Case Report

Harderoporphryia: Case of lifelong photosensitivity associated with compound heterozygous coproporphyrinogen oxidase (CPOX) mutations

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1. Introduction

The porphyrias are caused by altered activity of enzymes in the heme biosynthetic pathway, and result in the accumulation and excretion of intermediates of the pathway. Major symptoms are cutaneous photosensitivity due to elevations of photosensitizing porphyrins in plasma or erythrocytes, or neurological manifestations that are associated with elevations in the porphyrin precursors δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) [1].

Coproporphyrinogen oxidase (CPOX) is the sixth enzyme in the heme biosynthetic pathway, and is found as a homodimer in the mitochondrial intermembrane space [2]. CPOX mutations have been associated with hereditary coproporphyria (HCP), an acute hepatic porphyria that results from a single heterozygous CPOX mutation and is an autosomal dominant inherited disorder with low penetrance. HCP causes acute attacks of neurological symptoms with associated increases in the porphyrin precursors ALA and PBG, and less commonly chronic cutaneous photosensitivity due to elevation of coproporphyrin III [3]. The disease is genetically heterogeneous, with more than 50 different CPOX mutations described [HGMD, Stinson et al. 2017]. Rare homozygous cases of HCP with two pathogenic CPOX mutations present with childhood onset of severe photosensitivity and neurological symptoms [1,3,4]. Harderoporphryia, a clinically distinct variant of homozygous HCP, presents with neonatal hemolytic anemia and jaundice, and results from certain mutations within the substrate-binding cleft of CPOX, which reduce enzyme activity and also lead to premature release of hardyoporphyrinogen, the tricarboxyl intermediate in the two-step oxidative decarboxylation of coproporphyrinogen III to protoporphyrinogen IX [2,4]. The excess harderoporphyrinogen undergoes autoxidation to hardyoporphyrin, which may be found in erythrocytes and is excreted in large amounts in feces. Harderoporphryia was first described in 1983 in three siblings from Lille, France [5]. Since then, there have been only five additional published cases, to our knowledge [4–10]. The present case with two previously undescribed CPOX mutations is the first with symptoms continuing to an advanced age.

1.1. Case study

A 78-year-old Caucasian male presented for evaluation of chronic cutaneous photosensitivity. He had a history of neonatal jaundice and anemia requiring blood transfusions. Thereafter he had cutaneous blistering on sun exposed areas, mild anemia and splenomegaly but developed normally and was able to participate normally in sports and other activities. Urine porphyrins were elevated in his 40s, and porphyria cutanea tarda was suspected but no diagnosis established. He consumed alcohol moderately, but was abstinent after age 43, and stopped smoking cigarettes at age 53. He underwent cholecystectomy at age 75 for symptomatic gallstones. At age 76 an enlarging but asymptomatic pancreatic cyst and a massively enlarged spleen were removed at laparotomy without sequelae. His parents were unrelated and had no
similar symptoms, and a sister, his only sibling, is asymptomatic. His only offspring, a son has unexplained cutaneous blistering, has not consented to evaluation.

Biochemical findings are summarized in Table 1. Urine porphyrins were markedly elevated to 2420 nmol/g creatinine (normal < 300), with a predominance of highly carboxylated porphyrins; ALA and PBG were normal. Plasma porphyrins were elevated to 13.4 μg/dL (< 0.9), with a peak fluorescence of diluted plasma at neutral pH at 620 nm [11]. Fecal porphyrins were increased to 875 μmol/g dry weight (< 200), with harderoporphyrin comprising 48%, coproporphyrin III 39% and coproporphyrin I 1% of the total (Fig. 1). Erythrocyte total porphyrin was elevated at 591 μg/dL (< 80), of which 24% was harderoporphyrin and 22% protoporphyrin (predominantly metal-free) (Fig. 2, Table 1). Erythrocyte uroporphyrinogen decarboxylase activity was normal.

Sequencing of the CPOX coding region and adjacent intronic boundaries revealed two previously unreported mutations in trans. The first was c.698A > G (p.D233G), which changes an aspartate to a neutral glycine (Fig. 3(a), which is predicted to be a minor change. The second was c.1207_1218del 12 (Fig. 3(b), which results in the in-frame deletion of 12 nucleotides encoding four amino acids at 403 to 406 in the active site of CPOX, which presumably prevents substrate binding and may impair catalysis. Sequencing of the UROS, FECH and ALAS2 genes demonstrated no abnormalities.

2. Discussion

The diagnosis of hardendorporphyria was established in this man later in life than in the other eight reported cases of hardendorporphyria, all of whom were diagnosed during childhood or adolescence. However, his history of neonatal jaundice and anemia requiring transfusions and splenomegaly, followed later by good health was quite typical. An early demise (at 5 months) occurred in only one of the cases reported to date, and in this and the other cases reported to date early severe manifestations improved after early childhood. Therefore, hardendorporphyria is compatible with prolonged survival, and should be considered along with other more common porphyrias as a possible cause of chronic cutaneous photosensitivity at any age. The disease can be difficult to diagnose accurately unless patterns of porphyrins in feces are examined for an unusual pattern that includes a substantial increase in hardendorporphyrin. As in this patient, hardendorporphyrin may also be elevated in erythrocytes, along with erythrocyte protoporphyrin. The latter is substantially elevated in all homozygous porphyrrias because after heme synthesis is complete, marrow reticulocytes still contain the heme biosynthetic pathway enzymes needed to convert accumulated intermediates to protoporphyrin [12]. Unfortunately, family members have not been available for study. The patient's son with blistering skin lesions on the dorsal fingers inherited one of his father's CPOX mutation, and is likely to have HCP, as reported in some heterozygous relatives of other patients with hardendorporphyria [4]. His sister is also presumably heterozygous for one of the patient's CPOX mutations, and is likely to have HCP, as reported in some heterozygous relatives of other patients with hardendorporphyria [4].
expressed no enzyme activity, again pointing to the K404E mutation as important in causing harderoporphyria [6]. The fifth patient was also homoallelic for the K404E mutation. The sixth patient had typical clinical features and was homozygous for a mutation, c980A > G (p.H327R), in another region of the gene, and died at 5 months of age [7]. Recently, 2 siblings were initially diagnosed as having osteopetrosis, but found by whole exome sequencing 15 years after successful bone marrow transplantation to be homozygous for the K404E mutation, and were belatedly diagnosed as having harderoporphyria [8]. Site-directed mutagenesis studies, and studies of a R401W CPOX mutation identified in a patient with HCP, have indicated that mutation of any five amino acids (D400-K404), which are encoded by exon 6, can lead to accumulation of harderoporphyrin and may cause harderoporphyria if homoallelic or heteroallelic to a CPOX null mutation, whereas mutations elsewhere lead to accumulation only of coproporphyrin III and cause HCP [4].

This patient with clinical and biochemical features of harderoporphyria was found to have two previously unreported CPOX mutations (Fig. 3). The first, encoding D233G, changes aspartate to a neutral glycine. Analysis of the predicted severity of the D233G change, located on a solvent exposed loop, was performed using the PolyPhen2 software [13], and was predicted as “benign”. The second, c.1207_1218del 12, results in removal of the four amino acids T403, L404, F405 and G406, a region of the gene that includes two of the five amino acids (T403, L404) known to be important in binding harderoporphyrin to the CPOX active site [4]. The region including amino acids at 400–404 is important for association between dimers as well as for enzyme activity. Because some mutant CPOX may dimerize to act in a dominant-negative fashion and lead to decreased enzyme activity and accumulation of harderoporphyrinogen [14], it may be important to study these mutations as mutant heterodimers. We were not able at present to assess the composition of the associated CPOX dimers formed in vivo in this case. The alteration of the active site pocket with deletion of four key residues is likely to facilitate early release of the substrate, and might also result in a partially stable protein. As shown for uroporphyrinogen decarboxylase (the preceding enzyme in the pathway and also active as a homodimer) [15], the active sites of each CPOX monomer may not directly interact, and it is possible that combinations of dimers where at least one subunit has the deletion

Fig. 2. Fractionation of erythrocyte porphyrins by HPLC in a patient with harderoporphyria.

Fig. 3. Changes in the CPOX monomer in a patient with harderoporphyria heteroallelic for mutations (a) D233G and (b) c.1207_1218del 12.

Table 2
Coproporphyrinogen oxidase mutations reported in 9 cases of harderoporphyria.

| Case | Sex | CPOX Mutations | Author Year |
|------|-----|----------------|-------------|
| 1    | M   | c. 1210A > G (p.K404E) - homozygous | Nordmann et al. [5,9] 1983 |
| 2    | M   | c. 1210A > G (p.K404E) - homozygous | Lamoril et al. [6] 1998 |
| 3    | F   | c. 1210A > G (p.K404E) - homozygous | Schmitt et al. [4] 2005 |
| 4    | M   | c. 1210A > G (p.K404E)/IVS6 + 3 | Hosanoglu [7] 2011 |
| 5    | M   | c.980A > G (p.H327R) - homozygous | Mendoza et al. [8] 2013 |
| 6    | ?   | c.1210A > G (p.K404E) - homozygous | This case 2018 |
| 7    | ?   | c.1210A > G (p.K404E) - homozygous | |
| 8    | ?   | c.1210A > G (p.K404E) - homozygous | |
| 9    | M   | p.D233G (c.698A > G)/c.1207_1218del 12 | |

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of residues 403–406 will lack the functionality to maintain hard-
eroporphyrinogen in the active site.

In conclusion, harderoporphyria is a variant of homozygous HCP with prominent hematological features, especially neonatal anemia and jaundice, with recovery usually followed by a benign course. It should be considered as the cause of increased porphyrins and chronic photosensitivity at any age, and is recognized by finding increases in harderoporphyrin in feces and, in many cases, in erythrocytes. CPOX mutations affecting exon 6 that encodes amino acids D400-K404 have been found in most but not all cases.

Conflicts of interest

The authors have no significant conflicts.

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