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Heerfordt, Ida M.; Fontenete, Silvia; Lerche, Catharina M.; Wulf, Hans Christian

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Distribution of protoporphyrin IX in erythrocytes in a case of acquired erythropoietic protoporphyria

Ida M. Heerfordt a,*, Silvia Fontenete b, Catharina M. Lerche a, c, Hans Christian Wulf a

a Department of Dermatology, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Denmark
b Department of Biology, University of Copenhagen, Denmark
c Department of Pharmacy, University of Copenhagen, Denmark

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ABSTRACT

Background: Erythropoietic protoporphyria (EPP) is a rare genetic photodermatosis caused by loss-of-function mutations in the gene for ferrochelatase leading to accumulation of the fluorescent protoporphyrin IX (PpIX) in erythrocytes. The mutations are most often inherited mutations present in all cells causing inherited EPP. In very rare cases EPP are acquired in association with myelodysplastic syndromes or myeloproliferative neoplasms, conditions with genetic instability.

Case report: We report a case of acquired EPP in association with hematological disease. We followed erythrocyte PpIX concentration over a year and measured PpIX fluorescence in individual erythrocytes in a blood sample from the case using flow cytometry. The major proportion of erythrocytes did not fluoresce (84%), whereas 13% contained low PpIX fluorescence, 1% contained medium fluorescence, and 2% contained high fluorescence.

Discussion: Our observation of the very skewed PpIX distribution in erythrocytes supports the description that acquired EPP is caused by a somatic mutation effecting a clone of hematopoietic cells.

1. Introduction

Erythropoietic protoporphyria (EPP) is a rare genetic photodermatosis caused by loss-of-function mutations in the gene for ferrochelatase (FECH) [1]. FECH is the last enzyme in the heme biosynthetic pathway converting protoporphyrin IX (PpIX) into heme [1]. Reduced activity of FECH in erythroid cells in the bone marrow results in accumulation of PpIX in the cells and in the mature erythrocytes entering the bloodstream [1,2]. PpIX is transferred to the skin by exposure to light, causing severe photosensitivity [1,3].

The mutation in FECH is most often an inherited mutation. Although the mutation is present at birth many EPP patients do not have symptoms in early childhood, as the PpIX accumulation increases through childhood and adolescence for unknown reasons [4]. Thus, EPP with late onset of symptoms may be inherited but can also be acquired. In very rare cases EPP are due to an acquired somatic mutation in the FECH gene seen in association with myelodysplastic syndromes or myeloproliferative neoplasms, both conditions with genetic instability [5,6]. In one case the somatic FECH mutation was found in a subclone of cells compromising 30% of the nucleated bone marrow cells [5]. A new case of acquired EPP is presented in this report.

By extraction method a series of mean value of PpIX in erythrocytes from the case was followed over a year and during hematological treatment. Using flow cytometry, the PpIX level in each individual erythrocyte can be determined. We aimed to investigate the distribution of PpIX in individual erythrocytes in blood from a case with acquired EPP.

2. Case report

A 48-year-old woman was referred to dermatology during the spring of 2020 due to new-onset photosensitive skin. The past year the patient had experienced painful skin reactions, erythema, edema, and blistering upon exposure to sunlight. The patient was diagnosed with autoimmune hypothyroidism in 2017 but has otherwise previously been in good health and had no family history of photosensitivity. A blood test from May 2020 showed elevated metal-free PpIX in erythrocytes of 25.0 µmol/L (normal range 0–0.5 µmol/L) and zinc PpIX of 3.8 µmol/L (normal range 0–1.5 µmol/L) suggesting a diagnosis of EPP. Hemoglobin concentration (6.5 mmol/L, normal range 7.3–9.5 mmol/L) and platelet

* Corresponding author at: Department of Dermatology, Copenhagen University Hospital - Bispebjerg and Frederiksberg, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark.
E-mail address: ida.marie.heerfordt@regionh.dk (I.M. Heerfordt).

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count (150 × 10^9/L, normal range 165–390 × 10^9/L) were low. White cell count, serum alanine transaminase, and aspartate transaminase tests were normal. Genetic examination of peripheral blood leukocytes found a heterozygous mutation in exon 4 (364C>T) in the FECH gene. The mutation has previously been reported in EPP patients [2]. The case did not carry the low-expression variant allele IVS3–48C in peripheral blood. After evaluation of a bone marrow biopsy the patient was diagnosed with acquired erythropoietic protoporphyria associated with clonal cytopenia of undetermined significance in the fall of 2020. The patient was followed monthly. In November 2020 the patient started treatment with the methyltransferase inhibitor, azacitidine (AZA) by hematology from November 2020 to February 2021. PpIX accumulation and photosensitivity almost resolved in the months after AZA treatment (see Fig. 1). During the late summer of 2021, PpIX began to accumulate again in patients’ erythrocytes and photosensitivity returned.

3. Materials and methods

Metal-free PpIX fluorescence in individual erythrocytes were examined in two blood samples from the reported patient with acquired EPP using flow cytometry. The first blood sample was collected in October 2020 before AZA treatment. The second blood sample was collected in May 2021 after AZA treatment. In addition, a blood sample was examined from one non EPP patient attending routine biochemical control at Department of Dermatology, Bispebjerg Hospital during the fall of 2020. Blood samples were analysed in a FACS Aria III (Becton Dickinson, Franklin Lakes, NJ, USA). Excitation wavelength was set at maxima at 407 nm. Emission was measured at maxima at 711 nm corresponding to the spectral features of PpIX. Data was analyzed using FlowJo software version 10 (FlowJo, Ashland, OR, USA). PpIX fluorescence was divided in 4 categories; (i) negative (PpIX fluorescence <10); (ii) low (PpIX fluorescence 10–200); (iii) medium (PpIX fluorescence 200–400); and (iii) high (PpIX fluorescence >400). A minimum of 8000 erythrocytes was examined from each blood sample. In addition, mean metal-free PpIX in erythrocytes of the case was monthly analyzed by high pressure liquid chromatography (HPLC) and subsequently quantified fluorometrically by comparison to a PpIX standard measuring the mean erythrocyte PpIX concentration [4]. Written informed consent was obtained from the patient to publish this case report.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Acquired EPP, before treatment</th>
<th>Acquired EPP, after treatment</th>
<th>Non EPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean erythrocyte PpIX concentration (µmol/L)</td>
<td>22</td>
<td>1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Erythrocytes with low fluorescence level (%)</td>
<td>13%</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>Erythrocytes with medium fluorescence level (%)</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Erythrocytes with high fluorescence level (%)</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Overall, proportion of fluorescent erythrocytes (%)</td>
<td>16%</td>
<td>2%</td>
<td>0%</td>
</tr>
</tbody>
</table>

4. Results

Before AZA treatment most erythrocytes from the patient with acquired EPP contained no fluorescence (84%), whereas 13% contained low fluorescence, 1% contained medium fluorescence, and 2% contained high fluorescence. The PpIX distribution in erythrocytes appeared skewed. Results on PpIX distribution in individual erythrocytes are presented in Table 1. After the AZA treatment the number of fluorescent cells decreases substantially in the patient with acquired EPP. Fluorescence negative erythrocytes accounted for 98%, 2% contained low fluorescence, and no cells contained medium or high fluorescence. Erythrocytes from the non EPP healthy patient did not fluorescence. Results on monthly mean PpIX erythrocyte concentration are presented in Fig. 1.

5. Discussion

Worldwide, a total of 20 cases with acquired EPP have previously been reported [6]. We here describe a new case of acquired EPP where...
the hematological disease was diagnosed following presentation of cutaneous symptoms.

Inherited EPP is caused by affected function of both \textit{FECH} alleles: One allele with a severe \textit{FECH} mutation in combination with a low-expression variant allele \cite{1,2}. A severe \textit{FECH} mutation makes the allele incapable of producing functional \textit{FECH} enzyme. The low-expression variant allele produces reduced amounts of functional \textit{FECH} enzyme. In peripheral blood, our case has only affection of one \textit{FECH} allele with a severe mutation. In accordance with our case, cases with acquired EPP present PpIX accumulation and photosensitivity due to a single severe \textit{FECH} mutation in combination with a normal \textit{FECH} allele \cite{7,10}. There may be several reasons why it takes only a single mutation to accumulate PpIX in acquired EPP in association with a myeloid neoplasm for example, e.g. more acquired mutations in the unstable clones.

We report the first investigation of the PpIX distribution in erythrocytes in a case with acquired EPP. Our observation of the very skewed PpIX distribution in erythrocytes supports the explanation that acquired EPP is caused by a somatic mutation affecting a clone of hematopoietic cells.

The case of acquired EPP were treated with AZA which resolved PpIX accumulation and photosensitivity for a while (Fig. 1) indicating that the \textit{FECH} mutated clone was reduced substantially but not completely eradicated. It seems that especially the sickest erythroid cells that produced erythrocytes with high PpIX level were damaged by AZA (see Table 1). The latest PpIX measurement (see Fig. 1), however, suggests a resurgence of the porphyria clone. Our results propose that measurement of erythrocyte PpIX concentration can be used to follow treatment effect and recurrence of acquired EPP.

Previously, 3 cases of EPP associated with myelodysplastic syndromes have been treated with AZA after which skin photosensitivity resolved \cite{6,8,9}. A different case experienced spontaneous remission of PpIX accumulation which was explained with possible replacement of the porphyria clone by another myelodysplastic clone \cite{10}. Otherwise substantial decrease in PpIX accumulation in EPP patients has only been reported during pregnancy \cite{4,11}.

**Declaration of Competing Interest**

None declared.

**Acknowledgements**

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**References**

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