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 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].

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.
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 erythematosus patches with crusting and pigmentation were seen on the dorsal hands.

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’–AGCCCCACCCCTCATGCC–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.
Conflict of interest
The authors have no conflict of interest to declare.

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References

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Letter to the Editor

Dysbiosis of oral microbiota in palmoplantar pustulosis patients

Palmoplantar pustulosis (PPP), a chronic inflammatory skin disease, is characterized by sterile pustular eruptions and erythematous scaling on the palms and soles [1]. Nearly 10% of patients suffer from joint manifestation, pustulotic arthro-osteo-sis (PAO) [1]. Both skin and joint symptoms are triggered or worsened by focal infections including tonsillitis and dental infection [1]. Recently, we retrospectively evaluated the efficacy of dental infection control and tonsillectomy on the clinical outcomes of 85 PPP patients, and demonstrated that oral focal infections were closely associated with disease severity [2]. These findings led us to hypothesize that changes in oral microbiota based on focal infections may correlate to disease severity and clinical characteristics of PPP. Here, oral microbiota of PPP patients and healthy controls were comparatively analyzed by next generation sequencing and statistical analysis are described in Supplementary materials and methods. First, we compared the bacterial communities of the pts regarding the following characteristics: joint manifestation (PAO) (n = 7), periodontitis (n = 6), smoking habit (n = 9) and higher disease severity according to Palmoplantar Pustulosis Area and Severity Index (PPPSASI) score (n = 6). From these results, subjects in the pts with these characteristics showed a different oral bacterial community (Fig. 1C). Third, we analyzed the relative bacterial abundance in all subjects in the pts, subjects in the pts with PAO, periodontitis, smoking habit and higher PPPASI score, and subjects without these characteristics. Six major phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and TM7, and 13 genera selected as the majority of 16S readings were analyzed (Fig. 1D and Table S1). Results of comparison of the pts and HCs demonstrated that the pts had less Proteobacteria (pts = 24%, HCs = 37%, P < 0.05) at the phyla level, and less Haemophilus (pts = 10%, HCs = 17%, P < 0.05) and more Prevotella (pts = 17%, HCs = 10%, P < 0.05) at the genus level. Interestingly, all of these features were shared only in subjects in the pts with PAO (Proteobacteria; pts with PAO = 21%, HCs = 37%; Haemophilus: pts with PAO = 8%, HCs = 17%; Prevotella: pts with PAO = 20%, HCs = 10%; P < 0.05). According to previous oral microbiome studies in other autoimmune diseases by two Japanese groups, these changes in bacterial abundance (less Proteobacteria, less Haemophilus and more Prevotella) were also observed. The oral microbiota among the pts regarding the following characteristics: joint manifestation (PAO) (n = 7), periodontitis (n = 6), smoking habit (n = 9) and higher disease severity according to Palmoplantar Pustulosis Area and Severity Index (PPPSASI) score (n = 6). These results indicated that oral microbiota of the pts significantly differed from that of the HCs, and suggested oral dysbiosis in PPP patients. Second, we evaluated the difference in the oral microbiota among the pts regarding the following characteristics: joint manifestation (PAO) (n = 7), periodontitis (n = 6), smoking habit (n = 9) and higher disease severity according to Palmoplantar Pustulosis Area and Severity Index (PPPSASI) score (n = 6). These results indicated that oral microbiota of the pts significantly differed from that of the HCs, and suggested oral dysbiosis in PPP patients. Second, we evaluated the difference in


Table 1
Characteristics of patients with Palmoplantar Pustulosis (pts) and healthy controls (HCS).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pts (n = 12), n(%)</th>
<th>HCs (n = 10), n(%)</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (67%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Male</td>
<td>4 (33%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Age (yrs), mean ± SD</td>
<td>53.7 ± 14.6</td>
<td>29.5 ± 2.87</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>9 (75%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>6 (50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Joint manifestation</td>
<td>7 (58%)</td>
<td>NA a</td>
</tr>
<tr>
<td>PPPASI c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.4–19.2</td>
<td>NA b</td>
</tr>
<tr>
<td>Low score group (range)</td>
<td>6 (0.4–4.9)</td>
<td>NA b</td>
</tr>
<tr>
<td>High score group (range)</td>
<td>6 (5.4–19.2)</td>
<td>NA b</td>
</tr>
</tbody>
</table>

a Periodontitis was diagnosed using CDC-AAP.
b NA: not applicable.
c PPPASI: Palmoplantar Pustulosis Area and Severity Index.