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ERYTHROPOIETIC PROTOPORPHYRIA

CLINICAL AND EPIDEMIOLOGICAL ASPECTS

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SUMMARY

Erythropoietic protoporphyria (EPP, OMIM 177000) is an inherited disease in which mutations in the gene encoding the last enzyme in heme biosynthesis, ferrochelatase, results in deficient enzyme activity. The characteristic biochemical abnormality in EPP is accumulation of protoporphyrin, the substrate for ferrochelatase. The main clinical manifestation is painful cutaneous sensitivity to intense light which is caused by the photoactive properties of protoporphyrin. A minority of EPP patients develop liver disease which in some progress to a serious condition which requires liver transplantation for survival.

The work with this thesis started with questions that originated in the management of an EPP patient who developed cholestatic liver failure. The ideas that motivated the five studies all aimed to answer some of these questions and advance the perceived limitations in knowledge that surrounded decisions that were made in the management of this patient. This first contact with an EPP patient also stimulated a survey of the whole Swedish EPP population.

Study I describes the reversal of EPP-related liver failure in an EPP patient. It details a novel concept for treating liver failure by combining several previously suggested treatments. These treatment modalities and their modes of action are compiled in a comprehensive literature review. The reversal of liver disease presented a unique opportunity to attempt curative hematopoietic stem cell transplantation (HSCT) which had previously never been attempted in this context. The successful HSCT strengthened the concept that the accumulated protoporphyrin in EPP originates in the erythroid tissue.

Study II is a follow-up to study I. It confirms that HSCT indeed leads to long-standing genotypic correction in the erythroid tissues and that this is enough to prevent the phenotypic manifestations. The EPP genotype that is still present in cultured fibroblasts from a skin biopsy does not cause photosensitivity. We discuss differences between EPP mouse models and human EPP and conclude that some of the findings in transplantation studies on EPP mice models can not be applied to human EPP.

Study III explores the risk for and the protection from phototoxic injuries caused by surgical luminaires. Several publications had reported burn injuries from the lights used during liver transplantation in EPP patients, in some despite the use of shielding light filters. In this study we explored: 1) the risk for burn injuries in non-transplant surgery, 2) the characteristics of optimal filters for use during liver transplant surgery, and 3) different models to study light induced phototoxicity *in vitro*. We collected data on the experience from all types of surgery among Swedish EPP patients, the characteristics of the light emitted during medical interventions that require special light-sources, the characteristics of various light filters and asked surgeons to assess the safety and visibility while working in different types of filtered light.

We concluded that: 1) surgical light is harmless for EPP patients that are not in cholestatic liver failure and undergoing liver transplantation, 2) a yellow filter which blocks wavelengths below 470 nm seems optimal for use during liver transplantation and that 3) an *in vitro* erythrocyte assay was a good model for studying phototoxicity.

Study IV is a comprehensive review of the Swedish EPP cohort including demographic, clinical, genetic and biochemical features. An extensive questionnaire, genotyping and testing of a large number of biochemical parameters were used to collect data. In 2008 the prevalence in Sweden was 1:180,000. The ferrochelatase mutation was established in all but one and nine novel mutations were found. Women had a lower erythrocyte protoporphyrin concentration than men and tended to have a higher vitamin D concentration. Alterations in red blood cell parameters, iron parameters and liver function tests were common and 20% had previously been diagnosed with gall stone disease. Life quality was markedly reduced by the disease and available treatments for phototoxicity had poor efficacy for most. The most common reported age at onset of symptoms was the first year of life, yet the mean age at diagnosis was 22 years. This conspicuous diagnostic delay had worsened in the 21st century.

Study V is a retrospective study including all EPP patients that have been liver transplanted in Europe. Thirty-seven transplants in 33 patients were identified in a literature search and a search of the European liver transplant registry. We compiled the overall outcome, the frequency of EPP specific complications such as disease recurrence in the graft, phototoxic burn injuries and postoperative neuropathy, any EPP specific treatments that had been used preoperatively and background characteristics including EPP genotype and erythrocyte protoporphyrin concentrations. This study is the largest compilation of transplanted EPP patients to date. The overall survival was 80 percent at one year and 66 percent at five and ten years. Recurrent EPP-related graft disease was common but did not seem to affect long-term survival. None of the patients that had been protected by light filters were burnt, in contrast to 20 % of the unprotected patients. Fifteen percent had prolonged postoperative motor neuropathy. The limited data on ferrochelatase mutations and protoporphyrin concentrations did not permit any hypothesis about risk factors for developing severe liver disease in EPP.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers which are referred to in the text by their Roman numerical:

I.   **Curative bone marrow transplantation in erythropoietic protoporphyria after reversal of severe cholestasis.**
     Wahlin S, Aschan J, Bjornstedt M, Broome U, Harper P.
     J Hepatol. 2007 Jan;46(1):174-9.

II. **Skin Ferrochelatase and Photosensitivity in Mice and Man.**
     Wahlin S, Harper P.
     J Invest Dermatol. 2010 Feb;130(2):631-3.

III. **Protection from phototoxic injury during surgery and endoscopy in erythropoietic protoporphyria.**
     Wahlin S, Srikanthan N, Hamre B, Harper P, Brun A.
     Liver Transpl. 2008 Sep;14(9):1340-6.

IV   **Erythropoietic Protoporphyria in Sweden: Demographic, clinical, genetic and biochemical features.**
     Wahlin S, Floderus Y, Stål P, Harper P.
     Manuscript under review, 2010

V   **Liver transplantation for erythropoietic protoporphyria in Europe.**
     Wahlin S, Stål P, Adam R, Porte RJ, Samuel D, Seehofer D, Gunson B, Hillingsø J, Klempnauer JL, Schmidt J, Clavien PA, Alexander G, Salizzoni M, Paul A, Ericzon BG, Harper P.
     Manuscript. 2010.

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LIST OF ABBREVIATIONS

ADP	ALA-dehydratase deficiency porphyria
AIP	Acute intermittent porphyria
ALA	5-Aminolevulinic acid
ALAS	5-Aminolevulinic acid synthase
ALAS1	The ubiquitous form of ALAS
ALAS2	The erythroid form of ALAS
BMT	Bone marrow transplantation
CDCA	Chenodeoxycholic acid
DMSO	Dimethyl sulfoxide
CEP	Congenital erythropoietic porphyria
CYP	Cytochrome
ELITA	the European Liver and Intestine Transplant Association
ELTR	the European Liver Transplantation Registry
EPP	Erythropoietic protoporphyria
FECH	Ferrochelatase
FEP	Free erythrocyte porphyrin
HCP	Hereditary coproporphyria
HEP	Hepatoerythropoietic porphyria
HGVS	Human Genome Variation Society
HMB	Hydroxymethylbilane
HO-1	Heme oxygenase 1
HSCT	Hematopoietic stem cell transplantation
INR	International Normalized Ratio
LDL	Low density lipoprotein
LT	Liver transplantation
MELD	Model for End-stage Liver Disease
OMIM	Online Mendelian Inheritance in Man
OR	Operating room
PBG	Porphobilinogen
PCT	Porphyria cutanea tarda
PPIX	Protoporphyrin IX
UDCA	Ursodeoxycholic acid
VP	Variegate porphyria
UVA	Ultraviolet A
UVB	Ultraviolet B
XLDPP	X-linked dominant protoporphyria

CONTENTS

1 INTRODUCTION

1.1 THE PORPHYRIAS

The human porphyrias are inherited metabolic diseases that result from partial deficiencies in the activities of enzymes involved in the heme biosynthetic pathway (*fig. 1*). A specific type of porphyria has been ascribed to deficiencies in all enzymes except for the ubiquitous form of the initial enzyme, ALA synthase 1 (ALAS1) [1]. No disease related mutations have been described for ALAS1, which is found in all tissues but is most active in the liver. Mutations in the X-linked erythroid-specific form of ALA synthase (ALAS2) have been associated with sideroblastic anemia. Recently, gain-of-function mutations that enhance ALAS2 activity were identified [2]. These lead to a condition termed X-linked dominant protoporphyria (XLDPP) which has a phenotype similar to that of erythropoietic protoporphyria.

In each porphyria, the enzyme deficiency gives rise to a characteristic accumulation of heme precursors that cause the particular symptoms associated with that porphyria. The increase in precursor concentration and excretion also enables an accurate diagnosis based on heme precursor measurement in erythrocytes, plasma, urine or feces [3].

The chromosomal locations and genomic sequences have been established for each of the genes coding for the enzymes in the heme biosynthetic pathway and numerous disease related mutations have been identified for each of the porphyrias.

The porphyrias are often grouped into hepatic or erythropoietic. Most of the porphyrias are classified as hepatic; in which excess production and accumulation of intermediates occur in the liver, while three of the porphyrias are classified as erythropoietic (*table 1*). Clinically, the porphyrias are divided into acute or cutaneous depending on whether they primarily present with acute neurovisceral symptoms or cutaneous photosensitivity (*table 1*). Mixed porphyrias can present with both acute and cutaneous symptoms.
According to the pattern of inheritance, the porphyrias are grouped into autosomal dominant or autosomal recessive disorders with the exception of XLDPP. The label for inheritance pattern is not fully applicable for EPP. In the majority of patients, EPP is inherited in what has been called a pseudo-dominant fashion [4]. This is further discussed below.
The five autosomal dominant porphyrias (*table 1*) have low clinical penetrance, and symptoms are often triggered by specific endogenous or environmental porphyrogenic factors. Residual enzyme activity is usually enough to satisfy the physiologic heme demands [5]. The recessive conditions exhibit very low residual enzyme activity and high clinical penetrance, often with phenotypic manifestations from early childhood [5]. The same is true for the extremely rare homozygous or compound heterozygous variants of AIP, HCP, and VP.
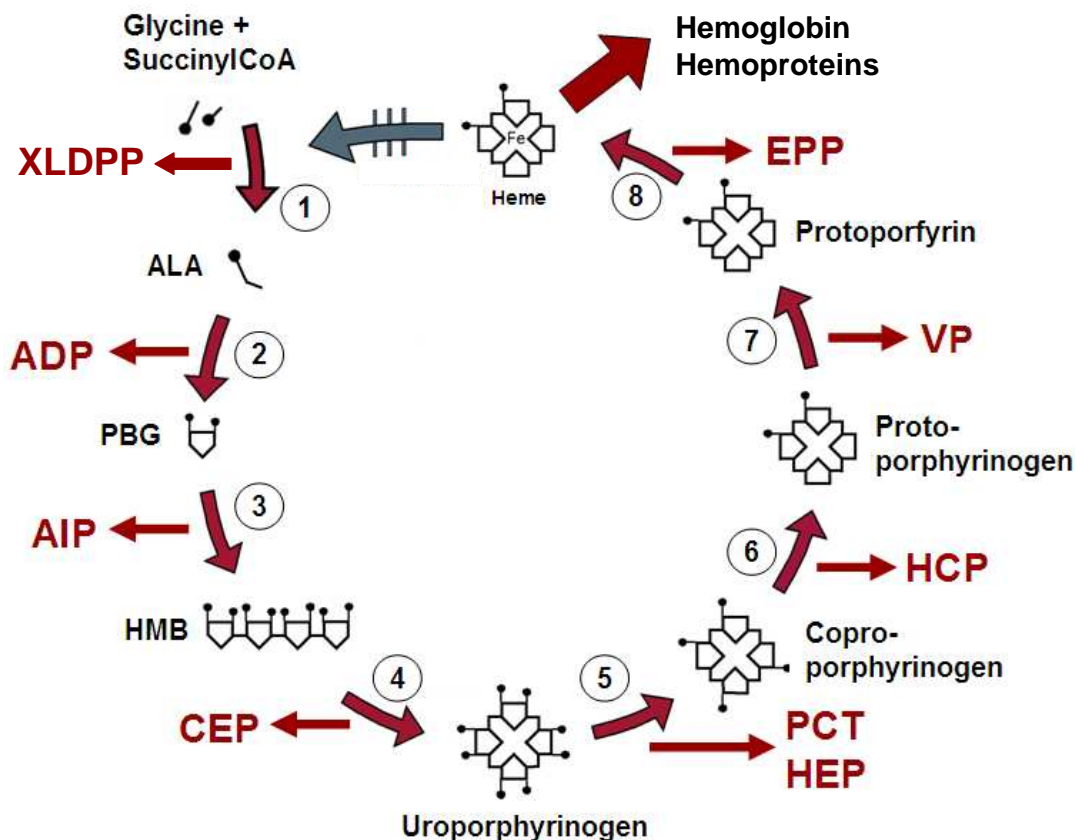
Figure 1

The heme biosynthesis and its associated diseases; the porphyrias.

A specific type of porphyria has been ascribed to deficiencies in all enzymes except for the ubiquitous form of first enzyme in the pathway, ALAS1. Mutations in the erythroid form, ALAS2, may lead to sideroblastic anemia or to the recently described new form of porphyria, XLDPP. If the function of an enzyme is deficient (e.g. number 8, ferrochelatase), the metabolite up-stream from the enzyme (e.g. protoporphyrin) is accumulated.

Enzyme names:

①; ALA synthase, ②; ALA dehydratase, ③; HMB synthase (also known as PBG deaminase), ④; Uroporphyrinogen III synthase, ⑤; Uroporphyrinogen decarboxylase, ⑥; Coproporphyrinogen oxidase, ⑦; protoporphyrinogen oxidase, ⑧; Ferrochelatase

Abbreviations:

XLDPP; X-linked dominant protoporphyria, ADP; ALA dehydratase deficiency pophyria, AIP; acute intermittent porphyria, CEP; congenital erythropoietic porphyria, PCT; porphyria cutanea tarda, HEP; hepatoerythropoietic porphyria (homozygous PCT), HCP; hereditary coproporphyria, VP; variegate porphyria, EPP; erythropoietic protoporphyria, ALA; 5-aminolevulinic acid, PBG; porphobilinogen, HMB; hydroxymethylbilane

		Prevalence	Inheritance pattern	Clinical features
Hepatic	ADP	1 case	Autos. recessive	Acute neurovisceral
	AIP	1:10,000	Autos. dominant	Acute neurovisceral
	VP	1:130,000	Autos. dominant	Acute neurov. & cutaneous
	HCP	1:180,000	Autos. dominant	Acute neurov. & cutaneous
	PCT	1:10,000	Autos. dominant*	Cutaneous
Erythropoietic	EPP	1:170,000	Pseudodominant	Cutaneous
	CEP	2 cases	Autos. recessive	Cutaneous
	XLDPP	None	X-linked dominant	Cutaneous
Hepatoerythropoietic	HEP	1 case	Autos. recessive	Cutaneous

* >70% sporadic, not associated with uroporphyrinogen decarboxylase gene defect

Table 1
Approximate prevalences of the porphyrias in Sweden 2010 and categorization according to the organ where most of the metabolite overproduction takes place, the inheritance pattern and main clinical features; acute neurovisceral or cutaneous symptoms

1.1.1 The heme biosynthetic pathway

Heme biosynthesis involves eight enzymes that sequentially convert glycine and succinyl coenzyme A into heme (*fig.1*). The first and the final three enzymes are situated in the mitochondria and the remaining in the cytosol. The heme biosynthetic pathway includes four basic processes: formation of the pyrrole, assembly of the tetrapyrrole, modification of the tetrapyrrole side-chains followed by oxidation of protoporphyrinogen IX to protoporphyrin IX and insertion of a single ferrous iron to form heme. This final step, the insertion of iron into the protoporphyrin molecule, is catalyzed by the enzyme ferrochelatase. Deficient ferrochelatase function causes an accumulation of protoporphyrin, the biochemical hallmark of EPP.

Heme serves as a prosthetic group for hemoproteins involved in an array of crucial biological functions [1]. In humans, at least 80 percent of the daily heme synthesis takes place in the bone marrow [6, 7] for the formation of hemoglobin, the transporter of oxygen. Around 15 percent takes place in the liver for the formation of microsomal cytochromes and many other essential enzymes [8]. Heme is also involved in the transfer of electrons in the cytochromes of the respiratory chain and serves as prosthetic group of hemoproteins that synthesize regulatory and signaling molecules, including cyclic guanosine monophosphate and nitric oxide.

1.1.2 Regulation of the heme biosynthetic pathway

The cellular heme concentration is tightly controlled by a fine balance between heme biosynthesis, the demands for heme protein synthesis and the catabolism of heme by heme oxygenase. Heme requirements vary considerably among different cells and

tissues. The four initial enzymes in the heme biosynthesis pathway are tissue-specific enzymes that are regulated differently, mainly by tissue-specific genes or by single genes with tissue-specific promotor regions [1, 9]. Separate genes encode the erythroid and the hepatic ALAS isozymes. While the hepatic (ubiquitous) ALAS1 gene has been assigned to chromosome 3p21.1, the erythroid-specific ALAS2 gene is situated in a distal subregion of Xp11.21 [10, 11].

In non-erythroid cells, the rate of heme biosynthesis depends on the activity of ALAS1 but in erythroid cells it is determined by the availability of iron for ferrochelatase.
In hepatocytes, heme biosynthesis is controlled by the intracellular heme pool via feed-back inhibition of ALAS1 and is mainly regulated by the demand for heme as prosthetic group for cytochrome P450. The heme dependent mechanisms controlling ALAS1 activity include increased degradation of ALAS1 mRNA, inhibition of the translocation of cytosolic ALAS1 pro-enzyme into the mitochondria and repression of ALAS1 transcription [9, 12-16].

In contrast, heme does not inhibit ALAS2 activity but does inhibit cellular iron acquisition from transferrin in erythroid cells [17]. Several erythroid-specific factors interact in the regulation of ALAS2 gene expression, such as iron-related elements, erythropoietin and the oxygen tension [18]. The response to stimuli for heme biosynthesis occurs during cell differentiation. Several regulatory mechanisms allow for the production of the very large amounts of heme needed for hemoglobin synthesis. Unlike the liver, the stimulation of heme biosynthesis in erythroid cells is not only accompanied by increases in ALAS2 but also by sequential induction of other heme biosynthetic enzymes, with heme having a stimulatory role in hemoglobin formation [1]. Heme also regulates the rate of its own synthesis in erythroid cells by controlling the transport of iron into reticulocytes [19].
Control of heme biosynthesis in tissues other than the liver and erythroid cells may be different but has been less studied [1].

1.1.3 **Porphyrin precursors and porphyrins**

The intermediaries in the heme biosynthesis pathway (*fig. 1*) are the porphyrin precursors aminolevulinic acid (ALA) and porphobilinogen (PBG) and the porphyrins, the latter mainly in the reduced forms known as porphyrinogens. Heme biosynthesis is remarkably efficient, with near-complete utilization of porphyrin intermediates. Free porphyrins have no biologic utility in humans, and are generally produced only in small amounts as side-products of heme biosynthesis.
All the heme biosynthesis intermediates are potentially toxic. If they accumulate, they cause the characteristic symptoms of the porphyrias. The accumulation of porphyrin precursors, ALA and PBG, causes the characteristic neurovisceral symptoms of the acute porphyrias and the accumulation of porphyrins leads to the photosensitivity symptoms of the cutaneous porphyrias.

1.1.4 Photochemistry

Porphyrins, the oxidized tetrapyrrole intermediates, are large molecules with unique electronic structures that enable them to interact with light energy in several ways and give rise to their characteristic colors as well as their biochemical properties.

Porphyrins absorb radiant energy intensively in the UV region, in the Soret band (400-410 nm) and to a lesser extent within the long visible bands (580-650 nm) (*fig. 2*). The energy is released via several mechanisms; by emission of light energy as fluorescence or phosphorescence, or by transfer of energy to appropriate acceptor molecules.
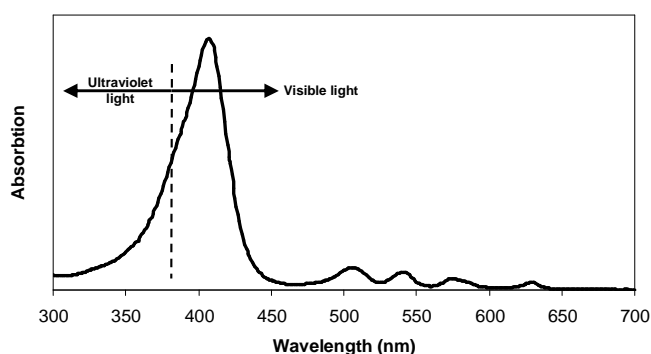
Figure 2

The protoporphyrin absorption spectrum. Protoporphyrin absorbs light energy mainly in the visible light spectrum, most intensely in the Soret band (400-410 nm).

Absorption of light energy converts stable ground-state porphyrin molecules to unstable excited compounds by promoting electrons from their ground-state orbital to the unstable singlet and triplet states (*fig. 3*). The excited porphyrins will give off their absorbed energy when promoted electrons return to their ground-states and may react directly with biological structures (type I reactions) or via activation of molecular oxygen (type II reactions). Oxygen has been implicated as the key intermediary in the photochemical processes by which excited porphyrins give rise to biological damage. Singlet oxygen damages tissues through several mechanisms including oxidation of membrane lipids and amino acids in proteins, cross-linking of proteins and oxidative damage to nucleic acids. In addition to singlet oxygen, other reactive oxygen species (ROS) are formed. These are also important in the photosensitization process [20].
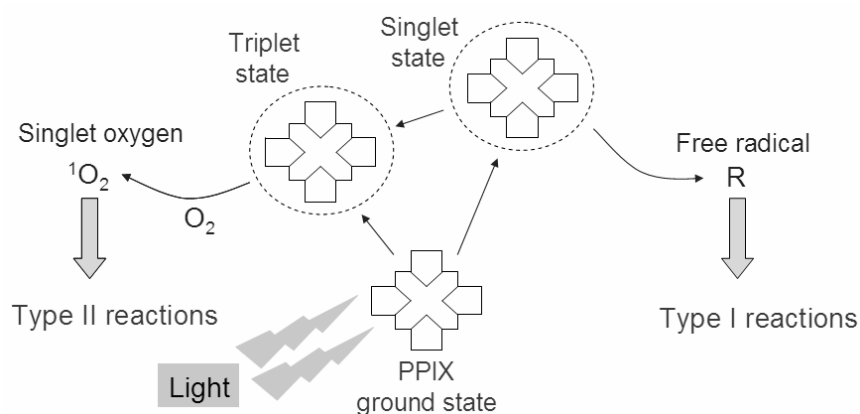
Figure 3

Protoporphyrin (PPIX) energetic transactions

Which structures that are damaged by the photodynamic reaction is determined by the localization of the specific porphyrin and thus the proximity to potential target molecules [21]. Since the accumulated protoporphyrin in EPP is hydrophobic, it is mainly localized to lipid membranes. The most susceptible structure to protoporphyrin-mediated damage seems to be certain amino acids in membrane proteins yielding cross-linked proteins. This has been demonstrated in e.g. erythrocyte membranes [22], subcellular organelles [23], cell cultures [24] and hepatic proteins [25].

1.2 ERYTHROPOIETIC PROTOPORPHYRIA

Erythropoietic protoporphyria (EPP, OMIM 177000) is the most common erythropoietic porphyria and in many countries the third most common form of porphyria after acute intermittent porphyria (AIP) and porphyria cutanea tarda (PCT).

1.2.1 History

EPP has a rather short history as a disease entity with a unique name. The clinical features of a condition characterized by an immediate form of photosensitivity and fluorescent erythrocytes were first reported in 1953 [26]. In 1961 the description was extended by Magnus and his co-workers [27] and the condition was termed erythropoietic protoporphyria. It was not until 1975 [28] that the condition was demonstrated to be caused by deficient function of ferrochelatase (FECH; EC 4.99.1.1). The cDNA for the human form of ferrochelatase was cloned in 1990 [29], the gene mapped to chromosome 18q21.3 in 1991 [30] and the genomic structure was delineated in 1992 [31]. Once the gene was identified and a large number of mutations were found, it became apparent that the disease had low clinical penetrance, with less than ten percent of mutation carriers developing overt symptoms [32]. In an extensive report on 200 EPP patients in 91 Dutch families in 1984 [33], only a single patient was discovered in 46 of these families. The mode of inheritance appeared to be primarily autosomal-dominant but the exact mechanisms of inheritance remained a mystery into the 21st century.

1.2.2 Inheritance

In 1996 Gouya et al [34] demonstrated that the phenotype in one EPP family resulted from the co-inheritance of both a mutant and a low-expressing ferrochelatase allele. The mechanism responsible for the low expression variant was later shown to be an IVS3-48T/C polymorphism that modulates the use of a constitutive aberrant acceptor splice site 63 base pairs upstream from the normal site [35]. The intronic single nucleotide polymorphism C at position IVS3-48 results in 40 percent aberrantly spliced mRNA by nonsense-mediated mRNA decay. This finding could thus explain the low enzyme activity, usually 10-30 %, seen in EPP patients [36] which is less than the 50 % of normal which would be expected from an autosomal-dominant disease.

The C polymorphism has been found in frequencies ranging from <1 to 45 % in different populations (*table 2*). The phylogenetic origin of the IVS3-48C haplotype strongly suggested that the IVS3-48C allele arose from a single recent mutational event.

Population	Frequency	Source
Japanese	45%	[37]
Chinese	41%	[38]
Southeast Asian	31%	[39]
British	13%	[40]
French	11%	[39]
Swedish	8%	[41]
Swiss	7%	[42]
Spanish	5%	[43]
North African	3%	[39]
Italian	1%	[44]
West African	<1%	[39]

Table 2

Reported frequencies of the low-expression *FECH* allele (IVS3-48c) in different populations

Estimation of the age of the IVS3-48C allele from haplotype data in white and Asian populations yielded an estimated age 3 to 4 times younger in the Japanese than in the white population. This difference could be attributable to differing demographic histories or to positive selection for the IVS3-48C allele in the Asian population. Haplotype analysis suggested that the mutation occurred after the population had moved out of Africa [39].

The mysterious inheritance pattern of a disease that in some families appears in individuals separated by several generations and in some families appears in siblings thus seemed to have been solved. Incomplete penetrance was explained by the inheritance of a mutated *FECH* allele and a 'low expressed' normal allele in a patient with overt disease, and the inheritance of a mutated allele and a normally expressed allele in an asymptomatic *FECH* mutation carrier.

The IVS3-48C polymorphism results in a reduced enzyme activity but is not enough to cause EPP symptoms, not even in individuals with the IVS3-48C/C genotype [35]. Clinical expression of pseudo-dominant EPP requires the hypomorphic IVS3-48C allele to be *trans* to the deleterious mutation but alone neither is enough to cause the EPP phenotype.

Around 150 different *FECH* mutations have been associated with EPP ([4, 45] and Study IV). There are reports on other genetic explanations. Less common cases with two deleterious *FECH* mutations, compound heterozygotes, have been reported in European EPP cohorts [4, 39, 40]. Rarely, an acquired mutation or deletion in the ferrochelatase gene secondary to myelodysplastic or myeloproliferative disorders leads to adult-onset EPP [46-49]. Mutation-negative patients meeting diagnostic criteria in the form of photosensitivity and elevated erythrocyte protoporphyrin concentration constitute up to seven percent in some case series [50]. Some of these may display mutations in the ALAS2 gene [2, 4] but other explanation that are yet to be revealed are likely to exist in rare cases.

There is no clear relationship between genotype and phenotype with the possible exception of compound heterozygosity which may result in a markedly reduced residual enzyme activity and an increased risk for liver disease at young age [40]. It seems likely that other genetic, epigenetic or acquired factors influence disease severity and ferrochelatase enzyme activity but these factors remain to be identified.

1.2.3 Prevalence

EPP has been described in patients worldwide and is probably equally common in women and men [51]. The reported prevalence in Caucasian populations ranges from 1:75,000 to 1:200,000 (*table 3*). Whether hyperpigmentation prevents phenotypic penetrance or the very low prevalence of the IVS3-48C polymorphism explains the reported rarity of black African cases is unknown. The IVS3-48C allele frequency may correlate to the varying EPP prevalence but additional studies on the EPP prevalence and the IVS3-48C prevalence in different populations are needed to substantiate this hypothesis.

Population	Prevalence	Source
The Netherlands	1:75,000	[33]
Northern Ireland	1:79,000	[52]
United Kingdom	1:143,000	[51]
South Africa*	1:152,000	[53]
Switzerland	1:170,000	[54]
Sweden	1:180,000	Study IV
Wales	1:200,000	[55]

Table 3
Reported prevalences erythropoietic protoporphyria in different populations

* The European immigrant population

1.2.4 Diagnosis

The diagnosis of EPP is based on symptoms, porphyrin biochemistry and DNA-based genotyping [3]. EPP is characterized by cutaneous photosensitivity that characteristically begins in childhood, affects sun-exposed areas and is generally worse in spring and summer. The typical biochemical finding is a plasma fluorescence marker around 632 nm and elevated concentrations of free protoporphyrin in erythrocytes (FEP). This protoporphyrin is not chelated to any metal in contrast to the elevated protoporphyrin concentrations in lead poisoning or iron-deficiency where zinc-protoporphyrin is found in elevated concentrations [56]. Most patients have an increased fecal excretion of protoporphyrin although some patients may have a normal fecal concentration [57]. Urinary excretion of porphyrin precursors is normal, and the urinary porphyrin concentration is generally normal in EPP patients without protoporphyric liver disease [58].

Since ferrochelatase is a mitochondrial enzyme, measurement of enzyme activity requires isolation of e.g. reticulocytes or lymphocytes. Results are method-specific and can seldom be compared between laboratories. EPP patients typically display an enzyme function of 10-30 percent of normal [36], while the asymptomatic mutation carrier generally exhibits an activity above 50 percent. With modern DNA analyses the diagnosis can be confirmed by molecular techniques in almost all patients [4].

1.2.5   Clinical manifestations

1.2.5.1   *Photosensitivity*

EPP manifests mainly by cutaneous pain within minutes of exposure to sunlight [59]. The first onset of symptoms is usually in childhood [51] (Study IV & *fig. 12*), sometimes upon the first sun exposure in early infancy. Stinging, itching, prickling and burning sensations on the exposed areas are initial symptom. These symptoms may have a variable intensity and duration depending on the intensity and duration of the exposure. Patients have described photosensitivity symptoms as "holding a lit match or a hot iron against the skin". Skin lesions are often absent despite severe pain [60]. If the exposure has been long enough, erythema, swelling or even blistering may be seen. The painful reaction can persist for hours or days after a photosensitivity episode. Often photosensitivity is more intense the day or days after the initial episode; the so called "priming phenomenon"[61]. Repeated photosensitivity episodes may result in chronic skin changes such as skin thickening with a leathery appearance or hyperkeratosis, often on the dorsal side of the hands (*fig. 4*), the forehead or on the nose bridge. The lips may show linear furrows and pseudo-rhagades. Seasonal palmar keratoderma has been reported in some EPP patients, usually in compound heterozygotes [62]. The milia, hyperpigmentation, hypertrichosis, vesicles and bullous lesion that are characteristic for some of the other cutaneous porphyrias are generally not seen in EPP.

Figure 4
The left hand of a Swedish EPP patient displaying chronic leathery skin changes caused by repeated photosensitivity episodes

1.2.5.2   Hepatobiliary disease

One quarter to one third of EPP patients display some degree of clinically evident liver damage [58, 63]. Usually the liver disease is mild and manifests only in slight elevation of liver function tests or mild histologic abnormalities [63, 64]. Gallstone disease, thought to result mainly from protoporphyrin precipitation in bile, may occur even during childhood [54, 65, 66]. The prevalence of gall stone disease in EPP varies in different publication from four [54, 67] to twelve [68] to twenty percent (Study IV). The mode of diagnosis varies between publications and no study has yet compared the age-adjusted incidence with background populations in order to see if the incidence or prevalence of gall-stone disease truly is markedly increased in EPP.

Less than five [69], up to 25 percent [70, 71] develop advanced liver disease. The high frequencies in these latter studies probably reflect the referral pattern in one [70] and in the other the liver disease was not well defined [71].

The most serious liver complication in EPP is cholestatic liver failure. In some patients, the course of the liver disease is insidious and cirrhosis is eventually detected but in some a sudden and rapidly progressive cholestatic liver failure characterizes the symptomatic debut. In most of these patients, a previously undetected liver cirrhosis is already present [58] but in a few only some degree of fibrosis is seen (Study I & V). Liver transplantation is the only treatment option with durable effects for patients with advanced liver disease.

1.2.5.3   Associated signs and symptoms

General

Photosensitivity is often associated with non-cutaneous symptoms such as disturbed sleeping, irritability, fatigue, nausea, headache or feeling generally unwell [51]. Increased sensitivity to other external factors such as high or low temperatures, draft, wind or physical exercise is also frequent (Study IV). Apart from hepatobiliary disease, no particular concurrent medical condition or disease has been reported to be over-represented among EPP patients.

Anemia and iron metabolism

Anemia and disturbed iron metabolism are common findings in EPP. Although it is likely that the anemia in EPP reflects reduced heme formation by ferrochelatase deficiency, the mechanisms and the relationship to disordered iron metabolism remain unclear. In contrast to congenital erythropoietic porphyria (CEP), the anemia is not dyserythropoietic, there is no iron overload and there is evidence of iron deficiency without iron loss [72]. Suboptimal iron stores may be frequent [73]. In study IV, we found a downward shift in erythrocyte parameters and ferritin which is in agreement with previous observations [72, 74].

Diagnosing iron deficiency in EPP poses particular difficulties. Common indicators of iron deficiency are frequently altered in the absence of deficient iron stores. Whether calculation of the percentage of hypochromic red blood cells or cellular hemoglobin in reticulocytes would add information to the interpretation of iron deficiency anemia in EPP remains to be studied. The downward shift in hemoglobin and erythrocyte indices

suggests that different reference values should be utilized in EPP. However, large cohorts of patients need to be studied to make alternative cut-off values reliable.

At present, in the absence of reliable biochemical parameters, it may be reasonable to attempt iron supplementation under clinical and biochemical surveillance if iron deficiency is suspected. However, caution is warranted since both increased photosensitivity and elevation of erythrocyte protoporphyrin concentrations [75, 76] as well as clinical improvement [77, 78] has been reported during oral iron therapy.

Vitamin D

During exposure to sunlight, the solar UVB photons are absorbed by 7-dehydrocholesterol in the skin and converted to pro-vitamin D_3 [79]. Under-exposure to ultraviolet B radiation leads to a risk of vitamin D deficiency. It is not surprising that EPP patients, who avoid direct sunlight, may exhibit low levels of 25-OH vitamin D [80, 81]. In study IV, we found that a majority of our patients had insufficient levels and half of them were vitamin D deficient. The mean vitamin D concentration in the cohort was 53.1 nmol/L. For comparison, previous studies on adult Swedish background populations reported average 25-OH vitamin D levels of 84.8 [82] and 75-102 nmol/L [83]. In recent years many publications have reported associations between vitamin D deficiency and different conditions such as malignancies, neurologic and psychiatric diseases [84]. Possible clinical consequences of low vitamin D levels among EPP patients remain to be studied. As for other populations with a very low sun exposure, oral supplementation of 800-1000 IU vitamin D_3 per day [84] should be recommended in EPP (Study IV).

1.2.6 Pathophysiology of clinical manifestations

Photosensitivity

The wavelengths that are preferentially absorbed by porphyrins (*fig. 2*) can penetrate through the epidermis and reach the level of cutaneous blood vessels [85]. The protoporphyrin in circulating erythrocytes is then released into the plasma and into endothelial cells [86] that are subsequently photo-damaged [87]. As discussed above, protoporphyrin generates reactive oxygen species upon exposure to radiation. From studies on EPP mice, we know that this causes lipid peroxidation of the cell membranes, resulting in cell lysis [88]. Radiation causes the release of mast cell derived mediators [89] and activation of the complement system [90, 91]. Polymorphonuclear cells also seem to play a role as the phototoxic reaction is suppressed in leukopenic animals [92].

Studies on human skin biopsies [87, 93, 94] largely substantiate *in vitro* and rodent findings. Some of the characteristic findings are thickened vessel walls caused by concentric reduplication of basal lamina and excess of fine granular material at the basal membrane zone in the superficial dermis. Direct immunofluorescence shows immunoglobulin and complement deposits in the vessel walls and, in immunohistochemistry, collagen IV and laminin at the vascular basal membrane. The perivascular deposits are thought to be secondary phenomena resulting from the leakage of serum components. These changes are not found in the non-exposed skin which indicates that the interaction of protoporphyrin and solar radiation is indeed needed for the cutaneous manifestations in EPP.

The priming-phenomenon may be explained by a two-phased phototoxic reaction in the endothelial cells of the skin vessels [86]. The skin is "primed" by light-induced transfer of protoporphyrin from erythrocytes to the endothelial cells during the first exposure. Subsequent exposures promptly cause symptomatic damage in the protoporphyrin-enriched endothelial cells [61, 86].

Hepatobiliary disease

At least 80 % of protoporphyrin originates in the bone marrow [6] and enters circulation in red blood cells where it diffuses across the red cell membrane and binds to albumin, hemopexin and lipoproteins [59]. Due to pronounced hydrophobicity, protoporphyrin is not present as an active monomer in aqueous solution. The intra-erythrocyte concentration of protoporphyrin is at least 10 times higher than that of plasma [95] but hepatic uptake is mainly from the plasma compartment [96]. Transfer in both directions between erythrocytes and plasma takes place [95] and an individual balance between compartments has been described [97] that is influenced by conditions related to EPP liver disease [98]. Protoporphyrin is the most lipophilic of the porphyrin molecules and is therefore not soluble in urine. The excess protoporphyrin leaves the body unaltered via hepatic excretion into bile. Although possible receptors that mediate the active cellular uptake and efflux of protoporphyrin have been identified [96], the understanding of these processes is incomplete.

Ultrastructural studies [99-102] have demonstrated alterations in many hepatic structures such as depolymerization of pericanalicular actin filaments, loss of bile canalicular microvilli, and protoporphyrin deposits in hepatocytes and sinusoidal endothelial cells. Alterations of nuclei, endoplasmatic reticulum and lateral plasma membrane are also seen.

The mechanisms by which protoporphyrin causes this wide range of hepatobiliary alterations that lead to inflammation, cell death and fibrosis have only partially been delineated. Excited protoporphyrin may react directly with biological structures or with molecular oxygen, generating singlet oxygen. Sub-cellular functions are affected, such as mitochondrial membrane alterations, lipid peroxidation and cytochrome inhibition [103-105], involving reactive oxygen species, hydroxyl radicals and hydrogen peroxide [106]. The important balance between antioxidant activities and free radical reactions is altered in these processes. The gene for heme-oxygenase I, which is induced in response to oxidative stress, is up-regulated in EPP liver disease [107]. Many effects of protoporphyrin are light independent [20, 108]. In cholestatic liver disease, impaired secretion of bile acids can cause malabsorption of the scavenger vitamin E [109], which may also be of importance in late stages of EPP liver disease. Protoporphyrin aggregates to form solid hepatocyte deposits [63] which in polarizing microscopy are seen as birefringent crystals [110] (Study I). In addition to alterations in hepatocytes, Kupffer cells and bile duct epithelial cells are also affected [100]. In cholestasis, bile acid induced apoptosis has been demonstrated [111].

A course of events with functional excretory disturbance followed by biliary micromechanical obstruction, causing hepatocyte damage due to protoporphyrin accumulation may be a reasonable hypothesis for the course of events that eventually leads to liver severe damage. A change in bile composition with an inter-individual difference in bile formation has been proposed as one of the possible factors contributing to the variability of liver disease [112].

1.2.7 Prediction of severe liver disease

The exact incidence of significant liver disease is unclear and the determinants of clinically overt hepatic dysfunction in EPP remain elusive. Factors that have been suggested to be of prognostic importance are null mutations [113] or autosomal recessive disease [114], a family history of EPP-related liver disease [115], the presence of other risk factors for liver disease [116], abnormal liver function tests [117], persistently high erythrocyte protoporphyrin concentrations [59] and histologic abnormalities in the liver .

It is, however, not possible to accurately predict which individuals will develop severe liver affection. A prudent strategy is probably to be vigilant and to have a low threshold for performing liver biopsies [116, 118]. Progressive liver fibrosis in likely to be an ominous prognostic finding but the determinants of progression in EPP liver disease also remain to be delineated.

1.2.8 Treatment options

1.2.8.1 Photosensitivity

The cornerstone of prophylactic treatment is avoidance of direct sunlight exposure and using protective clothing.

The benefit of non-reflecting topical sunscreens formulated to protect against ultraviolet radiation is limited. These sunscreens only partially block the wavelengths that are mainly responsible for provocation of symptoms in EPP (*fig. 2*). Sunscreens containing reflecting or pigmented blocking agents such as topical zinc or titanium oxide or tanning agents containing dihydroxy-acetone, a physical reflecting agent, offer better protection but may be less cosmetically appealing [119]. Another alternative is yellow light filters (Study III) that are especially suitable for application on windows in houses and cars, but there are today only anecdotal data available that report very good protective effects of yellow filters.

Apart from avoiding exposure to bright sunlight and using covering clothing, the longstanding mainstay of prophylactic tolerance-enhancement is oral beta-carotene; the most used and best studied treatment. To achieve recommended serum concentrations of 11 to 15 µmol/L [3], daily doses of 180 to 300 mg [120] or a dose sufficient to cause a yellow discoloration of the skin [53] may be required in adults. Beta-carotene re-directs radiation energy by isomerization from the cis-carotenoid to a trans-carotenoid [121, 122], but the main photoprotective function is explained by quenching of singlet oxygen and inhibition of free radical reactions [123, 124].

The protective effect is limited for the majority of patients [125] (Study IV), although study results are contradictory. The least impressive results are found in the few randomized controlled studies [125].

Cysteine [126, 127], N-acetylcysteine [128, 129], Vitamin C [130] and narrow band UVB [131, 132] or UVA phototherapy [133] have also been explored in a few studies but results have been contradictory or have suggested a lack of efficacy [125]. Better treatment options are clearly needed.

Afamelanotide, an analogue of α-Melanocyte Stimulating Hormone (MSH), is a potentially exciting new therapeutic option for EPP. Afamelanotide induces increased skin pigmentation by enhancing eumelanin formation when administered in a sustained release formulation. Favorable results were reported from a pilot study on five patients; both tolerance to artificial light and melanin density increased significantly after 120 days [134].

An interim analysis of data from a multicenter study comparing afamelanotide and placebo in 100 patients showed an overall reduction in the average number of phototoxic reactions and that pain severity was positively correlated with treatment (Clinuvel newsletter Dec 22, 2009). Participating patients in our own centre reported good subjective effects without significant side-effects. A full analysis of study data is not yet completed but preliminary results seem promising.

1.2.8.2 Cutaneous pain

Few effective pain-reducing treatments are available once cutaneous symptoms have arisen after exposure to sun-light. Patients usually try whatever they think might help on a trial and error basis. Cold baths, showers, wet towels, oral analgesics and antihistamines are often used [51], seldom with more than limited effects (Study IV).

1.2.8.3 Hepatobiliary disease

Prophylaxis

Considering that the incidence of significant liver disease is unclear and that the determinants of the presence and progression of hepatic dysfunction in EPP remain elusive, reports on prophylactic treatments are difficult to interpret. Treatment with ursodeoxycholic acid was reported to "stabilize" a patient in one study [135] and reduced the amount of hepatic protoporphyrin deposits in another study [100]. It seems that the development of liver disease can be monitored but it cannot be prevented [63].

Liver failure

The proposed pathophysiologic events responsible for progressive cholestatic liver failure in EPP provide a number of possible treatment targets. Hence treatments may be aimed at: 1) reducing protoporphyrin production, 2) reducing the circulating pool of protoporphyrin, 3) ameliorating intrahepatic disease mechanisms, 4) increase the excretion of protoporphyrin into bile and 5) to interrupt the enterohepatic re-uptake of protoporphyrin into the liver (*fig. 5, table 4 &* Study I).

Several experimental studies and case studies support proposed treatment effects and many of these treatments were concomitantly or serially used in Study I. The clinical value remains uncertain but many patients are reported to have been successfully bridged to liver transplantation by an active treatment strategy and in Study I we suggest that a combination of treatments reversed cholestatic liver failure in a patient which enabled us to offer curative hematopoietic stem cell transplantation (HSCT) instead.

The use of exogenous heme (hemin) in the treatment of EPP liver disease is perhaps especially controversial. The rational for using hemin in this context is discussed in the discussion section below.

Figure 5

The route of excess protoporphyrin in EPP; from its origin in the marrow erythropoiesis to hepatobiliary excretion and possible sites for treatment interventions
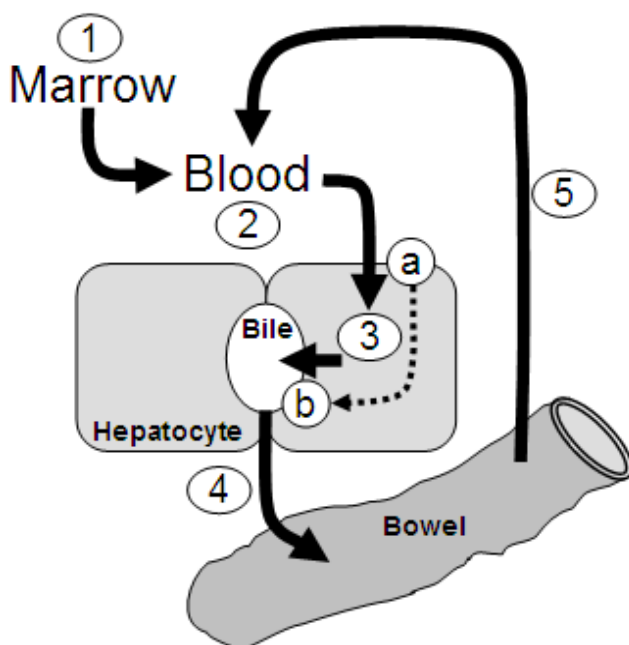
① Erythrocyte transfusion to reduce protoporphyrin production via feed-back inhibition of erythropoiesis, ② Reduction of the protoporphyrin pool in transit by afereses, ③ Protection of hepatocytes from toxic damage, ④ Enhancement of biliary excretion and altering bile composition into a less toxic composition, ⑤ Interruption of enterohepatic re-uptake of proto-porphyrin into the liver.
Whether any of the putative transporters that mediate the active cellular uptake and efflux (a→b) of protoporphyrin can be subject to therapeutic manipulation is presently unknown.

1. Inhibition of protoporphyrin production	
Erythrocyte transfusion	[136-139]
2. Removal of protoporphyrin in transit	
Exchange transfusion/ erythrocyte apheresis	[95, 136, 138-141]
Plasmapheresis	[95, 136, 140, 142-144]
Albumin dialysis	[145]
LDL apheresis	[146]
Hemodialysis (unsuccessful)	[147]
3. Amelioration of toxic hepatocyte injury	
Hemin	[142-144, 148-150]
N-acetylcysteine	[151]
Vitamin E	[152]
UDCA, CDCA	[100, 153, 154]
4. Alteration of bile composition and excretion	
UDCA, CDCA	See above
5. Inhibition of enterohepatic circulation	
Cholestyramine	[155, 156]
Charcoal	[157, 158]

LDL, low-density lipoprotein; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid

Table 4

Treatments described for EPP-related liver disease; aim of treatment, modality used and selected references

1.2.8.4 Liver transplantation

Liver transplantation is life-saving for patients with advanced liver disease and the only treatment option with durable effects. After the first EPP patient was transplanted in 1979 [159], more than 40 patients have been reported world-wide [116]. The US cohort of 20 patients was reported in 2005 [160] but European patients have never been compiled except for sporadic case reports and case series until we conducted Study V.

Three frequent problems have been identified in liver transplantation for EPP liver disease: phototoxic tissue injury from surgical luminaires [161], post-operative neuropathy [162] and graft disease recurrence [160]. All these problems may influence the short and long-term results. Several efforts aiming at minimizing these problems have been reported, such as using light filters to prevent burn injuries and measures to reduce the circulating load of protoporphyrin. These measures include many of the treatment options that are outlined above.

Preoperative optimization

The different treatment modalities used and discussed in EPP liver failure have been described before or after liver transplantation. Treating patients in the preoperative period aims at ameliorating the failing liver function in order to bridge the patient successfully to transplantation, at preventing perioperative complications and at reducing the load of protoporphyrin that the transplanted graft will have to handle. In this situation, treatment efforts primarily attempt to inhibit protoporphyrin synthesis and to reduce the amount of circulating protoporphyrin (*fig. 5 & table 4*). Combinations of measures have both reversed liver failure [98] and been unable to reverse or prevent progressive hepatic deterioration despite a significant decrease in protoporphyrin concentrations [95]. The clinical effects of these measures remain to be elucidated.

Perioperative measures

In addition to the treatment options that may be used in the preoperative optimization, an exchange transfusion during the anhepatic phase may offer some initial protection against protoporphyrin induced graft damage but the potential benefits may be outweighed by the risks of this procedure. Phototoxic burn injuries induced by surgical luminaires can be prevented by the use of light filters [138, 145, 163] (Study III).

Postoperative treatment

The same treatments that are described above have been used in the postoperative phase in some patients, mainly to treat histologically evident recurrent EPP-related graft disease. A feared complication in the postoperative period is severe neuropathy [162] (Study V) that leads to motor weakness necessitating prolonged mechanical ventilation. This is an uncommon complication to liver transplantation [164] but has been reported in many EPP recipients [160-162, 165, 166]. The mechanisms for neuropathy in EPP are not entirely clear but high concentrations of circulating protoporphyrin may contribute [167]. If so, attempts to reduce the amount of circulating protoporphyrin and to keep erythropoietic production of protoporphyrin low make sense. The mechanisms for neuropathy need to be further delineated before effective prophylactic therapy can be designed.

1.2.8.5   HSCT

Hematopoietic stem cell transplantation has been done in a few cases after liver transplantation and in one patient as single organ transplantation (Study I). It cures all phenotypic manifestations but the role for HSCT in EPP is presently intimately related to EPP-related liver disease. This is further discussed in the discussion section below.

1.2.8.6   Gene therapy

The genetic correction of autologous hematopoietic stem cells in theory represents a highly attractive alternative to HSCT. This approach could resolve the search for a donor and eliminate the risk of graft versus host disease and graft rejection associated with HSCT. EPP mouse models have successfully been treated by gene therapy [168-170]. Proof of principle in animal models may be spectacular, but safe clinical applications for human EPP remain to be developed. Based on preclinical and clinical data, evidence is accumulating that gene therapy may induce several kinds of unexpected side effects [171]. Safety and toxicology studies are at the forefront of current investigations in anticipation of future clinical studies but there is still a long way to go before gene therapy can be regarded a real option in human EPP.

1.2.9   Life perspective in EPP

No studies have indicated that life expectancy is reduced in EPP. For most patients EPP is grueling and for many also a psychosocially disabling condition. Except for those who are struck by severe hepatobiliary disease, EPP has not been associated with other concurrent conditions that may reduce the expected life span. Whether the deficient concentration of vitamin D that many patients display increases the risk for e.g. malignancies has never been studied in EPP.

2 AIMS OF THE STUDIES

The ideas behind the studies selected for this thesis all sprung from the questions and the perceived limitations in the knowledge about several aspects of EPP that surrounded decisions that were made in the management of the patient described in Study I.

We specifically wanted to study:

1. If treatments aimed at the different mechanisms thought to progress the cholestatic liver failure in EPP could reverse the liver disease.

2. Whether EPP is truly an erythropoietic porphyria and thus curable by HSCT

3. The long-term effects of HSCT on marrow and dermal *FECH* genotype

4. Whether findings in rodent transplantation studies are applicable to human EPP

5. Optimal characteristics of light filters for protection from phototoxic injuries during liver transplantation.

6. Whether there is a risk for phototoxic injury from surgical luminaires in non-transplant surgery

7. The demographic, genetic, biochemical and clinical features of the Swedish EPP population

8. The outcome and complications in a large cohort of liver transplanted EPP patients

3 PATIENTS, MATERIALS AND METHODS

3.1 STUDY SUBJECTS

Two different patient populations were involved in the studies. In study III and IV, data from the Swedish EPP cohort were used. All individuals known to have EPP in 2008 contributed data to Study IV while Study III also included data from EPP patients that had died during the last 15 years. Study V comprised data from all identified European patients that had undergone liver transplantation. Data from a single Swedish patient were used for Studies I and II.

3.2 ETHICS

All studies were conducted in accord with the ethical principles in the World Medical Association Declaration of Helsinki. Two separate regional ethics review board approvals, for the genetic study and for the cross-sectional questionnaire and biochemistry study, were applicable in Study IV. Study V was approved by the ELITA (European Liver and Intestine Transplant Association) review board. Study I and II did not require ethical board approvals since the patient data presented in these studies represent clinically motivated procedures.

3.3 EPIDEMIOLOGICAL METHODS

All patients in the present studies had a confirmed EPP diagnosis. For all the Swedish patients in Study I-V, the diagnosis was established by a typical plasma emission scan peak around 632 nm, elevated erythrocyte protoporphyrin levels and symptoms of painful photosensitivity. A typical underlying genotype confirmed the diagnosis in all but two patients. A detailed description of these two patients is presented below.

For Study III we retrieved information on surgical procedures and endoscopies that patients had been subjected to. Patients were asked to report procedures that they had undergone, whether they had experienced any objective or subjective complications or discomfort related to light and whether they had been protected from light during the procedures. Information on three patients who expired during the last fifteen years from EPP liver failure was also included.

We used a questionnaire to retrieve data for Study IV (also used in Study III). The questionnaire included 73 questions that covered ten main areas: diagnosis, symptoms, treatment, coping strategies, female endocrine factors, concomitant conditions, surgical procedures, life style aspects, quality of life and porphyria heredity.

For Study V, we used both registry data and information from data forms. These forms were created for the study. One form for each identified patient was sent to and completed by the transplant center that had performed the liver transplantation.

3.4   BIOCHEMISTRY

Hemoglobin level, platelets, erythrocyte indices and liver function tests were determined by standard automated methods by the Karolinska University Hospital Laboratory. Ferritin was determined by an immuno-enzymatic assay, iron by colorimetry, transferrin by turbidemitry, soluble transferrin receptor by nephelometry and 25-OH vitamin D analyses were done using a commercial radioimmunoassay. Porphyrin concentrations in erythrocytes, plasma, urine and feces were determined by standard methods [3, 172-174] at the Porphyria Centre Sweden.

The laboratory parameters described in Study V were retrieved from case reports or obtained from data forms that were completed by the participating centers.

3.5   GENETIC ANALYSES

For *ferrochelatase genotype analysis* in peripheral blood and dermal fibroblasts we used sequencing analysis*,* denaturing gradient gel electrophoresis (DGGE) and restriction enzyme cleavage analysis with standard methods as described previously [41]. Large deletions were identified by multiple ligation-dependent probe amplification (MLPA).

3.6   PHOTOCHEMISTRY AND PHOTOTOXICITY

The spectral irradiances of luminaires were determined by using a Spectrascan [PR]705 spectrophotometer or a Ramses ACC Hyperspectral Irradiance sensor. We measured both operating room luminaires, xenon head light, laparoscope and endoscopes. Measurements were done at distances resembling those used in clinical practice.

Filter transmittance was measured spectrophotometrically.

The protoporphyrin absorption spectrum was determined by dissolving Protoporphyrin in dimethyl sulfoxide and scanning the absorption spectrum on a spectrophotometer.

The experimental erythrocyte model, based on previous experimental work [175, 176], was constructed from normal red blood cells that were made light sensitive by adding protoporphyrin. The suspension of protoporphyrin-loaded erythrocytes was exposed to light from an operating room luminaire with and without different filters between the light source and the cuvette containing the suspension. The degree of cell damage was measured as the amount of hemoglobin released from the erythrocytes to the medium. The small amount of spontaneous hemolysis observed in parallel samples that were kept in dark for the same duration was subtracted from observed total light induced hemolysis in each sample.

For the *surgeon survey*, surgical luminaires were equipped with three different filters during a liver transplantation pause. Four present surgeons were asked to assess visibility and working conditions. Furthermore, an endoscopy light source was equipped with a yellow glass filter and two gastroenterologists were asked to assess visibility during gastroscopy and colonoscopy. Two surgeons evaluated visibility and working conditions in the same manner during laparoscopy.

3.7   STATISTICAL AND MATHEMATICAL METHODS

In Study IV, the Mann-Whitney nonparametric test was used for analysis of differences between quantitative variables and the Spearman rank correlation was used to test the significance of relationships between pairs of variables. In study V, statistical analyses of patient survival and graft disease recurrence were done by Kaplan-Mayer and log-rank tests.

In Study III we used mathematical processing in a theoretical model to predict biological activity for different filtered and unfiltered light doses. Weighted spectral irradiance was created by the product of irradiance and normalized protoporphyrin absorption (*fig. 2*) at each wavelength. The biologically active irradiance for the light that is used during a liver transplantation was estimated by calculating the product of the sum of weighted spectral irradiance for the three main operating room luminaires (head light and two main lights) and filter transmittance at each wavelength.

4 RESULTS

4.1 STUDY I-II

Treatment of liver disease and hematopoietic stem cell transplantation
By serially or concomitantly applying several treatment modalities aiming at proposed possible targets (*fig. 5 & table 4*) implicated in the progression of EPP liver failure, the condition was successfully reversed. As described in the paper, there was a remarkable response to treatment, both biochemically and in liver histology. The evolution of the erythrocyte protoporphyrin concentrations is displayed in *figure 6* and the excretion patterns of protoporphyrin in feces and porphyrin in urine in *figure 7*
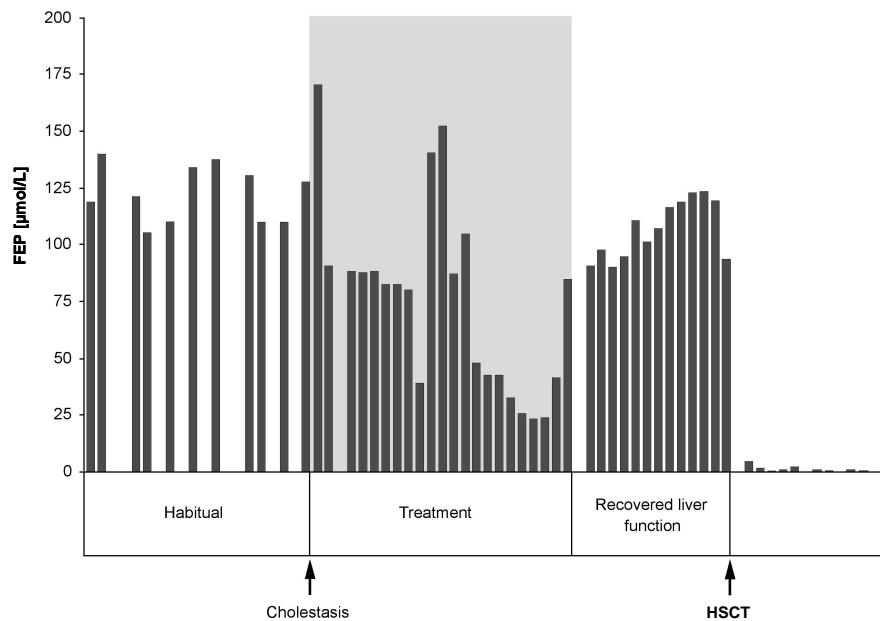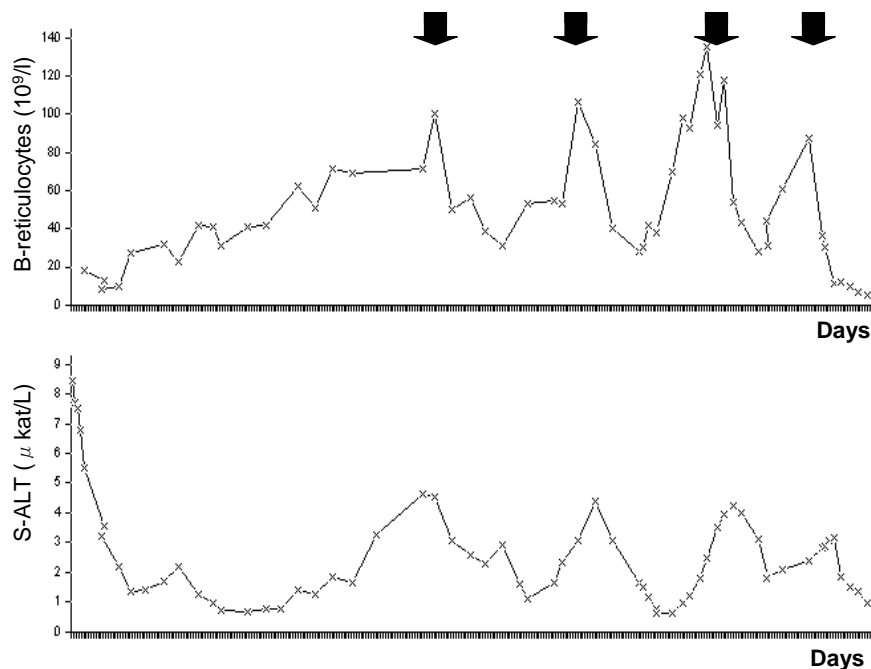

Figure 6

Total free erythrocyte porphyrin (FEP) concentrations (normal 1.2 μmol/L) during different phases
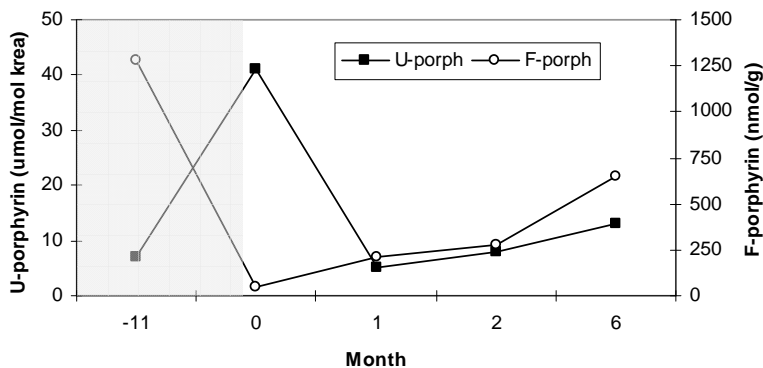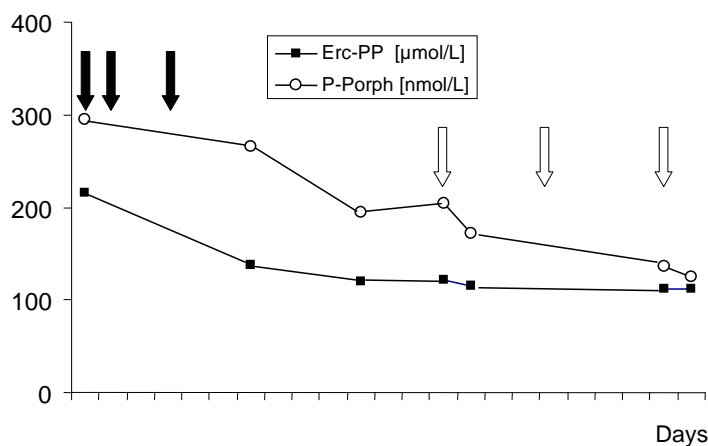

Figure 7

Porphyrin excretion in urine and faeces before liver failure (grey area) and during treatment
U-porphyrin; normal <25 μmol/mol creatinine, F-porphyrin; normal <200 nmol/g dry weight

The clinical effects of individual treatment modalities are difficult to interpret, but some biochemical effects were more obvious. *Figure 8* displays a temporal relationship between the blood reticulocyte concentration and the plasma alanine aminotransferase activity which indicates that suppression of erythropoiesis by erythrocyte transfusion ameliorates the disturbed liver function by reducing the protoporphyrin load that the liver has to handle and that the circulating pool of protoporphyrin does indeed constitute a significantly harmful burden to the liver.
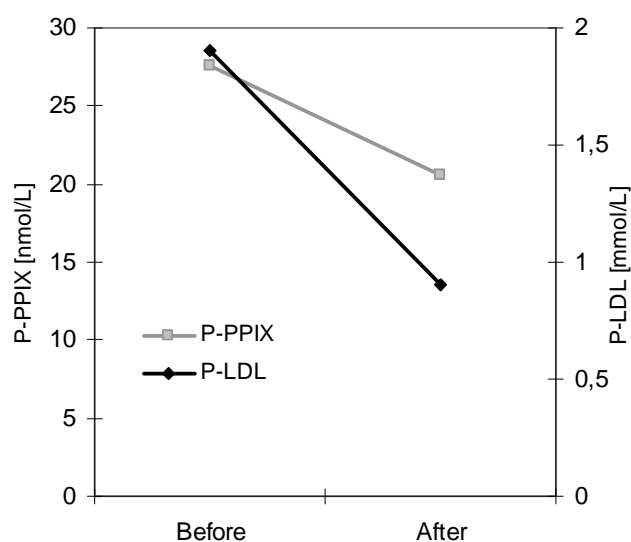

Figure 8
Temporal relationship between the reticulocyte concentration in peripheral blood and serum alanine aminotransferase (ALT, normal <1.2 μkat/L) activity
Filled arrows represent erythrocyte transfusions.

Figure 9 describes the effect on the erythrocyte and plasma concentrations of protoporphyrin when the patient was treated with erythrocyte transfusions and plasmapheresis in the initial phases of treatment.

Figure 9
Erythrocyte trans-fusions (filled arrows) and plasmapheresis (open arrows)
Erc-PP; normal <1.2 μmol/L, P-Porph; normal <10 nmol/L

We also used another, in this context novel, apheresis modality to attempt reduction of the plasma protoporphyrin concentration; low-density lipoprotein (LDL) apheresis. *Figure 10* provides an indication that LDL is an important carrier of protoporphyrin in the plasma compartment. By lowering the plasma LDL concentration by 53 percent, the plasma protoporphyrin concentration was reduced by 25 percent. This finding was reported in a later publication [146].

Figure 10
The effects of LDL-apheresis on plasma concentrations of low-density lipoprotein and protoporphyrin (PPIX)

The patients normalized liver function appeared unstable and required intensified treatment with hemin infusions, plasmapheresis and erythrocyte apheresis to remain biochemically silent. He thus displayed signs of advanced EPP-related liver disease, and recurrent cholestatic crisis developing into liver failure could be regarded as a likely event in the near future. This provided both motivation and a unique opportunity to correct the disordered heme biosynthesis in erythroid tissue by HSCT.

A first HSCT was uneventful but the patient lost his graft and had autologous recovery. After careful considerations, a second HSCT was done. The second transplant course was also uncomplicated and he was discharged with normal liver function tests. The EPP genotype is no longer present in peripheral blood cells but remains in skin fibroblasts. Liver biochemistry as well as porphyrin biochemistry in blood, urine and feces has remained normal for the more than four years that have now passed since HSCT. There are no symptoms of photosensitivity. For the first time in his life, he is now able to be outdoors on a sunny day without pain and has taken several trips to Mediterranean beach resorts, spending long days in the sun without experiencing any photosensitivity.

Ferrochelatase in mice and man

The EPP mouse model has provided results that are different from those in Study I-II. HSCT does not entirely correct circulating protoporphyrin levels or reverse liver damage in mice. This might be expected due to the dual production sites in mice. In contrast, the patient in Study I-II suggests that EPP is indeed only caused by protoporphyrin from the marrow. Although numerical evidence is still small, this finding strengthens the concept of EPP as an erythropoietic disease in man, unlike the hepato-erythropoietic EPP in mice.

Transplantation of bone marrow from EPP mice to normal recipients does not cause photosensitivity and photosensitivity does not develop in skin transplanted from normal mice to EPP mice. The accumulated experience from corresponding human situations suggests that this would not be the case in human EPP.

4.2 STUDY III

Protection from phototoxic injury in surgery

The protoporphyrin absorption spectrum (*fig. 2*) explains the ability of different wavelengths to excite protoporphyrin and cause photochemical tissue injury.

There was a notable difference in output between operating room (OR) luminaires and laparoscopy on one hand and endoscopy as well as background OR light on the other hand. Mathematical processing of the protoporphyrin absorption spectrum in combination with the measured output from different luminaires provided an understanding of the biological effects of light in different clinical situations. Data from this model suggested that the risk for phototoxic injuries from surgical luminaires in situations other than liver transplantation is very small. This hypothesis was confirmed by the data provided by EPP patients. They reported 60 surgical and 38 endoscopic procedures of which none had resulted in any identified complications. We concluded that recommendations for protective light filters are not warranted in endoscopy, laparoscopy or open surgery other than liver transplantation.

Viewing filter transmittance spectra together with protoporphyrin absorption spectrum facilitated an understanding of the extent to which filters reduce or block wavelengths that excite protoporphyrin and thus may cause phototoxic burn injuries. Mathematical processing of the protoporphyrin absorption spectrum in combination with the measured total output from the OR luminaires and also mathematically weighting for filter transmissions provided an understanding of the relative protective effects for different filters. The more efficient a filter blocks the wavelengths that can excite protoporphyrin, the better protection it offers but the more it also distorts the color perception. Surgeons found the red filter unacceptable due to pronounced distortion of color perception. The orange filter caused a moderate influence on color perception but caused no significant restrictions to working conditions and was readily accepted for use. The yellow filter caused minimal visual distortion. Altogether, the surgeons did not consider visibility or working conditions significantly impaired when working under the yellow or orange filters.

The experimental erythrocyte model that was used to assess the protective effects of different filters, suggested that the irradiation time needed to cause comparable degrees of tissue injury was prolonged by 25, 100 and 150 percent respectively for a clear, a yellow and an orange filter. We concluded that the yellow filter offered the best balance between protection and visibility and should be recommended for use during liver transplantation surgery.

4.3 STUDY IV

Demography
In 2008, 51 individuals resident in Sweden were known to have EPP. This suggested a minimum prevalence of 1:180,000. Twenty-nine (57%) were men and 22 (43%) women. The mean age of individuals was 38.4 years (range 8-83) at the beginning of 2008. The geographic distribution of EPP patients in Sweden corresponds well to that of the general population (*fig. 11*). The study participation rate was high; 92 % participated in the questionnaire study and 82 % participated in the subsequent biochemistry study.
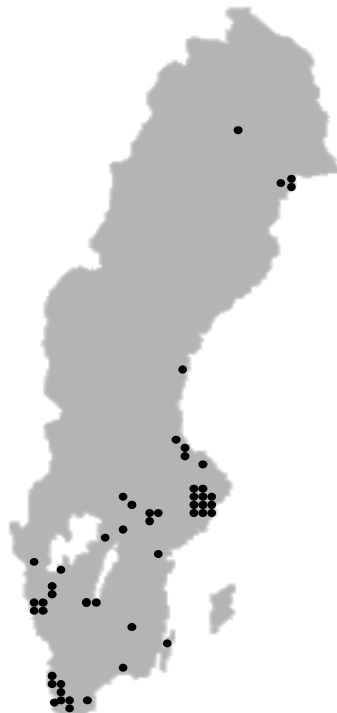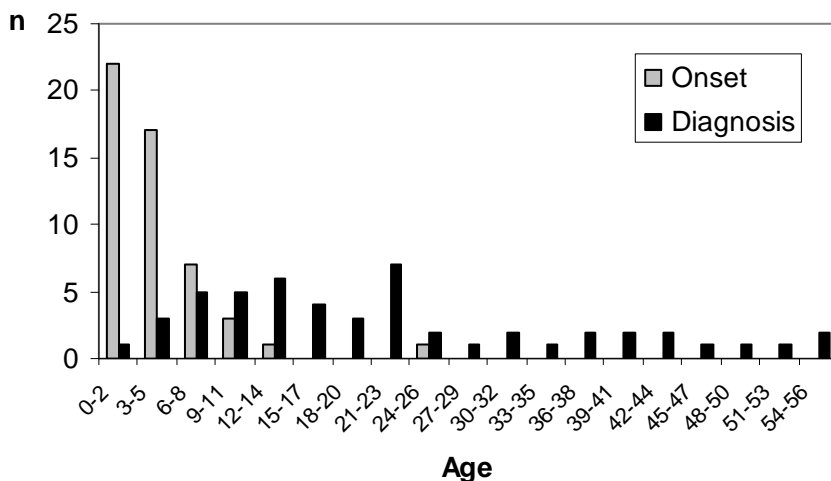

Figure 11
Geographic distribution of 51 Swedish EPP patients in 2008

Symptoms and diagnosis

While the most common age at onset of symptoms was the first year of life, the mean age at diagnosis was 22 and the mean delay from the onset to diagnosis was 18 years *(fig. 12)*. The mean age at diagnosis had risen from 19 to 27 years and the mean delay between the onset of symptoms to diagnosis from 15 to 24 years when we compared those diagnosed in the 20th to those diagnosed in the 21st century.

Figure 12

The age at first onset of symptoms and the age at diagnosis

Up to 60 percent reported an increased cutaneous sensitivity to different external factors, both with and without a preceding photosensitivity episode. Thirty-six percent of women reported an improvement in photosensitivity during pregnancy. The effects of prophylactic treatments to reduce photosensitivity were meager. As might be expected, non-reflecting sun creams offered little help *(fig. 13)* and beta-carotene offered a good protective effect in only six percent even if a sufficient dose, adjusted for intake per body weight, was used. A multitude of measures to ameliorate the pain experienced after a photosensitivity episode was reported, none of which stood out as especially effective.
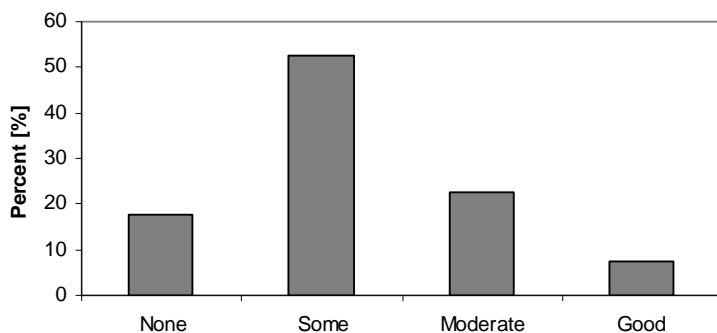
Figure 13

Reported effectiveness of non-reflecting sun creams

Ninety-one percent reported that their quality of life was affected by the disease and 73 percent that they experienced limitations in everyday life (*table 5*). Twenty-two percent had no regular follow-up with a physician.

Measure		Frequency (%)
Quality of live affected:	Much	44
	Some	47
	None	9
Affects relations with:	Friends	58
	Family	40
Feelings of detachment		40
Influenced choice of profession		24
Limitations in everyday life		73

Table 5

The degree to which EPP influences different quality of life measures negatively

Molecular diagnosis

All but two patients displayed the most frequently reported constellation of a *FECH* mutation on one allele and the IVS3-48C polymorphism on the other allele. The only patient in whom the *FECH* mutation was not identified had the IVS3-48C allele. No mutations in the ALAS2 gene were found in this patient. One other patient had a *FECH* mutation but not the IVS3-48C low-expression allele. Both these cases had elevated erythrocyte protoporphyrin concentrations and reported marked photosensitivity. No one was compound heterozygous for *FECH* mutations. Nine novel *FECH* mutations were found in the Swedish population (*fig. 14*).
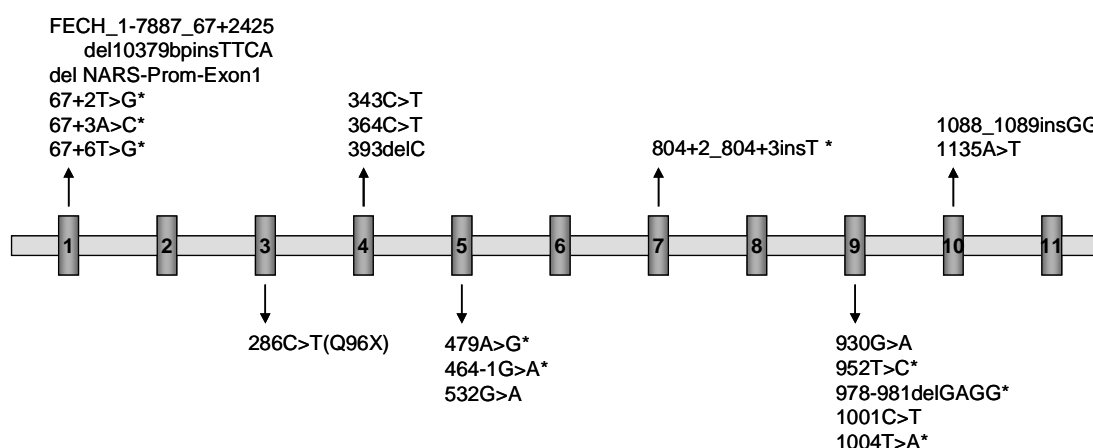
Figure 14

Mutations in the ferrochelatase gene in Swedish EPP patients; novel mutations are marked by an asterisk. The ferrochelatase (*FECH*) gene is located on chromosome 18q21.3, spans 45 kb and contains 11 exons

Biochemistry

The inter-individual distribution of erythrocyte protoporphyrin concentrations was large, with individual means ranging from 4.8 to 124.6 μmol/L. In contrast, the intra-individual distribution was small. Three of the patients that displayed a significant intra-individual spread had a history of bleeding episodes that probably contributed to this finding.

Anemia was found in 21 percent. A subnormal ferritin concentration was a finding in 44 percent and subnormal transferrin saturation was found in 12 percent of the cases. The downward shift in the distribution of red blood cell indices and iron parameters in the cohort is illustrative. It remains to be established whether this finding supports the formulation of different reference values designed to correctly diagnose iron deficiency and anemia in EPP.

As might be expected from a population that avoids direct sunlight in the summer months, insufficient or deficient circulating vitamin D concentrations were frequent findings (*table 6*).
Twenty-five percent had altered serum liver biochemistry and 20 percent reported having been diagnosed with gallstone disease.

	Thresholds	%	
Sufficient	>75 nmol/L >30 ng/mL	16	
Insufficiency	51-74 nmol/L 21-29 ng/mL	34	a
Deficiency	<50 nmol/L <20 ng/mL	47	b
Severe deficiency	<25 nmol/L <10 ng/mL	3	

Table 6
Serum 25-OH vitamin D concentrations in the Swedish EPP cohort
The mean concentrations were 47.6 nmol/L for men and 59.8 nmol/L for women. Insufficiency (a) was found in 84 percent and deficiency (b) in 50 percent.

4.4   STUDY V

Liver transplantation for EPP liver disease
In this retrospective study we identified 33 European patients that had undergone a total of 37 liver transplantations. Sixty-one percent were men and the mean age at the time of the first transplantation was 39 years. Overall survival was 80 percent at one year and 66 percent at five and ten years. The outcome was similar to that of European patients that had been transplanted for other cirrhotic liver diseases during the same time period. Those with concurrent hepatic malignancies were excluded from the control group.

The elevated serum bilirubin concentration of cholestasis (280 μmol/L) was the driving force behind the elevated mean MELD (Model for End-stage Liver Disease) score of 20, as seen by a modestly elevated mean INR of 1.5 and a low mean serum creatinine of 55 μmol/L.

Active preoperative treatments aiming at ameliorating the different mechanisms thought to progress EPP-related liver disease were reported in 17 patients. The efficacy of these treatments could not be evaluated in the study.

Data on post-transplant liver histology was incomplete which yielded two different interpretations concerning the frequency of EPP-related graft disease recurrence. If we used the first instance of histologically proven recurrence in patients with available liver histology data for interpretation, a recurrence rate of 43 percent at one year and 68 percent overall was seen. Transient signs of recurrence were noted in 33 percent of the 21 patients with available data on histology. If these patients together with all patients with just clinically assessed recurrence were included in the analysis as non-recurrent, a recurrence rate of 25 percent at one year and 30 percent overall was instead noted. Four patients received a second transplant due to graft failure which was attributed to EPP-related graft recurrence in one patient. Two transplantations were done with a partial graft from a live donor and three patients received bone marrow transplantation after the liver transplantation in order to prevent future graft loss from EPP-related recurrence.

Phototoxic burn injuries from the surgical luminaires were noted in 22 percent of the patients that were not protected by light filter but in none of those that were protected. Severe motor neuropathy was seen in 15 percent in the post-transplant period.

5 DISCUSSION

There have been significant advances in our understanding of EPP in recent years. Still, most of the factors governing clinical expression and disease progression remain elusive. This thesis, with its associated five papers, has hopefully contributed in a few but significant ways.

We now know more about how to handle the risk for phototoxic injuries during surgery, about the outcome and risks for complications in liver transplantation, about the characteristics of a national cohort of EPP patients, about ways EPP-related liver failure may be treated, about the effects and the role for hematopoietic stem cell transplantation in EPP and why results from studies on EPP mice should be interpreted with caution.

The studies and the literature review of course have limitations. EPP is an uncommon disease and EPP-related liver disease is a rare complication with a variable clinical demeanor. Controlled and randomized studies will most likely never be possible undertake in EPP liver disease. We are therefore left with a number of case reports, such as Study I, or at best case series that show different effects from different combinations of treatments. In Study I several concomitant treatments were utilized. The outcome was good but the treatment concept difficult to assess regarding what treatment or treatments were primarily beneficial.

The subsequent HSCT cured the EPP phenotype and strengthened the concept of the erythropoiesis as the main culprit causing the EPP phenotype. The case is unique since no other EPP patient has undergone HSCT as single organ transplantation. This also calls for caution. Although the notion that the liver may contribute significantly to the excess production of protoporphyrin in some patients is not supported by much evidence, the absolute proof consists of this single case.

The discussion in Study II was elicited by a publication that reported surprising results in an EPP mouse model which contradicted what we thought we knew about the relative contribution of the erythroid, dermal and hepatic *FECH* genotype and ferrochelatase enzyme deficiency to dermal photosensitivity and liver disease. A number of publications cited the results from the mouse studies and suggested therapies in human EPP based on the conclusions drawn from the EPP mice model. This provided reasons for a commentary. In Study II we therefore discussed experiences from human EPP, including organ transplantation, that clearly indicate significant differences between EPP in mice and man. Results from EPP mice models should be interpreted with caution before conclusions about the human disease are drawn.

The recommendations stated in Study III are based on a more sound evidence base. Collectively our findings showed that endoscopy, laparoscopy and surgical procedures other than liver transplantation are safe in the non-cholestatic EPP patient and that protective filters are not needed for these procedures. The recommendation was supported by epidemiologic, experimental and theoretic evidence. The same may be said for the recommendation that a yellow filter is a good choice for protection during liver transplantation.

Study IV is a descriptive population study which adds to the understanding of EPP. It provides a comprehensive characterization of the Swedish EPP cohort. It clarifies the prevalence and genetic characteristics and provides many details on clinical features. The biochemical review provides mainly confirmatory data concerning the recently reported prevalent vitamin D deficiency, altered iron metabolism and red cell parameters in EPP. The visualization of an abnormal left shift in iron and red cell parameters and nine new mutations are novel aspects. The delay in diagnosis points to a need for increased awareness of this disease and many of the biochemical discoveries once again point to the need for further exploration of the mechanism that determine clinical expressions in EPP.

Study V is the largest retrospective study to date on EPP patients that have undergone liver transplantation. Liver transplantation is life saving and has good long-term effects in EPP. In contrast to a previous report on the US cohort [160], data suggest that disease recurrence in the transplanted liver does not necessarily involve a poor graft prognosis. That all patients did not provide liver biopsy data limits the interpretation to some extent, but graft failure due to recurrence was a rare event in one patient and there were no deaths attributed to recurrence. The study confirms findings in previous case reports and case series concerning EPP specific complications.

What remains obvious is that the mechanisms and determinants of liver injury caused by protoporphyrin remain elusive and that prognostication is difficult. This needs to be delineated if patients suitable for curative HSCT are to be identified early, before the liver fails and a life-saving liver transplantation is required. Effective treatments to prevent and treat cutaneous photosensitivity need to be devised. HSCT is the only curative option in human EPP but is presently mainly an option for patients with progressive liver disease.

5.1 SPECIFIC COMMENTS

5.1.1 Hemin treatment in EPP

Hemin therapy is controversial in the context of EPP-related liver disease and therefore deserves a more detailed discussion.

The current understanding of the detailed effects of hemin therapy is mainly derived from non-EPP studies. In the acute hepatic porphyrias, hemin therapy mainly aims to inhibit hepatic ALAS1 to suppress the over-production of porphyrin precursors. Hemin also repletes deficient pools of hemoproteins, enhances cytochrome P450 activity and has an isoenzyme-specific influence on cytochrome P450 mediated drug metabolism [177-179]. Heme is the prosthetic group in many important liver enzymes and is involved in regulating the amount and activity of cytochrome P450 [180]. A heme-deficient cytochrome can partly re-acquire new heme [181].

There are very limited data supporting that hemin can inhibit ALAS2 synthesis or activity in erythroid cells [17, 18]. Whether hemin even enters the bone marrow is unknown [182].

While the presence of excess heme in cells is highly toxic [183], reduced heme concentrations in non-erythroid cells cause oxidative stress, impaired mitochondrial function, iron accumulation and cell death [184-186]. Heme oxygenase 1 (HO-1) catalyzes heme to generate biliverdin and carbon monoxide (CO). These metabolites protect cells and tissues by interfering with mitogen-activated protein kinase and cGMP pathways [187] and regulates bile formation and bile flow [188]. Possibly, the up-regulation of HO-1 by hemin provides yet another mechanism by which hemin may ameliorate protoporphyrin induced disease processes.

Since hemin probably does not affect erythropoiesis, the rationale for its use in EPP would be to down-regulate hepatic ALAS1 to reduce a possible hepatic contribution to the protoporphyrin surplus, to ameliorate a relative hepatic heme deficiency and protoporphyrin induced inflammation in EPP-related liver disease. It is reasonable to assume that the effects demonstrated in the acute hepatic porphyrias are similar in EPP but this remains to be proven.

No clinical trial has ever been done on hemin therapy in EPP. The evidence of putative effects remains clinical. Thirteen reports describing 23 EPP patients receiving hemin infusions have been published and some of the patients are included in more than one report. Narrowing down to articles presenting biochemical data that may reflect therapeutic effects, seven reports including 11 patients remain [142-144, 148-150] (Study I). These describe the effects of hemin given at different times during the course of EPP-related liver disease, with or without a preceding liver transplantation. Concomitant treatments were often given, yielding ambiguous results.
If reported, a temporal association between hemin infusions and a decrease in protoporphyrin and bilirubin concentrations was often noted. A decrease in erythrocyte protoporphyrin content was seen in three patients but no difference was noted in most. The observed decrease in serum bilirubin suggests improved hepatic function.

Long term effects have been described in three reports [95, 143, 144], in which hemin infusions were given intermittently for 7-10 months. Hemin therapy seems safe and convenient but carries a risk of iron overload. In the case of frequent hemin therapy, as in some cases of acute intermittent porphyria, hyperferritinemia is sometimes seen. Iron positively regulates erythroid ALAS2 [17, 18]. Hemin may thus, if it enters the marrow, even stimulate erythroid heme synthesis in EPP.

Available evidence suggests that hemin acts in the liver but does not reduce erythroid heme synthesis. Biochemical improvement in case reports imply positive effects on liver function and no hemin toxicity has been described. Together with indirect evidence indicating possible hepatic targets for hemin therapy, these data provide a reasonable rationale for using hemin in EPP-related liver disease but clinical trials under close biochemical surveillance are needed to clarify the effects of hemin in EPP.

5.1.2 The role for marrow and liver transplantation in EPP

Although HSCT has been discussed as a potential treatment for many years, it was not utilized until recently. Apart from fear of the inherent HSCT risks, there has been an uncertainty about whether EPP is a strictly erythropoietic porphyria. Possibly there may be individual heterogeneity with significant hepatic contribution to the excess protoporphyrin in some individuals [148]. A temporary [189] or permanent [190] loss of photosensitivity has been described after liver transplantation (LT). If, as suggested by these publications, excess protoporphyrin originates in both the liver and the bone marrow in some patients, erythroid ferrochelatase replacement by HSCT might not be sufficient to cure photosensitivity and prevent future liver disease.

The experience from HSCT was very limited when the patient in Study I underwent HSCT. The first case was reported in 2002 [49]. A woman with adult onset of severe photosensitivity was diagnosed with myelodysplastic syndrome (MDS). HSCT was indicated when she later developed acute myelogenous leukemia. HSCT cured photosensitivity and she became single *FECH* mutation carrier, like her donating brother. In this case [49], the second *FECH* mutation was caused by MDS [48]. She was thus a heterozygous mutation carrier outside the marrow and conclusions about the role of the liver could not be drawn. The case did however pave the way for subsequent cases.

In addition to the case in Study I, four other cases were treated by HSCT around the same time. An American boy was treated with HSCT following LT [191]. A 20-year-old-man in France, a 57-year-old man in England and a 9-year-old-boy in Holland [192] underwent sequential LT and HSCT to prevent recurrent liver disease. HSCT in an American patient after two LT has also been briefly mentioned [160]. In our own case, HSCT was done without antecedent LT after medical reversal of EPP-related liver failure (Study I).

These cases may be used as background for discussing the role for HSCT in EPP (*fig. 15*). In view of the benign nature of uncomplicated EPP, it is not reasonable to use HSCT in the treatment of photosensitivity. Even if reduced conditioning is used, HSCT risks are significant. The role for HSCT in EPP is therefore intimately connected to liver disease [193].

The majority of those who develop liver failure already have cirrhosis [160] which precludes HSCT. In an older patient it may be reasonable to wait for significant recurrence of EPP-related liver disease in the graft after LT, before HSCT is contemplated to save the graft and prolong life. The likelihood of significant recurrence is probably high in the young liver transplant recipient with a long anticipated life span. This justifies the rational of pre-emptive HSCT as secondary prophylaxis. The young American [191], French and Dutch [192] cases illustrate this strategy.

Cholestatic liver failure may strike before advanced liver fibrosis is present, but this appears uncommon. We have seen two such patients in our center (mentioned in Study I). These patients have clearly demonstrated a propensity for potentially fatal EPP-related liver disease. If the failing liver function can be reversed, HSCT should be

contemplated in order to avoid an eventual liver transplantation and possibly subsequent HSCT. Our case (Study I) illustrates this strategy.

Ideally, patients with progressive liver disease are identified before advanced fibrosis that precludes HSCT is present. As discussed above, this is presently not possible. Postulated prognostic risk factors are hampered by inconsistent evidence, but with vigilant surveillance programs [116, 118] [www.porphyria-europe.com] progressive fibrosis might be possible to detect in time.

Different scenarios (*fig.15*) offer possible indications for HSCT in EPP:
1. After reversal of liver failure in patients without advanced liver fibrosis
2. After liver transplantation in older patients with progressive recurrent graft disease
3. After liver transplantation in young patients
4. Patients with progressive liver disease
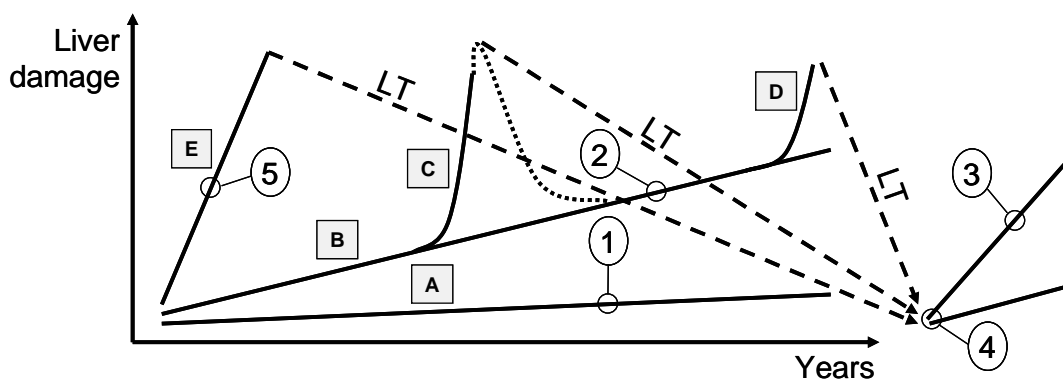
Figure 15
Schematic displaying different scenarios of liver disease in erythropoietic protoporphyria and possible indications for stem cell transplantation

A majority of the patients have no clinically relevant liver disease [A], while a minority have a progressive fibrosis presenting in middle-age or later [B]. Among these, some may develop protoporphyric liver failure before advanced fibrosis is present [C]. Cholestatic liver failure in the cirrhotic liver [D] is a typical scenario for the majority of liver transplanted patients. A small number of patients develop a rapidly progressive fibrosis at young age [E].

Indications for HSCT that may be discussed are indicated by numbers: ① Risks are in our view too high to consider HSCT in uncomplicated EPP. Reasonable indications are: ② Reversed (---) cholestatic liver failure in a patient without advanced fibrosis. ③ Progressive graft disease after liver transplantation. ④ After liver transplantation in the young patient given the long life expectancy and reported high incidence of recurrent disease in the liver graft. ⑤ The ideal candidates for HSCT are the young patients with progressive liver disease, if the condition can be detected before advanced liver fibrosis is present. (Figure reprinted from [193])

The cases above illustrate issues concerning indications, timing and patient selection. If the index case with MDS is excluded for stated reasons, the total experience answering the concern for a significant hepatic contribution to the phenotype is limited to one case (Study I) since this concern is eliminated when serial LT-HSCT is done. Additional cases are needed to alleviate the theoretical concerns regarding HSCT efficacy as mono-therapy. Ideally, HSCT is done in the EPP patient prone for severe liver disease before the need for liver transplantation occurs. Better prognostic markers are needed to identify these patients in time.

5.1.3   The importance of population studies

On a theoretical note, the genotypic and phenotypic heterogeneity and distribution of mutations across a population are essential components for the understanding of a genetic disease and can only be obtained by population studies. Whether cross-sectional or longitudinal, population studies are important to understand the prevalence and incidence of disease related complications. These studies are not only concerned with populations variables but also with the relationships between variables such as psychological, biologic, genetic, geographical variables and also interrelationships between these variables. Both qualitative and quantitative aspects may be included and phenomena that constitute risks for the well-being of the population can be identified and valued.

There is a relative scarcity of population studies in EPP. For low prevalent orphan diseases like EPP, population studies may be especially important to attain a better general understanding of the disease, of both collective and individual features. Clinical samples such as case reports and case series are not always representative for the population and are prone to selection biases.

When the study on Swedish EPP patients (Study IV) was planned, the comprehensive U.K. study [51] was not yet published. Both studies point to a need for information in the medical community and for further study of many aspects.

Study IV has led to a better appreciation of both clinical, genetic, biochemical and epidemiological features of the Swedish EPP population. Already, results from the study have had an impact on counseling and surveillance programs, management and treatment protocols as well as on teaching and research. Results from this population study have been passed on to the EPP population via the patient organization and in the form of news letters. Population education is important for rare diagnoses like EPP, with a lack of knowledgeable physicians, as it may convey a feeling of empowerment to patients. The study noted that many patients did not have a regular health care contact. Many of these have now been referred to nearby physicians with an interest in porphyria. Hopefully Study IV will have national impact enough to address the notably delayed diagnosis of EPP in Sweden.

The enhanced understanding of EPP and the application of the findings in the management practices point to the great value of population studies.

6 CONCLUSIONS

The present studies aimed to clarity some of the questions and perceived limitations in the current knowledge that accompanied the management of the case described in Study I.
Some of these questions have been elucidated.

1. Cholestatic liver failure in EPP is not necessarily an irreversible condition. It may be reversed by applying several concomitant treatment modalities, at least in the non-cirrhotic EPP patient.

2. Hematopoietic stem cell transplantation offers phenotypic cure. This finding confirms the concept of EPP as an erythropoietic protoporphyria.

3. Genotypic correction of the bone marrow alone prevents photosensitivity. The remaining dermal EPP genotype was not enough to cause photosensitivity.

4. Results from studies on EPP mouse models should be interpreted with caution. The EPP mice models differ significantly from human EPP in many ways.

5. In liver transplantation, light filters are necessary and yellow filters that block wavelengths below 470 nm offer a good balance between protection and utility.

6. Surgical luminaires or light sources used in endoscopy, laparoscopy and surgery other than liver transplantation do not cause phototoxic tissue injuries. Recommendations for protective light filters are not warranted in these procedures.

7. The Swedish EPP cohort comprised 51 individuals in 2008. There was a notable delay in diagnosis and quality of life was significantly affected by the disease. The ferrochelatase mutation was identified in all but one patient and nine novel mutations were found. Altered red blood cell indices, iron analyses and liver function tests as well as vitamin D deficiency were common findings. Good ameliorating therapies are lacking and better alternatives are necessary to devise.

8. Liver transplantation is a life-saving treatment for cases with advanced EPP liver disease. Long-term outcome is good but light filters are essential to avoid phototoxic injuries from surgical luminaires and postoperative motor neuropathy is overrepresented in liver transplantation for EPP. Graft disease recurrence is common but not necessarily as ominous a finding as previously thought.

7 FUTURE OUTLOOK

Porphyrias are complex, fascinating and in some aspects unexplored diseases. They provide opportunities for exciting and attractive research projects. Many limitations remain in the current understanding of the porphyrias. My focus for studies in the near future is primarily on hepatic complications in the porphyrias, both in EPP and in acute intermittent porphyria, AIP.

At Karolinska, a network of competent and passionate people from different clinical and preclinical disciplines with an interest in porphyria has evolved in the last few years. This provides a unique possibility for clinical and translational research projects. The Swedish porphyria centre at Karolinska is a centralized national competence centre that provides both national patient registries, a laboratory for biochemical and genetic analyses for the porphyrias and a close cooperation with the patient organization. This also provides excellent opportunities for epidemiological research and national cohort studies.

Some initiated studies represent spin-offs to the work presented in this thesis. One study which is already under way concerns the incidence, prevalence and possible determinants of hepatobiliary complications in EPP. The study on the Swedish cohort has provided a database on which to build this work. Another study concerns the controversial role for hemin, exogenous heme, in the treatment of EPP liver disease. The liver disease in AIP, the high incidence of hepatocellular carcinoma in AIP and studies on an AIP hepatocyte model are areas in which studies are also already under way. Possible secondary complications to deficient vitamin D concentrations in EPP deserve further attention.

The consequence of vitamin D deficiency is a research area that has attracted an increasing amount of attention in the last few years. Hepatology research has lagged behind since the hepatic vitamin D receptor, situated in stellate cells, was discovered only recently. The clinical importance of vitamin D deficiency for hepatic conditions, including the liver disease in EPP, is still largely unclear. This and many other aspects remain to be clarified. I hope to realize at least some of a large number of ideas for studies in this field.

The variable phenotypic expressions in EPP and other porphyrias are interesting topics. Some mutation carriers suffer from sometimes lethal complications while others are virtually asymptomatic. Since the porphyrias are monogenetic diseases, of which some have different distinct phenotypic expressions, they may be especially well suited for study with some of the new research tools that are emerging: e.g. genome-wide association studies, molecular network studies and studies on epigenetic mechanisms. Hopefully the imperfect understanding of determinants for phenotypic expression in the porphyrias will abate in the years to come.

8 SUMMARY IN SWEDISH FOR LAYMEN

Denna avhandling handlar om erytopoetisk protoporfyri (EPP), en ganska ovanlig ärftlig sjukdom. I Sverige finns idag 54 kända fall. Denna bok beskriver i början översiktligt först ämnesområdet; porfyrierna, hemsyntesen och framförallt olika aspekter av EPP. Sedan beskrivs arbetet med och resultaten av de fem studier som ingår som delarbeten i avhandlingen och på slutet finns dessa arbeten med som särtryck.

Porfyri-sjukdomarna, varav EPP är en, beror på bristfällig funktion i något av enzym-stegen i hemsyntesen, den kemiska process i kroppen som leder till bildandet av hem som ingår i t.ex. blodkropparnas hemoglobin, musklernas myoglobin och i en mängd olika viktiga leverproteiner. Vid porfyri-sjukdomarna ansamlas ett överskott i kroppen av något av de ämnen som utgör delsteg i hemsyntesen. Vid EPP ansamlas protoporfyrin.

Protoporfyrin är ett ämne med två speciella egenskaper. Protoporfyrin absorberar energin i blåviolett ljus och kan bara utsöndras från kroppen via levern och gallan. Detta förklarar de två huvudsakliga besvären vid EPP; smärtsam ljuskänslighet hos alla och hos några svår leversjukdom som kräver levertransplantation för överlevnad.

Det första arbetet (*Study I*), beskriver först den framgångsrika behandlingen av allvarlig leversjukdom hos en EPP-patient och den teoretiska bakgrunden till att vi använde just detta nya, sammansatta behandlingskoncept. Behandlingen var på ett unikt sätt framgångsrik och gav en möjlighet att ge en annan behandling som också den var unik för denna situation; benmärgstransplantation (hematopoetisk stamcellstransplantation, HSCT). HSCT var lyckad och ledde till bot från EPP. Efter transplantationen har patientens svåra ljuskänslighet försvunnit och leversjukdomen har aldrig återkommit.
Det var under den långa behandlingstiden och förberedelserna för levertransplantation för denne patient som brister i kunskaperna om EPP noterades och idéerna för de följande studierna i denna avhandling väcktes.

Det andra arbetet (*Study II*), kan ses som dels en uppföljning till det första arbetet eftersom det dels ger information om en lite längre uppföljningstid och dels beskriver hur HSCT verkligen bara korrigerade arvsmassan i benmärgen och inte i huden. Det fyndet står i kontrast till olika studier på EPP-möss där man t.ex. sett att ljuskänsligheten inte bara verkat bero på benmärgens överproduktion av protoporfyrin. Studien diskuterar utifrån vår och övrig publicerad erfarenhet skillnader mellan möss och människor med EPP och varför musforskning måste tolkas med försiktighet.

Det tredje arbetet (*Study III*), handlar om risken för brännskador av operationsljus vid EPP. Användning av skyddande ljusfilter har rekommenderats vid både levertransplantationskirurgi, där man tidigare noterat brännskador på patienter, och vid annan kirurgi. Vi använde information från Sveriges EPP-patienter (se *Study IV*), analyserade ljuset från alla förekommande operationsljus-typer och studerade vävnadsskador av ljus både i en experimentell laboratoriemodell och i en matematisk modell.

Slutsatserna blev att ett gult filter som helt blockerar de blåvioletta våglängderna från operationslamporna är det mest optimala för levertransplantationskirurgi men att ljusfilter inte behöver användas vid annan kirurgi. Laboratoriemodellen fungerade utmärkt för det som den avsåg att studera.

Det fjärde arbetet (*Study IV*), beskriver hela den Svenska patientgruppen med EPP; de 51 fall som var diagnostiserade fram till 2008. Gruppen hade tidigare inte sammanställts och studerats. Vi använde en omfattande enkät och samlade blodprover från alla som ville vara med. För det fåtal som inte kunde eller ville vara med så kompletterade vi med information som redan fanns i Porfyricentrums databas. Vi konstaterade att EPP i hög grad påverkar livskvaliteten hos de drabbade och att ingen bra mildrande behandling finns för flertalet. Vi såg att den vanligaste debutåldern för ljuskänslighetssymptom var det första levnadsåret men att den genomsnittliga åldern då man fick sin diagnos var väl över tjugo år. I den genetiska genomgången hittade vi nio mutationstyper som inte tidigare hade beskrivits i världen. Vi såg att D-vitaminbrist var mycket vanligt och att tecken på järn- och blodbrist var vanligt. Förändringar i leverprover och förekomst av gallstenssjukdom var också vanliga fynd. EPP är en besvärlig sjukdom som är svår att mildra och som tyvärr upptäcks sent. Det finns ett stort behov av information till både sjukvården och patienter liksom ett stort behov av bättre behandlingar.

I den sista och femte studien (*Study V*) så sammanställdes alla levertransplantationer som har gjorts för EPP leversjukdom i Europa. Vi hittade 37 levertransplantationer bland 33 patienter genom att söka bland publicerade artiklar och i det Europeiska levertransplantations-registret. Detta är den största sammanställningen av levertransplantation vid EPP någonsin. I kontrast till en tidigare mindre studie från USA så såg vi att risken för och konsekvenserna av att den nya lever också drabbas av EPP leversjukdom inte verkade så betydande. Överlevnaden efter levertransplantation skiljde sig inte från överlevnaden efter levertransplantation för andra jämförbara sjukdomar. Vi studerade också förekomsten av komplikationer som är specifika för EPP, brännskador av operationsljus och utdragen muskelsvaghet, liksom en rad blodprovsvärden samt olika medicinska behandlingar som använts i anslutning till transplantationen.

Vår förhoppning är att dessa studier kommer att bidra till både ökad kunskap och en bättre behandling av dem som har denna ovanliga sjukdom, inte minst för dem som drabbas av den ovanliga men fruktade leverkomplikationen vid EPP.

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Maire Kotajärvi, for dedicated work with all the mutation analyses

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