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REVIEW

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New insights into the pathogenesis of erythropoietic protoporphyria and their impact on patient care

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Abstract Erythropoietic protoporphyria (EPP, MIM 177000) is an inherited disorder caused by a partial deficiency of ferrochelatase (FECH) which catalyses the chelation of iron into protoporphyrin to form haem. The majority of EPP patients experience solely a painful photosensitivity whereas a small number of them develop liver complications due to the accumulation of excessive amount of protoporphyrin in the liver. EPP is considered to be an autosomal dominant disorder, however, with a low clinical penetrance. To date, a total of 65 different mutations have been identified in the FECH gene of EPP patients. Among the 89 EPP patients who carry a ''null allele'' mutation which results in the formation of a truncated protein, 18 of them developed EPP-related liver complications. None of the 16 missense mutations identified among 19 patients on the other hand, have been associated with liver disease ($P = 0.038$). The allelic constellation of an overt patient consists of a mutated FECH allele and a ''low expressed'' normal allele and that of an asymptomatic carrier, a combination of a mutated and a normally expressed FECH allele. The identification of the ''low expressed'' allele is facilitated by haplotype analysis using two single nucleotide polymorphisms, −251 A/G in the promoter region and IVS1−23C/T. At the current time when only partially effective therapies are available, the disclosures of both ''null allele'' and the ''low expression'' mechanisms will improve patient management.

Conclusion While covering the important clinical aspect of erythropoietic protoporphyria, this article emphasises the latest achievements in the molecular genetics of the disorder.

Key words Erythropoietic protoporphyria · Ferrochelatase · Mutation · Inheritance · Therapy

Abbreviations *EPP* erythropoietic protoporphyria · *FECH* ferrochelatase

Introduction

Erythropoietic protoporphyria (EPP, MIM 177000) is one of the inborn errors of porphyrin metabolism first described by Magnus et al. in 1961 [15]. The prevalence of EPP is estimated to range between 1:75,000 to 200,000 [32]. EPP is caused by a partial deficiency of ferrochelatase (FECH) (or haem synthase, EC 4.99.1.1), the terminal enzyme of the haem biosynthetic pathway which catalyses the insertion of iron into protoporphyrin

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IX to form haem [2, 4]. Since the publication of the cDNA sequence and the genomic structure of the human FECH gene, numerous mutations have been described underlining the molecular basis of EPP [23]. In most of the studied EPP cases, a single mutation affects only one FECH allele. The transmission of EPP follows an autosomal dominant trait, however, the disease has a low clinical penetrance with less than 10% of the mutation carriers developing clinical symptoms. Although the prognosis quo ad vitam is generally good, fatal liver complications occur in about 2% of patients [32].

Besides mutation studies, two questions concerning the pathogenesis of EPP have been the focus of the recent research. First, why only a small number of individuals with enzyme deficiency develop clinical symptoms while the majority of them remain asymptomatic. Second, why some overt EPP patients develop liver complications while others exhibit only symptom of photosensitivity. The latest results from molecular studies have unveiled fascinating new insights into the pathogenesis of EPP. Their impact on actual patient management will be the main focus of this article.

Clinical symptoms

The main clinical symptom of EPP is photosensitivity commencing in childhood. Patients complain of a severe burning pain in the skin, affecting mostly the face and hands after a short exposure to bright light. The painful attacks, which usually last several days, can be ameliorated by taking cold water baths or touching a cold metal surface. Visible skin change in the form of a discrete oedema and paleness can be seen in the light-exposed areas. In more severe attacks, the skin changes can be associated with small, eventually haemorrhagic vesicles; the face is so swollen that the eyes can barely be seen. During the latent period, the patients' skin tone appears extremely pale, incompatible with the pigmentation in their eyes or hairs. Thickening of the skin, especially over the knuckles can be observed in long-standing cases [4]. Female patients repeatedly describe a disappearance or at least alleviation of symptoms during pregnancy.

The clinical manifestations in EPP are directly related to the overproduction and accumulation of protoporphyrin as a result of FECH deficiency. Cutaneous photosensitivity is caused by accumulation of protoporphyrin in the skin. Upon excitation by light at 410 nm, protoporphyrin becomes phototoxic by generating reactive oxygen species that cause cell damage. As protoporphyrin is a strongly hydrophobic molecule, it is cleared exclusively by the liver and is secreted into the bile. The two major complications of EPP are the result of an insufficient elimination of the excessive amount of protoporphyrin from the body. Firstly, gallstone formation is fairly common in EPP and often occurs at an early age. Secondly, in less than 2% of EPP patients, a massive deposition of protoporphyrin in the liver tissue and bile canaliculi causes cholestatic liver damage that leads to rapidly progressive and life-threatening liver failure.

Diagnosis

Biochemical analysis

The diagnosis of overt EPP is based on the clinical symptoms mentioned above and a significantly increased (more than five times the normal level) concentration of free protoporphyrin in peripheral erythrocytes. Direct measurement of FECH activity in peripheral lymphocytes ensures the diagnosis of individuals with a defective enzyme, both symptomatic patients and asymptomatic carriers. Typically, FECH activity is reduced to 30%–40% of the normal level in patients and around 50% in asymptomatic carriers [8].

The comparably subtle objective signs in EPP renders diagnosis difficult. Among the 26 Swiss EPP patients that are currently under our care, the average time between the first clinical symptoms and the establishment of EPP diagnosis was 15.5 years (range: 0–74 years). Indeed, the reason for such delay in several older patients was due to the lack of knowledge on EPP at the time when they developed the symptoms, namely before the 1960s. However, even among the younger patients, a delay of several years in diagnosis is common. The extent of the delay among our patients apparently correlates to a certain degree with the protoporphyrin concentration in red cells in that the higher the protoporphyrin concentration, the earlier the diagnosis (unpublished data).

Mutation analysis

Information that is essential to the molecular diagnosis of EPP became available in the early 1990s. The human FECH gene contains 11 exons spanning over 45 kb. It is located on chromosome 18q21.3 [30, 40]. Two mRNAs, the housekeeping form and the erythroid specific form differing in their 3′-end, are transcribed from the same FECH gene. The mRNAs encode a FECH precursor of 423 amino acid residues which is processed into the mature enzyme of 369 amino acid residues based on the cDNA sequence published in 1990 [18].

The first report of two causative mutations in the FECH gene of an EPP patient was published in 1991 [13]. At the time of writing, 65 different mutations have been identified in 89 unrelated families and individuals with EPP, among whom a considerable number of mutations were described by our group (Table 1). The highly heterogeneous FECH mutations include virtually every existing type of mutation: point mutations (missense and nonsense), short nucleotide (less than 6 bp) deletions and insertions and exon deletions due to point mutations in the intron sequence of the FECH gene. In one case, the

Table 1 Mutations in the human FECH gene. (*DS* donor splice-site mutation, *AS* acceptor splice-site mutation, *del* deletion, *ins* insertion)

Exon	Mutation	Sequence modification	Relationship to liver disease	Reference
1	IVS1 + 5, g → a	DS (del exon 1)		[38]
	Del G40	Frameshift		[31]
2	IVS1−1, g → c	AS (del exon 2)		[22]
	G163T	G55C		[13]
	C175T	Q59X	Present/absent	[27, 23]
	Del AA168	Frameshift		[8]
	IVS2 + 11, a → g	DS (del exon 2)	Present	[1]
3	Ins A205	Frameshift		[37]
	T212A	I71K		[23]
	Ins T213	Frameshift	Absent/present	[37, 23]
	C286T	Q96X		[9]
	IVS3 + 2, t → g	DS (del exon 3)	Absent/present	[24, 1]
	IVS3 + 6, a → c	DS (del exon 3)	Present	[1]
4	Del A338	Frameshift		[5]
	C343T	R115X	Absent/present	[9, 23]
	Del ATCAA393	Frameshift		[5]
	Del A400	Frameshift		[37]
	T451C	S151P		[23]
	G463C	DS (del exon 4)		[36]
	IVS4 + 1, g → c	DS (del exon 4)		[23]
	IVS4 + 1, g → t	DS (del exon 4)		[38]
5	Del T488	Frameshift		[5]
	T557C	I186T		[10, 7]
	T571C	Y191H		[23]
	C574A	P192T		[23]
	Del TACAG580	Frameshift	Absent/present	[27, 23, 1]
	IVS5 + 1, g → t	DS (del exon 5)		[5]
6	Del G678	Frameshift		[37]
	IVS6 + 1, g → a	DS (del exon 6)		[38]
7	IVS6−2, del a → g	DS (del exon 7)		[5]
	Del T709	Frameshift	Present	[23]
	738del7ins37	Frameshift		[5]
	Del GAGAA751	Frameshift		[9]
	T790C	S263P		[8]
	G801A	M267I		[13]
	IVS7 + 1, g → a	DS (del exon 7)		[19]
	IVS7 + 5, g → a	DS (del exon 7)		[34]
8	Del C843	Frameshift		[7]
	C848T	T283I		[23]
	T863A	M288K		[23]
	Del TG899	Frameshift		[27, 23]
	G903A	W301X		[23]
9	IVS8−1, g → t	AS (del exon 9)		[36]
	IVS8−2, a → g	AS (del exon 9)		[23]
	Del AG970	Frameshift		[8]
	C1001T	P334L		[23]
	G1077 A	DS (del exon 9)		[38]
	IVS9 + 1, g → a	DS (del exon 9)	Present	[20]
	IVS9 + 2, t → a	DS (del exon 9)		[38]
10	IVS9−1, g → a	AS (del exon 10)		[8]
	IVS9−2, a → g	AS (del exon 10)		[23]
	T1088G	DS (del exon 10)	Present	[25]
	Del T1122	Frameshift		[9]
	A1135T	K379X	Absent/present	[33, 1]
	Del A1135	Frameshift	Present	[1]
	IVS10 + 1, g → t	DS (del exon 10)		[23]
	IVS10 + 1, del g	DS (del exon 10)		[6]
	IVS10 + 3, a → g	DS (del exon 10)	Present	[24]
11	A1157C	H386P		[7]
	G1217A	C406Y		[29]
	G1217C	C406S		[29]
	T1231B	C411G		[29]
	T1250C	F417S		[3]
	Del CTT1248	F417		[23]
Complete deletion of the FECH gene (del 18q)				[14]

entire FECH gene was absent in a patient as a result of a chromosome 18q deletion [14]. The molecular defects in the FECH gene are spread over the entire gene with no particular mutation "hot spots" observed so far.

In the majority of the studied cases, a single mutation was found in the FECH gene. There are only two known cases of the so called "compound heterozygosity" in which each FECH allele of the patients was affected by a deleterious mutation [13, 25]. Although most of the FECH mutations are family-specific, there are a number of reports on identical mutations being found among patients with the same or even different ethnic backgrounds. Mutation del TACAG580 has been identified in patients from France, Switzerland and the USA [1, 23, 28]. Point mutation T557C (I186T) was first reported in a Japanese and later in a Spanish patient [7, 10]. The efficiency of mutation analysis of the FECH gene has been improved significantly in the past few years due to the introduction of screening methods such as denaturing gradient and conformation-sensitive gel electrophoresis which enabled a systematic examination of large numbers of patients [5, 23]. The sensitivity of the denaturing gradient gel electrophoretic screening method in combination with sequencing is over 90% [23]. Mutation analysis of the FECH gene has an advantage over biochemical analysis in that it provides a precise diagnosis of the molecular defect causing EPP. Because of its complexity, however, molecular diagnoses of EPP are performed only in a few specialised research laboratories.

Inheritance

The mode of inheritance of EPP is mainly autosomal dominant with incomplete penetrance. An autosomal recessive trait has so far only been observed in two EPP cases as mentioned earlier. In the dominant type of EPP, different degrees of enzyme deficiency can be seen between patients and asymptomatic gene carriers, i.e. patients usually have less than 50% of normal activity whereas the carriers show approximately 50% of normal activity. This indicates that factors in addition to mutations are involved in the clinical manifestation of EPP. In an extensive family study published in 1984, Went and Klasen examined the parent-to-offspring transmission of the disease among 200 patients from 91 Dutch families [39]. The result showed that the overt disease of EPP occurs in approximately 25% of the siblings of the index patient which resembles an autosomal recessive disorder. A "three-allele" hypothesis was proposed by the authors to describe the inheritance of EPP.

Low expressed "normal" FECH allele in EPP patients

The "three-allele" hypothesis was first verified at the molecular basis by Gouya et al. in 1996 [6]. In the study, steady-state concentration of FECH mRNAs transcribed from each FECH allele were quantified in an EPP family in which the symptomatic EPP patient and asymptomatic carriers exhibited 25% and 50% residual FECH activity respectively. The result demonstrated that while both subjects carried the same FECH mutation, the patient and the asymptomatic carrier differed by their "normal" alleles in trans to the mutated allele. The mRNA output from the "normal" allele of the patient was around 50% lower than that of the carrier and controls. The mechanism for clinical expression of EPP in this family could therefore be explained at the molecular level by the co-inheritance of a gene defect from the biochemically abnormal parent and a "low-expressed" allele from the other apparently "normal" parent. In a recent publication, Gouya et al. [8] extended the study to additional EPP families and demonstrated that the low expression phenomenon is a general mechanism for the clinical expression of EPP (Fig. 1). While N depicts the functionally normal FECH gene allele, M represents the allele affected by a deleterious FECH gene mutation. Allele n denotes the "low expressed" FECH gene allele. The combination of NM results in a 50% FECH deficiency, however, the individual is asymptomatic, whereas Nn is silent. The combination of nM results in a less than 50% residual FECH activity and a symptomatic disease. No sequence abnormalities have so far been identified in the n alleles. The exact mechanism by which the "low expressed" allele operates is yet to be explored.

In the normal population, the predominant genotype is NN whereas genotype Nn appears much less frequently. Among 39 control subjects, mRNA quantification unveiled 9 individuals bearing one "low-expressed" FECH allele [8]. The frequency of the "low-expressed" FECH gene allele n in the French population was estimated to be 6%–11.5%. At such a high heterozygote frequency, about 1% of the population should be homozygote nn (not indicated in Fig. 1). Indeed, in their study, Gouya et al. [8] identified one homozygote for the low-expressed allele (nn) with decreased FECH activity who was a member of an EPP family, however, neither showing any clinical symptoms nor abnormal protoporphyrin excretion and devoid of the family-own FECH mutation. Since the "low-expressed" allele alone, even in the homozygous state, does not cause overt EPP, the inheritance of EPP does not fit to the classical autosomal recessive trait based on

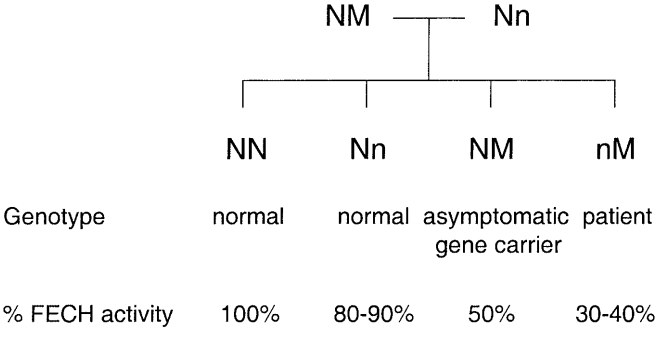

Fig. 1 A schematic presentation of the inheritance of EPP. (*N* normal FECH allele, *M* mutated FECH allele, *n* "low expressed" normal FECH allele. *NN*, *NM*, *Nn* and *nM* represent the two FECH alleles of individuals)

Mendelian rules. ''Autosomal dominant with a low clinical penetrance'' remains an appropriate denotation for the hereditary trait of EPP.

Intragenic markers for determining the risk of overt EPP

One of the most significant findings in the latest publication of Gouya et al. [8] was the identification of a common haplotype associated with the ''low-expressed'' FECH allele that enables an estimation of the risk of overt EPP among individuals carrying a FECH mutation. Six intragenic polymorphisms have been reported in the human FECH gene spreading from the 5′ promoter to the 3′ UTR [6, 23, 41]. Five were shown to have a relatively high heterozygosity in the general population: one dinucleotide repeat and four single nucleotide polymorphisms. Two single nucleotide polymorphisms namely, −251 A/G in the promoter region and IVS1−23C/T were in strong linkage disequilibrium [8]. These informative polymorphisms allowed two types of genetic studies: (1) the allelic segregation study among five EPP families demonstrated that the ''low expressed'' FECH allelic variant was systematically transmitted from the ''normal'' parent to the EPP patient and was associated with the 5′ haplotype −251G/IVS1−23T [8] and (2) the case control association study. Henriksson et al. [9] first observed that the −23T allele was more frequently expressed among EPP patients (total 82 alleles studied, $P = 0.01$) [9]. Gouya et al. [8] analysed the allelic distribution of five FECH intragenic polymorphisms in 39 EPP nuclear families. The subjects were divided into three groups: 39 EPP patients, 22 transmitter parents (i.e., the asymptomatic parents who carried a specific FECH mutation) and 16 normal parents. As shown in Table 2, the allelic frequencies of −251A/G and IVS1−23C/T in the patient group and the normal parent group are statistically significantly different from that in the transmitter parent group and the normal control group. This study confirmed the transmission of the −251G/IVS1−23T allele – the major 5′ haplotype associated with the ''low expressed'' allele, from the normal parents to their EPP children in a large group of EPP families. Based on the frequency of the ''low expressed'' alleles bearing haplotype −251G/IVS1–23T in a control population, the risk for a carrier of a FECH mutation to develop the disease was estimated to be 60% if the person bears the same haplotype. However, the risk decreases to only about 2% if the person bears the haplotype −251A/IVS1−23C, which is the most frequent one (75%) in the French population [8]. No evidence has so far indicated a direct involvement of −251G/IVS1–23T in the low expression of the FECH gene. They merely serve as markers for the ''low-expressed'' allele.

Association between ''null-allele'' mutation and liver complications

Despite their different locations, mutations identified among patients with liver complications have a common feature, namely they all lead to the formation of a shortened mRNA and as a result, to a truncated protein. The term ''null allele'' used by Cox [4] to describe the nonsense mutation can be extended to outline all mutations that lead to a truncated protein. Of the 65 known FECH mutations, 49 are ''null allele'' mutations. The remaining 16 mutations are the so called missense mutations that generate a functionally impaired, however, intact FECH by substitution of a single amino acid residue in the protein molecule (Table 1).

In order to study the association between ''null allele'' mutation and liver complications, we combined our data with those in the literature. A total of 108 EPP patients were counted among 92 EPP families and individuals (89 published cases and 3 unpublished cases [17]) with a known FECH mutation. As shown in Table 3, these 108 EPP patients were divided into four groups based on the type of mutation they carried (''null allele'' versus missense) and the clinical symptoms they presented (with versus without liver complication). Two interesting facts can be observed. First, all 18 EPP patients who had severe liver complications carried a ''null allele'' mutation (including two siblings of compound heterozygote carrying a ''null allele'' mutation on both FECH alleles [25]). Second, none of the 19 patients who carried a missense mutation (including one compound heterozygous case of two missense mutations [13]) had

Table 3 Association between null-allele mutation and liver complications

Mutation category	Liver complications in EPP patients	
	Without	With
Missense	19	0
''Null allele''[a]	71	18

[a] Including nonsense mutations, short nucleotide deletions and insertions, and exon deletions. Fisher's exact test, $P = 0.038$

Table 2 Allelic frequency at FECH polymorphic sites; a case-control association study

Polymorphic sites	Controls ($n = 70$)	Transmitter parents ($n = 22$)	Patients ($n = 39$)	Normal parents ($n = 16$)
−251 A/G ($P < 10^{-6}$)[a]	A	A	G	G
−23 C/T ($P < 10^{-6}$)[b]	C	C	T	T

[a] Promoter
[b] IVS1

developed liver complications up to the time of the study (Fisher's exact test, $P = 0.038$). These data indicate a significant genotype-phenotype correlation between the ''null allele'' mutation and protoporphyrin-related liver disease in EPP. Although the risk for a EPP patient with a missense mutation to develop liver disease cannot be totally eliminated based on these data, it is comparably low.

Therapy

Therapeutic measures are limited to EPP. Beta-carotene is often prescribed to ease the acute photosensitivity. Beta-carotene reduces the damage of protoporphyrin in the skin by acting as a free radical quenching agent and increases the skin coloration. However, the effect of β-carotene is highly variable among EPP patients. Many patients reject the therapy because of the poor effect and the undesired skin coloration [11]. Application of reflective sunscreen preparations, a more preferred treatment, remains the most important measure in managing acute photosensitivity. Normal sunscreens, even those with high UV absorption, do not block the visible light and therefore have no protective effect. The light tolerance is different from patient to patient, whereby those with higher levels of protoporphyrin in the erythrocytes tend to tolerate less. Most of our adult patients have learned over the years to cope with the disease by adjusting their life-styles without any medication and are well integrated into society.

Liver transplantation, although it does not correct the metabolic error, is the only choice of therapy for patients with terminal liver failure and was successfully conducted in a number of patients [1, 26]. Caution must be taken, however, in preventing photodamage of the exposed area by surgical lights during the operation. Liver function of all EPP patients should be tested regularly since the majority of them carry a ''null-allele'' mutation as discussed earlier; the number of patients with missense mutation is still small and their observation period is rather limited so that their remaining risk to develop liver failure cannot be estimated at this time. Bone marrow transplantation (including the red cell line) could theoretically eliminate the protoporphyrin over-production. So far, there is only one report of allogenic bone marrow transplantation in a patient who suffered from a severe aplastic anaemia as a result of a combination of both viral hepatitis and EPP [12]. In vitro gene transfer using human FECH cDNA was able to correct the enzyme deficiency in cultured fibroblasts from an EPP patient [16]. In a recent study, Pawliuk et al. successfully conducted gene transfer into haematopoietic stem cells and achieved a long-term correction of skin photosensitivity in a mouse EPP model [21]. Despite the differences between murine EPP and the human disease, gene therapy in the mouse model has set the stage for human studies in the future.

Since EPP is a rare disorder, we hope this review will raise the attention among paediatricians to the early recognition and the correct diagnosis of the disease. At present, when no effective therapy can cure or even alleviate the symptoms of EPP patients, genetic counselling plays an important role in management. Once the information regarding the causal mutation and the FECH haplotype is available by way of molecular analysis, the relative risk for an individual to develop an overt disease as well as the prognosis with respect to liver disease can be estimated.

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