Clinical Trial Watch

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Current and innovative emerging therapies for porphyrias with hepatic involvement

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Key points

All nucleated cells synthesise haem. About 80% of total daily haem synthesis occurs in the erythropoietic lineage for haemoglobin synthesis and about 15% in hepatocytes, mainly for synthesis of cytochrome P450 enzymes.

Summary

Porphyrias are rare inherited disorders caused by specific enzyme dysfunctions in the haem synthesis pathway, which result in abnormal accumulation of specific pathway intermediates. The symptoms depend upon the chemical characteristics of these substances. Porphyrins are photoreactive and cause photocutaneous lesions on sunlight-exposed areas, whereas accumulation of porphyrin precursors is related to acute neurovisceral attacks. Current therapies are suboptimal and mostly address symptoms rather than underlying disease mechanisms. Advances in the understanding of the molecular bases and pathogenesis of porphyrias have paved the way for the development of new therapeutic strategies. In this Clinical Trial Watch we summarise the basic principles of these emerging approaches and what is currently known about their application to porphyrias of hepatic origin or with hepatic involvement.

Molecular bases and pathogenesis of the porphyrias

Abnormalities in the activity of the 8 enzymes in the haem(/heme) biosynthesis pathway cause diseases with specific biochemical profiles of metabolite accumulation that are important for diagnosis.[1,2] Clinical features of each porphyria also result from the chemical characteristics of these metabolites and their tissue accumulation. The accumulation of the early haem precursors, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG), is associated with acute neurovisceral attacks in the acute hepatic porphyrias, whereas accumulation of the heterocyclic aromatic porphyrins is associated with phototoxic lesions affecting sunlight-exposed areas of the skin (Table 1). Liver involvement results from accumulation of highly carboxylated porphyrins in liver parenchymal cells in porphyria cutanea tarda (PCT) or from hepatic uptake and biliary excretion of protoporphyrin IX, a water insoluble dicarboxyl porphyrin overproduced by the bone marrow in protoporphyrias.

Although the haem biosynthesis pathway is present in all cell types, the major sites of haem production are erythroblasts and hepatocytes (80% and 15% of total haem synthesis, respectively).[1] The first and rate-limiting step in haem biosynthesis is the conversion of glycine and succinyl-coenzyme A to ALA, catalysed by ALA-synthase (ALAS) (Fig. 1). This enzyme is encoded by 2 separate genes: the erythroid-specific *ALAS2*, and the ubiquitously expressed *ALAS1*. In the liver where haemoproteins turn over rapidly in response to metabolic needs, ALAS1 activity is regulated by the end-product haem through several mechanisms: i) transcriptional repression via a haem-responsive element, ii) post-transcriptional destabilisation of *ALAS1* mRNA, iii) post-translational inhibition of mitochondrial transfer via a haem regulatory motif, iv) direct inhibition of the activity of the enzyme and v) breakdown of ALAS1 protein via haem-mediated induction of the protease Lon peptidase 1.[3] In erythroid cells, ALAS2 is a gatekeeper for the production of the very large amounts of haem needed for haemoglobin synthesis. The rate of ALAS2 synthesis is determined by trans-activation of nuclear factor *GATA1*, CACC box and *NFE2*-binding sites in the promoter areas. *ALAS2* mRNA translation is also regulated by the iron-responsive element/iron regulatory protein binding system.[4,5]

Porphyrias are classified either as hepatic or erythropoietic based on the primary source of the excess production of metabolic intermediates, or as acute neurovisceral or photocutaneous based on clinical features (Table 1).

Acute hepatic porphyrias

Acute hepatic porphyrias (AHPs) include acute intermittent porphyria (AIP), hereditary coproporphyria (HCP) and variegate porphyria (VP), which result from autosomal dominant loss-of-function mutations in the third, sixth and seventh enzymes of the haem biosynthesis pathway, respectively. The estimated prevalence of patients with AIP, VP and HCP is 5.9, 3.2 and <1 per million, respectively.[6,7] Very few cases of severe homozygous dominant AHPs have been reported, with affected patients presenting with severe, infantile-onset symptomatology.[8] The AHPs also include an autosomal recessive disorder, ALA dehydratase porphyria (ADP), with only 8 confirmed cases reported.[9–11]

Table 1. Classification of the human porphyrias associated with deficiencies of specific enzymes of the haem biosynthetic pathway.

Type	Main symptoms	Origin	Inheritance	Deficient enzyme	Gene location	Interventional drug trials
Acute hepatic porphyrias						
ADP	Acute neurovisceral	Hepatic	AR	ALA dehydratase (EC 4.2.1.24)	*ALAD* 9q34	No
AIP	Acute neurovisceral	Hepatic	AD	PBG deaminase (EC 4.3.1.8)	*HMBS* 11q23.3	Yes (Table 2)
HCP	Acute neurovisceral and/or skin lesions	Hepatic	AD	COPROgen oxydase (EC 1.3.3.3)	*COPROX* 3q12	No
VP	Acute neurovisceral and/or skin lesions	Hepatic	AD	PROTOgen oxydase (EC 1.3.3.4)	*PROTOX* 1q23	No
Hepatic cutaneous porphyrias						
PCT[1]	Chronic bullous skin lesions	Hepatic	AD/sporadic	URO decarboxylase (EC 4.1.1.37)	*UROD* 1p34	Yes (Table 3)
HEP	Chronic bullous skin lesions	Erythro-hepatic	AR	URO decarboxylase (EC 4.1.1.37)	*UROD* 1p34	No
Erythropoietic cutaneous porphyrias						
XLP	Acute photosensitivity occasional hepatobiliary	Erythropoietic	X-linked	ALA synthase (ES) (EC 2.3.1.27)	*ALAS2* Xp11.21	Yes (Table 4)
CEP	Chronic bullous skin lesions and/or anaemia	Erythropoietic	AR	UROIII synthase (EC 4.2.1.75)	*UROS* 10q25.2	No
EPP	Acute photosensitivity rarely hepatobiliary	Erythropoietic	AD[2]	Ferrochelatase (EC 4.99.1.1)	*FECH* 18q21.3	Yes (Table 4)

[1] PCT is essentially an acquired disease, only 20% of patients exhibit mutations in the *UROD* gene and in all cases clinical signs are always linked to additional exposure to precipitating factors. [2]95% of patients with EPP have a low-expression pathogenic variant c.315-48T>C in trans with a loss a function ferrochelatase mutation. AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; EPP, erythropoietic protoporphyria; ES, erythroid specific; PCT, porphyria cutanea tarda.

Most gene carriers of the dominant forms of AHPs are asymptomatic. Population studies have revealed a high prevalence of PBG deaminase (PBGD [or HMBS]) mutations (~1 in 1,700 individuals)[7,12] but with markedly low penetrance (≤1%). Although precipitating factors can trigger acute attacks, unknown modifying genes and/or epigenetic factors are likely to be largely responsible for the pathological phenotype.[13,14] Common precipitants of acute attacks include medications and other chemicals (see network resources), excess alcohol intake, certain steroid hormones, acute illness, infection, stress, physical exhaustion, caloric deprivation and rapid weight loss. These factors are all linked through the induction of hepatic *ALAS1* mRNA expression, either directly or via PGC1α (*e.g.* starvation);[15] or through the suppression of the negative feedback mechanism, caused by an increased need for haem for hepatic haemoproteins (*e.g.* inducible cytochrome P450 enzymes); or by increasing hepatic haem degradation through induction of heme oxygenase-1 (HMOX-1, EC 1.14.14.18).[7,16,17]

Clinical picture and prognosis

The most common symptoms during attacks of AHPs are abdominal pain often accompanied by nausea, vomiting, hyponatremia, constipation, hypertension, tachycardia, insomnia and anxiety. More severe symptoms may include sensory loss, motor neuropathy (sometimes progressing to life-threatening respiratory muscle failure), seizures, hallucinations and depression.[18,19] An estimated two-thirds of patients with VP and one-third of HCP cases also develop bullous photocutaneous lesions on sunlight-exposed areas of skin, due to porphyrins produced by the liver and circulating at high levels in the plasma.[1]

Attacks are associated in particular with excess accumulation of porphyrin precursors and porphyrins. Patients with AIP, VP and HCP exhibit increases in both ALA and PBG, whereas ALA but not PBG is elevated in ADP. The liver has been confirmed as the major source of ALA and PBG in AIP, and orthotopic liver transplantation (OLT) effectively resolves the disease.[20,21]

The current goal of treatment for acute attacks is to reduce hepatic ALAS1, thus resulting in decreased production of ALA, PBG and porphyrins. Both carbohydrate loading, acting through PGC1α, and intravenous haemin/hemin (haem arginate, Normosang® in Europe and lyophilised haematin, Panhematin® in the US, both from Recordati Rare Diseases, Milan, Italy) downregulate hepatic *ALAS1* transcription. Haemin replenishes the regulatory haem pool in hepatocytes and is more effective than glucose.[16] The decrease in ALA and PBG excretion often becomes pronounced on day 3 of haemin treatment (usually after the second or third daily infusion). Pain and nausea typically resolve on day 4.[22]

Human haemin treatment has also been effective in several ADP cases.[23] However, it was recently reported that a patient achieved clinical remission through suppression of erythroid haem synthesis, indicating that ADP has both hepatic and erythroid origins.[11]

About 3–5% of patients with AHPs suffer more severe or recurrent acute attacks, resulting in markedly impaired quality of life.[24–26] Long-term complications in AHPs, particularly in AIP, include chronic pain, hepatocellular carcinoma[27] and chronic renal failure.[28,29] In this regard, a common variant of peptide transporter 2 (PEPT2, UniProtKB - Q16348) with increased activity has been identified as predictive of the severity of kid-

Key points

Acute hepatic porphyrias are characterised by neurovisceral attacks associated with porphyrin precursor accumulation. Current and emerging therapies for these porphyrias target the liver and they include end-product (*i.e.* haem) supply, RNA interference and gene or mRNA addition.

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ney disease in AIP.[30] Given the central role of PEPT2 in ALA tubular reabsorption, the angiotensin II receptor antagonist losartan, which is a potent inhibitor of this transporter, has been suggested as a potential nephroprotective strategy in AIP.[30]

Common therapeutic options for preventing frequent attacks are the off-label use of prophylactic intravenous haemin infusions, and hormonal suppression therapy in catamenial-associated attacks.[1,31] However, repeated haemin administration can lead to loss of venous access, and be complicated by sepsis, secondary iron overload and induction of hepatic HMOX-1.[16] OLT represents a curative strategy for patients with severe, progressive neurological symptoms or who fail other therapies.[20,21]

Combined liver and kidney transplantation has been successful in patients with AIP and renal failure.[32] Although the rates of post-OLT survival in patients with OIP are similar to those transplanted for other aetiologies, significant complications such as hepatic artery thrombosis have been reported in some[21] but not all series.[17] Given the shortage of donors, novel curative treatments are needed before neurological, kidney or liver complications become irreversible.

Recent clinical trials and emerging therapies for AHPs

Three liver-directed strategies are currently under development (Fig. 1 and Table 2):

A. Reducing the expression of liver ALAS1 protein using a synthetic small interfering RNA (siRNA) targeting *ALAS1* mRNA.[33]
B. Increasing the expression of the deficient protein by recombinant adeno-associated virus (rAAV)-mediated transfer of human *PBGD* cDNA.[34]
C. Administering human *PBGD* mRNA packaged into lipid nanoparticles.[35]

Hyper-induction of hepatic ALAS1 is a common feature of all AHPs. Givosiran (Alnylam Pharmaceuticals, Cambridge, MA, USA) is an ALAS1-specific siRNA covalently linked to a triantennary N-acetyl galactosamine (GalNAc) that facilitates its delivery to hepatocytes via the asialoglycoprotein receptor (ASGPR).[36] The siRNA target sequence (ALN-60519) is well conserved across species.[33] A phase I, randomised, placebo-controlled study of givosiran was conducted in 40 patients with AIP, and consisted of 3 separate studies (NCT02452372, Table 2).[37] In Part A (single ascending dose) and Part B (2 injections of 0.35 or 1 mg/kg 28 days apart), subcutaneous givosiran administration induced a durable (weeks or months) dose-dependent decrease in urinary levels of *ALAS1* mRNA, ALA and PBG. In Part C, 17 patients with recurrent acute attacks were randomised (treatment-placebo, 3:1) to 2.5 mg/kg or 5 mg/kg, monthly or quarterly for 12 weeks. Monthly givosiran administration (n = 6) resulted in a significant silencing of hepatic *ALAS1* mRNA (~60% reduction compared to baseline levels) as measured in plasma and urine, a sustained lowering of ALA and PBG urinary excretion (75% of baseline values) and a reduction (56%) in the annualised attack rate (AAR) compared to the run-in period among the patients who received givosiran. Serious adverse events occurred in 6 of 33 patients who received givosiran (18%), including 1 spontaneous abortion and 1 case of haemorrhagic pancreatitis. The most common adverse events (abdominal and back pain, fatigue, headache, nausea and diarrhoea) occurred with similar frequencies in the placebo and active treatment groups.[37] It can be assumed that recovery from damage from porphyrin precursor-mediated toxicity to neural tissues may require several months, as previously reported in patients after liver transplantation.[38] In this regard, an open-label extension (OLE) study (NCT02949830, Table 2) is underway to continue monitoring patients enrolled in Part C (dose: 2.5 mg/kg, monthly over 37 months).

A reduction of the liver's detoxification capacity is a potential complication of long-term down-regulation of hepatic ALAS1; this is being analysed in a new clinical trial (NCT03505853, Table 1). The safety, pharmacokinetics and pharmacodynamics of a 5-drug probe cocktail (midazolam, caffeine, losartan, omeprazole, and dextromethorphan) are also being studied in 10 patients with AIP after a single dose of givosiran.

A larger, multinational phase III clinical trial (NCT03338816; EudraCT Number: 2017-002432-17) of the safety and efficacy of givosiran for preventing frequent attacks of acute porphyrias, including 94 patients, is underway with an estimated completion date of September 2021. Patients in this study were randomised 1:1 to placebo (n = 46) or givosiran (n = 48). Significant reductions in urinary ALA and PBG levels, and in AARs (defined as attacks requiring hospitalisation, urgent healthcare visit, or haemin administration) were evident after 6 months of treatment with givosiran (2.5 mg/kg monthly, subcutaneously), when compared to placebo.[39] However, adverse events and serious adverse events were similar or higher in the givosiran arm than the placebo arm. Alanine aminotransferase increases (≥3 times upper limit of normal) were observed in 7 patients on givosiran (14.6 %), of whom 1 discontinued treatment due to a >8-fold increase. Only 1 patient in the placebo arm showed such an elevation of alanine aminotransferase. Chronic kidney disease was reported in 5 givosiran-treated patients who showed renal dysfunction at baseline (as it is the case for many patients with AIP), whereas no changes in renal function were reported in patients included in the placebo group.[39]

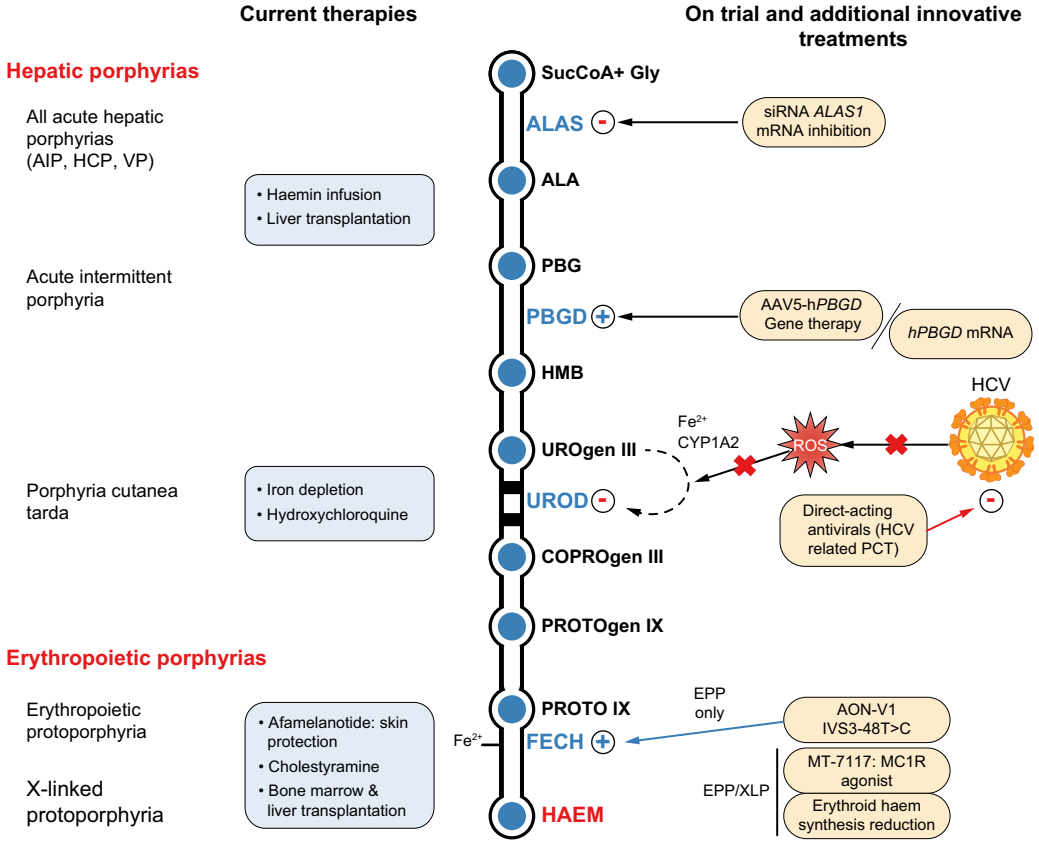

Fig. 1. Site of action of common and emerging therapies for acute hepatic porphyrias and for chronic photocutaneous porphyrias with hepatic involvement. The specific enzyme abnormality underlying each porphyria determines which intermediates of the pathway accumulate and the resulting symptoms. Accumulation of early pathway intermediates (*i.e.* ALA and PBG) leads to neurologic symptoms in acute hepatic porphyrias. Haemin infusions downregulate the over-expression of ALAS1 in the liver by negative feedback mechanisms, reducing the accumulation of these potentially neurotoxic metabolites. A synthetic interfering RNA specific for ALAS1 (givosiran, Alnylam Pharmaceuticals, Inc.) has an inhibitory effect with a longer duration, as demonstrated in recent clinical trials. The restoration of the enzymatic defect by gene addition therapy (UniQure, Amsterdam, The Netherlands) or by the administration of h*PBGD* mRNA encapsulated in lipid nanoparticles targeting the liver (Moderna Therapeutics, Inc.) is being explored for treatment of acute intermittent porphyria. When UROgen, COPROgen or PROTOgen accumulate in cutaneous porphyrias they are largely oxidised to UROporphyrin, COPROporphyrin and PROTOporphyrin, respectively, which are fluorescent molecules that are responsible for phototoxic manifestations. Mild to moderate iron overload and HCV infection are among the most important susceptibility factors in PCT. Controlled clinical trials are needed in PCT associated with HCV infection to clarify whether direct-acting antiviral treatment alone is as rapidly effective as phlebotomy or low-dose hydroxychloroquine. Oral adsorbents such as cholestyramine have been used in the protoporphyrias to reduce circulating protoporphyrin levels by interrupting the enterohepatic circulation of porphyrins, but published experience is limited. Skin protection using an analogue of α-melanocyte-stimulating hormone (afamelanotide, Clinuvel Pharmaceuticals, Ltd.) or an agonist of melanocortin 1 receptor (MT-7117, Mitsubishi-Tanabe Pharma Development America, Inc.) are also applicable for both XLP and EPP. The AON-V1 IVS3-48 T>C allele redirects splicing from the cryptic to the physiological acceptor site of the ferrochelatase mRNA. This offers a novel therapeutic approach for the 95% of patients with EPP that carry the hypomorphic IVS3-48T allele. AAV5, adeno-associated virus 5; AIP, Acute intermittent porphyria; ALA, δ-aminolevulinic acid; ALAS, ALA-synthetase; EPP, erythropoietic protoporphyria; FECH, ferrochelatase; Gly, Glycine; HCP, hereditary coproporphyria; HCV, hepatitis C virus; HMB, hydroxymethylbilane; MCR1, melanocortin 1 receptor; PBG, porphobilinogen; PBGD, PBG deaminase; PCT, porphyria cutanea tarda; ROS, reactive oxygen species; SucCoA, Succinyl-CoA; VP, variegate porphyria; UROD, uroporphyrinogen decarboxylase; XLP, X-linked protoporphyria.

All eligible patients (n = 93) continued dosing with givosiran in an OLE of this study, which will provide additional information on safety after longer term treatment and its effects, particularly in the liver and kidneys. The ASGPR is expressed in additional cell types, such as renal tubular cells, peripheral monocytes, peritoneal macrophages and cells in the endometrium and placenta.[40–43] Conceivably, long-term inhibition of ALAS1 might reduce the availability of haem and some of its metabolic end products (*e.g.* carbon monoxide, biliverdin and bilirubin) and compromise their cytoprotective, antioxidant and anti-inflammatory effects.[44,45]

The livers of most patients with AHP function normally despite the specific inherited enzyme deficiencies and periodic dysregulation of haem biosynthesis. Thus, transfer of a normal copy of cDNA to hepatocytes by means of an rAAV vector is a potentially durable approach to restore the specific enzyme deficiency. Proof-of-concept for this approach was achieved in AIP mouse

Key points

Porphyrias can be classified into erythropoietic and hepatic types, depending on where haem intermediates are predominantly overproduced, or clinically as acute hepatic or photocutaneous porphyrias. Notably, in most porphyrias the liver is the site of overproduction of pathway intermediates or a potential major site of tissue damage.

Table 2. Recent clinical trials for treatments of acute hepatic porphyrias.

Identifier	Participant condition (n) [start-end dates]	Study type	Intervention	Primary outcome measures [time frame]	Secondary outcomes	Ref.
NCT02082860	AIP (n = 8) [Nov12-Nov14]	Interventional (phase I), randomised, SGA, OL	rAAV2/5-*PBGD* vector (dose-ranging)	Safety, maximum therapeutic safe dose [<48 weeks]	Urine ALA and PBG level, frequency of hospitalisations and treatments, QoL	34
NCT02452372	AIP (n = 8) [May15-Sep17]	Interventional (phase I), randomised, parallel assignment	Givosiran (ALN-AS1) SAD, MAD and MD studies	Safety [days 0–42 for SAD, days 0–70 for MAD, days 0–168 for MD]	PK, change in ALA and PBG levels	37
NCT02943213	(n = 20) [Nov16-Dec16]	Interventional (phase I), SGA, OL	Chlorpromazine hydrochloride	PK of chlorpromazine and 7-hydroxy-chlorpromazine [from 0–94 h]	Tmax λz and $t_{1/2}$ of chlorpromazine and 7-hydroxy-chlorpromazine	
NCT02935400	**AHP (n = 50) [Jan14-Dec21]**	**Observational, prospective**	**Haemin**	**Potential biomarkers in blood, urine and stool samples [10 days]**	**None**	
NCT02180412	**AHP (n = 40) [Apr14-Aug20]**	**Interventional (phase II), randomised with placebo**	**Lyophilised haematin**	**Pain scale [4 days]**	**Porphyrin precursor and porphyrin levels**	
NCT02922413	**AHP (n = 20) [Aug15-Sep21]**	**Interventional (phase II), randomised with placebo**	**Haemin**	**Occurrence of acute attacks and adverse events [1–4 weeks]**	**Effects on PBG levels, age, nature of PBGD mutation and frequency of injection site complications**	
NCT02949830; EudraCT 2016-002638-54	**AIP (n = 17), not recruiting [Oct16-Aug20]**	**Interventional (phase I/II), SGA, OL**	**Givosiran (ALN-AS1)**	**Safety [through month 37]**	**Urine levels of ALA and PBG, frequency of attacks and haematin administrations**	
NCT03338816; EudraCT 2017-002432-17	**AHP (n = 74), not recruiting [Nov17-Sep21]**	**Interventional (phase III), randomised with placebo**	**Givosiran (ALN-AS1)**	**Annualised rate of attacks [at 6 months]**	**Urine ALA and PBG levels, annualised rate of hemin doses, pain, nausea, fatigue**	
NCT03505853	**AIP (n = 10), not recruiting [Apr18-Jan19]**	**Interventional (phase I), SGA, OL**	**Givosiran, probe cocktail: midazolam, caffeine, losartan, omeprazole and dextromethorphan**	**PK CytP450 (Cmax and AUC) probe cocktail [days 1–36]**	**PK and PD, safety**	

Status: bold denotes active studies.
rAAV, recombinant adeno-associated virus; AHP, acute hepatic porphyrias; AIP, acute intermittent porphyria; MAD, multiple ascending dose; MD, multi-dose; QoL, quality of life; OL, open label; PD, pharmacodynamics; PK, pharmacokinetics; SAD, single ascending dose; SGA, single group assignment.
NCT identifier for https://clinicaltrials.gov/; EudraCT identifier for European Clinical Trials Database (https://eudract.ema.europa.eu).

models.[46,47] However, a clinical trial (NTC02082860) in patients with AIP who had experienced frequent attacks failed to reduce levels of porphyrin precursors, due to insufficient liver transduction at the doses tested.[34] Although rAAV5-preimmunisation was ruled out in the 8 patients with AIP enrolled in the trial, it is likely that the more evolved human immune system effectively targeted viral capsid antigens and thereby precluded efficacy.[48,49] Ongoing efforts to improve the efficacy of rAAV-gene therapy vectors include: (i) the use of inducible promoter sequences responsive to porphyrinogenic factors that trigger acute attacks,[50] (ii) the development of new PBGD variants with increased catalytic capacity,[51] (iii) the elimination of the innate immune response by antigen-selective modulation of vector immunogenicity[52] and (iv) the blockade of B and T cell activation against the rAAV vector capsid.[53]

Administration of *PBGD* mRNA formulated in lipid nanoparticles[35] may be a cheaper and less immunogenic strategy than rAAV-gene therapy. Packaging of a single strand of *PBGD* mRNA into biodegradable lipid nanoparticles allows fast and efficient delivery to the liver. These nanoparticles are composed of ionisable cationic lipids and polyethylene glycol-conjugated lipids, providing for escape from early capture by phagocytic cells. The process of lipid nanoparticle internalisation by hepatocytes is mediated by the low-density lipoprotein receptor. The ligands that allow receptor interaction are apolipoprotein E and other opsonins. Endosome escape is then induced by the ionisable

cationic lipids, allowing release of mRNA cargo into the cytoplasm, which is soon followed by synthesis of human PBGD (hPBGD) protein.[54] In proof-of-concept studies in AIP mice, more than 90% of hepatocytes over-expressed PBGD protein for 10 days.[35] ALA and PBG levels were reduced in a dose-dependent manner within a few hours after treatment. Similar reductions in ALA and PBG levels were seen in a chemically induced acute porphyria model in rabbits, demonstrating efficacy in a larger animal. Additionally and most importantly, a single dose of h*PBGD* mRNA prevented mitochondrial dysfunction, hypertension, delayed drug metabolism, pain, nerve dysfunction and motor impairment in AIP mice,[35] all signs present in acute attacks in human AIP. Finally, multiple administrations of the mRNA packaged in lipid nanoparticles in AIP mice, rabbits and non-human primates confirmed safety and translatability across species. Moreover, the preventive administration of this formulation during the prodromal phase was fully protective against a porphyria attack in mouse and rabbit models. These observations support evaluation of this therapeutic strategy in placebo-controlled clinical trials.[54,55]

Photocutaneous porphyrias

Cutaneous hepatic porphyrias

These include PCT, the most common porphyria world-wide, with a mean prevalence of around 50 per 1 million and an incidence of about 2–5 per million,[7] and hepato-erythropoietic porphyria (HEP), the very rare homozygous form of familial-PCT, with less than 100 reported cases. PCT responds readily to specific treatments that are not effective in other types of porphyria.

PCT and HEP result from acquired and/or inherited hepatic deficiency of URO decarboxylase (UROD) activity, the fifth enzyme of the haem pathway. HEP is caused by a biallelic mutation of UROD. A heterozygous *UROD* mutation is found in only about 20% of PCT cases and is a predisposing factor. UROD catalyzes the 4-step decarboxylation of uroporphyrinogen to coproporphyrinogen (Table 1). Deficient activity of hepatic UROD leads to accumulation of uroporphyrinogen and the porphyrinogen intermediates of this 4-step decarboxylation reaction (hepta-, hexa, and pentacarboxyl porphyrinogen). These intermediates are then oxidised and excreted as the corresponding porphyrins. Chronic phototoxic lesions on sun-exposed areas are due to photo-activation of porphyrins.[1,2]

Unlike all other human porphyrias, which are monogenic disorders, PCT should be considered as an acquired disease. PCT can be divided into 2 types that are often clinically indistinguishable based on the absence (type I) or presence (type II) of a *UROD* mutation.

In type I PCT (about 80% of patients), also termed sporadic PCT, UROD activity is deficient due to liver-specific inhibition of the enzyme.[56] Any genetic contribution to the pathogenesis of PCT Type I will come from outside the *UROD* locus. Rarely, PCT cases indistinguishable from the sporadic form are clustered in families in the absence of *UROD* mutations. These patients have been called Type III PCT and may represent sharing of other inherited or acquired susceptibility factors among family members.

In familial type II PCT (comprising about 20% of all patients with PCT), a 50% reduction in UROD activity is found in all tissues including erythrocytes from birth. *UROD* mutations are inherited in an autosomal dominant fashion with extensive allelic heterogeneity at the *UROD* locus. However, the heterozygous mutation is insufficient to reduce hepatic UROD activity to 20% of normal, the level required for porphyrin accumulation and cutaneous lesions to occur.

As in sporadic type I PCT, patients with familial-PCT type II need additional acquired or as yet unknown inherited susceptibility factors to develop the disease phenotype. These factors may include iron overload, alcohol, smoking, chronic hepatitis C, oestrogen use, and perhaps other liver diseases such as non-alcoholic fatty liver disease,[57] which are common in the general population and are highly prevalent in type I PCT.[57,58] These factors are also important in type II PCT, since a heterozygous *UROD* mutation is not sufficient to cause the disease. These factors may act by inducing oxidative stress and iron accumulation in hepatocytes, and subsequently contribute to production of a porphomethene UROD inhibitor.[59]

Key points

Cutaneous hepatic porphyrias are characterised by chronic bullous skin lesions associated with accumulation of uroporphyrin and other porphyrins. Current therapies in porphyria cutanea tarda (the most common porphyria) reduce iron or mobilise porphyrins, or remove contributing factors such as HCV, alcohol, *etc.*

Clinical picture and current therapies

Patients with PCT present with chronic, blistering lesions on sun-exposed areas of skin, with marked fragility, bullae, vesicles, erosions, crusts and milia.[57] Uroporphyrin and other highly carboxylated porphyrins that have accumulated in the liver circulate in plasma and become activated by sunlight in skin capillaries, resulting in the release of oxygen free radicals and immune-mediated damage of the lower dermis and basement membranes.[60] Advanced liver disease is unusual, but mild abnormalities of liver function tests are common, with increases in serum aminotransferases in over 50% of patients with PCT.[57]

PCT is an iron dependent disorder. Mild to moderate iron overload with hepatic siderosis is reported in up to 90% of PCT cases.[57,61] Serum ferritin levels are always normal or increased. Associated susceptibility factors such as mutations in the hemochromatosis gene (*HFE*)[62,63] and downregulation of hepcidin from alcohol use and HCV infection[64] are documented in PCT. The underlying mechanisms are hypothesised to rely on increased oxidative stress in hepatocytes, hepcidin gene (*HAMP*) downregulation[60,65] and induction of hepatic cytochrome P450 enzymes.[66]

Notably, depletion of hepatic iron stores leads to clinical and biochemical remission in both Types I and II PCT, even when total body iron stores are not increased (Fig. 1). Repeated phlebotomy (350–450 ml of blood approximately every 2 weeks) reduces hepatic iron levels and serum ferritin, leading to normal porphyrin levels.[57] Resolution of skin fragility and blistering is expected in 6–9 months, depending on compliance with the phlebotomy schedule and the degree of iron overload. An oral iron chelating agent can be used in patients with anaemia or those who do not tolerate phlebotomies.[67] However, this approach is much less efficient than repeated phlebotomy and might induce kidney- or liver-related adverse events.[68]

Therefore, the preferred alternative to iron depletion is a low-dose regimen of a 4-aminoquinoline antimalarial (oral hydroxychloroquine 100 mg or chloroquine 125 mg per week), which mobilises excess porphyrins from hepatocytes and leads to their excretion in urine. In a prospective, comparative study, time to remission with low-dose hydroxychloroquine was comparable to that with phlebotomy.[69] Although comparably effective, the former is more convenient and less costly. Relapse rates may be higher after treatment with 4-aminoquinoline antimalarials than after phlebotomies, but this has not yet been determined by long-term follow-up studies. Relapse is likely to be influenced by the continued presence of susceptibility factors, such as alcohol or oestrogen use, smoking and HCV infection.

Cutaneous manifestations of HEP usually appear in the early years of life and are generally severe and persistent, resulting in scarring with photomutilation in some patients. The treatment for HEP is based on photoprotection because low-dose hydroxychloroquine or phlebotomy are usually not beneficial.[57] Autologous haematopoietic-stem-cell-based gene therapy using lentiviral vectors has been proposed.[70] but clinical trials have not been conducted.

Key points

Erythropoietic cutaneous porphyrias are characterised by chronic, often severe skin blistering, and particularly in protoporphyrias, painful skin photosensitivity and hepatobiliary involvement due to hepatic uptake and excretion of protoporphyrin. Current and emerging therapies include modulation of iron supply, protoporphyrin-binding resins, antisense oligonucleotide therapy and increased light tolerance with antioxidants or stimulators of skin melanin synthesis.

Treatment of chronic hepatitis C in patients with PCT

HCV is among the most important susceptibility factors in PCT, although with a wide geographic variation.[58,71,72] The mechanisms linking HCV and PCT are unclear but may include oxidative stress and dysregulation of hepcidin expression.[64]

Given that PCT symptoms are usually more severe than those of chronic HCV and are easily treated by phlebotomy or low-dose hydroxychloroquine or chloroquine,[69,73] it has been considered preferable to treat PCT first and hepatitis C later.[74] However, the emergence of direct-acting antivirals (DAAs) has revolutionised the treatment of hepatitis C and revived the notion of treating the viral infection first (Fig. 1), which might even make treatment of PCT by phlebotomy or low-dose hydroxychloroquine unnecessary.[75–77] Single case reports of resolution of PCT using new DAAs do not determine the success rate and durability of this approach. Results of controlled clinical trials are needed to clarify whether DAA therapy alone can be curative for both conditions.[78] The US Porphyrias Consortium is studying (NCT03118674, Table 3) whether time to remission of PCT with DAA treatment is non-inferior to that published for phlebotomy and low-dose hydroxychloroquine.[69]

It seems likely that eradication of hepatitis C will prevent recurrence of PCT, but long-term observations to document this are lacking. Single arm DAA treatment trials can be extended to assess PCT recurrence rates, which may be influenced by alcohol and other susceptibility factors in individual patients.

Erythropoietic protoporphyrias

Both erythropoietic protoporphyria (EPP) and X-linked protoporphyria (XLP) are characterised by variable degrees of painful phototoxicity, erythema, swelling and scarring in areas of sun-exposed skin.[79] The estimated prevalence of these protoporphyrias in Caucasians is 9.2 cases per million individuals.[6,7]

EPP arises from reduced activity of ferrochelatase (FECH) to less than 30% of normal activity, causing accumulation of metal-free protoporphyrin IX primarily in bone marrow reticulocytes, circulating erythrocytes, plasma, liver and other tissues. The enzymatic defect is transmitted in 95% of cases as an autosomal recessive trait promoted by the combined inheritance of a null/loss-of-function mutated allele and an allele carrying the hypomorphic IVS3-48C polymorphism.[80] This hypomorphic allele is found in approximately 10% of Caucasians, is even more common in eastern Asia, and very rare in Africa. In rare families, EPP is due to 2 loss-of-function *FECH* mutations in the absence of the hypomorphic allele. An additional molecular mechanism underlying the development of EPP was recently identified in affected families. A dominant heterozygous mutation in the ATPase active site of the human *CLPX* gene (ATP-dependent CLP protease ATP-binding subunit CLPX-like, UniProtKB O76031; encoded by *CLPX* gene) increased post-translational stability of ALAS2 and promoted pathological accumulation of protoporphyrin IX.[81,82] A recent observational clinical study (NCT01880983, Table 4) confirms mitoferrin-1 (*SLC25A37*) mRNA levels are positively correlated with ferrochelatase enzymatic activity in all cell types.[83] Mitoferrin-1 is a mitochondrial transporter of ferrous iron used in the final step of haem synthesis and in the formation of 2Fe-2S clusters. Clusters bound to the C-terminal region of ferrochelatase are essential for enzymatic activity and stability of FECH. Thus, low mitoferrin-1 expression in EPP and XLP suggests that the ability to import iron, which can be toxic in excess, may in some manner be tied to the capacity to produce haem.

Table 3. Recent clinical trials for treatments of porphyria cutanea tarda.

Identifier	Participant condition (n) [start-end dates]	Study type	Intervention	Primary outcome measures [time frame]	Secondary outcomes	Ref.
NCT00599326	n = 10 [Jan08-April10]	Interventional (phase III), SGA, OL	Deferasirox	Participants showing reduction or elimination of skin blistering [6 months]	Participants showing decrease in ferritin and urinary porphyrin level	
NCT01284946	n = 45 [Jan11-Dec12]	Interventional (phase II), SGA, OL	Deferasirox	Safety [6 months]	Efficacy: iron burden and porphyrin level changes, improvement in clinical symptoms	67
NCT01573754	**n = 100 [April03-Aug22]**	**Interventional (phase III), randomised assignment, OL**	**Drug: Hydroxychloroquine Procedure: Phlebotomy**	**Plasma porphyrin levels [6 months]**	**Susceptibility factors on responses to treatment and recurrence rates**	**69**
NCT03118674	**n = 49 [Aug17-Aug22]**	**Interventional (phase II), SGA, OL**	**Ledipasvir + sofosbuvir**	**Resolution of active PCT [12 months]**	**Time to resolution of active PCT**	

Status: bold denotes active studies.
OL, open label; PCT, porphyria cutanea tarda; SGA, single group assignment.
Clinical trial identifier: NCT for clinicalTrials.gov (https://clinicaltrials.gov/); EudraCT for European Clinical Trials Database (https://eudract.ema.europa.eu).

Approximately 5–10% of patients with the protoporphyria phenotype have XLP which results from *ALAS2* gain-of-function mutations. Both metal-free- and zinc-protoporphyrin are greatly elevated in erythrocytes in XLP. Males are uniformly and severely affected. Clinical expression in XLP heterozygous females ranges from asymptomatic to severe, due primarily to random X-chromosomal inactivation, and also to somatic mosaicism resulting from tissue-specific variations in X chromosome inactivation.[79]

Clinical picture and liver involvement

Painful photosensitivity is the most common clinical presentation in both EPP and XLP. Progressive accumulation of lipophilic protoporphyrin in cell membranes in the skin and dermal blood vessels leads to the formation of reactive oxygen species upon exposure to visible light, causing subsequent vascular and tissue damage.

The lipid solubility of protoporphyrin also results in its uptake in the liver and excretion in bile. It may accumulate and precipitate in hepatocytes and bile, causing liver damage and biliary stones.[84,85] Although up to 40% of patients display some degree of parenchymal liver damage, only 3–5% develop severe liver dysfunction with cholestatic liver failure.[86] The acute onset of this condition may be followed by rapid progression, requiring OLT for survival.[20,87] To date, blood protoporphyrin levels and biochemical liver tests have not been predictive of liver disease development, while liver biopsy is diagnostic and can better assess the degree of hepatic injury.[86] The overall survival of patients with EPP after OLT is comparable to that of patients transplanted for other types of liver disease, with a survival rate of 77% in the first year and between 46% and 66% at 10 years.[88] However, biliary complications were found in over 45% of cases, a significantly higher proportion than in the general liver transplant population (15–30%).[89] This complication may impair bile flow, resulting in protoporphyrin accumulation and graft damage. Because the excessive bone marrow production of protoporphyrin continues, liver disease recurs in 69% of patients with EPP after OLT. Bone marrow transplantation is curative for EPP, and can prevent liver graft damage and loss after OLT.[90]

Recent clinical trials and emerging therapies

Although patients learn to remove themselves from exposure to sunlight and to wear protective clothing in order to avoid painful photosensitivity, this significantly impairs quality of life. Beta-carotene and antioxidants may quench reactive oxygen species formed by light-activated porphyrins, although their efficacy is limited.[91] Afamelanotide (Clinuvel Pharmaceuticals Ltd., Melbourne, Australia) is an analogue of α-melanocyte-stimulating hormone (α-MSH). This tridecapeptide binds to the melanocortin 1 receptor (MC1R) in dermal cells, including melanocytes, and increases the formation of eumelanin in the patient's skin (Table 4). Melanin may also provide anti-inflammatory, and free radical scavenging effects that contribute to decreased symptom severity. Multicentre, randomised, double-blinded, placebo-controlled phase II and phase III trials in both the US (NCT01097044 and NCT01605136, Table 4) and Europe (EudraCT Number: 2007-000636-13 and NCT00979745/ EudraCT Number: 2009-011018-51, Table 4) have shown that afamelanotide significantly reduces the frequency of phototoxic reactions, increases tolerance to direct sunlight and improves quality of life compared to placebo.[92] This occurs without changes in plasma or erythrocyte protoporphyrin levels or hepatic function.

In cases with severe liver damage, therapeutic plasma exchange, erythrocyte exchange, hypertransfusion, haemin infusions, and N-acetyl-

Key point

Human porphyrias illustrate multiple modes of inheritance (*e.g.* autosomal dominant and recessive, X-linked, loss- and gain-of-function mutations, hypomorphic allele and an acquired enzyme inhibition), with variable clinical expressivity ranging from affected to unaffected.

Table 4. Recent clinical trials for treatments of erythropoietic protoporphyria/ X-linked protoporphyria.

Identifier	Participant condition (n) [start-end dates]	Study type	Intervention	Primary outcome measures [time frame]	Secondary outcomes	Ref.
NCT00979745 & EudraCT 2009-011018-51	n = 70 [Sep09-May11]	Interventional (phase III), randomised with placebo	Afamelanotide	Severity of phototoxic reaction [9 months]	Phototoxic reactions, QoL, free PPIX level, AE	92
EudraCT 2007-002863-28	n = 21 [unkown-Nov11]	Observational, cohort, prospective	Colestyramine	Plasma and RBCs PPIX levels	None	
NCT01422915	n = 4 [May11-Mar12]	Interventional (phase I/II), SGA, OL	Colestipol	PPIX level in RBC and photosensitivity	None	94
NCT01550705	n = 11 [Mar12-Dec15]	Interventional, SGA, OL	Isoniazid	PPIX levels in plasma [baseline and 3 months]	Sun sensitivity	
NCT01605136	n = 93 [May12-Apr13]	Interventional (phase III), randomised with placebo	Afamelanotide	Duration of direct sunlight exposure [6 months]	Phototoxic pain, QoL, safety and tolerability	92
EudraCT 2007-000636-13	**n = 80, not recruiting [May08-hitherto]**	**Interventional (phase III), randomised with placebo**	**Afamelanotide (CUV1647) implants**	**Number and severity of phototoxic reactions**	**Duration of sunlight, melanin density, safety and tolerability, QoL**	**92**
NCT02979249	**n = 20 [Dec16-Aug19]**	**Interventional, SGA, OL**	**Oral iron**	**PPIX level in RBC [baseline and 12 months]**	**QoL**	
NCT03520036	**n = 102 [July18-Mar19]**	**Interventional (phase II), randomised with placebo**	**Immunomodulator MT-7117**	**Duration of sunlight exposure [week 0 and 16]**	**Sunlight exposure episodes, change in pigmentation, QoL**	

Status: bold denotes active studies. AE, adverse event; OL, open label; PPIX, protoporphyrin IX; QoL, quality of life; SGA, single group assignment.
NCT identifier for https://clinicaltrials.gov/; EudraCT identifier for European Clinical Trials Database (https://eudract.ema.europa.eu).

cysteine may decrease protoporphyrin levels but may not improve liver function.[93] These and other interventions, such as ursodeoxycholic acid, vitamin E and cholestyramine, have improved liver function in some cases or bridged patients to liver transplantation. Controlled studies in these rare and severely ill patients are lacking.

Treatments that address the underlying mechanisms that cause increased porphyrin levels in protoporphyrias are still under development. Developing better strategies for protecting either the skin from phototoxicity or the liver from high levels of circulating protoporphyrin is also a priority. Some current and emerging therapies include the following (Fig. 1):

A) The enterohepatic circulation of porphyrins may be interrupted and their faecal excretion increased by oral intake of adsorbents, thereby reducing circulating protoporphyrin levels; although substantial benefits remain to be demonstrated (NCT01422915, Table 4).[94] Resins such as cholestyramine may have similar effects and deserve further study.

B) MT-7117 (Mitsubishi-Tanabe Pharma Development America, Inc. Jersey City, NJ) is an orally administered, non-peptide small molecule, which acts as an agonist of MC1R and increases skin melanogenesis, as well as having immunomodulatory properties. It is being investigated in an ongoing placebo-controlled phase II clinical trial (NCT03520036) in patients with EPP and XLP.

C) Giving oral iron to protoporphyria patients with low body iron stores is being investigated (NCT02979249) as a potential approach to decrease erythrocyte and plasma protoporphyrin levels and improve EPP symptoms. EPP and XLP are commonly associated with unexplained mild iron deficiency, and it is generally agreed that XLP with iron deficiency should be treated with iron. Some patients with EPP have described worsening of photosensitivity after iron administration, but associated changes in protoporphyrin levels have not been studied prospectively.

D) Alternatively, reducing haem and haemoglobin synthesis through erythrocyte transfusion, haem infusion, hydroxyurea, iron suppression or direct inhibition of ALAS2[95] might ameliorate all erythropoietic porphyrias by reducing synthesis of porphyrin intermediates.[5] In this regard, improvement was reported using haem transfusion and hydroxycarbamide (hydroxyurea) in ADP[11] and congenital erythropoietic porphyria,[96] and iron reduction in a patient with congenital erythropoietic porphyria.[97] Thus, other erythropoietic porphyrias could profit from approaches aimed at reducing the erythroid production of haem precursors.

E) Gouya and colleagues have developed an antisense oligonucleotide (AON) technique that uses specific AONs that target the hypomorphic IVS3-48C allele,[98] which is found in 95% of patients with EPP. This novel therapeutic approach redirects splicing from the

cryptic to the physiological acceptor site, generating the wild-type mRNA. The resulting higher level of normal messenger is expected to increase FECH activity and ultimately, reduce protoporphyrin IX accumulation. These authors have further developed a novel strategy to deliver a specific and efficient AON (AON-V1) to erythroid progenitors using transferrin receptor 1 (TRF1). A bifunctional peptide (P1-9R) including a TFR1-targeting peptide coupled to a 9-arginine cell-penetrating peptide facilitates the release of the AON-V1 into erythroid progenitor cells. Proof-of-concept has recently been demonstrated in primary cultures of differentiating erythroid progenitors from a patient with EPP.[99] Work is ongoing to ensure a constant supply of this antisense oligonucleotide to erythroid progenitors in a humanised mouse model of EPP.

Conclusions and future directions

The various symptoms of the different porphyrias depend on the chemical characteristics of the pathway intermediates that accumulate. Their variable clinical expression depends on the severity of the underlying enzymatic abnormality and the activity of the first and rate-limiting enzymes of the pathway, the housekeeping ALAS1 in the liver and ALAS2 in the bone marrow.

Activation of AHPs results from induction of hepatic ALAS1 by exogenous or exogenous factors in the presence of inherited deficiency of 1 of 4 downstream enzymes in the pathway. This results in the accumulation of porphyrin precursors and porphyrins, leading to neurological symptoms. A therapeutic interference RNA that targets hepatic ALAS1 is currently undergoing clinical trials in AHPs. Meanwhile, trials aimed at rescuing the metabolic blockage by gene or mRNA therapies are ongoing for AIP.

Porphyrins accumulate and may cause photosensitivity when there is an enzymatic deficiency after the third enzyme in the pathway. PCT, the most common porphyria, is readily treated with repeated phlebotomies or low-dose hydroxychloroquine, which are equally effective. Nowadays hepatitis C is a major susceptibility factor in many patients with PCT, and the possibility that DAAs can be effective as initial treatment for both conditions is under investigation.

In erythropoietic porphyrias resulting from abnormalities in other pathway enzymes, compensatory induction of erythroid ALAS2 or gain-of-function ALAS2 mutations, lead to porphyrin accumulation and cutaneous photosensitivity. Protection from sunlight exposure is the cornerstone of management for these conditions. In EPP and XLP, the most common erythropoietic porphyrias, increased skin melanin after treatment with afamelanotide can increase light tolerance and quality of life. However additional novel therapies based on the aetiologies and pathogenesis of these conditions are under development, such as the AON strategy to increase expression of the hypomorphic *FECH* allele (IVS3-48 T>C, found in the great majority of patients with EPP), which is now in preclinical development. Alternatively, inhibitors of erythroid ALAS2 and suppression of erythroid haem synthesis are additional innovative approaches with potential for treating all erythropoietic porphyrias.

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Conflict of interest

AF and MAA have no conflict of interest to declare. JCD is an occasional consultant for Alnylam Pharmaceuticals and Recordati Rare Diseases. KEA reports grants and personal fees from Alnylam Pharmaceuticals during the duration of the study; grants and personal fees from Recordati Rare Diseases, personal fees from Moderna Therapeutics, and personal fees from Mitsubishi-Tanabe Pharma, outside the submitted work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors made an equal contribution.

Network resources

Online Mendelian Inheritance in Man (OMIM): http://www.ncbio.nlm.nih.gov/Omim/
Human Gene Mutation Database (HGMD). http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html/
Drugs database and acute porphyrias. http://www.drugs-porphyria.org/; http://www.porphyriafoundation.com/drug-database.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.05.003.

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Author names in bold designate shared co-first authorship

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