Variegate porphyria (VP) is an autosomal dominant disease caused by mutations of the protoporphyrinogen oxidase (PPOX) gene. This porphyria has unique characteristics which can induce acute neurovisceral attacks and cutaneous lesions that may occur separately or together. We herein report a 58-years-old VP patient complicated with cholelithiasis. A sequencing analysis indicated a novel c.40G>C mutation (p.G14R) in the PPOX gene. His cutaneous photosensitivity had been worsening for 3 years before the emergence of cholecystitis and it then gradually improved after cholecystectomy and ursodeoxycholic acid treatment with a slight decline in the porphyrin levels in his blood, urine and stool. In VP patients, a worsening of photosensitivity can thus be induced due to complications associated with some other disease, thereby affecting their porphyrin-heme biosynthesis.

Key words: variegate porphyria, photosensitivity, cholelithiasis, PPOX gene, protoporphyrin
Figure 1. Chronic cutaneous symptoms before (a) and after (b) cholecystectomy. (a) Blisters, erosions, crusts and milia on the back of the hands are observed. (b) The cutaneous symptoms improved except for pigmentation at 3 years after cholecystectomy.

Table 2. Porphyrin Concentrations before and 3 Years after Cholecystectomy.

| Porphyrins       | before | after | reference |
|------------------|--------|-------|-----------|
| Urine (µg/g creatinine) |        |       |           |
| Uroporphyrin     | 29     | 18    | <36       |
| Coproporphyrin   | 367    | 128   | <170      |
| Blood (µg/dL RBC)|        |       |           |
| Coproporphyrin   | <1     | <1    | <1        |
| Protoporphyrin   | 90     | 75    | 30-86     |
| Faeces (µg/24h)  |        |       |           |
| Uroporphyrin     | <1     | <1    | <170      |
| Coproporphyrin   | 799    | 497   | <500      |
| Coproporphyrin III | 6,550 | 4,575 | <400      |
| Protoporphyrin   | 12,087 | 11,204 | <1,500   |
| Copro III/Copro I | 8.19  | 9.21  | <1.20     |

Case Report

A 58-year-old male was referred to our hospital for surgical treatment of cholecystolithiasis. He had been diagnosed as having acute porphyria because of an acute attack after the administration of an antifungal agent 7 years previously (though the diagnosis was not made at that time). Until recently, there had been no cutaneous symptoms except for two episodes of the transient formation of blisters over his entire body during sunbathing when he was in his thirties. He presented with a 3-year history of chronic blistering on the backs of his hands due to photosensitivity, and he had developed skin fragility, blisters and scarring (Fig. 1a). Facial hyperpigmentation was also present. Liver function and inflammatory reaction tests did not show abnormal findings (Table 1). The serum levels of iron and ferritin were also within the normal limits. A computed tomography (CT) scan of the abdomen demonstrated 30 mm and 4 mm calcified gallstones in the neck of the gallbladder and distal bile duct, respectively. Despite three episodes of acute cholecystitis in the previous 6 months, no elevations in urinary levels of δ-Aminolevulinic acid (ALA) or porphobilinogen (PBG) had been observed. A stool examination suggested the excessive excretion of protoporphyrin consistent with a biochemical diagnosis of VP (Table 2). Sequencing analyses using polymerase chain reaction primers (4), identified a heterogeneous novel c.40G>C mutation in exon 2 of the PPOX gene (Fig. 2a).

Owing to the characteristics of the acute attack and photosensitivity, cholecystectomy was done avoiding use of porphyrinogenic agents for anaesthesia and protection the patient from phototoxic injury due to surgical luminaires. Lights in the operating room were covered with orange filters (Lumicool 1905; Yamahira, Saitama, Japan), and a yellow filter (Dichroic Filter-Y; Koshin, Kanagawa, Japan) was used for the headlight, and emitted light at wavelengths < 500 nm and 460 nm, respectively (6). There was no clinical evidence of any exacerbation of the patient’s illness throughout the perioperative period.

Postoperatively, he was given UDCA. The urinary excretion of uroporphyrin and coproporphyrin and protoporphyrin in the erythrocytes fell to normal limits after 3 years (Table 2). In the patient’s faeces, porphyrin excretion also decreased slightly. Chronic blistering on the backs of his hands due to photosensitivity had also completely improved at this time (Fig. 1b). Skin pigmentation remained but there was no fragility, blistering or pronounced scarring.

Table 1. Laboratory Data.

|                | before | after | reference |
|----------------|--------|-------|-----------|
| WBC            | 5.090  | 4.680 | 5.130     |
| Neut           | 2.890  | 2.490 | 2.250     |
| RBC            | 464×10⁶| 486×10⁶| 482×10⁶  |
| Hb             | 14.0   | 12.4  | 12.8      |
| Ht             | 41.9   | 42.3  | 41.8      |
| Plt            | 13.9×10⁶| 15.2  | 13.6      |
| TP             | 7.4    | 7.2   | 7.0       |
| Alb            | 4.0    | 4.8   | 4.2       |
| T.Bil          | 0.7    | 0.6   | 0.4       |
| AST            | 18     | 17    | 16        |
| ALT            | 22     | 20    | 19        |
| LDH            | 157    | 148   | 139       |
| BUN            | 128    | 138   | 140       |
| Cr             | 79     | 81    | 79        |
| Ferritin       | 105    | 125   | 115       |
| Ferrum         | 68.1   | 65.2  | 64.5      |
| ALP            | 258    | 267   | 270       |
| CRP            | 0.1    | 0.2   | 0.1       |

WBC: white blood cell, Neut: neutrophil, RBC: red blood cell, Ht: hematocrit, Plt: platelet, TP: total protein, Alb: albumin, T.Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, γ-GTP: γ-glutamyl transferase, BUN: blood urea nitrogen, Cr: creatinine, TIBC: total iron binding capacity, UIBC: unsaturated iron binding capacity, CRP: C-reactive protein.
In VP patients, excess porphyrins accumulate in the skin and dermal vessels. They produce reactive singlet oxygen molecules when they are activated by excessive exposure to sunlight, resulting in skin fragility and a tendency to develop blister formation (7). However, not all VP patients show these cutaneous symptoms. The number of skin lesions in VP deteriorates with sunlight exposure, but it is not known which factors determine the emergence of chronic cutaneous symptoms. Our patient experienced two episodes of transient, acute photosensitive dermatitis after excessive exposure to sunlight while in his thirties, but did not present with chronic cutaneous lesions until 3 years previously. Since a 30 mm calcified gallstone was observed in the cystic duct at the first visit, he would have been complicated with cholelithiasis for several years. Therefore, it is supposed that his cutaneous symptoms became apparent with the progression of his gallstone. Moreover, His cutaneous lesions improved with no specific therapy except for cholecystectomy and UDCA treatment, so we hypothesised that cholelithiasis induced these symptoms.

The prevalence of cholelithiasis has been reported to increase in VP patients, and some patients exhibit chronic skin symptoms before cholelithiasis becomes apparent (8). It has been suggested that a disturbance of porphyrin excretion to bile due to cholestasis might cause increases in porphyrin accumulation. However, there was no sign of cholestasis or liver dysfunction in laboratory data of this patient (Table 1). Several VP cases presented with cutaneous symptoms before the definitive complications of another disease became apparent (8-14). Most of these cases had liver-related disease, but some did not (Table 3). Therefore, the exacerbation of
skin lesions is due not only to liver dysfunction, but is also dependent upon systemic stress, which may alter heme biosynthesis. Furthermore, in patients with homozygous VP, severe cutaneous symptoms were reported from childhood even though they had experienced no acute attacks (15), suggesting that a severe deficiency of protoporphyrinogen oxidase (PPOX) can cause chronic cutaneous lesions rather than acute attacks. In most cases, increases in the levels of protoporphyrins in erythrocytes are observed, but not for patients with heterozygous cases. Therefore, increased levels of protoporphyrin in the blood may predict the emergence of chronic cutaneous symptoms in VP patients. During chronic stress (as seen in our patient), the suppression of PPOX activity and increases in protoporphyrinogen levels (including other porphyrin metabolites) may be induced. Then, autooxidation of water-soluble protoporphyrinogen to insoluble other porphyrin metabolites) may be induced. Finally, autooxidation of water-soluble protoporphyrinogen to insoluble protoporphyrin may occur in erythrocytes and peripheral tissues, resulting in their accumulation in the skin and thus leading to the onset of cutaneous symptoms.

In our case, we identified a c.40G>C mutation in exon 2 of the PPOX gene, which resulted in a substitution of a nonpolar glycine by a polar arginine (p.G14R). This glycine residue is evolutionarily highly conserved in humans, mice, Rattus norvegicus, Xenopus laevis, Drosophila melanogaster and Nicotiana tabacum, attesting to its importance (Fig. 2b), and lies in the flavin adenine dinucleotide (FAD)-binding domain in the amino-terminal α1 helix of the PPOX (16). Modelling of the p.G14R mutation reveals conformational changes at the canonical FAD binding site (Fig. 2c). Consequently, this mutant seems to have a reduced activity. Moreover, 28 amino acids in the amino terminus of PPOX contain a functioning signal for mitochondrial targeting (19). A positively charged and hydrophilic arginine substitution disrupts the hydrophobic face in this lesion, and this affects the interaction with the mitochondrial outer membrane receptor Tom20 (translocase of outer mitochondrial membrane 20). Therefore, a p.G14R mutation may disrupt the correct transport of PPOX into mitochondria.

The use of protective light filters during surgical procedures for VP patients is not commonly recommended. However, phototoxic injury due to operating-room lights in a Japanese VP patient was reported recently (14). That patient presented with chronic blistering due to photosensitivity and increased levels of protoporphyrins in erythrocytes with a complication of cervical cancer. Thus, in our patients, we could not exclude the possibility of phototoxic injury due to surgical luminaires, and therefore used filters to avoid that risk. Photosensitivity may be dependent upon the extent of accumulation of cutaneous porphyrins, light quality or duration of irradiation. After cholecystectomy and the dosage of UDCA, the porphyrin levels in his blood, urine and stool decreased and cutaneous photosensitivity improved. Therefore, the onset of photosensitivity should be considered to be a potential and transient symptom in some VP patients who have complications.

The authors state that they have no Conflict of Interest (COI).

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