ERYTHROPOIESIS AND IRON METABOLISM IN DOMINANT
ERYTHROPOIETIC PROTOPORPHYRIA

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ABSTRACT

Erythropoietic protoporphyria (EPP) results from deficiency of ferrochelatase (FECH). Accumulation of protoporphyrin IX causes life-long acute photosensitivity. Microcytic anemia occurs in 20 – 60% of patients. We investigated 178 patients with dominant EPP confirmed by molecular analysis. Erythropoiesis was impaired in all patients; all had a downward shift in haemoglobin (Hb), the mean being decreased in males by 1.2g/dL. By WHO criteria, 48% of women and 33% of men were anemic. Iron stores, assessed by serum ferritin (sFn), were decreased by two-thirds but normal serum soluble transferrin receptor-1 and iron concentrations suggested that erythropoiesis was not limited by iron supply. FECH deficiency in EPP appears to lead to a steady state in which decreased erythropoiesis is matched by reduced iron absorption and supply. This response may in part be mediated by protoporphyrin but we found no correlation between erythrocyte protoporphyrin and Hb, sFn, total iron binding capacity or transferrin saturation.
INTRODUCTION
Erythropoietic protoporphyria (EPP, MIM 177000) is an inherited disorder caused by partial deficiency of ferrochelatase (FECH; EC 4.99.1.1) which catalyses the chelation of ferrous iron by protoporphyrin IX. FECH deficiency leads to accumulation of protoporphyrin in normoblasts, erythrocytes, plasma, skin and liver, causing lifelong acute photosensitivity and, in about 2% of patients, severe liver disease\textsuperscript{1,2}.

Microcytic anemia occurs in 20 – 60% of patients\textsuperscript{3-6}. In contrast to other inherited disorders of erythroid heme biosynthesis,\textsuperscript{7} the anemia is not dyserythropoietic, there is no iron overload, and there is evidence for iron-deficiency\textsuperscript{5,6,8,9}, without iron loss\textsuperscript{9}. A mouse model of EPP, the homozygous \textit{Fech}\textsuperscript{m1Pas} mutant, develops a similar microcytic anemia\textsuperscript{10,11}. Although it is probable that the anemia of EPP reflects limitation of heme formation by FECH deficiency, its incidence, mechanism and relationship to disordered iron metabolism remain unclear.

SUBJECTS AND METHODS
Patients and control subjects
Blood samples were obtained from 210 patients with EPP during a cross-sectional study of EPP in the United Kingdom\textsuperscript{12}. 192 patients had one \textit{FECH} mutation with one or two \textit{FECH IVS3-48C} alleles and were classified as dominant EPP (dEPP)\textsuperscript{13}; 14 of these were excluded because they had diseases likely to affect iron metabolism. Ethical approval was obtained from the North West Multicentre Research Ethics Committee and 84 local research ethics committees. All patients or their parents gave informed consent.
Hematological and biochemical measurements

All analyses were carried out in the same laboratory. Serum iron (sFe)\textsuperscript{14}, total iron binding capacity (TIBC)\textsuperscript{14}, serum ferritin (sFn) (Elecsys 2010, Roche Diagnostics, IN, USA), soluble transferrin receptor-1 (sTfR) (R&D Systems, Abingdon, UK) and erythrocyte protoporphyrin\textsuperscript{15} were determined as described. Other measurements were by standard automated methods. Data obtained previously for 611 male first time blood donors were used for comparisons\textsuperscript{16}.

Statistical methods

Results were expressed as mean ± SD for normally distributed data and median and range for data (sFn, protoporphyrin) with a log-normal distribution. Differences between quantitative variables were assessed by the Mann-Whitney test. Spearman rank correlation (r_s) was used to test the significance of relationships between pairs of variables and the chi-square test for differences between proportions.

RESULTS AND DISCUSSION

Red cell indices

By WHO criteria, 73 (41%; 95% CI: 34 – 48%) of our patients with dEPP were anemic. All had a mild microcytic, hypochromic anemia; 48% of females and 33% of males being affected. The anemic patients did not form a separate subgroup. In both sexes, hemoglobin (Hb) (females: 11.9 ± 1.0 g/dL; males: 13.3 ± 1.0), mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) were normally distributed with a shift in their
means towards lower values (Table S1; supplemental data); the mean Hb for males being 1.2 g/L lower than in the general population (Fig 1A). A similar shift has been noted in Dutch EPP patients\textsuperscript{17}. This downward shift in Hb leads to some patients falling within the definition of anemia; part of the wide variation in reported incidences can be explained by use of different definitions\textsuperscript{2-6,9}. Erythrocyte protoporphyrin concentrations (females: 21.9, 4.1 – 75.3 µmol/L; males: 25.5, 8.9 – 77.3 µmol/L) showed no correlation with Hb.

FECH activity in dEPP is about 35% of normal\textsuperscript{13}. Our data show that this decrease is sufficient to produce in all patients a mild defect of erythropoiesis that impairs hemoglobinisation. Defective erythropoiesis persists throughout life and our findings (supplemental data, legend to table S1) and previous reports\textsuperscript{8,18} suggest that it may not be corrected by oral iron unless there is evidence of co-existing iron loss.

**Iron status**

Both sexes showed evidence of iron depletion (Table S2; supplemental data). Differences in sFn and transferrin saturation (TS) between women and men suggested that more of the former had iron depletion due to iron loss in addition to abnormalities caused by EPP. Therefore, we restricted detailed analysis of iron indices to the 67 male patients (Hb 13.5 ± 0.9 g/dL) aged over 15 years who had never received iron supplements.

The main abnormality was a marked shift in sFn towards lower values (Fig 1B) (Table 1); sFn correlated with Hb (r\textsubscript{s} = 0.415; p < 0.001). Because protoporphyrin is hepatotoxic and accumulates in the liver in EPP, and liver cell damage may increase sFn,
we assessed liver cell function by measuring liver enzymes. One or more of these was increased in 17 (25%) patients; sFn correlated with γ-glutamayl transpeptidase ($r_s = 0.507; P < 0.001$) and alanine aminotransferase ($r_s = 0.392; P < 0.001$) but not with aspartate aminotransferase. Since sFn correlates with mobilisable iron stores $^{19}$, the downward shift in sFn by about two-thirds (Fig 1B) (Table 1) suggests that iron stores in dEPP are decreased to a similar extent or a little more if the effect of liver dysfunction is taken into account. Turnbull et al. $^8$ found that storage iron, determined by venesection, was less than 250 mg in 3 patients; otherwise, quantitative measurements of tissue iron have not been reported in EPP. However, in contrast to iron deficiency due to iron loss, stainable iron is present in erythroblasts $^9$.

In homozygous $Fech^{mlPas}$ mice, total body iron is normal but iron is redistributed from peripheral tissues to an enlarged hematopoietic spleen $^{11}$. Though these mice have liver disease, lower FECH activity and more severe anaemia $^{11}$, it seems unlikely that FECH deficiency limits erythropoiesis and disturbs iron metabolism by different mechanisms in the two species. The anomalous observation in EPP of accumulation of iron in erythroblasts $^9$ suggests that there may also be redistribution of iron stores towards the site of erythropoiesis in EPP. Thus, in both species, FECH deficiency appears to provoke a response that leads to accumulation of protoporphyrin IX but prevents accumulation of the other, more toxic, substrate, iron.

A second notable feature of iron depletion in dEPP is our finding that sFe (Table 1) and sTfR ($18.6 \pm 5.1$ nmol/L) (Fig 1C) concentrations are normal. The normal sTfR in our patients is consistent with the degree of depletion of iron stores indicated by sFn and,
together with the normal sFe, suggests that erythropoiesis is not limited by iron supply. This indicates that the reduction in iron stores has not led to iron deficient erythropoiesis. Furthermore, the rate of erythropoiesis is not increased as this would also increase sTfR levels. These findings suggest FECH deficiency in dEPP leads to the establishment of a steady state in which iron absorption and supply is diminished but matches the requirement for reduced erythropoiesis.

The mechanism of these changes in iron metabolism has not been established. Iron metabolism is also altered in griseofulvin-induced protoporphyria. Because serum transferrin is increased in \textit{Fech}^{m1Pas} BALB/c mice and correlates with erythrocyte protoporphyrin concentration, it has been suggested that protoporphyrin may act as a signal to increase hepatic transferrin synthesis when iron supply to erythroid cells is insufficient and thus modulate iron metabolism. We found only a slight increase in TIBC (Table 1) and no correlation with erythrocyte protoporphyrin. Alternatively, FECH deficiency within enterocytes might affect duodenal iron transport by altering enterocyte mitochondrial iron status.

Finally, measurement of sTfR, in addition to sFn, may help to distinguish those patients in whom the anemia of EPP is exacerbated by iron loss and who might benefit from iron replacement.
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S.A.H collected the clinical data and patient samples; M.W and M.N.B. supervised laboratory analyses; A.V.A. supervised patient contact and clinical aspects; G.H.E and M.W. wrote the paper; all authors participated in the design of the research and checked the final version of the manuscript.

The authors declare no competing financial interests.
REFERENCES


Figure 1. Hemoglobin, serum ferritin and serum soluble transferrin receptor-1 concentrations in male patients with dominant EPP.

(A) Hemoglobin concentrations in 66 male patients with dEPP aged 16 or over (■) and in a sample of 5206 men aged 16 or over from the English population^24 (□).

(B) Serum ferritin concentrations in 66 male patients with dEPP aged 16 or over (■) and in 612 male first-time blood donors from south Wales aged 17 – 62 years^16 (□).

(C) Serum soluble transferrin receptor-1 concentrations in 61 male patients with dEPP aged 16 or over (18.6 ± 5.1 nmol/L) (■) and in 225 hematologically normal male and female subjects from the United States aged 17 – 97 years^25 (□) assayed using the same method.
Figure 1 (A)
Figure 1 (B)
Figure 1 (C)
Table 1. Comparison of indicators of iron status in male patients with dEPP and male first-time blood donors

<table>
<thead>
<tr>
<th>Serum iron indices</th>
<th>EPP patients (n = 67)</th>
<th>First-time blood donors (n = 611)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sFe (µmol/L)</td>
<td>15.1 ± 6.6&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>16.7 ± 6.0</td>
</tr>
<tr>
<td>TIBC (µmol/L)</td>
<td>63.0 ± 6.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>54.5 ± 10.0</td>
</tr>
<tr>
<td>TS (%)</td>
<td>23.9 ± 10.3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>31.1 ± 10.9</td>
</tr>
<tr>
<td>sFn (µg/L)</td>
<td>37 (10 – 119)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>101 (35 – 220)</td>
</tr>
</tbody>
</table>

Figures are means ± SD or, for sFn, medians and 95% ranges. EPP patients are males aged 16 – 77 years who have never been prescribed iron supplements. Blood donors are male, first time donors aged 17 – 62 years from South Wales<sup>15</sup>; samples for analysis were obtained prior to first donation. *P < 0.001 compared with donors; ns not significant. Only TIBC showed any correlation with sFn ($r_s = -0.412$, $P < 0.001$).
Erythropoiesis and iron metabolism in dominant erythropoietic protoporphyria

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