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Recent Advances on Porphyria Genetics: Inheritance, Penetrance & Molecular Heterogeneity, Including New Modifying/ Causative Genes

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Abstract

The inborn errors of heme biosynthesis, the Porphyrias, include eight major disorders resulting from loss-of-function (LOF) or gain-of-function (GOF) mutations in eight of the nine heme biosynthetic genes. The major sites of heme biosynthesis are the liver and erythron, and the underlying pathophysiology of each of these disorders depends on the unique biochemistry, cell biology, and genetic mechanisms in these tissues. The porphyrias are classified into three major categories: 1) the acute hepatic porphyrias (AHPs), including Acute Intermittent Porphyria (AIP), Hereditary Coproporphyria (HCP), Variegate Porphyria (VP), and 5-Aminolevlulinic Acid Dehydratase Deficient Porphyria (ADP); 2) a hepatic cutaneous porphyria, Porphyria Cutanea Tarda (PCT); and 3) the cutaneous erythropoietic porphyrias, Congenital Erythropoietic Porphyria (CEP), Erythropoietic Protoporphyria (EPP), and X-Linked Protoporphyria (XLP). Their modes of inheritance include autosomal dominant with markedly decreased penetrance (AIP, VP, and HCP), autosomal recessive (ADP, CEP, and EPP), or X-linked (XLP), as well as an acquired sporadic form (PCT). There are severe homozygous dominant forms of the three AHPs. For each porphyria, its phenotype, inheritance pattern, unique genetic principles, and molecular genetic heterogeneity are presented. To date, >1000 mutations in the heme biosynthetic genes causing their respective porphyrias have been reported, including low expression alleles and genotype/phenotype correlations that predict severity for certain porphyrias. The tissue-specific regulation of heme biosynthesis and the unique genetic mechanisms for each porphyria are highlighted.

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Conflicts of Interest

MY and RJD are past recipients of research grants from Alnylam Pharmaceuticals and Recordati Rare Diseases. They are co-inventors of a patent licensed to Alnylam Pharmaceuticals for RNAi therapy of the AHPs. RJD is a consultant for Alnylam Pharmaceuticals, Mitsubishi Tanabe Pharma Development America and Recordati Rare Disease. BC has no conflicts.

1. Introduction

The porphyrias are a group of eight metabolic disorders, each resulting from the defective activity of a specific enzyme in the heme biosynthesis pathway (1, 2). The genetics of these inborn errors are of particular interest as they encompass many genetic mechanisms and principles (Table 1), including multiple modes of inheritance, molecular genetic heterogeneity, genotype/phenotype correlations, high prevalence and low penetrance, modifying genes, pharmacogenetics, as well as environmental factors.

Heme biosynthesis occurs in almost all cell types, but most heme is produced in the bone marrow erythron (~85%) primarily for hemoglobin synthesis, and in hepatocytes (~15%) for critical hemoproteins, including the cytochrome P450 enzymes for drug metabolism (1). Heme biosynthesis in each of these major sites is under different regulatory mechanisms, which account for the unique pathophysiology of the hepatic and erythropoietic porphyrias. As shown in Figure 1, there are eight enzymatic steps of the heme biosynthesis pathway that are encoded by nine genes, as the first step in the pathway has two genes, 5-aminolevulinic acid synthase 1 (*ALAS1*), a housekeeping gene located at chromosome 3p21.1, and an erythroid-specific gene (*ALAS2*) on the X-chromosome at Xp11.21. The cDNAs and genes encoding all the heme biosynthetic enzymes have been cloned and sequenced (Table 2), and the 3-dimensional structures of their respective encoded enzymes have been solved for human, eukaryotic, and/or prokaryotic species (3-10).

Unique to this pathway is the fact that there is a negative-feedback regulation of *ALAS1* expression in the liver depending on the cellular “free heme pool” concentration (See Phillips *et al.* in this volume; Fig. 1). This is analogous to cholesterol biosynthesis where the first enzyme in the pathway (HMG-CoA reductase) is regulated by the hepatic cholesterol concentration (11). The hepatic negative-feedback regulation of *ALAS1* is particularly relevant to the genetics, biochemistry, and pathogenesis of the acute hepatic porphyrias (AHPs). Analogously, the unique regulation of the erythroid–specific expression of *ALAS2* is relevant to the erythropoietic porphyrias. *ALAS2* has several regulatory elements in its promoter, including a GATA1-binding site (12), and iron response elements (IREs). The 5’ IRE binds the iron response proteins (IRPs) when cellular iron levels are low, thereby inhibiting the translation of ALAS2. Mutations in the terminal exon of the *ALAS2* gene lead to truncation or elongation of the mature ALAS2 polypeptide resulting in its gain-of-function (GOF) and subsequent accumulation of erythrocyte protoporphyrin IX (PPIX), resulting in XLP [(13); see below]. Conversely, *ALAS2* loss-of-function (LOF) mutations result in X-linked sideroblastic anemia.

The porphyrias can be categorized based on their major clinical features and primary sites of abnormal porphyrin or porphyrin precursor accumulation. As shown in Table 3, there are four acute hepatic porphyrias (AHPs), each due to LOF mutations that decrease the activity of a particular heme biosynthetic enzyme. These include three autosomal dominant disorders, Acute Intermittent Porphyria (AIP), Hereditary Coproporphyria (HCP), Variegate Porphyria (VP), and the ultra-rare autosomal recessive 5-Aminolevulinic Acid Dehydratase Deficient Porphyria (ADP), due to mutations in the hydroxymethylbilane synthase (*HMBS*), coproporphyrinogen oxidase (*CPOX*), protoporphyrinogen oxidase (*PPOX*), and 5-

Aminolevulinic acid dehydratase (*ALAD*) gene, respectively. The most common porphyria, Porphyria Cutanea Tarda (PCT), is a hepatic disorder that has primarily cutaneous manifestations, and therefore, is classified as a hepatic cutaneous porphyria. This disorder has sporadic and familial subtypes, both postulated to be due to the induced deficiency of uropophyrinogen decarboxylase (UROD) by an enzyme inhibitor, uroporphomethene, which occurs in patients with hepatic iron overload (14-16). The third group is the erythropoietic cutaneous porphyrias, which include autosomal recessive Congenital Erythropoietic Porphyria (CEP) and Erythropoietic Protoporphyria (EPP) and the X-linked disorder, X-Linked Protoporphyria (XLP). CEP and EPP result from LOF mutations in the uroporphyrinogen synthase (*UROS*) and ferrochelatase (*FECH*) genes, respectively, while XLP results from GOF mutations in the *ALAS2* gene. Below, the inheritance, genetic principles, and molecular genetics of the AHPs, PCT, and the erythropoietic porphyrias are presented.

2. The Porphyrias Illustrate Many Genetic Principles

2.1. Multiple Modes of Inheritance, Prevalence and Penetrance:

As noted above, the eight major porphyrias are inherited as autosomal dominant (AIP, HCP, and VP), autosomal recessive (ADP, CEP, and EPP), and X-linked (XLP) traits. Notably, the autosomal dominant AHPs have remarkably low penetrance. For example, the estimated prevalence of AIP is remarkably high, 1 in ~1,700 Caucasians, whereas the estimated penetrance of an acute attack is about 1% (17, 18). Also, there are very rare and severe homozygous dominant forms of AIP (19-22), HCP (23-25), and VP (26-28). Notably, PCT has two major subtypes that are clinically indistinguishable: sporadic Type 1 and familial Type 2. Type 2 patients have half-normal UROD activity in all cells, whereas the Type 1 sporadic patients only have deficient hepatic UROD activity secondary to iron overload, porphyrin accumulation, and the formation of an UROD inhibitor (14).

2.2. Multiple Mutations Underlying Each Disease

In each of the eight major porphyrias, there is remarkable molecular genetic heterogeneity with all mutation types represented (see Table 4). Only a few mutations are relatively common due to CpG dinucleotide mutational hotspots in the gene (29) or due to founder effects, e.g. *HMBS* W198X in Sweden and Norway (30). An exception is the most common mutation in CEP, c.217T>C (p.C73R), which occurs in about 30% of unrelated patients and is not at a CpG dinucleotide, nor is it a founder mutation in a certain population (31).

2.2.1. Loss and Gain of Function Mutations—Most mutations cause LOF (i.e., decreased enzymatic activity) of its encoded heme biosynthetic enzyme. These mutations alter the enzyme's kinetics or stability or both. The exception is the GOF mutations in the terminal exon of the *ALAS2* gene, which increase the ALAS2 enzymatic activity and result in high levels of PPIX, causing XLP (13, 32, 33).

2.2.2. Common Low Expression Allele—In EPP, over 95% of patients are compound heterozygotes for a pathogenic LOF allele and the common low expression allele, c. 315-48T>C [also known as IVS3-48T>C (34, 35)]. This low expression (or hypomorphic)

allele causes alternative splicing and markedly reduces the amount of normal *FECH* mRNA it expresses (34). This allele is relatively common, occurring in ~5% of Europeans, about ~2% of Africans, and up to 33% of Asians (GnomAD).

2.2.3. Promoter Alterations—The ALAD, HMBS, and UROS enzymes are each encoded by a single gene with unique housekeeping and erythroid promoters (Fig. 2). For HMBS, the housekeeping and erythroid-specific promoters encode two isozymes. The upstream housekeeping promoter directs a transcript containing exons 1 and 3-15, while the downstream erythroid-specific promoter produces a transcript containing the exon 2-15 sequence. The two mRNAs encode the 42-kDa housekeeping and 40-kDa erythroid-specific HMBS isozymes, due to the housekeeping isozyme having 17 additional *N*-terminal amino acids. Therefore, mutations that alter exon 1 of the housekeeping gene or the splicing of exon 1 to exon 3 result in a variant form of AIP in which the liver enzyme has half-normal activity, but erythroid cells have normal activity (36, 37) (Fig. 3). This form of AIP is known as the “erythroid variant”, whereas mutations in exons 3-15 result in half-normal activity of both isozymes, referred to as “classic” (or “non-erythroid variant”) AIP. In contrast, the *ALAD* and *UROS* promoters encode the same enzyme (Fig. 2).

Regulatory gene defects in the 5’-promoter regions of the *HMBS* (38), *UROS* (39), and *FECH* genes (40) have been reported in patients with AIP, CEP, and EPP, respectively. For example, the pathogenicity of four promoter lesions in patients with CEP that involved GATA1 or CP2 erythroid transcription binding sites (Fig. 4) have been confirmed by *in vitro* expression, using luciferase assays (39).

Additionally, hypermethylation of CpG sites within the *FECH* gene promoter was reported to decrease its transcriptional activity, perhaps leading to a more severe form of EPP with liver complications. Family members who had overt EPP but lacked hypermethylation of the promoter region had milder disease, suggesting that this modulated disease severity (41).

2.3. Benign Variants:

Most published mutations causing the porphyrias and other Mendelian diseases are catalogued in the Human Gene Mutation Database [HGMD, (42)]. This publically available database is updated quarterly, is available online (www.hgmd.cf.ac.uk), and provides a reference for each mutation. The database lists published mutations by disease and mutation type. However, many of these mutations have not been validated clinically and/or biochemically. In the early days of gene sequencing (1980’s and 90’s), benign variants were identified and many were thought to be pathogenic, as the entire coding region (or gene) was not sequenced to find the causative pathogenic mutation as it is today. Moreover, until recently, there were no available databases that provided the frequencies of all detected variants in various racial and ethnic populations such as GnomAD (gnomad.broadinstitute.org). Typically, benign variants are missense mutations or are located near the consensus splice-site but further into the introns (e.g., >4 nucleotides). Such mutations have been reported in HGMD as pathogenic in the past, and some have been reclassified when data indicating that they are benign variants are published [e.g., for *HMBS* benign variants and AIP, see (17)].

Genomic databases provide allele frequency data for variant nucleotides in all genes based on whole exome and genome sequencing of thousands of individuals in different racial and ethnic populations. They are particularly useful in identifying relatively frequent variants and are informative for classifying a variant as pathogenic or benign. For example, *HMBS* IVS10-31A>G was identified in a French AIP patient of Caribbean origin and originally reported as pathogenic in 1997 (43). Later, it was reclassified as a common variant that was frequent in African and Hispanic populations (44) with minor allele frequencies (MAF) of up to ~43% and ~2% in Black and Hispanic populations, respectively (based on GnomAD data).

2.4. X-Chromosomal Inactivation (XLP)

XLP, resulting from GOF mutations located in the terminal exon of *ALAS2*, is inherited as an X-linked trait. Affected males who inherited the X-chromosome with the GOF mutation from their heterozygous mothers (X^{XLP}/X^{normal}) have markedly increased erythrocyte PPIX levels and are clinically indistinguishable from male or female patients with EPP. However, female heterozygotes for XLP have variable clinical expressivity ranging from asymptomatic to as severely affected as their male relatives. This variability results from random X-chromosomal inactivation (45).

Since females have two X-chromosomes and males have only one, a mechanism to equalize the expression of the genes on the X-chromosomes of females occurs in early embryogenesis. One of the female's X-chromosomes is inactivated. This "dose compensation" may vary in different cell types, but once a particular X-chromosome is inactivated, the same X will be inactivated in its daughter cells, cell division after division. Thus, if the cell inactivated the X-chromosome with the *ALAS2* mutation causing XLP, only normal levels of the *ALAS2* enzyme would be expressed by the active normal X-chromosome. One might expect that since the inactivation process is random, it would be 50:50. In effect, half of the cells would inactivate the X with the *ALAS2* mutation and half of the cells would inactivate the normal *ALAS2* on the other X-chromosome. If a 50:50 ratio for X-chromosomal inactivation occurred, the ALAS2 enzymatic activity would be increased in erythroid cells by the amount overexpressed by the GOF *ALAS2* gene.

However, as one may have experienced at a casino, the 50:50 expectation may only occur about 50% of the time. The other 50% of the time, the ratio may be 60:40, 70:30, even 95:5. It is estimated that these ratios occur 40%, 30%, and 5% of the time, respectively. It has been suggested that random X-chromosomal inactivation occurs in various cell types independently, making the expression of an X-linked mutation variable by cell type, tissue, and organ. For XLP, the erythroid cells are the major cell type involved. So assume that in the earliest erythroid precursor cell in a given XLP female heterozygote, the ratio of inactivation of the chromosome with the mutant *ALAS2* was 100% (a rare event!), then only the wild-type *ALAS2* on the active X-chromosome would be expressed. This woman would have totally normal erythroid *ALAS2* activity, normal erythrocyte PPIX levels, and no clinical manifestations, but still have a risk to pass the mutant gene to half (on average) of her male and female offspring. She would be diagnosed only by sequencing her *ALAS2* gene. If the opposite occurred, and a woman had only the chromosomes with the *ALAS2*

mutation actively expressed, then she would have elevated ALAS2 activity, increased levels of erythrocyte 'free' and zinc PPIX, and would be as severely affected as her male relatives. The variation in clinical severity results from when the ratios are between the above two extreme scenarios. For example, say its 70:30 in favor of the mutation being expressed, this woman would likely have some disease manifestations including increased erythrocyte PPIX levels and photosensitivity, but likely would not be as severe as her affected male relatives. Conversely, if only 30% of erythroid cells expressed the mutant enzyme, it is likely that the erythrocyte PPIX would only be somewhat increased, but the woman may not have erythrocyte PPIX levels that caused clinical photosensitivity, or if she did, her sun exposure time would be longer until she felt the first prodromal symptoms than that of affected male relatives.

CEP resulting from *GATA1* mutations also is inherited as an X-linked trait. Therefore, heterozygotes may be asymptomatic or as affected as male relatives [(46, 47); see Section 2.5 below.]

2.5. New Causative Genes

Two other genes involved in porphyria pathogenesis have been identified to date. The ATP-dependent CLP protease ATP-binding subunit (*CLPX*) gene located at chromosome 15q22.31 encodes a protein with mitochondrial ATPase activity, which forms a complex with the ATP-dependent CLP protease proteolytic subunit (CLPP) protein. This multimeric complex, termed CLPXP, is an ATP-dependent serine protease that degrades specific proteins, including the ALAS enzymes. A LOF mutation in *CLPX* reduces the proteasome activity of CLPXP and leads to increased posttranslational stability and activity of ALAS2, and consequentially, the accumulation of erythrocyte PPIX, thereby causing a form of EPP (48). The second is the *GATA1* gene, which encodes a transcription factor important for erythropoiesis. Rarely, a mutation that directly alters the expression of the *GATA1* gene transcription factor protein can cause an X-linked form of CEP (46, 47).

2.6. New Modifying Genes and Environmental Factors

The severity of the different porphyrias can be affected by modifying genes and the environment. To date, only a few modifying genes have been identified for the erythropoietic and hepatic porphyrias. A terminal exon mutation in the *ALAS2* gene that increases ALAS2 activity was found in a family with CEP and shown to increase the severity of the patient's photosensitivity (49). More recently, a LOF mutation in *CLPX*, which is involved in the degradation of the ALAS enzymes, was found to stabilize ALAS2 and increase its activity, promoting erythroid porphyrin synthesis, and consequentially, increasing erythrocyte PPIX levels and photosensitivity (48) (see Section 2.2.4). Thus, if *CLPX* or *CLPP,* which it is associated with, is mutated in any erythropoietic porphyria, it is expected to make the disease more severe. Similarly, as noted above, a very rare X-linked form of CEP resulted from a particular mutation in the X-linked GATA1 erythroid transcription factor. Hence, mutations in this gene in an erythropoietic porphyria most likely would increase disease severity, particularly in males.

In the AHPs, it was recently reported that a polymorphism in the *PEPT2* gene, which is responsible for the transport of ALA from the cerebrospinal fluid (50), is also involved in the re-uptake of ALA from the urine into renal cells, which can be toxic and cause renal insufficiency (51). If these findings are confirmed, all patients with an AHP, particularly those with elevated ALA levels, should be screened for the higher affinity *PEPT2* polymorphic genotypes, 1*1 or 1*2, which in this single study were associated with increased risk for renal disease.

Finally, it is clear that environmental factors play an important role in the pathogenesis of the porphyrias. For the AHPs, there is a list of porphyrinogenic drugs which patients should avoid as they can trigger an acute attack (see the Porphyrias Consortium website : https://www.rarediseasesnetwork.org/cms/porphyrias). In addition, AHP attacks can be triggered by fasting or dieting and by various hormonal changes including the use of high progesterone birth control medications. With respect to PCT, it is now appreciated that environmental agents and genetic factors that cause hepatic iron overload are precipitating factors for the cutaneous manifestations. These include significant alcohol consumption, infections such as HCV and HIV, smoking, and various cytochrome P450 variants. For the erythropoietic porphyrias, the amount of sun exposure is directly related to the degree of phototoxicity experienced by these patients.

It is likely that in the future, additional modifying genes will be identified that exacerbate AHP acute attacks or increase the severity of PCT or an erythropoietic porphyria. In addition, there may be protective variants that decrease the likelihood of an AHP attack or increase the amount of sun exposure an EPP patient can endure before the first prodromal symptom. Such advances in the identification of modifying genes will lead to better clinical management and possibly to new safe and effective treatments.

3. The AHPs

3.1. Clinical Manifestations of the AHPs

The AHPs include the three autosomal dominant disorders, AIP, HCP, VP, and the ultra-rare autosomal recessive ADP (Table 3). All four acute porphyrias present with acute neurovisceral attacks, which typically start with a prodome of ‘brain fog’, insomnia, and fatigue, and then crippling abdominal pain that progresses to include tachycardia, hypertension, motor weakness, and seizures. The attacks are triggered by various porphyrinogenic factors, including fasting, cytochrome P450-inducing drugs, and hormonal fluctuations, all of which induce the hepatic expression of *ALAS1*, the first and rate-limiting enzyme of the heme biosynthetic pathway. When hepatic *ALAS1* is induced, the respective enzyme deficiency becomes rate-limiting, leading to decreased heme production and depletion of the hepatic ‘free’ heme pool. This leads to derepression and further induction of hepatic *ALAS1*, resulting in the marked elevation of the putative neurotoxic porphyrin precursors, ALA and PBG. Patients with HCP and VP may also present with cutaneous photosensitivity.

3.2. Genetics of the AHPs

As noted above, three of the AHPs, AIP, HCP, and VP, are autosomal dominant disorders with incomplete penetrance, while ADP is inherited as an autosomal recessive disease with variable clinical severity depending on the amount of residual ALAD activity (Table 3). Of the three autosomal dominant AHPs, AIP is by far the most common, with an estimated prevalence of 1 in ~1600-1700 (17, 18). Each disorder develops clinical manifestations due to LOF mutations in the respective heme biosynthetic gene, *HMBS*, *CPOX*, *PPOX*, and *ALAD* (Tables 2 and 3). For the three autosomal dominant AHPs, LOF mutations lead to half-normal activity of the respective enzyme, whereas in ADP, patients typically have LOF mutations that result in markedly decreased, but not absent ALAD activity. Prokaryotic expression studies have shown that patients with clinically severe ADP had two *ALAD* mutations with <8% of expressed wild-type activity, whereas those with less severe disease had one mutation with significant residual activity (ranging from 19-70% of expressed wild-type activity) and a severe *ALAD* mutation (<8% of expressed wild-type activity) on the other allele (52).

To date, >685 mutations for the four AHPs have been reported in their respective genes [HGMD version 2018.2; (42)], (see Table 4 for types of AHP mutations)]. There are 415 *HMBS* mutations causing AIP, 69 *CPOX* mutations causing HCP, and 182 *PPOX* mutations causing VP. While most AHP mutations are “private,” identified in a single or few families, certain mutations are more common as they occur at CpG dinucleotides, hot spots for mutations (29). For the *HMBS* gene, these include the mutations encoding p.R173W and p.R167Q. Table 5 indicates the most common mutations causing AIP, HCP, and VP, based on the Mount Sinai Porphyria Diagnostic Laboratory’s experience. Founder mutations occur in certain populations and are inherited for generations. For example, in Sweden and Norway, *HMBS* c.593G>A (p.W198X) is a common founder mutation dating back multiple generations (53). There are also founder mutations for AIP in the Netherlands [c.346C>T, p.R116W; (54)], Switzerland and France [c.848G>A, p.W283X; (55)], Argentina [c. 331G>A, p.G111R; (56)], and the Murcia region of Spain [c.669_698del; (57)]. Although there are no specific founder or common mutations for HCP, for VP, there are an estimated 30,000 to 40,000 patients in South Africa with the founder mutation c.175C>T (p.R59W), which was brought to the Cape by a Dutch couple who arrived in 1680 (58). It should be noted that AIP patients who have *HMBS* exon 1 mutations have normal erythrocytic HMBS activity, as this exon is not included in the alternatively-spliced erythroid *HMBS* transcript (Figs. 2 and 3; also see Principles Section above).

Several previously reported pathogenic *HMBS* mutations causing AIP have been shown to be benign variants (17). To verify the pathogenicity of the genes causing the AHPs, an International Collaborative has been established between the Porphyrias Consortium of the NIH-supported Rare Disease Clinical Research Network (https://www.rarediseasesnetwork.org/cms/porphyrias) and the European Porphyrias Network (EPNET: http://porphyria.eu). All previously reported and new mutations causing the AHPs are being validated both clinically and biochemically (i.e. in patients with acute attacks and markedly elevated ALA and/or PBG values). Each mutation will be classified as pathogenic,

likely pathogenic, or benign, based on clinical and biochemical evidence (see Chen *et al.*, 'Collaborative' in this issue). This information will be available in a public database.

Of particular importance, clinical manifestations for the three autosomal dominant AHPs do not occur in most mutation carriers. These asymptomatic individuals are termed 'latent heterozygotes,' and although they have never had an acute attack, they should be counseled to avoid the precipitating factors that can trigger an attack, including the use of porphyrinogenic drugs, dieting or fasting, and certain hormonal changes resulting from the use of high progesterone birth control medications. Previous studies have estimated that the penetrance of symptomatic AIP acute attacks ranged from <10% to ~30% (59-61). More recently, using samples from the blood bank or large population-based exome/genome databases, the prevalence of *HMBS* mutations was estimated to be 1 in ~1700 Caucasians (17, 18). In contrast, the prevalence of symptomatic AIP patients, based on data from porphyria specialist centers in Europe, was estimated to be 1 in ~200,000 Caucasians (17, 18). Together, these studies indicate that the penetrance of AIP is estimated to be only ~1% of *HMBS* heterozygotes (17, 18). The fact that the penetrance of clinical manifestations is so low among unrelated AHP heterozygotes, but much higher (~23%) within AHP families who have at least one member with recurrent attacks, strongly suggests that there may be important modifying genes and/or other environmental factors that trigger the severe acute attacks (18). To date, little serious effort has been undertaken to systematically search for such modifying genes and other factors.

3.3. Homozygous Dominant (HD)-AHPs

Rare cases of patients with biallelic mutations in their *HMBS, PPOX,* and *CPOX* genes, causing HD-AIP, HD-VP, and HD-HCP, respectively, have been reported (19-25, 27, 62). These patients have profound deficiency of their respective AHP enzyme, resulting in severe disease with marked neurological and/or cutaneous manifestations that begin in infancy or early childhood with growth retardation and short stature. Interestingly, with the exception of one HD-HCP patient (24), acute neurovisceral attacks have not been reported in HD-AHP patients.

3.3.1. HD-AIP—To date, five HD-AIP patients have been reported (19-22, 63). These patients had low levels of residual HMBS activity, typically <4% of normal (64), and constitutively elevated urinary ALA and PBG. In addition to gross psychomotor retardation, these patients have early-onset ataxia, nystagmus, and dystonia. The neurological impairment is progressive and patients typically die in childhood. Interestingly, of the five HD-AIP patients reported to date, four had mutations encoding p.R167Q, p.R167W, or p.R173Q. One was homozygous for p.R167W (19), two siblings were heterozygous for p.R167Q and p.R167W (21), while one was a compound heterozygote for p.R167W and p.R173Q (20).

3.3.2. HD-VP—HD-VP patients who have biallelic *PPOX* mutations have been reported to have between 5 and 20% of normal PPOX activity (27). They present in infancy with psychomotor delay, nystagmus, severe cutaneous photosensitivity and photomutilation, and skeletal deformities of the hand. Of the 11 HD-VP patients reported to date, eight have had

both of their *PPOX* mutations identified (28, 65). Most were compound heterozygotes, while two unrelated HD-VP patients, both with consanguineous parents, were homozygous for the *PPOX* mutations encoding p.D349A and p.A433P (65). Of interest, although the p.R59W mutation is frequent in South Africa, no homozygotes have been identified to date, presumably because this mutation is severe and nearly abolishes PPOX activity, and therefore, is not compatible with life (58).

3.3.3. HD-HCP—Only two biochemically-confirmed HD-HCP patients have been reported to date, both young females with <10% of normal CPOX activity and marked elevation of coproporphyrins, with predominance of the III isomer (23, 24). One presented with CEP-like symptoms, including skin fragility and erythrodontia (23), while the other had skin pigmentation, hypertrichosis and episodic acute neurovisceral attacks with markedly elevated urinary ALA and PBG during the attacks (24). A clinically distinct subtype of HD-HCP, Harderoporphyria, has been described in four families to date (25, 62, 66, 67). Patients with Harderoporphyria present with neonatal jaundice and hemolytic anemia and accumulate harderoporphyrin in their feces, an intermediate metabolite in the conversion of coproporphyrin to PPIX. Of interest, HD-HCP patients identified to date were either homozygous for the *CPOX* mutation encoding p.K404E or carried this mutation in trans- with another pathogenic *CPOX* mutation (62, 66, 67). Only one patient was homozygous for the *CPOX* mutation encoding p.H327R (25). Mutagenesis studies have shown that CPOX amino acids residues Y399 to K405 are directly involved in the enzyme's oxidative decarboxylation of harderoporphyrinogen (62), while molecular modeling studies based on the CPOX crystal structure support that the p.H327R change also interferes with this enzymatic step (68).

4. Hepatic Cutaneous Porphyria: Porphyria Cutanea Tarda (PCT)

4.1. Clinical Manifestations of PCT

PCT, the most common human porphyria, is classified as a hepatic cutaneous porphyria since it presents with cutaneous bullous lesions, but the primary site of porphyrin accumulation is the liver. Clinically, PCT is characterized by the development of blisters on sun-exposed skin, similar to those of CEP. The disease becomes active when patients are exposed to predisposing factors that cause hepatic iron overload, including excess alcohol consumption, estrogen use, infections (HCV, HIV, etc.), and smoking (1, 2, 69). It has been postulated that the hepatic activity of UROD is markedly reduced during active disease due to the formation of uroporphomethene, an iron-oxidized product of uroporphyrinogen, which acts as a reversible inhibitor of UROD activity (14-16). This leads to marked accumulation of porphyrins, predominantly consisting of uroporphyrin and 7-carboxylate porphyrin in the liver and urine.

4.2. Genetics of PCT

PCT occurs both in a Type 1 sporadic subtype, in the absence of a *UROD* mutation, and a Type 2 familial subtype, in which a pathogenic *UROD* mutation is inherited in an autosomal dominant pattern (Table 3). To date, over 120 *UROD* mutations have been reported to cause PCT in HGMD version 2018.2 (see Table 4 for mutation types). Of clinically and

biochemically documented cases of PCT, about 75-80% have the Type I sporadic subtype with no *UROD* mutation, while the remaining 20-25% have the Type 2 familial form with a pathogenic *UROD* mutation (see Weiss et al., this volume). Type 1 sporadic PCT patients have normal hepatic UROD activity except during active disease when the UROD inhibitor is formed, while Type 2 familial PCT patients had half-normal UROD activity systemically (70). Clinically, the two forms are indistinguishable. While the half-normal activity of UROD predisposes to PCT, additional susceptibility factors are required to activate disease in Type 2 familia l patients, like the Type 1 sporadic patients.

As iron overload is a major predisposing factor, patients who have hemochromatosis (*HFE*) gene mutations are at higher risk for developing PCT. In fact, >50% of PCT patients with active disease are reported to carry *HFE* mutations, most commonly the mutation encoding p.C282Y or p.H63D (69).

4.3. Hepatoerythropoietic Porphyria

Biallelic *UROD* LOF mutations cause Hepatoerythropoietic Porphyria (HEP), a rare condition that presents with CEP-like manifestations, including hemolytic anemia, severe skin blistering that begins in infancy, discolored teeth (erythrodontia), and reddish-colored urine (1). Patients typically have 15-20% of normal UROD activity.

4.4. Dual Porphyrias

To date, >15 patients have been identified to have clinical and biochemical features of two major porphyrias (71-73). The majority of these patients have combined deficiency of UROD and HMBS, CPOX, or PPOX, consistent with the fact that PCT is the most common porphyria. Other combinations, including dual deficiencies of CPOX and HMBS; UROS and CPOX; CPOX and ALAD; UROS and UROD; CPOX and PPOX, have been reported. However, it should be noted that only a few cases have been confirmed by genetic testing.

5. Erythropoietic Cutaneous Porphyrias

The erythropoietic porphyrias include three disorders, CEP, EPP, and XLP (Table 3). These disorders are characterized by cutaneous photosensitivity that results from the massive accumulation of photoreactive porphyrins in the bone marrow erythroid cells and circulating erythrocytes, which when released during erythrocyte senescence gain access to blood vessel endothelial cells and other organs, particularly the liver in EPP and XLP, as PPIX is excreted via the hepatobiliary system.

5.1. CEP

5.1.1. Clinical Manifestations of CEP—CEP is a rare disorder, with less than 250 cases reported to date. Clinically, CEP is characterized by marked cutaneous photosensitivity, with blistering and formation of vesicles on sun-exposed skin. Recurrent vesicles and secondary infection can lead to cutaneous scarring as well as skin and bone loss that is disfiguring. Other prominent symptoms include red urine since birth, reddish-brownish discoloration of the teeth (erythrodontia) and hemolytic anemia. Clinical severity can vary, from non-immune hydrops fetalis *in utero* to transfusion-dependent severe disease,

or later-onset disease with only moderate or mild cutaneous photosensitivity in adulthood. Biochemically, CEP patients display markedly elevated erythrocyte and urinary uroporphyrin I and coproporphyrin I isomers, which are nonphysiologic and phototoxic porphyrins that accumulate, as they are not further metabolized to heme.

5.1.2. Genetics of CEP: CEP is an autosomal recessive disorder due to LOF mutations in the *UROS* gene (Table 3). To date, > 50 *UROS* mutations have been reported in HGMD, of which ~60% are missense (Table 4). The most common missense mutation encoding p.C73R occurs in ~35% of genotyped CEP patients, which can result in non-immune hydrops fetalis or newborns with severe anemia, particularly when homozygous (31). Among the mutations causing CEP, there are four verified regulatory mutation in the promoter (39). These mutations, located within a 20 bp region of the promoter (−90 to −70 from the initiation ATG), involve transcription factor binding sites for GATA1 and CP2 (Fig. 4). They have been expressed in luciferase constructs to demonstrate their pathogenicity (39). Genotype/phenotype correlations predict disease severity, as the activity of the encoded UROS enzyme deficiency correlates with clinical severity. For example, the mutation encoding p.C73R has <0.1% of wild-type UROS activity when prokaryotically expressed, while L4F, which causes transfusion-independent later-onset CEP, has >3% of wild-type UROS activity [Table 6; (74)]. These genotype/phenotype correlations are particularly useful to predict transfusion dependency and the need for early hematopoietic stem cell transplantation, when feasible.

To date, three cases of the CEP phenotype have been reported to be caused by a mutation encoding p.R216W in the X-linked *GATA1* gene (46, 47). GATA*1*, a transcriptional factor that is critical for normal erythropoiesis and megakaryocyte development, regulates the expression of various erythroid-specific genes, including *UROS*, *ALAS2*, and the α- and β-globins. All three unrelated CEP male patients were hemizygous for the p.R216W *GATA1* mutation that is located in the *N*-terminal zinc finger domain, which mediates the interaction with cofactors to stabilize the binding of the GATA1 transcription factor to promoter binding sites of its target genes. Two of the patients did not have *UROS* mutations, while one was heterozygous for the *UROS* mutation encoding p.D113V (46). Clinically, these patients had a photosensitive bullous dermatosis, scarring of the skin, microcytic anemia, and markedly increased levels of uroporphyrin I, similar to CEP patients with *UROS* mutations. However, they had additional hematological abnormalities, including increased fetal hemoglobin and thrombocytopenia, which were unique to the *GATA1* mutation-positive patients (46, 47).

Additionally, a non-inherited mild form of CEP secondary to myeloid malignancy, most commonly myelodysplastic syndrome (MDS), has been reported in a small number of patients (75, 76). Sarkany *et al.* demonstrated in four of these patients that germline *UROS* and *GATA1* mutations were not detectable, erythrocyte UROS activities were normal, and that only a small fraction of their circulating erythrocytes were uroporphyric (75). As MDS causes genomic instability, it is highly probable that a minor clone of erythropoietic cells contained an acquired somatic *UROS* mutation that was not detected due to the small number of mutation-positive cells relative to normal erythroid cells.

5.2. Erythropoietic Portoporphyria

5.2.1. Clinical Manifestations of EPP—EPP, the most common childhood porphyria, is primarily characterized by extremely painful photosensitivity that is accompanied by marked elevation of free PPIX in plasma and erythrocytes. A mild microcytic anemia occurs in ~20 to 30% of patients. ~20% of patients experience cholelithiasis, while ~5% of patients develop liver failure due to hepatobiliary involvement, requiring liver transplantation. Since the PPIX released from erythroid cells is lipophilic, it accumulates in the vascular endothelial cells and liver and is excreted via the hepatobiliary system. The accumulation of PPIX in the liver and biliary tract leads to cholestasis and hepatobiliary fibrosis, and eventually, liver failure.

5.2.2. Genetics of EPP—EPP is an autosomal recessive disorder due to mutations in the ferrochelatase (*FECH*) gene (Table 3). Its prevalence is estimated at 1 in ~50,000 Caucasians (61). To date, >190 LOF *FECH* mutations have been reported, including ~7% that are large deletions, which are sequencing cryptic and require gene dosage analysis [Table 4; (77)]. Notably, ~95% of EPP patients have a pathogenic LOF *FECH* mutation on one chromosome and the common ‘low expression allele’, IVS3-48A>G, on the other (32-34). As shown in Figure 5, the IVS3-48T>C change leads to the increased use of an alternative 3’-splice site that is 63 nucleotides upstream of the normal site, leading to an aberrant transcript with 63 additional nucleotides. Two in-frame stop codons in this additional sequence target this aberrant transcript for nonsense-mediated decay. As a result, the ‘low expression allele’ has ~30% of normal FECH activity (34). The allele frequency of the ‘low expression allele’ ranges from ~2% in Africans to 6% in Caucasians and 30-35% in East Asians and Hispanics, based on GenomAD data. Only a small number of patients with EPP have two LOF *FECH* mutations, which leads to a more severe form of the disease and seasonal palmer keratoderma. The hyperkeratosis ranged in severity from a waxy keratoderma over the whole palm to mild hyperkeratosis of the first interdigital web (78). To date, over 20 patients have been reported in 16 unrelated EPP families. In these patients, ~85% of the mutations were missense, and most patients were compound heterozygotes (78).

Recently, a dominant mutation in the *CLPX* gene was reported that caused an EPP-like phenotype (48). CLPX is a mitochondria AAA+ (or ATPase associated with a variety of cellular activities) unfoldase that promotes heme biosynthesis by activation of ALAS 1 and 2. Importantly, CLPX associates with CLPP to form CLPXP, an ATP-dependent serine protease that mediates the heme-induced turnover of ALAS 1 and 2 (79, 80). Within the CLPXP complex, CLPX binds substrate, unfolds stable tertiary structure in the substrate, and then traslocates the unfolded polypeptide chain into the proteolytic compartment of CLPP. A dominant *CLPX* mutation encoding p.G98D in the active site was shown to reduce its ATPase activity and to weaken its interaction with CLPP, resulting in reduced proteasome activity. This leads to increases the post-translational stability of ALAS2, leading to the abnormal accumulation of PPIX and clinical photosensitivity (48).

A non-congenital late-onset form of EPP also may develop as a rare complication of MDS or a myeloproliferative disorder (MPD) (81-83). One patient with MPD had clonal

expansion of hematopoietic cells with an acquired somatic mutation that resulted in the complete deletion of one *FECH* allele. This patient had the IVS-48T>C 'low expression allele' on the remaining *FECH* allele, resulting in the EPP phenotype (82).

5.3. XLP

XLP is clinically indistinguishable from EPP, although biochemically, XLP patients accumulate both Zn-PPIX and free protoporphyrin, whereas EPP patients predominantly accumulate free-PPIX. As XLP is an X-linked disorder, most symptomatic patients are males. Females can be asymptomatic or as severely affected as their male relatives, due to random X-chromosomal inactivation (see Genetic Principles Section). Unlike the other porphyrias, XLP is due to GOF mutations in the erythroid-specific *ALAS2* gene [Table 3; (13)]. To date, six XLP mutations have been reported, all of which reside in the terminal exon 11 of *ALAS2* (13, 32, 84). These mutations are nonsense or frameshift lesions that prematurely truncate or extend the carboxy-terminal residues (Fig. 6). The truncations and extensions lead to structural alterations of the carboxyl-terminus, which normally shutters the active site, to abnormally remain open (4, 84). This results in increased ALAS2 activity [2 to 3–fold in *in vitro;* (84)], and consequentially, increased production and accumulation of PPIX.

As noted above, s GOF mutation in the *ALAS2* gene has been shown to increase the severity of other porphyrias, including CEP. In four unrelated CEP patients with *UROS* mutations encoding p.C73R/p.P248Q, one had moderately severe disease and a novel *ALAS2* exon 11 gain-of-function mutation (c.1757A>T), while the other three lacking *ALAS2* mutations had milder disease (49).

6. Summary

The porphyrias, as a group of eight metabolic disorders, illustrate many genetic principles of Mendelian disorders. There are multiple modes of inheritance in the eight disorders: three AHPs are inherited as autosomal dominant traits with markedly reduced penetrance, while most erythropoietic cutaneous porphyrias are inherited as fully penetrant autosomal recessive or X-linked traits, the latter in XLP males. Although about 20% of patients with PCT have a LOF mutation in their *UROD* gene, most patients have the sporadic or acquired form secondary to factors that induce hepatic iron overload and the formation of the UROD inhibitor, uroporphomethene. Together, the eight porphyrias result from a total of >1000 mutations in the eight heme biosynthetic genes. Multiple mutations of various types occur in each disease, giving rise to the molecular genetic heterogeneity and variable severity in a given porphyria due to the level of the mutation's residual activity *in vivo.* While most of these mutations are LOF mutations that result in decreased enzymatic activity, XLP is caused by GOF mutations that alter the carboxyl-terminal structure of the ALAS2 protein resulting in a more catalytically active enzyme. The three autosomal dominant AHPs have very low penetrance, suggesting that modifying genes (predisposing and/or protective) and/or environmental factors are responsible for the penetrance as well as the onset and severity of these disorders. While genetic sequencing of the heme biosynthetic genes has identified the disease-causing mutations in almost all porphyria patients, the nature of the

primary genetic defects for a few patients with AHPs and erythopoietic porphyrias remain to be determined, perhaps by whole genome sequencing. Such studies identified the roles of the *ALAS2* and *CLPX* genes. Future studies of the porphyrias may identify other heme transport and/or degradation genes that may provide additional understanding of the pathophysiology of the porphyrias and identify new targets for their therapy.

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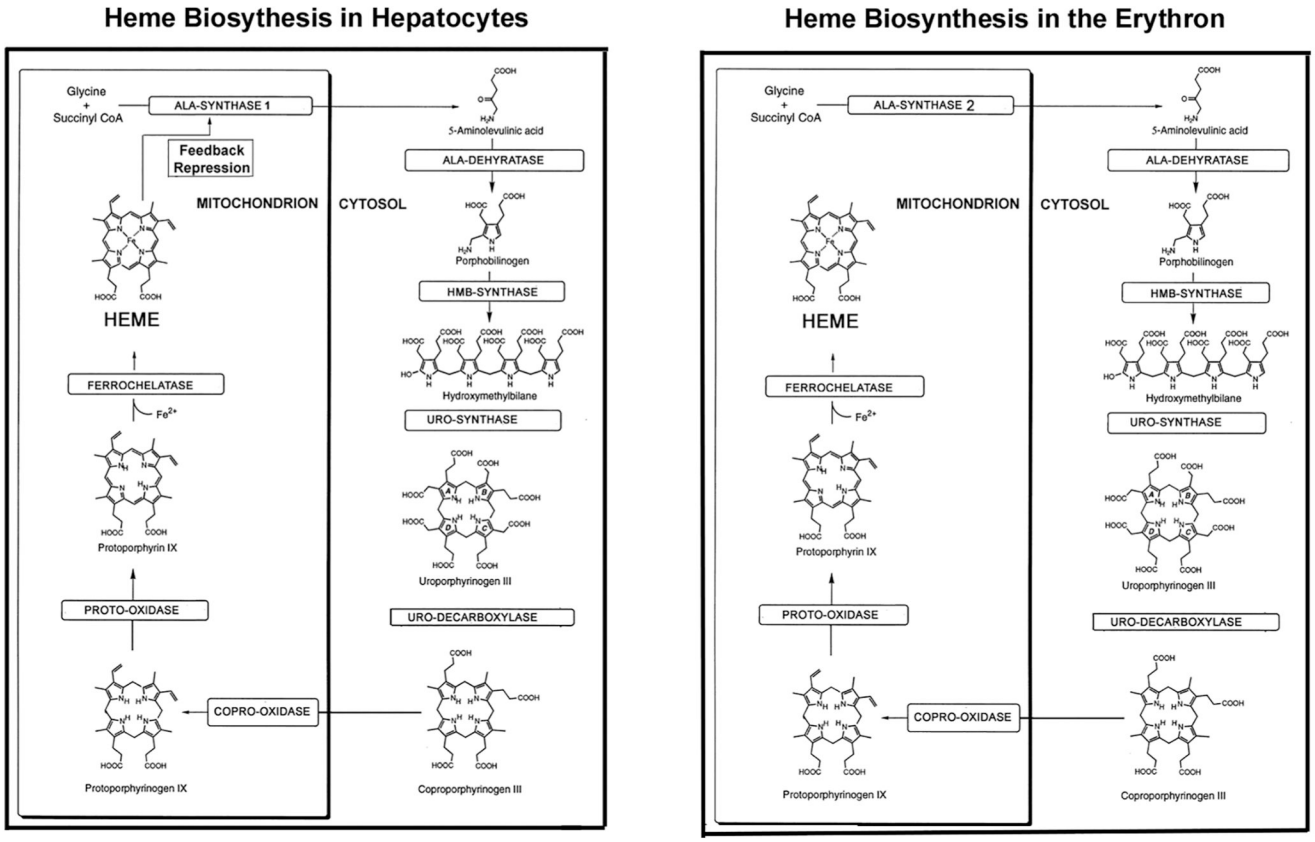


Fig 1.

The human heme biosynthetic pathway in hepatocytes (left) and erythroid cells (right). The eight enzymatic steps, their porphyrin precursor and porphyrin substrates, and metabolic products are shown. Note that the first enzyme in the pathway in hepatocytes, ALAS1 is regulated by negative feedback, repression by the concentration of the end product heme in the free heme pool. In contrast, the erythroid-specific ALAS2 enzyme is responsible for synthesis of 5'Aminolevulinic acid (ALA) in the erythron and its expression is under control of erythroid regulatory elements. The subsequent enzymatic steps in the pathway are similar, some of the enzymes having unique housekeeping and erythroid specific promoters. Modified from Anderson *et al.:* Disorders of heme biosynthesis: X-Linked sideroblastic anemia and the Porphyrias. Online Metabolic and Molecular Bases of Inherited Diseases (https://ommbid.mhmedical.com).

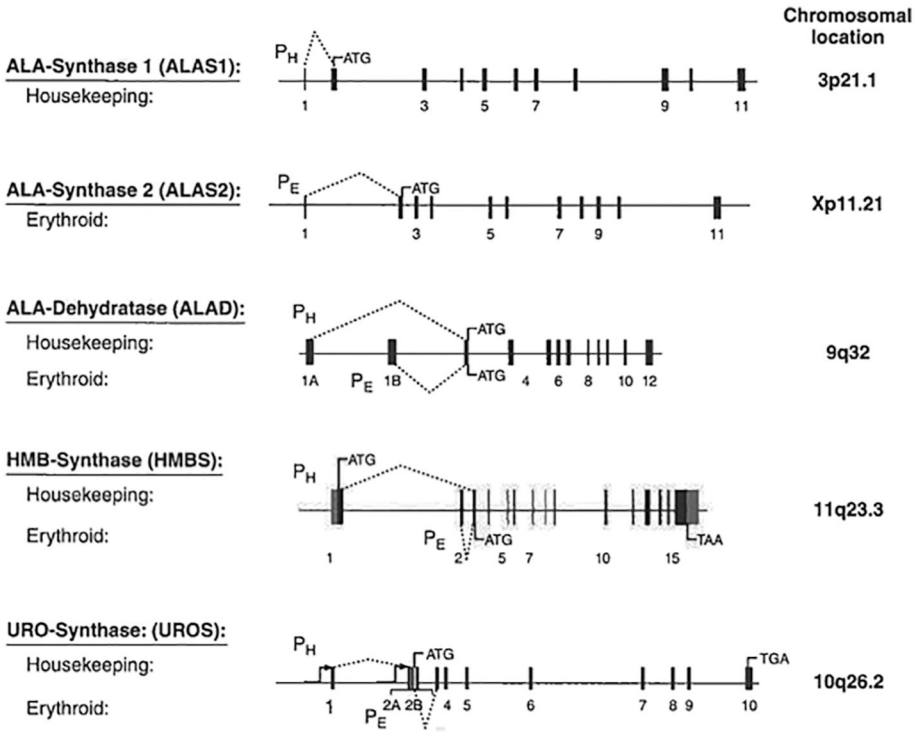


Fig 2.

The first four genes in the human heme biosynthetic pathway have unique housekeeping (P_H) and erythroid-specific (P_E) promoters. The dotted lines indicate the exons transcribed by each promoter. The *ALAS1* and *ALAS2* genes are on different chromosomes and are regulated by negative feedback repression (*ALAS1*) or by erythroid transcription factors, and the iron response element (*ALAS2*).

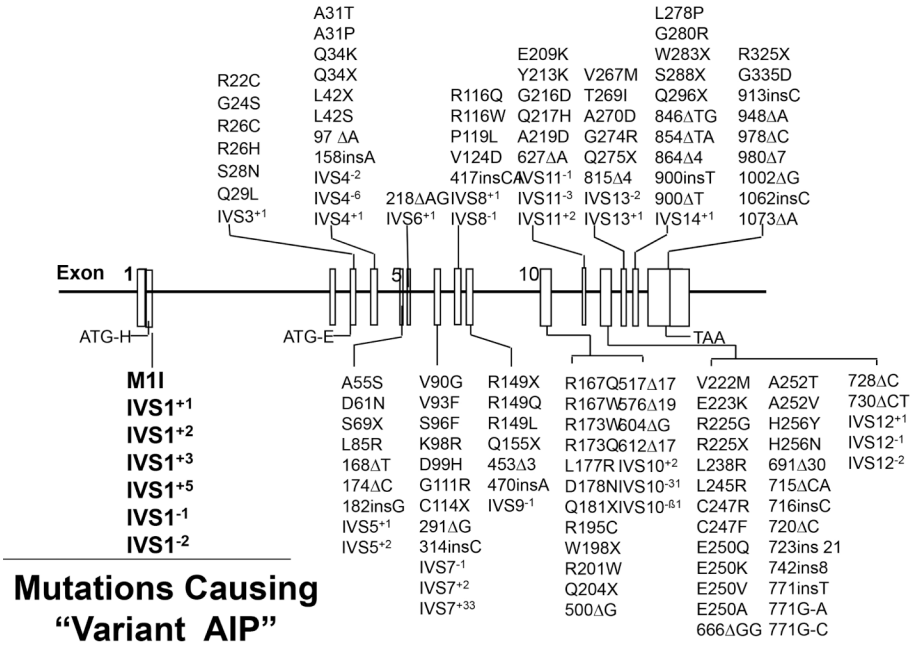


Fig 3.

Schematic of the *HMBS* gene indicating the numbered exons (black bars) and introns. Various known mutations are indicated by their exonic/intronic positions. Note that mutations that alter the sequence of the housekeeping gene’s initiation of transcription in exon 1 (ATG-H) or that alter the normal splicing of exon 1 to exon 3, result in a “variant form of AIP” in which the housekeeping enzyme is not synthesized, whereas, the erythroid-specific enzyme is expressed at normal levels. Thus, patients who have mutations causing “variant AIP” will have half-normal activity of the housekeeping HMBS isozyme and AIP, but normal activity of the HMBS enzyme in circulating erythrocytes.

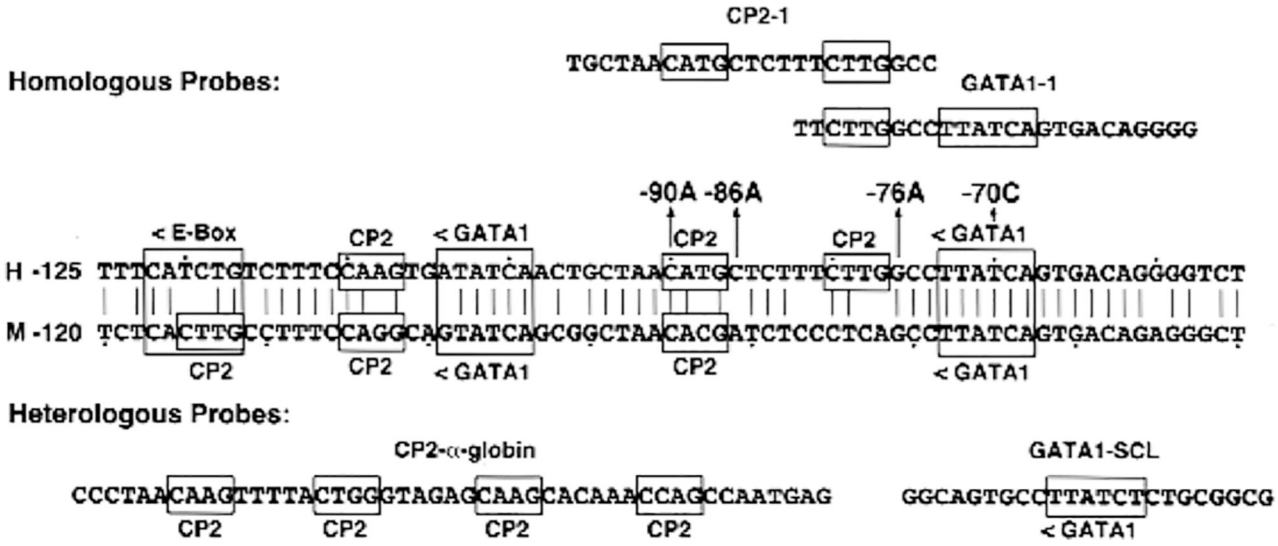
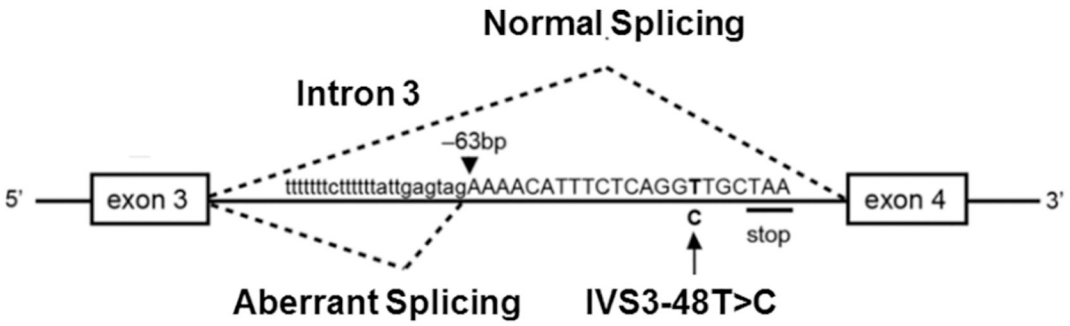


Fig 4.

Partial sequence of the human and murine UROS erythroid-specific promoters showing the GATA1 and CP2 transcription factor binding sites. The location and orientation (<, >) of the GATA1, E-box, and CP2 erythroid binding elements are indicated, as are the four novel promoter mutations causing CEP. Dots are placed every tenth nucleotide. The homologous CP2 site from the α- globin gene promoter and the GATA1 site from the human stem cell leukemia (SCL) gene promoter are shown.



~Allele Frequency in gnomAD

Racial or Ethic Group	% C Allele
Hispanic	0.348
East Asians	0.326
South Asians	0.089
Ashkenazi Jews	0.063
Caucasians	0.056
Africans	0.019

Fig 5.

Alternative splicing of the *FECH* IVS-48T/C intronic sequence. The presence of the IVS-48T/C variant modulates the splicing efficiency of a constitutive cryptic acceptor splice-site. Note that the IVS-48C variant alters splicing such that 63 intronic base pairs are included in the mutant allele resulting in an abnormal transcript and decreased expression (35% of normal) for this allele. The minor allele frequency for the IVS-48C mutation in various racial and ethic groups from gnomAD is shown. Gouya *et al., Nature Genet.* 30:27-28, 2002.

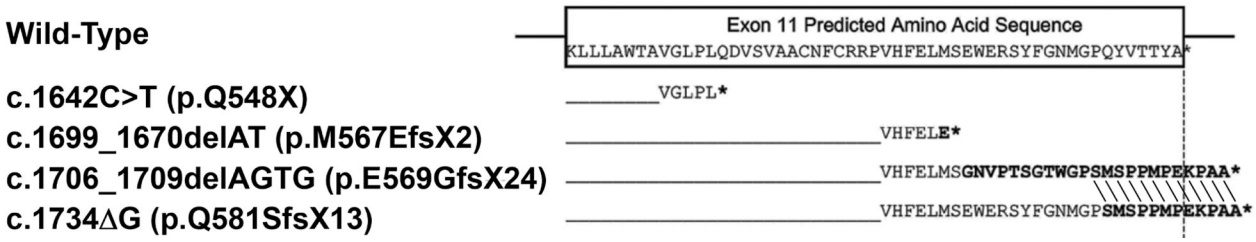


Fig 6.

Variations in the carboxy-terminal mutant sequences of XLP mutations. The partial wild-type exon 11 *ALAS2* sequence is boxed. The hybrid sequences of wild-type *ALAS2* and the sequence of several GOD mutations are aligned below the wild-type sequence. The termination codons are denoted by asterisks. Note that for the pE569GfsX24 and pQ581SfsX13 mutations, the last 12 mutated residues bolded are identical and different from the wild-type sequence. Balwani *et al., Molec. Med.* 19:26-35, 2013.

Table 1.

The Porphyrias Illustrate Many Genetic Principles

1. Multiple Modes of Inheritance:
Autosomal Dominant (AIP, HCP, & VP) & Recessive (AHPs, CEP, & EPP)
X-Linked (XLP, Rarely CEP)
Rare Homozygous Dominant Types (AIP, HCP, & VP)
Sporadic/Acquired (PCT)
Prevalence & Penetrance (AHPs)
2. Multiple Mutations Causing Each Disease (All)
Loss- or Gain-of-Function Mutations (All)
Common Low Expression Allele (EPP)
Regulation of Heme Biosynthesis & Regulatory Mutations
Negative Feedback Repression (AHPs)
Variable Expressivity (All)
Genotype/Phenotype Correlations (CEP)
Benign Variants (All)
3. X-Chromosomal Inactivation (XLP)
4. Modifying Genes Responsible for Expression & Severity (AHPs, EPP)
5. Pharmacogenetic/Ecogenetic Disorders (AHPs)

Table 2.

Characteristics of The Heme Biosynthesis Genes

Gene	Gene Symbol	Chromosome Position	Gene Length (kb)	Exons	cDNA (bp)	Encoded Protein Amino Acids
Aminolevulinic Acid Synthase 1	*ALAS1*	3p21.1	~20	12	~2458	641
Aminolevulinic Acid Synthase 2	*ALAS2*	Xp11.21	~26	12	~2044	588
ALA-Dehydratase Deficiency	*ALAD*	9q33.1	~19	12	~3200	331
Hydroxymethylbilane Synthase	*HMBS*	11q23.3	~13	15	~1526	362
Uroporphyrinogen Synthase	*UROS*	10q25.2-q26.3	~39	10	~1371	266
Uroporphyrinogen Decarboxylase	*UROD*	1p34.1	~8	10	~1408	368
Coproporphyrinogen Oxidase	*CPOX*	3q12	~18	7	~2728	455
Protoporphyrinogen Oxidase	*PPOX*	1q22	~9	13	~1716	478
Ferrochelatase	*FECH*	18q21.3	~46	11	~7277	424

Human Gene Mutation Database 2018.2: www.hgmd.org | Aug 15, 2018

*Genes listed in pathway sequence for ALAS to FECH

Table 3.

Classification of the Porphyrias: Their Enzyme Defects & Inheritance

Classification	Enzyme Deficiency	Inheritance*
Acute Hepatic Porphyrias (AHPs):		
Acute Intermittent Porphyria	HMB-Synthase	AD
Hereditary Coproporphyria	COPRO-Synthase	AD
Variegate Porphyria	PROTO-Oxidase	AD
ALA-Dehydratase Deficiency	ALA-Dehydratase	AR
Hepatic Cutaneous Porphyria (PCT):		
Porphyria Cutanea Tarda Type 1	Hepatic UROD Deficiency	Sporadic
Porphyria Cutanea Tarda Type 2	Systemic UROD Deficiency	AD
Erythropoietic Cutaneous Porphyrias:		
Congenital Erythropoietic Porphyria	URO-Synthase	AR
Erythropoietic Protoporphyria	Ferrochelatase	AR
X-Linked Protoporphyria	ALA-Synthase 2	X-L

*AD = Autosomal Dominant; AR = Autosomal Recessive; X-L= X-Linked

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Table 4.

Reported Mutations Causing The Porphyrias (HGMD v2018.2)

HGMD Porphyria	ALAD ADP	HMBS AIP	CPOX HCP	PPOX VP	UROS CEP	UROD PCT	FECH EPP	ALAS2 XLP
Mutation Type:								
Missense	9	140	43	69	30	71	54	1
Nonsense	0	33	6	18	1	8	26	1
Splicing substitutions (Consensus)	0	76	2	22	2	10	39	0
Splicing substitutions (Cryptic)	2	15	2	7	2	3	7	0
Regulatory substitutions	0	7	0	4	6	1	2	0
Small deletions	1	84	7	36	2	16	40	3
Small insertions/duplications	0	41	7	21	2	7	10	0
Small indels	0	8	1	3	1	1	1	0
Gross deletions	0	13	3	3	2	5	13	1
Gross insertions/duplications	0	2	0	1	2	0	0	0
Complex rearrangements	0	2	0	0	1	0	3	0
Repeat variations	0	0	0	0	0	0	0	0
TOTAL	**12**	**421**	**71**	**184**	**51**	**122**	**195**	**6**

Table 5.

Most Common Mutations: Acute Porphyrias – Mount Sinai Experience Since 1/1/07

AIP / *HMBS*			HCP / *CPOX*			VP / *PPOX*		
Mutation		% of 315	Mutation		% of 29	Mutation		% of 54
cDNA	Amino Acid	Unrelated Probands	cDNA	Amino Acid	Unrelated Probands	cDNA	Amino Acid	Unrelated Probands
c.517C>T	p.R173W	13.0	c.601G>A	p.E201K	13.8	c.1303C>T	p.Q435X	5.6
c.500G>A	p.R167Q	7.6	c.565G>A	p.G189S	6.9	c.503G>A	p.R168H	5.6
c.848G>A	p.W283X	6.3	c.626T>A	p.V209D	6.9	c.503G>T	p.R168L	5.6
c.331G>A	p.G111R	3.2	c.395C>T	p.A132V	3.4	c.565C>T	p.Q189X	5.6
c.673C>T	p.R225X	2.9	c.520G>A	p.A174T	3.4	c.217C>G	p.L73V	3.7
c.499C>T	p.R167W	2.5	c.980A>G	p.H327R	3.4	c.454C>T	p.R152C	3.7
c.973C>T	p.R325X	2.2				c.40G>C	p.G14R	1.9
c.76C>T	p.R26C	1.9						
c.992C>T	p.A331V	1.9						
c.1084delT	c.1084delT	1.9						
TOTAL:		**45.5%**			**52%**			**34%**

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Table 6.

Genotype/Phenotype Correlations in CEP

Phenotype	Genotype	Residual Activity Expressed in E.coli Alleles/Total	
Hydrops Fetalis/ Newborn Demise:			
	C73R/C73R	<0.1 / <0.1	= <0.1
Transfusion Dependent:			
Severe	C73R/T228M	<0.1 / <0.1	= <0.1
Transfusion Independent:			
Moderate	T62A/E249X	<0.1 / 1.8	= 1.8
	G225S/T228L	2.1 / <0.1	= 2.1
	L4F/C73R	2.9 / <0.1	= 2.9
	L4F/Deletion	2.9 / 0	= 2.9
	L4F/IVS2+1	2.9 / ?	= 2.9
Mild	Y19C/G225S	2.7 / 2.1	= 4.8
	V99A/Ins211A	3.7 / 1.7	= 5.4
	C73R/A104V	<1.0 / 5.6	= 5.6
	A66V/C73R	14.5 / <0.1	= 14.5
	L4F/V82F	2.9 / 24.2	= 27.1

Desnick, RJ & Astrin, KH: *Br. J. Haematol.* 117:779–795, 2002