

# Iron in erythropoietic protoporphyrias: Dr. Jekyll or Mr. Hyde?

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## Article Info

### Article Notes

Received: April 16, 2017

Accepted: June 02, 2017

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### Keywords

Erythropoietic protoporphyria

Anemia

Iron supplementation

Heme biosynthesis

Porphyrin synthesis

Regulation

Liver disease

## ABSTRACT

Erythropoietic protoporphyria consists of two different genetic diseases, erythropoietic protoporphyria (EPP) and X-linked erythropoietic protoporphyria (XLEPP). Both of them are often accompanied by iron deficiency. Iron supplementation appears to be beneficial in XLEPP, although the clinical experience until to date is limited. In EPP, iron supplementation is discussed ambiguously and may cause harm in the majority of cases.

This minireview summarizes the limited knowledge on the connections of iron metabolism to regulation of porphyrin and heme synthesis and the influence these regulations may have on disease severity in the protoporphyrias. Further, we propose clinical guidelines, how to manage iron deficiency in both XLEPP and EPP.

## Introduction

Two inborn errors of heme biosynthesis, erythropoietic protoporphyria (EPP, OMIM #177000) and X-linked erythropoietic protoporphyria (XLEPP, OMIM #300752) lead to accumulation of protoporphyrin IX (PPIX) during erythropoiesis<sup>1</sup>. EPP is caused by inactivating mutations in the gene for the last enzyme of heme biosynthesis, ferrochelatase (FECH). FECH catalyzes the incorporation of ferrous iron into protoporphyrin IX (PPIX) to produce heme.

XLEPP is caused by activating mutations in the aminolevulinatase synthase 2 (ALAS2) gene<sup>2</sup>. ALAS2 catalyzes the conversion of glycine and succinyl-CoA to delta-aminolevulinic acid - the exclusive heme precursor in the initial and rate-limiting step of heme biosynthesis during erythropoiesis. In both disorders, PPIX accumulates in the body and leads to acute and severely painful phototoxic reactions of the skin upon exposure to light, both sunlight and artificial light. Excess PPIX is excreted through the liver and cause damages to hepatocytes and cholangioles. In 2-5% of the patients, the cholestatic liver damage is so severe that liver transplantation - preferentially together with bone marrow transplantation to prevent relapse by ongoing PPIX overproduction in the native erythropoietic tissue - is needed to save patients' lives<sup>3,4</sup>.

The clinical description of EPP was first made by Magnus et al. in 1961<sup>5</sup> and its unique inheritance was however resolved 40 years later by Gouya et al.<sup>6</sup>. In over 95% of the patients, a combination of a loss-of-function mutation in the *FECH* gene with a splice altering single nucleotide polymorphism (SNP) c.315-48C *in trans* reduces

the enzyme activity to below a critical threshold of 35% and leads to *overt* disease. Homozygous or compound heterozygous mutations are rare<sup>7</sup>.

EPP patients frequently show signs of iron deficiency e.g. a mild microcytic, hypochromic anemia and low serum iron, low transferrin saturation and ferritin and a slightly elevated zinc-protoporphyrin<sup>8-13</sup>. Female patients, who are physiologically more likely to develop iron deficiency, tend to have lower PPIX levels than male patients<sup>13,14</sup>.

In 2008, gain-of-function mutations affecting the first enzyme of heme biosynthesis, ALAS2, were found responsible for a second form of protoporphyria, XLEPP<sup>2</sup>. The mutations result in an over activity of ALAS2 and in turn, leads to accumulation of PPIX and to a similar extent, also zinc-protoporphyrin (ZnPP). XLEPP is suspected in patients with both high PPIX and high ZnPP concentration in the erythrocytes, and the diagnosis has to be confirmed by genetic testing. Patients with XLEPP also show signs of iron deficiency<sup>15</sup>. In XLEPP, the amount of PPIX accumulation is on the average more pronounced and liver damage is more frequent compared to EPP<sup>15</sup>.

The prevalence of protoporphyria is 1:100.000 – 1:200.000 in Europe and the US, 2- 10% of all cases are XLEPP<sup>15</sup>. Before 2008, XLEPP was included in the statistic of EPP, as the two diseases are clinically indistinguishable.

### Ambivalent experiences with iron substitution

As patients of both types of protoporphyria show signs of an iron deficient anemia<sup>8</sup>, iron substitution appears to be an obvious therapeutic approach for treatment of both anemia and possibly even the photosensitivity. The hypothesis in this approach is that substituted iron, as a co-substrate of FECH, may be able to deplete excess amount of PPIX to form heme. The clinical experience however is ambiguous: Iron substitution was shown to indeed increase hemoglobin and decrease photosensitivity in some cases and was even able to reverse liver damage<sup>16,17</sup>. In the majority of published case reports however, iron substitution was accompanied by an increase in photosensitivity and / or PPIX levels<sup>18-20</sup>.

The ambiguity is illustrated by a peculiar case with severe iron deficiency<sup>21,22</sup>, who prior to iron supplementation exhibited a PPIX concentration of 18  $\mu\text{mol/L}$ . Oral iron substitution improved the photosensitivity and had initially no influence on his PPIX levels, but later under intermittent oral iron PPIX concentrations varied between 30-40  $\mu\text{mol/L}$ , which again dropped to 21.4 comparable to the initial value after intensified intravenous iron therapy. The general well-being improved each time by intensified iron therapy (seasonal fluctuations of protoporphyrin concentrations which may be significant<sup>5</sup> have not been discussed in this publications).

In contrast, our study on longitudinal data of three EPP-patients found that iron availability (as assessed by hemoglobin levels) positively correlated with the amount of PPIX in the individual patient. In two patients, iron substitution increased liver damage as seen by an increase in liver transaminases, which led to discontinuation of the treatment<sup>23,24</sup>. In the same direction points the observation of lower PPIX levels in females than in males, the former being more prone to iron deficiency<sup>13</sup>. Additionally, the majority of EPP-patients under our care, who received iron supplementation at some point in their life from their family doctor or other physicians for suspected iron deficiency, informally reported worsening of photosensitivity after iron substitution. How can iron substitution in protoporphyria be benevolent and maleficent at the same time, comparable to characters in the famous novel of Robert Louis Stevenson “Strange Case of Dr. Jekyll and Mr. Hyde”<sup>25</sup>?

### Beneficial effects of iron substitution in XLEPP

In XLEPP, the overproduction of PPIX is caused by the overly active rate-limiting enzyme ALAS2, while the activity of the last enzyme, FECH, is normal. Human FECH is able to use iron and, with a lesser affinity, zinc, as substrates. Overly active ALAS2 increases one of the substrates of FECH reaction, PPIX. The other substrate, iron, might become depleted and the second best substrate, zinc, is used. Substitution of iron therefore might help to further reduce the pool of metal free PPIX, thereby reducing phototoxic reactions, at the same time reducing the liver damage by PPIX and further correct iron-deficiency including anemia. In our experience with three XLEPP cases from our Swiss porphyria center, iron substitution indeed reduced PPIX and light sensitivity and improved general health (unpublished results). A recent case report described a boy with confirmed XLEPP: his PPIX dropped from 128  $\mu\text{mol/l}$  (reference <0,09) at the time of diagnosis to between 20 to 40  $\mu\text{mol/l}$  under oral iron substitution<sup>26</sup>. The authors also raise the question, whether case reports regarding beneficial iron substitution published before the discovery of XLEPP in 2008 might contained this subgroup of protoporphyria. The current knowledge is that iron is only beneficial for patients with XLEPP, however the pathophysiology of the iron deficiency and the long-term effects of iron substitution, such as a possible iron overload, are still unknown.

### Adverse effects of iron substitution in EPP

As stated above, the role of iron supplementation is more ambivalent for EPP and our group was intrigued by our clinical observations that iron supplementation increased photosensitivity, the amount of PPIX and even adversely affected liver parameters in EPP patients. A potential explanation of this unexpected findings is the fact that the expression of the rate-limiting enzyme in erythropoietic

heme synthesis, ALAS2, connects iron to heme metabolism<sup>27</sup>: The mRNA of ALAS2 carries an iron-responsive element (IRE) at its 5' untranslated region (UTR). Under low iron condition, iron-responsive protein 1 or 2 (IRP1/ IRP2) bind to these IREs and thereby prevent translation of ALAS2 mRNA into active enzyme. This process adapts heme precursor synthesis to iron availability.

Our investigations support an effect of enhanced ALAS2 expression on the rate of heme precursor and specifically PPIX synthesis in EPP, as we found elevated ALAS2 mRNA and protein concentrations in peripheral blood samples of patients. Moreover, *in-vitro* FECH inhibition was accompanied by induced ALAS2 mRNA expression in cultures of erythroleukemic K562 cells<sup>24</sup>. And, thirdly, we expect that the regulation of the porphyrin biosynthesis in EPP behaves similarly to the pathophysiologically related congenital erythropoietic porphyria (CEP, OMIM #263700). In these condition, elevated ALAS2 expression exacerbates porphyrin intermediates production and disease severity, which can be improved by iatrogenic-induced iron deficiency<sup>28,29</sup>. Therefore, our hypothesis is that the translation of increased expressed ALAS2 mRNA into enzyme protein is stimulated by an unlimited iron availability and that this mechanism accounts for the increase in PPIX after iron supplementation that we observed in the EPP patients.

On the one hand, slight iron deficiency apparently protects against exacerbated phototoxic symptoms. On the other hand, iron is necessary for the expression and stability of FECH: The enzyme carries a FeS-Cluster, which stabilizes the newly translated protein and is necessary for the enzymatic function<sup>30,31</sup>. Under iron depleted condition, the half-life of FECH was shown to be reduced in pulse chase and cell culture experiments<sup>32,33</sup>. In addition, iron deficiency increases the usage of the aberrant splice acceptor site in FECH intron 3 (c.315-63), reducing the amount of correctly spliced FECH mRNA and protein<sup>34</sup>. This aberrant acceptor splice site also happens to be activated by the disease defining SNP as discussed earlier<sup>6</sup>. Indeed, EPP-patients with replete iron stores as defined by a normal serum ferritin, show normal hemoglobin concentrations<sup>13</sup> indicating that iron availability, rather than FECH activity, is the limiting factor in the heme synthesis of EPP patients. EPP patients with normal hemoglobin also exhibit normal iron stores as accessed by ferritin<sup>13</sup>. Therefore, the question remains unsolved, why are many EPP patients iron deficient?

### The fate of iron in EPP

As no controlled mechanism to excrete iron is known, iron homeostasis is thought to be regulated by its absorption only. The observed iron deficiency in protoporphyrias is either caused by a block in intestinal

absorption or an unidentified mechanism of iron loss. The hepatic peptide hepcidin is considered to be the master regulator of iron metabolism and controls intestinal iron absorption by degradation of the iron transporter protein ferroportin—thereby preventing dietary iron uptake<sup>35</sup>. Hepcidin is upregulated in iron overload, infection and chronic disease. In addition, hepcidin hinders the export of iron from its stores such as macrophages to the circulation. The question arose whether the iron deficiency in both EPP and XLEPP is due to an inadequately elevated hepcidin, similar to other chronic diseases. Bossi et al. investigated iron uptake in a group of EPP patients with normal iron status and found no block of intestinal absorption and normal hepcidin expression in urine and serum<sup>12</sup>. However, the patients selected by Bossi et al. had a normal iron status without prior supplementation and therefore in our opinion, do not qualify to investigate the pathophysiology of iron deficiency in EPP. In our own investigation, a cohort of 67 EPP patients, which comprised both anemic and not-anemic individuals, exhibited a significantly decreased hepcidin concentration compared to healthy volunteers<sup>13</sup>. The results of both studies finally excluded an inadequately elevated hepcidin to be responsible for the observed iron deficiency in EPP patients.

Bossi et al. further investigated in the same iron-replete EPP-subgroup the expression of erythropoietin and of the serum cytokines G-CSF, IFN, IL-8, MCP-1, MIP-1b or TNF- $\alpha$ , which all did not differ from the control group. However, the same limitation as mentioned above applies.

The main conundrum about the observed iron deficiency remains, why do EPP patients show iron deficiency? In this respect it is interesting to mention that inactivating mutations of ALAS2 in sideroachrestic anemia (OMIM #300751) lead to iron accumulation, whereas activating mutations in XLEPP or an increased expression of ALAS2 in EPP lead to iron deficiency. We wonder whether this facts point to a direct physiological link between ALAS2 expression and iron metabolism by a hitherto unidentified feedback regulation.

### Proposed clinical guidelines for the management of iron deficiency in the protoporphyrias

Iron is an essential factor for human life, but toxic in excess. In both types of protoporphyria, all studies describe an increased frequency of iron deficiency. The current knowledge is that iron supplementation is beneficial for patients with XLEPP and our guideline includes a generous iron substitution based on general health, anemia and ZnPP-concentrations.

In EPP, we prefer to apply iron supplementation only to patients with severe iron deficiency, especially those suffering from severe fatigue, low ferritin and microcytic and hypochromic anemia with a hemoglobin concentration

of below 10g/dL. During iron supplementation, preferably on small doses and applied during seasons with short daylight, the patients are monitored closely for their PPIX concentrations and signs of liver damage. Current trials on iron supplementation in both XLEPP and EPP<sup>36</sup> will help to further clarify the pathophysiology of the iron deficiency and the effect of iron substitution on symptoms, PPIX-concentration and hemoglobin synthesis.

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