Review Article

Role of genetic testing in the management of patients with inherited porphyria and their families

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Abstract

The porphyrias are a group of mainly inherited metabolic conditions that result from partial deficiency of individual enzymes in the haem biosynthesis pathway. Clinical presentation is either with acute neurovisceral attacks, skin photosensitivity or both, and is due to overproduction of pathway intermediates. The primary diagnosis in the proband is based on biochemical testing of appropriate samples, preferably during or soon after onset of symptoms. The role of genetic testing in the autosomal dominant acute porphyrias (acute intermittent porphyria, hereditary coproporphyria and variegate porphyria) is to identify presymptomatic carriers of the family specific pathogenic mutation so that they can be counselled on how to minimize their risk of suffering an acute attack. At present the additional genetic factors that influence penetrance are not known, and all patients are treated as equally at risk. Genetic testing in the erythropoietic porphyrias (erythropoietic protoporphyria, congenital erythropoietic porphyria and X-linked dominant protoporphyria) is focused on predictive and preconceptual counselling, prenatal testing and genotype–phenotype correlation. Recent advances in analytical technology have resulted in increased sensitivity of mutation detection with success rates of greater than 90% for most of the genes. The ethical and consent issues are discussed. Current research into genetic factors that affect penetrance is likely to lead to a more refined approach to counselling for presymptomatic gene carriers.

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Introduction

The inherited porphyrias are caused by mutations in the genes that code for the enzymes of the haem biosynthetic pathway or, in one case, by mutation of an interacting transcriptional protein.[1,2] They can be divided into two groups on the basis of their clinical features (Table 1). The acute porphyrias are characterized by episodic, life-threatening neurovisceral attacks that are always accompanied by increased urinary excretion of 5-aminolevulinate (ALA) and, except in the very rare ALA-dehydratase deficiency porphyria (ADP), porphobilinogen (PBG) (Table 1). The non-acute porphyrias are characterized by photosensitization of the skin by porphyrins, the absence of acute attacks and normal excretion of PBG and ALA at all times (Table 1).

Each porphyria results from overproduction of haem precursors secondary to partial deficiency or, in X-linked dominant protoporphyria (XLDPP), increased activity of one of the enzymes of haem biosynthesis (Figure 1; Table 1). These result in specific patterns of accumulation and excretion of haem precursors that define each disorder (Table 1). Diagnosis is normally straightforward, and requires only biochemical investigation, provided the appropriate analytes are measured in the appropriate samples while symptoms are present, or soon after.[7,8] Indeed, since symptoms may be non-specific, porphyria cannot be established as their cause unless haem precursor concentrations are shown to be increased. Genetic testing is rarely required to make a diagnosis of porphyria and, by itself, may be misleading if a mutation is not found or unclassified variants are identified. Genetic analysis does not identify mutations in all unequivocally diagnosed cases and therefore cannot be used to exclude a diagnosis of porphyria. In addition, low clinical penetrance in the autosomal dominant porphyrias means that identification of a mutation does not necessarily indicate active porphyria. However, DNA analysis is now the method of choice for presymptomatic diagnosis, family studies and for predictive counselling. This review summarizes current knowledge of the molecular genetics of the porphyrias and describes the use of genetic testing in these disorders.

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Table 1 The porphyrias

Porphyria	Estimated UK prevalence	Defective enzyme	Enzyme activity[Ref]	Inheritance	Main clinical features	Biochemical findings			
						Urine	Faeces	Erythrocyte porphyrins	Plasma fluorescence emission peak (nm)
Acute									
ADP	Unknown	5-Aminolevulinate dehydratase	<5%[3]	AR	Acute attack	ALA>>PBG, Copro III	Not increased	Zn + free proto	Not increased
AIP	1–2:100,000	Hydroxymethyl bilane synthase	50%*	AD	Acute attack	ALA, PBG, Uro I	Not increased	Not increased	615–620
HCP	1–2:10^6	Copropophyrinogen oxidase	50%†	AD	Acute attack, skin fragility, bullae	ALA, PBG, Copro III	Copro III	Not increased	615–620
VP	1:250,000	Protoporphyrinogen oxidase	50%†	AD	Acute attack skin fragility, bullae	ALA, PBG, Copro III	Proto>Copro	Not increased	624–627
Non-acute									
CEP	1:0.33 × 10^6	Uroporphyrinogen III synthase	2–30%[4–6]	AR	Skin fragility, bullae, haemolysis	Uro I, Copro I	Copro I	Zn + free proto Copro I, Uro I	615–620
PCT	1:25,000	Uroporphyrinogen decarboxylase	50%	AD in 25%	skin fragility, bullae	Uro I + III, Hepta	Isocopro, Hepta	Not increased	615–620
EPP	1:140,000	Ferrochelatase	5–30%†	AR	Acute painful photosensitivity	Not increased	Proto	Free proto	626–634
XLDPP	0.15:10^6	5-Aminolevulinate synthase 2	Increased‡	X-linked	Acute painful photosensitivity	Not increased	Proto	Zn + free proto	626–634

ALA, 5-aminolevulinic acid; PBG, porphobininogen; Uro I or III, uroporphyrin I or III; Copro I or III, coproporphyrin I or III; Hepta, heptacarboxylic porphyrin; Isocopro, isocoproporphyrin; ADP, ALA dehydratase deficiency porphyria; AIP, acute intermittent porphyria; HCP, hereditary coproporphyria; VP, variegate porphyria; PCT, porphyria cutanea tarda; CEP, congenital erythropoietic porphyria; EPP, erythropoietic protoporphyria; XLDPP, X-linked dominant protoporphyria

*PBG deaminase

†Lymphocytes; other activities are for erythrocytes

‡Assessed *in vitro*

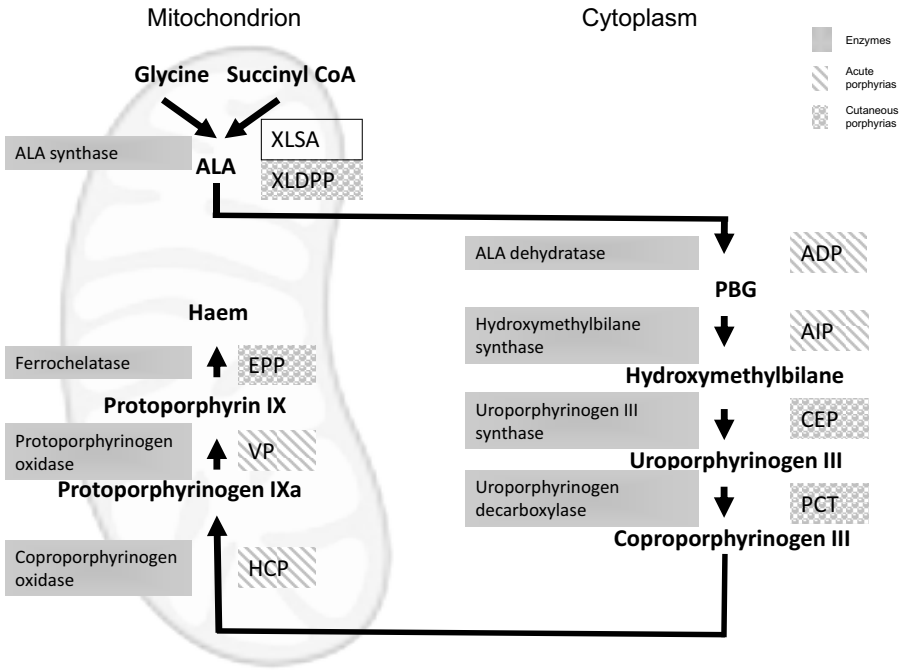

Figure 1 Haem biosynthetic pathway. Enzymes are shown in solid fill, acute porphyrias (striped background) ADP, 5-aminolevulinic acid dehydratase deficiency porphyria; AIP acute intermittent porphyria; HCP, hereditary coproporphyria; VP, variegate porphyria. Cutaneous porphyrias (circles background): XLDPP, X-linked dominant protoporphyria; CEP, congenital erythropoietic porphyia; PCT, porphyria cutanea tarda; EPP, erythropoietic protoporphyria. Clear background: XLSA, X-linked sideroblastic anaemia

Table 2 Human genes encoding enzymes of haem biosynthesis

Gene symbol	Chromosomal location	No. of exons	Genomic DNA (kb)	Tissue expression
ALAS1	3p21.1	11	17	Ubiquitous
ALAS2	Xp11.21	11	22	Erythroid cells
ALAD	9q34	12 or 13 (1A 1B)	15 (UCSC genome browser)	Ubiquitous and erythroid-specific mRNAs
HMBS	11q24.1–q24.2	15	10	Ubiquitous and erythroid-specific mRNAs and isoforms
UROS	10q25.2–q26.3	10	34	Ubiquitous and erythroid-specific mRNAs
UROD	1p34	10	3	Ubiquitous
CPOX	3q12	7	14	Ubiquitous
PPOX	1q21–23	13	5.5	Ubiquitous
FECH	18q21.3	11	45	Ubiquitous

ALAS, 5-aminolevulinic acid synthase; ALAD, 5-aminolevulinic acid dehydratase; HMBS, hydroxymethylbilane synthase; UROS, uroporphyrinogen synthase; UROD, uroporphyrinogen decarboxylase; CPOX, coproporphyrinogen oxidase; PPOX, protoporphyrinogen oxidase; FECH, ferrochelatase

Gene structure and expression

The first genetic information relating to human haem synthesis was the characterization of the complementary DNA (cDNA) sequence for hydroxymethylbilane synthase (*HMBS*).[9] Subsequently, all the genes of the pathway have been characterized and their chromosomal locations identified (Table 2).[10–16]

ALAS has both erythroid and non-erythroid genes[17,18] with the next three genes of the haem biosynthetic pathway (*ALAD*, *HMBS*, *UROS*) having dual promoters[18–20] (Figure 2). This enables the genes in the liver and the erythroid cells to be regulated according to the organs differing haem requirements. Most haem synthesis takes place in the developing red cells in the bone marrow with about 15% produced in the liver for the formation of haem-containing enzymes.[21] In the liver, most haem biosynthetic enzymes are turned over rapidly enabling the liver to respond effectively to changing metabolic requirements. ALAS1 is the rate-limiting enzyme in the production of hepatic haem[17] and is controlled by negative feedback regulation by the intracellular haem pool. In erythroid cells the rate of ALAS2 synthesis is regulated to permit a high level of haem synthesis and is linked to the availability of iron[22] and is not inhibited by haem.[23]

Autosomal dominant porphyrias

Four porphyrias are inherited in an autosomal dominant pattern: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) and familial porphyria cutanea tarda (F-PCT). At the molecular level, all show extensive allelic heterogeneity with most mutations being restricted to one or a few families although founder mutations have been identified in some countries, notably those that underlie the high prevalence of AIP in Sweden,[24] VP in South Africa[25] and F-PCT in Norway.[26]

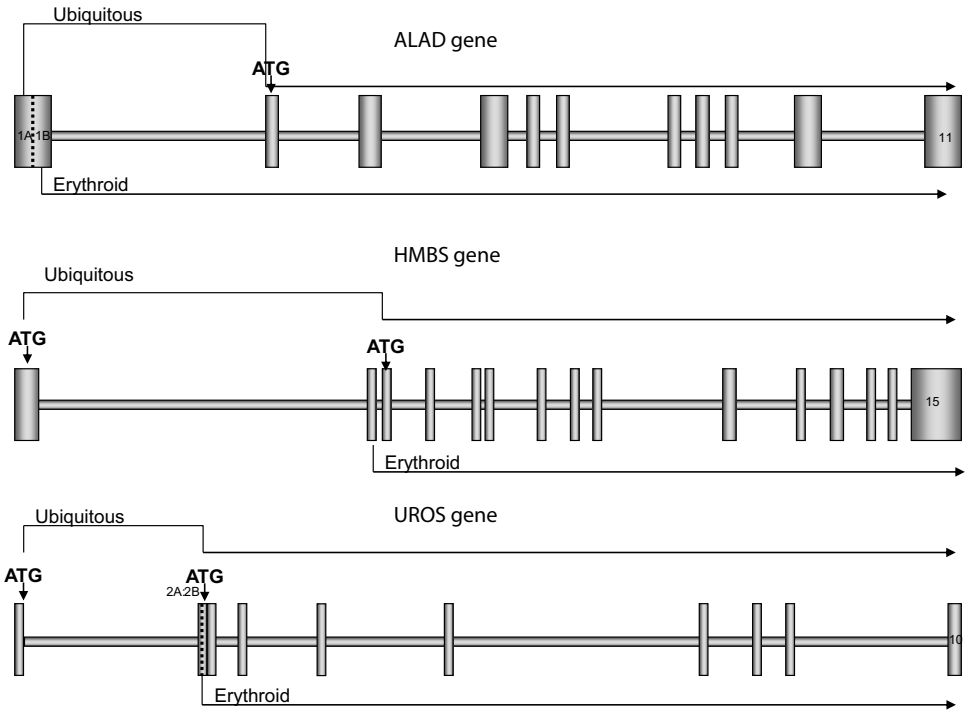

Figure 2 5-Aminolevulinic acid dehydratase (*ALAD*), hydroxymethylbilane synthase *(HMBS)* and uroporphyrinogen synthase (*UROS)* genes are transcribed from different promoters to produce ubiquitous and erythroid isoforms of the enzymes in these different tissues

Enzyme activities are reduced to around 50% of normal, indicating near or complete haplodeficiency.[27,28] All four disorders show low clinical penetrance indicating that environmental factors and, probably, genes at other loci are important in determining their presentation. Family studies in France[1] and the UK suggest that about 10–20% of affected individuals develop symptoms but figures as high as 50% have been reported for AIP from Sweden[29] and, when minor skin lesions are taken into account, 40% for VP from South Africa.[30,31] As might be predicted for a heterogeneous haplodeficient disorder, no clear genotype–phenotype correlation has yet been established in any autosomal dominant porphyria though there are reports that some mutations may be associated with high penetrance.[29,31]

There is evidence that mutations for all four disorders are more common in western European populations than the prevalence of the diseases would suggest. In France, one in 1675 blood donors carries an *HMBS* gene mutation.[27] Consistent with this high gene frequency, rare homozygous or compound heterozygous forms, so-called 'homozygous' variants, have been described for each autosomal dominant porphyria.[32–36] Also consistent with a high prevalence of asymptomatic heterozygotes in the population are the high frequency of sporadic presentation, with less than 50% of patients having a family history of overt porphyria, unexplained by *de novo* mutation, and the rare occurrence of co-inheritance of two porphyrias, so-called dual porphyria.[37,38]

Autosomal dominant acute porphyrias

General features

AIP, HCP and VP are characterized by the episodic occurrence of life-threatening acute neurovisceral attacks (Table 1) which are identical in all three conditions. In VP and HCP, bullous skin lesions are present during an acute attack in 10–50% of patients or may be the only clinical manifestation (Table 1).[1] This is rare in HCP but, in the UK, about 60% of patients with VP present only with skin lesions, identical to those of PCT.[39] Acute attacks are rare before puberty and often provoked by identifiable precipitants, notably certain drugs, endocrine factors and alcohol. Their management and prevention has recently been reviewed.[1] Preventing acute attacks by advising patients to avoid porphyrogenic drugs [European Porphyria Network: www.porphyria-europe.org] and other potential precipitants is an essential part of the management of families with AIP, HCP or VP.

Molecular genetics

More than 342 mutations have been identified in the *HMBS* gene in AIP, about 52 in the *CPOX* gene in HCP and more than 150 in the *PPOX* gene in VP (Human Gene Mutation Database: www.hgmd.org). Mutations are distributed throughout the genes; most are point mutations but a few large deletions have been detected in the *HMBS* and *CPOX* genes.[40–42] Mutations decrease enzyme activities in all tissues with the important exception of about 3% of AIP families (variant AIP) which have *HMBS* mutations that alter the N-terminus of the ubiquitous isoform and therefore do not impair activity in erythroid cells.[43,44]

Indications for molecular investigation

Index patient: The main indication for molecular investigation of a patient with biochemically proven AIP, HCP or VP is to identify a mutation as an essential preliminary to molecular investigation of that patient's family. As noted,

Table 3 Sensitivity of mutation detection

Porphyria	Patients	Sensitivity of mutation detection including gene dosage (%)	95% confidence interval (%)	Reference
AIP	260	98.1	95.6–99.2	41
HCP	31	96.9	84.3–99.5	
VP	152	100	95.7–100	
EPP	191	93.9	89.4–96.6	45
CEP		See text		46
PCT		See text		

AIP, acute intermittent porphyria; HCP, hereditary coproporphyria; VP, variegate porphyria; PCT, porphyria cutanea tarda; CEP, congenital erythropoietic porphyria; EPP, erythropoietic protoporphyria

mutational analysis is not required to confirm the diagnosis and is not useful for assessing prognosis. However mutational analysis of an index patient may be indicated if clinical features and/or biochemical findings suggest the presence of a 'homozygous' or dual porphyria or are otherwise atypical. All exons and their flanking regions should be sequenced. Where no mutation is found sequencing should be extended to include the 5 prime untranslated region (5′UTR) and if necessary dosage analysis to detect large intragenic deletions/duplications. Sensitivities of mutation detection in the *HMBS, CPOX* and *PPOX* genes are 97–100%, where all these procedures have been followed (Table 3).[41]

Family studies: Presymptomatic diagnosis of affected relatives is an essential part of the management of families with AIP, HCP or VP. Provided the family mutation can be identified, DNA analysis is now the method of choice, having 100% sensitivity for this purpose.[1,47] It has greater diagnostic accuracy than erythrocyte HMBS activity assay for AIP, plasma fluorescence scanning for VP and faecal porphyrin analysis for HCP and, unlike metabolite assays, is applicable before puberty.[48] In contrast to biochemical investigations, it can exclude inheritance of the family mutation with a high degree of certainty but, since the rest of the gene is not sequenced there is a theoretical possibility of the patient having a different mutation within the same gene. Biochemical investigation can be used to decrease the number of adult relatives that require DNA analysis, particularly in VP where plasma fluorescence scanning has been reported to identify 76% of asymptomatic gene carriers.[49] Faecal coproporphyrin isomer analysis may similarly reduce the need for mutation testing in adult relatives of HCP patients.[50]

Retrospective investigation of suspected acute porphyria: Occasionally, patients who are currently asymptomatic may require investigation to confirm or exclude a past history of acute porphyria in themselves or in a relative who is no longer available for investigation. Because symptoms may be followed by full biochemical remission, normal metabolite measurements do not exclude the diagnosis, particularly in AIP, and enzyme measurements lack sufficient diagnostic accuracy.[51]

In this situation, DNA analysis may identify a disease-specific mutation or, for mutation-negative patients, in combination with biochemical investigation, allow the patient's risk of being affected to be assessed.[41] Our current practice does not include analysis of all three genes as this is not considered cost-effective.

Homozygous acute porphyrias

The number of gene carriers for all three autosomal dominant acute porphyrias is sufficiently high for patients to inherit either two acute porphyrias or mutations for the same disease on each allele. The latter patients are either homozygotes or compound heterozygotes and at least one of the mutations must be associated with some residual enzyme activity in order to sustain life. Homozygous AIP is usually associated with severe, progressive central and peripheral neurological deterioration after birth.[33] Homozygous VP presents with bullous skin lesions in childhood, often associated with skeletal and neurological defects.[52,53] Homozygous HCP may present with acute neurovisceral attacks in childhood[54] while a variant, harderoporphyria, causes neonatal haemolytic anaemia and mild photosensitivity.[55] Patients are either heteroallelic or homoallelic for a missense mutation, p.Lys404Glu, in exon 6 of the *CPOX* gene that impairs the sequential decarboxylation of coproproporphyrinogen III resulting in increased faecal excretion of the tricarboxylic intermediate, harderoporphyrin. Molecular genetic analysis is essential to confirm the diagnosis in these rare conditions.

Porphyria cutanea tarda

General features

PCT is by far the commonest porphyria with over 100 new cases per year in the UK. Patients usually present with skin fragility and bullae on sun-exposed skin.[56] Evidence of liver dysfunction and some degree of iron overload is common. Associated risk factors include high alcohol intake, hepatitis C infection, haemachromatosis and use of oestrogens.[57–60] Treatment is by depletion of iron stores, usually by repeated phlebotomy, or with low-dose oral chloroquine. Both are equally effective in all patients and produce prolonged remission or cure.[60–62] The disease results from reversible inactivation of uroporphyrinogen decarboxlase (UROD) in the liver; symptoms do not occur until activities are below 50% of normal. Inactivation is reported to be due to an inhibitor formed by iron-dependent oxidation of uroporphyrinogen.[63]

Molecular genetics

Enzymatic and molecular investigations have revealed the existence of two main types of PCT.[56] Most patients have the sporadic form (S-PCT) in which UROD deficiency is restricted to the liver and the *UROD* gene is normal. About 25% of patients have the autosomal dominant form, familial PCT (F-PCT) in which UROD activity is decreased in all tissues. Over 108 disease-specific mutations have been identified in the *UROD* gene in F-PCT and its rare 'homozygous' variant, hepatoerythropoietic porphyria (HEP).[64,65] Mutations in F-PCT decrease UROD activity by 50%. An additional decrease in hepatic UROD is required to produce symptoms which is brought about by the inactivation

mechanism described above which is common to both types of PCT. The need for additional inactivation and the frequent presence of the same risk factors in both types of PCT explains, at least in part, the low clinical penetrance of F-PCT. The sensitivity of mutation identification in F-PCT probably exceeds 95% but is difficult to determine because there is no method for unequivocally distinguishing F-PCT from S-PCT which may itself rarely cluster in families.[66]

Indications for molecular investigation

Molecular analysis of the haemochromatosis gene should be part of the initial investigation of all patients with PCT in the UK where about 20% are homozygous for the p.Cys282Tyr (C282Y) mutation.[59,60] Homozygotes should be treated by phlebotomy, not with chloroquine, and subsequently monitored for re-accumulation of iron.

Mutational analysis of *UROD* is not needed for the diagnosis of PCT except in the rare instances when HEP is suspected.[65] There is currently debate about whether all patients with PCT should be offered testing to identify those with F-PCT.[1,26,67] Mutational analysis of *UROD* has greater diagnostic accuracy than measurement of erythrocyte UROD activity for this purpose.[26,68] Differentiation of F-PCT from S-PCT has little benefit for the individual patient since the response to treatment and prognosis of the two forms is similar.[67] However, subsequent screening of their families would allow asymptomatic affected relatives to be counselled about the need to avoid risk factors. As yet, there is no published evidence for the benefits of managing families in this way. Some patients and relatives may wish to know whether their disease is inherited.[26] In these, DNA analysis of *UROD* in the proband may be justified after appropriate genetic counselling. Current practice in the UK is to undertake only biochemical analyses except in exceptional circumstances.

Autosomal recessive porphyrias

Two rare porphyrias, ADP[69] and congenital erythropoietic porphyria (CEP) are inherited in an autosomal recessive pattern and a third, erythropoietic protoporphyria (EPP), is a recessive disorder which due to a prevalent hypomorphic allele behaves in a pseudodominant fashion.[70] XLDPP, although an X-linked disorder, is included in this section as it is convenient to consider it as a form of EPP.

Enzyme activities in the autosomal recessive porphyrias are decreased in all tissues to around 30% of normal or less. In contrast to the dominant porphyrias, all three disorders are fully penetrant with only very rare exceptions[71] and, in common with other autosomal recessive disorders, some genotype–phenotype correlation is apparent.

ALA dehydratase deficiency porphyria

ADP is a very rare acute porphyria; only six families have been reported worldwide.[69] Patients are usually compound heterozygotes for *ALAD* mutations. Up to 2% of the population may be heterozygotes, who although asymptomatic, may be more susceptible to toxicity from lead and other chemicals that inhibit this enzyme.[72]

Congenital erythropoietic porphyria

General features

CEP is the most severe of the cutaneous porphyrias, affecting about three per 10 million of the UK population. It usually presents with red urine, severe blistering and haemolytic anaemia in infancy. Patients may develop photomutilation and become transfusion-dependent. As with all recessive conditions, the phenotype can vary depending on residual enzyme function and more severe forms presenting *in utero* with hydrops foetalis as well as mild, later onset forms clinically resembling PCT are described.[73–75] Treatment depends on photo-protection and other supportive measures.[76,77] Allogeneic bone marrow transplantation is curative but is usually reserved for the more severe patients with haemolytic anaemia and should be undertaken at a young age wherever possible.

Molecular genetics

Affected individuals are homozygous or compound heterozygous for *UROS* mutations of which about 45 have been reported (HGMD: www.hgmd.org). Rarely deficient UROS activity is due to mutations in the gene encoding the transcriptional regulator GATA1.[2] There is some evidence for genotype–phenotype correlations in CEP.[77] For example, the mutation Cys73Arg, especially in homozygotes, may be associated with early onset of severe disease and transfusion-dependent haemolytic anaemia. Conversely, patients with the IVS9 + 4delA mutation, which is associated with significant residual activity, present with non-progressive cutaneous manifestations in mid-childhood or early teens. The correlation is weakened by the striking impact of lifestyle and varying sunlight exposure on the phenotypic expression and possibly due to mutations in modifier genes such as *ALAS2*.[78] In the largest series of biochemically proven CEP patients, *UROS* mutations were identified on 25 (74%) of 34 alleles; no mutation being identified on either allele in three patients.[46] The low sensitivity of mutation detection in this and other case series of CEP is unexplained and may be due to as yet unknown genes.

Indications for molecular investigation

Mutation analysis of the *UROS* gene, although not required for diagnosis, may be useful for assessing prognosis, particularly in helping to decide whether allogeneic bone marrow transplantation is indicated. Family members who are heterozygotes for CEP mutations are asymptomatic and have successfully acted as donors.[79] Prenatal diagnosis in families who already have an affected family member can be helpful, but is dependent on identification of both mutations. Where these are not identified, measurement of uroporphyrin I in amniotic fluid may be informative.[80]

Erythropoietic protoporphyria

General features

EPP is a disorder in which accumulation of protoporphyrin in erythrocytes, skin, liver and other tissues leads to lifelong, acute painful photosensitivity and, in about 2% of patients,

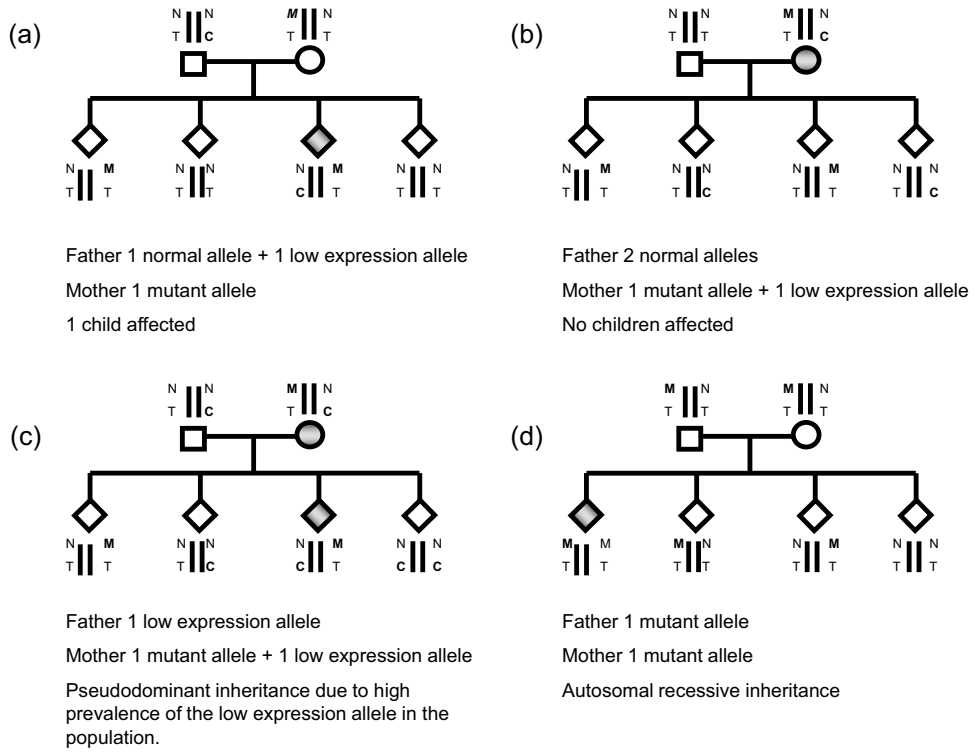

Figure 3 The inheritance of EPP. (a) Family with an affected child who has inherited a mutation (M) from the mother and a low expression allele (C) from the father. Both parents are asymptomatic. (b) Affected mother but no children have inherited overt EPP. (c) Affected mother, father has a low expression allele (C). The risk of having a child with overt EPP is one in four. (d) Mother and father both have mutations in the *FECH* gene (M). The risk of having a child with overt EPP is one in four. Both parents are asymptomatic. N denotes 'normal' *FECH* allele. T denotes thymine at position IVS3-48, C denotes cytosine at position IVS3-48 (the low expression polymorphism). Shaded box/circle denotes symptomatic

severe liver disease. Symptoms usually start in early childhood. Chronic skin lesions are minor and skin fragility is absent.[81,82] The most effective treatment is the prevention of the photosensitive reaction by avoiding sunlight, skin protection with clothing and sunscreen ointments and by increasing skin pigmentation.[83,84] Severe liver disease usually requires transplantation.[85,86]

Molecular genetics

Excess accumulation of free protoporphyrin results from partial deficiency of ferrochelatase (FECH) activity. In the UK, most EPP patients are compound heterozygotes for a hypomorphic IVS3-48C allele that produces a truncated unstable mRNA, reducing activity by 20–30%,[87] and a deleterious *FECH* mutation (Figures 3a and b) that abolishes or markedly decreases enzyme activity.[45] This combination reduces overall FECH activity below a threshold of 35% of normal at which symptoms occur.[88] The population frequency of the IVS3-48C allele varies, ranging from less than 1% in West Africa to 45% in Japan,[88] and correlates with the prevalence of EPP and the frequency of parent to offspring transmission. In the UK, where 13% of the population carry the low expression allele,[45] 30–35% of unrelated patients have an affected relative and EPP is present in more than one generation in about 5% of families (Figure 3c).[81]

Patients from about 4% of families are homozygous or compound heterozygous for deleterious mutations (Figure 3d) and characteristically have residual FECH activities of around 10% of normal. The frequency of the IVS3-48C allele in this group of patients does not differ significantly from the general population.[45]

More than 130 *FECH* mutations have been identified in EPP (Human Gene Mutation Database: http://www.hgmd.org). Less than half the mutations are missense; the rest are nonsense, frameshifts, large deletions or affect RNA splicing.[45] In the UK, allelic heterogeneity is less than in other porphyrias, largely due to one mutation being present in 24% of EPP families.[45]

In 2% of UK families, FECH activity is normal and a gain of function mutation in *ALAS2*, the gene encoding the rate-limiting enzyme of erythroid haem synthesis, results in increased flux through the pathway. The resultant accumulation of free and zinc-chelated protoporphyrin, which may be due to reduced availability of iron and or zinc within the mitochondrion, gives rise to a distinct protoporphyria, named X-linked dominant protoporphyria (XLDPP).[89] Interestingly, loss of function *ALAS2* mutations cause X linked sideroblastic anaemia (XLSA).[90]

Genotype:phenotype correlations

A molecular explanation has been sought for two unusual phenotypes of EPP: severe liver disease and palmar keratoderma. The risk of liver disease is increased in the rare patients who are homozygous or compound heterozygous for deleterious mutations, even though they are clinically indistinguishable from the more common form of EPP with regard to severity of photosensitivity. Five of 21 (24%) reported patients with this molecular type of EPP

had severe liver disease. However, the majority of patients with severe liver disease come from the much larger group of patients with one IVS3-48C allele. There is evidence to suggest that missense mutations that retain some residual activity carry a lower risk of liver disease than mutations that abolish FECH activity.[91,92] However, mutations associated with liver disease are found more frequently in patients without liver disease. Mutational analysis currently has no role in predicting the risk of liver disease in an individual patient except when it leads to identification of deleterious mutations on both *FECH* alleles or an *ALAS2* mutation (see below). To date, all EPP patients with palmar keratoderma have been homozygote/compound heterozygotes for deleterious *FECH* mutations, although interestingly they had lower total erythrocyte porphyrin concentrations than patients with a single mutation and a low expression allele and none had protoporphyric liver disease.[93,94] XLDPP, which is clinically indistinguishable from EPP, has a higher risk of severe liver disease than the common form of EPP in those patients so far identified.[89]

Indications for molecular investigation

Patients with EPP: Mutational analysis of *FECH* is not required to establish the diagnosis of FECH-deficient EPP, except for presymptomatic diagnosis in infants from affected families and in the rare patients, for example, those with keratoderma, in whom erythrocyte protoporphyrin concentrations are only marginally increased.[93] However, there are arguments for including it in the investigation of all new patients, mainly to identify patients with two deleterious mutations who are at increased risk of liver disease but also for subsequent genetic counselling (see below). An additional benefit is the collection of clinical and genetic information from sufficient numbers of patients for detection of genotype–phenotype correlations that may be clinically useful in the future. The sensitivity of detection of *FECH* mutations in EPP is 94% (Table 3).[45] In mutation-negative patients, decreased lymphocyte FECH activity and the presence of the IVS3-48C allele infer the presence of a *FECH* mutation undetectable by current methods.

Mutational analysis of *ALAS2* is essential to confirm the diagnosis of XLDPP and is recommended for all EPP patients where zinc protoporphyrin comprises 10% or more of the total erythrocyte porphyrin. In addition, there are very rare patients with acute photosensitivity, and increased erythrocyte free and zinc-protoporphyrin but no detectable *ALAS2* mutations; the cause of their disease has yet to be determined.[45]

Family studies: Screening all asymptomatic relatives for *FECH* mutations is not needed for the management of all families with EPP. However, mutation analysis is required for an asymptomatic adult relative who requests predictive counselling and requires prior genetic testing of the proband. This has become increasingly important given increased awareness of the pattern of inheritance of EPP and the relatively high frequency of the low expression polymorphism in the UK population.

In XLDPP, all males and most female heterozygotes have photosensitivity. Family studies by testing for *ALAS2* mutations are required to identify the few asymptomatic female carriers.

Predictive counselling: It is common for patients with EPP and their asymptomatic relatives to ask for an assessment of the risk that their children will inherit EPP. For families with a child with EPP, the risk of a subsequent child having the disease is likely to be one in four, since the unaffected parent is much more likely to be heterozygous than homozygous for the IVS3-48C allele; DNA analysis is not normally required. The risk that the first child of a parent, who either has EPP or is an asymptomatic carrier of a *FECH* mutation, will inherit the disease depends mainly on whether the unaffected partner carries the IVS3-48C allele (Figures 3a and b).[95] If he or she is hetero-allelic for the hypomorphic IVS3-48C allele, the risk is one in four; increasing to one in two if the unaffected partner is homozygous. If this allele is absent the risk relates to the carrier frequency of *FECH* mutations in the general population, which although not formally assessed, is likely to be less than 1:1000. In addition, there may be other rare, as yet unidentified, hypomorphic variants. Their existence is suggested by the finding that about 1% of EPP patients with one *FECH* mutation are IVS3-48T homozygotes.[45]

Acquired somatic mutations

A small number of patients have been described in whom either EPP or CEP has developed in association with myelodysplasia or myeloproliferative disorder.[96,97] The myeloid disorder usually precedes the onset of porphyria, patients are aged 40 y or more and there is no family history of porphyria. Most of the EPP patients have an acquired full or partial deletion of chromosome 18 which results in loss of heterozygosity for *FECH* in a clone of haematopoietic cells.[96,98] The association of CEP with myelodysplasia remains unexplained but may result from a similar mechanism.[97,99]

Prenatal diagnosis in the porphyrias

Prenatal diagnosis is rarely indicated in the porphyrias. In most, the clinical prognosis is not generally considered sufficiently poor to warrant termination and specialist treatment at birth, requiring prenatal planning, and therefore is rarely required. In the autosomal dominant porphyrias, transmission of a severe phenotype is unlikely; indeed, most who inherit the genes for these conditions remain asymptomatic throughout life. However, prenatal diagnosis by DNA analysis of chorionic villous or amniotic cells may be justified in the more severe, and phenotypically predictable, autosomal recessive porphyrias and 'homozygous' variants when the family already has a child in whom the pathogenic mutations have been identified. Prenatal diagnosis by molecular analysis has been reported in CEP and HEP.[100,101]

Methods for mutation detection

Most mutations in the porphyria genes are found in the regions that code for the protein that is the exons or in the

areas flanking the exons causing defects in the RNA processing. It is these regions that are targeted for analysis in the first instance. Analysis of the promoter region at the 5′ end of the gene and analysis for the presence of large deletions may also be needed. Other parts of the intronic sequence are not usually analysed.

Identification of a mutation in the proband

The regions of interest are initially amplified from genomic DNA using polymerase chain reaction (PCR) to produce a product that is then used for the investigation. Screening methods may be used to identify parts of the gene that contain variants, for example denaturing high-performance liquid chromatography,[102,103] high resolution melting[104] or denaturing gradient gel electrophoresis[105,106] before sequencing using dideoxy terminators. As the cost and speed of sequencing improves, screening is becoming less widely used. Direct sequencing of all the regions of interest is now often the preferred option. Because a number of large deletions encompassing one or more exons have been identified in the porphyrias,[40–42,107–109] gene dosage analysis by quantitative fluorescent PCR[110] or multiple ligation dependent analysis[40] should always be carried out if a mutation is not detected by sequencing of genomic DNA. Single exon deletions must always be confirmed as variants under primers or probes can mimic these. Confirmation of deletions can be carried out by repeat analysis with different primers or probes or the identification of the breakpoints although the latter may be very laborious. Confirmation of a duplication may be difficult as the size of the duplication may be greater than that easily achieved by long range PCR. This strategy is very similar to that used for a large number of other genes, e.g. *BRCA*, *PMS2*, *RB1*, *VHL*, *ATP7B* (http://www.ukgtn.nhs.uk/gtn).

When no mutation can be identified in a patient with biochemically proven porphyria, RNA analysis using a fresh blood sample may be worthwhile. A heterozygous exonic polymorphism will become homozygous in cDNA, or gene dosage[111] analysis will show a 50% reduction in signal, if there is loss of heterogeneity caused by nonsense mediated decay due to a functional intronic mutation.

DNA methods for family screening

When a pathogenic mutation has been identified in a family, analysis of samples from family members is straightforward. The most common method is to sequence the area that contains the mutation although RFLP is used in some centres.[112] Those families with large deletions encompassing primer sites will require dosage analysis or PCR across the breakpoints.

Differentiating a mutation that is causative of porphyria from a harmless sequence variant

As the amount of molecular genetic testing increases more novel sequence variants are identified. Nonsense, frameshift and consensus splice site changes are likely to be pathogenic.[113] However if the pathogenicity of a variant is uncertain guidelines are available from the clinical molecular genetics society (http://www.cmgs.org) to assist in the determination of the clinical significance of variants identified in routine testing. In brief when a sequence variant is identified, disease specific databases are checked, when available, for information regarding the variant. The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) is commonly used although the access to the most recent data is only available to subscribers. Care needs to be taken that there is evidence of pathogenicity. In most cases the original publication describing the variant will need to be checked. The most useful information is that of functional assay studies which may be available in scientific publications. Bioinformatic programs are available, e.g. AlaMut (Interactive Biosoftware USA) which provide genetic information that may help to interpret pathogenic status. Further investigations may be required such as family studies. If a variant does not track with the disease in a family then it is likely to be non-pathogenic. However segregation studies have to be used with caution in porphyria families as many family members who have a pathogenic mutation will be asymptomatic. RNA studies may be needed to determine the effect of a variant on the splicing of RNA transcripts.

Annual participation in a specific molecular external quality assessment scheme such as the European Molecular Genetics Quality Network (EMQN: Website; www.emqn.org) is essential in order to improve the services for the benefit of patients with porphyria. Without this errors may be perpetuated with the risk of acute attacks going unrecognized or unnecessary lifelong restrictions being practiced both with a potential of litigation.

The European Porphyria Network (EPNET; www.porphyria-europe.org) has encouraged communication among the specialist porphyria laboratories. One outcome of the close links that have developed between laboratories is the exchange of information on many subjects including pathogenicity data on specific variants. A central diagnostic database with the participation of the wider porphyria community inputting novel mutations and their evidence for pathogenicity would be a huge advantage for diagnosis of the porphyrias ensuring that laboratories do not repeat the same work and preventing misdiagnoses.

Ethical considerations

Consent

The regulations governing genetic testing vary in each country. In September 2011 the Joint Committee on Medical Genetics in the UK published the second edition ‘Consent and confidentiality in genetic practice’. The guidance given in this report was that consent should be obtained prior to a laboratory test with genetic implications being undertaken. It also placed the onus for gaining consent on the clinician requesting the genetic information; so that the laboratory is not required to confirm or document consent.

When a sample is submitted to a laboratory the Joint Committee suggests that the laboratory can assume that

consent has been obtained for testing, possible storage and for the use of the sample and the information generated from it to be shared with the members of the donor's family and their health professionals (if appropriate). As a safeguard for the laboratory and to ensure that the patient's wishes are complied with, a customized consent form may be used. One specifically designed for the porphyrias is available on the Cardiff Porphyria Service website http://www.cardiff-porphyria.org/ which the clinician may find helpful and is very useful for the laboratory.

Testing of children

It is recommended that where genetic testing is primarily predictive of an illness or impairment that testing should be delayed until the young person can decide for themselves. However testing may occur when there are specific reasons not to wait until the child is older (Report on the genetic testing of children British Society for Human Genetics 2010). In the acute porphyric disorders it is very rare that acute attacks occur before puberty with only a few documented cases[48,114,115] in the world literature. However in order to pre-empt this possibility children in families with acute porphyria, should be offered testing with appropriate consent from parent or guardian. This enables advice on avoidance of precipitating factors to be provided and ensures rapid diagnosis with prompt treatment should an attack occur.

The clinical benefit of genetic diagnosis in EPP is unclear. Presymptomatic diagnosis may be requested in response to parental anxiety. However if a child has not developed symptoms by the age of three years, genetic testing, which is likely to be for carrier status, should be delayed until the child is competent to make an informed decision.

Prenatal or postnatal genetic testing of a sibling in a family with CEP should be carried out so that the child can be protected from light exposure including operating theatre lights during caesarean section and phototherapy for neonatal jaundice.

Conclusions

In summary, the role of genetic testing in the management of patients and their families is not always straight forward and differs among the types of porphyria. In complex cases the advice of a specialist laboratory should be sought.

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REFERENCES

1 Puy H, Gouya L, Deybach J-C. Porphyrias. *Lancet* 2010;**375**:924–37
2 Phillips JD, Steensma DP, Pulsipher MA, Spangrude GJ, Kushner JP. Congenital erythropoietic porphyria due to a mutation in GATA1: the first trans-acting mutation causative for a human porphyria. *Blood* 2007;**109**:2618–21
3 Plewinska M, Thunell S, Holmberg L, Wetmur JG, Desnick RJ. delta-Aminolevulinate dehydratase deficient porphyria: identification of the molecular lesions in a severely affected homozygote. *Am J Hum Genet* 1991;**49**:167–74
4 Grandchamp B, Deybach JC, Grelier M, de Verneuil H, Nordmann Y. Studies of porphyrin synthesis in fibroblasts of patients with congenital erythropoietic porphyria and one patient with homozygous coproporphyria. *Biochim Biophys Acta* 1980;**629**:577–86
5 Romeo G, Levin EY. Uroporphyrinogen 3 cosynthetase in human congenital erythropoietic porphyria. *Proc Natl Acad Sci USA* 1969;**63**:856–63
6 Tsai SF, Bishop DF, Desnick RJ. Coupled-enzyme and direct assays for uroporphyrinogen III synthase activity in human erythrocytes and cultured lymphoblasts. Enzymatic diagnosis of heterozygotes and homozygotes with congenital erythropoietic porphyria. *Anal Biochem* 1987;**166**:120–33
7 Anderson KE, Bloomer JR, Bonkovsky HL, *et al.* Recommendations for the diagnosis and treatment of the acute porphyrias. *Ann Intern Med* 2005;**142**:439–50
8 Deacon AC, Elder GH. ACP Best Practice No 165: front line tests for the investigation of suspected porphyria. *J Clin Pathol* 2001;**54**:500–7
9 Raich N, Romeo PH, Dubart A, Beaupain D, Cohen-Solal M, Goossens M. Molecular cloning and complete primary sequence of human erythrocyte porphobilinogen deaminase. *Nucleic Acids Res* 1986;**14**:5955–68
10 Potluri VR, Astrin KH, Wetmur JG, Bishop DF, Desnick RJ. Human delta-aminolevulinate dehydratase: chromosomal localization to 9q34 by in situ hybridization. *Hum Genet* 1987;**76**:236–9
11 Namba H, Narahara K, Tsuji K, Yokoyama Y, Seino Y. Assignment of human porphobilinogen deaminase to 11q24.1––q24.2 by in situ hybridization and gene dosage studies. *Cytogenet Cell Genet* 1991;**57**:105–8
12 Cacheux V, Martasek P, Fougerousse F, *et al.* Localization of the human coproporphyrinogen oxidase gene to chromosome band 3q12. *Hum Genet* 1994;**94**:557–9
13 Roberts AG, Whatley SD, Daniels J, *et al.* Partial characterization and assignment of the gene for protoporphyrinogen oxidase and variegate porphyria to human chromosome 1q23. *Hum Mol Genet* 1995;**4**:2387–90
14 Dubart A, Mattei MG, Raich N, *et al.* Assignment of human uroporphyrinogen decarboxylase (URO-D) to the p34 band of chromosome 1. *Hum Genet* 1986;**73**:277–9
15 Taketani S, Inazawa J, Nakahashi Y, Abe T, Tokunaga R. Structure of the human ferrochelatase gene. Exon/intron gene organization and location of the gene to chromosome 18. *Eur J Biochem* 1992;**205**:217–22
16 Astrin KH, Warner CA, Yoo HW, Goodfellow PJ, Tsai SF, Desnick RJ. Regional assignment of the human uroporphyrinogen III synthase (UROS) gene to chromosome 10q25.2––q26.3. *Hum Genet* 1991;**87**:18–22
17 May BK, Dogra SC, Sadlon TJ, Bhasker CR, Cox TC, Bottomley SS. Molecular regulation of heme biosynthesis in higher vertebrates. *Prog Nucleic Acid Res Mol Biol* 1995;**51**:1–51
18 Chretien S, Dubart A, Beaupain D, *et al.* Alternative transcription and splicing of the human porphobilinogen deaminase gene result either in tissue-specific or in housekeeping expression. *Proc Natl Acad Sci USA* 1988;**85**:6–10
19 Kaya AH, Plewinska M, Wong DM, Desnick RJ, Wetmur JG. Human delta-aminolevulinate dehydratase (ALAD) gene: structure and

alternative splicing of the erythroid and housekeeping mRNAs. *Genomics* 1994;**19**:242–8
20 Aizencang G, Solis C, Bishop DF, Warner C, Desnick RJ. Human uroporphyrinogen-III synthase: genomic organization, alternative promoters, and erythroid-specific expression. *Genomics* 2000;**70**:223–31
21 Kauppinen R. Porphyrias. *Lancet* 2005;**365**:241–52
22 Ajioka RS, Phillips JD, Kushner JP. Biosynthesis of heme in mammals. *Biochim Biophys Acta* 2006;**1763**:723–36
23 Smith SJ, Cox TM. Translational control of erythroid delta-aminolevulinate synthase in immature human erythroid cells by heme. *Cell Mol Biol* 1997;**43**:103–14
24 Lee JS, Anvret M. Identification of the most common mutation within the porphobilinogen deaminase gene in Swedish patients with acute intermittent porphyria. *Proc Natl Acad Sci USA* 1991;**8**:10912–5
25 Meissner PN, Dailey TA, Hift RJ, *et al.* A R59W mutation in human protoporphyrinogen oxidase results in decreased enzyme activity and is prevalent in South Africans with variegate porphyria. *Nat Genet* 1996;**13**:95–7
26 Aarsand AK, Boman H, Sandberg S. Familial and sporadic porphyria cutanea tarda: characterization and diagnostic strategies. *Clin Chem* 2009;**55**:795–803
27 Nordmann Y, Puy H, Da Silva V, *et al.* Acute intermittent porphyria: prevalence of mutations in the porphobilinogen deaminase gene in blood donors in France. *J Intern Med* 1997;**242**:213–7
28 Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. *J Inherit Metab Dis* 2005;**28**:277–86
29 Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. *Scand J Clin Lab Invest* 2009;**69**:612–8
30 Hift RJ, Meissner D, Meissner PN. A systematic study of the clinical and biochemical expression of variegate porphyria in a large South African family. *Brit J Dermatol* 2004;**151**:465–71
31 von und zu Fraunberg M, Timonen K, Mustajoki P, Kauppinen R. Clinical and biochemical characteristics and genotype-phenotype correlation in Finnish variegate porphyria patients. *Eur J Hum Genet* 2002;**10**:649–57
32 Llewellyn DH, Smyth SJ, Elder GH, Hutchesson AC, Rattenbury JM, Smith MF. Homozygous acute intermittent porphyria: compound heterozygosity for adjacent base transitions in the same codon of the porphobilinogen deaminase gene. *Hum Genet* 1992;**89**:97–8
33 Solis C, Martinez-Bermejo A, Naidich TP, *et al.* Acute intermittent porphyria: studies of the severe homozygous dominant disease provides insights into the neurologic attacks in acute porphyrias. *Arch Neurol* 2004;**61**:1764–70
34 Hessels J, Voortman G, van der Wagen A, van der Elzen C, Scheffer H, Zuijderhoudt FMJ. Homozygous acute intermittent porphyria in a 7-year-old boy with massive excretions of porphyrins and porphyrin precursors. *J Inherit Metab Dis* 2004;**27**:19–27
35 Martasek P, Nordmann Y, Grandchamp B. Homozygous hereditary coproporphyria caused by an arginine to tryptophane substitution in coproporphyrinogen oxidase and common intragenic polymorphisms. *Hum Mol Genet* 1994;**3**:477–80
36 Palmer RA, Elder GH, Barrett DF, Keohane SG. Homozygous variegate porphyria: a compound heterozygote with novel mutations in the protoporphyrinogen oxidase gene. *Br J Dermatol* 2001;**144**:866–9
37 Doss MO. Dual porphyria in double heterozygotes with porphobilinogen deaminase and uroporphyrinogen decarboxylase deficiencies. *Clin Genet* 1989;**35**:146–51
38 Harraway JR, Florkowski CM, Sies C, George PM. Dual porphyria with mutations in both the UROD and HMBS genes. *Ann Clin Biochem* 2006;**43**:80–2
39 Whatley SD, Puy H, Morgan RR, *et al.* Variegate porphyria in Western Europe: identification of PPOX gene mutations in 104 families, extent of allelic heterogeneity, and absence of correlation between phenotype and type of mutation. *Am J Hum Genet* 1999;**65**:984–94
40 Di Pierro E, Besana V, Moriondo V, *et al.* A large deletion on chromosome 11 in acute intermittent porphyria. *Blood Cells Mol Dis* 2006;**37**:50–4
41 Whatley SD, Mason NG, Woolf JR, Newcombe RG, Elder GH, Badminton MN. Diagnostic strategies for autosomal dominant acute porphyrias: retrospective analysis of 467 unrelated patients referred for mutational analysis of the HMBS, CPOX, or PPOX gene. *Clin Chem* 2009;**55**:1406–14
42 Barbaro M, Kotajärvi M, Harper P, Floderus Y. Identification of an AluY-mediated deletion of exon 5 in the CPOX gene by MLPA analysis in patients with hereditary coproporphyria. *Clin Genet* 2012;**81**:249–56
43 Grandchamp B, Picat C, Mignotte V, *et al.* Tissue-specific splicing mutation in acute intermittent porphyria. *Proc Natl Acad Sci USA* 1989;**86**:661–4
44 Whatley SD, Roberts AG, Llewellyn DH, Bennett CP, Garrett C, Elder GH. Non-erythroid form of acute intermittent porphyria caused by promoter and frameshift mutations distant from the coding sequence of exon 1 of the HMBS gene. *Hum Genet* 2000;**107**:243–8
45 Whatley SD, Mason NG, Holme SA, Anstey AV, Elder GH, Badminton MN. Molecular epidemiology of erythropoietic protoporphyria in the U.K. *Br J Dermatol* 2010;**162**:642–6
46 Katugampola R. *Assessment of Clinical and Laboratory Data to Inform Management Decisions in Congenital Erythropoietc Porphyria*. MD thesis. Cardiff University, UK, 2010
47 Sassa S. Modern diagnosis and management of the porphyrias. *Br J Haematol* 2006;**135**:281–92
48 Hultdin J, Schmauch A, Wikberg A, Dahlquist G, Andersson C. Acute intermittent porphyria in childhood: a population-based study. *Acta Paediatr* 2003;**92**:562–8
49 Hift RJ, Davidson BP, van der Hooft C, Meissner DM, Meissner PN. Plasma fluorescence scanning and fecal porphyrin analysis for the diagnosis of variegate porphyria: precise determination of sensitivity and specificity with detection of protoporphyrinogen oxidase mutations as a reference standard. *Clin Chem* 2004;**50**:915–23
50 Allen KR, Degg TJ, Barth JH. Hereditary coproporphyria: comparison of molecular and biochemical investigations in a large family. *J Inherit Metab Dis* 2005;**28**:779–85
51 Santos JL, Fontanellas A, Batlle AM, Enriquez de Salamanca RE. Reference values of 5-aminolevulinate dehydrase and porphobilinogen deaminase in the Spanish population from Madrid. *Ecotoxicol Env Saf* 1998;**39**:168–71
52 Norris PG, Elder GH, Hawk JL. Homozygous variegate porphyria: a case report. *Br J Dermatol* 1990;**122**:253–7
53 Roberts AG, Puy H, Dailey TA, *et al.* Molecular characterization of homozygous variegate porphyria. *Hum Mol Genet* 1998;**7**:1921–5
54 Grandchamp B, Phung N, Nordmann Y. Homozygous case of hereditary coproporphyria. *Lancet* 1977;**2**:1348–9
55 Schmitt C, Gouya L, Malonova E, *et al.* Mutations in human CPO gene predict clinical expression of either hepatic hereditary coproporphyria or erythropoietic harderoporphyria. *Hum Mol Genet* 2005;**14**:3089–98
56 Frank J, Poblete-Gutierrez P. Porphyria cutanea tarda–when skin meets liver. *Best Pract Res Clin Gastroenterol* 2010;**24**:735–45
57 Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in porphyria cutanea tarda: systematic review and meta-analysis. *J Hepatol* 2003;**39**:620–7
58 Egger NG, Goeger DE, Payne DA, Miskovsky EP, Weinman SA, Anderson KE. Porphyria cutanea tarda: multiplicity of risk factors including HFE mutations, hepatitis C, and inherited uroporphyrinogen decarboxylase deficiency. *Dig Dis Sci* 2002;**47**:419–26
59 Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH. Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. *Lancet* 1997;**349**:321–3
60 Sarkany RP. The management of porphyria cutanea tarda. *Clin Exp Dermatol* 2001;**26**:225–32
61 Valls V, Ena J, Enriquez-De-Salamanca R. Low-dose oral chloroquine in patients with porphyria cutanea tarda and low-moderate iron overload. *J Dermatol Sci* 1994;**7**:169–75
62 Badminton MN, Elder GH. Management of acute and cutaneous porphyrias. *Int J Clin Pract* 2002;**56**:272–8
63 Phillips JD, Bergonia HA, Reilly CA, Franklin MR, Kushner JP. A porphomethene inhibitor of uroporphyrinogen decarboxylase causes porphyria cutanea tarda. *Proc Natl Acad Sci USA* 2007;**104**:5079–84
64 Elder GH, Smith SG, Herrero C, *et al.* Hepatoerythropoietic porphyria: a new uroporphyrinogen decarboxylase defect or homozygous porphyria cutanea tarda? *Lancet* 1981;**1**:916–9

65 Moran-Jimenez MJ, Ged C, Romana M, *et al.* Uroporphyrinogen decarboxylase: complete human gene sequence and molecular study of three families with hepatoerythropoietic porphyria. *Am J Hum Genet* 1996;**58**:712–21

66 Elder GH. Porphyria cutanea tarda. *Semin Liver Dis* 1998;**18**:67–75

67 Sarkany RPE. Making sense of the porphyrias. *Photodermatol Photoimmunol Photomed* 2008;**24**:102–8

68 Badenas C, To-Figueras J, Phillips JD, Warby CA, Munoz C, Herrero C. Identification and characterization of novel uroporphyrinogen decarboxylase gene mutations in a large series of porphyria cutanea tarda patients and relatives. *Clin Genet* 2009;**75**:346–53

69 Akagi R, Kato N, Inoue R, Anderson KE, Jaffe EK, Sassa S. delta-Aminolevulinate dehydratase (ALAD) porphyria: the first case in North America with two novel ALAD mutations. *Mol Genet Metab* 2006;**87**:329–36

70 Zschocke J. Dominant versus recessive: molecular mechanisms in metabolic disease. *J Inherit Metab Dis* 2008;**31**:599–618

71 Ged C, Megarbane H, Chouery E, Lalanne M, Megarbane A, de Verneuil H. Congenital erythropoietic porphyria: report of a novel mutation with absence of clinical manifestations in a homozygous mutant sibling. *J Invest Dermatol* 2004;**123**:589–91

72 Scinicariello F, Murray HE, Moffett DB, Abadin HG, Sexton MJ, Fowler BA. Lead and delta-aminolevulinic acid dehydratase polymorphism: where does it lead? A meta-analysis. *Env Health Perspect* 2007;**115**:35–41

73 Verstraeten L, Van Regemorter N, Pardou A, *et al.* Biochemical diagnosis of a fatal case of Gunther's disease in a newborn with hydrops foetalis. *Eur J Clin Chem Clin Biochem* 1993;**31**:121–8

74 Deybach JC, de Verneuil H, Phung N, Nordmann Y, Puissant A, Boffety B. Congenital erythropoietic porphyria (Gunther's disease): enzymatic studies on two cases of late onset. *J Lab Clin Med* 1981;**97**:551–8

75 Murphy A, Gibson G, Elder GH, Otridge BA, Murphy GM. Adult-onset congenital erythropoietic porphyria (Gunther's disease) presenting with thrombocytopenia. *J R Soc Med* 1995;**88**:357P–8P

76 Fritsch C, Bolsen K, Ruzicka T, Goerz G. Congenital erythropoietic porphyria. *J Am Acad Dermatol* 1997;**36**:594–610

77 Desnick RJ, Astrin KH. Congenital erythropoietic porphyria: advances in pathogenesis and treatment. *Br J Haematol* 2002;**117**: 779–95

78 To-Figueras J, Ducamp S, Clayton J, *et al.* ALAS2 acts as a modifier gene in patients with congenital erythropoietic porphyria. *Blood* 2011;**118**:1443–51

79 Lagarde C, Hamel-Teillac D, De Prost Y, *et al.* Allogeneic bone marrow transplantation in congenital erythropoietic porphyria. Gunther's disease. *Ann Dermatol Venereol* 1998;**125**:114–7

80 Deybach JC, Grandchamp B, Grelier M, *et al.* Prenatal exclusion of congenital erythropoietic porphyria (Gunther's disease) in a fetus at risk. *Hum Genet* 1980;**53**:217–21

81 Todd DJ. Erythropoietic protoporphyria. *Br J Dermatol* 1994;**131**: 751–66

82 Cox T. *Protoporphyria*. In: Kadish KM, Smith KM, Guilard R, eds. *The Porphyrin Handbook*. Amsterdam: Academic Press, 2003: 121–50

83 Holme SA, Anstey AV, Finlay AY, Elder GH, Badminton MN. Erythropoietic protoporphyria in the U.K.: clinical features and effect on quality of life. *Br J Dermatol* 2006;**155**:574–81

84 Minder EI. Afamelanotide, an agonistic analog of alpha-melanocyte-stimulating hormone, in dermal phototoxicity of erythropoietic protoporphyria. *Expert Opin Invest Drugs* 2010;**19**:1591–602

85 McGuire BM, Bonkovsky HL, Carithers RL Jr, *et al.* Liver transplantation for erythropoietic protoporphyria liver disease. *Liver Transplant* 2005;**11**:1590–6

86 Dowman JK, Gunson BK, Bramhall S, Badminton MN, Newsome PN. Liver transplantation from donors with acute intermittent porphyria. *Ann Intern Med* 2011;**154**:571–2

87 Gouya L, Puy H, Robreau A-M, *et al.* The penetrance of dominant erythropoietic protoporphyria is modulated by expression of wildtype FECH. *Nat Genet* 2002;**30**:27–8

88 Gouya L, Martin-Schmitt C, Robreau A-M, *et al.* Contribution of a common single-nucleotide polymorphism to the genetic predisposition for erythropoietic protoporphyria. *Am J Hum Genet* 2006;**78**:2–14

89 Whatley SD, Ducamp S, Gouya L, *et al.* C-terminal deletions in the ALAS2 gene lead to gain of function and cause X-linked dominant protoporphyria without anemia or iron overload. *Am J Hum Genet* 2008;**83**:408–14

90 Ducamp S, Kannengiesser C, Touati M, *et al.* Sideroblastic anemia: molecular analysis of the ALAS2 gene in a series of 29 probands and functional studies of 10 missense mutations. *Hum Mutat* 2011;**32**:590–7

91 Bloomer J, Wang Y, Singhal A, Risheg H. Molecular studies of liver disease in erythropoietic protoporphyria. *J Clin Gastroenterol* 2005;**39**:S167–75

92 Elder GH, Gouya L, Whatley SD, Puy H, Badminton MN, Deybach JC. The molecular genetics of erythropoietic protoporphyria. *Cell Mol Biol (Noisy-le-grand)* 2009;**55**:118–26

93 Holme SA, Whatley SD, Roberts AG, *et al.* Seasonal palmar keratoderma in erythropoietic protoporphyria indicates autosomal recessive inheritance. *J Invest Dermatol* 2009;**129**:599–605

94 Mendez M, Poblete-Gutierrez P, Moran-Jimenez MJ, *et al.* A homozygous mutation in the ferrochelatase gene underlies erythropoietic protoporphyria associated with palmar keratoderma. *Br J Dermatol* 2009;**160**:1330–4

95 Lecha M, Puy H, Deybach J-C. Erythropoietic protoporphyria. *Orphanet J Rare Dis* 2009;**4**:19

96 Goodwin RG, Kell WJ, Laidler P, *et al.* Photosensitivity and acute liver injury in myeloproliferative disorder secondary to late-onset protoporphyria caused by deletion of a ferrochelatase gene in hematopoietic cells. *Blood* 2006;**107**:60–2

97 Kontos AP, Ozog D, Bichakjian C, Lim HW. Congenital erythropoietic porphyria associated with myelodysplasia presenting in a 72-year-old man: report of a case and review of the literature. *Br J Dermatol* 2003;**148**:160–4

98 Aplin C, Whatley SD, Thompson P, *et al.* Late-onset erythropoietic porphyria caused by a chromosome 18q deletion in erythroid cells. *J Invest Dermatol* 2001;**117**:1647–9

99 Sarkany RPE, Ibbotson SH, Whatley SD, *et al.* Erythropoietic uroporphyria associated with myeloid malignancy is likely distinct from autosomal recessive congenital erythropoietic porphyria. *J Invest Dermatol* 2011;**131**:1172–5

100 Ged C, Moreau-Gaudry F, Taine L, *et al.* Prenatal diagnosis in congenital erythropoietic porphyria by metabolic measurement and DNA mutation analysis. *Prenatal Diagn* 1996;**16**:83–6

101 Phillips JD, Whitby FG, Stadtmueller BM, Edwards CQ, Hill CP, Kushner JP. Two novel uroporphyrinogen decarboxylase (URO-D) mutations causing hepatoerythropoietic porphyria (HEP). *Transl Res* 2007;**149**:85–91

102 Lam CW, Poon PM, Tong SF, *et al.* Novel mutation and polymorphisms of the HMBS gene detected by denaturing HPLC. *Clin Chem* 2001;**47**:343–6

103 Christiansen L, Bygum A, Kaehne M, Jensen A, Horder M, Petersen NE. Mutation screening of the entire coding region of the protoporphyrinogen oxidase gene using denaturing gradient gel electrophoresis and denaturing hplc. *Clin Chem* 2001;**47**:1115–7

104 Ulbrichova-Douderova D, Martasek P. Detection of DNA variations in the polymorphic hydroxymethylbilane synthase gene by high-resolution melting analysis. *Analyt Biochem* 2009;**395**:41–8

105 Puy H, Deybach JC, Lamoril J, *et al.* Molecular epidemiology and diagnosis of PBG deaminase gene defects in acute intermittent porphyria. *Am J Hum Genet* 1997;**60**:1373–83

106 Robreau-Fraolini AM, Puy H, Aquaron C, *et al.* Porphobilinogen deaminase gene in African and Afro-Caribbean ethnic groups: mutations causing acute intermittent porphyria and specific intragenic polymorphisms. *Hum Genet* 2000;**107**:150–9

107 Magness ST, Tugores A, Christensen SR, *et al.* Deletion of the ferrochelatase gene in a patient with protoporphyria. *Hum Mol Genet* 1994;**3**:1695–7

108 Wood LH, Whatley SD, McKenna K, Badminton MN. Exonic deletions as a cause of erythropoietic protoporphyria. *Ann Clin Biochem* 2006;**43**:229–32

109 Whatley SD, Mason NG, Holme SA, Anstey AV, Elder GH, Badminton MN. Gene dosage analysis identifies large deletions of

the FECH gene in 10% of families with erythropoietic protoporphyria. *J Invest Dermatol* 2007;**127**:2790–4
110 Yau SC, Bobrow M, Mathew CG, Abbs SJ. Accurate diagnosis of carriers of deletions and duplications in Duchenne/Becker muscular dystrophy by fluorescent dosage analysis. *J Med Genet* 1996;**33**:550–8
111 Aurizi C, Schneider-Yin X, Sorge F, Macri A, Minder EI, Biolcati G. Heterogeneity of mutations in the ferrochelatase gene in Italian patients with erythropoietic protoporphyria. *Mol Genet Metab* 2007;**90**:402–7
112 Parker M, Corrigall AV, Hift RJ, Meissner PN. Molecular characterization of erythropoietic protoporphyria in South Africa. *Br J Dermatol* 2008;**159**:182–91
113 Spurdle AB, Couch FJ, Hogervorst FBL, Radice P, Sinilnikova OM, Group IUGVW. Prediction and assessment of splicing alterations: implications for clinical testing. *Hum Mutat* 2008;**29**:1304–13
114 Sykes RM. Acute intermittent porphyria, seizures, and antiepileptic drugs: a report on a 3-year-old Nigerian boy. *Seizure* 2001;**10**:64–6
115 Elder GH. Hepatic porphyrias in children. *J Inherit Met Dis* 1997;**20**:237–46

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