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Heme biosynthesis and the porphyrias

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Abstract

Porphyrias, is a general term for a group of metabolic diseases that are genetic in nature. In each specific porphyria the activity of specific enzymes in the heme biosynthetic pathway is defective and leads to accumulation of pathway intermediates. Phenotypically, each disease leads to either neurologic and/or photo-cutaneous symptoms based on the metabolic intermediate that accumulates. In each porphyria the distinct patterns of these substances in plasma, erythrocytes, urine and feces are the basis for diagnostically defining the metabolic defect underlying the clinical observations.

Porphyrias may also be classified as either *erythropoietic* or *hepatic*, depending on the principal site of accumulation of pathway intermediates. The erythropoietic porphyrias are congenital erythropoietic porphyria (CEP), and erythropoietic protoporphyria (EPP). The acute hepatic porphyrias include ALA dehydratase deficiency porphyria, acute intermittent porphyria (AIP), hereditary coproporphyria (HCP) and variegate porphyria (VP). Porphyria cutanea tarda (PCT) is the only porphyria that has both genetic and/or environmental factors that lead to reduced activity of uroporphyrinogen decarboxylase in the liver.

Each of the 8 enzymes in the heme biosynthetic pathway have been associated with a specific porphyria (Table 1). Mutations affecting the erythroid form of ALA synthase (ALAS2) are most commonly associated with X-linked sideroblastic anemia, however, gain-of-function mutations of ALAS2 have also been associated with a variant form of EPP. This overview does not describe the full clinical spectrum of the porphyrias, but is meant to be an overview of the biochemical steps that are required to make heme in both erythroid and non-erythroid cells.

1. Introduction

Heme (iron protoporphyrin IX, Fig. 1) is essential for all cells and functions as the prosthetic group of numerous hemoproteins such as hemoglobin, myoglobin, respiratory cytochromes, cytochromes P450 (CYPs), catalase, peroxidase, tryptophan pyrrolase, and nitric oxide synthase. The largest daily output of heme is from the bone marrow to meet the requirement for hemoglobinization of the red blood cells [1]. Heme synthesized in the liver is mainly required for CYPs, which are located primarily in the endoplasmic reticulum where they turn over rapidly and oxidize a variety of chemicals, including drugs, environmental carcinogens, endogenous steroids, vitamins, fatty acids, and prostaglandins [2].

Heme is a term used for ferrous protoporphyrin IX (PPIX) that is easily oxidized *in vitro* to hemin, termed- ferric PPIX. In heme the ferrous iron atom (Fe^{2+}) has 6 electron pairs, 4 are bound to the pyrrolic nitrogens of the porphyrin macrocycle, leaving two unoccupied electron pairs, one above and the other below the plane of the porphyrin ring. One of the unoccupied pairs is coordinated to a histidine residue of globin chains, and the other coordination site is used to bind oxygen.

1.1. Physiological heme biosynthesis

The eight enzymatic steps involved in heme biosynthesis in eukaryote cells are shown in Fig. 1. The first and last three enzymes steps are carried out in the mitochondria while the intermediate four steps are performed in the relatively reducing environment of the cytosol. The erythroid compartment has evolved to accommodate a burst of heme production for 3–5 days to form a red blood cell (RBC) while the genes expressed in the liver need to adjust heme synthesis over the life of the hepatocyte for the ever changing demand of the detoxifying enzymes of the cytochrome P450 class, each containing a heme molecule as a cofactor [3].

1.1.1. δ -Aminolevulinatase synthase (succinyl CoA: glycine C-succinyl transferase, decarboxylating; EC 2.3.1.37)—The first and rate limiting enzyme in the heme biosynthetic pathway is the condensation of glycine and succinyl-CoA to form ALA (Fig. 1), pyridoxal 5'-phosphate is a required cofactor. Mammalian ALAS is located in the mitochondrial matrix [4], is synthesized as a precursor protein in the cytosol and transported into mitochondria. Distinct ALAS genes encode the housekeeping (tissue-nonspecific, OMIM 125290) and erythroid specific forms of the enzyme (erythroid specific, OMIM 301300) [5,6]. The human *ALAS* genes appear to have evolved due to a gene duplication event, where each gene went on to develop independent regulatory control [7]. The nucleotide sequences for the ALAS2 and the ALAS1 isoforms are approximately 60 percent similar. Human ALAS2 encodes a precursor protein of 587 amino acids, with high homology (approximately 73 percent) between proteins after residue 197 of the housekeeping form [8].

In the liver ALAS1 activity is regulated by the rate of synthesis (transcription), import into the mitochondria (post-translational), folding and cofactor insertion. Heme works to provide negative feedback on each of these steps through differing mechanisms. Many chemicals, hormones and drugs increase the synthesis of hepatic CYPs, which in turn increases the demand for heme that is met by induction ALAS1 gene [9]. The ALAS1 gene contains upstream enhancer elements that are responsive to inducing chemicals through an interaction with the pregnane X receptor (PXR). Therefore, ALAS1 and CYPs are subject to direct induction by xenobiotics and steroid hormones [10]. Chemical exposures that induce hepatic heme oxygenase and accelerate the destruction of hepatic heme, or inhibit heme formation, can also induce hepatic ALAS1.

Thus, hepatic heme availability is balanced between synthesis, which is controlled primarily by ALAS1, and degradation by heme oxygenase, both of which are regulated by heme at different intracellular concentrations. ALAS1 is also up-regulated by the peroxisomal

proliferator-activated cofactor 1 α (PGC-1 α) [11], a co-activator of nuclear receptors and transcription factors [12]. Transcriptional regulation of ALAS1 by PGC-1 α is mediated by interaction of Nrf-1 (nuclear regulatory factor 1) and FOXO-1 (a forkhead family member) with the ALAS1 promoter [13]. When glucose levels are low, transcription of PGC-1 α is up-regulated [14,15] in turn increasing ALAS1, which might precipitate an attack of acute porphyria in an individual with the appropriate inherited enzyme deficiency. This form of regulation is also the basis of therapeutic use of glucose loading to suppress a porphyric attack [16,17].

The promoter for *ALAS2* contains several erythroid-specific cisacting elements including GATA-1 and an NF-E2 binding sites [7,18]. Both GATA-1 and NF-E2 are erythroid transcription factors that also bind other DNA sites, such as the promoters of the human β -globin, PBGD and uroporphyrinogen synthase (UROS) genes [19]. Synthesis of ALA is tied to the availability of iron in erythroid cells. A system of mRNA secondary structures and proteins that bind to these structural elements has evolved to couple iron availability to protoporphyrin IX production. The ALAS2 mRNA contains an Iron Responsive Element (IRE) in the 5'-untranslated region, this secondary structure forms a stem-loop structure (REF). There are two proteins that are able to bind to these IREs, named Iron Responsive Element Binding Protein 1 and 2 (IRP1 or IRP2). When cellular concentrations of iron are low the IRPs bind the 5'-IRE and prevent the ribosome from translating the message, decreasing ALA production. When cellular iron concentrations rise the IRPs are either degraded (IRP2) or modified by the insertion of an 4Fe-4S cluster (IRP1) that prevents the IRE-binding proteins from interacting with the IRE, allowing the mRNA to be translated into protein. This regulatory loop is designed to feed back so that only when iron is sufficient will protoporphyrin IX be made [20–22].

Mutations in ALAS2 are associated with X-linked sideroblastic anemia (XLSA); many are in exon 9, which encodes the binding site for pyridoxal 5'-phosphate. At least one mutant (D190V) identified in a patient with pyridoxine-refractory XLSA [23], failed to associated with SCS- β A. The mature D190V mutant protein, but not its precursor protein, underwent abnormal processing, indicating that appropriate association of SCS- β A and ALAS2 is necessary for functioning of ALAS2 in mitochondria [24]. Gain-in-function mutations of ALAS2 have recently been identified in patients with a variant form of EPP [25].

1.1.2. δ -Aminolevulinatase (PBG synthase; δ -aminolevulinatase hydrolase; EC 4.2.1.24)—ALA dehydratase (ALAD) is a cytosolic enzyme that catalyzes the condensation of two molecules of ALA to form the monopyrrole PBG, with removal of two molecules of water (OMIM 125270) (Fig. 1). The enzyme functions as a homooctomer, and requires an intact sulfhydryl group and zinc for activity. Mutations associated with ALAD porphyria favor formation of the less active hexamer [26–28]. Lead inhibits ALAD activity by displacing the catalytically important zinc [29], leading to urinary ALA and coproporphyrin excretion. In cases of lead poisoning the urinary ALA is in significant excess to the amount of urinary PBG, a laboratory difference that distinguishes AIP from lead poisoning. Neurologic symptoms are thought to be due to the increased ALA in plasma, however calcium containing enzymes may also be affected providing a unique clinical picture when compared to the acute porphyrias [30]. Succinylacetone is a potent inhibitor of

ALAD, [31,32] and is a biproduct of the enzyme deficiency in hereditary tyrosinemia type I, this acts as a substrate analogue for ALA [33,34].

Human ALAD mRNA has an open-reading frame of 990 bp, encoding a protein with an M_r of 36,274, and has a high degree of homology to the rat enzyme [35,36]. The gene for human ALAD is localized at chromosome 9p34 [37]. The mRNA for ALAD has two splice variants, a housekeeping (1A) and an erythroid-specific (1B) form [37]. Both humans and mice the promoter region upstream of exon 1B contains GATA-1 sites, providing for significant tissue-specific control of these transcripts [38].

1.1.3. PBG deaminase (hydroxymethylbilane synthase; PBG ammonialyase (polymerizing), EC 4.3.1.8)—Deamination and condensation of four molecules of PBG to yield the linear tetrapyrrole hydroxymethylbilane (HMB) [39] (Fig. 1) are performed by PBG deaminase (PBG-D) (OMIM 609806). This activity was previously known as *preuroporphyrinogen I synthase*. Enzyme activity is measured using a coupled reaction that converts HMB to uroporphyrin I, a cyclic tetrapyrrole that has intrinsic fluorescent properties.

PBG-D uses a unique cofactor, dipyrromethane, a dipyrrole that binds at the catalytic site and allows four additional pyrrole units to concatamerize until six pyrroles (including the dipyrrole cofactor) are assembled in a linear fashion. The terminal four tetrapyrroles, hydroxymethylbilane (HMB) is the cleaved and released [40]. Studies have shown that the apo-deaminase generates the dipyrrole cofactor forming the holo-enzyme, and this occurs more readily from HMB than from PBG [41].

The human PBG-D gene maps to chromosome 11q23→11qter, [42] and consists of 15 exons spanning 10 kb of DNA [43]. Erythroid-specific and housekeeping specific mRNAs are produced by alternative splicing. Two distinct primary mRNA transcripts are derived from individual promoters within the gene [44]. The housekeeping promoter is upstream of exon 1 and is active in all tissues, while the erythroid-specific promoter, upstream of exon 2, is active only in erythroid cells. Proteins encoded by the housekeeping and erythroid-specific isoforms contain 361 and 344 amino acid respectively [45]. Erythroid-specific transcription factors, GATA-1 and NF-E2, recognize sequences in the erythroid promoter [46]. An additional in-frame AUG codon present 51 bp upstream from the initiating codon of the erythroid cDNA accounts for the additional 17 amino acid residues at the N-terminus of the housekeeping isoform. Accordingly, a splice site mutation at the last position of exon 1, or a base transition in intron 1, in certain patients with AIP results in decreased PBG deaminase expression in nonerythroid tissues including the liver, but not in erythroid cells, because transcription of the gene in erythroid cells starts downstream of the site of [47].

1.1.4. Uroporphyrinogen III synthase (uroporphyrinogen III cosynthase; EC 4.2.1.75)—Uroporphyrinogen III synthase (UROS), a cytosolic enzyme, catalyzes the formation of uroporphyrinogen III from hydroxymethylbilane (OMIM 606938). The process involves an intramolecular rearrangement that affects only ring D of the porphyrin macrocycle [39] (Fig. 1). When activity of UROS is limited, HMB spontaneously closes and forms uroporphyrinogen I, where the acetate and propionate sidechains of each pyrrole unit

are symmetric. All isomers of uroporphyrinogen are substrates for UROD. However, because coproporphyrinogen I is not a substrate for coproporphyrinogen oxidase the type I porphyrinogen isomers can't be further metabolized. Only the type III isomers are precursors of heme, this reaction imparts the stereo specificity that is maintained throughout the remainder of heme biosynthesis.

The UROS cDNA has an open-reading frame of 798 bp, and the predicted protein product consists of 263 amino acid residues, with an M_r of 28,607 [6]. The amino acid compositions of the hepatic and the purified erythrocyte enzyme are essentially identical, and no tissue-specific isoforms have been described. Among all the gene comparisons Homology of the UROS protein between differing species is below 10%, depending on the number and divergence of the species being compared. Despite the high primary sequence variation between species the crystal structures of uroporphyrinogen III cosynthase from several eukaryotic and bacterial (*Thermus thermophilus*) have been solved and they are very similar [48,49]. The structure supports a mechanism that includes the formation of a spirolactam intermediate by positioning the A and D rings such that the non-catalytic closure, to form uroporphyrinogen I, is not possible [49].

1.1.5. Uroporphyrinogen decarboxylase (EC 4.1.1.37)—UROD is a cytosolic enzyme that catalyzes the sequential removal of the four carboxylic groups of the carboxymethyl side chains in uroporphyrinogen to yield coproporphyrinogen (OMIM 613521) (Fig. 1). Human UROD is a 42-kDa polypeptide encoded by a single gene containing 10 exons spread over 3 kb and functions as a homodimer [50]. The gene has been mapped to chromosome 1p34 [51]. Decarboxylation reactions yield 7-, 6-, 5-, and 4-carboxylated porphyrinogens, starting at the asymmetric D-ring and proceeding in a clockwise manner to the A ring. Partially decarboxylated intermediates are oxidized to the corresponding porphyrins and are eliminated from the cell into the plasma. These intermediates are primarily produced in liver, and are eliminated through the plasma, urine and stool in human porphyria cutanea tarda (PCT). An inhibitor of UROD activity, a partially oxidized substrate molecule [52], is produced in response to halogenated polycyclic aromatic hydrocarbons such as hexachlorobenzene, dioxin and polychlorinated biphenyls and other compounds able to activate the Ah receptor [53], and is believed to explain UROD inhibition in human PCT [52].

Although the UROD gene contains two initiation sites, both sites are used with the same frequencies in all tissues, and the gene is transcribed into a unique mRNA [54]. Recombinant human UROD has been purified to homogeneity and crystallized, the crystal structure was determined at 1.60-Å resolution [55]. The purified protein is dimeric with a dissociation constant of 0.1 μ M [56]. Both of the biologically produced isomers of uroporphyrinogen I and III can be metabolized by UROD, however, only the III isomer is able to be further metabolized to heme [57].

1.1.6. Coproporphyrinogen oxidase, (EC 4.1.1.37)—Coproporphyrinogen oxidase (CPO) is associate with the inner mitochondrial membrane of the mitochondria in mammalian cells [58]. The enzyme catalyzes the removal of the carboxyl group and two hydrogens from the propionic side chains of pyrrole rings A and B, forming vinyl groups at

these positions (OMIM 612732). The enzyme is isomer specific for coproporphyrinogen III, yielding protoporphyrinogen IX (see Fig. 1). Human CPO has been cloned and the gene is assigned to chromosome 3q12, spans approximately 14 kb, and consists of seven exons and six introns [59–61]. A mitochondrial targeting sequence, which is removed during transport into the intermembrane space of mitochondria yields a mature protein of 354 amino acid residues ($M_r = 36,842$). A similar protein structure is present in the mouse where the pre-protein is 354 amino acid residues in length ($M_r = 40,647$), with a mitochondrial targeting sequence of 31 amino acid residues, that is processed on import. The mature protein is 323 amino acid residues ($M_r = 37,225$) and is associated with the innermitochondrial membrane facing the mitochondrial matrix [62]. Transcriptional regulatory elements present in the GC-rich promoter include six Sp1, four GATA-1, one CACCC site, and the specific *CPO* gene promoter regulatory element (CPRE) [63]. CPRE binds specifically to a CPRE-binding protein, which has a leucine-zipper-like structure and serves as a DNA sequence-specific transcription factor that regulates gene expression [63]. Tissue-specific expression of CPO is highly regulated, proteins bound to the Spl-like element, CPRE and GATA-1, function in a cooperative fashion to ensure proper *CPO* gene expression in multiple cell types. The CPRE-binding protein by itself plays a principal role in basal expression of CPO in nonerythroid cells [64]. *CPO* mRNA increases during erythroid cell differentiation [60,61]. A five-base insertional mutation in the middle of this presequence has been described in one patient with HCP [65]. The structure of the mature CPO protein has been solved from several organisms [66,67] confirming the dimeric interface and location of the active site pocket. It has been proposed that the coproporphyrinogen III substrate normally remains bound for both of the decarboxylation reactions.

1.1.7. Protoporphyrinogen oxidase (EC 1.3.3.4)—The penultimate step in heme biosynthesis is the oxidation of the fully reduced porphyrinogen, protoporphyrinogen IX, to the fully oxidized protoporphyrin IX, by the removal of six hydrogen atoms (OMIM 600923). This reaction is mediated by the mitochondrial enzyme protoporphyrinogen oxidase (PPO) (see Fig. 1). Human gene for PPO and the mature cDNA have been cloned and has been mapped to chromosome 1q22 [68,69]. The protein encoded by PPO consists of 477 amino acids with an M_r of 50,800 [70]. Sequences required for import into the mitochondria have been identified [71,72]. Expression of PPO is upregulated, approximately four-fold, in the developing erythron from two GATA-1 binding sites located in exon 1 [73]. The deduced protein from many mammalian species exhibits a high degree of homology over its entire length to the amino acid sequence of protoporphyrinogen oxidase encoded by the *HEMY* gene of *Bacillus subtilis*. Protoporphyrinogen oxidase has been crystallized and the structure shows that the enzyme is a homodimer. The protein has been shown to be part of a larger complex that includes ABCB10, Mitoferrin-1, Ferrochelatase and TMEM14 [74,75]. This complex is thought to be involved in coordinating the import of substrates, protoporphyrin IX, and iron to prevent the “free” association within the mitochondrial matrix since inherent properties of each of the substrates makes them potentially toxic.

1.1.8. Ferrochelatase (EC 4.99.1.1)—The final step of heme biosynthesis is the insertion of iron into protoporphyrin IX (OMIM 612386). This reaction is catalyzed by the mitochondrial enzyme ferrochelatase (see Fig. 1). Ferrochelatase utilizes protoporphyrin IX,

rather than its reduced form, as substrate, but requires the reduced ferrous form of iron [76]. The gene encoding human ferrochelatase has been assigned to chromosome 18q [77,78]. The human ferrochelatase gene contains a total of 11 exons and has a minimum size of approximately 45 kb [77]. Two ferrochelatase mRNA species, approximately 2.5 kb and approximately 1.6 kb in size, are produced from the same gene by utilization of two alternative polyadenylation sites in the mRNA. A major site of transcriptional initiation originates from an adenine, 89 bp upstream from the translation-initiating ATG. The promoter region contains a potential binding site for several transcription factors, Sp1, NF-E2, and GATA-1, but not a typical TATA or CAAT sequence. Alternative splicing due to mutations within the third intron produce a frame shift, this allele is present in the population at varying frequency [79–81].

The crystal structure of *B. subtilis* ferrochelatase has been determined at 1.9-Å resolution [82]. Subsequently the structure of human ferrochelatase was solved and the location of the substrate binding site determined. The enzyme functions as a homodimer and is associated with the matrix side of the inner mitochondrial membrane [82]. The mechanism of catalysis has not been identified nor has a function been assigned to the 2Fe-2S cluster that is present in human ferrochelatase. Lead inhibits ferrochelatase, and a structure of the protein-lead complex has been solved indicating a critical role for the pi helix in catalysis [83]. Ferrochelatase seems to have a structurally conserved core region that is common to the enzyme from bacteria, plants, and mammals.

2. Pathologic heme biosynthesis

Defects in any one of the eight steps in heme biosynthesis may lead to a *porphyric* disorder. The diseases can be classified depending on if they are erythroid or hepatic in nature as well as if they have neurologic manifestations or photo-toxic phenotypes, and in some cases both (Table 1).

Two of the porphyrias have an erythroid etiology; Congenital Erythropoietic Porphyria (CEP) or Günther Disease, and Erythropoietic Protoporphyrinemia (EPP). CEP is one of the least common porphyrias but the severe photomutilation on sun exposed areas of the body (head, hands, feet) often leads to a very dramatic disfigurement and is thus often referenced. EPP is the most common porphyria in children, and third in most common porphyria overall.

An erythropoietic component may be important in ALA-Dehydratase Porphyria (ADP) and in cases where the subject is homozygous for mutations in one of the genes normally associated with a hepatic porphyria, such as Hereditary Erythropoietic Porphyria (HEP), the homozygous form of Porphyria Cutanea Tarda (PCT), Acute Intermittent Porphyria (AIP), Hereditary Coproporphyrinemia (HCP) and Variegated Porphyria (VP), as indicated by substantial increases in erythrocyte zinc protoporphyrin. The involvement of the erythroid compartment is often dependent on the amount of residual enzymatic activity present in a homozygous or compound heterozygous state.

2.1. Acute porphyrias

Defects in four of the steps in heme biosynthesis (steps 2, 3, 6 and 7, see Fig. 1) can be termed “acute porphyrias” and are distinctive for intermittent neurological symptoms that usually occur acutely due to accumulation of the intermediates ALA and PBG. Lead poisoning can also present with a similar neurologic phenotype; as can hereditary tyrosinemia type B [33].

2.1.1. Step 2) δ -Aminolevulinatase (deficiency) porphyria (ADP)—It

is an autosomal recessive disorder resulting from severe deficiency of ALA dehydratase activity (Fig. 1). This is the rarest of the porphyrias, with few cases documented at the molecular level [84]. The molecular defect in five cases was compound heterozygosity for two distinct ALA dehydratase mutations of the ALA dehydratase gene [84,85]. Four were males (3 in Germany and one in the U.S.) with onset of symptoms in their teens, whereas one Swedish case developed severe symptoms in infancy [86]. The sixth patient was a Belgian male who developed ADP at age 63 years and was found to have two inherited base transitions in one allele, and was therefore heterozygous for ALA dehydratase deficiency [87,88]. He also developed polycythemia and his erythrocyte ALAD activity was <1 percent of normal, while lymphocyte ALAD activity was greater >20 percent, suggesting that clonal evolution was present that led to the observed phenotype. In this case, the ALAD deficiency was clinically silent until there was expansion of a clone of erythroid cells that carried the mutant ALAD allele [89].

The highly heterogeneous nature of this very rare disease is observed even at the molecular level, with a total of 11 mutant alleles identified in these 6 patients [85]. Additional mutation have been found in healthy individuals with significantly decreased ALAD activity (~12 % normal), that was detected by ALAD measurement during neonatal screening for hereditary tyrosinemia [90]. With the advent of genome sequencing additional polymorphisms and mutations have been identified however, in heterozygosity there is no associated phenotype.

Human ALAD is a homo octamer, each subunit has two zinc binding sites. Lead can bind and displace zinc in at least one of these sites resulting in diminished enzymatic activity. Some mutations found in ADP may affect zinc binding, or favor formation of a hexameric enzyme with decreased activity rather than the fully active octomer. Thus, ADP has been described as a conformational disease [26].

ADP is classified as one of the hepatic porphyrias because it closely resembles the other acute porphyrias. However, the site of overproduction of ALA has not been clearly established [91]. Substantial increases in erythrocyte zinc protoporphyrin also suggests an erythroid component. The excess urinary coproporphyrin III in ADP may originate from metabolism of ALA to porphyrinogens in a tissue other than the site of ALA overproduction. ALA loading in normal subjects was shown cause to substantial coproporphyrinuria [92] The pathogenesis of the neurologic symptoms is poorly understood, as in other acute porphyrias.

2.1.1.1. Diagnosis: A biochemical diagnosis of ADP includes demonstration of markedly deficient erythrocyte ALA dehydratase activity, marked elevation in urinary ALA and

coproporphyrin III and erythrocyte zinc protoporphyrin, with little or no increase in urinary porphobilinogen. Erythrocyte ALA dehydratase activity is approximately half-normal in both parents. Lead poisoning is differentiated by increased blood lead and restoration of ALAD activity *in vitro* by reduced glutathione or dithiothreitol [84,93]. Although biochemical measurements can strongly suggest ADP, the diagnosis must be confirmed by DNA studies.

Patients with hereditary tyrosinemia type I may also have ALAD inhibition and increased excretion of ALA [33] Succinylacetone, a structural analogue of ALA and a potent ALAD inhibitor, accumulates as a result of an inherited deficiency of fumaryl acetoacetate hydrolase in these patients. A diagnosis of tyrosinemia can be made by demonstrating succinylacetone in urine by measuring ALAD activity in normal blood after addition of a patient's urine. ALA dehydratase protein is not reduced in this disease [94].

2.1.2. Step 3) Acute Intermittent Porphyria (AIP)—It is an autosomal dominant disorder due to a partial deficiency of PBG deaminase (Fig. 1). The acute attacks present with severe abdominal pain that is of neurological origin and are manifest after puberty. While this is a dominantly transmitted disease, less than 10% of individuals who inherit the enzyme deficiency never develop symptoms. The first case of acute porphyria was described in 1889 by Stokvis [95] who noted a relationship of the symptoms to the drug sulfonal, which is related to the barbiturates, these early drugs were strong inducers of cytochrome P450 enzymes (CYPs).

Up to 300 PBGD mutations have been described in AIP. The disease occurs in all races, but clusters due to founder effects occur in some countries. The prevalence of AIP was estimated to be 1–2 per 100,000 in Europe, [96] and 2.4 per 100,000 in Finland [97]. A notable cluster due to a prevalent founder mutation in northern Sweden is associated with a disease prevalence of one per 1500 [98]. The prevalence of low PBG deaminase activity, which includes latent gene carriers of AIP, is as high as one per 500 in the general population of Finland [99]. Based on DNA studies, the minimal prevalence of the AIP-associated genes in France has been calculated to be one per 1,675 [100].

Low activity of PBGD is *not sufficient* to cause clinical expression of AIP, and most individuals who inherit this enzyme deficiency remain healthy with normal porphyrin precursor excretion throughout life. Most hepatic heme synthesis is utilized for as a cofactor for the abundant CYPs that are used in the detoxification of most drug metabolites. Therefore, many drugs and hormones are inducers of ALAS because they are inducers of CYPs and increase the demand for heme synthesis [101].

Concentrations of ALA in the plasma and urine are increased in a number of disorders with similar neurological manifestations, including the acute porphyrias, lead poisoning and hereditary tyrosinemia type 1 [33]. These observations support the conclusion that ALA accumulation is the cause of the symptoms. Cells readily take up ALA that is then converted to porphyrin, which in turn may have toxic potential. This is the basis for much of the photodynamic therapy [102]. ALA has a very similar structure to γ -aminobutyric acid (GABA) and has been shown to interact with GABA receptors [103,104]. When patients are given

ALA orally or intravenously in therapeutic doses there have been no reported side effects that mimic the acute attack, however, they can become photosensitive due to accumulation of tetrapyrroles [105].

Several theories have been proposed to explain neurological dysfunction in the acute porphyrias include; [1] heme pathway intermediates or derivatized intermediates that are neurotoxic yet none are conclusive; [2] deficiency of PBG deaminase, in the nervous system, limits heme synthesis and formation of important hemoproteins; [3] mal-regulation of heme and hemoprotein synthesis in nervous tissue, is difficult to study, and there is little direct evidence for this hypothesis; [4] impaired hepatic heme synthesis during an attack may lead to decreased activity of hepatic tryptophan pyrrolase, which might increase levels of tryptophan in plasma and brain, leading to increased synthesis of the neurotransmitter 5-hydroxytryptamine [106]. Liver transplantation in patients with severe AIP has been shown to be beneficial, a clear indication that the liver plays an essential role in producing the neurotoxic agent associated with acute porphyria [107, 108]. Evidence strongly implicating excess ALA and PBG produced from the liver is based in reports of domino liver transplants where the recipient of a liver from an AIP patient developed signs and symptoms of AIP shortly after transplant including the associated neuropathy [109].

The majority of gene carriers for AIP never experience an acute attack, however, attacks can be provoked by exposure to environmental factors and drugs that induce heme biosynthesis. Many inducing factors also cause increased production of hepatic ALAS1, which is closely associated with induction of CYPs in response to detoxification of drugs by the liver [20]. Drug safety databases are available at the websites of from the American Porphyria Foundation (www.porphyrifoundation.com/) and the European Porphyria Initiative (www.porphyrria-europe.com/). Ethanol and other alcohols are inducers of some CYPs and can lead to acute attacks [110,111].

2.1.2.1. Diagnosis: The lack of phenotypic penetrance in gene carriers for AIP requires that an initial diagnosis have a high index of suspicion. One of the acute porphyrias should be considered in patients with unexplained abdominal pain or other characteristic symptoms and ruled in or out by assessment of urinary ALA and PBG. A substantial increase in urinary PBG confirms that a patient has a defect in one of the genes leading to AIP, HCP or VP, and in very rare cases ADP. Quantitative measurements of ALA, PBG, and total porphyrin can be done in most commercial laboratories. If PBG is substantially increased, samples of plasma, erythrocytes and feces should also be obtained prior to treatment with hemin. This approach provides for rapid initial diagnosis of AIP, HCP and VP, subsequent biochemical differentiation of these conditions may be required for the diagnosis of ADP.

PBG excretion is generally 50–200 mg/day (normal range 0~4 mg/day) during acute attacks of AIP. Excretion of ALA is usually about half that of PBG (expressed as mg/day). ALA and PBG can remain elevated for prolonged periods between acute attacks, especially in AIP.

Urinary porphyrins may be increased in AIP, are predominantly uroporphyrin and may lead to a reddish urine if concentrations are significantly elevated. Uroporphyrin forms non-enzymatically from PBG in urine prior to excretion. However, there is evidence that

porphyrins in these conditions are predominantly type III, suggesting enzymatic processing, [112] perhaps from ALA transported to tissues other than the liver [102]. Total fecal porphyrins and plasma porphyrins are normal or slightly increased in AIP, and erythrocyte zinc protoporphyrin concentrations may be nonspecifically increased.

A confirmed diagnosis of AIP and subsequent identification of the underlying PBGD provides the basis for screening for the mutation in direct family members. Other confirmed gene carriers can be educated to avoid the factors known to precipitate porphyric attacks.

2.1.3. Step 6) Hereditary Coproporphyria (HCP) and Step (7) Variegate Porphyrin (VP)

—These hepatic porphyrias are due to defects in CPO and PPO, respectively, can cause neurovisceral symptoms, as in AIP, and/or skin lesions similar to those seen in PCT. The cutaneous phenotype is more common in VP than in HCP. HCP and VP are inherited as autosomal dominant traits with variable penetrance. As in AIP, most heterozygous individuals remain asymptomatic. The neurovisceral phenotype observed in these disorders is nearly identical to AIP and manifest only when levels of ALA and PBG are elevated. The prevalence of HCP was estimated to be 0.2 per 100,000 [113] and the prevalence of VP is reported to be approximately 1.3 per 100,000 [114]. The presence of “founder effect” mutations that are present in certain populations lead to higher than expected frequencies as are seen for VP (*PPO* mutation (R59W)) in South Africa. The reported frequency of VP in the descendants of the Dutch settlers have an estimated prevalence of 300 per 100,000, due to a founder effect [115].

Mutation in *CPO* and *PPO* include missense, nonsense, splicing defects, and indels. Clinical expression is variable and onset of neurological manifestations is influenced by the same factors that are important in AIP [116, 117].

CPO catalyzes the 2-step oxidative decarboxylation of coproporphyrinogen III at a single active site to yield protoporphyrinogen IX, with intermediate formation of harderoporphyrinogen, a tricarboxyl porphyrinogen. A variant form of HCP termed harderoporphyria is due to *CPO* mutations (in the region of residues 400–405) that favor premature release of the tricarboxylic porphyrinogen from the enzyme prior to the decarboxylation of the B ring [118].

2.1.3.1. Diagnosis: Plasma and urinary PBG levels are elevated during an acute attack, however, less than what is measured in patients with AIP. Fecal porphyrin levels, coproporphyrin (HCP) or protoporphyrin (VP), are markedly increased while they are normal or only slightly elevated in AIP. Fecal porphyrins in HCP are predominantly coproporphyrin III, whereas in VP both coproporphyrin III and protoporphyrin are both approximately equally increased. The fecal coproporphyrin III/I ratio is sensitive for diagnosis of HCP, even in asymptomatic stages of the disease [119].

Plasma porphyrin concentrations are increased in VP, seldom increased in HCP (unless there are cutaneous manifestations), and are normal or only slightly increased in AIP. A specific feature of VP is a plasma porphyrin fluorescence maximum at neutral pH of ~626 nm, which is believed to represent protoporphyrin bound covalently to plasma proteins [120].

Diagnostically, fluorometric scanning of plasma is quicker and more effective than extraction and HPLC analysis of fecal porphyrins for detecting asymptomatic VP [121]. This plasma fluorescence scanning is also useful for rapidly differentiating VP from PCT, which displays a fluorescence peak at ~620 nm. Erythrocyte PBG deaminase activity is normal in HCP and VP, and usually deficient in AIP. Assays for CPO and PPO require cells containing mitochondria and are not widely available. DNA studies are the most reliable methods for identifying asymptomatic carriers, if family members are known mutation carriers.

Increases in porphyrin precursors and porphyrins may be more severe in homozygous HCP and VP, with substantial increases in erythrocyte zinc protoporphyrin. Harderoporphyria is identified by elevations in both coproporphyrin III and harderoporphyrin III in feces [118].

2.2. The cutaneous porphyrias

2.2.1. Step 4) Congenital Erythropoietic Porphyria (CEP)—It is an autosomal recessive disorder resulting from severe deficiency of Uroporphyrinogen III Synthase activity (UROS) (Fig. 1). Clinically there is an accumulation and excretion of type I porphyrins, especially uroporphyrin I and coproporphyrin I. Phenotypically, CEP presents as a chronic, severe photosensitivity with hemolytic anemia evident in early childhood. Onset during adult life may be a milder disease that resembles PCT, this has been associated with clonal myeloproliferative disorders [122]. One of the most famous cases of CEP is that of Mr. Petry, who started to work with the porphyrin chemist Hans Fisher in 1915 as a lab aid. He provided samples for early studies of porphyrin chemistry that culminated in Fisher receiving the Nobel Prize in 1930 for his elucidation of heme synthesis [123].

Mutations observed in UROS are remarkably heterogeneous at the molecular level, with at least 51 different mutations of the UROS gene, and one GATA-1 mutation reported to date (<http://www.hgmd.cf.ac.uk/ac/>) [124,125]. The UROS mutations include deletions, insertions, rearrangements, splicing abnormalities, and both missense and nonsense mutations. Twenty-eight of the mutations are missense mutations that are well distributed throughout the gene. Of the 16 single base substitutions, four (T228M, G225S, A66V, A104V) were hot spot mutations, occurring at CpG dinucleotides [126]. With the exception of V82F, all CEP missense mutations occurred in amino acid residues that are conserved in both the mouse and the human enzyme.

Clinically a cutaneous photosensitivity is noted shortly after birth, and severity is inversely proportional to residual enzymatic activity of UROS. In toddlers, the teeth may be stained brown by deposited porphyrins. This is termed erythrodontia and the teeth fluorescence pink when illuminated with long-wave ultraviolet light. Skin lesions in CEP resemble those associated with PCT, but are usually much more severe, reflecting the much higher porphyrin levels in patients with CEP. Bullous lesions are characteristic, resolving with scabbing that scars on complete healing. Areas of involved skin often have either hyper- or hypo-pigmentation. Severe disfigurement including loss of facial features and digits are common, and result from recurrent blisters, infection and scarring. Porphyrins are also deposited in bone.

Hemolytic anemia can be severe, leading to transfusion dependence. The underlying anemia can increase erythropoietic demand, which in turn stimulates porphyrin production by the erythropoietic cells in the marrow. Erythrocytes exhibit polychromasia, poikilocytosis, anisocytosis and basophilic stippling, and reticulocytes and nucleated red blood cells are increased. Reducing iron availability has been reported to be beneficial in suppressing porphyrin production by limiting ALAS2 activity in the erythron [127].

2.2.1.1. Diagnosis: As a recessive trait, a family history is often negative in CEP. In cases of hydrops fetalis, CEP should be considered since the disease can be diagnosed and treated *in utero*. A sample of amniotic fluid is visually dark brown in color and contains large amounts of porphyrins. If a diagnosis of CEP is suspected shortly after birth checking urine in the diaper with a long wave ultraviolet light will produce a pink fluorescence that is characteristic of the excreted urinary porphyrins.

Urinary porphyrin excretion is markedly increased, and often in the range of 50–100 mg/day (normal up to ~0.3 mg/day). Uroporphyrin I and coproporphyrin I account for most of the increase, although the III isomers and hepta-, hexa-, pentacarboxylate porphyrins may also be increased. Fecal porphyrins are generally increased, and are predominantly the less water soluble coproporphyrin I. Total plasma porphyrins are significantly increased as well, with a pattern of individual porphyrins similar to that in urine. Markedly increased erythrocyte porphyrins are predominantly uroporphyrin I and coproporphyrin I, although protoporphyrin IX may predominate especially in milder cases.

Diagnostically DNA confirmation can identify causative mutations in almost all cases. Genetic counseling for prenatal diagnosis and in subsequent pregnancies is often presented. A GATA-1 mutation has been identified as the causative mutation for the phenotypic expression of CEP in one case, this illustrates that on occasion a genetic defect outside the eight heme biosynthetic pathway can cause CEP [125].

2.2.2. Step 5A) Porphyria Cutanea Tarda (PCT)—It is the only porphyria that can develop in absence of the mutation of the affected enzyme [128]. Environmental risk factors are responsible approximately 2/3 of cases of PCT [129]. These risk factors vary considerably by geographic location, so non-familial case frequency is variable. In all cases of PCT there is an enzymatic deficiency of UROD in the liver. UROD sequentially decarboxylates uroporphyrinogen III (with eight carboxyl side groups) to coproporphyrinogen III (with four carboxyl groups). Both isomer series, uroporphyrinogen I and III are substrates, but uroporphyrinogen III is preferred [130]. The disease causes chronic, blistering lesions on sun-exposed areas of skin such as the hands feet and face. Multiple susceptibility factors have been identified, most of which contribute in some manner to generation of a UROD inhibitor [52].

PCT develops when hepatic UROD activity is reduced to ~20% of normal [128]. The enzyme is inhibited, and the amount of enzyme protein remains at its genetically determined level in the liver, as measured immunochemically [131,132]. Mutations at the UROD locus are found in 20–30% of cases [129]. Although PCT is an autosomal dominant disease it is incompletely penetrant. Subjects with a damaging mutation in UROD are more susceptible

to develop PCT because their UROD activity is approximately half-normal in all tissues. Phenotypic expression of the PCT is not observed in these individuals unless there is further reduction of UROD activity in the liver.

A UROD inhibitor, characterized as an *uroporphomethene*, was identified in a mouse model that spontaneously develops biochemical features of PCT [52]. This inhibitor is a product of partial oxidation of uroporphyrinogen, possibly generated by one or more CYPs [52]. When hepatic UROD is inhibited, uroporphyrinogen I and III and the partially decarboxylated intermediates in the reaction (i.e. hepta-, hexa-, and pentacarboxylporphyrinogen) accumulate in the liver and auto-oxidize to the corresponding porphyrins these are eventually transported from hepatocytes to plasma. These porphyrins, present in the micro capillaries of the skin, are activated by light and generate reactive oxygen species that cause mast cell degranulation, complement activation and damage to the subepidermal layers of skin [133,134]. This makes the skin fragile and easy to blister.

PCT most commonly develops in the fourth or fifth decade of life and is more common in men. This is a chronic rather than acute disease that responds to treatment and may recur. Fluid-filled bullae are common on the backs of the hands, and other sun-exposed area of the body, often arising after minor and inapparent trauma, reflecting increased skin fragility. Blisters also occur on the forearms, face, ears, neck, legs, and feet. They rupture easily, crust over and are prone to infection before healing slowly. Milia may precede or follow vesicle formation. Facial hypertrichosis and hyperpigmentation are common and cosmetically problematic especially in women. Scarring, and hyper- or hypopigmentation occur with prolonged disease. Severe thickening of affected areas of skin can resemble systemic sclerosis and is termed *pseudoscleroderma*. Identical skin lesions can occur in adults with VP and HCP, and usually beginning in childhood in CEP and HEP. There are no neurological symptoms in PCT.

PCT associated with end-stage renal disease is usually more severe, because porphyrins are poorly dialyzed and lack of urinary porphyrin excretion results in higher concentrations in plasma [135]. The disease occasionally develops during pregnancy, perhaps related to effects of estrogen. Reported associations with cutaneous and systemic lupus erythematosus are unexplained.

Mild abnormalities in liver function tests are found in almost all cases, but cirrhosis is unusual at the onset of PCT. Fresh hepatic tissue shows strong red fluorescence on exposure to long-wave ultraviolet light, reflecting massive accumulation of porphyrins. Liver histopathology is nonspecific and usually includes increased iron, increased fat, hepatocyte necrosis and inflammation. The risk of HCC is increased, especially with more prolonged disease [136–138]. Patients very often have coexisting factors that cause liver damage and an increased risk for HCC.

Skin biopsy reveals subepidermal blistering and deposition of periodic acid–Schiff-positive material around blood vessels and fine fibrillar material in the upper dermis and at the dermoepithelial junction. Deposits of immunoglobulins and complement are also found

[139]. These histologic changes are not diagnostic, and are seen in other cutaneous porphyrias as well as pseudoporphyria.

2.2.2.1. Risk factors associated with PCT: Human PCT is a heterogeneous disorder, with many susceptibility factors that clearly contribute to the disease, but none of which is essential. These include genetic factors, viral infections and chemical exposures. Multiple such factors are often found in any individual patient [140]. In a series of 143 patients with PCT in the U.S., the most common susceptibility factors were ethanol use (87%), smoking (81%), chronic hepatitis C virus (HCV) infection (69%), and HFE mutations (53%) [141].

2.2.2.2. UROD mutations: Approximately 1/3 of patients have a heterozygous predisposing *UROD* mutation. These individuals have an autosomal dominant trait with low penetrance and are said to have familial PCT. Most familial PCT patients have no known relatives with PCT. Hepatoerythropoietic porphyria (HEP) is the homozygous form of PCT and resembles CEP clinically, (see **Step 5B**). More than 120 different mutations of the *UROD* gene, including 80 missense mutations, have been identified in PCT and HEP. The remaining mutations include splicing, deletions and insertions. PCT without *UROD* mutations (~2/3 of patients) is described as sporadic PCT.

2.2.2.3. Iron and hemochromatosis gene (HFE) mutations: Iron stores may be normal however they are generally increased in PCT, and iron deficiency is protective. The importance of iron has been confirmed in animal models. For example, mice with disruption of one *UROD* allele (*UROD*^{+/-}) and two disrupted *HFE* alleles (*HFE*^{-/-}) develop uroporphyrin without administration of exogenous chemicals [142]. Prevalence of the *C282Y* mutation of the *HFE* gene, the major mutation causing hemochromatosis in Caucasians, is increased in both sporadic and familial PCT. Up to 10–20% of patients may be *C282Y* homozygotes, and may experience earlier onset of disease [143,144]. In southern Europe, where the *C282Y* is less prevalent, the *H63D* mutation is more commonly associated with PCT [145]. Iron does not directly inhibit *UROD* activity. An imbalance in iron homeostasis may contribute to *UROD* inhibition by providing an oxidative environment in hepatocytes, contributing to generation of a *UROD* inhibitor. *HFE* mutations impair serum iron sensing so hepatic hepcidin production is decreased, intestinal iron absorption and transfer across the gut enterocytes [146]. Hepatic hepcidin may also be altered in PCT patients without *HFE* mutations, suggesting that other susceptibility factors reduce expression of this hormone and cause hepatic siderosis in PCT [147]. PCT has also been associated with mutations in *GNPAT* a gene shown to associate with alterations in iron homeostasis [148].

2.2.2.4. Alcohol: PCT has long been associated with excess alcohol use. Metabolism of alcohol by induction of CYPs for detoxification and the co-induction of *ALAS1* feeds more precursors into the pathway. Increased reactive oxygen species, mitochondrial injury, lipid deposition, depletion of reduced glutathione and stressing of other antioxidant defenses, are all associated with the metabolism of alcohol.

2.2.2.5. Smoking and cytochrome P450 enzymes: Smoking is commonly associated with alcohol use and is less extensively studied as a risk factor in PCT [140]. Smoking may

increase oxidative stress in hepatocytes, and induces hepatic CYPs, including CYP1A2 which in rodent models is important for development of uroporphyrin [149,150].

2.2.2.6. Estrogens: Oral estrogen use has been associated with women and PCT [140,151,152]. The disease also developed in some men treated with estrogen for prostate cancer [151]. Transitioning from an oral estrogen to a transdermal estrogen has been shown to be safe, it is thought that higher concentrations of estrogen are seen by the liver when it is administered orally. The mechanism is not established, although estrogens can generate reactive oxygen species in some experimental systems [128,153].

2.2.2.7. Hepatitis C: Reported prevalence of chronic hepatitis C in PCT has ranged from 21–92% in various countries, reflecting the considerable geographic variation in prevalence of this viral infection, but is always far greater than the prevalence in healthy subjects. Hepatitis C is associated with hepatocyte steatosis, iron accumulation, mitochondrial dysfunction, oxidative stress and dysregulation of hepcidin expression [154,155]. Resolving the hepatitis C infection with targeted antivirals is beneficial to reduce the phenotypic expression of PCT [156].

2.2.2.8. Chemical exposure and drugs: A large outbreak of PCT occurred in eastern Turkey in the 1950s during a period of food shortage, when a population consumed seed wheat treated with the fungicide hexachlorobenzene [157]. Smaller outbreaks of the disease and individual cases have occurred after exposures to other chemicals such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) [158]. These and related chemicals were subsequently shown to cause uroporphyrin in laboratory animals, providing models that have greatly increased our understanding of PCT [128,159]. But such exposures have not been convincingly documented in other PCT patients. Hepatic ALAS1 is less induced in PCT than in the acute porphyrias, and drugs that induce ALAS1 are only occasionally reported to play a role in this disease [160].

2.2.2.9. Diagnosis: A diagnosis of PCT is initially established by evaluation of porphyrins in urine or plasma. Highly carboxylated porphyrins, 8-COOH and 7-COOH, uroporphyrin and heptacarboxylporphyrin respectively are significantly elevated. Levels of PBG are normal, and urinary ALA is normal or slightly increased. An increase in isocoproporphyrin, is part of the complex pattern of fecal porphyrins in PCT. This is formed when pentacarboxylporphyrinogen accumulates in liver and is decarboxylated by CPO, leading to formation of dehydroisocoproporphyrinogen, which is excreted in bile and undergoes oxidation by intestinal bacteria to isocoproporphyrin [161].

Measurement of plasma porphyrins and determination of the fluorescence emission peak at neutral pH may also be a useful screening method if PCT is suspected. A substantial increase with a peak at ~620 nm is most commonly due to PCT. Although not a specific finding, it can rapidly exclude VP and pseudoporphyria, other porphyric conditions with skin manifestations resembling PCT [162]. A plasma porphyrin determination is essential for diagnosis of PCT in patients with advanced renal disease, keeping in mind that the reference range is higher in this setting [163]. Occasionally mild cases of CEP or HEP (see below) can mimic PCT. Therefore, measurement of erythrocyte porphyrins, which are

normal or only modestly increased in PCT and markedly increased in CEP and HEP, is recommended. Once the biochemical diagnosis of PCT has been established, determination if there is a genetic component should be established by identification of a *UROD* mutation.

2.2.3. Step 5B) Hepatoerythropoietic Porphyria (HEP)—It is the homozygous form of PCT, where each allele of *UROD* is affected and there is minimal enzymatic activity from the protein(s) that are produced. HEP resembles CEP clinically, it is evident in early childhood and the low enzyme activity is present in all cells. (OMIM 176100) The same scope of mutations present in PCT have been identified in HEP. Erythrocyte *UROD* activity is 5–30% of normal in HEP. Homozygosity for a *UROD* null allele is lethal [142]. Therefore, in HEP at least one of the mutant *UROD* alleles must produce some enzyme protein with catalytic activity. Expression studies in eukaryotic cells suggest that some mutations may destabilize the enzyme protein in a tissue-specific manner [164].

Excess porphyrins originate mostly from the liver in this condition. However, zinc protoporphyrin accumulates in the marrow and is markedly elevated in erythrocytes, as in all other autosomal recessive porphyrias except CEP, in which uroporphyrin I and coproporphyrin I are usually the most abundant erythrocyte porphyrins. As in CEP, onset of blistering skin lesions, hypertrichosis, scarring, and red urine usually begins in early childhood. Sclerodermoid skin changes are sometimes prominent. Unusually mild cases have been described [165].

2.2.3.1. Diagnosis: Biochemical findings in HEP resemble PCT, with predominant accumulation and excretion of uroporphyrin, heptacarboxyl porphyrin and isocoproporphyrins. In contrast to PCT, erythrocyte zinc protoporphyrin may be substantially increased.

2.2.4. Step 8) Erythropoietic Protoporphyrin (EPP) and Step 1, X-linked Protoporphyrin (XLP)—Results from diminished activity of ferrochelatase (*FECH*), allowing the accumulation of the substrate protoporphyrin-IX (PPIX) in the developing erythroid compartment; or a variant form of the disease due to gain of function mutations in *ALAS2*, that also results in accumulation of PPIX [25]. The protoporphyrins are characterized by onset of nonblistering cutaneous photosensitivity in early childhood. Combined these are the most common type porphyria in children and the third most common in adults. Reported prevalence varies between 5 and 15 cases per million individuals [166–168]. Hepatopathy due to excess PPIX accumulation is a potentially fatal complication of either protoporphyrin, estimated to occur in less than 5% of patients.

More than 75 different mutations, including nonsense, missense, splice site mutations, nonsense mutations, and deletions, insertions and rearrangements have been described in the *FECH* gene. EPP is an autosomal recessive disorder, most cases involve a loss of function mutation coupled with an allele that leads to mis-splicing. Typically, EPP patients have approximately 30% or less of normal ferrochelatase activity. The low-expression (hypomorphic) intronic polymorphism (a –23C→T transition, termed IVS 3 –48C) is present in trans to mutant allele of patients with EPP [79–81]. The intronic polymorphism favors the use of a cryptic acceptor splice site 63 bases upstream of the normal splice site.

The aberrantly spliced mRNA contains a premature stop codon and is degraded by a nonsense-mediated decay mechanism [81]. The result is a lower steady state level of wild type FECH mRNA. The frequency of the IVS 3 –48C hypomorphic allele is common in the Caucasian population, and by itself has no phenotype. Its frequency varies widely in different populations and relates to the observed differences in the prevalence of EPP [166–168].

Other underlying genetic mechanisms must be considered in newly identified EPP families. In a few families, true autosomal recessive inheritance, with a FECH mutation inherited from each parent has been described. Interestingly, autosomal recessive EPP is sometimes associated with seasonal palmar keratoderma. Other features in some patients with this unexplained association include neurological symptoms, less than expected increases in erythrocyte protoporphyrin and absence of liver dysfunction [169].

Patterns in families with a variant form of EPP in which FECH mutations were not found suggested sex-linked inheritance, and this led to discovery of a gain-in-function mutation of ALAS2 (the only heme pathway enzyme found on the X chromosome) [25]. This was the first demonstration that a mutation of ALAS, the first enzyme in the pathway, can be associated with a type of porphyria. In XLP there is an approximate 3 fold increase in enzymatic activity of ALAS2. This then uncovers the next rate limiting step in the pathway in erythrocytes, ferrochelatase. In all cases of XLP there is a truncation of the ALAS2 protein where 18–22 residues are lost, in essence removing a regulatory function of the protein [170,171].

Bone marrow reticulocytes are thought to be the primary source of the excess protoporphyrin in EPP [172–174]. Free PPIX in these cells declines much more rapidly with red cell age than it does in conditions associated with increased erythrocyte zinc protoporphyrin [172,174]. Free PPIX, but not zinc-PPIX, is released from erythrocytes following irradiation, which may explain why lead intoxication and iron deficiency, which are associated with elevated erythrocyte zinc-PPIX levels, are not associated with photosensitivity [175]. Excess protoporphyrin is taken up from plasma by hepatocytes and excreted in bile and feces, and may undergo enterohepatic circulation. Hepatocytes may also be a limited additional source of excess protoporphyrin, when activity of FECH is severely limited.

Light-excited porphyrins generate free radicals and singlet oxygen, [176] which in EPP can lead to peroxidation of lipids [177] and crosslinking of membrane proteins [178]. Skin irradiation in EPP patients leads to complement activation and polymorphonuclear chemotaxis, which contributes to the development of skin pathology [179].

Protoporphyrin hepatopathy is the most significant complication and develops in <5% of patients. The insoluble nature of PPIX is thought to contribute to the cholestatic effects of excess PPIX as it is metabolized by the liver. This complication may begin with chronic abnormalities in liver function tests and then progress rapidly as a vicious cycle of increasing plasma PPIX and worsening liver function with increased photosensitivity. Hepatopathy is sometimes precipitated by another cause of liver dysfunction such as viral or

alcoholic hepatitis. Protoporphyrin is cholestatic, and can form crystalline structures in hepatocytes and impair mitochondrial function, leading to decreased hepatic bile formation and flow [180,181]. Accumulated PPIX appears as brown pigment in hepatocytes, Kupffer cells, and biliary canaliculi and are doubly refractive with a Maltese cross appearance under polarizing microscopy [182]. Significant changes in expression of several genes involved in wound-healing, organic anion transport, and oxidative stress were found in DNA microarray studies in explanted livers of patients who underwent liver transplantation for this complication [183].

Cutaneous photosensitivity in EPP and XLP is acute and nonblistering, which is distinctly different from the more chronic, blistering skin manifestations of the other cutaneous porphyrias. Skin symptoms are usually worse during spring and summer and affect light-exposed areas, especially of the face and hands. Characteristically, stinging or burning pain develops upon sunlight exposure, and is followed by erythema and edema – described as solar urticaria and sometimes petechiae or less commonly purpura. A history of these symptoms may occur in the absence of objective cutaneous signs. Artificial lights may contribute to photosensitivity [184]. Over time, chronic changes may include onycholysis, leathery hyperkeratotic skin especially on the dorsae of the hands and finger joints, and mild scarring. Bullae, skin fragility, hypertrichosis, hyperpigmentation, severe scarring, and mutilation are very unusual.

Precipitating factors that are important in the hepatic porphyrias do not appear to play an important role in EPP. Although more long-term follow-up studies observations are needed, porphyrin levels and symptoms typically do not change over time, unless liver dysfunction develops. Concurrent iron deficiency or other bone marrow problems might also lead to further increases in porphyrin levels and photosensitivity. Pregnancy is reported to lower erythrocyte protoporphyrin levels somewhat and increase tolerance to sunlight [185]. Neurovisceral manifestations are absent in uncomplicated EPP. Patients with severe protoporphyrin hepatic pathology may develop a severe motor neuropathy similar to that seen in the acute porphyrias [186]. Autosomal recessive EPP associated with palmar keratoderma has also been associated with unexplained neurological symptoms [169].

Gallstones containing large amounts of protoporphyrin are common, and may require cholecystectomy at an unusually early age [187]. Liver function and liver protoporphyrin content are usually normal in EPP. However, protoporphyrin hepatic pathology, which is the most life-threatening complication of EPP, develops in 1–5% of patients. This complication results from the cholestatic effects of protoporphyrin presented in excess amounts to the liver. It is sometimes the major presenting feature of EPP, [188] and may be chronic or progress rapidly to death from liver failure. Unnecessary surgery for suspected biliary obstruction can be detrimental and should be avoided [181]. Operating room lights during liver transplantation or other surgery especially in patients with hepatic pathology can cause marked photosensitivity with extensive burns of the skin and peritoneum and photodamage of circulating erythrocytes [189].

2.2.4.1. Diagnosis: Painful, nonblistering photosensitivity suggests the diagnosis. A substantial elevation of erythrocyte PPIX is expected, but is not specific, since erythrocyte

zinc-PPIX is predominantly increased in conditions such as homozygous porphyrias (other than most cases of CEP), iron deficiency, lead poisoning, anemia of chronic disease, [190] hemolytic conditions, [191] and many other erythrocytic disorders. A unique finding in EPP is increased erythrocyte PPIX with a predominance of free rather than zinc-PPIX. This occurs because FECH, which can utilize metals in addition to iron, catalyzes the formation of zinc-PPIX, and this activity is deficient when FECH is deficient in EPP. In cases of XLP where FECH activity is normal there is a significant increase in zinc-PPIX. Therefore, the diagnosis of EPP requires demonstration of an increase in free PPIX in red cells, which can be measured by an ethanol extraction or HPLC method.

The plasma porphyrin concentration is almost always at least somewhat increased in EPP, but often less than in other cutaneous porphyrias, and may be normal especially in mild cases. Plasma porphyrins are particularly subject to photodegradation in EPP during sample processing unless great care is taken to shield the sample from natural or fluorescent light [192]. For these reasons, measurement of erythrocyte rather than plasma porphyrin should be the primary screening test for EPP and XLP. Fecal porphyrins are increased in most cases and consist mostly of PPIX. Urine porphyrins are normal, except after hepatopathy develops, which causes increases in urinary coproporphyrin as is typical for other forms of liver diseases.

Conclusions

The spectrum of porphyrias is indeed due to the accumulated intermediates in heme biosynthesis rather than deficiency of heme. Accumulation of early intermediates in the pathway lead to the observed neurologic signs and symptoms. Once the porphyrin macrocycle has been formed the clinical presentation typically changes to one of a phototoxicity on sun exposed areas of the skin. This is due to the release of a high energy photon (fluorescence) that produces reactive oxygen species that results in damage to the skin and subdermal layers to the extent of 400 nm light penetration. The approved treatments for these diseases are generally directed towards the relief of symptoms and or shielding of light. While a Nobel prize was awarded to Dr. Hans Fisher for his work on heme biosynthesis much is still yet to be determined. This highlights the need for additional research in the areas heme synthesis and the porphyrias.

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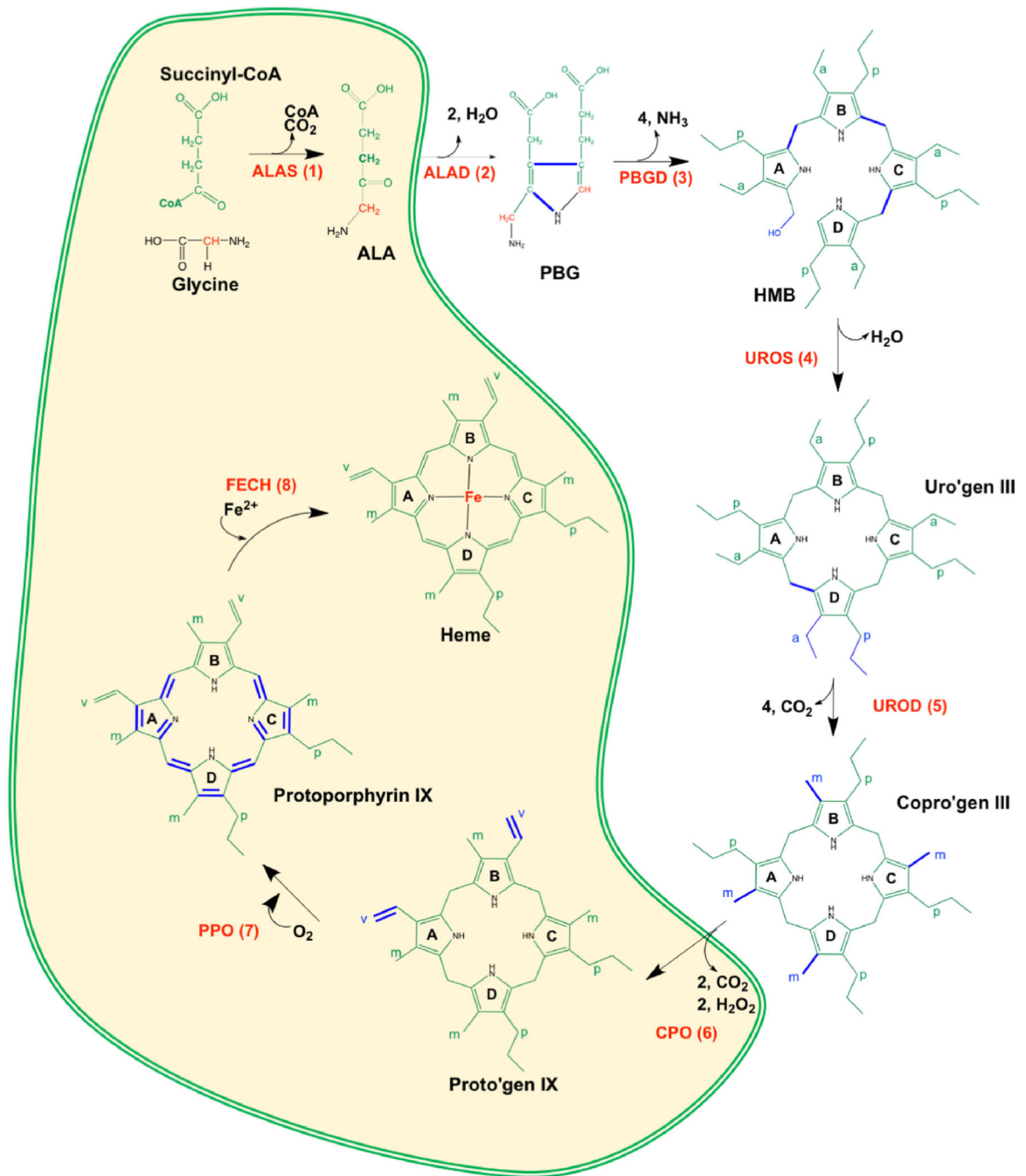


Fig. 1. Heme biosynthesis. The eight committed steps in heme biosynthesis are labeled in red; ALAS, d-aminolevulinic acid synthase; ALAD, aminolevulinic acid dehydratase; PBGD, porphobilinogen deaminase; UROS, uroporphyrinogen III synthase; UROD, uroporphyrinogen decarboxylase; CPO, coproporphyrinogen oxidase; PPO, protoporphyrinogen oxidase; FECH, ferrochelatase. The bonds that change between substrates are colored blue. The number after the enzyme name is the enzymatic step. Substrates and

enzymes within the yellow shading with double green line are located within the mitochondria.

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Table 1

Classification schema for the porphyrias

	RBC	Liver	Neurologic	Photosensitive	Inheritance
ALAS	X			X	X
ALAD		X	X		R
PBGD		X	X		D
UIIS	X			X	R
UROD		X		X	D
CPO	X		X	X	D
PPO	X		X	X	D
FECH	X			X	R

X = X-linked, R = autosomal recessive, D = autosomal dominant

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