See related commentary on pg 1467

Late-Onset X-Linked Dominant Protoporphyria: An Etiology of Photosensitivity in the Elderly

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TO THE EDITOR

EPP is a rare inherited disorder in which accumulation of free protoporphyrin (PP) IX leads to the childhood onset of acute photosensitivity (Puy *et al.*, 2010). Over 95% of EPP cases are due to co-transmission of a loss-of-function allele of ferrochelatase (*FECH*) in *trans* to a common hypomorphic *FECH* allele (IVS3-48C), resulting in enzymatic activity of ~35% of normal (Gouya *et al.*, 2006, Elder *et al.*, 2009). We described another type of childhood-onset protoporphyria, X-linked protoporphyria (X-linked dominant protoporphyria (XLDPP), MIM no. 300752), caused by gain-of-function mutations within the C-terminal region of 5-aminolevulinate synthase 2 (*ALAS2*), the erythroid-specific isoform of the first enzyme of the heme biosynthetic pathway (Whatley *et al.*, 2008). Biochemically, XLDPP only differs from EPP in that much of the excess PP in erythroid cells is zinc-PP rather than free PP.

Late-onset cases of EPP have occurred in association with myeloproliferative or myelodysplastic disorders (MDS; Aplin *et al.*, 2001, Berroeta *et al.*, 2007, Blagojevic *et al.*, 2010). In some of these cases, there has been partial or complete loss of chromosome 18, including the *FECH* locus, in the abnormal hematopoietic clone. Defects of chromosome 18 are frequent in MDS, but PP-induced photosensitivity is rare. This suggests that additional factors affecting hematopoietic clones are required for the expression of myelodyplasia-associated EPP.

An 89-year-old Caucasian man developed acute painful photosensitivity on all sun-exposed areas in the summer of 2008. There were no other photosensitivity cases in the patient's family (Figure 1a). Within 12 hours of sun exposure he developed burning edema and erythema (Figure 1b). The condition worsened progressively, and in 2011 he also suffered from photosensitivity in winter. Provocative phototesting was performed. The polychromatic minimal erythematous dose (Dermolum UM-Müller, Moosinning, Germany) was normal. Simple UVA phototest (13 J cm^{-2}, UVA-700 lamp-Waldmann, Reichstett, France, 340–400 nm) was positive at 24 hours for erythema.

Initially, a possible drug-associated photosensitivity was suspected because the patient was taking *perindopril* (Coversyl, Neuilly-sur-Seine, France) for hypertension, but cessation of *perindopril* resulted in no improvement. The suspicion of porphyrin-induced photosensitivity was confirmed by porphyrin measurements, establishing a diagnosis of EPP (Table 1). The PP levels in erythrocytes correlated with the clinical exacerbation of photosensitivity in 2011. A mild macrocytic anemia in the context of late-onset protoporphyria led to bone marrow aspiration in April 2010. It showed dyserythropoiesis in a few erythroblasts without obvious myelodysplastic changes. In 2011, the macrocytic anemia progressed without evidence of folates or vitamin deficiency (Table 1). Another bone marrow aspirate showed karyorrhexis in <10% of erythroblasts (Supplementary Figure S1). There was no excess of myeloblasts. Molecular and enzymatic studies including *FECH* and *ALAS2* DNA sequencing and karyotyping on the marrow aspirate were performed. Both FECH activity in lymphocytes and *FECH* gene sequencing were normal. Karyotyping revealed no abnormalities of chromosome 18. Collectively, these findings demonstrate that PP accumulation was not caused by *FECH* deficiency. These data strongly suggested late-onset XLDPP. A nonsense *ALAS2* mutation (c.1642C4T [p.Q548X] resulting in a 39-amino-acid, truncated protein, and to our knowledge previously unreported, was identified in DNA extracted from peripheral blood (Figure 1c). The mutation was found in some alleles but not others, strongly suggesting somatic mosaicism in the bone marrow. The mutated allele was predominant in DNA extracted from blood but was absent in hair bulbs (Figure 1c). The pedigree data showed that no *ALAS2* genomic mutations were found in the one daughter who was investigated. Cytogenetic analysis of the marrow aspirate was normal in 2010, but in 2011 a 20q deletion was found in 20% of cells, suggesting clonal evolution of the hematological disease. The del(20q) is not included in the list of recurring chromosomal abnormalities considered as presumptive evidence of MDS for the World Health Organization (Vardiman *et al.*, 2009), but could be an early cytogenetic event in the context of myelodysplasia (Braun *et al.*, 2011). The progressive macrocytic anemia, the dyserythropoiesis, the karyorrhexis, and the 20q deletion were consistent with evolution into refractory anemia. The genotyping and pedigree studies were conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki for medical research involving human subjects, plus its subsequent amendments; all available relatives provided their written informed consent.

Only 12 patients have been described in whom EPP developed after the age of 40 years. In all cases, clinical symptoms

Abbreviations: ALA, aminolevulinate acid; ALAS2, 5-aminolevulinate synthase 2; FECH, ferrochelatase; MDS, myelodysplastic syndrome; PP, protoporphyrin; XLDPP, X-linked dominant protoporphyria

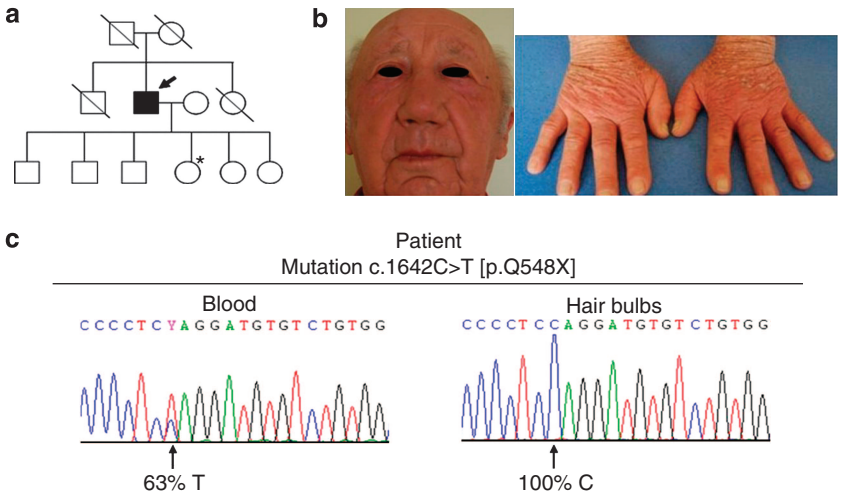

Figure 1. Pedigree, clinical presentation, and C-terminal *ALAS2* mutation in a late-onset protoporphyric patient. (**a**) *ALAS2* (5-aminolevulinate synthase 2) mutation in the family of the late-onset protoporphyric patient. Pedigree of the family with an *ALAS2* mutation (X-linked allele). Surprisingly, we noted the absence of mutation transmission from the father to the one daughter who was investigated (*). (**b**) Clinical presentation of a late-onset X-linked dominant protoporphyria case: lesions of photosensitivity. Edema and erythema developed immediately after exposure to sunlight. Chronic lesions, such as thickening of the skin on hands and face, were visible; the patient consented to his image being used. (**c**) C-terminal mutation in *ALAS2*. Sequence analysis of genomic DNA extracted from peripheral blood and hair bulbs. The c.1642C>T (p.Q548X) mutation was identified in the X-chromosome in the patient. The percentage of mutated allele was determined by PeakPicker software: http://genomequebec.mcgill.ca/EST-HapMap (Ge *et al.*, 2005).

Table 1. Biological data of the late-onset XLDPP patient

	April (2010)	February (2011)	September (2011)
PP in red blood cells ($\mu mol\,l^{-1}$ red blood cells; *N*: <1.9)	22.3	48.4	23.4
Free/ZnPP (%ZnPP)	45	52	NA
Total porphyrins in plasma ($nmol\,l^{-1}$; *N*: <20.0)	51	77	NA
Fluorescence emission peak (in nm)	634	634	NA
Total porphyrins in urine ($nmol\,mmol^{-1}$ creatinine; *N*: <30)	10	NA	NA
Total porphyrins in stool ($nmol\,g^{-1}$ of dry weight; *N*: <200)	560	750	NA
% PP (*N*: 70–75%)	98	99	NA
Fech enzyme activity ($nmol\,mg^{-1}$ protein per hour; *N*: >3.5)	5.2	NA	NA
Hb (*N*:13–17 $g\,dl^{-1}$)	10.5	9.0	7.9
MCV (*N*: 80–100 fl)	100	105	110
MCH (*N*: 27–32 pg)	34.1	38.4	39.2
White cell count (*N*: 4–10 × 10^9 per l)	4.99	5.22	4.23
Platelet count (*N*: 150–450 × 10^9 per l)	123	132	129
Folates (*N*: >5.38 $ng\,l^{-1}$)	3.43	5.48	5.82
B12 vitamin 210–920 $pg\,ml^{-1}$	200	586	572
γ-GT (*N*: <60 $UI\,l^{-1}$)	61	867	176
Total bilirubin (*N*: 2–21 $\mu mol\,l^{-1}$)	28	32	22
ALP (*N*: 100–200 $UI\,l^{-1}$)	221	320	210
AST (*N*: 3–35 $UI\,l^{-1}$)	34	32	30
ALT (*N*: 3–45 $UI\,l^{-1}$)	10	20	29

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; MCH, MC hemoglobin; MCV, mean corpuscular volume; NA, not available; PP, protoporphyrin; ZnPP, zinc PP; γ-GT, γ-glutamyl transferase.
Porphyrin measurements were performed according to the European Porphyria Network guidelines and quality-control schemes (http://www.porphyria-europe.org).

were associated with MDS. Most of these patients have complete or partial deletions of chromosome 18 in hematopoietic cells responsible for loss of *FECH* alleles. We present here a case of late-onset XLDPP, which to our knowledge is previously unreported and clinically indistinguishable from late-onset EPP. The high percentage of

zinc-PP led us to suspect XLDPP, which was confirmed by *ALAS2* sequencing. The presence of the X-linked ALAS2 mutation in the blood of a man with a normal marrow karyotype, the absence of transmission of the mutation to his daughter, and the absence of the mutation in the DNA from his hair bulbs strongly supports our conclusion that a somatic hematopoietic mosaicism was present. Late-onset XLDPP was revealed by expansion of the abnormal clone and by the dyserythropoiesis secondary to an emerging MDS as already described in other X-chromosome genes such as *ATRX* gene in α-thalassemia MDS (Gibbons *et al.*, 2003).

Somatic mosaicism in erythroid cells may account for the late presentation and for the mild severity. In adults whose disease became manifest in childhood, XLDPP leads to the production of PP in sufficient quantities to cause severe liver damage (Puy *et al.*, 2010). In our patient, compared with most XLDPP patients, late onset of XLDPP was associated with gallstones and mild cholestasis without cytolysis (Table 1). These relatively mild symptoms compared with some of the other XLDPP patients may be related to both its late onset and the acquired hematopoietic mosaicism. Two frameshift *ALAS2* mutations in the C-terminal region have been identified in XLDPP families (Whatley *et al.*, 2008). The p.Q548X mutation found here is predicted to result in the deletion of the C-terminal region, resulting in a marked increase in ALAS2 activity as shown in prokaryotic expression studies (data not shown).

In conclusion, we demonstrate that late-onset photosensitivity can be caused by XLDPP, and that both EPP and XLDPP should be considered when adverse reactions to sunlight occur in the elderly. Analysis of *ALAS2* alleles in marrow DNA would seem worthwhile in patients with late-onset photosensitivity associated with high PP levels in the absence of FECH deletions or mutations.

CONFLICT OF INTEREST

The authors state no conflict interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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