CHAPTER 27

Haem biosynthesis and the porphyrias

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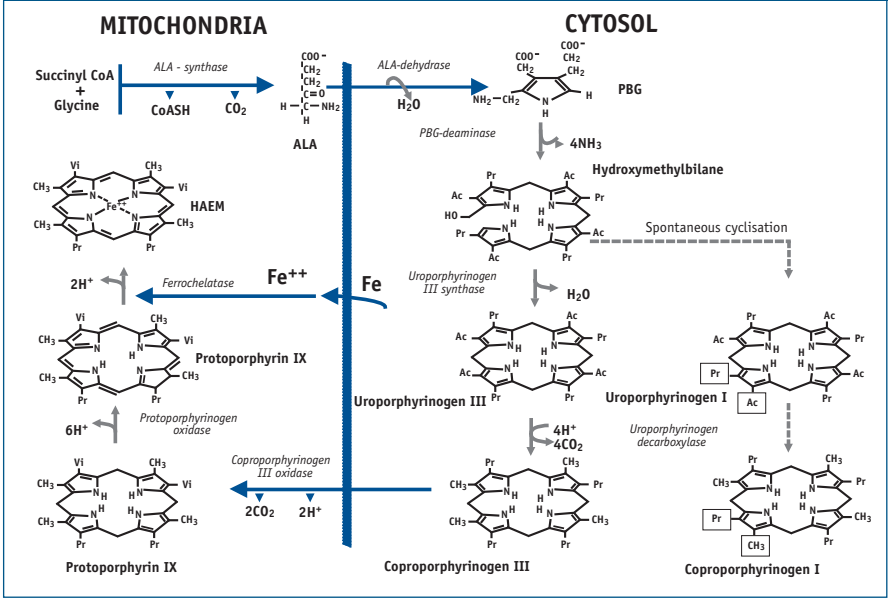
1. Normal haem synthesis and catabolism

1.1 Haem synthesis

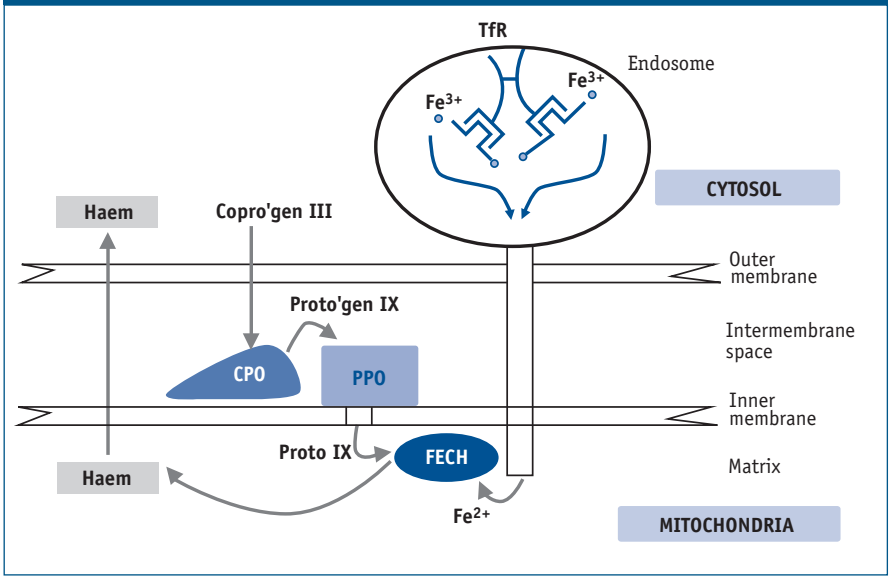
Haem is required for the synthesis of haemoproteins such as haemoglobin, myoglobin, mitochondrial or microsomal cytochromes, catalase, peroxidase, nitric oxide synthase, prostaglandin endoperoxide synthase, guanylate cyclase or tryptophan pyrrolase, which play very important roles in electron and oxygen transport. The two types of cells in the body that are responsible for synthesising most of the haem are the erythropoietic cells (80%) and the liver parenchymal cells (15%). Porphobilinogen (PBG) and δ-amino-levulinic acid (ALA) are linear porphyrin precursors whereas porphyrins are cyclic haem precursors. Eight enzymes are involved in haem synthesis from succinyl CoA and glycine; the biosynthetic pathway starts in the mitochondria and, after passing through three cytoplasmic stages, re-enters the mitochondria for the final steps of haem formation (1). The first and last three enzymes are found in mitochondria and the others in the cytosol (Figures 1 and 2). δ-aminolaevulinic

Figure 1: **Enzymes and intermediates of the haem biosynthetic pathway**



The subcellular distribution of enzymes and intermediates in the synthesis of haem is shown. ALA: δ-aminolaevulinic acid; PBG: porphobilinogen; Ac: acetyl (CH_2-COOH); Pr: propionyl (CH_2-CH_2-COOH); Vi: vinyl (CH=CH_2)

Figure 2: **Schematic representation of the three terminal steps of the haem biosynthetic pathway**



The three terminal enzymes (coproporphyrinogen oxidase CPO, protoporphyrinogen oxidase PPOX, ferrochelatase FECH) are associated with the inner mitochondrial membrane. Copro'gen: coproporphyrinogen; Proto'gen: protoporphyrinogen; Proto: protoporphyrin.

acid synthase (EC 2.3.1.37), the first enzyme in the pathway, is a mitochondrial protein that requires pyridoxal phosphate as a cofactor. It is encoded for by two different genes: one erythroid specific (ALAS-2 on chromosome X) and one ubiquitous (ALAS-1 on chromosome 3). It catalyses the condensation of glycine and succinyl-CoA, which is produced by the tricarboxylic acid cycle, to form ALA, which is exclusively committed to the synthesis of haem. ALA dehydrase (EC 4.2.1.24) then catalyses the condensation of two molecules of ALA to form the monopyrrole porphobilinogen (PBG). The ALA dehydrase gene, situated on chromosome 9, has two codominant alleles, 1 and 2 (2). The isoenzymes are produced in the liver and in erythroid tissue through tissue-specific alternative splicing. The ALA dehydrase-2 isoenzyme is more electronegatively charged than ALA dehydrase-1, and its affinity for lead, which inhibits its activity by competing with the zinc atoms needed for catalytic action, is therefore higher. As a consequence, individuals with the ALA dehydrase-2 genotype are more vulnerable to lead exposure.

Two cytoplasmic enzymes, porphobilinogen deaminase (EC 4.3.1.8) and

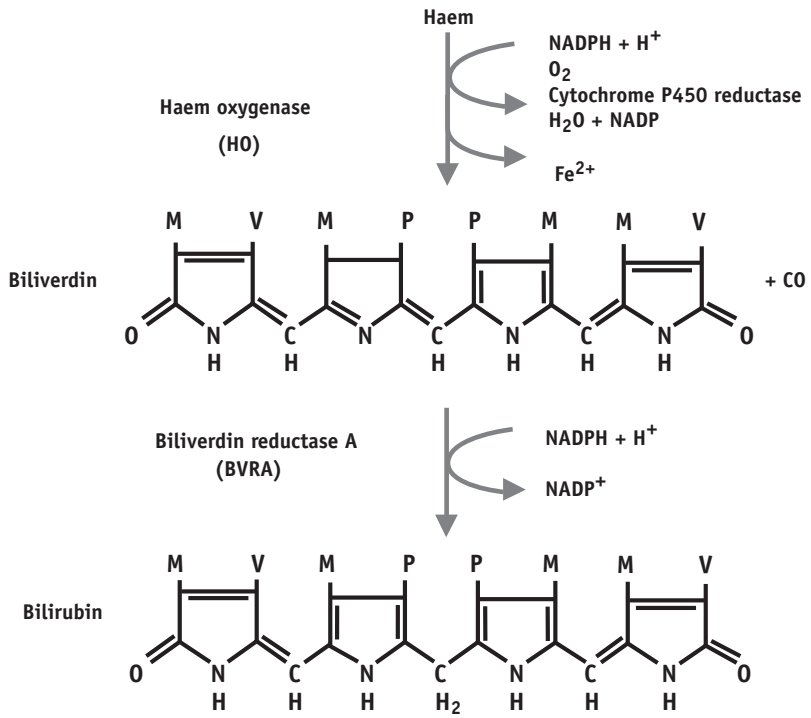
uroporphyrinogen III cosynthetase (cosynthetase; EC 4.2.1.75), convert four molecules of porphobilinogen (PBG) to uroporphyrinogen III. In the absence of the ring-closing and side-chain rearranging cosynthetase enzyme, an abortive I isomeric uroporphyrin is formed spontaneously, and, after partial decarboxylation to I isomeric coproporphyrinogen, is excreted via the hepatobiliary route as well as in urine. Uroporphyrinogen decarboxylase (EC 4.1.1.37), encoded by a gene on the short arm of chromosome 1, catalyses the stepwise decarboxylation of uroporphyrinogen III to form coproporphyrinogen III (1).

The three terminal enzymes (coproporphyrinogen oxidase CPO, protoporphyrinogen oxidase PPOX, ferrochelatase FECH) are associated with the inner mitochondrial membrane (Figure 2). In the intermembrane space, coproporphyrinogen oxidase (EC 1.3.3.3) selectively converts the III isomeric form of coproporphyrinogen to protoporphyrinogen IX. Then, protoporphyrinogen oxidase (EC 1.3.3.4) catalyses the oxidation of protoporphyrinogen IX to protoporphyrin IX (PPIX). This implies that protoporphyrinogen oxidase spans the inner membrane and facilitates the translocation of PPIX into the mitochondrial matrix where iron will be incorporated. This final enzymatic step in haem synthesis is catalysed by the enzyme ferrochelatase (EC 4.99.1.1) which is localised to the inner membrane of mitochondria. Only the reduced form of iron (Fe^{2+}), and not Fe^{3+}, is incorporated into PPIX by the enzyme. Co^{2+} and Zn^{2+} are more efficient substrates than Fe^{2+} for the enzyme but iron is more abundant. However, iron deficiency leads to Zinc-PPIX accumulation whereas ferrochelatase deficiency leads to free PPIX accumulation.

1.2 Haem catabolism

All cells are able to handle haem left over from the breakdown of haemoproteins, thus preventing toxic accumulation of the compound. Furthermore, spleen and liver macrophages have a special role in degrading haem and recycling iron following phagocytosis of senescent erythrocytes. Haem oxygenase 1 (HO-1, EC 1.14.99.3) situated in the endoplasmic reticulum, is ubiquitously expressed but is present in especially large amounts in liver and spleen. Combined with NADPH cytochrome-450 reductase, it cleaves one of the methene bridges of the porphyrin ring and generates carbon monoxide, biliverdin and iron (Figure 3). The linear tetrapyrrole, biliverdin, is reduced to bilirubin and excreted via the liver-bile route, while the liberated iron is stored into ferritin or recycled back to the plasma. HO-1 activity is induced at the transcriptional level by a variety of stimuli, including haem itself, heavy metals, organic chemicals, endotoxins, hyperthermia, hypoglycaemia, burns and oxidative stress. Stimuli that increase HO-1 gene expression could deplete the “uncommitted” haem pool, release the negative feedback on ALAS1 and stimulate the haem biosynthetic pathway. This mechanism can contribute to

Figure 3: **Catabolism of haem: role of haem oxygenase and biliverdin reductase**



M: methyl ($-CH_3$); V: vinyl ($-CH=CH_2$); P: propionyl ($-CH_2-CH_2-COOH$)

triggering clinical symptoms in some forms of porphyria by inducing accumulation of metabolites upstream of the deficient enzyme (3). The isoenzyme haem oxygenase 2 (HO-2), encoded by another gene, is not induced by the same agents that increase the transcription of the ubiquitous gene. This HO-2 gene is especially strongly expressed in brain, where it may serve to supply the tissue with carbon monoxide, a neuronal messenger which binds to the haem prosthetic group of guanylate cyclase generating cyclic GMP.

1.3 Regulation of haem synthesis

The mechanisms implicated in the control of haem biosynthesis differ between the two tissues that make haem in largest amounts: liver and bone marrow. Both erythroid-specific and non-erythroid or "housekeeping" transcripts have been

identified for each of the first four enzymes in the pathway. ALA is the first intermediate in the haem biosynthetic pathway exclusively committed to haem synthesis, and the rate of ALA synthesis is an important controlling step for haem formation. Erythroid-specific and housekeeping transcripts for the first step enzyme ALAS are encoded by two separate genes (ALAS-1 on chromosome 3 and ALAS-2 on chromosome X), whereas for ALA dehydrase, porphobilinogen deaminase and uroporphyrinogen synthase each transcript is produced from the same gene via an alternative splicing (2).

In the liver, the haemoproteins formed, including cytochrome P450s, are rapidly turned over in response to current metabolic needs. The "free" cellular haem pool retroinhibits ALAS-1 synthesis and activity via a negative feedback regulation. ALAS-1 is the rate-limiting enzyme in the production of haem. Increased hepatic ALAS activity is a secondary phenomenon that results from exposure to several factors (such as drugs and hormones). This would account for the especially marked increase in ALA and PBG production and excretion in acute hepatic porphyrias (4).

In erythroid cells, synthesis of the enzymes participating in the formation of haem is regulated at the transcriptional level during erythroid differentiation in response to erythropoietin and is finely tuned by iron availability. Furthermore, in contrast to the liver, the erythron responds to stimuli for haem synthesis also by increasing its cell numbers to meet changing requirements for haemoglobin. The haemoglobinisation of the erythroid cell is controlled by ALAS-2 which exhibits a 75% identity in the C terminal part with ALAS-1. This isoform is not inducible by the drugs that induce ALAS-1 in the liver, and is not repressed by exogenous haem treatment. ALAS-2 activity is induced only during the period of active haem synthesis in red cells, and its rate of synthesis is regulated by the amount of free iron present. The ALAS-2 mRNA contains an IRE in its 5' untranslated region. In conditions of iron deficiciency, ALAS-2 synthesis is repressed by interaction with cytosolic iron sensors IRP1 and IRP2, thereby preventing the formation of PPIX when iron is limited (see Chapter 19 for details).

ALA dehydrase and porphobilinogen deaminase are other sites of regulation mediated by alternative splicings. The single PBG deaminase gene is located at chromosome 11q24.1-24.2 and contains 15 exons. It encodes erythroid-specific and ubiquitous isoforms of PBG deaminase that are generated by the use of separate promoters and alternative splicing of the two primary transcripts. The isoforms differ only at their NH_2 ends where the ubiquitous isoform extends for an additional 17 residues making a polypeptide of 361 amino acids (5). The upstream promoter is active in all tissues, and thus the enzyme encoded by the larger transcript was termed the "housekeeping PBG deaminase". The other promoter, located 3 kb downstream, is active only in erythroid cells. It displays some structural homology with the β-globin gene promoter,

suggesting that some common trans-acting factors may coregulate the transcription of these genes during erythroid development. The expression of ALAS-2, ALA dehydrase, and PBG deaminase erythroid isogenes is determined by trans-activation of nuclear factor GATA-1, CACC box and NF-E2 binding sites in the promoter areas (3). Ferrochelatase, the final enzyme of haem biosynthesis also play a significant role in controlling the rate of haem formation in erythroid cells. Ferrochelatase deficiency in human protoporphyria results in the accumulation of protoporphyrin almost exclusively in erythroid tissue, even though ferrochelatase is deficient in all other tissues in these patients. Thus, ferrochelatase activity can become rate limiting in erythroid cells, but not in other tissues, when the enzyme itself or its substrate, iron, is partially deficient. Although protoporphyrin is excreted only in the bile and accumulates in the liver in some patients, it originates in the bone marrow.

1.4 Excretion of porphyrins and of porphyrin precursors

Each enzyme defect will give rise to a characteristic biochemical profile of porphyrins and porphyrin precursors (ALA and PBG) accumulation in urine, faeces, plasma and/or erythrocytes, which alone allows the accurate identification of type of porphyria in patients (Table 1). Enzyme or DNA analysis are only needed for family studies. ALA and PBG are excreted only in the urine. Coproporphyrin and uroporphyrin are

Table 1: **Diagnosis in symptomatic porphyric patients**

Porphyria		Urine	Stool	Red Cells	Plasma*
Acute hepatic	ALA dehydratase porphyria	**ALA**, Copro III	-	Zn-Proto	-
	Acute intermittent porphyria	**ALA**, **PBG**, URO III	-	-	615-620
	Hereditary coproporphyria	**ALA**, **PBG**, Copro III	Copro III	-	615-620
	Variegate porphyria	**ALA**, **PBG**, Copro III	Proto > Copro	-	624-627
Non-acute hepatic	Porphyria cutanea	Uro III, Hepta**	Isocopro, Hepta	-	615-620
Erythropoietic	Congenital erythropoietic porphyria	Uro I, Copro I	Copro I	Uro I, Copro I	615-620
	Erythropoietic protoporphyria	-	Proto	Free Proto	615-620
	X linked dominant protoporphyria	-	Proto	Zn Proto	626-634

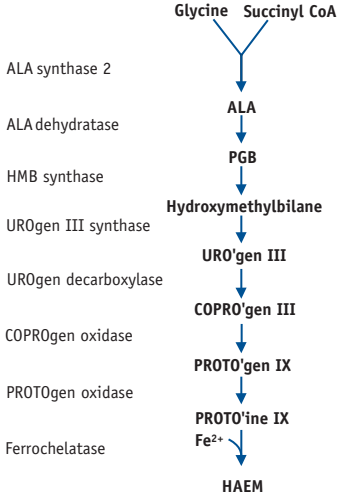
** Fluorescence emission peak in nm ** Heptacarboxyl-porphyrin.*

the predominant porphyrin in normal human urine (1). Protoporphyrin and 70% of coproporphyrin are excreted in faeces. Among haem-forming tissues, the bone marrow is the major source of PPIX, a very poorly water-soluble compound. Hepatic uptake may occur through a process similar to that of other organic anions (such as bilirubin) that are bound to albumin. The rate-limiting step for the overall transport of PPIX from plasma to bile appears to be canalicular secretion, since less than 5% of the protoporphyrin extracted by liver is secreted into bile. This secretion should be mediated by the ABCG2/BCRP transporter, a member of the ATP-binding cassette (ABC) family (2).

2. Porphyrias

The porphyrias are a group of inherited metabolic disorders of haem biosynthesis (Figure 4) in which specific patterns of overproduction of haem precursors are associated with characteristic clinical features: acute neurovisceral attacks, skin lesions or both.

Figure 4: **Classification of the major human porphyrias**



PORPHYRIAS		
DISEASE	**INHERITANCE**	**CLASSIFICATION**
X-linked proto-porphyria **(X-L DPP)**	Gain of function/ X-linked	Erythropoietic
ALA dehydratase deficiency porphyria (ADP)	Autosomal recessive	Acute hepatic
Acute intermittent porphyria (AIP)	Autosomal dominant	Acute hepatic
Congenital erythropoietic porphyria (CEP) *	Autosomal recessive	Erythropoietic
Porphyria cutanea tarda Familial/Sporadic/HEP	Variable**	Non-acute hepatic
Hereditary coproporphyria (HC)	Autosomal dominant	Acute hepatic
Variegate porphyria (VP)	Autosomal dominant	Acute hepatic
Erythropoietic protoporphyria (EPP)	Autosomal dominant***	Erythropoietic

ALA: δ-aminolaevulinic acid; PBG: porphobilinogen; HEP: hepatoerythroporphyria.
** X-linked erythroid-specific transcription factor GATA binding protein 1 mutation has been reported in a few CEP cases. ** Autosomal dominant inheritance has been documented in familial porphyria cutanea and recessive inheritance has been documented in HEP. *** Erythropoietic protoporphyria is mainly related to the co-inheritance of both a ferrochelatase gene mutation and a weak normal ferrochelatase allele; autosomal recessive inheritance has been also reported in a few families.*

Each type of porphyria is the result of a specific enzymatic defect in the haem pathway (Figure 4; Table 1; (6)). The porphyrias are generally broadly classified as **acute (hepatic) porphyrias** and **cutaneous (erythropoietic) porphyrias**, based on the site of the overproduction and accumulation of the porphyrins (or their chemical precursors). Acute intermittent porphyria (AIP) (7) and the rare ALA dehydratase deficiency porphyria (ADP) (8) are associated with acute attacks only. Variegate porphyria (VP) (9) and hereditary coproporphyria (HC) (10) are associated with both acute attacks and/or skin lesions. Congenital erythropoietic porphyria (CEP), porphyria cutanea tarda (PCT), erythropoietic protoporphyria (EPP) and its variant, X-linked dominant protoporphyria (X-LDPP), present with skin lesions only. The pattern of pigment excretion in the different porphyrias is shown in Table 1, a summary of treatment approaches in Table 2 and a list of information resources in Table 3.

2.1 Acute hepatic porphyrias (AHP)

Acute attacks are identical in four of the hepatic porphyrias: acute intermittent porphyria (AIP), hereditary coproporphyria (HC), variegate porphyria (VP), and ALA dehydratase deficiency porphyria (ADP) (Figure 5). Except for ADP, an autosomal recessive disorder, the acute hepatic porphyrias are autosomal dominant conditions in which a 50% reduction in enzyme activity is brought about by a mutation in one of the alleles of the corresponding gene (8). The penetrance is low and about 90% of affected individuals never experience an acute attack (11). VP and HCP may also be associated with skin lesions, which are the only manifestation of the condition in 60% of VP patients (Figure 5). In most countries AIP is the commonest of the acute porphyrias (5).

2.2 Non-acute hepatic porphyria: porphyria cutanea tarda

PCT is due to decreased activity of urodecarboxylase (UROD, Figures 4 and 5; Table 1). There is photosensitivity leading to lesions areas exposed to light such as the backs of hands, face, neck and also in women the legs and backs of the feet (Figure 6A). Skin fragility is perhaps the most specific feature: minimal trauma is followed by superficial erosion which is soon covered by a crust. Bullae or vesicles usually appear after exposure to sun and take several weeks to heal, leaving hypo- or hyper-pigmented atrophic scars. White papules (milia) may develop in areas of bullae, particularly on the backs of the hands. Hypertrichosis is often seen on the upper cheeks, ears and arms. Increased pigmentation of sun-exposed areas is common.
This most common form of porphyria is a heterogeneous group including three types:

- The sporadic type (sPCT, type I; 80% of cases) is more often observed in male patients without a family history of the disease; its development appears to be

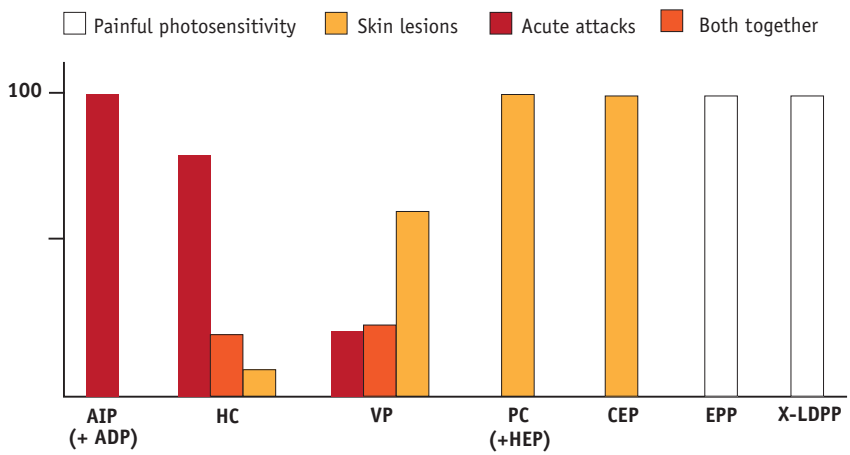
Table 2: **Management summary for acute attacks and cutaneous porphyria**

	Treatment	Measure	Safe drugs
Acute attacks (AIP, VP, HC)	Preventive	Prescribe drugs from safe drug list Avoid alcohol Avoid smoking and "soft drugs" Avoid dieting, fasting	-
	Specific	Repress haem synthesis	Haem arginate, haematin
	Supportive	Stop porphyrinogenic drugs Maintain fluid, calorie intake Treat symptoms - *Pain* - *Vomiting/sedation* - *Constipation* - *Hypertension/tachycardia* - *Convulsions*	 2 litres normal saline containing 5% dextrose or glucose Aspirin, paracetamol, dihydrocodeine, pethidine, morphine, diamorphine Promazine, chlorpromazine, cyclizine, ondansetron Bulk laxatives, senna Propanolol, atenolol, labetalol Monitor and correct hyponatraemia Diazepam (IV 10 mg once only), clonazepam, magnesium sulphate
Cutaneous porphyria *PCT*	Supportive	Sunlight avoidance Protective clothing Sunblocks (opaque sunscreens)	
	Preventive	Prescribe drugs from safe drug list Avoid alcohol Avoid smoking and "soft drugs" Avoid dieting, fasting	
	Specific	Iron and porphyrin depletion	Venesection 350-450 mL 2-weekly until remission Chloroquine 100 mg twice weekly until remission
CEP	Specific	For haemolytic anaemia To restore erythroid haem synthesis	Blood transfusion Bone marrow transplantation
EPP and X-LDPP	Specific	Increase tolerance to sunlight Deplete hepatic protoporphyrin Hepatic failure	β-carotene 100-300 mg daily Activated charcoal 40-50 g daily Liver transplantation

Table 3: Porphyria information resources

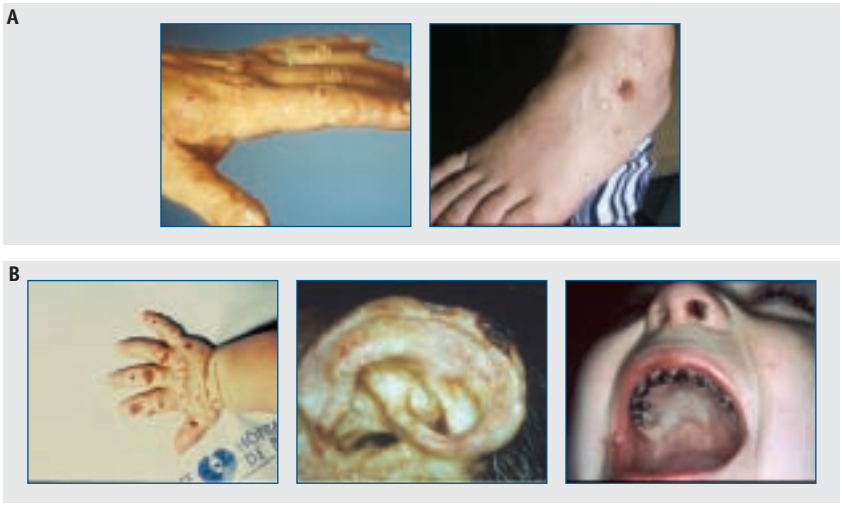
USA	American Porphyria Foundation PO Box 22712 Houston, Texas 77227 Website: www.enterprise.net/apf/index.html
Europe	European Porphyria Initiative Website: www.porphyria-europe.com
France	Centre Français des Porphyries CHU Louis Mourier 92701 Colombes Cedex Website: www.porphyrie.net (including a list of safe drugs: www.drugs-porphyria.org)
South Africa	Liver Research Centre University of Cape Town Cape Town; Website www.uct.ac.za/depts/liver/porphpts.htm

Figure 5: Clinical features in porphyrias



AIP: acute intermittent porphyria; ADP: ALA dehydratase porphyria; HC: hereditary coproporphyria; VP: variegate porphyria; PC: familial and sporadic porphyria cutanea tarda; HEP: hepatoerythropoietic porphyria; CEP: congenital erythropoietic porphyria; EPP: erythropoietic protoporphyria; X-LDPP: X-linked dominant protoporphyria

Figure 6: **Cutaneous symptoms in porphyrias**



***A**. Cutaneous symptoms (bullae) found in porphyria cutanea tarda, variegate porphyria, and hereditary coproporphyria. **B**. Clinical presentation of congenital erythropoietic porphyria (Günther's disease)*

related to some inducing compound such as alcohol, oestrogens, iron overload, or the hepatitis C virus (HCV). In this sporadic type, UROD activity is deficient only in the liver.

- The familial type (fPCT, type II, 20% of cases) has an earlier onset and is observed equally in both genders. In fPCT, there is a 50 per cent reduction of UROD activity in all tissues and this defect is inherited in an autosomal dominant pattern.
- Hepato-erythropoietic porphyria (HEP) is the rare autosomal recessive form that presents in infancy or childhood with severe blistering skin lesions accompanied by haematological abnormalities (e.g. haemolysis) which resemble congenital erythropoietic porphyria *(12)*.

Several risk factors are known to predispose patients to develop the enzyme deficiency associated with PCT. Variable degrees of liver dysfunction are common among patients with PCT, particularly in association with excessive alcoholic intake. The incidence of hepatic cancer among patients with PCT seems to be greater than in the general population. Among the other precipitating factors, oestrogens, iron overload, HCV, and to a lesser extent hepatitis B virus and HIV, are most frequently incriminated. Many drugs classified as porphyrinogenic in acute porphyrias will also precipitate or exacerbate PCT; however, most patients may receive these drugs (or

alcohol) over several years before developing PCT. Abnormal iron metabolism appears to be another factor precipitating clinical onset, probably related to oxidative radicals produced by reactive intracellular iron. Mutations of the HFE gene associated with haemochromatosis are found in fPCT and sPCT more commonly than in control populations, indicating that genetic factors unrelated to the haem biosynthesis pathway can predispose to PCT (13). A strong association has been found between HCV and PCT in several countries. Hepatitis B virus and HIV are not as closely associated with PCT as HCV but antibodies to HCV, HBV and HIV should be evaluated in each PCT patient at the time of diagnosis. These precipitating factors act either alone or in combination.

Urine contains increased concentrations of uroporphyrin and 7-carboxy-porphyrin; coproporphyrin, 5- and 6-carboxylic-porphyrins are moderately increased (Table 1). In the faeces, the dominant porphyrin excreted is often isocoproporphyrin. During clinical remission total porphyrin excretion decreases progressively and measurement of urinary porphyrins and ferritin are one of the best methods for following the effects of treatment. After a few months, urinary porphyrin levels appear normal but in the faeces copro- and isocoproporphyrin may remain increased for a long period.

All patients with PCT should first be treated for infectious disease (e.g. HCV, HIV), and advised to avoid precipitating factors (e.g. alcohol, pills, porphyrinogenic drugs) and exposure to sunlight until clinical and biological remission has been obtained by treatment.

Phlebotomy is at present the treatment of choice (Table 2), even when serum iron or ferritin levels are not increased. A unit of blood (350-450 mL) is removed at weekly intervals until transferrin saturation falls to 16% or less or the ferritin level is reduced to the lower limit of normal. Urine porphyrin levels are monitored every three months: clinical and biological remissions are usually obtained within 3-6 months.

When phlebotomy is contraindicated (anaemia, cardiac or pulmonary disorders, age) low-dose chloroquine therapy (100 mg twice weekly), which complexes with porphyrin and slowly mobilises it from the liver, is the favored alternative. Duration of treatment and relapse rate are only marginally greater than with venesection. High-dose chloroquine treatment must be avoided because it causes a hepatitis-like syndrome in patients with PCT. In severe cases, the combination of phlebotomy and chloroquine therapy is often used with good results. Because of the high incidence of liver disease, liver function should be followed.

Skin blisters in patients on chronic dialysis may be caused either by PCT or pseudoporphyria. The differential diagnosis should be performed by porphyrin analysis in plasma or faeces. In these cases, erythropoietin supplementation, in a gradually increased dosage, is given to increase erythropoiesis, thereby depleting excessive body iron stores.

2.3 Erythropoietic porphyrias (CEP, EPP and X-LDPP)

2.3.1 Congenital erythropoietic porphyria (CEP)

CEP is a rare autosomal recessive disorder resulting from a marked deficiency of uroporphyrinogen III synthase activity (Figure 1). Skin blisters are observed in the neonatal period or in early infancy (Figure 6B). It is are serious, chronic progressive, and mutilating disorders associated with haemolytic anaemia. Urine has a reddish brown color from the first day of life and exhibits a purple fluorescence under long UV light. The diagnosis is confirmed by a characteristic porphyrin pattern in urine, plasma and faeces (Table 1). Treatment of CEP involves skin protection and blood transfusions to maintain the haemoglobin concentration. Allogenic bone marrow transplantation has been successful in several patients with moderate to severe disease (Table 2).

Very recently, a 3-year-old boy with the clinical phenotype of CEP was described (14). No mutation or rearrangement in the *UROS* gene was identified. Instead, a novel R216W germ line mutation in the X-linked erythroid-specific transcription factor GATA binding protein 1 (GATA-1) was observed. The R216 residue is located in a region of the N-terminal zinc finger of the GATA-1 protein that is highly conserved. It might impair the function of the alternative erythroid-specific promoter of the UROS gene that contains several GATA-1 binding sites (15). Interestingly, a pathogenic mutation in one of these GATA-1 binding elements was found in a CEP patient (16).

2.3.2 Erythropoietic protoporphyria (EPP)

Erythropoietic protoporphyria (EPP) (MIM 177000) is an inherited disorder caused by partial deficiency in mitochondria of ferrochelatase (FECH) (EC 4.99.1.1), the terminal enzyme of haem biosynthesis (Figure 5; Table 1). Accumulation of PPIX in erythrocytes and other tissues leads to life-long photosensitivity and, in about 2% of patients, severe liver disease. Most patients have autosomal dominant EPP in which clinical expression normally requires co-inheritance of a *FECH* mutation that abolishes or markedly reduces FECH activity *trans* to a hypomorphic *FECH IVS3-48C* allele carried by about 11% of Western Europeans (17). About 4% of families have autosomal recessive EPP. Clinical manifestation of EPP begins in childhood with acute and severely painful photosensitivity and history of burning in areas of skin exposed to sunlight. Pain is usually followed by oedema, erythema and swelling. Repeated exposures lead to chronic changes giving the skin a waxy, thickened appearance with faint linear scars.

Urine porphyrin levels are normal and the diagnosis is based on increased free PPIX levels in erythrocytes and in plasma, identified by its characteristic fluorescent

emission peak (Table 1). Patients often exhibit a slight microcytic, hypochromic anaemia. Liver dysfunction has been reported in up to 20% of EPP patients and hepatic failure in less than 5%. The liver dysfunction is caused by the accumulation of protoporphyrin in hepatocytes resulting in cell damage, cholestasis and further retention of protoporphyrin. EPP patients may develop gallstones formed from protoporphyrin and are at increased risk of cholelithiasis.

Acute burning pain is ameliorated by application of cold water. Avoidance of sunlight is the mainstay of management. Oral β-carotene (75-200 mg per day; optimal blood concentration of 11-15 µmol/L), which acts as a singlet oxygen trap, improves light tolerance in about one third of patients (Table 2). It is impossible to predict those patients who will develop severe liver disease, and management should include annual biochemical assessment of liver function. When liver dysfunction appeared, treatment with cholestyramine which deplete hepatic protoporphyrin, or activated charcoal, which binds protoporphyrin in the gut, interrupting the enterohepatic circulation, should be attempted but their efficacy is not proved.

Once liver failure is advanced, transplantation is usually the only treatment likely to ensure survival.

2.3.3 X-linked dominant protoporphyria (X-LDPP)

Each of the seven inherited porphyrias previously described results from a partial deficiency of an enzyme of haem biosynthesis. Recently we have described a previously unrecognised form of porphyria in 8 families with a clinical presentation very similar to EPP but with no ferrochelatase mutation. In these patients, we identified deletions in *ALAS2* resulting in frameshifts that lead to replacement or deletion of the 19-20 C-terminal residues of the enzyme (18). ALAS2 is essential for haemoglobin formation by erythroid cells and ALAS1 cannot replace this function. No mutations have been identified in *ALAS1* but pathogenic mutations in the 14.4 kb, 11 exon-containing *ALAS2* cause X-linked hereditary sideroblastic anaemia (XLSA) (MIM 301300) with iron overload. Patients with these C-terminal deletion or replacement in the ALAS2 protein had high levels of erythrocyte PPIX and acute photosensitivity clinically indistinguishable from that of typical EPP (Figure 5). Prokaryotic expression studies showed that mutations markedly increase ALAS2 activity. Gain of function mutations had not been previously identified in genes of the haem biosynthetic pathway. This discovery strongly suggests that protoporphyrin and its zinc chelate accumulate in erythrocytes because the rate of ALA formation is increased to such an extent that insertion of Fe^{2+} into PPIX by FECH becomes rate-limiting for haem synthesis. The disruption of the C-terminal region of ALAS2

leads to the production of protoporphyrin in excess of the amount required for haemoglobinisation and in quantities sufficient to cause photosensitivity and liver damage, in spite of normal FECH activity. In these families, both sexes were affected and patients had neither anaemia nor iron overload. Instead, there was some evidence for diminished iron stores particularly in males. Similar abnormalities have been observed in typical EPP and may result from accumulation of PPIX rather than FECH deficiency. Excretion of ALA and other protoporphyrin precursors is normal in X-LDPP indicating that most of the ALA produced by erythroid cells is metabolised to PPIX. Some is used for haemoglobin synthesis but the fate of the rest is uncertain. Since FECH activity in erythroid cells exceeds that required for haemoglobin synthesis, some may be converted to free haem and exported from the cytoplasm. However, the accumulation of zinc protoporphyrin in X-LDPP, indicating utilisation by FECH of its alternative metal substrate, suggests that formation of excess haem may be prevented by lack of available iron. These patients showed a phenotype of iron deficiency and microcytic anaemia. The 26 C-terminal amino acids of ALAS2 are highly conserved and have diverged from ALAS1 which suggests that this sequence may have an important, but unknown, erythroid-specific function. During erythropoiesis, tight co-ordination of substrate supply to FECH normally prevents accumulation of toxic amounts of PPIX. Co-ordination is largely achieved through iron-dependent post-transcriptional regulation of synthesis of ALAS2. The discovery of gain of function mutations in *ALAS2* defines a new type of human porphyria, provides new information about the regulation of substrate supply for haem synthesis during erythroid differentiation and identifies a potential tool for increasing erythroid haem synthesis in experimental systems.

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Multiple Choice Questionnaire

To find the correct answer, go to http://www.esh.org/iron-handbook2009answers.htm

1. What is the main molecule involved in the regulation of erythroid haem biosynthesis?

a) Globin chains ☐

b) Protoporphyrin ☐
c) Haem itself ☐
d) Iron ☐

2. Among these porphyrias which one is not erythroid?
a) Acute intermittent porphyria ☐
b) Günther disease ☐
c) XLDPP ☐
d) Protoporphyria ☐

3. Regarding excretion of porphyrins and haem precursors, which one of the following is never found in urine of porphyric patients?
a) δ-aminolaevulinic acid ☐
b) Porphobilinogen ☐
c) Protoporphyrin ☐
d) Coproporphyrin ☐

4. Iron overload is a common feature in which one of the following?
a) Erythropoietic protoporphyria ☐
b) Sporadic porphyria cutanea ☐
c) Hereditary coproporphyria ☐
d) Congenital erythropoietic porphyria ☐

5. All the following porphyrias *except one* result from a specific decrease in the activity of one of the enzymes of the haem biosynthetic pathway; which one is the exception?
a) Erythropoietic protoporphyria ☐
b) X-linked dominant protoporphyria ☐
c) Variegate porphyria ☐
d) Congenital erythropoietic porphyria ☐