Erythropoietic protoporphyria A short review

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SUMMARY

Erythropoietic protoporphyria (EPP) is caused by the accumulation of protoporphyrin in the red blood cells and is characterized by extreme photosensitivity. Large scale clinical investigations of EPP patients became possible in the mid 60s, when relatively simple spectrophotometric laboratory tests for porhyrins in biological material were introduced. Patients with EPP have been studied in most European countries and in the USA. It was considered to be an autosomal dominant hereditary disorder, but research in the last few years has shown that two alleles are involved: one mutated allele and a second wild-type allele that is only partially active. In symptomatic patients the level of enzyme activity is 10-30% of the normal, and latent carriers characteristically display around 50%.
Common absorbent sunscreens offer very little protection compared to reflectant preparations, and particularly those that contain very small particles of TiO_2 and ZnO.

KEY WORDS

erythropoietic protoporphyria, review, clinical, manifestations, laboratory tests, molecular biology, treatment

History

The first unequivocal clinical report on erythropoietic protoporphyria (EPP – OMIM 177000) appeared in 1953 by Kosenow and Treibs who described one patient (1). Two further reports were published independently in 1961: Langhof et al (2) described multiple cases in one family. The first well-known paper was published by Magnus(3) who was the first to coin the current diagnosis *erythropoietic protoporphyria*. In the following years larger numbers of patients were described in Europe: Haeger Aronsen and Krook in Sweden (4), Suurmond in Holland (5) , Schmidt in Denmark (6), Heilmeyer and Clotten in Germany (7) and Mirzoeva in Russia (8). Reports from the USA were by Peterka (9), Harber (10), Redeker and Bronow (11), Reed (12) and De Leo (13). The first report from Slovenia was in 1972 by Kansky (14) and the next by Kansky and Berčič (15). The common conclusion of these studies was that EPP was a hereditary autosomal dominant disease.

EPP is the commonest erythropoietic porphyria and occurs throughout the world with no racial predilection. Prevalence in UK is estimated at 1 in 130 000 and in Northern Ireland at 1 in 79 000.

Clinical features

The most prominent symptom is an extreme photosensitivity, and in principle, acute and chronic lesions can be observed.

Acute symptoms may appear shortly after exposure to UV radiation, within a period ranging from 15 minutes to a few hours. The first symptoms are distressing sensations such as itching, pricking and pain in the exposed skin areas. The involved skin becomes edematous and reddened, and small vesicles, oozing and small crusts may appear. Within a few days purpuric spots (petecchiae) may be observed. Sometimes urticaria-like lesions may occur. These lesions may be seen even in infants. Figure 1.

Chronic lesions develop gradually on exposed areas, mostly on the cheeks, the forehead, the nose and on the backs of the hands. Usually they already occur in early childhood, but they may become milder or even disappear after the patients become conscious of the deleterious effects of sun radiation. The skin appears lichenified due to deposits of an amorphous substance (hyaline) in the upper dermis and around the superficial blood vessels. Tiny scars and small grooves are expressed (skin texture resembles orange peel). On the lips creases are ordered radially while verruciform lesions may be seen on the back of the hands. Figures 2 and 3.

Histopathology. Usually a mild hyperkeratosis and an irregular acanthosis are expressed. In the papillary dermis deposits of amorphous PAS positive material are seen around the capillaries and under their basal lamina. This material, which consists of glycoproteins also surrounds the arterioles and sweat ducts.

Inheritance

Those authors that described larger groups of EPP patients tended to emphasize the autosomal dominant mode of inheritance with incomplete penetrance (4-12). A more careful clinical analysis of larger groups of patients has, however, cast some doubt on this conclusion, because the direct transmission of EPP across two or three generations has been observed rather rarely. Additional investigations, despite in principle sanctioning the autosomal dominant mode of inheritance, have demonstrated that the mechanism of inheritance is rather complex. The presence of fluorescing erythrocytes in the blood has always been considered a reliable marker for EPP. It has however been postulated that for the clinical expression of EPP the coinheritance of two alleles is necessary: namely, both a mutated allele and a low-expressed (or less active) wild-type allele whose activity is less than 50% of normal levels.. A wild-type allele whose activity exceeds 50% of the normal value, will compensate for the mutated one and such patients will be symptomless carriers (16,17,18).

An autosomal recessive heredity is assumed in certain EPP cases with severe involvement of the liver (19,20).Went and Klasen also expressed the hypothesis that the presence of an additional factor (a third allele) is necessary for clinical expression of EPP (16,17).

The latest investigations have confirmed that EPP is genetically very heterogeneous. In the majority of families co-inheritance of a mutant ferrochelatase allele from one parent and a low-output "normal" ferrochelatase allele from the other parent is required for expression of the disease. The molecular basis of protoporphyric hepatic failure has not yet been resolved. The important mechanism of the low expression of FECH was revealed by Gouya et al (17,21). The presence of a C at position IVS3-48 was shown to cause a 40% aberrantly spliced mRNA, compared with just 20% for the T allele. The reduced level of FECH was due to degradation of the aberrantly-spliced mRNA by the mechanism of nonsense-mediated mRNA decay. Individuals who were homozygous for C showed the lowest FECH activity.

In 9 Swedish families with EPP, 4 novel and 2 previously reported FECH mutations were detected. They found that all individuals carrying a mutated allele and IVS3-48C in trans position to each other were affected by overt EPP (22).

Laboratory investigations

The most relevant laboratory test for diagnosing EPP is the *quantitative assessment* of *protoporphyrin (PP)* in *erythrocytes* (RBCs). Analytic methods for the assessment of PP in erythrocytes have been reported since 1930, such us Vigliani and Waldenström (23), but they were rather complicated. A new impetus for clinicians was given by the introduction of the relatively simple spectrophotometric method by Rimington (24). It included:

- a collection of RBCs from peripheral blood treated with anti-coagulants;
- repeated extractions;
- spectrophotometric readings at three wavelengths of 405, 400.5 and 380 nm.

Normal values depend on the method applied, mostly up to 534 moles/l packed RBCs (30 μg/100 ml packed RBCs). Analogue coproporphyrin values are up

to 24.5 moles/l RBCs (1.6 μg/ 100 ml packed RBCs). In EPP patients the PP values are significantly increased, up to one hundred times. Moderately increased PP values can be found in sideropenic or sideroachrestic anemia, persons exposed to or intoxicated with lead, pellagroid and further conditions (15). The newer methods have been, essentially, developed from Rimington's spectrophotometric test (25).

An interesting observation is that a part of the RBCs in the peripheral blood display a transient bright red fluorescence on investigation with a fluorescent microscope. The test which is only semi-quantitative due to the short duration of fluorescence was already used by Treibs and Kosenow (1), but is suitable for the detection of new cases and especially for the identification of symptomless. For a detailed description of this procedure, see Rimington and Cripps (26,27). In this author's own experience up to 23,5% of RBCs may fluoresce (25). The results must be handled cautiously however, as the test is not specific and fluorocytes may be present in peripheral blood due to other unrelated reasons such as intoxication with lead, in sideropenic anemia or pellagra.

Even when the values of hemoglobin and of RBCs are somewhat low, true anemia remains rare.

After EPP was defined as a separate nosologic entity, and believed to be a pure skin condition, a severe involvement of the liver ending eventually in cirrhosis has been observed in certain cases. (28,29)

Studies of patients supected of having porphyria should include several steps:

- clinical evaluation,
- biochemical study to confirm the diagnosis,
- an enzymatic test is suggested,
- genetic studies to detect the causal genetic mutation are recommended.

Molecular biology

In a study of 112 EPP patients with a known FECH mutation, no correlation was found between protoporphyrin blood level and the type of mutation if patients with overt liver disease were excluded from the sample. Furthermore, no significant association of the liver complication with the location of the mutation within the FECH gene was found. There remains, however, a risk that an EPP patient with a missense mutation will subsequently develop liver disease, if not respecting the guide lines. According to Minder (30) 2% of EPP sufferers develop a liver involvement that can lead to cirrhosis or liver failure.

A short review of porphyrin metabolism is presented in figure 4. The increase of PP in RBCs is due to the deficient activity of the enzyme *ferrochelatase (FC, hemsynthase)*, which catalyses the incorporation of the ferrous ion into the protoporphyrin IX molecule. The enzyme FC is located on the inner membrane of the mitochondria. The FC gene is on chromosome 18q21.3 and has a size of about 45 kb, including 11 exons ranging in size from 108 to 1293 bp, and 10 introns (17,31). The gene for FC has been cloned, sequenced and mapped to the long arm of chromosome 18 by Nakahashi in 1990 (32) and Taketani in 1992 (33).

By the year 2000 already 65 different FC mutations had been reported in medical literature: missens mutations, nucleotide deletion, nonsense mutations; splicing and exon skipping. In Swiss EPP patients the following - mutations were reported: point mutations G1217A (cystin to tyrosin) and G1217C Q59X, nucleotide deletion TACAG 580-84 (also detected in patients from France and the USA) and TG 899-900 or insertion T 213 (34,35). Patients with liver complications share the common feature ("null allele mutation") which results in the formation of a truncated protein. None of the 16 missens mutations have been associated liver disease (30).

Human FC is a homodimeric (85 kDa) mitochondrial membrane associated enzyme that catalyzes the insertion of the ferrous ion into protoporphyrin to form heme. The enzyme contains two NO-sensitive and uniquely coordinated [2 Fe - 2S] clusters (31), (21,22, 36).

Treatment

The majority of EPP patients do not experience, except for the photosensitivity, any major discomforts. They are, however, strongly advised to avoid substances which may interfere with the porphyrin metabolism. Among the so called *porphyrinogenic substances* that deserve to be mentioned are alcohol, barbiturates and other hypnotics, estrogens including contraceptives, analgetics, and synthetic antimalarials.

The major emphasis must be placed upon *local photoprotection*. Porphyrin phototoxicity in EPP is triggered mainly by UVA radiation. Unfortunately almost all commercially available sunscreens offer strong protection against UVB, but fail to provide sufficient protection against UVA. Among the few UVA competent *absorbents* (filter substances) that deserve to be mentioned are *dibenzoylmethanes* (methoxydibenzoylmethane) and *terephtalidene dicamphor sulfonic acid* and their derivatives.

The most efficient photoprotectives are mineral oxides that contain reflecting preparations *(reflectants)*, and particularly the recently introduced micro TiO_2 and the micro fine ZnO. Non-micronized TiO_2 and ZnO, although efficient, are cosmetically less acceptable as they cover the skin with white layers; in contrast, the micronized substances are not visible and cosmetically acceptable (37,38).

Good protection is also offered by combined prepa-

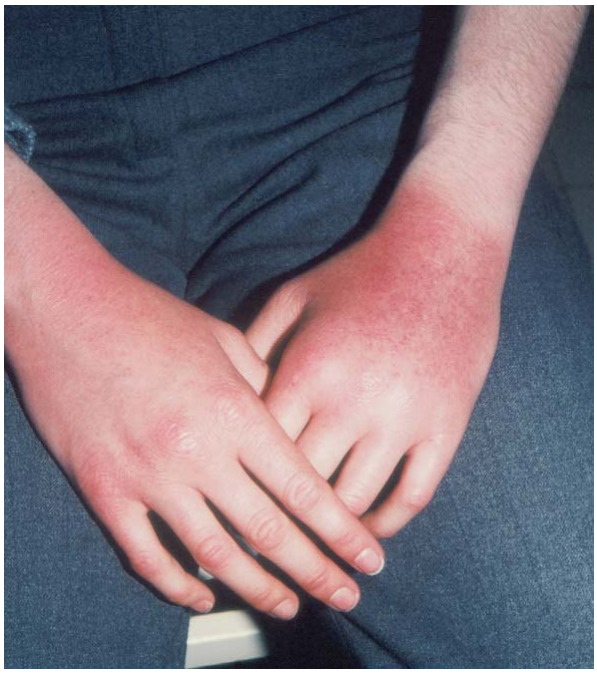
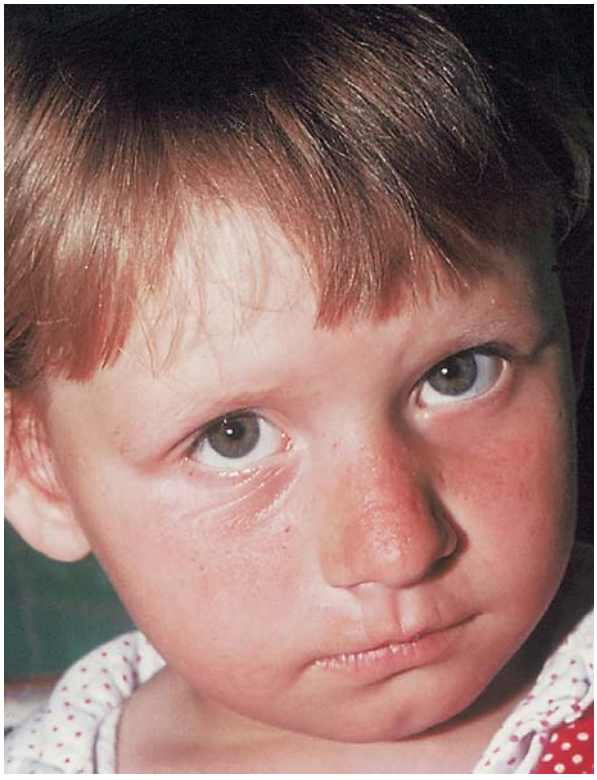


Figure 1. Acute symptoms: on the exposed parts the skin is erythematous and edematous.

Figure 2. Chronic and partially subacute lesions: the skin is lichenified, hyper pigmented and also reddened. On the exposed skin tiny grooves and scars are visible.



rations that contain UVA absorbents as well as mineral reflectants.

Systemic photoprotection has not yet proved worthy of the hope invested in it. It has been claimed that *Beta carotene*, a precursor of vitamin A, provides protection when taken in a dose up to 200 mg daily; but in our experience it is of limited value and does not protect manifest EPP patients. *Cystein 500 mg* taken twice daily has a similar effect (39).

Gene therapy may hold the best prospects for future treatments. Encouraging experiments were made in a mouse model in which a self-inactivating lentiviral vector containing human FC cDNA was introduced (40).

Conclusion

The great majority of EPP patients have only photosensitivity problems; and a further 2-5% of them may experience involvement with the liver. The reflectants, especially the micronized TiO_2 and ZnO offer the best protection aganst UV rays. Systemic treatment with photoprotectives like beta carotenes are of limited value. In order to avoid possible liver complications, substances that can interfere with the porphyrin metabolism such as barbiturates, estrogens, lead, alcohol and others have to be strictly avoided.

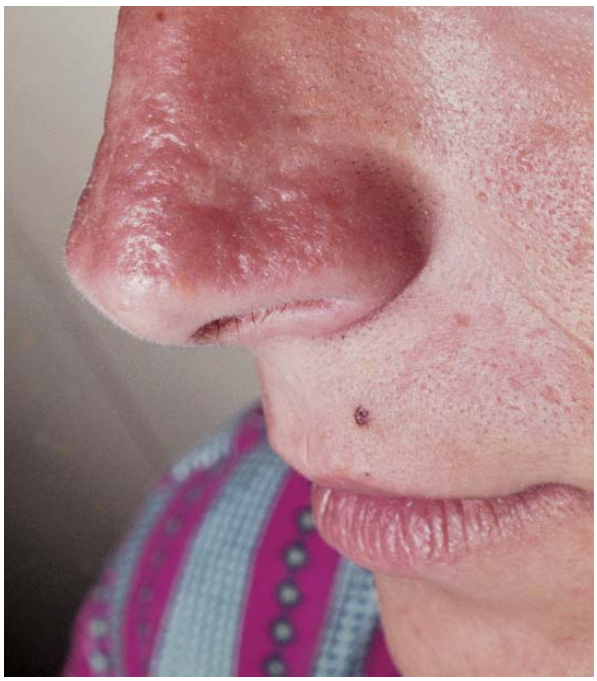


Figure 3. Typical chronic lesions: lichenification and hyperpigmentation, small grooves or scars (orange peel-like apperance), radial creases on the lower lip.

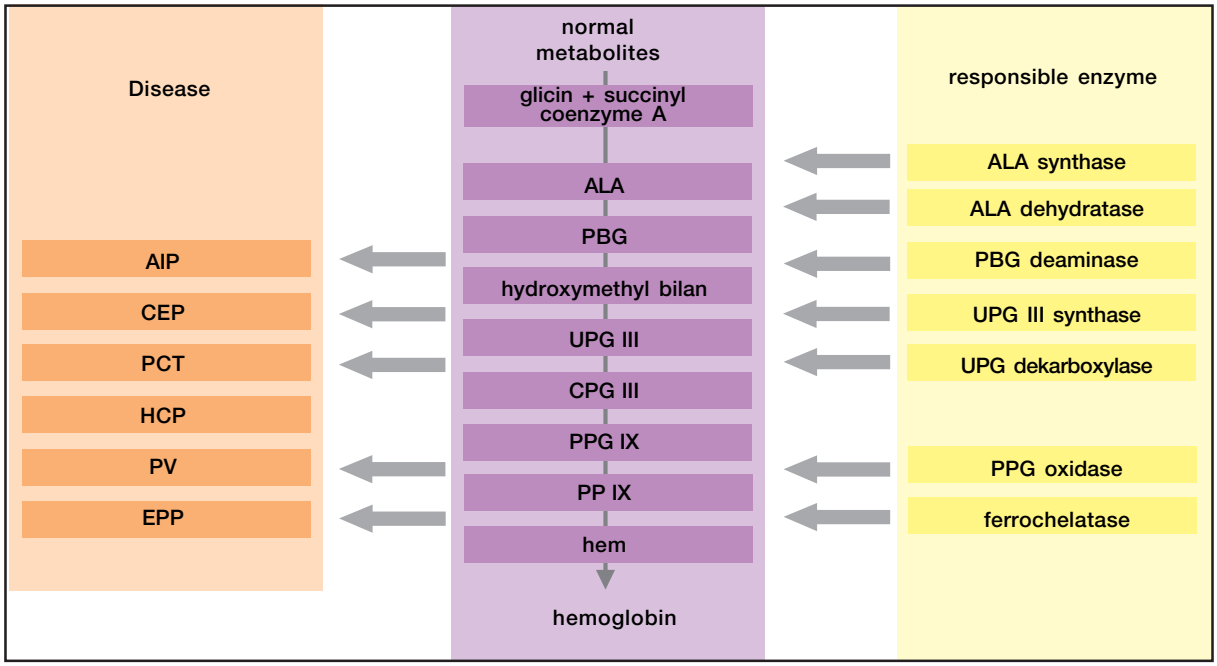


Table 1. A symplified presentation of heme synthesis and pahogenesis of porphyrias
Legend:
ALA - delta aminolevulinic acid, AIP - acute intermittent porphyria, CEP – congenital erythropoietic porphyria, CPG III – coproporphyrinogen III, EPP - erythropoietic protoporphyria, HCP – hepatic coproporphyria, PBG – porphobilinogen, PCT – porphyria cutanea tarda, PP – protoporphyrin, PPG – protoporphyrinogen, PV – porphyria variegata, UPG – uroporphyrinogen

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