SUBMITTED ABSTRACTS

ORAL PRESENTATIONS AND POSTERS

(ALPHABETICALLY SORTED BY FIRST AUTHOR SURNAME)

SHORT-TERM AND LONG-TERM WITHIN-SUBJECT BIOLOGICAL VARIATION OF URINARY ALA, PBG AND PORPHYRINS IN ACUTE INTERMITTENT PORPHYRIA

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Many patients with acute intermittent porphyria (AIP) have a baseline excretion of porphobilinogen (PBG) 10-20 fold the upper reference value even when in remission. In order to distinguish the natural variation of porphyrin excretion from that which is assumed to accompany an acute attack, it is thus important to know the within-subject biological variation of porphyrins and porphyrin precursors in urine.

Fifteen AIP patients without symptoms for the past two years were included. Short-term variation was calculated based on urine samples collected for ten consecutive weeks whereas the long-term variation was calculated based on samples collected through a two year period.

The short-term within-subject biological coefficients of variation (CVBw) of urinary ALA, PBG and total porphyrins per creatinine for latent AIP are 18%, 20% and 28% respectively.

Calculating the level of ALA, PBG and total porphyrins per mmol creatinine significantly reduces the within-subject biological variation. The long-term CVBw of 25.1% for PBG is significantly larger than the short-term, whereas the long-term and short-term CVBw for ALA and total porphyrins are not significantly different. This means that when using the level of urinary PBG in the assessment of AIP-related symptoms, not only must it be compared to the patient's level of urinary PBG when in remission, the time lapse since the previous urine sample must also be taken into consideration.

HUMAN X-LINKED SIDEROBLASTIC ANAEMIA (XLSA) CAUSING MUTATIONS EXPLAINED BY THE CRYSTAL STRUCTURE OF 5-AMINOLEVULINATE SYNTHASE FROM RHODOBACTER CAPSULATUS

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The first dedicated enzyme in heme biosynthesis in humans is 5-aminolevulinate synthase (ALAS). eALAS (e for erythroid) is one of two isoforms of ALAS expressed in mammals and is responsible for approximately 90% of body heme production.

Naturally occurring mutations in human eALAS directly cause a class of porphyrias known as X-linked sideroblastic anaemia (XLSA). These disorders are characterized by inadequate formation of heme and accumulation of iron in erythroblast mitochondria.

Both human and bacterial (*Rhodobacter capsulatus*) ALAS were cloned and overexpressed in *E. coli* and purified to homogeneity. The bacterial ALAS, 50% identical by sequence to its human counterpart, crystallized successfully. The crystal structure was solved and refined to a resolution of 2.1 Å.

The high resolution of the crystal structure of ALAS allows us to locate most naturally occurring mutations with high precision. Hence we can determine why these mutations affect the enzyme efficiency in producing aminolevulinic acid (ALA), a prerequisite intermediate in synthesizing heme.

Several mutations occur in the binding pockets of the two substrates (glycine and succinyl Co-A) and of the cofactor PLP (pyridoxal-5'-phosphate, product of Vitamin B_6). Others are located in the channels leading from the protein surface to the active site, while yet others are located at the interface of the two subunits making up the functional ALAS dimer.

We are therefore now in a position to interpret the clinical XLSA-cases in terms of the three dimensional structure of the enzyme involved. Thereby new impetus is given to finding ways of treating XLSA other than by increasing levels of Vitamin B_6 or the product aminolevulinc acid.

References:

I. Astner, J.O. Schulze, W.-D. Schubert, D. Jahn, D.W. Heinz (2004) Human X-linked sideroblasic anaemia causing mutations explained by crystal structure of 5-aminolevulinate synthase of *Rhodobacter capsulatus*. *In preparation.*

UROD R193P – A NORWEGIAN FOUNDER MUTATION FOR PORPHYRIA CUTANEA TARDA

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The South African PPOX mutation R59W and the HMBS W198X found in the northern parts of Sweden and Norway, are archetypes of founder mutations. In contrast, although the UROD mutation IVS6+1G>C is demonstrated in most published series of PCT patients, UROD mutations are mostly private.

In a Norwegian series of PCT patients, we have so far identified UROD mutations in 86 seemingly unrelated cases. Two mutations made up for some 70% of all Norwegian mutations, viz. IVS6+1G>C (44%) and R193P (27%). The mutation R193P was first described in a single patient by JD Phillips et al in Utah (Blood 2001;98:3179-85), but it has not been reported since. Studies of 17 microsatellite markers in a 9Mb genomic region encompassing the UROD locus, clearly indicated that all R193P mutations found in the Norwegian, as well as the Utah PCT patients, are likely to be identical by descent. It is therefore likely that we are dealing with a Norwegian UROD founder mutation. In preliminary studies, the genetic length of the most common shared haplotype ranged from

less than 3.1cM to more than 6.2cM. The results of extended marker studies as well as attempts at genealogical studies, will be presented.

PORPHYRIA DIAGNOSIS - PROBLEMS AND PITFALLS

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The porphyrias are uncommon so that general physicians as well as specialists rarely acquire familiarity with these disorders. Moreover, in recent times minimal teaching about them occurs in United States medical schools, residencies and fellowships. Thus it is not surprising that those of us more focused on porphyrias encounter the problems of over-diagnosis, misdiagnosis and under-diagnosis. To some extent, misconceptions also surface among patients who surf the web.

This presentation will review the experience with referrals and diagnosis of the various porphyrias in Oklahoma. The data indicate that over-diagnosis and misdiagnosis, with occasional incorrect treatment, have been more common than under-diagnosis. Key factors leading to the former were: borderline test data were over-interpreted, most relevant tests for specific porphyrias were not obtained, or tests were not performed accurately. A diagnosis was mainly overlooked when not thought of. This experience may be mirrored by that of other colleagues and would provide a basis for organizing a referral site with expertise in porphyrias in at least the majority if not in each of the United States as well as for fostering better physician education.

RENAL CLEARANCE OF PLASMA PORPHYRINS IN PCT PATIENTS

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The ratio between renal clearance of porphyrins and creatinine clearance was determined in 18 PCT patients with normal or slightly impaired renal function (plasma creatinine 47 – 187 micromol/L). Plasma concentrations of uroporphyrin ranged from 1.6 to 137 nmol/L. Independently of plasma concentrations, renal clearance of 8-COOH, 7-COOH, 5-COOH and 4-COOH porphyrins was found to be round half of creatinine clearance (clearance ratios: 8-COOH 0.49 (SD 0.25), 7-COOH 0.36 (SD 0.13), 5-COOH 0.27 (SD 0.14), 4-COOH 0.84 (SD 0.61)). Renal clearance of 6-COOH porphyrin was significantly lower ($p < 0.05$) than renal clearance of the other porphyrins and only 8% of that of creatnine (clearance ratio: 0.08 (SD 0.04)).

EFFECT OF HIGH LEVELS OF HEMIN ON THE ANTIOXIDANT DEFENSE SYSTEM OF *TRYPANOSOMA CRUZI*

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Trypanosomatid protozoa need heme compounds for growth in vitro. For T. cruzi epimastigotes grown under different hemin concentrations in the medium, we have previously observed that 5 mg/l hemin was the concentration yielding optimum growth, whilst concentrations above 15 mg/l clearly decreased growth rate, producing undesiderable morphologic changes.

Because porphyrins are an important source of oxidative stress in biologycal systems, we have evaluated the oxidant response to the presence of high levels of hemin in the culture medium of T. cruzi. Employing concentrations between 0-30 mg/l we have measured: 1) total protein content, 2) the antioxidant defense system including the enzymes: superoxide dismutase (SOD), ascorbate peroxidase (APx), trypanothione reductase (TryR) and total thiols content.

Level of total proteins was determined for hemin concentrations 0, 10 and 30 mg/l between 3-7 days of growth. Without hemin, protein concentration progressively increased from 6.41 ± 0.18 to 8.73 ± 0.25 $\mu g/10^6$cel, whilst for hemin 10 mg/l the values were 10-15% higher, showing a maximum level at day 5 of growth, without changing thereafter. For hemin 30 mg/l a different behaviour was obtained, the concentration of proteins increased within 3-5 days (from 9.26 ± 0.10 to 10.12 ± 0.08 $\mu g/10^6$cel), and then progressively decrease to 7.24 ± 0.25 $\mu g/10^6$cel. The antioxidant enzymes showed a maximum activity at hemin 5 mg/l (SOD 4.50 ± 0.05 UE/mg, APx 9.50 ± 0.04 UE/mg and TryR 3.72 ± 0.08 UE/mg). For hemin 5-30 mg/l TryR and SOD activity decreased about 30-35% whilst APx activity diminished 20%. Thiols levels showed a constant value (15.53 ± 0.89 nmoles/mg prot) for hemin 0-10 mg/l and then decreased to 9.54 ± 0.46 nmoles /mg prot for hemin 30 mg/l.

These findings provide strong evidence for the existence of a direct correlation between high extracellular hemin concentrations and cellular oxidative damage.

ACTIVE SITE OF HUMAN FERROCHELATASE

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Ferrochelatase catalyzes the terminal step in heme biosynthesis, the insertion of ferrous iron into protoporphyrin to form protoheme IX. In eukaryotes this enzyme is bound to the matrix side of the inner mitochondrial membrane. In animal cells ferrochelatase is a

homodimer with monomer molecular weight of approximately 43,000. It possesses a [2Fe-2S] cluster coordinated by four cysteine residues. The crystal structure of human ferrochelatase has previously been determined at 2.0 Á and clearly demonstrated the presence of the active site pocket that is present on a hydrophobic face of the molecule that is proposed to face the mitochondrial membrane

Currently two catalytic models for ferrochelatase exist. One proposes that deprotonation of the macrocycle and iron insertion are catalyzed by a single conserved active site histidine (H263). The second model proposes that deprotonation occurs via the histidine, but that metallation occurs from the opposite side of the active site pocket. Both models suggest that the metallation reaction involves distortion of the porphyrin macrocycle.

Here we have examined the crystal structures of three mutants of human ferrochelatase : H263C, H341C, and F337A. The mutant H263C has no enzyme activity while the other two mutants have significantly decreased activity. Mutation of either H263 or H341 results in the reorientation of M76, R164, H341, E343 and F337 in the active site. However, in the mutant F337A these residues retain their wild-type positions.

We propose that a hydrogen bond network existing among residues H263, H341 and E343, which is disrupted by mutation of any of these residues, is also disrupted during the normal catalytic cycle when H263 abstracts the pyrrolic proton from an incoming porphyrin molecule. Upon protonation of H263, the hydrogen bond between H263 and E343 is broken. This allows E343 and its hydrogen bonded partner, H341, to reorient. As H341 swings to one side, F337, which is normally restrained from movement by the presence of the side chain of H341, swings into the active site pocket. We propose that the resultant movement of F337 causes the distortion of the macrocycle and allows iron insertion.

THE IRISH NATIONAL PORPHYRIA DATABASE

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At present the Biochemistry Department, St James's Hospital, Dublin is the only laboratory providing a comprehensive porphyria diagnostic service for the Republic of Ireland (ROI). The laboratory, with over 30 years of biochemical and clinical diagnostic experience, retains the most comprehensive record relating to the nature and extent of porphyria in ROI. Consequently, to coordinate this archive, a bespoke database was commissioned with very specific functional requirements. These included (1) a standard patient module with unique family identification (2) family tree generation facility (3) porphyrin result module (4) capability to integrate with the current hospital and laboratory information systems, thus allowing biochemical result and clinical data download (5) capability to interface with current analytical systems (6) word processing feature and (7) access to current acute porphyria safe drug list. Wren Computing developed a system based on these criteria. Our database contains a full audit trail and is stored on a Microsoft SQL 2000 server with inbuilt FTP capability for data import. Analytical traces can be directly captured from laboratory screens and historical data can be scanned into the system. The family tree facility is highly visual in a rich windows environment. The presentation will elaborate on the practical usefulness of this system.

REVIEW OF PORPHYRIA IN THE REPUBLIC OF IRELAND

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Porphyrias are a heterogenous group of inherited disorders of haem biosynthesis, in which the clinical manifestations can vary from life-threatening acute neurovisceral episodes to photosensitive cutaneous lesions. The prevalence of each porphyria varies between ethnic groups, and also shows extreme variation between the different types of porphyria. Thus, it is important for the optimal clinical management of these disorders that the clinical, biochemical and molecular characteristics of porphyria is defined for each specific population. At present the Biochemistry Department, St James's Hospital, Dublin is the only laboratory offering a specific porphyria diagnostic service for the Republic of Ireland (ROI). The Porphyrin Laboratory, with over 30 years of biochemical and clinical details relating to all diagnosed porphyria probands, and subsequent pedigree studies, has the most comprehensive record concerning porphyria in ROI. Recently, a bespoke database was configured to manage this invaluable archive. In this presentation we will outline the current knowledge relating to the nature and extent of porphyrias in the unique ecosystem of ROI. A brief summary is provided in Table 1.

Table 1:

Diagnosis	Families/Cases	Genetic Diagnosis
Porphyria Cutanea Tarda (PCT)	150	1
Acute intermittent porphyria (AIP)	16	2
Variegate Porphyria (VP)	9	1
Hereditary Coproporphyria (HCP)	6	2
Erythropoietic Protoporphyria (EPP)	12	2
Congenital Erythropoietic Porphyria (CEP)	1	
Total	194	7

ANCESTRAL FOUNDER OF MUTATION R116W IN THE PORPHOBILINOGEN DEAMINASE GENE AMONG DUTCH ACUTE INTERMITTENT PORPHYRIA PATIENTS

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Introduction: Acute intermittent porphyria (AIP), the most common acute hepatic porphyria, is an autosomal dominant inborn disorder of heme biosynthesis caused by mutations in the porphobilinogen deaminase (PBGD) gene. The prevalence of AIP in Europe is estimated as 1/10.000-1/20.000.

The majority of the known AIP mutations are restricted to only one or just a few AIP families, however with the exception of the frequent occurring R116W mutation which is found in 19/80 Dutch AIP families. This mutation has also been reported in 6 other populations (Norway, Sweden, Finland, France, Spain and South Africa). Recent haplotype analysis of Norwegian and Swedish patients with the R116W mutation show high heterogeneity. The conclusion of that report is that this mutation is abundant due a high mutability of CpG dinucleotides. The Dutch R116W families are well documented with extended pedigrees (up to 1750) which makes it possible to study the haplotypes in these families.

Aim of this study: Haplotyping of the PBG-deaminase gene in Dutch R116W families.

Methods: To investigate the haplotype heterogeneity of the Dutch R116W families, intragenic single nucleotide polymorphism's (SNPs) which cover the whole PBGD gene of 8.6 kb were selected. In addition to the intragenic SNPs, microsatellite markers were selected, flanking the genomic region of the PBGD gene covering a distance of 7.48cM in chromosome 11(Schneider-Yin, X et al, Hum Hered 2002;54:69-81).

The 7 SNPs were first analysed in 4 out of 19 R116W families selected for their most complete and informative pedigree. The 7 analysed SNPs revealed a distinctive R116W haplotype and were used to analyse the other 14 families in this study cohort, which mainly consisted of DNA from single patients or families with limited members.

Results: The informative SNPs reveal a distinctive haplotype which segregates with the R116W mutation present in the Dutch AIP families (-64T, 1345 G, 2479 G, 3581 G, 6479 T, 7064 C and 8578 A).The microsatellite haplotype was found less conserved observed in these AIP families.

Conclusion: This common R116W haplotype based on 7 SNP's strongly suggest that the relatively high frequency of the R116W mutation in Dutch AIP patients is due a founder effect (eldest parent in pedigree is born in 1750 !!).

The high mutability of CpG dinucleotides, as suggested in previous studies, is not a likely explanation for the abundant presence of the R116W mutation, since it is only reported in a few western countries.

The haplotype heterogeneity described in the Sweden and Norwegian patients and the homogeneity found in the Dutch R116W carriers is compatible with a mutation from Scandinavia founded in the Netherlands.

Due to the high frequency of this R116W mutation within the Dutch AIP families it may be applied to refine estimations of the prevalence of AIP in The Netherlands.

AUTOSOMAL RECESSIVE ERYTHROPOIETIC PROTOPORPHYRIA WITHOUT IVS3-48C IN TWO BROTHERS WITH LIVER DYSFUNCTION IN EARLY CHILDHOOD

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Erythropoietic protoporphyria (EPP) is characterized by acute photosensitivity of sun-exposed skin from childhood. It results from partial deficiency of ferrochelatase (FECH), which leads to accumulation of protoporphyrin IX. Most patients with EPP have the combination of a low expression *FECH* allele (IVS3-48C/T in intron 3) *trans* to a severe mutation. Mutations on both alleles have been identified in only a small number of patients.

Case reports: A young boy (A) presented at 3 years of age with painful erythema and swelling of sun-exposed skin during the summer months. There was no family history of photosensitivity. On examination, there was erythema of the face and back of the hands with areas of scarring and hypopigmentation. His erythrocyte protoporphyrin (PP) concentration was increased. His younger brother (B) also presented with similar complaints during the summer months from the 1st year of age. He too was found to have varioliform scars on his face and hands. Sunblock creams and protective clothing were advised and beta-carotene prescribed. Although initially normal, biochemical tests of liver function became progressively abnormal at 3 and 2 years of age respectively. Erythrocyte PP concentrations, *FECH* gene analysis, FECH activity in lymphoblastoid cell lines and transaminase levels are given in the table.

	Ery. PP µmol/l	FECH pmol/mg	IVS3-48	*FECH* allele 1	*FECH* allele 2	AST U/l	ALT U/l
A	112,8	78	tt	Cys441Phe	His338Asn	163	259
B	136	46	tt	Cys441Phe	His338Asn	161	205
Mother	1,5	404	tt	Cys441Phe	n	-	-
Father	1,6	469	tt	n	His338Asn	-	-
Controls	< 1,5	>355	(tt)	n	n	< 56	< 39

Neither child, nor their parents, has the *FECH* IVS3-48C low expression allele. Both children are compound heterozygotes for mutations in the *FECH* gene, while their parents are carriers of one mutation each. Both children have markedly reduced FECH activity, and high erythrocyte free PP levels. In addition both children have a microcytic anemia (hemoglobin 6,6-6,8 mmol/l, n = 6,8 – 8,1; MCV 65 fL, n = 75 – 93), and low ferritin levels (12-13 µg/l, n = 30 - 240).

Conclusion: These two children have severe EPP, with markedly reduced ferrochelatase activity and abnormal liver function tests developing at a very early age. These findings support the suggestion that autosomal recessive EPP might carry a greater risk for liver damage than the more usual combination of a mutation and a low expression allele.

EUROPEAN PORPHYRIA INITIATIVE (EPI): A PLATFORM TO DEVELOP A COMMON APPROACH TO THE MANAGEMENT OF PORPHYRIAS AND TO PROMOTE RESEARCH IN THE FIELD.

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Porphyrias are uncommon inherited diseases of haem biosynthesis for which the diagnosis and treatment varies in individual countries. Despite the existence of guidelines, recommended by porphyria experts, concerning the diagnosis and management of the acute porphyrias, and of specialist centres in most European countries, many clinicians still do not apply these guidelines. The European Porphyia Initiative (EPI) network was formed in 2001 in order to compare experience between countries, attempt to develop a common approach to the management of the porphyrias, particularly concerning recommendation of safe and unsafe drugs, and to facilitate international collaborative clinical and biological research.

The main achievements of EPI during this period have been:

- Drafting and agreeing consensus protocols for the diagnosis and management of acute hepatic porphyrias
- Creation of a multilingual website, particularly focusing on guidelines for common prescribing problems in acute porphyria and on providing information for patients that is now available in 10 languages: www.porphyria-europe.org.

EPI's current objectives are to develop the EPI platform, expand to new countries, extend to non-acute porphyrias and design European research and clinical trials in porphyria. The project will focus on:

1. Setting up a European laboratory external quality assurance scheme (EQAS) for biochemical and molecular investigations and their interpretation
2. Establishing a consensus drug list in collaboration with the Nordic porphyria network
3. Improving patient counseling.
4. Developing large multi-centre, multi-national research projects. Due to the rareness of the porphyrias, it would be very difficult for any one country to provide this data, with a sufficient number of patients and within a reasonable timescale.

The progress achieved will facilitate improvements in treatment and the development of new therapeutic strategies. It will set a pattern for establishing, and subsequently harmonising, between countries, best clinical practice for a rare but important group of diseases, and will help to develop the optimal therapy and ensure its cost effectiveness.

ALA-PDT SENSITIVITY OF CELL LINES RESISTANT TO NITRIC OXIDE CYTOTOXICITY

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In an attempt to elucidate interactions between aminolevulinic acid-based photodynamic therapy (ALA-PDT) and nitric oxide (NO) cytotoxicity, we employed a murine mammary adenocarcinoma cell line LM3 and the NO resistant variant LM3-SNP.

LM3 –SNP cells were more sensitive to ALA-PDT treatment, and by multiple exposures to ALA-PDT treatment, two cell lines resistant to ALA-PDT identified as Clon 1 and Clon 3 were isolated, which were 9.2 and 12.5 times more resistant to the photosensitizing treatment than the parental cells.

In addition, the degree of resistance to the NO donor sodium nitroprussiate (SNP) was similar to that obtained for the parental LM3 line, and they did not show modifications in NO production, although the sensitivity to NO decreased in Clon 1 and increased in Clon3.

These results are indicating that resistance to ALA-PDT is not correlated with different degrees of resistance to NO toxicity, suggesting that NO resistance and ALA-PDT resistance are controlled by independent pathways.

PORPHYRIA CONSULTATION: FORTY YEAR EXPERIENCE IN GERMANY

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Starting porphyria research in1965 with in vitro and in vivo systems, an investigative diagnostic laboratory was established with a scientific and consiliary approach.

Acute versus non-acute hepatic porphyrias: In a forty-year period 840 patients with acute hepatic porphyrias were diagnosed (female:male ~ 2:1): AIP > variegate porphyria > hereditary coproporphyria > doss porphyria (compound heterozygous); relation 81 : 12 : 7 : 0.3. In ~5% of AIP cases RBC PBG deaminase activity was normal reflecting a subtype with normal PBG deaminase gene in erythrocytes. Primary liver tumor was found in 5 patients with acute hepatic porphyrias.

Only the metabolite excess reflects the pathobiochemical disease process and clinical expression of an acute or non-acute porphyria. The severity of clinical symptoms correlates with the dimension of metabolic aberration. Both for acute and non-acute porphyrias a phase concept was established, recognizing the stage of the subclinical and clinical disease process. Regulatory treatment of the acute hepatic porphyria syndrome by glucose and/or heme was highly effective, but fails in patients with persistent pareses. The background of this complicated courses was belated diagnosis in most cases. Latent phases and ovulocyclic forms were stabilized by intervall therapy with heme arginate.

60 cases of severe acute and chronic lead intoxication were detected. Most of them reached us primarily misdiagnosed as AIP or HCP due to a similar acute clinical syndrome.

PCT or chronic hepatic porphyria associated with liver injury was observed two times more than acute hepatic porphyrias. Acute porphyrias are induced by cumulatively acting manifestation factors as drugs, fasting, sex hormones, whereas PCT is triggered by alcohol, estrogens, iron and hepatitis virus infection. Homozygous PCT as hepatoerythropoietic porphyria was observed in three males.

Erythropoietic porphyrias: A quarter of patients with protopophyria develop hepatobiliary liver involvement ranging from mild to severe cholestatic liver disease as studied from 170 cases in the forty year period. About 10% of the patients developed protoporphyrin-induced cholestatic liver cirrhosis with excessive protoporphyrinemia, isomer I-coproporphyrinuria and decrease of fecal protoporhyrin. 11 patients underwent liver transplantation between 1987 and 2002. According to protoporphyria also in congenital erythropoetic porphyria (Günther's disease: 30 cases) an interdependence between degree of porphyrin excess and disease severity has been elucidated. Two children with Günther`s disease were treated succesfully by bone marrow transplantation.

Dual porphyrias: Four types of dual porphyrias with the coexistence of two different enzyme deficiencies in one patient were investigated. Family studies have shown that they do not segregate together. Three dual forms were described first: AIP and PCT, CEP and HCP as well as PCT and HCP.

Porphyrinurias: Coproporphyrin isomer studies are important for the early diagnosis of the hepatobiliary involvement in protoporphyria, for the recognition of gene carriers of VP and HCP, and for the characteriziation of hereditary hyperbilirubinurias.

Secondary coproporphyrinurias were observed four times more than acute porphyrias. They lack clinical significance, but represent the most frequent diagnostic pitfall suggesting porphyria.

Molecular genetics: Molecular genetic analyses of the genes from all enzymes of the heme biosynthetic chain were carried out. The aims were ensuring the diagnosis, family studies, detection of compound heterozygosity, investigation of AIP family members with normal RBC PBG deaminase or normal metabolite excretion and analysing the mutations of the genes from the mitochondrial enzymes in HCP, VP and protoporphyria. Molecular heterogeneity is evident in all types of porphyrias.

Conclusion: A competence center for porphyria investigation and consultation should be responsible for elaborationg and ensuring the diagnosis porphyria as well as for therapeutic management. (www.porphyria.com).

ERYTHROPOIETIC PORPHYRIA AND HAEMATOLOGICAL MALIGNANCY.

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Rare patients have been reported in whom the late onset of an erythropoietic porphyria has occurred in association with the preleukaemic disorders, myelodysplasia (MDS) and myeloproliferative disorder. We have investigated 4 patients aged over 40 years who developed cutaneous porphyria and overproduction of uroporphyrin I and coproporphyrin I, in a pattern typical of congenital erythropoietic porphyria (CEP), in association with MDS or myelofibrosis.

Findings in these patients have been compared with those in patients with classical CEP. Features distinguishing the 4 patients with late-onset disease from classical CEP were haemorrhagic bullae and thrombocytopenia, lower erythrocyte porphyrin concentrations (mean 4.2 μmol/L vs. 17.5 μmol/L), normal erythrocyte uroporphyrinogen III synthase (UROS) activity, and absence of mutations in the *UROS* gene in germline DNA. Examination of bone marrow aspirates showed porphyrin fluorescence in some erythroid cells. No abnormalities of chromosome 10 were detected by conventional bone marrow cytogenetics. No *UROS* mutation was identified by sequencing DNA extracted from unfractionated bone marrow. Only a minority of erythroid blast forming units cultured from peripheral blood showed porphyrin fluorescence, indicating that porphyrin overproduction was restricted to a minor clone of erythropoietic cells. These observations characterize the form of late-onset erythropoietic porphyria associated with preleukaemic disorders as a distinct syndrome, analogous to other disorders, such as the α-thalassaemia-myelodysplastic syndrome, in which a normally inherited condition arises in association with MDS. Like them it is probably caused by expansion of a clone of haematopoietic cells containing an acquired somatic mutation that has arisen as a consequence of the genomic instability that is one of the characteristic features of these haematological disorders.

QUANTITATION OF PLASMA PORPHYRINS:VALIDATION OF LIQUID-LIQUID EXTRACTION METHOD

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We have developed a sensitive and specific method for the measurement of porphyrins in plasma by a one-step liquid-liquid extraction followed by HPLC separation with fluorometric detection. The method has been validated in terms of extraction recovery, linearity, detection limit, analytical impression and sample stability.

Stock solutions of the I or III isomers of uroporphyrin, heptacarboxylporhyrin, hexacarboxyl-porphyrin, pentacarboxylporphyrin and coproporphyrin (Frontier Scientific Inc. Logan Utah, USA) were prepared by dissolving each compound in concentrated HCl and then diluted by water to a final concentration of 1 mol/L HCl. To prepare calibrators the I or III isomer porphyrins were spiked in plasma in concentrations from 0 to 500 nmol/L and pH was adjusted to 7.40.

Porphyrins were extracted by adding 250 µL dimethylsulfoxide and 250 µL 15% thrichloroacetic acid to 500 µL sample (calibrator or patient sample) in a glass tube. The mixture was vortexed for 1 min and then centrifuged at 3000 g for 10 min.

The assay was linear from 0 - 500 nmol/L and the detection limit was 1.0 nmol/L.

Extraction recovery of porphyrins from plasma and albumin solutions (30-50 g/L) was 0.97 (uroporphyrins), 0.94 (heptacarboxylporhyrins), 0.87 (hexacarboxylporphyrins), 0.74 (pentacarboxylporphyrins) and 0.50 (coproporphyrins).

The analytical imprecission (CV) based on duplicate measurements was found to be <5%.

Plasma porphyrins were stable for 3 days at 20°C, 6 days at 4°C and at least one month at –20°C when stored in dark.

VARIEGATE PORPHYRIA IN ARGENTINEAN POPULATION. CHARACTERIZARION OF THE MOLECULAR DEFECT

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Variegate Porphyria (VP) is a low penetrance, autosomal dominant disorder of heme biosynthesis that results from partial deficiency of Protoporphyrinogen oxidase (PPOX). At present 120 different mutations in PPOX gene causing VP have been described. VP prevalence is much higher in South Africa (1:300) due to a founder effect. In Europe, the higher prevalences are found in Finland (2:100.000) and Sweden (1:100.000).

Up to date, about 60 individuals representing 49 apparently unrelated Argentinean families have been biochemically diagnosed with VP, giving a prevalence of approximately 1:600.000 inhabitants.

Genetic studies have been performed in 15 of these families. Sequencing analysis identified 8 different mutations in 7 of 12 probands. In other 3 families the mutation was not yet found. Three nucleotide substitutions (R168H, L178V and H106P), a small deletion (delG745), a small insertion (insT1320) and 3 splicing defects (G810→A, G749→A, g3912→c) were found. R168H and delG745 mutations have been previously reported. The occurrence of the missense mutation R168H had been reported in American (Chile), German and Dutch families, representing the first demonstrable hot spot mutation in VP. All mutations were each specific for an individual family except the small insertion (insT1320) which was found in 5 unrelated families suggesting that it might represent a common mutation in Argentina. So, the initial screening to elucidate the genetic defect in VP patients from Argentina includes the insertion search. Haplotype analysis should still be performed to elucidate if the high occurrence of this mutation would be due to a founder effect.

Molecular analysis in those available family members revealed 5 adults and 4 children who were silent carriers of VP trait assessing that molecular techniques represent the most accurate approach to identify unaffected carriers and to also provide accurate genetic counseling for asymptomatic affected individuals.

EFFECTS OF REPEATED ADMINISTRATION WITH CP-55,940, A CANNABINOID CB_1 RECEPTOR AGONIST ON THE METABOLISM OF THE HEPATIC HEME

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Drugs metabolised by cythochrome P450s such as analgesics may induce acute attacks in patients with hepatic porphyrias. In recent years, preclinical and clinical studies suggest that cannabinoid pharmaceutical preparations may potentially be considered to treat pain. The purpose of the study was to examine the effects of CP-55,940, a cannabinoid CB1 receptor agonist, on the hepatic heme metabolism in mice.

To this aim, hepatic activities of aminolevulinic acid synthase (ALAS1) and heme oxygenase (HO), and cytochrome P-450s (CYPs) levels were determined in mice treated with CP-55,940 (0.5 mg/kg/day; i.p.; 5 or 24 days) .

The results revealed that treatment with CP-55,940 decreased (80%) CYPs concentrations and increased (158%) HO activity, however, ALAS1 activity decreased (37%) suggesting that regulatory free heme pool was not modified.

Our findings suggest that CP-55,940 and its metabolites do not behave as porphyrinogenic drugs and may potentially be safe to treat pain in patients with acute porphyrias.

CONTRIBUTION OF A COMMON SINGLE SNP TO THE GENETIC PREDISPOSITION TO ERYTHROPOIETIC PROTOPORPHYRIA

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Erythropoietic protoporphyria (EPP) is an inherited disorder of heme biosynthesis resulting from partial deficiency of ferrochelatase (FECH) and characterized by early onset of lifelong acute photosensitivity. Individuals who are heterozygous for a FECH mutation are asymptomatic, despite having half-normal FECH activity. Recently, we have shown that the inheritance of the common SNP IVS3-48C allele trans to the mutation appears to explain photosensitivity in most EPP patients. Then, FECH activity is reduced below a critical threshold of about 35% of normal. We evaluate the contribution to the occurrence of photosensitivity of the IVS3-48C allele compared to alternative mechanisms in the 113 unrelated French Caucasian EPP families. We also studied the frequency of IVS3-48C allele in 347 unrelated healthy subjects from 4 ethnically diverse human populations and examined whether this allelic frequency may influence the prevalence of clinical EPP in a specific population. Haplotypes analysis of alleles carrying the IVS3-48C allele was performed, the phylogenic origin of these haplotypes was determined and the level of functional constraint of the FECH gene was estimated. In the EPP cohort, the autosomal dominant disease with co-inheritance of the IVS3-48C allele is the usual explanation for overt EPP in 95 % of the cases (95 % confidence interval: 93 to 98). The IVS3-48C allele is not involved in 6 families: autosomal recessive disease is found in 4 families, the co-inheritance of a deleterious mutation and a mild Y191H mutation is found in one family, and partial hepatectomy is a co-precipitating factor in one overt EPP patient. The frequency of the IVS3-48C allele widely differs between East Asian (35%), European Caucasian (11%), North African (2.3%), and West African (<1%) populations. EPP prevalence strongly correlates to IVS3-48C allele frequency in Caucasian population. This IVS3-48C allele frequency should be related to most EPP cases reported in Asia and could explain the absence of EPP patients reported so far in black Afrikaners. Haplotypes analysis based on 12 polymorphic loci identified five common haplotypes closely linked altogether. The phylogenic origin of these haplotypes strongly suggest that the IVS3-48C allele originates from a recent single mutational event. The alignment of orthologous FECH sequences between human and P. troglodytes chimpanzee indicates that FECH gene is under strong negative functional constraint. Altogether these results improve the risk prediction and genetic counselling in EPP families and shed new light on genetic predisposition to a Mendelian disorder which depends on the frequency of a single common polymorphism in the population under study.

CONGENITAL ERYTHROPOIETIC PROTOPORPHYRIA: APPARENT AMELIORATION OF SYMPTOMS AND SUCCESSFUL PREGNANCY

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Congenital erythropoietic porphyria (CEP) is a rare autosomal recessive disorder of haem biosynthesis that usually presents in infancy with severe life-long bullous photosensitivity. We report the case of a female patient who presented in infancy but whose condition has subsequently proven to be a milder phenotype, and who has recently been through an uncomplicated pregnancy and surgical delivery.

The patient was noted to pass pink urine from birth and suffered from severe erythema and blistering after sun exposure. CEP was confirmed at six months with an elevated plasma porphyrin of 6992nmol/l. Mutational analysis identified two mutations in the uroporphyrinogen III synthase gene; C73R and IVS8-23A→G. Severe photosensitivity occurred throughout childhood and extreme photo-protective measures were used including reglazing the house with glass opaque to UVA and short wave visible light, wearing full head and face-mask. Other treatment modalities tried were, ß-carotene, narrowband UVB desensitisation and hypertransfusion but all were unsuccessful. Bone marrow transplant was considered but not attempted due to unacceptable risk and lack of a suitable donor. At age 15, she reported reduced photosensitivity although her plasma porphyrin remained elevated. She became pregnant at age 19 and had an elective caesarean section for breech presentation following an uncomplicated pregnancy. Care was taken to minimise light exposure to the patient and her infant. Plasma porphyrin on cord blood was elevated but had decreased significantly after 48-hours.

PLASMA AND URINARY HEME PRECURSOR PATTERNS AFTER I.V. RHPBGD ADMINISTRATION TO ASYMPTOMATIC PBGD-DEFICIENT SUBJECTS WITH HIGH EXCRETION OF URINARY PBG

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The combined Phase I and II trials demonstrating the safety, tolerability and pharmacodynamics of single and repeated doses of i.v. rhPBGD were reported in Prague 2003 (Sardh et al.). It was shown that i.v. administration of rhPBGD produced an instant and almost complete removal of PBG in plasma, lasting for at least 2 hours. Hereby we present the second part of the trials, studying the fate of plasma PBG.

Results: After i.v. administration of rhPBGD the rate of reduction in plasma PBG concentration corresponded closely to the rate of reduction in urinary PBG concentration. No effects were noted on plasma or urinary ALA concentrations. The concentrations of plasma and urinary porphyrins increased, reaching the highest peak at 1 hour after i.v. rhPBGD administration. The principal porphyrin moiety was uroporphyrin I.

Conclusion: The increase in the amounts of porphyrins in plasma and urine was synchronized (with some delay) with the disappearance of PBG from plasma and urine. The increment in plasma porphyrin is interpreted as a result of condensation of surplus PBG. The plasma and urinary porphyrin concentrations returned to normal levels within 24 hours, suggesting a complete renal clearance of porphyrins formed.

BIOCHEMICAL AND GENETIC CHARACTERIZATION OF FOUR CASES OF HEREDITARY COPROPORPHYRIA IN SPAIN

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Hereditary coproporphyria (HCP) is a rare disease that results from the inheritance of mutations in the CPO gene that encodes the mitochondrial enzyme, coproporphyrinogen oxidase. We report four cases of HCP in Spain. The initial biochemical analyses showed in all cases an increased excretion of fecal coproporphyrin and an inverted isomeric ratio (I:III < 1). Two of the patients showed increased urinary excretion of heme precursors porphobilinogen (PBG) and aminolevulinic acid (ALA) at the moment of the diagnosis and were treated with heme arginate. The other two, including a patient with the highest excretion of coproporphyrin III, showed PBG and ALA within normal limits at the moment of the diagnosis and during the follow-up. The existence of patients with a highly increased coproporphyrin accumulation and consistently low PBG and ALA over the time, suggests a complex mechanistic interdependence between both phenomena. The biochemical HCP profile was confirmed by a molecular analysis of the CPO gene that revealed the existence in three cases of novel mutations : V135A (404T>C; exon 1); L214R (641T>G, exon 2) and P249R (746C>G; exon 3) and in a fourth case of a previously described R426X mutation (1276C>T) in exon 6. Further biochemical (ratio coproporphyrin III:I in feces) and genetic analyses allowed to detect several non-symptomatic carriers among the close relatives. This is the first study to report a full biochemical and genetic characterization of HCP cases in Spain. (supported by grant FIS/03/ 0489/Spain).

UNITED KINGDOM PROSPECTIVE CLINICOPATHOLOGICAL STUDY ON ERYTHROPOIETIC PROTOPORPHYRIA

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There are no large-scale, prospective studies on erythropoietic protoporphyria (EPP). Previous research examining liver dysfunction in EPP is hard to interpret due to retrospective design of studies, small sample size and/or selection bias. The aim of this UK-wide study was to collate clinical and biochemical features (molecular features, reported elsewhere) in a large cohort of EPP patients in order to better define the condition and to identify possible risk factors for liver disease. Ethical approval was obtained prior to the onset of the study. Patients with biochemically proven EPP were identified from records held at units in Cardiff, Dundee, Leeds, London and Manchester. All study subjects were seen by a single study investigator. 223 EPP patients (114 female; 109 male) were recruited to the study over a six-month period, 41 of whom were siblings and 6 other relations. Median age was 34 years (range 5-87 years). 203 complete blood sample sets were available for analysis. After initial descriptive statistics, six patients' results were withdrawn from further analysis due to: metastatic malignancy (one colonic carcinoma, one testicular seminoma), liver failure, orthotic liver transplant recipient, and two pronounced alcoholic liver disease. All independent factors were analysed using Spearman's correlation coefficient for continuous quantitative data, or the Mann-Whitney test for nominal data, to identify significant associations (where significance was taken to be $p<0.05$, or where the association approached significance, $p<0.1$). Linear regression analysis was subsequently used to investigate associations between these factors. 34% of individuals were anaemic, affecting similar proportions of males and females (34 females, 36 males), 37% had a low MCV, and 47% a low MCH. A related-samples t-test showed significance between low haemoglobin and MCV ($p<0.005$) and MCH ($p<0.005$). Low ferritin was seen in 42% individuals, low serum iron and transferrin saturation in 35%, and a raised TIBC in 17%. Low ferritin ($p<0.0005$), low serum iron ($p=0.027$) and low transferrin saturation ($p=0.019$) were all significantly associated with anaemia. Abnormal liver function, taken to be an elevated ALT, was seen in 15% individuals. It was significantly associated with male gender ($p>0.0005$), total porphyrin ($p=0.016$), haemoglobin ($p>0.0005$), ferritin ($p=0.006$), albumin ($p=0.009$) and γGT ($p<0.0005$).

In summary, this study has identified abnormal liver function in 15% of subjects. Male gender, raised γGT and total porphyrin were all significantly associated with abnormal liver function. More surprisingly, haematological features of iron deficiency anaemia were identified in one third of cases, with no sex difference. The occurrence of iron deficiency anaemia in males is highly unusual, and suggests a fundamental defect in iron metabolism in EPP. However, it is unclear from this study what role, if any, iron is playing in the abnormal liver function seen in EPP.

UNITED KINGDOM PROSPECTIVE STUDY ON ERYTHROPOIETIC PROTOPORPHYRIA: QUALITY OF LIFE ASSESSMENT

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Skin conditions may profoundly affect quality of life (QOL) through disruption of relationships, and interference with sport, leisure and work. Despite advances in understanding the molecular genetics and treatment of erythropoietic protoporphyria (EPP), little has done to assess the psychosocial consequences of this condition.[1] A number of specifically-designed tools have been developed to measure health-related QOL for adults and children with skin disease. The aim of this study was to assess quality of life parameters in a large cohort of EPP patients in the UK. Two validated dermatology-specific quality of life measures were used: dermatology life quality index (DLQI)[2] and the children's dermatology life quality index (CDLQI)[3]. Each index consists of ten questions each scored

up to 3, giving a maximum possible score of 30. 223 EPP patients (114 females, 109 males) were recruited, and seen by the study investigator over a six-month period. 176 DLQIs and 44 completed CDLQIs were obtained. The mean total DLQI score was 13.95 (SD +/- 6.715, range 0-29). The highest scoring (highest disability) DLQI questions related to clothing, social/leisure activities and sport. The mean total CDLQI score was 13.02 (SD +/- 4.027, range 5-21). The highest CDLQI questions related to social/leisure activities, skin symptoms and sleep. These mean scores are in the upper range when compared to mean scores for other dermatological conditions such as acne (4.3-17.7), eczema (4.4-21.4), psoriasis (1.7-18.2), rosacea (6.3-7.8) and normal populations (0.3-0.5)[4].

In conclusion, this is the first large-scale research project to have formally assessed quality of life parameters in EPP. Results obtained in this study show that EPP ranks as one of the most disabling of skin conditions, despite the relative paucity of visible signs.

References

Millward LM, Kelly P, Deacon A et al Self-related psychosocial consequences and quality of life in the acute porphyrias. J Inherit Metab Dis 2001; 24: 733-747.

Finlay AY, Khan GK. Dermatology life quality index (DLQI): a simple practical measure for routine clinical use. Clin Exp Dermatol 1994; 19: 210-6.

Holme SA, Man I, Sharpe JL et al. The children's dermatology life quality index: validation of the cartoon version. Br J Dermatol 2003; 148 (2): 285-90.

Lewis V, Finlay AY. 10 years experience of the dermatology life quality index (DLQI). J Invest Dermatol Symp Proc 2004; 9: 169-180.

DOES SCREENING FOR HEPATOCELLULAR CARCINOMA BENEFIT PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA?

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Objective. Evaluate the benefit from the screening of hepatocellular carcinoma (HCC) in gene carriers of acute intermittent porphyria (AIP) - a high-risk group for developing HCC – as early diagnosis and resection of small HCC may be curative.

Design. A prospective study in northern Sweden 1994 – 2004.

Methods. AIP gene carriers aged > 55 yrs were invited at intervals of 1-2 yrs for examination of the liver (comuterized tomography, ultrasonography or magnetic resonance imaging, and relevant blood laboratory tests). All 15 AIP patients with HCC during the study period, verified by Cancer Registry in Sweden, were studied in detail.

Results. On average 64 AIP gene carriers (78%) regularily participated in the screening, sex was irrelevant. 63% of the women and 50% of the men had manifest AIP. 12 (80%) of the 15 patients (m:w 9:6) with HCC had manifest AIP. The PAD revealed cirrhosis in 3 cases. In 10 cases the tumors were well-differentiated. Aminotransferases or alphafetoprotein was of no diagnostic use in the screening. Hepatitis B or C was not found. The levels of U-ALA or U-PBG were increased in all but one case. Eight cases of HCC were diagnosed by screening; 2 patients at first screening and 6 patients at repeated screening. Seven cases of HCC detected due to symptoms had not been screened. The 6 patients detected after repeated screening (A) were compared with the group of 9 patients comprising HCC patients not screened and those detected at first screening (B). The mean age at HCC diagnosis was the same in both groups, 67 yrs (54 – 80 yrs). Surgery (liver resection) was an option in 5 of 6 in group A and in 3 of the 9 patients in group B. In total 11 patients with HCC died during study period. Great variations in survival time were registered, 2 – 179 months. A two-year survival was more common in group A (repeated screening) compared with group B (p= 0.04).

Conclusions. Screening for HCC in AIP gene carriers for early diagnosis, combined with surgical options, can improve the prognosis of this condition. Annual screening with ultrasonography or computorized tomography is recommended from the age of 50 yrs.

STRUCTURE AND FUNCTION OF HEME BIOSYNTHETIC ENZYMES

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The initial step of heme biosynthesis in humans, animals, fungi and a few bacteria is catalyzed by aminolevulinic acid synthase. The solved crystal structure of the enzyme in complex with substrates and cofactor explains in molecular detail the enzymatic mechanism and the surprising structural basis of various enzyme defects causing X-linked sideroblastic anemia. Furthermore, the two enzymes catalyzing the steps prior iron insertion, coproporphyrinogen III and protoporphyrinogen IX oxidase, were investigated for their structure-function relationsship. Data concernng the molecular basis of their catalytic mechanisms will be presented. Finally, an outlook concerning the application of heme biosynthetic enzymes in the treatment of maleria in our laboratory will be given.

ADENOVIRAL-MEDIATED EXPRESSION OF PORPHOBILINOGEN DEAMINASE IN LIVER RESTORES THE METABOLIC DEFECT IN A MOUSE MODEL OF ACUTE INTERMITTENT PORPHYRIA

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Current treatment of acute intermittent porphyria (AIP) using heme therapy is only palliative and does not prevent acute attacks. New therapeutic alternatives ought to be explored and since AIP is caused by a defect in the porphobilinogen deaminase (PBGD) gene, one obvious strategy would be to use gene therapy.

Here we present the first experiments *in vivo* using adenoviral vectors to replace the deficient PBGD enzyme in liver of an AIP mouse model. These mice mimic AIP biochemically by increased accumulation of porphyrin precursors 5-aminolevulinic acid (ALA) and porphobilinogen (PBG) after induction of heme synthesis with phenobarbital. By using an adenoviral vector carrying the luciferase cDNA in wild-type mice it was confirmed that transgene expression after intravenous administration was mainly found in liver.

When PBGD-deficient mice were administered with an adenoviral vector carrying mouse housekeeping PBGD cDNA, the hepatic PBGD activity increased in a dose- and time-dependent manner. Highest activity was found 7 days after injection and remained high after 29 days. The PBGD enzyme did also restore the metabolic defect in the AIP mouse as no accumulation of ALA or PBG was found after induction of heme synthesis in gene therapy treated animals.

This study demonstrates that hepatic PBGD expression prevents accumulation of porphyrin precursors, suggesting a future potential for gene therapy in AIP.

INVESTIGATION OF THE EFFECTS OF AN AROMATIC RESIDUE AT CODON 59 OF HUMAN PROTOPORPHYRINOGEN OXIDASE

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Variegate porphyria (VP) is a low penetrant autosomal dominant disorder of haem metabolism resulting from defects in protoporphyrinogen oxidase (PPOX), the penultimate enzyme in the haem biosynthetic pathway (1). VP is highly prevalent in South Africa, the result of a founder effect, now confirmed genetically as a single point mutation (R59W), and is present in the majority of VP patients in South Africa (2,3). Missense mutations may affect substrate specificity, stability or electronic catalysis, the ability to bind and utilise the FAD cofactor or the ability to translocate to the mitochondria or the correct compartment within. As these factors may impair normal porphyrin and haem biosynthesis, their study may yield important insights into VP.

A recent study in our laboratory investigated the effect of Arg59 on catalysis and/or cofactor binding (4). The R59W founder mutation, in addition to a conservative mutation (R59K) and two non-conservative mutations (R59S and R59I) were generated, expressed, purified and partially characterised. All mutants had reduced PPOX activity but were able to bind FAD, apart from R59W in which the binding was dramatically reduced. The findings suggested that the positive charge at Arg59 is probably involved in catalysis directly and not only in FAD binding. It is speculated that the reduced FAD binding in Arg59 may be due to the bulky nature of the aromatic tryptophan. The recently solved crystal structure of the mitochondrial form of tobacco PPOX(5) revealed that Asn67(≡R59 in human) is positioned on a loop between the isoalloxazine ring of FAD and the substrate binding site. Hence, a bulky tryptophan at this position may indeed interfere with FAD and substrate binding.

Here we examine the effects of aromaticity at codon 59 by a partial characterisation of recombinant wild type and 2 R59 mutant human PPOXs. The R59F and R59Y mutant PPOXs were constructed by site-directed mutagenesis (GeneEditor kit). Wild type and mutant human PPOXs were expressed in *E. coli* using the pTrc-His vector and purified to homogeneity by metal affinity chromatography (Talon resin).

R59F human PPOX had a negligible specific activity (20.8±2.4nmol/mg/min) and a markedly reduced k_{cat}(0.02±0.002sec^{-1})when compared to wild type (2471.4±381.6 nmol/mg/min and 2.19±0.32sec^{-1}, respectively) and similar to that reported for the clinically relevant, R59W (4). The relatively invariant K_ms for the wild type (0.92± 0.19 μM) and R59F (1.42± 0.12 μM) suggests that the reduction in specific activity for the R59F mutant PPOX is not attributed to a reduced affinity for substrate. We speculate that the rigidity of the aromatic phenylalanine may disrupt the structural integrity of the active site. Examination of cofactor binding in wild type and R59F and R59Y mutant PPOX revealed that the presence of an aromatic residue at codon 59 alone is not sufficient to prevent FAD binding and that the degree of 'molecular bulk' in this position may be responsible for the severely compromised FAD binding previously reported for the R59W mutant.

References

1. Deybach J-C, Puy H, Robreau A-M, Lamoril, J, Da Silva, V, et al (1996). Mutations in the protopporphyrinogen oxidase gene in patients with porphyria. Hum Mol Genet 5:407-410.

2. Meissner PN, Dailey TA, Hift RJ, Ziman M, Corrigall AV et al (1996). A R59W defect in human protoporphyrinogen oxidase results in decreased enzyme activity and is prevalent in South Africans with variegate porphyria. Nat Genet 13:95-97.

3. Warnich MJ, Kotze IM, Groenewald JZ, Groenewald MG, van Brakel CJ et al (1996). Identification of the three mutations and associated haplotypes in the protoporphyrinogen oxidase gene in South African families with variegate porphyria. Hum Mol Genet 5:981-984.

4. Maneli MH, Corrigall AV, Klump HH, Davids LM, Kirsch RE et al (2003). Kinetic and physical characterisation of recombinant wild-type and mutant human protoporphyrinogen oxidases. Biochim Biophys Acta 1650 10-21.

5. Koch M, Breithhaupt C, Kiefersauer R, Freigang J, Huber R. et al.(2004). Crystal structure of protoporphyrinogen –IX oxidase: a key enzyme in haem and chlorophyll biosynthesis.

NOVEL 19 BP DELETION OF EXON 15 IN THE PBGD GENE AND NORMAL ERYTROCYTE PORPHOBILINOGEN ACTIVITY IN A PATIENT WITH ACUTE INTERMITTENT PORPHYRIA

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A 36-year old Estonian patient experienced recurrent acute attacks related to the irregular menstrual cycle since 15 years of age. She had had one normal pregnancy and delivery at the age of 27, which normalised her menstrual cycle and she became symptom-free. During the last four years she experienced two miscarriages and an ovarian cyst, which precipitated several monthly attacks. The diagnosis of AIP was established only recently when she was taken to the emergency area because of confusion, rhabdomyolysis (S-myoglobin 2144 mmol/l, CK up to 21806 mmol/l), severe hyponatremia (S-Na 108 mmol/l) because of SIADH and hypokalemia (S-K 2.8 mmol/l). Transaminases were increased (ALT 128,AST 601 mmol/l). The acute attack had started 2 weeks earlier with abdominal pain, restlessness and constipation. Sensomotor polyneuropathy was detected, and brain CT was normal and EEG showed predominance of slow waves in occipital areas suggesting diminished electrical activity. Two weeks later when serum sodium level was normal, brain MRI showed enlarged internal and external liquor space and decreased signal from neural hypophysis. After a month nerve conduction studies were normal despite slightly decreased muscle strength. She was treated with heme arginate twice because of a subsequent attack. Contraceptive pills were started to prevent acute attacks and she recovered fully from polyneuropathy within 6 months and has been symptom-free for a year. Erythrocyte PBGD activity was repeatedly normal (81-79-77 Upor/h/mg protein, normal 50-100) both during an acute attack (U-PBG 900 umol/l, U-DALA 570 umol/l) and in remission (U-PBG 300 umol/l, DALA 150 umol/l). Activities of Ly-PPOX and E-CoproOX were normal, and E-UROD was low (54 coproporph/h/mg protein, normal 65-100). Plasma fluorescence emission maximum was at 619nm, faecal porphyrins were normal and in urinalysis the amount of uroporphyrins exceeded that of coproporphyrins (9000 vs 2000 nmol/l). The direct sequencing of the genomic DNA sample revealed only a 19 bp deletion in exon 15 (GAACTTGGGC ATCAGCCTGG CCAACTTGTT), which introduced an early stop codon (L A A Q N T C C X) and caused a loss of function when evaluated using expression studies.

HOMOZYGOUS VARIEGATE PORPHYRIA PATIENT WITH SEVERE IGA NEPHROPATHY

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A 24-year-old patient, whose homozygous form of variegate porphyria was diagnosed early in the childhood, manifested renal insufficiency accompanied by hypertension at the age of 20. Kidney biopsy, which was performed because of haematuria, proteinuria (6gr/d) and increased creatinine value (130-150 umol/l, normal <115) , revealed mesangioproliferative IgA glomerulonephritis. Creatinine value increased rapidly within a few years and the patient became uraemic (s-urea 15-30 mmol/l, normal 3-8.5) and haemoglobin decreased (70 g/l). The patient responded well to iron and erythropoietin therapy (Hb 136 g/l) but microsytosis (MCV 67, MCH 21) did not change. Activities of E-ALADT, E-PBGD, E-UROD and Ly-FECH were normal, but E-COPROX activity was increased (110 pmol, normal 2.2-6.5) and E-FECH was low (2.4 pmol haem/h/E6retic, normal 6-20). The amount of reticulocytes were low (0.1%, normal 0.6-2%). Ly-PPOX has been constantly low (1.5 nmol, normal 3.9-6). He tolerated well a calsium blocker, ACE inhibitor and diuretics and blood pressure has been normal. The skin symptoms such as blistering and fragility lindered slightly with the age but scarring and disfiguration of sun exposed skin have been a constant finding. Mild sensomotor-neuropathy and glaucoma have been stable, and no acute attacks have occurred. Cholecystolithiasis was observed by ultrasound but liver transaminases have been normal. Urinary excretion of porphyrin precursors has been normal throughout the follow-up, and increased urinary excretions of coproporphyrin and uroporphyrin (510 and 83 nmol, normal <230 and <36) normalised (uroporphyrin 27, coproporphyrin 0) as renal impairment proceeded. At the same time E-protoporphyrin level has been increased (8000 nmol/l red cells, normal 250-1050) as previously but plasma protoporphyrin level has increased 1.5 fold (141-255 nmol/l, normal <2 nmol/l, ratio of Zn-chelate- and free-protoporphyrin 1.5). Simultaneously faecal excretion of protoporphyrin has diminished to half of the previous values (670-200 nmol/l). These results indicate that ineffective erythropoiesis and high circulating protoporphyrin causing photosensitivity are the main findings in this patient and no liver disease is present. Moreover, renal impairment changed the porphyrin profile but has no effect on the patient's porphyric symptoms. Peritoneal dialysis was started a year ago with no effect to the porphyrin profile and cholecystectomy before kidney transplantation is planned.

SPECTRUM OF MUTATIONS IN THE FERROCHELATASE GENE IDENTIFIED AMONG FINNISH ERYTRHROPOIETIC PROTOPORPHYRIA PATIENTS INCLUDING A NOVEL SPLICING DEFECT

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Currently 36 patients from 15 families with erythropoietic protoporphyria have been identified in Finland. Most of the patients have been children diagnosed usually at the five years of age. The majority of them have been sporadic cases appearing throughout the country. 30% (n=11) of the patients were symptom-free, 70% (n=25) experience photosensitivity and 14% (n=5) severely. None of our patients have experienced fatal liver failure although elevated transaminases (ALT:AST 2:1) have occurred especially during puberty. When currently 10 mutations in the ferrochelatase gene has been identified among Finnish EPP patients, new asymptomatic patients have been identified. However, all symptomatic EPP patients have been diagnosed using biochemical analyses such as measuring blood protoporphyrin concentration (more than 10 fold) and positive plasma fluorescence spectrum. In some cases despite a gene defect and low FECH activity, only a mild increase in blood protoporphyrin levels (up to 3-fold increase) is not sufficient for the diagnosis of symptomatic EPP, but should alert a doctor to look for other causes of photosensitivity. Of the polymorphisms in the ferrochelatase gene, -23T in intron 1 (symptomatic patients 0.70 vs symptom-free 0.70 vs healthy controls 0.30) and -48C in intron 3 (symptomatic patients 0.70 vs symptom-free patients 0.22 vs healthy control 0.07) were common among symptomatic patients. The novel mutation identified in a EPP family was IVS4+1 G-A causing a splicing defect.

LIVER BIOPSY MORPHOLOGY AND HFE-GENE STATUS IN CUTANEOUS PORPHYRIA (PCT)

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Porphyria cutanea tarda (PCT) is caused by decreased activity of hepatic URO-D that is mainly mediated by an iron-dependent pathway. The HFE, C282Y and H63 mutations are the most common cause of hemochromatosis. We investigated semiquantitatively the hepatic iron content by Perls´ prussian blue staining of liver biopsy material in 38 PCT patients in relation to their HFE-gene status.

29 (76 %) of 38 patients showed liver siderosis of variable severity. In 28 patients the HFE-gene status was also available. Thereby two groups could be differentiated: group 1 = without mutations, group 2 = with mutations (C282Y/WT, H63D/WT, C282Y/H63D, C282Y/C282Y).

		Group 1	Group 2
No siderosis	-	4	0
Mild siderosis	+	5	8
Medium severe siderosis	++	4	2
Severe siderosis	+++	1	4

$p < 0,05$

The present study clearly demonstrates that the risk of iron deposition in hepatic tissue is influenced by the HFE-gene status. PCT patients with HFE-mutations show a significant higher risk of developing liver siderosis than those without HFE-mutations ($p < 0,05$; exact Fisher-test). While PCT in general is a complex disease triggered by both endogenous and exogenous factors, HFE-status is of importance since it demonstrates a risk factor for liver siderosis (and cirrhosis).

INVOLVEMENT OF FASE I DRUG METABOLIZING SYSTEM IN THE METABOLIZATION OF PORPHYRINOGENIC AGENTS IN BRAIN. A COMPARATIVE STUDY IN LIVER AND KIDNEY

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The presence of the monooxygenase enzymes in brain, suggests that they have more specialized functions than the hepatic enzymes which participate in xenobiotic detoxification. Brain cytochrome P-450 (CYP) levels are only 4-10% of the corresponding hepatic content. CYP is mainly localized in mitochondria although a small quantity was found in the microsomal fraction.

The aim of this work was to evaluate the involvement of mitochondrial and microsomal mice brain CYP in the metabolization of known porphyrinogenic agents and to compare their response in liver and kidney.

Some of the porphyrinogenic agents studied altered mitochondrial brain CYP but not the microsomal CYP. Thus, Gris induced a 70% ($p<0.05$) and chronic anaesthesia with Isoflurane produced a 30% ($p<0.01$) reduction. Instead, AIA diminished both microsomal and mitochondrial brain CYP content.

Hepatic CYP levels were altered in the most of the groups studied, in some cases a similar pattern was detected for the mitochondrial and microsomal CYP. However, after chronic Isoflurane administration, a 36% ($p<0.05$) diminution was only detected in the mitochondrial fraction.

Kidney CYP levels were only modified after Veronal administration and this effect was only observed in mitochondria.

Moreover, taking into account that ALA could be responsible for the neurological symptoms of acute porphyrias, the effect of this metabolite on tissues CYP levels was investigated. CYP reductase activity was also evaluated in all groups.

Our findings showed differential tissue response to the different xenobiotics assayed. Although the liver is the main tissue involved in the metabolization of exogenous drugs, extrahepatic tissues can also participate. Brain CYP was more affected than kidney CYP and in all cases mitochondrial CYP was mainly involved.

The alterations provoked on brain CYP levels could be attributed to a direct effect on the Fase I drug metabolization system or indirectly, due to changes in the activities and expression of ALA-S and Heme oxygenase produced by some porphyrinogenic agents, as we have previously reported.

INSULIN AND VANADATE ACTION ON HEME BIOSYNTHESIS GENES TRANSCRIPTION IN DIABETIC MICE

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Treatment of porphyria acute attacks with carbohydrates diminishes the production and excretion of heme precursors such as 5´aminolevulinate acid (ALA). In spite of its therapeutic use, the molecular and biochemical basis of the known glucose effect are yet to be dilucidated.

Diabetes-induced alterations on the transcription of heme biosynthesis genes were examined in male CF1 mice. Animals were diabetized with a single dose of streptozotocin (STZ, 170 mg/Kg *ip*) and Northern blot analysis were performed at different times after treatment. Animals with serum glucose levels higher than 300 mg/ml were considered diabetic and were treated with vanadate (0.2 mg/ml in drinking water) during 16 days (STZ+V group), or insulin (30 U/100g, *sc*) during 9 days (STZ+I group) or only STZ

(STZ group). Ferrochelatase (FQ) and ALA Synthetase (ALA-S) mRNA levels increased 100% 32 days after STZ injection. Insulin and vanadate treatment restored ALA-S RNAm to basal levels. However, FQ mRNA induction in diabetic animals was not modified by insulin treatment although vanadate administration was able to restore basal levels. No changes were detected in ALA dehydratase (ALA-D), Uroporphyrinogen Decarboxylase URO-D) and Heme Oxygenase (HO-1) mRNA. The increase in ALA-S mRNA was due to higher transcription levels as the mRNA stability was not altered in diabetic animals compared to controls (ALA-S mRNA half life = 25 min).

Employing an experimental model of diabetes we have demonstrated that insulin regulates the *in vivo* transcription of ALA-S. By using an insulin-mimetic agent we have corroborated that insulin is involved in the downmodulation of this gene transcription.

A CASE OF ALAD PORPHYRIA IN NORTH AMERICA: RESPONSE TO HEMIN THERAPY

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Porphyria due to 5-aminolevulinic acid (ALA) dehydratase (ALAD) deficiency is termed ALAD porphyria (ADP) and was first described by Doss in 1979. Of ~7 cases described five have been documented at the molecular level. ADP is classified as one of four acute hepatic porphyrias, although the finding of increased erythrocyte zinc protoporphyrin suggests a disturbance of heme biosynthesis in bone marrow erythroblasts as well. Treatment with intravenous hemin is widely recommended, but a biochemical response has been documented in only two cases (Gross et al 1998). There is a need for further study of therapy in this very rare form of human porphyria.

Here we describe the clinical features and response to hemin therapy a 14 year old boy who presented with abdominal pain, tachycardia, hypertension and low grade fever. ADP was suggested by markedly increased excretion of urinary ALA and total porphyrins (mostly coproporphyrin III) and erythrocyte zinc protoporphyrin, without significant increases in urinary porphobilinogen and fecal porphyins. Plasma ALA (Lee et al 2004) was also markedly elevated. Erythrocyte ALAD activity was <8% of normal, and approximately half-normal in both parents and a brother. Two novel ALAD mutations, one inherited from each parent, were documented and studied in detail (Kato et al, submitted). There was no response to intravenous glucose after one day. Administration of lyophilized hemin (Panhematin®, Ovation) 3 mg/kg body weight reconstituted with human albumin once daily for 4 days resulted in rapid decreases in urinary and plasma ALA. There was also rapid clinical improvement, and he was discharged 2 days after the course of treatment was completed. Two attacks developed during the next year, and one was treated with hemin. These findings support the early use of intravenous hemin for treatment of attacks of ADP.

THE 1.58 Å CRYSTAL STRUCTURE OF HUMAN COPROPORPHYRINOGEN OXIDASE REVEALS THE STRUCTURAL BASIS OF HEREDITARY COPROPORPHYRIA

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Hereditary coproporphyria (HCP) is an autosomal dominant disorder resulting from the half-normal activity of coproporphyrinogen oxidase (CPO), a mitochondrial enzyme catalyzing the antepenultimate step in heme biosynthesis. Although several mutations in the CPO gene have been described, the structural basis for how these alterations diminish enzyme activity is incompletely understood. Moreover, the mechanism by which CPO catalyzes the extraordinary metal- and cofactor-independent oxidative decarboxylation is also unknown. Here, we show that CPO has a novel tertiary topology comprising an unusually flat seven-stranded β-sheet that is sandwiched by α-helices. The quaternary state is defined by a homodimer (KD = 5.3 ± 1.2 μM, ΔGdiss ≈ 7.0 kcal mol-1) in which one monomer rotates relative to the second by approximately 40º to create an inter-subunit interface in close proximity to the two independent active sites. Consequently, deletion of the highly conserved region encoded by exon six, detected in the CPO gene of a HCP patient, will generate a protein that can neither dimerize nor sustain activity. Furthermore, most of the disease-causing mutations occur in regions that are indispensable for maintaining the structural integrity of CPO. The unexpected finding of citrate at the active site helps to demarcate the residues critical for substrate binding and catalysis. Thus, Arg262 and Arg413 mediate carboxylate recognition; Gly406 and Leu407 entertain non-bonded contacts; His258 and Asp282 constitute a catalytic diad; Ser244, Asn260, and Ser416 facilitate proton abstraction or stabilize an intermediate. A transition metal center is also absent. Based on these findings, we propose a mechanism in which triplet oxygen serves as the immediate electron acceptor and a substrate radical or a carbanion intermediate with substantial radical character participates in catalysis. Together, our results have broad implications for deciphering the mechanistic puzzle of CPO and for understanding structure-function relationships in HCP. [This work is supported by the Pew Charitable Trusts via a Pew Scholar Award (C.S.R.), the Robert A. Welch Foundation (C.S.R., AU-1574), and GAUK 25/04 (M.B. and P.M.)]

EXPERIMENTAL PROTOPORPHYRIA: EFFECT OF BILE ACIDS ON GRISEOFULVIN-INDUCED HEPATIC DAMAGE

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We have previously reported that oral administration of Griseofulvin (Gris) (0.5% in the diet) produces a great redox imbalance in mice liver due to the accumulation of porphyrins, which are known to generate reactive oxygen species.

The major route of hydrophobic porphyrin elimination is their biliary excretion. Administration of Gris induces a reduction of bile flow parallel to an increase in hepatic porphyrin accumulation. It has been reported that protoporphyrin (PPIX) impairs the biliary secretion of phospholipids and cholesterol, but not that of bile acids. Since both PPIX and lipid secretion into bile are coupled processes, biliary excretion of porphyrins can also be diminished.

Because alterations induced by Gris as a consequence of the high accumulation of porphyrins in liver were so important, they could not be reverted by traditional antioxidants such as α-tocopherol, β-carotene or ascorbic acid. So we decided to study the effect of some bile acids, such as deoxycholic acid (DXA), dehydrocholic acid, chenodeoxycolic acid, and ursodeoxycholic acid, which could accelerate the porphyrin excretion through the bile, in an attempt to diminish liver damage.

Administration of Gris alone induced in liver increased activity of Glutathione reductase (GRed) (144.60%), Superoxide dismutase (SOD) (167.93%), Alkaline phosphatase (Aph) (147.27%), γ-GT (130.28%) and GST (133.28%); and high levels of total porphyrins (155.33%), glutathione (GSH) (142.39%), and P450 (130.86%).

DXA reduced the accumulation of porphyrins induced by Gris, and the hepatic levels of GSH were within control values, although GRed activity was still enhanced (150.59%). Also lipid peroxidation and the hepatic enzymes γ-GT and Aph were about control values. The drug metabolizing system was not altered either when mice were fed with Gris plus DXA. These findings would indicate that the hepatic damage induced by Gris can be partially prevented by administration of DXA.

FREQUENCY OF INTRON 1 C/A POLYMORPHISM OF THE CYP1A2 IN SPANISH PATIENTS WITH PORPHYRIA CUTANEA TARDA

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Porphyria cutanea tarda (PCT) is characterized by partial deficient activity of the hepatic uroporphyrinogen decarboxylase enzyme (UROD). The enzymatic defect of the UROD alone is not sufficient to provoke clinical symptoms of PCT. An increased frequency of the highly inducible A/A genotype of cytochrome CYP1A2 has been described in patients with PCT and inheritance of this genotype was suggested as a susceptibility factor in development of overt-PCT.

The aim of this study was to determine the frequency of the CYP1A2 C/A polymorphism in intron 1 in a series of 110 patients with PCT and in 146 normal subjects from Spain. Patients were divided into familial-PCT, which are associated with mutations in the UROD gene and decrease erythrocyte UROD activity, and sporadic-PCT who exhibited normal UROD activity in erythrocytes. The rare familial occurrence of sporadic-PCT cases were classified as Type III-PCT. CYP1A2 genotype was determined after PCR amplification plus *Apa*I restriction enzyme digestion. No differences were found between the three PCT groups. Among the PCT patients 4 were C/C homozygous while 57 were C/A heterozygous and 49 A/A homozygous. The comparison with the control population (18 C/C, 68 C/A, and 60 A/A) revealed statistical differences (contingency χ^2=6.044; 2 d.f.; $p<0.05$). This supports the idea that the "A" allele could be a susceptibility factor in the development of PCT.

EFFECTS OF THE HERBICIDE ACIFLUORFEN IN THE R59W PROTOPORPHYRINOGEN OXIDASE MUTANT MOUSE MODEL FOR VARIEGATE PORPHYRIA

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Variegate porphyria (VP) is inherited as an autosomal dominant disorder with variable penetrance. The prevalent mutation underlying the disease in South Africa is the R59W mutation in protoporphyrinogen oxidase (PPOX). The clinical features of the disease are photosensitivity and acute neurovisceral attack. Drugs, chemicals and infections can precipitate acute attacks. There is no truly satisfactory lab. or animal model of VP. The R59W mouse model is established in our lab. However, pilot experiments in our labs suggest that the R59W defect alone is not sufficient to induce typical VP biochemistry in mice and further inhibition by chemical means (such as feeding the mice a known PPOX inhibitor, acifluorfen) may be necessary to provoke a useful porphyrin phenotype. In this study we establish the R59W VP mouse model further by the measurement of ALA synthase (ALAS), PBG deaminase (PBGD) and PPOX enzyme activities in the haem biosynthetic pathway. The affect of further inhibition of PPOX on the R59W heterozygous mice and wild type mice (controls) was also studied by adding aciflurofen (AF) to the mice diet as appropriate.

In this study we have developed assays for measuring mouse liver ALAS, PBGD and PPOX activities from a single liver. We have used groups of control mice, R59W heterozygous mice, with and without AF.

PPOX activity was decreased by 50% in the R59W mouse model, as expected. AF (0.125%) resulted in a 42% decrease in PPOX in control mice, and reduced PPOX activity to 29% of control mice when added to R59W VP mice. ALAS was uninduced in VP mice, induced by 170% in control mice on AF, and induced by 530% in R59W VP mice. PBGD gave a similar pattern of induction as ALAS in the various mice groups, but to a lesser extent.

Interestingly baseline hepatic ALAS activity was not routinely upegulated in "normal" VP mice, without added AF. ALAS was induced when AF added to both control and VP mice. As both R59W and WT+AF have 50% PPOX activity, AF appears porphyrinogenic in it's own right. In the VP mice with added AF the PPOX deficiency appears to be severe enough to engender a haem deficiency, which leads to further ALAS up-regulation, in addition to independent porphyrinogenic effect of AF. As there was

a smaller relative increase in PBGD activity, compared to induction of ALAS, either there is PBGD molar insufficiency, or PBGD is being (relatively) inhibited by protoporphyrinogen when PPOX activity is severely diminished, such as occurred in the VP mice with added AF.

MOLECULAR HETEROGENEITY OF PORPHYRIA CUTANEA TARDA IN SPAIN: IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF MUTATIONS IN THE UROPORPHYRINOGEN DECARBOXYLASE GENE

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Porphyria cutanea tarda (PCT) is resulting from decreased activity of hepatic uroporphyrinogen decarboxylase (UROD). We investigated the molecular basis of PCT by screening the UROD DNAs of 71 unrelated Spanish patients with PCT, using polymerase chain reaction, heteroduplex analysis, automated sequencing, and restriction enzyme digestion.

Ten novel and 4 previously described mutations (of which, two of them were detected in Spanish patients) were identified in 15 patients (21%). The probability of each novel missense mutation in decreasing UROD activity was assessed by prokaryotic expression studies. Other 52 patients (73%) showed no mutation or polymorphism and were classified as sporadic-PCT. Four patients (6%) exhibiting normal erythrocyte UROD activity with family history of the disease were classified as Type III-PCT. No mutation was found in the UROD gene from Type III-patients, and confirms that inherited factors other than mutations in the UROD gene, may predispose to PCT.

Our results indicate that the frequency of familial-PCT in Spain is 21%. Among the 14 molecular defects observed in our patients, 12 of them seem to be restricted to the Spanish population and confirm the molecular heterogeneity in familial-PCT.

ATYPICAL RED CELL INCLUSIONS IN A PATIENT WITH CONGENITAL ERYTHROPOIETIC PORPHYRIA

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We show the morphological finding of some atypical inclusions in mature red cells and their precursors in a 28 years-old patient with congenital erythropoietic porphyria.

We evaluated the red cell morphology in a 28 years-old women with cutaneous photosensitivity that began in early infancy and manifested by increased friability and blistering of the epidermis on the hands, face and other sun-exposed areas, with secondary loss of digits. She also showed hyperpigmentation, hypertrichosis, severe hemolytic anemia that required splenectomy and erythrodontia. Leukocyte and platelet counts were normal. Nevertheless the red cell count was low ($2{,}74 \times 10^{12}$/L) with reticulocytosis, increased nucleated red cells, absence of haptoglobin and increased ferritin values (647 ng/mL). Chromatography (h.p.l.c.) analyses showed that erythrocytes contained significant amounts of uroporphyrin I (7,4 µmol/L red cells) and coproporphyrin I (11,2 µmol/L red cells). The patient showed a characteristic CEP profile in urine with high concentrations of uroporphyrin I (5510 nmol/mmolcreatinine) and coproporphyrin I (1278 nmol/mmol creatinine).

In the morphological red cell analysis (May-Grünwald-Giemsa staining) we found anisocitosis, poikilocytosis, spherocytosis, polychromasia, basofilic stippling and Howell-Jolly bodies. We also found in the red cells many purple-violet, slender, straight or slightly curved needle-like inclusions, mostly aligned in radial orientation. With transmission electron microscopy we found a) erythrocytic sideroacrestic phenomenon and b) deposits or needle-like inclusions in the red cells.

Similar atypical inclusions that we describe here were previously found in the hepatocytes in patients with erythropoietic porphyria. Hepatic deposits correspond to pigment crystals and are composed of protoporphirin. This phenomenon in the erythrocytes is exceptional and probably in the present case may be due to the lack of the pitting function of the spleen.

STUDY OF THE OLIGOMERIC STATUS OF COPROPORPHYRINOGEN OXIDASE (CPO) USING ANALYTICAL ULTRACENTRIFUGATION (AU)

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CPO catalyzes the sixth enzymatic step of the biosynthesis of the critical cofactor heme. The CPO deficiency in human causes a severe metabolic disorder, hereditary coproporhyria. CPO is active as a homodimer.

We purified human CPO using GST Fusion System (Pharmacia) to electrophoretic homogeneity (>95%). The data from the sedimentation equilibrium experiments were analyzed with the new version of UltraScan software. Monte-Carlo analysis was used to determine 95% confidence intervals of all parameter estimates. All scans were fitted to a global model describing either a single ideal species, or a reversibly self-associated monomer-dimer or monomer-dimer-tetramer system.

All three models strongly suggest that CPO is almost exclusively present in the dimeric form. We found the monomer-dimer-tetramer model as the most accurate, with the monomer MW = 37.6 kDa. Using this model, the K_D for monomer-dimer was calculated to be 4.31e-7 M and for monomer-tetramer 1.39e-17 M, respectively.

AU proved to be powerful tool to address CPO oligomerization behaviour. Our results suggest monomer only being present in low nanomolar range, while minuscule amount of tetramer being present only in the highest concentrations examined. Disease causing CPO mutants will be tested now using AU to address their effect on self-association of the enzyme.

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DISEQUILIBRIUM IN EXPRESSION OF APOPTOSIS FACTORS BETWEEN SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS WITH ACUTE INTERMITTENT PORPHYRIA

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Deficiency in hydroxymethylbilane synthase (HMBS) is the primary cause of acute intermittent porphyria (AIP) – a dominantly inherited disorder. However, not all individuals with a defective enzyme develop AIP symptoms. To explore the mechanism of incomplete penetrance in AIP, we generated gene expression profiles from AIP patients (n=6), asymptomatic mutation carriers (n=3) and control subjects (n=3). Among the 6 patients studied, 3 suffered from more than 3 episodes of acute attacks in the past and were categorized as "severe symptomatic". RNA samples were prepared from peripheral blood and then hybridized with Microarray U133A chips. Expression data obtained from Microarray correlated with clinical statuses as analyzed by hierarchical tree clustering. If the samples are grouped based on clinical statuses, 1040 genes (p=0.05), 2005 genes (p=0.1) respectively, out of 15'914 genes tested were found to be differentially expressed. The highest percentage of differentially expressed genes were found in the categories "apoptosis regulators" and "cell death regulators" from genebank "Simplified Gene Ontology". Factors from both intrinsic and extrinsic apoptosis pathways were involved. These results suggest that expression level of various apoptosis factors correlate with the clinical penetrance of AIP.

CONCURRENT UPREGULATION OF HAEM OXYGENASE 1 AND CYTOCHROME P450 2A5: POSSIBLE ROLE OF Nrf2 AND hnRNP A1

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We have been investigating the molecular mechanisms by which cadmium (Cd) affects the regulation of cytochrome P450 2A5 (CYP2A5) and haem oxygenase 1 (HO-1). $CdCl_2$ treatment (16 μmol/kg body weight) in DBA/2J male mice resulted in significant ($P<0.05$) progressive decrease of total hepatic cytochrome P450 (CYP450) content as well as significant increase of haem oxygenase activity with time. In contrast, a late effect of the treatment showed progressive increase in coumarin 7-hydroxylase activity (catalysed by CYP2A5). Northern blot analysis suggests that the treatment concurrently induced expression of HO-1 and CY2A5 mRNAs with the expression of the former precedes that of the latter.

Western blot analysis and UV cross-linked assay using cytoplasmic and nuclear protein extracts from the liver of treated mice suggests that the treatment causes translocation of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) from the nucleus to the cytoplasm. Western blot analysis also showed that Nrf2 is translocated from the cytoplasm to the nucleus. The translocations of both hnRNP A1 and Nrf2 were observed at a time range corresponds to the progressive increase of HO-1 and CYP2A5 mRNA expression.

The pattern of concurrent induction of HO-1 and CYP2A5 by Cd suggests that the transcription factor, Nrf2 and the multifunctional protein hnRNP A1 may be involved in the up-regulation. To date, we have been unable to establish whether or not the proteins interact with each other in synchronising the transcription and post-transcription regulation of both enzymes.

PRODUCTION AND CHARACTERIZATION OF ERYTHROPOIETIC PROTOPORPHYRIC HETERODIMERIC FERROCHELATASES

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Mutations resulting in diminished activity of the dimeric enzyme ferrochelatase are a prerequisite for the inherited disorder erythropoietic protoporphyria (EPP). Patients with clinical EPP have only 10-30% of normal levels of ferrochelatase activity and while many EPP patients possess one mutant allele and one "low expression" normal allele, the possibility remains that for some, low ferrochelatase activity may result from an EPP mutation that has an impact on both subunits of the wild-type/mutant heterodimer. Herein we present data for twelve ferrochelatase wild-type/EPP mutant heterodimers showing that some mutations result in heterodimers with the residual activity anticipated from individual constituents, but other heterodimers had significantly lower activity than would have been predicted. While the data do not allow an a priori prediction of heterodimeric residual activity based solely upon in vitro activity of EPP homodimers or position of the mutated residue within ferrochelatase, mutations that affect the dimer interface or [2Fe-2S] cluster have a significantly greater impact on residual activity than would be predicted. These data suggest that some EPP mutations may result in clinically overt EPP in the absence of a low expression wild-type allele and this is of potential significance for genetic counseling of EPP patients.

THE MANAGEMENT OF PORPHYRIC PATIENTS WITH MALIGNANT DISEASE: A REVIEW OF THE LITERATURE AND RECOMMENDATIONS FOR THE FUTURE

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Porphyria is a collection of seven disorders caused by genetic defects in the biosynthesis of haem which clinically can be subdivided into the neuropsychiatric (acute), dermatological or mixed types. The mainstay of the treatment of porphyria is the prevention of acute attacks and the avoidance of skin lesions. The avoidance of porphyric drugs is a key part of this prevention strategy. It is known that certain types of porphyrias have an increased risk of malignant disease such as hepatocellular carcinoma in acute intermittent porphyria and porphyria cutanea tarda. In addition, an acute porphyric attack may be the manifestation of an underlying malignancy. Porphyric patients like any member of the general population may develop a malignant disease. The investigation and management of such porphyric patients requires particular care and attention to ensure that an acute attack is not precipitated, and that the patients receive optimal care and attention for their underlying malignancy. In addition treating units must be aware of the signs and symptoms of an acute attack and its management. We reviewed all the available English literature relating to the treatment of malignant disease in porphyric patients. Based on this literature the investigation of porphyric patients who may have an underlying malignant disease, as well as the treatment of such patients will be discussed. Drugs used for symptomatic treatment and in the palliative setting will also be reviewed. We have developed the Imperial College London-King's College Hospital guidelines for the management of oncology patients with a history of porphyria. It is hoped that these recommendations will help to improve the care and outcome of such porphyric patients.

CONGENITAL ERYTHROPOIETIC PORPHYRIA IN ARGENTINA

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Congenital Erythropoietic Porphyria (CEP) or Gunther's disease is an extremely rare metabolic disorder. CEP develops as a consequence of diminished activity of Uroporphyrinogen III Sinthetase (UROIII-S) which catalyses the synthesis of the physiological isomer Uroporphyrinogen III in Heme pathway. Large amounts of isomer I are accumulated and oxidized to Uroporphyrin I (URO I) producing red blood cells lysis and releasing URO I content which is excreted in urine and faeces.

Clinical manifestations including severe cutaneous lesions of exposed areas that could lead to tissues mutilation, hypertrichosis, alopecy, erythrodontia, are presented since birth. However later onset has also been described in 12 patients.

This report presents 3 cases of infantil CEP and 1 adult case in a 55 years old man. Urinary porphyrins excretion ranged between 37,000 and 59,500 μg/24 h (NV: < 250 μg/ 24 h). Fecal porphyrins were between 880 and 5,250 μg/g (NV: <130 μg/g) and plasmatic porphyrins between 5.80 and 11.50 (NV: < 1.30). HPLC analysis of urinary porphyrins showed a profile according to a typical CEP pattern (96-98% UROI – 2-4% UROIII).

UROIIII-S activity assayed in one of the children and in the adult revealed that the ability of the enzyme in converting isomer type I to III is diminished between 25-44%; nevertheless this value is greater than expected taking into account the autosomical recessive character of the pathology, these findings are in accordance with the mild clinical manifestation observed in both patients.

CHARACTERISATION OF ERYTHROPOIETIC PROTOPORPHYRIA IN SOUTH AFRICA

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Background: Erythropoietic protoporphyria (EPP) is an inherited disorder that has been described as autosomal dominant with incomplete penetrance (Todd 1994). Patients present in childhood with photosensitivity as a result of a defect in heme biosynthesis causing accumulation of protoporphyrin (DeLeo et al. 1976). Mutations in ferrochelatase (*FECH*), the terminal enzyme of the pathway, are observed in both affected and asymptomatic individuals. Affected individuals, however, appear to have an enzyme deficiency of >50%, which has recently been attributed to a common polymorphism (IVS3-48T/C) on the "wildtype" allele that leads to reduced expression of the allele (Gouya et al. 1996; Gouya et al. 2002). **Aim:** To characterise a cohort of 13 affected South African EPP individuals at molecular level and to assess the frequency of the IVS3-48C polymorphism in this cohort and a control population. **Methods:** PCR amplification and subsequent single-stranded conformational polymorphism (SSCP) analysis of the coding region and surrounding intronic sequences of *FECH* was carried out. Fragments showing an altered pattern were sequenced to identify the mutation. Various enzymes were employed in restriction enzyme digestion in order to screen for identified changes including the IVS3-48T/C polymorphism. **Results:** A novel 7bp deletion in exon 4 of *FECH* has been identified in a single individual and a previously described 5bp deletion in exon 7 has been observed in eight individuals in the cohort. Both cause a frameshift and a premature stop codon within 100 amino acids of the deletion. A novel change, namely IVS10-61G/A, was identified although the functional significance has yet to be determined. The frequency in the population has not been determined but unaffected control individuals have been shown to carry the change suggesting that it is a common polymorphism. The IVS3-48C polymorphism was found to be present in 11 of the 13 affected individuals and 10.78% of the matched control population. **Discussion:** The prevalence of the deletion identified in exon 7 for the majority of patients strongly suggests a founder effect in the South African EPP population. Family studies will allow us to determine whether this mutation is indeed the result of a single ancestral event. The low expression allele appears to play a role in the manifestation of the disease in our population as proposed for the French population.

References

Todd DJ (1994) Erythropoietic protoporphyria. Br J Dermatol 131: 751-66

DeLeo VA, Poh-Fitzpatrick M, Mathews-Roth M, Harber LC (1976) Erythropoietic protoporphyria. 10 years experience. Am J Med 60: 8-22

Gouya L, Deybach JC, Lamoril J, Da Silva V, Beaumont C, Grandchamp B, Nordmann Y (1996) Modulation of the phenotype in dominant erythropoietic protoporphyria by a low expression of the normal ferrochelatase allele. Am J Hum Genet 58: 292-9

Gouya L, Puy H, Robreau AM, Bourgeois M, Lamoril J, Da Silva V, Grandchamp B, Deybach JC (2002) The penetrance of dominant erythropoietic protoporphyria is modulated by expression of wildtype FECH. Nat Genet 30: 27-8

HEPATIC GENE EXPRESSION PROFILING IN HEPATITIC C VIRUS INFECTED PATIENTS WITH PORPHYRIA CUTANEA TARDA (PCT)

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Porphyria cutanea tarda (PCT) is the most common of the porphyric disorders affecting 1 per 5,000 Caucasians. In all cases of PCT the activity of uroporphyrinogen decarboxylase (URO-D) in liver cells is markedly reduced whether mutations in the URO-D gene are present (familial PCT) or not (sporadic PCT). Risk factors predisposing to PCT include alcohol abuse, hepatitis C infection (HCV), the use of medicinal estrogens, and mutant hemochromatosis alleles. In our experience hepatitis C is the most common risk factor as 86% of men with both the familial and sporadic forms of PCT have serologic evidence of HCV. The mechanism by which HCV leads to clinical expression of PCT is not known. We utilized microarray technology to probe the mechanism.

The expression levels of 13,026 unique cDNA clones, purchased from Agilent, were analyzed in liver biopsies samples from 6 HCV positive and 6 HCV negative men with sporadic PCT, and 8 HCV positive patients without PCT. Expression profiles in each group were compared to a pool of 8 normal livers. The microarray format and all protocols are described on the web site http://expression.microslu.washington.edu. A single experiment comparing two samples was done with four replicate arrays using the dye label reverse technique, thus providing mean ratios between the expression levels of each gene in the analyzed sample pair, standard deviations, and *P* values.

Thirty-seven genes were up-regulated specifically in PCT whether HCV was present or not. These 37 genes had in common an association with macrophage and hepatic stellate cell activation. One-hundred-forty-nine genes were up regulated specifically with HCV whether PCT was present or not. HCV specific genes fell into 5 groups: interferon inducible; proteosome mediated degradation; oxidative stress and lipid metabolism; immune response and chemokines; and genome modifiers. Expression of ALA-S1 was down regulated in all PCT patients and expression of ferrochelatase was up regulated in most. No other genes related to heme biosynthesis, including URO-D, were altered. Expression of heme oxygenase was down regulated in all patients. Expression of iron-related genes was generally unchanged even though all PCT patients had moderate iron loading. All patients with PCT up regulated expression of CYP1A2 whereas HCV alone produced up regulation in some, down regulation in some, and no effect in others. These data indicate a PCT specific response but do not provide a molecular explanation for the role of HCV as a risk factor for the development of PCT. We conclude that alterations in genes other than the synthesis in heme are required to produce the porphyric phenotype.

NATURAL COURSE OF AN ACUTE ATTACK IN ACUTE INTERMITTENT PORPHYRIA

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Objective: To assess the course of an acute attack by studying correlation of clinical manifestations with precipitating factors, and treatment of patients with acute intermittent porphyria (AIP).

Patients and methods: The study group included 15 Russian and Finnish AIP patients experiencing totally 41 acute attacks in 1995-2004, who were assessed clinically and biochemically during an acute attack and in remission.

Results: An acute attack could be dissected into several distinct phases. An initial phase of an acute attack included dysautonomia and mental symptoms lasting 3-29days (mean 14 days). If an additional precipitating factor was administrated, the second phase of an acute attack developed including paresis, epileptic seizures, unconsciousness and hyponatremia. If an attack was treated early and precipitating factors were eliminated, acute attacks rarely proceeded to the second phase. After pareses had developed, intensity of abdominal pain usually declined, but myalgia increased. Tachycardia correlated with activity of an acute attack. Mechanical lung ventilation, bulbar paresis, severe consciousness impairment and arrhythmia appeared only at the late stage of an acute attack (mean 45 days, range 13-83 days) and reflected the most severe impairment of the nervous system. In the majority of the cases it was provoked by additional precipitating factors and could be attributed to a separate phase of an acute attack. Transaminases were usually normal during early days of an acute attack but increased in the majority of cases when an acute attack proceeded. Heme arginate was more effective than glucose infusion leading to a plateau phase significantly earlier (mean 7.6±1.0 vs. 12.3±2.0 days in patients treated conventionally, p=0.001) even in severely affected patients.

Conclusions: Precipitating factors appeared usually in combination, and were responsible for several distinct phases of an acute attack, which could be observed in severe cases. Heme therapy should be started early but it is efficient even in severely affected patients. The delay of diagnosis and administration of additional precipitating factors were responsible for a poor outcome of an acute attack.

THE DRUG DATABASE FOR ACUTE PORPHYRIA

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Since certain drugs may precipitate attacks in acute porphyrias, it is essential to avoid prescribing them. Several porphyria drug lists containing very useful safety guidance, are available on the Internet, but for many drugs there are considerable discrepancies between the different lists and background information is usually lacking. In addition, most of the relatively new drugs on the market are not safety classified.

The Swedish and Norwegian national porphyria centres have evaluated the porphyria prescribing guidance. The evaluation has taken into account pharmacological traits as well as all retrievable published and unpublished clinical and experimental data concerning porphyrinogenicity. In a collaborative project, a comprehensive database containing the findings of this evaluation has been developed. Functionality and user-friendliness has been emphasised in the development of the drug database. Clinicians can easily use this database tool to quickly find the safest alternative. The system can also combine vulnerability data of the patient with the safety classification of the desired drug. Specific advice concerning follow up and possible prophylactic measures is then given.

In the Scandinavian countries, national versions of the database are either available on the web or being established. A drug working group in EPI (European Porphyria Initiative) has recently decided that this database shall be the platform for a European system. The aim is to establish national versions of the drug database in more European countries. The safety classification of the drugs is kept constant in the different national versions, but trade names and generic names vary and are adjusted to national needs. A preliminary UK version has recently been established. A practical demonstration of the use of this UK version will be given.

DRUG PORPHYRINOGENICITY MONOGRAPHS IN THE DRUG DATABASE FOR ACUTE PORPHYRIA

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The drug database for acute porphyria (as presented at this conference by the same authors) has been developed as a practical clinical tool to which can be used as an aid in prescribing problems for patients suffering from acute porphyrias. Establishing prescribing guidance for patients with acute porphyria is a very difficult task. All relevant information needs to be collected and evaluated in a systematic way. Porphyrinologists need a comprehensive source which provides insight in the evidence supporting prescribing guidance. This information can be found in The Drug Database for Acute Porphyria in documents called drug porphyrinogenicity monographs.

Each monograph contains a porphyrinogenicity classification (Porphyrinogenic - Probably porphyrinogenic - Possibly porphyrinogenic - Probably not porphyrinogenic - Not porphyrinogenic), as well as a rationale for the given classification and other background information. The following headings form the outline of a monograph: Substance - ATC code - Risk class - Rationale for risk classification - Most recent revision - Therapeutic use – Metabolism – Pharmacokinetics - Extent of hepatic exposure - Clinical observations - Porphyria drug lists – References.

To gather data, bibliographic searches are performed (standard literature searches include boolean combinations of the drug and an array of predefined elements). Several commercial drug databases are consulted. Summaries of product characteristics from drug manufacturers are consulted as well. Personal communications from porphyrinologists are of major importance, and these are therefore incorporated in the monographs. Available prescribing guidance concerning acute porphyries from other sources is referenced.

HAEM-MEDIATED DESTABILISATION OF HUMAN ALAS1 mRNA

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Haem is the prosthetic moiety of numerous haemoproteins critical for the function of all aerobic cells. Its biosynthesis is a tightly controlled process since high intracellular haem concentrations are cytotoxic, whilst haem deficiency impedes the activity of essential haemoproteins. In the liver and probably all other non-erythroid cells, haem supply is regulated primarily through feedback regulation of the stability of the mRNA encoding aminolaevulinic acid synthase 1 (ALAS1), the first and rate-limiting enzyme in the haem biosynthetic pathway. However, the underlying mechanism is unknown and consequently our investigations have set out to determine exactly how haem regulates ALAS1 mRNA stability to control its own synthesis in non-erythroid cells.

The experimental approach to delineate the ALAS1 *cis*-acting element(s) has involved RT-PCRs and heterologous reporter gene assays to analyse the stability of ALAS1-fusion mRNAs in response to haem in the human HepG2 hepatoma cells. The data demonstrates that the sequences that confer haem-mediated instability reside within the ALAS1 mRNA coding region and translation through the coding region is required for haem-mediated destabilisation. A minor alternatively-spliced ALAS1 mRNA species is poorly translated and consequently resistant to haem-mediated decay. These results provide a basis for identifying both the *cis*- and *trans*-acting factors, and their interactions involved in haem-mediated instability of ALAS1 mRNA.

URINARY COPROPORPHYRIN ISOMERS IN CONGENITAL AND ACQUIRED LIVER CHOLESTASIS

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Proven the key role of liver in heme synthesis and porphyrin metabolism and excretion, urinary porphyrin (and their metabolites) concentrations and/or profiles may result altered in the presence of liver impairment and cholestasis. The present study was aimed at comparing coproporhyrin isomer (CPI) urinary excretion profiles (measured by HPLC methods) in congenital and acquired liver cholestatic syndromes in order to assess the possible diagnostic-functional significance of these assays. The table resumes urinary CPI values and cholestasis parameters in controls and in patients affected by different acquired cholestatic and Dubin-Johnson syndromes.

	Total Plasma Bilirubin (mg/dl)	Alkaline Fosfatase (mU/ml)	γ-GT (mU/ml)	Urinary Total Coproporhyrin (nnmol/mmol creatinine)	Urinary Coproporphyrin I/III ratio	Plasma Tri-oh / Di-oh Bile acid ratio
Controls (n=16)	0.8 ± 0.4	142 ± 37	13 ± 1.7	71 ± 29	0.53 ± 0.1	3.5 ± 0.6
Intrahepatic Cholestasis of Pregnancy (n=12)	1.6 ± 1.2*	250 ± 26**	80 ± 7.5**	169 ± 50**	1.9 ± 0.2**	3.3 ± 1.2°
Viral Chronic Hepatitis (n=13)	6.5 ± 1.9**	249 ± 58**	351 ± 58**	248 ± 90**	1.5 ± 0.2**	2.3 ± 1.2*
Liver Cirrhosis (n=21)	5.7 ± 1.0**	377 ± 86**	85 ± 16**	268 ± 163*	1.23 ± 0.4**	0.9 ± 0.6**
Primary Biliary Cirrhosis (n=8)	4.9 ± 1.3**	386 ± 54**	479 ± 94**	304 ± 136**	1.9 ± 0.7**	3.7 ± 1.3°
Extrahepatic Biliary Obstruction (n=20)	17 ± 2.3**	382 ± 42**	435 ± 74**	311 ± 102**	1.4 ± 0.4**	4.2 ± 1.8°
Drug-Induced Cholestasis (n=10)	7.2 ± 3.4**	247 ± 36*	88 ± 8.1**	286 ± 86*	1.7 ± 0.2**	2.9 ± 1.6°
Dubin-Johnson Syndrome (n= 4)	2.7 ± 0.2**	167 ± 53°	15 ± 3.1°	106 ± 11*	5.1 ± 1.5**	2.6 ± 0.9*

Statistical vs. control group : ° p=ns; *p<.05; **p<.01. Anova for independent samples.

The abnormal distribution of urinary CP isomers in different cholestatic diseases is suggested to be the consequence of a retention effect due to biliary excretion impairment, nevertheless a new synthetic active response to cholestasis may be also relevant and particularly important especially in Dubin-Johnson Syndrome. In cholestastic diseases the inversion of urinary CP isomers ratio may be also considered an early signal of entero-hepatic circulation derangement even in those conditions known as "dissociated cholestasis" (moderate or no jaundice), such as pregnancy cholestasis. The amount of isomer I production and secretion rate, seems also to be correlated to the functional status of hepatocyte: the higher tri-oh/di-oh ratio (an extremely sensible functional test) and the greater increase of copro I/copro III ratio in pregnancy cholestasis , in primary biliary cirrhosis and in obstructive forms (scanty liver derangement) with respect to liver cirrhosis, chronic hepatitis and drug-induced cholestasis, supports this hypothesis.

δ-AMINOLEVULINIC ACID ALTERS THE ANTIOXIDANT ENZYME SYSTEM IN MICE BRAIN

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δ-Aminolevulinic acid (ALA), a heme precursor accumulated during the clinical expression of Acute Intermittent Porphyria (AIP), seems to be responsible for the neuropsychyatric manifestations of this syndrome. ALA-generated oxyradicals have been shown to cause oxidative lesions in rat brain synaptic membranes.

We have previously observed that Heme oxygenase activity in the brain of ALA treated animals, was induced as an antioxidant response to protect this organ against the injury provoked by ALA. Moreover, ALA accumulation in whole brain was detected.

To determine if ALA provokes some alterations on the brain antioxidant system, we have investigated the effect of acute (a single dose of 40 mg/kg, ip.) and chronic (40 mg/kg every 48 hours during two weeks) ALA administration to mice on superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GRed) activities and TBARS and GSH levels.

A 56% ($p<0.01$) increase of TBARS levels was observed after acute ALA administration. Catalase activity was induced above 50% ($p<0.05$) after acute ALA treatment. Similar results were observed on GPx y GRed. No alterations were detected on SOD activity. In animals treated with chronic ALA, a minimal if any alteration was observed on the parameters assayed.

Data indicate that a rapid response to oxidative stress was developed against reactive oxidative species induced by ALA treatment. However, in long term intoxication, the

redox balance was probably restored minimising oxidative damage.

CLINICAL, BIOCHEMICAL AND PATHOHISTOLOGICAL ASPECTS IN 12 PATIENTS WITH ACUTE PORPHYRIA AND HEPATOCELLULAR CANCER

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The aim of the study was to retrospectively study 10 women and 2 men with acute porphyria (10 AIP, 1 HCP, 1 VP) who had developed hepatocellular cancer (HCC).

The mean age for diagnosis of HCC was 64.8 (range 53-76). Eight patients were investigated for HCC because of reactivation of porphyria symptoms and/or increased urinary excretion of porphyrin precursors and porphyrins. In two of them the liver tumour was already palpable. The tumour was discovered on abdominal palpation in two patients and in two others by routine hepatic ultrasound examination. The patients were treated surgically (7), with chemotherapy (1), with radiotherapy (1), palliative (2) or with a combination of ablation, alcohol injection and chemotherapy (1). Six patients have died due to HCC. The survival time was 4.2 years; range 0.3-13. Alfa feto protein was positive in two of 10 investigated patients. At the present a re-investigation of liver biopsy material and screening for various cancer markers is in process.

Conclusion: It is of primary importance to detect HCC as early as possible, allowing surgical intervention. Carriers of acute porphyria above 50 years should be submitted for control at least yearly using ultrasound technology to detect incipient HCC. This method shows so far the highest sensitivity and is cost effective.

RELIABLE BIOCHEMICAL CRITERIA FOR CONFIRMING AN ACUTE ATTACK OF NEUROPORPHYRIA

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Porphyria experts concur that acute attacks of AIP, VP and HCP, are invariably associated with increases in urinary PBG. Reports differ, however, as to the amount of increase indicative of an acute attack. Some authors consider excretion of at least 50 mg PBG/day, (25-fold the upper level of normal), as indicative, whereas, others regard a 10-fold or even a 2-fold increase, as sufficient indication.

An additional diagnostic difficulty arises from the fact that in many individuals known to have inherited one of the acute porphyrias, PBG is persistently raised also during remission. It may be markedly elevated even in asymptomatic carriers.

In the absence of a universally accepted standard for interpreting PBG results, attribution of neurovisceral or neuropsychiatric symptoms in porphyrics to an acute attack of porphyria rather than to other causes, depends largely on clinical assessment.

The aim of this work was to identify reliable criteria, which will enable establishing or excluding an acute attack, on a biochemical basis.

The study summarizes and interprets data obtained during classical neurovisceral acute attacks and latent phases in 20 patients (10 with AIP, 6 with VP, and 4 with HCP). Calculated increases in urinary PBG, with the upper level of normal excretion, (2 mg/day), defined as 100%, revealed an overlap between values in the acute and latent phases, (1 to 18.5-fold and 2.3 to 51-fold, respectively). This overlap indicates that the workup in each case needs to be individualized. We achieved this goal, by using another method of calculation, in which the PBG value measured during an acute attack in a particular patient was divided by the PBG value measured in that patient's latent phase. Increases of 2.3 to 50.5-fold were obtained, leading to the conclusion that any increase, calculated as above, of 2.3-fold and higher, may be taken as indicative of an acute attack.

An additional finding, demonstrated in the study, which might be useful for supporting the diagnosis of an acute attack, is the distinct emission peak observed at 404/621 nm, in the plasma fluorometric scan of AIP and HCP patients, during an acute attack.

We conclude that comparison of the urinary PBG level and plasma fluorometric scan in the acute phase to those of the latent phase in the individual patient is the key to correct, accurate and reliable biochemical diagnosis of an acute attack in a patient previously diagnosed as a porphyric. The additional tests required for confirming a patient's first acute attack, having no data to compare with, are discussed.

KISS AND RUN: THE ROUTE OF IRON FROM ENDOSOMES TO MITOCHONDRIA CAN BYPASS THE CYTOSOL IN HEMOGLOBIN-PRODUCING CELLS

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During differentiation, immature erythroid cells acquire vast amounts of iron at a breakneck rate. Proper coordination of iron delivery and utilization in heme synthesis is essential and disruption of this process likely underlies iron loading disorders such as sideroblastic anemia and myelodysplastic syndrome with ringed sideroblasts. Iron is taken up by the cells *via* receptor mediated endocytosis, a process whereby diferric transferrin (Tf) binds to its cognate receptor (TfR) on the erythroid cell plasma membrane, followed by internalization of the Tf-TfR complex. Subsequent to endocytosis, the endosome is acidified by a H^+-ATPase, allowing the release of iron from Tf. Through an unknown mechanism, iron is targeted to the inner membrane of the mitochondria, where the

enzyme that inserts Fe into protoporphyrin IX, ferrochelatase, resides. Although it has been demonstrated that the divalent metal transporter, DMT1, is responsible for the egress of reduced Fe from the vesicle, the immediate fate of the iron atoms after their transport across the vesicular membrane remains unknown. Because reduced iron is a strong pro-oxidant, contributing to free radical formation through Fenton chemistry, it has been predicted that an iron binding molecule shuttles Fe from the endosome to mitochondria. However, this much sought iron binding intermediate, that would constitute the labile iron pool (LIP), has yet to be identified. Thus, we hypothesize that, in hemoglobin-producing cells, there is a direct relaying of Fe from the endosomal machinery to that of the mitochondria. We have taken two strategies in examining this supposition: 1) a biochemical approach by which the cytoplasm of cells was loaded with an impermeant iron chelator, thus intercepting the delivery of Fe by the putative LIP intermediate, and 2) a morphological approach employing time-lapse confocal microscopy which permits the tracking of iron-loaded endosomes and mitochondria with high spatial and temporal resolutions. To examine whether iron delivered by Tf for heme synthesis can bypass the cytosol, we have loaded reticulocytes with a high-molecular weight version of desferrioxamine, hDFO, prior to incubation with ^{59}Fe-Tf. The incorporation of transferrin iron into heme was unaffected by hDFO when compared to controls. Importantly, iron delivered to these cells in a form that freely diffuses across the membrane, iron-salicylaldehyde isonicotinoyl hydrazone ($^{59}FeSIH_2$), was significantly prevented from being used for heme synthesis in hDFO-laden reticulocytes. Using confocal microscopy, as well as polarized light microscopy, we found that endosomes are very mobile organelles. Immediately following budding from the plasma membrane, these organelles continuously traverse the cytosol and touch a number of mitochondria multiple times. Experiments using various pharmacological agents indicate that these movements are mediated by components of the cytoskeleton which are essential for proper iron delivery for use in heme synthesis. Together, these data suggest that iron is directly delivered to mitochondria by endosomes in a "kiss and run" paradigm. Our current studies will examine the required components and regulation of this interaction using the same experimental strategies as well as a cell free system consisting of isolated organelles.

STRUCTURAL ASPECTS OF HUMAN PBG DEAMINASE IN RELATION TO ACUTE INTERMITTENT PORPHYRIA

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X-Ray structures of both erythroid and ubiquitous human PBGD will be presented and the differences between the human and *E. coli* enzyme, previously used as a human enzyme model, will be discussed. Several newly described human deaminase mutants have been expressed *in vitro*, all of which lead to acute intermittent porphyria (AIP). One mutant, Asp 99 Gly, involves the essential cataytic group Asp 99 and, although the enzyme is completely inactive, it is correctly folded and has self-assembled the dipyrromethane cofactor at the active site. Other mutations result in deaminases that cannot assemble the cofactor and exist as highly unstable, inactive, CRIM -ve apo-proteins. Studies on the mechanism of dipyrromethane cofactor assembly have shown that porphobilinogen strongly inhibits the formation of holo-deaminase from apo-deaminase and that the elevated level of porphobilinogen that accompanies an acute attack of AIP is high enough to inhibit the formation of holo-deaminase and dramatically reduce the level of hepatic deaminase *in vivo*. This is consistent with the observations on liver biopsy samples from the laboratory of Marver in the 1970s. A lowered level of hepatic porphobilinogen deaminase during an acute attack of AIP would contribute to maintaining the vicious cycle of low haem synthesis and raised 5-aminolaevulinate synthase in the liver and would also explain why erythrocyte levels of deaminase are not substantially affected during an acute attack.

MOUSE MODELS FOR STUDYING THE EFFECTS OF ALCOHOL AND ASCORBIC ACID IN PCT

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Although several risk factors in PCT have been recognized, consumption of alcoholic beverages is the main risk factor in PCT. The mechanism of the effect of alcohol remains unknown. One of the problems has been the lack of an animal model. Recently, we demonstrated that mice which are null mutants for the Hfe gene, and are used as models for studying hereditary hemochromatosis, accumulate large amounts of hepatic uroporphyrin after 5-7 months' continual consumption of ethanol in the drinking water (1). This was associated with increased hepatocyte Perl staining for iron. There was no hepatic uroporphyrin accumulation in wild-type mice. This work was initially performed in 129S6 mice but has now been investigated in other mouse strains.

Another risk factor in PCT has been suggested to be low plasma ascorbate, based on studies in hepatocyte cultures, rat models and an initial study in patients. This work has now been extended to mice carrying null mutations in an enzyme required for ascorbate synthesis. In the absence of iron loading, decreased hepatic ascorbate increased sensitivity for development of uroporphyria. However, when iron overload was produced by administration of iron dextran, there was no further increase in uroporphyrin accumulation in ascorbate-deficient mice, beyond that produced in mice with normal hepatic ascorbate levels. There also was no depletion of hepatic ascorbate by the administered iron.

These results will be discussed and compared with other known risk factors in mouse PCT models and their applicability to the human disease.

1. Gorman N, Trask HW, Bement WJ, Szakacs JG, Elder GH, Balestra D, Jacobs NJ, Jacobs JM, Sinclair JF, Gerhard GS, Sinclair PR. Genetic factors influence ethanol-induced uroporphyria in Hfe(-/-) mice. Hepatology. 2004,40:942-50.

VARIEGATE PORPHYRIA VERSUS PCT AND ACNE: PITFALLS IN DIAGNOSIS AND TREATMENT

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Porphyria cutanea tarda (PCT) was diagnosed in a case of a 44 year old woman with acne conglobata since 20 years, initiated as acne fulminans together with light in-duced cutaneous symptoms (mainly blisters) since 4 years. The PCT-diagnosis was made on basis of histologic examination of a skin biopsy and a mild increase of uri-nary uroporphyrin within a slightly elevated excretion of total porphyrins (168 µg/die).

Treatment with chloroquine was initiated. The patient mentioned periods of abdominal pain about 10 years ago.

The examinations in our laboratory revealed the following data: Moderate elevation of δ-aminolevulinic acid and porphobilinogen and an excessive porphyrin excretion (3223 µg/l, normal: <145) with dominance of coproporphyrin (70%) and an increase of isomer III (91%, normal: 69-83). Fecal porphyrins were increased up to 440 µg/g (normal: <85) mainly due to an elevated excretion of protoporphyrin. An inversion of faecal coproporphyrin isomers could be observed (62% isomer III, normal: 23-35). The activity of porphobilinogen desaminase was found normal. Therefore the diagno-sis of a variegate porphyria (VP) in a non-acute phase was established. Family studies showed normal urinary porphyrin precursors and porphyrin excretion in mother and son (15 years). The faecal porphyrin excretion of the mother was 25 µg/g. Even lower were the stool porphyrins of the son (<10 µg/g) stating no metabolic indication of a genetic protoporphyrinogen oxidase defect in both cases. The father of the patient is deceased, so there is no sample material available.

Sequence analysis of the protoporphyrinogen oxidase gene revealed a mutation(base exchange) in exon 9 leading to an exchange of leucin for prolin (L291P).

To our knowledge this mutation was not described so far as being responsible for an enzyme deficiency, but the short distance of the location to a known and significant base exchange (only 4 base pairs) suggests that this mutation can assumed to be responsible for the lack of enzyme activity leading to the discribed metabolic disorder.

Molecular genetic studies considering the other members of the family are in progress.

Conclusion: The determination of urinary and faecal porphyrin excretion patterns gave evidence for variegate porphyria (VP). The metabolic constellation found in the patient is characterstic for this type of acute hepatic porphyria. The preceding diagnosis of PCT could be ruled out. As a consequence the chloroquine therapy was suspended.

In Germany VP is the second frequent acute hepatic porphyria; 102 cases (62 males and 40 females) were diagnosed during 40 years of activity in the Marburg porphyria center. This case shows that histopathologic examinations of skin samples together with the clinical/dermatological aspect can lead to a presumption of a disorder in the porphyrin metabolism, but they are not at all sufficient in establishing the final diagno-sis or classification of the metabolic disease.

THE NORWEGIAN PORPHYRIA REGISTER

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The Norwegian Porphyria Centre (NAPOS) established a national porphyria register in 2002. The main objectives of the register are porphyria research, genealogical studies as well as prophylaxis or treatment of individual patients. It is also a useful tool for the informative work performed by the Porphyria Centre, which includes distribution of brochures, newsletters and porphyria ID cards. Data collection is based on questionnaires filled in by individuals diagnosed with porphyria, and is supplemented with biochemical and genetic laboratory results. All registered persons will also receive a follow-up questionnaire every second year. These questionnaires will be used to continuously update existing information in the database, and also to collect new data for prospective studies.

So far we have registered 312 individuals with latent or manifest porphyria. These are divided into 5 different diagnoses: 160 with acute intermittent porphyria (AIP), 124 with porphyria cutanea tarda (PCT), 17 with erythropoietic protoporphyria (EPP), 9 with variegate porphyria (VP), and 2 with hereditary coproporphyria (HCP). We have also collected genealogical data, and have currently registered more than 2000 family members.

Clinical expressions during attacks of AIP are self-reported by 85 individuals with manifest AIP. Abdominal pain is the most common symptom (87%), followed by fatigue (69%), dark/red-coloured urine (59%), muscle pain (58%), muscle weakness (51%), and tachycardia (51%). Other results from the register will be presented.

HFE-MUTATIONS, IRON, HEPATITIS C, AUTOIMMUNITY, LIVER DAMAGE AND THERAPEUTIC RESPONSE IN PORPHYRIA CUTANEA TARDA IN GERMANY

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Background: Porphyria cutanea tarda (PCT) is caused by decreased activity of the enzyme uroporphyrinogen decarboxylase in the liver. Alcohol, hormones, drugs, iron and viral infections are known trigger factors responsible for the precipitation of PCT and the associated hepatic alterations.

Methods: Over a period of 20 years we studied a cohort of 207 patients with PCT, skin symptoms, liver injury and urinary uro-and heptaporphyrin excess on whether hepatitis B and C, HFE mutations (C282Y and H63D), liver and serum iron, diabetes, autoantibodies, drugs and alcohol consumption might have an influence on the clinical course, urinary porphyrin excretion, liver enzyme activities (ALT, AST), serum collagen peptides (PNIIIP, CVI) and histological abnormalities. Most of the patients were treated with low dose chloroquine diphosphate 125-250 mg twice weekly.

Results: Eight % of the PCT patients tested positive for hepatitis C and 13% for hepatitis B virus antibodies. The majority (61%) carried HFE mutations. The prevalence of diabetes mellitus type II was increased (15%) and autoantibodies (ANA, ASMA) were found significantly less common than in controls.

Serum and liver iron, serum ferritin and collagen peptide (PNIIIP) levels, and liver enzyme activities (ALT, AST) correlated significantly with urinary porphyrins and the clinical stage (overt, remission, relapse). Using multivariate regression analysis urinary porphyrins were found to strongly predict liver damage as reflected by elevated liver enzymes ($p< 0.0001$).

Chloroquine therapy was accompanied by clinical remission and reduced urinary porphyrin excretion ($p < 0,001$) among patients with both HFE wild type as well as in HFE heterozygous patients with PCT. Patients homozygous for the C282Y mutation (5%) failed to respond to chloroquine treatment.

Conclusions: Our findings confirm the multi-factorial pathogenesis of PCT. Increased urinary porphyrins strongly correlate with serum markers of liver fibrosis and liver damage. Simple or compound heterozygosity of HFE mutations did not affect the therapeutic response to chloroquine in PCT. Since HFE C282Y homozygotes did not respond to chloroquine, phlebotomy should be first-line therapy in patients with PCT and HFE-mutations.

INDIVIDUAL REFERENCE VALUES OF URINARY ALA, PBG AND PORPHYRINS IN PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA.

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Some patients with acute intermittent porphyria (AIP) present recurrently increased urinary values of porphobilinogen (PBG) and aminolevulinic acid (ALA). Therefore, the diagnose of an acute attack and the optimization of heme therapy may be problematical given the difficulty to interpret the laboratory results. In order to determine individual reference values, we have selected six of these patients and we have (a): analyzed in duplicate the concentration of PBG, ALA and porphyrins in urine collected once a week for a period of seven consecutive weeks; (b): studied the recorded basal values of PBG and ALA in urine during a three-year period; In addition, we have analyzed during 45 consecutive days the urine of one patient treated with heme-arginate. These urinary data have been contrasted with the enzymatic (erythrocyte PBG deaminase); genetic (mutation in the AIP gene) and clinical status of the patients. The results show that, during long periods of time, the urinary time course of PBG and ALA does not present an intermittent peak/baseline pattern but a profile of regular high excretion. The within-subject biological variation (CV) of PBG, ALA and major urinary porphyrins was calculated; This allowed to identify some patients with PBG reference values 20-50 folds the upper normal limit with a CV < 15%. PBG and ALA concentrations showed a good correlation but some of the major porphyrins did not always correlated with the precursors. The three-year recorded data suggest that each AIP patient approaches a steady-state of high ALA/PBG production as a probable consequence of an enduring up-regulation of the ALA-S gene. The set point value of this steady-state appears to be independent of the activity of PBGD; the type of AIP mutation and even ,to a certain degree, the clinical status of the disease (supported by grant FIS/03/ 0489/Spain).

EXPERIENCE OF GENETIC COUNSELING IN NORWAY

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To protect the rights of individuals, genetic testing is regulated by law in Norway. Whereas diagnostic work-up for overt disease is exempt, carrier testing and predictive testing for possible future disease are not. Unless serious consequences may be avoided by predictive testing, the testing of children is prohibited until the legal age of consent (16 years). License to perform predictive testing is granted to regional medical genetics departments and a few other units for defined disorders. Informed consent, professional genetic counseling before and after testing, and annual reports of the activity are among the prerequisites for predictive testing. Health personnel may not contact other members of the family to offer testing ("unsolicited genetic activity"), but leave such distribution of information to patients ("cascade testing"). Third parties (e.g. employer, insurance companies) are barred from information resulting from predictive genetic testing, and may not even ask whether such tests have been performed.

Genetic testing in Norway is only performed at The National center for porphyria disorders (NAPOS). We attempt to identify the mutation specter of all porphyria disorders, opening for predictive testing on the DNA level. The physicians and genetic counselor at NAPOS also perform genetic counseling for predictive testing. We would like to present some of our experience from the counseling activity. The authors would particularly like to address some issues concerning genetic counseling for discussion in an international community of porphyria experts, to reveal similarities and differences in the way of thinking between countries. Among these issues are

- Is genetic counseling an equally useful activity for the various porphyria disorders?
- Should genetic counseling before biochemical testing of family members for porphyria be different from before DNA-testing?

- Only a few of the relatives of familial PCT patients (10%?) will ever develop symptoms. To what extent should genetic counseling and predictive DNA testing be offered to family members?
- The counseling and testing of children for possible future porphyria.

MUTATION SCREENING IN NEWLY (2004) DIAGNOZED PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA FROM CZECH AND SLOVAK REPUBLICS

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BACKGROUND: Acute intermittent porphyria (AIP; OMIM 176000) is an autosomal dominant disorder caused by the partial deficiency of porphobilinogen deaminase (PBGD; or hydroxymethylbilane synthase, HMBS; EC 4.3.1.8), the third enzyme in the heme biosynthetic pathway. It is manifested by life-threatening acute neurological attacks that can be provoked by various factors, including certain drugs, hormones, alcohol and starvation. Clinical expression is highly variable, and ~90% of AIP heterozygotes remain asymptomatic throughout life. To date, over 250 HMBS mutations have been identified.

OBJECTIVE: To identify the molecular lesions in newly diagnosed (2004) Czech and Slovak AIP patients.

DESIGN AND METHODS: Genomic DNA was isolated from members of seven unrelated AIP families from the Czech and Slovak Republics, and mutation screening was performed by PCR and denaturing gradient gel electrophoresis (DGGE). Subsequently, automated DNA sequencing was used to verify the mutated lesions. For each identified mutations, a restriction fragment length polymorphism (RFLP) assay was established, and a total of 36 individuals from seven families were analyzed to detect asymptomatic carriers.

RESULTS: Eight mutations were identified, including three novel mutations (610 C>A, 675 delA, 966 insA), and five previously reported mutations (76 C>T, 77 G>A, 518 G>A, 771+1 G>T, 973 insG). This is the first report of the 518 G>A mutation in the Czech and Slovak population. Of particular interest, one patient had two mutations, 518 G>A and 610 C>A, both located in exon 10.

CONCLUSIONS: Three novel mutations were identified in seven unrelated AIP families. These studies further emphasize the molecular heterogeneity of AIP, and provide accurate detection of asymptomatic carriers.

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CHARACTERISATION OF MITOCHONDRIAL TARGETING OF WILD TYPE AND MUTANT (L88Q) HUMAN COPROPORPHYRINOGEN OXIDASE

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Coproporphyrinogen Oxidase (CPO), the sixth enzyme in the haem biosynthetic pathway, is located in the mitochondrial intermembrane space. Partial deficiency of CPO underlies hereditary coproporphyria, an autosomal dominant acute porphyria. The 453 amino acid apo-CPO is imported into the mitochondrial intermembrane (IM) space through a cleavable 110 amino acid leader peptide, although this has not been confirmed in living cells. Analyses of hydrophobicity and secondary structure conformational parameters in both human and mouse CPO suggests that the leader sequence is a bipartite structure, consisting, in human CPO, of a positively charged matrix-targeting signal region (residues 1-69) followed by an extended hydrophobic sorting region (residues 70-103) that may direct the protein to the inter-membrane space. We have fused human CPOs, containing N-terminal and C-terminal deletions, to the amino terminus of yellow fluorescent protein (YFP) and have imaged mitochondrial targeting by fluorescence and confocal microscopy. Here we show that amino acid residues 1-69 contain all the information necessary to target YFP to the mitochondria in living cells. Residues 70-110 were not, by themselves, able target YFP. A pathogenic mutation, L88Q, identified in a patient with HCP does not prevent targeting. We are currently investigating the role of the hydrophobic region in localising CPO to the inter-membrane space.

FOUR CASES OF CHOLESTATIC LIVER FAILURE IN ERYTHROPOIETIC PROTOPORPHYRIA - VARYING CLINICAL COURSE AND EXAMPLE OF EFFECTIVE MEDICAL TREATMENT

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In the last decade, four Swedish patients with EPP have presented with cholestatic liver failure. All were male with several similarities in medical history. Liver histology differed, as did the clinical course, which may be described as acute, insidious and chronic liver failure, respectively. The first three had a fatal outcome, one after liver transplantation. When in cholestatic liver failure, the time window where treatment is meaningful is often short.

The fourth patient presented with acute onset cholestasis, reduced faecal protoporphyrin (PP) excretion and elevated urinary copropophyrin excretion. During transplant work up treatment was initiated aiming at; 1. reducing PP production, 2. reducing PP in

transit, 3. reducing hepatic PP production and ameliorating heme deficiency by haemarginate infusions, 4. reducing hepatic PP toxicity, 5. inducing bile flow and 6. interrupting enterohepatic circulation. The condition was successfully reversed, resulting in complete normalization of liver function test within 80 days. Afterwards, treatment was de-escalated and the patient is doing well 6 months after onset of liver failure. Around 10% of all EPP patients diagnosed in Sweden present with life threatening cholestatic liver failure. We report the characteristics of four patients and an example of successful treatment of severe cholestatic liver failure in EPP.

MUTATION DETECTION IN THE FECH GENE

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Identification of mutations in the *FECH* gene is necessary for the accurate predictive genetic counselling of some families with EPP. However, with current methods, *FECH* mutations are not detected in about 20% of families. In an attempt to increase the detection rate, we are assessing a three stage strategy for mutation analysis. Denaturing HPLC was used as a first line screening test. Each exon was amplified from genomic DNA and analysed without further purification. Positive and negative controls for each exon were included with each batch of samples. Shifts, in addition to those caused by known SNPs, were identified in 32 (74%) of 43 patients; all were confirmed as mutations by fluorescent sequencing. For patients in whom mutations were not identified, quantitative PCR was used to search for deletions that encompassed exons and were not detectable by sequencing. Exons 2-11 were amplified by PCR in a multiplex reaction, with incorporation of a fluorescent label and subsequent analysis by gene scanning. Exon dosage was determined by comparison with internal controls. Finally, mutation negative samples were analysed by bi-directional sequencing using different primers from those used for dHPLC to avoid the frequent problem of primer site polymorphisms. Using either this strategy, or bi-directional automated sequencing alone, we have so far identified *FECH* mutations in 61(77%) of 79 patients with EPP, only one of whom had a deletion undetectable by direct sequencing of PCR-amplified genomic DNA.

DRUG PRESCRIPTION IN PATIENTS WITH CRIGLER-NAJJAR SYNDROME

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Crigler-Najjar syndromes (CNS) type 1 and 2 are characterised by markedly raised levels of unconjugated bilirubin. Despite phototherapy, patients remain at risk of developing bilirubin encephalopathy if unbound bilirubin concentrations in plasma exceed the binding capacity of albumin, or if bilirubin is displaced from albumin. The pharmacokinetics of drugs that undergo glucuronidation may be abnormal in CNS. We have drawn up lists of potentially unsafe drugs for our patients with CNS according to the following classification:

Drugs that increase the risk of kernicterus in CNS

- Drugs that compete for albumin binding. Any drug is potentially dangerous if it occupies > 5% of the binding sites on albumin, i.e. has a bound concentration > 15 µmol/l. (e.g. sulfisoxazole, sulfamethoxazole, dicloxacillin, cefoperazone, ceftriaxone)
- Drugs which increase production of bilirubin by reducing red cell survival (e.g. dapsone).
- In patients with Crigler-Najjar type 2, drugs which interfere with the induction of bilirubin UDP-glucuronyl transferase by phenobarbital (e.g. probenecid, St. Johns Wort?)
- Bilirubin exits the brain, and possibly the enterocyte, via p-glycoprotein. Drugs which inhibit p-glycoprotein will increase brain bilirubin concentrations and increase intestinal bilirubin re-absorption (e.g. verapamil, ceftriaxone, rifampin and herbals such as curcumin, ginsenosides, piperine, some catechins and silymarin)
- Drugs which affect mitochondrial function, could possibly increase bilirubin toxicity (e.g. salicylates, valproate)

Drugs with abnormal pharmacokinetics in CNS

Ten of our 18 CNS patients have mutations in the first 4 exons of the UDPGT1A gene, and are predicted to have abnormal glucuronidation of drugs conjugated by the UDGT1A isoenzymes (e.g. irenotecan, paracetamol, mycophenolic acid)

It should be emphasized that increases in bilirubin levels are not only caused by drugs. Causes of exacerbations of jaundice reported in a recent survey of 42 patients with Crigler-Najjar syndrome type 1 were respiratory infections, febrile illnesses, vaccinations, fasting, surgery, emotional stress, and non-compliance with treatment. However as drug-induced increases in total or free unconjugated bilirubin concentrations are preventable, patients and their doctors should take the above considerations into account when treatment is needed.

PREGNANCY IN CRIGLER-NAJJAR SYNDROME

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Background: With intensive phototherapy patients with Crigler-Najjar syndrome (CNS) can survive to become jaundiced but otherwise healthy adults. A major concern is that if pregnancy occurs, unconjugated bilirubin, which can cross the placenta, might cause bilirubin encephalopathy in the foetus.

Case Report: A woman with CNS 2, compound heterozygote for the mutations 15R and S190F in the UDT1A1 gene, had bilirubin levels between 200 and 250 µmol/l with 1-2 hours of phototherapy (10 lamps of 100 W) per day. She does not take phenobarbital because of side effects. At 27 years she became pregnant. At 9 wks her serum bilirubin was 234 µmol/l and albumin 43 g/l. The phototherapy was increased to 3 hours per day resulting in bilirubin levels between 165 and 205 µmol/l. Her serum albumin dropped to 34 g/l. Because of mild proteinuria delivery was induced and forceps-assisted at 39 weeks. At delivery her bilirubin was 239 µmol/l. The healthy boy weighed 3440 g and had an APGAR score of 8 at 1 min. and 9 at 5 min. Cord blood bilirubin levels were the same as the mother's. Her bilirubin rose after delivery to 278 µmol/l and her albumin dropped to 30 g/l. Phototherapy was given to the child for three days. Two years later she gave birth to a healthy girl at 39 weeks, weighing 3150 g with an APGAR of 10 at 5 min. Bilirubin and albumin levels were similar to the first pregnancy. Both children have developed normally without neurological abnormalities.

Conclusions: With regular monitoring and adjustment of treatment to keep bilirubin levels below 200 µmol/l, pregnancy without foetal damage is possible in patients with Crigler-Najjar syndrome.

THE DIAGNOSIS OF ACUTE HEPATIC PORPHYRIA BY PLASMA ALA, PBG, FLUORESCENCE SCANNING AND ENZYME ASSAYS, USING DNA ANALYSIS AS REFERENCE

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Background: We have developed an enzymatic assay for plasma aminolevulinic acid (ALA) and porphobilinogen (PBG), which is a more sensitive test for acute intermittent porphyria (AIP) than urine ALA and PBG analysis. Plasma fluorescence scanning has been reported to be a more sensitive test for variegate porphyria (VP) than faeces analysis. To determine whether the combination of plasma ALA, PBG and fluorescence scanning could be used as a screening test for AIP, VP and hereditary coproporphyria (HCP), we assessed these assays in a large group of subjects characterized for AIP, VP and HCP at the DNA level.

Methods: We evaluated all patients with AIP, VP and HCP and their relatives for whom the genotype and a plasma ALA, PBG and fluorescence scan were available in our reference laboratory. In addition, hydroxymethylbilane synthase (HMBS), coproporphyrinogen oxidase (CPO) and protoporphyrinogen oxidase (PPOX) were determined in many subjects. No patients had acute porphyric complaints at analysis.

Results: Percentage with abnormal results and number of subjects tested in brackets.

Test	AIP	HCP	VP	Controls
Plasma ALA	83% (98)	56% (18)	65% (121)	12% (201)
Plasma PBG	89% (98)	56% (18)	57% (121)	3% (201)
Fluorescence	51% (88)	29% (21)	34% (121)	0% (201)
HMBS ery's	89% (228)	0% (20)	0% (87)	0,5% (218)
CPO lympho's	0% (11)	100% (24)*	7% (70)*	0% (97)
PPOX lympho's	18% (11)	30% (24)*	87% (75)*	15% (81)

* One family had combined HCP and VP mutations.

All patients who had previously had an acute porphyric attack, had abnormal plasma ALA, PBG or fluorescence results in the asymptomatic period.

Conclusions: The combination of plasma ALA, PBG, fluorescence scanning will detect all patients with AIP, HCP or VP who have previously had an acute attack. These tests, combined with erythrocyte HMBS, can be performed on a single blood sample and will detect 100% of asymptomatic individuals with an AIP mutation. CPO assays in lymphoblastoid cells are very reliable for detecting HCP. Mutation analysis remains the only accurate method of confirming VP in asymptomatic individuals.

A TRUNCATED FORM OF HEME OXYGENASE-1 IS FOUND IN THE NUCLEUS OF CULTURED CELLS IN RESPONSE TO INCREASES IN INTRACELLULAR HEME

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Heme oxygenase (HO)-1, the rate–limiting enzyme in heme degradation, is an integral membrane protein of the smooth endoplasmic reticulum. HO-1 is induced by various stimuli including oxidative stress, heavy metals, UV radiation and inflammation but also by heme and heme-hemopexin. Cytoprotective roles for HO-1 are established, although the mechanism by which this occurs is not yet fully defined. When mouse hepatoma cells are incubated either with exogenous heme or heme-hemopexin, a truncated form of HO-1 is detected in the nucleus that lacks the C-terminus. Exogenous heme also generates a nuclear form of HO-1 in NIH 3T3 cells. Transfection of 3T3 cells with an N-terminal 6His-tagged/C-terminal FLAG-tagged HO-1 expression construct followed by incubation with heme leads to co-localization of the His, but not the FLAG signal, with a nuclear DAPI stain. Transfection with a GFP-HO-1 cDNA with a deletion of the C-terminus results in nuclear localization of the protein. Deletion of a putative nuclear export sequence (NES) results in diffuse localization of HO-1 that is not enhanced by hemin. Thus, hemin-mediated modification of the NES is also required for nuclear translocation. This nuclear C-terminal truncated HO-1 lacks normal enzymic activity and may act as a transcriptional regulator.

5-AMINOLEVULINATE SYNTHASE SCAFFOLD AND CONVERSION INTO A MORE ACTIVE ENZYME

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5-Aminolevulinate synthase (ALAS), a pyridoxal 5'-phosphate-dependent enzyme, catalyzes the first step of the heme biosynthetic pathway in mammalian cells. This reaction entails the condensation of glycine with succinyl-coenzyme A to yield 5-aminolevulinate, carbon dioxide and CoA. The active site of the dimeric ALAS is located at the subunit interface with contribution of amino acids from the two subunits. Linking the two subunits into a single polypeptide chain dimer (2XALAS) yielded an enzyme with a ~7-fold greater turnover number than that of wild-type ALAS. Spectroscopic and kinetic properties of 2XALAS were investigated to explore the differences in the coenzyme structure and kinetic mechanism relative to those of wild-type ALAS that confer a more active enzyme. Transient kinetic analysis of the formation and decay of the quinonoid intermediate EQ_2 indicated that, although their rates were similar in ALAS and 2XALAS, there was a greater accumulation of this intermediate in the 2XALAS-catalyzed reaction. Collectively, these results suggest that ketoenamine is the active form of the coenzyme and forms a more prominent coenzyme structure in 2XALAS than in ALAS at pH ~7.5.