Modern diagnosis and management of the porphyrias

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Summary
Recent advances in the molecular understanding of the porphyrias now offer specific diagnosis and precise definition of the types of genetic mutations involved in the disease. Molecular diagnostic testing is powerful and very useful in kindred evaluation and genetic counselling when a disease-responsible mutation has been identified in the family. It is also the only way to properly screen asymptomatic gene carriers, facilitating correct treatment and appropriate genetic counselling of family members at risk. However, it should be noted that DNA-based testing is for the diagnosis of the gene carrier status, but not for the diagnosis of clinical syndrome or severity of the disease, e.g. an acute attack. For the diagnosis of clinically expressed porphyrias, a logical stepwise approach including the analysis of porphyrins and their precursors should not be underestimated, as it is still very useful, and is often the best from the cost-effective point of view.

Keywords: porphyria, porphyrin, haem, \(\delta\)-aminolaevulinate synthase, molecular diagnosis.

The porphyrias are uncommon, complex, and fascinating metabolic conditions, caused by deficiencies in the activities of the enzymes of the haem biosynthetic pathway. While most of them are inherited, some may also occur as acquired diseases. In addition, not all gene carriers of inherited porphyrias develop clinical disease and there is a significant interplay between the primary gene defect and the secondary acquired or environmental factors. The enzyme deficiencies can be either partial or nearly complete depending on the types of genetic mutations. Depending on deficient enzymatic steps, various porphyrins and their precursors are accumulated in tissues and are excreted in urine and/or stool. Porphyrins can be classified either as (i) erythropoietic porphryia, (ii) acute hepatic porphyria, or (iii) chronic hepatic porphyria. Both erythropoietic porphyria and chronic hepatic porphyrina accompany cutaneous photosensitivity, but they are not associated with neurological symptoms. In contrast, acute hepatic porphyrias are characterised by neurological symptoms. Some of them may have additional photosensitivity (Fig 1).

The haem biosynthetic pathway
The enzymatic steps and intermediates in the haem biosynthetic pathway are illustrated in Fig 2. In eukaryote cells, the first enzymatic step and the last three steps occur in mitochondria; the other four steps take place in the cytosol. The two major cell types that are active in haem synthesis are hepatocytes and bone marrow erythroblasts, and inherited enzymatic defects in the porphyrias are chiefly manifested in these cells.

The first intermediate of the haem biosynthetic pathway is \(\delta\)-aminolaevulinic acid (ALA), a 5-carbon aminoketone, which is formed in mitochondria by the condensation of glycine and succinyl CoA by \(\delta\)-aminolaevulinate synthase (ALAS). Two molecules of ALA are then condensed in the cytosol to form a monopyrrole, porphobilinogen (PBG), by ALA dehydratase (ALAD). Four molecules of PBG are combined by PBG deaminase (PBGD), to form the first cyclic tetrapyrrole, uroporphyrinogen I, which is then converted to uroporphyrinogen III by uroporphyrinogen synthase (UROS). Uroporphyrinogen III is decarboxylated by uroporphyrinogen decarboxylase (UROD) to form coproporphyrinogen III. Coproporphyrinogen III enters into the mitochondria, where it is oxidatively decarboxylated by coproporphyrinogen oxidase (CPO) to form protoporphyrinogen IX. Protoporphyrinogen IX is then oxidised to protoporphyrin IX by protoporphyrinogen oxidase (PPO). Finally, ferrous iron is inserted into protoporphyrin IX by ferrochelatase to form haem. Protoporphyrin IX is the immediate precursor of the various haems and also of the chlorophylls. Information on enzyme proteins, and genes for haem pathway enzymes is summarised in Table I.

There is significant tissue-specific regulation for enzymes in the haem biosynthetic pathway (Sassa, 2006a,b). For example, there are two separate genes for ALAS, i.e. the housekeeping and the erythroid-specific ALAS genes, which are termed ALAS1 (or ALAS-N) and ALAS2 (or ALAS-E) respectively. In addition, there are the housekeeping and the erythroid-specific mRNAs for ALAD, the gene for PBGD.
Review

Fig 1. Classification of porphyrias Enzymatic defects, associated diseases, major symptoms and principal accumulation products are shown. ALAS2 defect is responsible for X-linked sideroblastic anaemia (XLSA) but is not associated with any porphyria, since the enzymatic defect blocks production of ALA, the obligatory precursor for porphyrin formation. ALA dehydratase porphyria (ADP) and acute intermittent porphyria (AIP) are accompanied by acute hepatic porphyria but not by photocutaneous porphyria, because their enzymatic defects do not result in an increase in porphyrin synthesis. Enzymatic defects beyond uroporphyrinogen synthase (UROS) are all associated with photocutaneous porphyrrias, because they produce excessive amounts of various porphyrins. Hereditary coproporphyria (HCP) and variegate porphyria (VP) are additionally associated with acute hepatic porphyria. Suc.CoA, succinyl coenzyme A; P’gen, rotoporphyrinogen; Proto, protoporphyrin; U’gen, uroporphyrinogen; C’gen, coproporphyrinogen; Adapted from Sassa S & Shibahara S. Disorders of Heme Production and Catabolism. In Handin RI, Lux SE, and Stossel TP, eds, Blood: Principles and Practice of Hematology, 2nd ed, Philadelphia, Lippincott Williams & Wilkins, 2003, with permission).

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<tr>
<th>Enzyme</th>
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(HMBS), and UROS, and the housekeeping and the erythroid-specific enzymes for ALAS as well as for PBGD (Table I). Haem-mediated regulation of ALAS is also tissue-specific; namely, ALAS1 expression in the liver is repressed by haem, while ALAS2 in the erythroid bone marrow is not.

General considerations

Pathogenesis

- All of the haem pathway intermediates are potentially toxic. Their overproduction causes the characteristic neurovisceral and/or photosensitizing symptoms.
- Porphyrins produce free radicals when exposed to ultraviolet light (~ 400 nm). As a result, skin damage ensues in the light-exposed areas, resulting in cutaneous porphyrias.
- In contrast to porphyrins, their precursors, e.g. ALA and PBG, are associated with neurological symptoms of acute hepatic porphyrias.
- Porphyrins and their precursors are excreted in urine or stool depending on their solubility. The water solubility of porphyrins is directly attributable to the number of carboxyl groups in each molecule (Falk, 1964). Accordingly, the water-soluble uroporphyrin is excreted into urine, while the water-insoluble protoporphyrin is excreted into bile and stool. Coproporphyrin is excreted into both urine and stool because of its intermediate solubility. Porphyrin precursors are essentially all excreted into urine.
- During an acute attack, ALAS1, the hepatic isoform of the first enzyme in the haem biosynthetic pathway is induced. ALAS1 formation in normal hepatocytes is repressed by feedback inhibition by the final product, haem. Metabolic inhibition along the pathway in the liver leads to reduced production of haem, resulting in derepression of ALAS1. This leads to increased production of haem precursors in an effort to overcome the metabolic block, and contributes to the accumulation of intermediates prior to the deficient
enzymatic step. This abnormality continues until sufficient haem synthesis is restored.

- There is significant interaction between the primary gene defect in the haem biosynthetic pathway and environmental factors for ALAS1 induction. Namely, patients with acute hepatic porphyrias may not become symptomatic unless these subjects are exposed to certain drugs, liver damage, hormonal changes during the menstrual cycle, stress, or starvation, which result in the induction of ALAS1.

**Diagnosis**

- Demonstration of porphyrin precursors, such as ALA and/or PBG, is essential for the diagnosis of acute hepatic porphyrias. 
- Porphyrin analysis is necessary for the diagnosis of porphyrias with cutaneous photosensitivity.
- The logical stepwise approach is most useful when there are clinical symptoms of the porphyrias (Sassa, 2004).
- Molecular diagnostic testing is powerful and very useful in kindred evaluation and genetic counselling when a disease-responsible mutation has been identified in the family. It is also the only proper way to screen asymptomatic gene carriers.

**Treatment**

- Recognition and avoidance of precipitating events is the first key part of treatment.
- Acute attacks of hepatic porphyrias should be treated similarly by (i) avoiding the precipitating factors; (ii) providing sufficient amounts of calories as carbohydrates (glucose infusion); and (iii) intravenous infusion of haematin.

Fig 2. Haem biosynthetic pathway. Enzymes and intermediates of the haem biosynthetic pathway are shown. Step 7: ALAS. Step 3: ALAD. Step 4: PBGD. Step 8: UROS. Step 9: UROD. Step 10: CPO. Step 11: PPO. Step 12: Ferrochelatase. The carbon atom that is derived from the α carbon of glycine is shown as a bold red dot. The structure that is denoted by the brackets after step 4 is the presumed intermediate whose pyrrole ring D becomes rearranged to yield uroporphyrinogen III. At step 7, 1 mole of oxygen is consumed per 1 mole of water produced. CoA, coenzyme A. (Adapted from *Clinical Hematology*, Philadelphia, Mosby Elsevier. Sassa S. Porphyrias. In Young NS, Gerson SL, and High KA, eds, Copyright 2006, with permission from Elsevier).
Haemolytic anaemia in erythropoietic porphyrias may be treated by blood transfusion.
Cutaneous photosensitivity of erythropoietic protoporphyria may be treated by oral β-carotene, while that of porphyria cutanea tarda (PCT) by phlebotomy, or oral chloroquine.

Erythropoietic porphyrias

The characteristic features of each porphyric disorder are described below. Porphyrins in red cells can cause photosensitive cell lysis, resulting in haemolytic anaemia. The two homozygous erythropoietic porphyrias, congenital erythropoietic porphyria (CEP) and hepatoerythropoietic porphyria (HEP), are associated with haemolytic anaemia of varying degrees. In contrast, erythropoietic protoporphyria (EPP), a heterozygous disease, rarely has accompanying haemolytic anaemia. The effect of life-long anaemia in CEP or HEP may lead to compensatory expansion of erythroid marrow, which may result in pathological fractures, vertebral compression or collapse, and shortness of stature. The haemolysis is also associated with varying degrees of splenomegaly and the production of pigment-laden gallstones.

Congenital erythropoietic porphyria

Congenital erythropoietic porphyria is an erythropoietic porphyria inherited in an autosomal recessive fashion. It is one of the most severely affected photosensitive disorders. The primary abnormality is an almost complete absence of UROS activity, previously termed uroporphyrinogen III cosynthase, which results in massive accumulation and excretion of uroporphyrin I and coproporphyrin I. This is the only porphyria that produces type I isomers in excess.

Uroporphyrinogen synthase catalyses the cyclization of the linear tetrapyrrole, hydroxymethylbilane, to yield a tetrapyrrole, uroporphyrinogen III, the physiological isomer, which ultimately leads to the formation of haem. This step involves inversion of the pyrrole D ring of hydroxymethylbilane and cyclization to uroporphyrinogen III (Fig 2) (Battersby et al., 1980). In the absence of UROS, as in CEP, hydroxymethylbilane is converted non-enzymatically to the non-physiological porphyrin isomer, uroporphyrin I. Uroporphyrinogen I is then enzymatically converted to coproporphyrinogen I via the activity of UROD, but it cannot be metabolized further.

Mild to severe haemolysis in CEP is characterised by anisocytosis, poikilocytosis, polychromasia, basophilic stippling, reticulocytosis, increased nucleated red cells, absence of haptoglobin, increased unconjugated bilirubin, increased faecal urobilinogen and increased plasma iron turnover. Secondary splenomegaly may contribute to the anaemia, and may also result in leucopenia and thrombocytopenia. Anemia can be so severe that some patients are transfusion-dependent. Splenectomy may reduce the need for transfusions, although signs of ineffective erythropoiesis and gallstones may continue.

Severe cutaneous photosensitivity usually begins in early infancy and is manifested by increased friability and blistering of the epidermis on the hands and face and other sun-exposed areas. Pink or red-brown staining of nappies due to markedly increased urinary porphyrins may be the first clue to the disease. Bullae and vesicles contain serous fluid and are prone to rupture and infection. The skin may be thickened, with areas of hypo- and hyperpigmentation. Hypertrichosis of the
face and extremities is often prominent. Sunlight, other sources of ultraviolet light, and minor skin trauma increase the severity of the cutaneous manifestations. Recurrent vesicles and secondary infection can lead to cutaneous scarring and deformities, as well as loss of finger nails and digits and severe damage to the eyelids, nose and ears. Corneal scarring can lead to blindness. Porphyrins deposited in the teeth produce a reddish brown colour in natural light, termed erythrodontia. Erythrodontia shows intense red fluorescence of porphyrins on exposure to long wavelength ultraviolet light.

A variety of mutations that cause CEP have been identified in the UROS gene, including missense and nonsense mutations, large and small deletions and insertions, splicing defects and intronic branch point mutations (Fontanellas et al, 1996; Desnick et al, 1998). UROS knockout in mice is embryonic lethal, while some UROS mutants knocked into these animals not only support the survival of the animals, but also develop cutaneous photosensitivity similar to those observed in CEP (Bishop et al, 2006). These findings suggest that this animal model is useful for the study of the effects of UROS mutations.

Urinary porphyrin excretion is markedly increased (up to 50–100 mg/d, normal range: <0.2 mg, or 0.3 μmol/d), and consists mostly of uroporphyrin, heptacarboxyl porphyrin, and coproporphyrin, with lesser increases in hexa- and pentacarboxyl porphyrins (Fritsch et al, 1997). Urinary ALA and PBG excretion are not increased. Faecal porphyrins are markedly increased due to increased coproporphyrin I. Circulating erythrocytes contain large amounts of uroporphyrin I and lesser, but still excessive, amounts of coproporphyrin I. Red cell protoporphyrin may also be increased, which reflects increased erythropoiesis. HEP is clinically very similar to CEP but can be distinguished by the predominance of protoporphyrin in red cells and isocoproporphyrin in stool.

There are three main components in the treatment of CEP, (i) protection from sunlight and UV exposure; (ii) meticulous skin care; and (iii) red cell transfusions and other haematological supportive care. Other therapeutic interventions include, (i) treatment with hydroxyurea to reduce bone marrow porphyrin synthesis (Guarini et al, 1994); (ii) splenectomy to reduce transfusion requirements in patients with hypersplenism (Piomelli et al, 1986); and (iii) oral charcoal treatment to facilitate faecal excretion of porphyrins (Tishler & Winston, 1990). Allogeneic bone marrow transplantation has also proved curative for patients with CEP (Kauffman et al, 1991; Thomas et al, 1996; Zix-Kieffer et al, 1996; Tezcan et al, 1998). Successful marrow transplantation results in marked reduction of the photosensitivity and porphyrin levels.

Erythropoietic protoporphyria

Erythropoietic protoporphyria is characterised by a partial deficiency of ferrochelatase activity. Cutaneous photosensitivity characteristically begins in childhood, but there is no neurological involvement. This is probably the third most common porphyria after acute intermittent porphyria (AIP) and PCT, and the most common erythropoietic porphyria.

Molecular analysis of ferrochelatase mutations has revealed missense mutations, splicing abnormalities, intragenic deletions, and possible nonsense mutations associated with functional deficiency of ferrochelatase (Ostasiewicz et al, 1995; Toddd, 1998). Among them, exon skipping was the most predominant. It has been reported that (i) a C→T transition at position S-$23 in intron 1 (N_7) reduces ferrochelatase activity; but (ii) the N_7 allele alone is not sufficient to bring about EPP, even in its homozygous state; and (iii) both a mutant allele (M) and the Nr allele in trans are necessary for the clinical expression of EPP (Gouya et al, 1999). Thus, ferrochelatase activity in patients can be defined as M-M, or M-N_7, silent gene carriers as M-N_0, and normal controls as N_0-N_0, N_0-N_7, or N_7-N_7 (increasingly reduced ferrochelatase activity). This model accounts for the fact (i) why normal controls have a wide range of ferrochelatase activity; (ii) why silent gene carriers have higher ferrochelatase activity than patients; and (iii) why patients with EPP have less than 50% of ferrochelatase activity. This model explains the majority of EPP cases, the rest being true autosomal recessive disease.

Cutaneous photosensitivity begins in childhood, affects sun-exposed areas, such as face and dorsal of hands, and is generally worse in spring and summer. Common symptoms include itching, painful erythema and swelling, which can develop within minutes of sun exposure (Poh-Fitzpatrick, 1984). Diffuse oedema of the skin in sun-exposed areas may resemble angioneurotic oedema. On occasion, burning and itching can occur without obvious skin damage. Petechiae and purpuric lesions may occur. Skin lichenification, leathery pseudovesicles and nail changes can be pronounced.

Bone marrow reticulocytes are the primary source of the excess protoporphyrin that accumulates in tissues and is excreted in faeces in EPP. Bone marrow fluorescence is almost entirely in reticulocytes rather than nucleated erythroid cells. Protoporphyrin is markedly increased in erythrocytes and plasma, and excreted in stool. Unlike other porphyrias, there is no increase in porphyrin excretion in urine. In contrast to iron deficiency anaemia and lead poisoning, in which increased erythrocyte protoporphyrin is chelated with zinc, erythrocytic porphyrin in EPP is exclusively free protoporphyrin (Lamola et al, 1975). There is no increase in plasma porphyrin concentrations in the two former conditions (Poh-Fitzpatrick & Lamola, 1976).

Oral administration of β-carotene is useful for treating EPP (Mathews-Roth et al, 1977). β-Carotene doses of 120–180 mg daily in adults are usually required to maintain serum carotene levels in the recommended range of 11–16–14 88 μmol/l, but doses up to 300 mg daily may sometimes be needed. Sunbathing, resulting from better tolerance of sunlight, may lead to further protection. Cholestyramine and other porphyrin absorbents, such as activated charcoal, may be helpful. Other therapeutic options include red blood cell transfusions, exchange transfusion and intravenous haematin to suppress
erythroid and hepatic protoporphyrin production, as well as liver transplantation. While liver transplantation can be temporary beneficial, the new liver is susceptible to protoporphyrin-induced damage.

**Acute hepatic porphyrias**

The most common neurovisceral complaints in acute hepatic porphyrias are abdominal pain (occurring in 85–95% cases), vomiting (43–88%), constipation (48–84%), muscle weakness (42–60%), mental symptoms (40–58%), limb, head, neck, chest pain (50–52%), hypertension (36–54%), tachycardia (28–80%), convulsion (10–20%), sensory loss (9–38%), fever (9–37%), respiratory paralysis (5–12%) and diarrhoea (5–12%) (Waldenstrom, 1957; Goldberg, 1959; Stein & Tschudy, 1970). While the exact mechanism underlying these complaints is not yet well understood, various hypotheses have been put forward. For example, (i) excess amounts of PBG or ALA may cause neurotoxicity (Meyer et al., 1998); (ii) increased ALA concentrations in the brain may inhibit gamma-aminobutyric acid release (Mueller & Snyder, 1977; Brennan & Cantrill, 1979); (iii) haem deficiency may result in degenerative changes in the central nervous system (Whetsell et al., 1984); (iv) decreased haem synthesis in the liver results in decreased activity of hepatic tryptophan pyrrolase (TP), a haem-dependent enzyme, possibly resulting in increased levels of brain tryptophan and increased turnover of 5-hydroxytryptamine, a neurotransmitter (Litman & Correia, 1985); (v) as all of the porphyrias that are associated with neurovisceral complaints show increased urinary excretion of ALA (ADP), or of ALA and PBG [AIP, hereditary coproporphyria (HCP) and variegate porphyria (VP)], and ALA increases lipid peroxidation, ALA-mediated lipid peroxidation may underscore the acute crisis of porphyria (Bechara, 1996); and (vi) TP deficiency results in decreased plasma melatonin levels (Puy et al., 1996), which may result in the loss of protection against ALA-mediated lipid peroxidation.

**ALA dehydratase porphyria (ADP)**

ALA dehydratase porphyria (ADP) is the rarest form of the inherited porphyrias. Only six cases have been reported that were molecularly confirmed to be due to ALAD mutations: three German males (Ishida et al., 1992; Doss et al., 2004), one Swedish baby boy (Thunell et al., 1987; Plewinska et al., 1991), one elderly Belgian man (Hassoun et al., 1989; Akagi et al., 2000), and an American male (Akagi et al., 2006). It is an autosomal recessive disorder resulting from a homozygous deficiency of ALAD in five reported patients, while it was due to a clonal expansion of a mutant ALAD allele in a late-onset disease in the Belgian patient. While clinical ADP is rare, the frequency of genetic carriers for ADP deficiency, i.e. heterozygotes, in the normal population is as high as 2%, according to a study performed in Sweden (Thunell et al., 1987). Although subjects heterozygous for ADL deficiency are asymptomatic, they may be at a higher risk than normal individuals for developing ADP when exposed to environmental chemicals or conditions that further influence deficient ALAD activity (Akagi et al., 2000).

In addition to the inherited enzymatic deficiency, ALAD activity can be inhibited by a number of substances including divalent heavy metal ions. By virtue of its zinc displacing activity from the enzyme, lead potently inhibits ALAD activity (Granick et al., 1978). Over 99% of lead in blood is found in erythrocytes, of which over 80% is bound to ALAD (Bergdahl et al., 1997), and patients with lead poisoning are associated with marked inhibition of ALAD activity in red cells. They also develop neurological symptoms, some of which resemble those seen in ADP. The most potent inhibitor of ALAD is succinylacetone (Sassa & Kappas, 1983), a structural analogue of ALA, which is found in the urine and blood of patients with hereditary tyrosinaemia type 1 (Lindblad et al., 1977). As a result, approximately 40% of children with hereditary tyrosinaemia develop the signs and symptoms of ADP, suggesting that succinylacetone results in the significant inhibition of haem synthesis in the liver.

The ADP is characterised by the markedly increased production of ALA, such that ALA production is 10–20 times that of PBG. AIP, on the other hand, is characterised by increased production of both ALA and PBG, often to a comparable extent. The fact that AIP and ADP are clinically indistinguishable suggests that ALA or its metabolites, rather than PBG, may be the neurotoxic agent. This is further supported by the finding, that patients with hereditary tyrosinaemia frequently develop ADP along with its neurological abnormalities, most likely via the marked inhibition of ALAD by succinylacetone.

Treatment of ADP, as with other acute hepatic porphyrias, is two-fold: (i) acute attacks are treated by withdrawal of the offending agent (if any), infusion of glucose, and, if the patient is still symptomatic, intravenous infusion of haem preparations or administration of inhibitors of haem oxygenase, (ii) future attacks are prevented by avoiding agents known to stimulate ALAS activity and by avoiding those agents known to inhibit ALAD activity.

**Acute intermittent porphyria**

Acute intermittent porphyria is due to inherited (autosomal dominant) mutations of the PBGD gene (HMBS) leading to deficient activity of the enzyme. This is the most important acute hepatic porphyria, both in its incidence and clinical severity. The enzyme activity is ~50% of normal in those who inherit the genetic trait. There is no difference in erythrocyte PBGD activity between patients and latent gene carriers. The prevalence of AIP in the United States is thought to be 5–10 per 100 000. It is more common in northern European countries, such as Sweden (60–100 per 100 000), Britain and Ireland. More than 200 mutations of the PBGD gene have been described to date in AIP.
Acute intermittent porphyria is more common in women than in men, and very rare in children. Symptoms may appear at or any time after puberty. The major clinical manifestations of AIP, including abdominal pain and other neurovascular and circulatory disturbances, are due to effects on the nervous system. Neurological and visceral symptoms are almost always intermittent and usually occur in acute attacks that develop over a few hours or days. The disease can be disabling but is only occasionally fatal. Abdominal pain has been reported in 85–95% of cases, followed by tachycardia (80%). Abdominal pain is usually severe, steady and poorly localised, but may be cramping. A variety of mental symptoms, pain in limbs, head, neck, or chest, muscle weakness and sensory loss can occur. Weakness most commonly begins in the proximal muscles and more often in the arms than in the legs.

The course of the neurological manifestations is highly variable. Sudden death may occur, presumably due to cardiac arrhythmia. Acute attacks of porphyria may resolve quite rapidly. The disease may be complicated by electrolyte abnormalities. Hyponatraemia is common during acute attacks, and may help to suggest the diagnosis. This is sometimes due to the syndrome of inappropriate antidiuretic hormone secretion. The urine is often dark red in colour, due to the presence of porphobilin, the oxidised product of PBG.

Mice with PBGD deficiency induced by gene targeting display some of the symptoms observed in patients with AIP, such as impaired motor function, ataxia, increased levels of ALA in plasma and brain, and decreased haem saturation of liver tryptophan pyrrolase (Lindberg et al., 1996). This model should be useful to further define the mechanisms of neurological damage in acute porphyrias.

Precipitating factors. An inherited deficiency of PBGD is not in itself sufficient to cause clinical expression of AIP, as the great majority (~ 90%) of individuals who inherit a deficiency of PBGD never develop porphryic symptoms. There is considerable evidence that endocrine factors and steroid hormones play an important role in precipitating the acute attack of AIP (Sassa, 1996). Thus, acute hepatic porphyrias are rarely seen before puberty, but are quite common at puberty or after, and particularly often in the premenstrual phase in women.

Drugs. Drugs are among the most important factors that precipitate acute attacks of acute porphyria, with barbiturates and sulphonamide antibiotics being most common (Anderson et al., 2001). A significant number of commonly used drugs are widely agreed to be either safe or unsafe for use in patients with AIP. Many of these can be found on a dedicated web site (http://www.porphyria-europe.org). However, knowledge about the safety of many drugs and other over-the-counter preparations in acute porphyrias is yet incomplete. Most drugs that exacerbate AIP have the capacity to induce ALAS activity in the liver. As mentioned above, this process is closely associated with the induction of cytochrome P450 enzymes, a process that increases the demand for hepatic haem synthesis.

Nutritional factors. Reduced energy intake, usually instituted in an effort to lose weight, commonly contributes to attacks of AIP. Thus, even brief periods of starvation during weight reduction, postoperative periods, or intercurrent illness should be avoided. Starvation induces haem oxygenase-1 (HO-1) activity, which can lead to depletion of regulatory hepatic haem pools and contribute to ALAS induction. Transcription of ALAS1 can also be upregulated by the peroxisome proliferators-activated receptor γ-coactivator 1α (PGC-1α). Under conditions of low glucose, PGC-1α production increases, leading to increased levels of ALAS1, creating conditions for attacks of acute porphyrias (Handschin et al., 2005). Glucose and other forms of carbohydrate are effective in treating acute attacks of porphyria.

Smoking. Chemicals in tobacco smoke, such as polycyclic aromatic hydrocarbons, are known inducers of hepatic cytochrome P450 enzymes and haem synthesis. An association between cigarette smoking and repeated attacks of porphyria was found in a survey of 144 patients with AIP in Britain (Lip et al., 1991). As a result, smoking cessation may have particular health benefits in patients with acute porphyrias.

Infections, surgery and stress. Attacks of porphyria may develop during intercurrent infections and other illnesses, after major surgery, and psychological stress. Most of these conditions are known to increase hepatic HO-1 activity (Rodgers & Stevenson, 1990).

All individuals with AIP, whether active or latent, have a ~50% deficiency of PBGD activity in the liver. Urinary ALA and PBG are always markedly increased in symptomatic patients with AIP (Aarsand et al., 2006) and even in some asymptomatic individuals with the inherited enzyme deficiency (Floderus et al., 2006). PBG in urine is oxidised to porphobilin upon standing, which gives a dark-brown colour to urine, and often referred to as 'port-wine reddish urine'. Urinary ALA and PBG levels may decrease to normal if the disease becomes inactive for a prolonged period. Urinary porphyrins are also markedly increased. Circulating concentrations of ALA and PBG in plasma (or serum) are substantially increased (Floderus et al., 2006).

Diagnosis can be established by the demonstration of reduced PBGD activity in erythrocytes (about 50% of normal) in type I and type III AIP patients. Type II AIP patients show normal PBGD activity in erythrocytes, but have reduced PBGD activity in non-erythroid cells, such as fibroblasts or lymphocytes (Sassa, 1996). Patients with the clinically expressed disease excrete increased amounts of ALA and PBG in the urine, and often during clinical remission. During an acute attack of AIP, there are further massive increases in excretion of these precursors (ALA 25–100 mg/d; PBG 50–200 mg/d). Some latent gene carriers who are characterised by reduced PBGD activity similar to patients also show increased ALA and PBG excretion. The Watson–Schwartz test has been widely used as a screening test for urinary PBG (Watson & Schwartz, 1941). It is useful for evaluating the efficacy of treatment on acute crisis. While the test may occasionally be associated with false-positive
findings, it is quick and quite sufficient for the rapid diagnosis of an acute attack of AIP. The column method of Mauzerall and Granick is quantitative and more specific, and can determine the amounts of both ALA and PBG (Anderson et al, 2001). For this reason, the column method of Mauzerall and Granick is now more widely used than the Watson–Schwartz test to avoid possible biological false-positive findings. All participating laboratories in the UK External Quality Assurance now use the column method, instead of the Watson–Schwartz test. Elevated levels of urinary ALA may additionally be seen in ADP, HCP and VP, while elevated levels of both ALA and PBG may be seen in HCP and VP.

Detection of PBGD mutations in AIP provides 95% sensitivity and around 100% specificity (Kauppinen, 2004), and has quickly been incorporated into good clinical practice. It can definitively confirm or exclude the diagnosis of AIP. While it is powerful and specific, and is very useful in pedigree screening and in genetic counselling, its application at the population level is not recommended, unless the frequency of gene carriers is locally very high (Kauppinen, 2004). DNA testing of AIP also determines the gene carrier status, rather than the severity of the disease. The same consideration applies to the diagnosis of most other acute hepatic porphyrias.

The treatment of acute attack is essentially the same for AIP, ADP, HCP and VP. Intravenous administration of carbohydrate (as dextrose) should be given to provide a minimum of 300 g of carbohydrate per day. Glucose and insulin together might also be more effective than glucose alone, as insulin blunts the PGC-1α effect (Handschin et al, 2005). Intravenous haematin administration is the treatment of choice, which curtails urinary excretion of ALA and PBG, acute attacks, and perhaps the severity of neuropathy. Reconstitution of haematin for intravenous infusion should be made according to the protocol described recently (Anderson et al, 2006). Normosang [Orphan Europe (UK) Ltd, Henley-on-Thames, UK], is a stable haematin solution complexed with arginine (Mustajoki et al, 1989). It is known to cause lesser vascular complications, such as phlebitis than the conventional haematin solution.

Liver transplantation was attempted in a 19-year-old woman with severe AIP. It successfully reduced urinary porphyrin precursor excretion to normal, and improved her quality of life (Soonawalla et al, 2004). Thus liver transplantation might be considered for selected patients with the severest forms of AIP, though it has not been successful in ADP. Nasal or subcutaneous administration of long-acting agonist of luteinizing hormone-releasing hormone inhibits ovulation and greatly reduces the incidence of perimenstrual attacks of AIP in such women (Anderson et al, 1990a). Pain, which is invariably present and severe, can be treated with frequent regular doses of narcotic analgesics.

**Hereditary coproporphyria**

Hereditary coproporphyria is an autosomal dominant hepatic porphyria due to a deficiency of coproporphyrinogen oxidase (CPO) activity. HCP is markedly less frequent than AIP and VP. HCP has identical clinical symptoms as AIP, except for its cutaneous photosensitivity, which is entirely absent in AIP.

It is generally thought that HCP may be a milder disease than AIP (Brodie et al, 1977), but there have also been a few fatalities from respiratory paralysis in HCP. Molecular analysis of several families with HCP, including the homozgyous dominant form and the harderoporphyria variant, has revealed a variety of mutations in the CPO gene (Martasek, 1998). In a series of 53 cases of HCP in Germany, the incidence of symptoms was 89%, 33%, 28%, 25% and 14%, for abdominal pain, neurological, psychiatric, cardiovascular and skin symptoms respectively (Kuhn et al, 2000). The disease is latent before puberty, and symptoms are more common in adult women than men. This porphyria is exacerbated by many of the same factors that precipitate attacks in AIP, including barbiturates and other drugs, and endogenous or exogenous steroid hormones. Symptoms can occur in association with the menstrual cycle. The risk of hepatocellular carcinoma may be increased in this porphyria, as in AIP and VP. A few homozygous HCP cases, with cutaneous lesions beginning in early childhood, have also been reported.

The most prominent biochemical feature of HCP is a marked increase in urinary and faecal coproporphyrin, predominantly isomer type III, typically 10–200 times compared with controls (Martasek, 1998). Urinary ALA, PBG and uroporphyrin are also increased during acute attacks. In harderoporphyria, a variant form of HCP, faecal excretion of harderoporphyrin (tricarboxylate porphyrin) and coproporphyrin is increased. Treatment of acute attacks of HCP is identical to the treatment of AIP. The identification and avoidance of precipitating factors is also essential for HCP.

**Variegate porphyria**

Variegate porphyria is an autosomal dominant acute hepatic porphyria, due to a deficiency of PPO activity, the penultimate enzyme in the haem biosynthetic pathway. The disease is termed *variegate* because it can present itself either with neurological manifestations, cutaneous photosensitivity, or both.

In most countries this porphyria is less commonly recognised than AIP. However, VP is quite common in South Africa, where it was first reported in 1945. The high prevalence among South African whites (approximately 3 of 1000) is due to genetic drift or the ‘founder effect’, and most VP cases in South Africa can be traced to a man or his wife who emigrated from Holland and were married in 1688 (Dean, 1982). As many as 20 000 South Africans may carry this gene, which is now found to be R59W mutation of the PPO gene (Meissner et al, 1996).

The acute attack is identical to that seen in AIP, and may include abdominal pain, tachycardia, vomiting, constipation, hypertension, neuropathy, back pain, confusion, bulbar paralysis, psychiatric symptoms, fever, urinary frequency and
dysuria. Hyponatraemia with evidence of sodium depletion or inappropriate antidiuretic hormone secretion can occur during acute attacks. Attacks of VP are generally milder than in AIP, and recurrent attacks are less common. Cutaneous photosensitivity is much more common than in HCP. Skin manifestations generally occur, and are usually of longer duration. They are very similar to those seen in PCT and HCP, and include increased fragility, vesicles, bullae, erosions, milia, hyperpigmentation and hypertichrosis of sun-exposed areas. Photosensitivity may be less commonly associated with VP in northern countries, where sunlight is less intense (Kirsch et al., 1998).

Faecal protoporphyrin and coproporphyrin and urinary coproporphyrin are markedly increased when VP is clinically active. Urinary ALA, PBG and uroporphyrin are also increased during acute attacks but may be normal or only slightly increased during remission. Plasma porphyrin is commonly increased in VP, and its analysis is more sensitive than that of faecal porphyrins (Hift et al., 2004). Increased plasma porphyrin in VP is readily detected as a fluorescence emission maximum at 626–628 nm. This is a specific finding in VP (Enriquez et al., 1993), as fluorescence emission peaks in EPP and PCT are found at 636 nm and 618–622 nm respectively (Enriquez et al., 1993). Biliary porphyrins are also increased. The risk of gallstones may be increased in VP, which consist mainly of protoporphyrin. The X porphyrin fraction (ether-acetic acid-insoluble porphyrins extractable from faeces by urea-Triton) is increased in VP. Rare homozygous VP patients have markedly increased erythrocyte Zn-protoporphyrin (Whatley et al., 1999).

**Chronic hepatic porphyria**

Patients with chronic hepatic porphyria, such as PCT, are associated with significant chronic cutaneous photosensitivity, but they do not accompany neurological symptoms. Petechiae and purpuric lesions may occur. Skin lichenification, leathery pseudovesicles and nail changes can be pronounced. Chronic blistering lesions may develop; the fluid-filled vesicles rupture easily and the denuded areas become crusted and heal slowly; secondary infection is common. Previous areas of blisters may appear atrophic, or brownish. Facial hypertrichosis, scarring and hyperpigmentation may result.

**Porphyria cutanea tarda**

Porphyria cutanea tarda is due to a profound deficiency of uroporphyrinogen decarboxylase (UROD) activity in liver. The disease has been classified into three subtypes. Type I PCT has decreased hepatic UROD activity, but normal erythrocyte UROD activity, and is found in sporadic fashion without family history. Type II PCT has decreased UROD activity both in red cells and in liver, and occurs in multiples in a family. Type III PCT is similar to type II with respect to familial occurrence, but erythrocyte UROD activity is normal.

A variety of mutations of UROD have been identified in PCT (McManus et al., 1996; Mendez et al., 1998; Christiansen et al., 2000). All but two were each found in only one family. UROD mutations have also been found in HEP, the homozygous form of UROD deficiency, but they were mostly unique and usually not found in PCT (Meguro et al., 1994).

Chronic blistering lesions develop on sun-exposed areas of skin. These are more common in the summer than winter. The fluid-filled vesicles rupture easily and the denuded areas become crusted and heal slowly. Previous areas of blisters may appear atrophic, or brownish. Facial hypertrichosis and hyperpigmentation are also common. All types of PCT respond readily to repeated phlebotomy (Ippen, 1977). PCT can also be treated with of chloroquine or hydroxychloroquine (administered in very low dosage regimen). This is the preferred therapy at some centres, especially for patients without marked iron overload (Valls et al., 1994).

Porphyrins accumulate in large amounts in liver, and are increased in plasma. Uroporphyrin and heptacarboxylyporphyrin are predominantly increased in urine.

Multiple factors can contribute to inactivation or inhibition of hepatic UROD in this disease, probably by an iron-dependent oxidative mechanism, including alcohol (Grossman et al., 1979), hepatitis C infection (Fargion et al., 1992), oestrogen (Grossman et al., 1979), human immunodeficiency virus (HIV) (Egger et al., 2002), and factors that increase hepatic iron content, such as mutations of the HFE gene (HFE) associated with haemochromatosis (Roberts et al., 1997a,b). Liver biopsy frequently shows haemosiderosis, and serum iron and ferritin concentrations are increased. Patients often develop cirrhosis, and may occasionally develop hepatoma (Kordac, 1972).

HFE mutation is found in PCT more commonly than in control populations, indicating that a tendency to iron overload can predispose to PCT (Bonkovsky et al., 1998). There is no increase in ALA or PBG in this disorder. Faecal isocoproporphyrin is the biochemical stigmata of the UROD deficiency, and its detection establishes the diagnosis of PCT (or HEP) (Elder, 1998). Namely, isocoproporphyrin III is formed from dehydroisocoproporphyrinogen III that is normally a minor product but can accumulate from penta-carboxylate porphyrinogen III if UROD activity is reduced (Elder & Chapman, 1970).

The identification and avoidance of precipitating factors is the first line of treatment, particularly for patients with type I PCT. Abstinence from alcohol should be recommended in all types of PCT. Phlebotomy is usually effective in reducing urinary porphyrin concentration, serum ferritin concentration, serum transferrin saturation, and in induction of clinical remission (Ippen, 1977). Phlebotomy should be undertaken weekly or biweekly until serum transferrin saturation and serum ferritin levels are normalised. It usually requires blood withdrawal ranging from 2.5 l to 7 l. Serum transferrin saturation and ferritin levels are correlated with urinary porphyrin excretion, and the clinical syndrome, and should
be used to assess the exhaustion of hepatic iron stores during phlebotomy treatment, as well as to detect an early replenishment after remission (Rocchi et al., 1995). If phlebotomy is ineffective, or contraindicated because of the presence of other diseases, such as anaemia, low-dose chloroquine therapy may be considered (Valls et al., 1994). Chloroquine is thought to chelate porphyrins to facilitate their water-solubility, thus to enhance their excretion into urine. Treatment of PCT associated with end-stage renal disease can be difficult, because phlebotomy is contraindicated and chloroquine is not effective, presumably because kidney failure precludes excretion of excess porphyrins mobilized from liver. Recombinant erythropoietin administration is now the treatment of choice for these patients, as it can correct anaemia, mobilize iron and support phlebotomy in this condition (Anderson et al., 1990b; Yaqoob et al., 1992; Peces et al., 1994).

**Hepatoerythropoietic porphyria**

Hepatoerythropoietic porphyria is a rare form of porphyria, which is due to a homozygous or compound heterozygous deficiency of UROD. Only some 20 cases of HEP were reported (Meguro et al., 1994). HEP resembles CEP clinically and usually presents in infancy or childhood with red urine, blistering skin lesions, hypertrichosis and scarring. Sclerodermoid skin changes may be prominent. Erythrocyte porphyrins are increased, but they are predominantly type III isomers. Some patients may be associated with haemolytic anaemia and splenomegaly.

The biochemical findings in HEP, however, resemble those that are observed in PCT, and include predominant accumulation and excretion of uroporphyrin, heptacarboxylate porphyrin and isocoproporphyrins. Erythrocyte Zn-protoporphyrin concentration is increased in HEP. Treatment of HEP is identical to that for CEP. Unlike PCT, phlebotomy is not effective in this disease.

**References**


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