. 1A). Diffuse erythema was noted, and was most prominent on the upper eyelids. Variously sized erythematous patches with crusting and pigmentation were seen on the dorsal hands (Fig. 1B). Blood examination showed elevated levels of AST (55 IU/L; normal, 10–40), ALT (83; normal, 5–40), and γ-GTP (96 IU/L; normal, <70). Serum hepatitis B antigen and anti-hepatitis C antibody were negative. Porphyrin tests demonstrated increased urine uroporphyrin levels (604 mg/dL·creatinine; normal, <36), while urine coproporphyrin, erythrocyte protoporphyrin, and erythrocyte coproporphyrin levels were within normal limits. Based on these clinical findings, a diagnosis of PCT was made. After obtaining written informed consent and following the Declaration of Helsinki, we performed mutational analysis using genomic DNA extracted from peripheral blood leukocytes of the proband, his elder brother, and his parents. Direct sequencing demonstrated a heterozygous single nucleotide change at the intron 6 / exon 7 border of *UROD*, designated as c.673-2A>C (Fig. 2A). In the proband, while his elder brother and parents do not have the nucleotide change. This nucleotide change was located at the splicing acceptor site of intron 6, suggesting it would cause exon skipping. To confirm this, we determined the primary structure of *UROD* mRNA prepared from the proband's leukocytes. Gel electrophoresis showed that reverse transcriptase (RT)-PCR products of the proband demonstrated a shorter band (Fig. 2B, arrow) as well as a band with the expected size (Fig. 2B, arrowhead). Direct sequencing of the shorter band revealed the whole deletion of exon 7 (Fig. 2C), confirming the skipping of exon 7. This deletion mutation was predicted to result in an in-frame 46-amino-acid deletion, designated as p.A213_M258del. We then performed immunoblotting to identify the mutant UROD protein with a predicted size of 35 kDa. We detected the wild-type UROD protein bands at approximately 40 kDa in all the samples (Fig. 2D, arrowhead). The expression level of the proband's UROD was approximately half that of control, as determined by densitometry (data not shown). However, no band corresponding to the 35-kDa mutant UROD was detected (Fig. 2D). Instead, a single band at 30 kDa was observed non-specifically in both of the proband and control in all repeated blottings (Fig. 2D, arrow). The aberrant UROD protein may be unstable and be degraded at the protein level.

PCT is the most common type of porphyria, and is caused by decreased UROD activity. F-PCT occurs in 20–30% of PCT patients in Western countries and has the same symptoms as S-PCT [2]. A literature search using ICHUSHI in Japan and PubMed identified 355 prior cases of PCT in Japan. Unexpectedly, only four PCT cases with positive family histories have been reported, and none have been genetically analyzed [2,3]. F-PCT is therefore extremely rare in Japan compared with Western populations, but the reason for this remains unclear. We could not find F-PCT cases in Korea and China, suggesting a low incidence of F-PCT in East Asians [4]. So far, the splice-site mutation demonstrated here has been reported in neither PubMed nor Human Genome Mutation Database® Professional, suggesting the mutation is a novel one. All the unaffected family members consisted of his elder brother and parents do not have the mutation, suggesting that this is a *de novo UROD* mutation.

In F-PCT, heterozygous mutations in *UROD* reduce UROD activity to about 50% in all tissues, predisposing carriers to clinical manifestations. Immunoblotting showed a 50% reduction of the wild-type UROD protein level in the proband (Fig. 2D). F-PCT also requires precipitating factors such as excessive alcohol intake, drug use, and HCV infection [5]. In our patient, alcohol consumption beginning at an early age may have precipitated the development of F-PCT. Sun protection whenever possible and abstinence effectively improved the patient's photosensitivity and liver damage, respectively.

In conclusion, we describe the first Japanese case of F-PCT with a novel splice-site *UROD* mutation. Efforts to detect *UROD* mutation heterozygotes in PCT families are required to prevent these individuals from developing PCT by encouraging them to avoid known precipitating factors.

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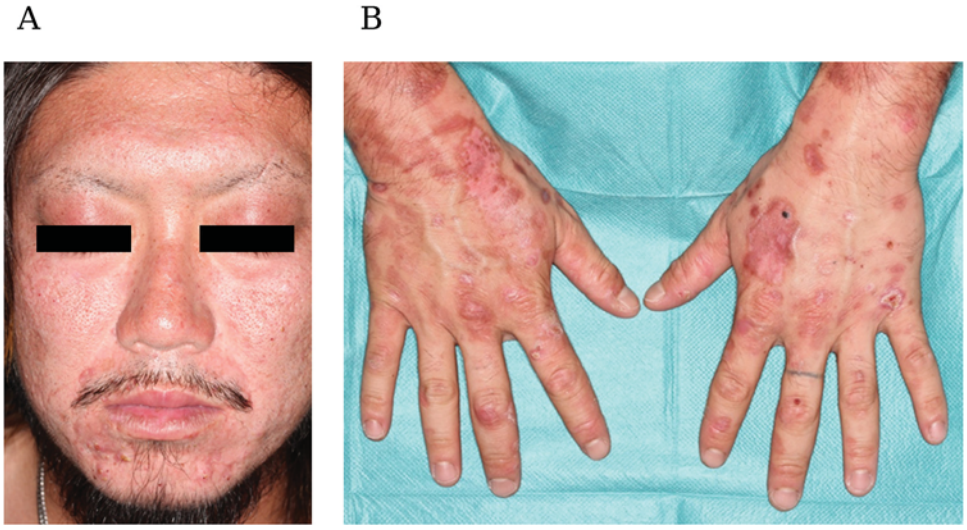

Fig. 1. Clinical Findings. (A) Physical examination revealed multiple fine pitted scars across the face. Diffuse erythema was noted, and was most prominent on the upper eyelids. (B) Variously sized erythematous patches with crusting and pigmentation were seen on the dorsal hands.

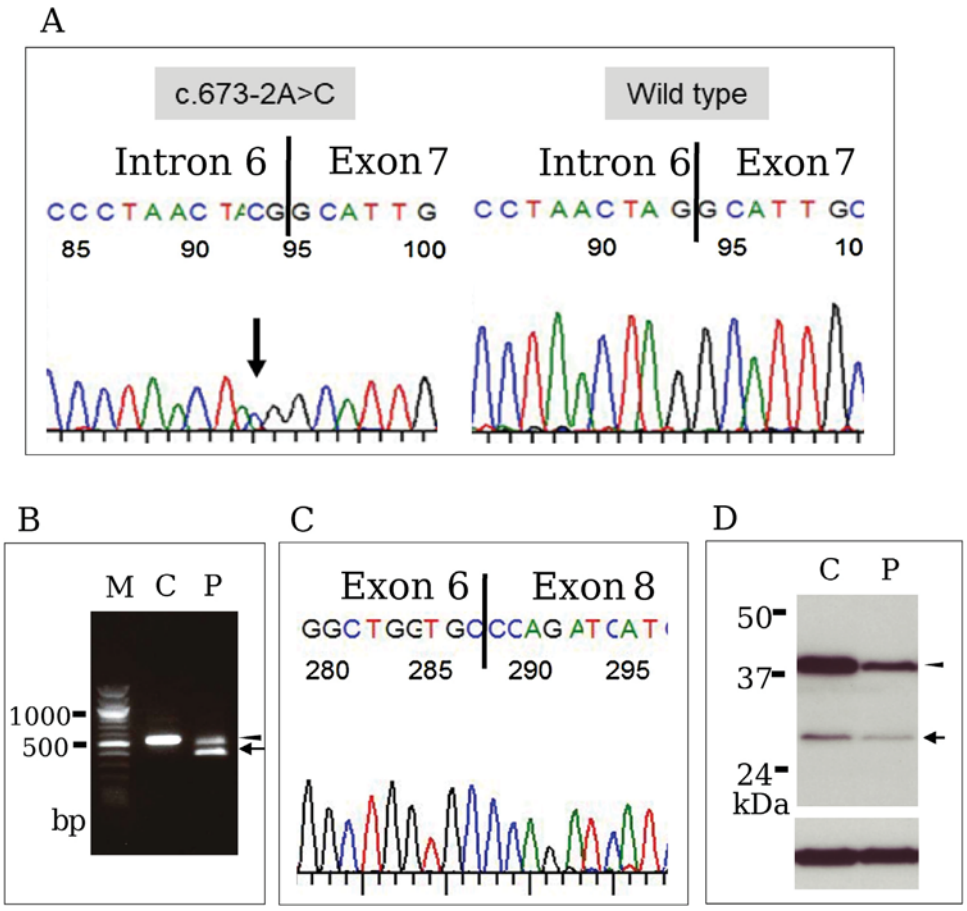

Fig. 2. Molecular analyses. (A) Direct sequencing demonstrated a heterozygous single nucleotide change at the intron 6 / exon 7 border of *UROD*, designated as c.673-2 A > C. (B) RT-PCR analysis of *UROD* mRNA from the proband. Gel electrophoresis showed that RT-PCR products of the proband demonstrated a shorter band (arrow) as well as a band with the expected size (arrowhead). M, molecular marker; C, control; P, proband. The primer sequences to amplify the specific *UROD* cDNA are as follows; 5′−CCCAGAGCCATTAAGAGAAG-3′ (forward) and 5′-AGCCCAACCACCTCATAGCC-3′ (reverse). (C) Direct sequencing of the shorter band revealed the whole deletion of exon 7, confirming the skipping of exon 7. (D) Immunoblotting using antibodies against UROD (Bioworld Technology, BS7604) (upper panel) and β-actin (lower panel). C, control; P, proband. These blots are representative of three independent experiments.

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Conflict of interest

The authors have no conflict of interest to declare.

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