Erythropoietic protoporphyria

CAUTON, CHUA, DOMINGO, ROSUMAN, VILLENA
What is EPP?

- disease of the porphyrin metabolism
- abnormally raised levels of protoporphyrin IX
  - erythrocytes, feces and plasma
- sensitivity to visible light
Incidence

- Third most common porphyria
- 2 to 5 per 1,000,000
  - most common in children
Cause

- Mutation of ferrocheletase gene
  - autosomal recessive
  - controls production of ferrocheletase
    - catalyze the insertion of ferrous into protoporphyrin in the heme synthesis pathway
Symptoms

- Burning sensation in skin
- Erythema
- Edema
Diagnosis

- Blood and stool test
  - check levels of protoporphyrin
  - use fluorescence microscope
    - red fluorescing erythrocytes
- Skin sample test
  - use light microscope
  - amorphous homogenous substance
Normal Heme Synthesis Pathway

• heme synthesis primarily takes place in:
  > bone marrow
    - ~80-85%
    - need heme for RBCs

  > liver
    - ~15%
    - need heme for the synthesis of other proteins (such as cytochrome P450)
• in mitochondria
• 1 succinyl CoA + 1 Glycine \( \rightarrow \) 1 ALA
• enzyme: ALA synthase
• cofactor: vitamin B6
• succinyl CoA is from TCA cycle
• in cytosol
• 1 ALA + 1 ALA → 1 porphobilinogen
• enzyme: ALA dehydrogenase
• 4 porphobilinogens cyclyze → 1 HMB
• enzyme: porphobilinogen deaminase
- HMB $\rightarrow$ URO III
- enzyme: URO synthase
- URO III → Copro III
- acetate side chains replaced with methyl groups
- enzyme: URO decarboxylase
• in mitochondria
• 2 propionate side chains replaced with vinyl groups
• Copro III $\rightarrow$ Protoporphyrinogen IX
• enzyme: copro-oxidase
- 4 hydrogens leave; remaining 2 propionate side chains replaced with ?
- Protoporphyrinogen IX → Protoporphyrin IX
- enzyme: proto-oxidase
- ferrous ion added
- Protoporphyrin IX → heme
- enzyme: ferrochelatase
- presence of heme – negative feedback
Ferrochelatase is affected by a negative feedback loop

↑ intracellular heme

↓ ALA synthase

↓ FECH
In Erythropoietic protoporphyria, there is deficient FCH or Ferrochelatase.

There is an accumulation of protoporphyrin IX; in blood and in liver.
Protoporphyrin pathway in the body

In EPP: free PP accumulates in RBCs

PP in erythroid fluoresce red 634 nm, excitation 405 nm

PP diffuses from RBCs into plasma, binds to albumin

PP hydrophobic, transported to liver

Enters enterohepatic circulation, accumulates in hepatocytes

Excreted in feces
Protoporphyrin pathway
Difference between Heme and Protoporphyrin IX

The only difference between heme and protoporphyrin is the insertion of an iron atom into the tetrapyrrole ring in heme.

This insertion increases the stability of the structure and results in the loss of its fluorescent properties.
Fluorescent property of protoporphyrin

Has fluorescent properties due to its conjugated pi bond system:

Porphyrin derivatives contain pyrrole rings, five membered heterocycles. These compounds have properties of a conjugated diene and of an amine.

Delocalization of π electrons stabilizes the ring and their ultra-violet absorption maximum peak shifts to a longer wavelength.

They can easily absorb visible light, and thus can utilize sunlight directly as a good photosensitizer.
On blood:

Photobleaches and produces "free radical" light oxidizes all forms of biomolecules causing unwanted effects.
Singlet Oxygen on biomolecules

**On lipids:** causes lipid peroxidation, which in turns affects cellular membranes of cells and causes the hemolysis of red blood cells

**On proteins:** reacts with amino acids histidine, tryptophan, methionine and tyrosine producing sulphoxides and endoperoxides that may be toxic to other cells

**On DNA:** oxidizes with guanine and produces strand breaks

All of these are more evident on parts exposed to light (skin), which causes the acute skin **photosensitivity** - which are forms of erythema and edema on face and hands
On acute skin photosensitivity

It is “acute” because eventually all singlet oxygens and the peroxides that it generated will be “quenched” by antioxidants that are found within the human body.

However they may have already affected tissues, though this only occurs from several hours to a few days after exposure to sunlight.

Over time - this leads to hyperkeratosis of skin - showing waxy and leathery appearance.
Protoporphyrin is hydrophobic and enters the hepatobiliary system. It is released into the intestine via the bile duct.

Too much protoporphyrin can cause crystallization and blocks the bile duct - causing **cholestasis**

Protoporphyrin enters the hepatocytes and induces oxidative stress (disrupting the Na, K and ATPase pumps) - **lesser secretions**

Toxic to biliary epithelium – **fibrosis, which leads to ESLD or End-Stage Liver Disease**
ESLD (End Stage Liver Disease)

Due to high porphyrin IX content in the blood and liver and the effects of singlet oxygen;

The liver produces more bilirubin and Alk Phos which causes jaundice, malaise and abdominal pain
Other symptoms connected to ESLD and EPP

There is **splenomegaly** - due to sequestering of erythrocytes (increase in hemolysis is also an abnormal increase in erythropoiesis).

Elevated protoporphyrin in RBCs and plasma

Fecal levels of Protoporphyrin decrease
Differential Diagnosis

HEPATIC PORPHYRIA

1. Acute hepatic porphyria (4)
   a. autosomal dominant: **AIP, HCP, VP**
   b. autosomal recessive: **ADP**

1. Hepatic cutaneous porphyria or **PCT** (1)

ERYTHROPOIETIC PORPHYRIA (3)

1. Congenital erythropoietic porphyria (**CEP**)
2. Erythropoietic protoporphyrria (**EPP**)
3. X-linked Protoporphyrria (**XLP**)
Differential Diagnosis

HEPATIC PORPHYRIA
overproduction and initial accumulation of ALA and PBG
primarily in the liver
Differential Diagnosis

1. **Acute Hepatic Porphyria**
   - life-threatening acute neurologic attacks
   - steady and localized **abdominal pain**
   - constipation, abdominal distention, decreased bowel sound
   - nausea; vomiting; tachycardia; hypertension; mental symptoms; extremity, neck, or chest pain; headache; muscle weakness; sensory loss; tremors; sweating; dysuria; bladder distention
Differential Diagnosis

1. Acute Hepatic Porphyria
   a. autosomal dominant
      i. AIP (most common)          ii. HCP                      iii. VP
         - urinary PBG (using Trace PBG kit)
         - confirmation: mutation analysis
   a. autosomal recessive
      ADP (rarest)
Differential Diagnosis

2. Hepatic Cutaneous Porphyria

- also Porphyria Cutanea Tarda (PCT)
- most common among all porphyria
- symptoms manifest when URO-decarboxylase activity is 20% or less than normal
- symptoms: blistering skin lesions → atrophy and scarring, skin friability; small white papules (milia); hypertrichosis; hyperpigmentation; skin in sun-exposed areas may severely be thickened with scarring and calcification
Differential Diagnosis

2. Hepatic Cutaneous Porphyria (PCT)
Differential Diagnosis

2. Hepatic Cutaneous Porphyria (PCT)

![Urine samples under daylight and UV light comparison](image)
2. Hepatic Cutaneous Porphyria (PCT)
- ALA (slight), normal PBG
- urinary porphyrins (ex. uroporphyrins, heptacarboxylate porphyrin)
- isocoporphyrin (in feces) = URO-decarboxylase deficiency
- URO-d activity:
  - if $\frac{1}{2}$ = type 2 PCT
  - if normal = type 1 PCT
2. Hepatic Cutaneous Porphyria (PCT)
   a. **Type 1: Sporadic** - no URO-decarboxylase mutations; normal enzyme activity when asymptomatic
   b. **Type 2: Familial** - heterozygous UROD mutations (autosomal dominant); half-normal enzyme activity when asymptomatic
Differential Diagnosis

ERYTHROPOIETIC PORPHYRIA

overproduction and initial accumulation of pathway intermediates in the bone marrow erythroid cells
Differential Diagnosis

1. **Congenital Erythropoietic Porphyria (CEP)**
   - deficient, but not absent, activity of URO-synthase → resultant accumulation of uroporphyrin I and coproporphyrin I isomers
   - associated with **hemolytic anemia** and **severe cutaneous photosensitivity**
   - skin over light-exposed areas is friable; bullae and vesicles prone to rupture and infection; skin thickening, focal hypopigmentation and hyperpigmentation, hypertrichosis of face and extremities
1. **Congenital Erythropoietic Porphyria (CEP)**

   - Porphyrins deposited in **teeth** (reddish-brown and fluoresce on exposure to long-wave **UV light**) and **bones** --> secondary infection and bone resorption --> disfigurement of **face** and **hands**

   - Hemolysis
Differential Diagnosis

Marked Skin photosensitivity, Scaring, Infection & Deformation

A

Erythrodontia

B
Differential Diagnosis
Differential Diagnosis

1. **Congenital Erythropoietic Porphyria (CEP)**
   - uroporphyrin and coproporphyrin (type I isomers) accumulate in bone marrow → circulates at **plasma, urine, and feces**
   - confirmation: deficient URO-synthase activity OR identification of specific mutations in the **UROS gene**
Differential Diagnosis

2. & 3. Erythropoietic Protoporphyria (EPP) and X-linked Protoporphyria (XLP)

- autosomal recessive
- due to mutations in *FECH* gene → reduced FECH activity
- symptoms: **skin photosensitivity** (pain, redness, and itching occurring within minutes of sunlight exposure)
- **liver disease** → abdominal pain, minor liver abnormalities or liver failure
Differential Diagnosis
Differential Diagnosis

2. & 3. Erythropoietic Protoporphyria (EPP) and X-linked Protoporphyria (XLP)

- erythroid cells: **red fluorescence** (under fluorescence emission microscope, at 620 nm); **erythrocyte protoporphoryn**
- urinary porphyrins and precursors: **normal**
- confirmation: analysis of **FECH** and **ALAS2** genes
Differential Diagnosis

2. Erythropoietic Protoporphyria (EPP)
   - erythrocyte protoporphyrin are almost all **free** (not complexed with zinc), mostly bound to **hemoglobin**

3. X-linked Protoporphyria (XLP)
   - free and zinc protoporphyrin in erythrocytes
<table>
<thead>
<tr>
<th>Porphyria</th>
<th>Deficient enzyme</th>
<th>Inheritance</th>
<th>Principal symptoms, NV or CP</th>
<th>Enzyme activity, % of normal</th>
<th>Increased porphyrin precursors and/or porphyrins*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute hepatic porphyrias</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>ALA-dehydratase</td>
<td>AR</td>
<td>NV</td>
<td>~ 5</td>
<td>Zn-protoporphyrin</td>
</tr>
<tr>
<td>AIP</td>
<td>HMB- synthase</td>
<td>AD</td>
<td>NV</td>
<td>~ 50</td>
<td>ALA, coproporphyrin III</td>
</tr>
<tr>
<td>HCP</td>
<td>COPRO-oxidase</td>
<td>AD</td>
<td>NV and CP</td>
<td>~ 50</td>
<td>ALA, PBG, coproporphyrin III</td>
</tr>
<tr>
<td>VP</td>
<td>PROTO-oxidase</td>
<td>AD</td>
<td>NV and CP</td>
<td>~ 50</td>
<td>ALA, PBG, coproporphyrin III, Cooporphyrin III</td>
</tr>
<tr>
<td><strong>Hepatic cutaneous porphyrias</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCT</td>
<td>URO-decarboxylase</td>
<td>Sporadic or AD</td>
<td>CP</td>
<td>&lt; 20</td>
<td>Uroporphyrin, 7-carboxylate porphyrin</td>
</tr>
<tr>
<td><strong>Erythropoietic cutaneous porphyrias</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEP</td>
<td>URO-synthase</td>
<td>AR</td>
<td>CP</td>
<td>1-5</td>
<td>Uroporphyrin I, coproporphyrin I</td>
</tr>
<tr>
<td>EPP</td>
<td>Ferrochelatase</td>
<td>AR†</td>
<td>CP</td>
<td>~ 20-30</td>
<td>Uroporphyrin I, coproporphyrin I</td>
</tr>
<tr>
<td>XLP</td>
<td>ALA-synthase 2</td>
<td>XL</td>
<td>CP</td>
<td>~ 100†</td>
<td>Free and zinc protoporphyrin</td>
</tr>
</tbody>
</table>

* ALA = delta-aminolevulinic acid, PBG = porphobilinogen.
## Differential Diagnosis

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene symbol</th>
<th>Chromosomal location</th>
<th>cDNA, bp</th>
<th>Size, kb</th>
<th>Exons*</th>
<th>Protein, amino acid</th>
<th>Subcellular location†</th>
<th>Known mutations‡</th>
<th>3D structure§</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA-synthase</td>
<td>ALAS1</td>
<td>3p21.1</td>
<td>2199</td>
<td>17</td>
<td>11</td>
<td>640</td>
<td>M</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ALAS2</td>
<td>Xp11.2</td>
<td>1937</td>
<td>22</td>
<td>11</td>
<td>587</td>
<td>M</td>
<td>62</td>
<td>R</td>
</tr>
<tr>
<td>ALA-dehydratase</td>
<td>ALAD</td>
<td>9q32</td>
<td>1149</td>
<td>15.9</td>
<td>12 (1A + 2-12)</td>
<td>330</td>
<td>C</td>
<td>12</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>ALAD</td>
<td>9q32</td>
<td>1154</td>
<td>15.9</td>
<td>12 (1B + 2-12)</td>
<td>330</td>
<td>C</td>
<td>0</td>
<td>Y, P, E</td>
</tr>
<tr>
<td>HMB-synthase</td>
<td>HMBS</td>
<td>11q23.3</td>
<td>1086</td>
<td>11</td>
<td>15 (1 + 3-15)</td>
<td>361</td>
<td>C</td>
<td>374</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>HMBS</td>
<td>11q23.3</td>
<td>1035</td>
<td>11</td>
<td>15 (2-15)</td>
<td>344</td>
<td>C</td>
<td>2</td>
<td>E</td>
</tr>
<tr>
<td>URO-synthase</td>
<td>UROS</td>
<td>10q26.2</td>
<td>1296</td>
<td>34</td>
<td>10 (1 + 2B-10)</td>
<td>265</td>
<td>C</td>
<td>35</td>
<td>H, T, S</td>
</tr>
<tr>
<td></td>
<td>UROS</td>
<td>10q26.2</td>
<td>1216</td>
<td>34</td>
<td>10 (2A + 2B-10)</td>
<td>265</td>
<td>C</td>
<td>4</td>
<td>H, A, N, B, F</td>
</tr>
<tr>
<td>URO-decarboxylase</td>
<td>UROD</td>
<td>1p34.1</td>
<td>1104</td>
<td>3</td>
<td>10</td>
<td>367</td>
<td>C</td>
<td>109</td>
<td>H, Y, E, L, D</td>
</tr>
<tr>
<td>COPRO-oxidase</td>
<td>CPOX</td>
<td>3q12.1</td>
<td>1062</td>
<td>14</td>
<td>7</td>
<td>354</td>
<td>M</td>
<td>64</td>
<td>H, Y, E, L, D</td>
</tr>
<tr>
<td>PROTO-oxidase</td>
<td>PPOX</td>
<td>1q23.3</td>
<td>1431</td>
<td>5.5</td>
<td>13</td>
<td>477</td>
<td>M</td>
<td>166</td>
<td>H, B, N, X, ES</td>
</tr>
<tr>
<td>Ferrochelatase</td>
<td>FECH</td>
<td>18q21.31</td>
<td>1269</td>
<td>45</td>
<td>11</td>
<td>423</td>
<td>M</td>
<td>138</td>
<td>H, Y, B</td>
</tr>
</tbody>
</table>
Treatment & Management

Table 2
Targets for Intervention

- Increase protoporphyrin excretion into bile: ursodeoxycholic acid
- Reduce protoporphyrin production: hematin and red blood cell transfusion
- Decrease the circulating pool of protoporphyrin
  - Removal: plasmapheresis, hemodialysis, and exchange transfusions
  - Interrupting enterohepatic circulation: cholestyramine and activated charcoal
- Use antioxidants or cytoprotective agents: vitamin E and ursodeoxycholic acid
- Perform liver transplantation
- Correct the underlying defect: bone marrow transplantation

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3096108/table/T2/
### Treatment & Management

1. **Increased protoporphyrin excretion**
   - Protoporphyrin is water-insoluble and therefore removed from the body only through hepatic excretion into bile or feces
   - **Ursodeoxycholic acid** - enhance the biliary excretion of protoporphyrin; cytoprotective properties

2. **Reduce protoporphyrin production**
   - Bone marrow
   - **Hematin and erythrocyte transfusions** - suppress heme production by decreasing protoporphyrin level
Treatment & Management

3. Decrease protoporphyrin circulation
   - **Exchange transfusions and plasmapheresis** have been used to remove the protoporphyrin in transit
   - Bile-acid binding agents (**cholestyramine**) for enterohepatic circulation
   - **Long-term activated charcoal** as a safe and cheap alternative for decreasing intestinal reabsorption

4. Antioxidants or cytoprotective agents
   - **Vitamin E** was reported to be effective for reversing liver disease
   - **Ursodeoxycholic acid** - early liver disease
5. Liver transplantation
   - Liver transplant recipients from US centers have been shown to have 1-, 5-, and 10-year survival rates of 85%, 69%, and 47%, respectively.
   - Recurrent EPP was found in 65% (11/17) of patients who survived more than 2 months post-transplant.
   - The transplant itself does not correct the primary cause and major source of protoporphyrin overproduction.

6. Correction of underlying defect
   - Successful bone marrow transplantation with or without liver transplantation, depending upon the severity of the liver disease, is considered the definitive treatment for EPP.
Treatment & Management

7. For lifelong cutaneous photosensitivity
   ◦ Shield skin from sunlight
   ◦ Sun-blocking formulations containing **zinc oxide or titanium dioxide** reflect visible light and may be helpful.
   ◦ Topical sunless tanning gels or creams containing **dihydroxyacetone**
   ◦ **Afamelanotide**, an alpha-melanocyte–stimulating hormone analogue that increases melanin production in the skin
   ◦ **Oral beta-carotene** reduces photosensitivity in some
   ◦ A pilot study of the **oral enteric sorbent colestipol** found less photosensitivity in 3 subjects with EPP after its use
Treatment & Management

Increased risk of developing chronic liver disease and should be vaccinated for hepatitis A and B.

Avoid hepatotoxins such as alcohol because of the risk of accelerating their liver disease.

Monitored with liver enzymes and porphyrin levels, both serum and RBC protoporphyrin to detect early signs of liver injury.

Screened regularly for hepatocellular carcinoma.

Treatment for minimizing complications from sun damage, monitoring for development of liver disease, and stabilizing cholestatic liver disease once it develops.
References

http://nopr.niscair.res.in/bitstream/123456789/23511/1/IJEB%2040(6)%20680-692.pdf
http://www.ojrd.com/content/4/1/19
http://themedicalbiochemistrypage.org/heme-porphyrin.php#introduction
https://www.rarediseasesnetwork.org/porphyrias/patients/EPP/
http://www.porphryiafoundation.com/for-healthcare-professionals/types-of-porphyria/EPP
http://www.porphryiafoundation.com/about-porphyria/types-of-porphyria/EPP
http://upload.wikimedia.org/wikipedia/commons/3/3c/Acute_photosensitivity_reaction_in_EPP.jpg
http://dualibra.com/wp-content/uploads/2012/04/037800~1/Part%2015.%20Endocrinology%20and%20Metabolism/Section%203.%20Disorders%20of%20Intermediary%20Metabolism/352_files/loadBinary.jpg
http://www.genecards.org/cgi-bin/carddisp.pl?gene=FECH
REFERENCES

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3096108/
http://emedicine.medscape.com/article/1104061-overview#a0104