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Porphyria in Switzerland, 15 years experience

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Summary

Background: The porphyrias, a group of seven metabolic disorders in the haem biosynthesis, can be classified into acute and non-acute porphyrias. A common symptom of acute porphyrias is severe acute abdominal pain, whereas cutaneous photosensitivity can occur in both acute and non-acute porphyrias. All porphyrias, except for sporadic porphyria cutanea tarda (sPCT), are hereditary disorders caused by mutations in the respective genes. We present porphyria cases documented in our porphyria centre during the past 15 years.

Methods: Diagnosis was based on clinical symptoms and biochemical analyses. Mutation analysis was performed in patients/families with a confirmed hereditary porphyria.

Results and conclusions: As the porphyria specialist centre of Switzerland, we perform the specialized analyses required for the diagnosis of all types of porphyrias, and give advice to patients, physicians and other laboratories. We therefore estimated that our data cover 80–90% of all diagnosed Swiss cases. A total of 217 patients from 170 families were diagnosed including, 111 acute intermittent porphyria, 45 erythropoietic protoporphyria, 30 variegate porphyria, 21 *s*PCT, five congenital erythropoietic porphyria, four hereditary coproporphyria and one hepatoerythropoietic porphyria patient. Systematic monitoring of the patients would allow early detection of the potential life-threatening complications such as hepatocellular carcinoma and renal insufficiency in acute porphyrias, and liver failure in EPP. Seventy-five percent of all families underwent genetic testing. Identification of pre-symptomatic mutation carriers so that these individuals and their physicians can be consulted with safety on drug use and other preventive measures, is important in managing acute porphyrias. The unique phenomenon of founder mutations in the Swiss population is also discussed.

Key words: porphyria, haem synthesis; enzyme deficiency; diagnosis; hepatocellular carcinoma; renal insufficiency; liver damage; DNA-testing, mutation

Introduction

Porphyrias are metabolic disorders in the haem biosynthesis. Haem is an essential constituent of the cellular haemoproteins such as haemoglobin, myoglobin and drug-metabolizing cytochrome P450. In most organisms including humans, haem is synthesised from amino acid

Abbreviations

ADP	5-Aminolevulinate dehydratase-deficient porphyria
AIP	acute intermittent porphyria
ALA	aminolevulinic acid
ALAD	ALA dehydratase
ALAS	ALA synthase
ALAT	alanine amino transferase
ASAT	aspartate amino transferase
CEP	congenital erythropoietic porphyria
CPOX	coproporphyrinogen oxidase
EPP	erythropoietic protoporphyria
FECH	ferrochelatase
GFR	glomerular filtration rate
HCC	hepatocellular carcinoma
HEP	hepatoerythropoietic porphyria
HCP	hereditary coproporphyria
LLN	lower limit of normal
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MDRD	modification of diet in renal disease
PBG	porphobilinogen
PBGD	porphobilinogen deaminase
PCT	porphyria cutanea tarda
PPOX	protoporphyrinogen oxidase
ULN	upper limit of normal
UROD	uroporphyrinogen decarboxylase
UROS	uroporphyrinogen III synthase
VP	variegate porphyria

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glycine and succinyl CoA. There are a total of eight enzymes in the haem biosynthetic pathway (fig. 1). The first and the last three enzymes are located in mitochondria and the others in the cytosol. Inherited deficiencies in seven of the eight enzymes (except ALA synthase) give rise to various porphyrias, a group of hereditary disorders. The only exception is the sporadic form of porphyria cutanea tarda (*s*PCT), in which not a hereditary, but rather an acquired hepatic defect of the enzyme uroporphyrinogen decarboxylase (UROD) is found [1].

There are different ways to classify porphyrias. The current classification which is preferred by most porphyrinologists, is to group porphyrias into acute porphyrias and non-acute porphyrias based on the clinical symptoms and treatment strategies. 5-Aminolevulinate dehydratase-deficient porphyria (ADP), acute intermittent porphyria (AIP), hereditary coproporphyria (HCP) and variegate porphyria (VP) comprise the group of acute porphyrias. Patients suffering from acute porphyrias share a common clinical symptom of acute attacks of severe abdominal pain due to a dysfunction of the autonomic nervous system. In addition, symptoms such as nausea, vomiting, hypertension, tachycardia and hyponatraemia are present in most porphyric crises. Severe or prolonged attacks may be accompanied by peripheral, predominantly motor and central nervous system dysfunction, which could eventually result in transient or prolonged tetraplegia and respiratory muscle dysfunction. Unlike Guillain-Barré Syndrome, acute porphyrias initially affect large muscles close to the trunk. All symptoms are believed to be caused by the accumulation of the porphyrin precursor aminolevulinic acid (ALA), as a result of various enzyme deficiencies.

Porphyric crises are often induced by precipitating factors such as inappropriate use of drugs including hormonal anti-conception, starving, excess of alcohol, infection and psychological stress. Females in the reproductive age are especially vulnerable and may develop spontaneous or regular premenstrual attacks. These attacks usually resolve within 1–2 days after the start of menstruation.

Light-induced skin symptoms, due to the dermal accumulation of the photosensitizing porphyrins, occur in both acute and non-acute porphyrias. There are two different forms of porphyric skin disease. One features blisters and skin fragility, the other is an acute painful dermatosis due to an acute phototoxic reaction. The porphyria-related photodamage of the skin is strictly limited to the light-exposed skin areas and is most prominent on the back of hands and face.

Blisters, ulcers and skin fragility are found in porphyria cutanea tarda (PCT), VP and HCP, as well as in two rare forms of non-acute porphyrias, congenital erythropoietic porphyria (CEP) and hepatoerythropoietic porphyria (HEP, the homozygous form of hereditary PCT). Patients with both CEP and HEP often develop symptoms early in life i.e., intrauterine hydrops fetalis, or photosensitivity during the neonatal period and infancy. However, cases with late clinical onset have also been described. The skin symptoms lead to light-induced mutilation of the face, mainly the nose and ears, the back of hands and of the fingers. In addition, various degrees of anaemia due to haemolysis or ineffective erythropoiesis are frequently observed [1].

Acute sunlight-induced pain in the skin is a characteristic symptom of erythropoietic protoporphyria (EPP) although it may also be observed in CEP and HEP. Initially, no objective signs in the affected skin areas, mostly on the back of hands and face, can be observed. After prolonged sun exposure, the acute phototoxic reaction progresses to incapacitating pain, swelling, redness, oedema and wheals in variable combinations.

In addition to the above mentioned porphyrias, rare forms of porphyria including homozygous hereditary coproporphyria (including harderoporphyria), homozygous acute-intermittent porphyria, homozygous variegate porphyria and dual porphyrias of which two different forms of heterozygous porphyrias are present in one individual, have also been described.

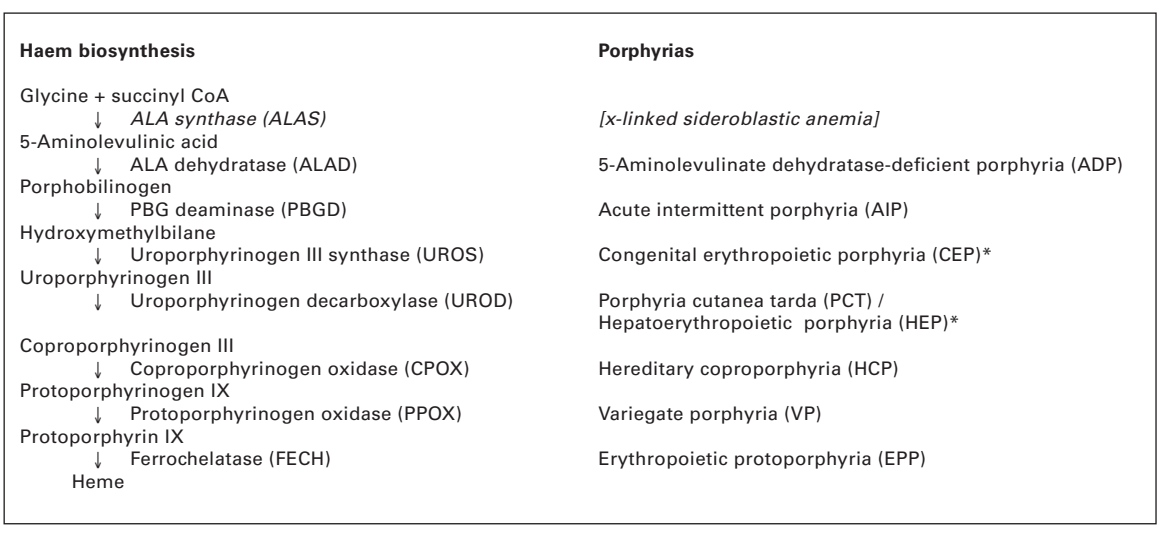


Figure 1

Haem biosynthesis and porphyrias. Recessive porphyrias are indicated by an *asterisk* (*).

Here we report the porphyria cases that have been documented in the central laboratory of Stadtspital Triemli during the past 15 years. We also outline the diagnostic strategy for porphyrias and compile the results of the genetic examinations. Our goal is to improve the awareness on porphyrias, seemingly rare disorders, among medical professionals in the wake of this presentation.

Patients and methods

Patients

This study presents data from patients who were diagnosed in our porphyria centre during the period from 1993 to 2007. The Swiss Registry on Porphyria Patients was approved by the "Eidgenössische Datenschutzkommission". Diagnosis of porphyria was established as outlined in the "Results and Discussion" section.

Laboratory diagnosis

In the central laboratory of Stadtspital Triemli, the following tests were routinely conducted for diagnosis and differential diagnosis of various types of porphyrias: urinary ALA and PBG, porphyrins (including uroporphyrin, heptacarboxyporphyrin, hexacarboxyporphyrin, pentacarboxyporphyrin, coproporphyrin isomers I and III) in urine and in plasma; porphyrins (including coproporphyrin isomers I and III, protoporphyrin) in faeces, porphyrins (including zinc-protoporphyrin and free protoporphyrin) in erythrocytes, and plasma fluorescence scan. Enzymatic assays were performed in three different enzymes, porphobilinogen deaminase, uroporphyrinogen III synthase and ALA dehydratase. All methods were established based on information in the literature and were accredited to ISO/IEC 17025 and EN ISO 15189 in 2006 (accreditation No. STS474) [2]. The glomerular filtration rate (GFR) was estimated according to the MDRD equation [3].

Laboratory specimens were obtained from our patients, and from other hospitals, private laboratories, and practitioners.

Genetic analysis

Mutation analysis was conducted in five types of porphyrias AIP, CEP, *f*PCT (or HEP), VP and EPP. The porphobilinogen deaminase (PBGD) gene (gene bank accession number NT033899, cDNA NM000190) is located in chromosome 11, the uroporphyrinogen III synthase (UROS) gene (NT035040; NM000375) in chromosome 10, the uroporphyrinogen decarboxylase (UROD) gene (NT032977; NM000374) in chromosome 1, the protoporphyrinogen oxidase (PPOX) gene (NT004487; NM000309) in chromosome 4 and the ferrochelatase (FECH) gene (NT025028; NM000140) in chromosome 18. All coding exons of the five genes, as well as the promoter region of the *UROS* gene, were sequenced in PCR products obtained by amplification of genomic DNA from porphyria patients.

In addition, cDNA of the *FECH* gene was quantified by real-time PCR using TaqMan reagents from Applied Biosystems.

Results and discussion

Consultation in the porphyria outpatient clinic

Our porphyria outpatient clinic began to operate in 1993. As shown in the statistics, around 100 consultations were performed each year (table 1). Most of the patients were referred by primary physicians or physicians from general hospitals. Some patients were members of known porphyria families who contacted us directly. More recently, a number of patients approached us through internet search.

Laboratory analyses in the diagnosis of porphyria

In 1993 a diagnostic laboratory was set up to ensure accurate diagnosis of all porphyrias. A comprehensive range of biochemical, enzymatic and genetic analyses, as outlined in the "patient and methods" section, is currently available in our laboratory. To further improve the analytical sensitivity of genetic testing, additional analyses such as real-time PCR quantification of ferrochelatase mRNA, were recently established. Since 1993, we were able to diagnose all symptomatic patients. The diagnostic sensitivity among asymptomatic individuals was greatly improved after the introduction of genetic testing. As shown in figure 2, the total number of tests increased from 8 in the year 1993 to 1539 in 2007. These numbers reflected a trend of an increasing number of specimens sent by private laboratories, hospitals and general practitioners from different parts of Switzerland.

International affiliations: in 1996, the central laboratory of Stadtspital Triemli was designated as "Swiss Porphyria Reference Laboratory" by the "International Federation of Clinical Chemistry". E. M. is a board member of the "European Porphyria Initiative" and a collaborating member of Epnet, a European Health Initiative (www.porphyria-europe.org; www.epnet.org).

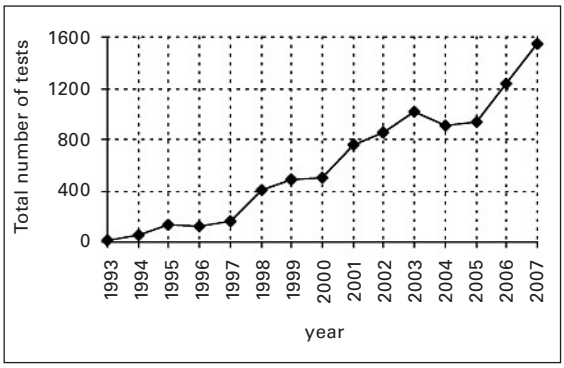
Table 1

Activities in the porphyria out-patient clinic.

number of	year 2004	year 2007
consultations	104	103
patients in total	55	62
porphyria patients	32	44
newly diagnosed patients	13	27
percentage of new cases	41% (13/32)	61% (27/44)

Figure 2

Number of laboratory tests on porphyria performed in the central laboratory of Stadtspital Triemli from 1993 to 2007.



Diagnostic strategy for porphyrias

The combination of a porphyria clinic and a diagnostic laboratory enabled us to conduct activities including diagnosis, differential diagnosis, counselling, prevention and treatment of all porphyrias.

Diagnosis of porphyrias is based on the clinical symptoms, family history and biochemical quantification of porphyrins and their precursors in different body fluids. The starting point of a cost-effective diagnostic strategy for symptomatic patients is the main clinical symptom. In the case of acute abdominal pain, the first-line test is urinary porphobilinogen (fig. 2). Urinary 5-aminolevulinic acid should be also included in order to detect lead intoxication with symptoms very similar to that of acute porphyria, or to diagnose the extremely rare form of porphyria, ADP. It is important to understand that the diagnosis of acute porphyrias can be hampered by their episodic nature. Therefore, particular attention should be given to the correct timing of specimen sampling in suspected cases of acute porphyrias i.e., within one week after the onset of acute symptoms.

To diagnose or to exclude acute porphyrias during the latent phase, we propose measurements of porphobilinogen in urine and PBGD activity for detection of AIP (both sensitivity and specificity for the enzymatic assay were estimated to be 95% [4]); faecal porphyrins for detection of VP (77% sensitivity; specificities of 87% and 80% with coproporphyrin and protoporphyrin, respectively [5]) and HCP (unknown sensitivity and specificity), and plasma-fluorescence scan for detection of VP (96% sensitivity [5]). Genetic tests are performed, only after a specific porphyria has been biochemically verified, or rarely, to establish a diagnosis in cryptic constellations.

Genetic testing is the most sensitive method for diagnosis of asymptomatic mutation carriers provided that a particular mutation has been identified in the index patient, or in another symptomatic individual within a family. Family screening is especially recommended in acute porphyrias since preventative measures can be

Figure 3

A flowchart illustrates the diagnostic strategy for porphyrias (except for rare homozygous forms; modified according to Minder and Schneider-Yin [27]).
[1] ALA may be added to detect lead intoxication and the very rare ADP as mentioned in the text.

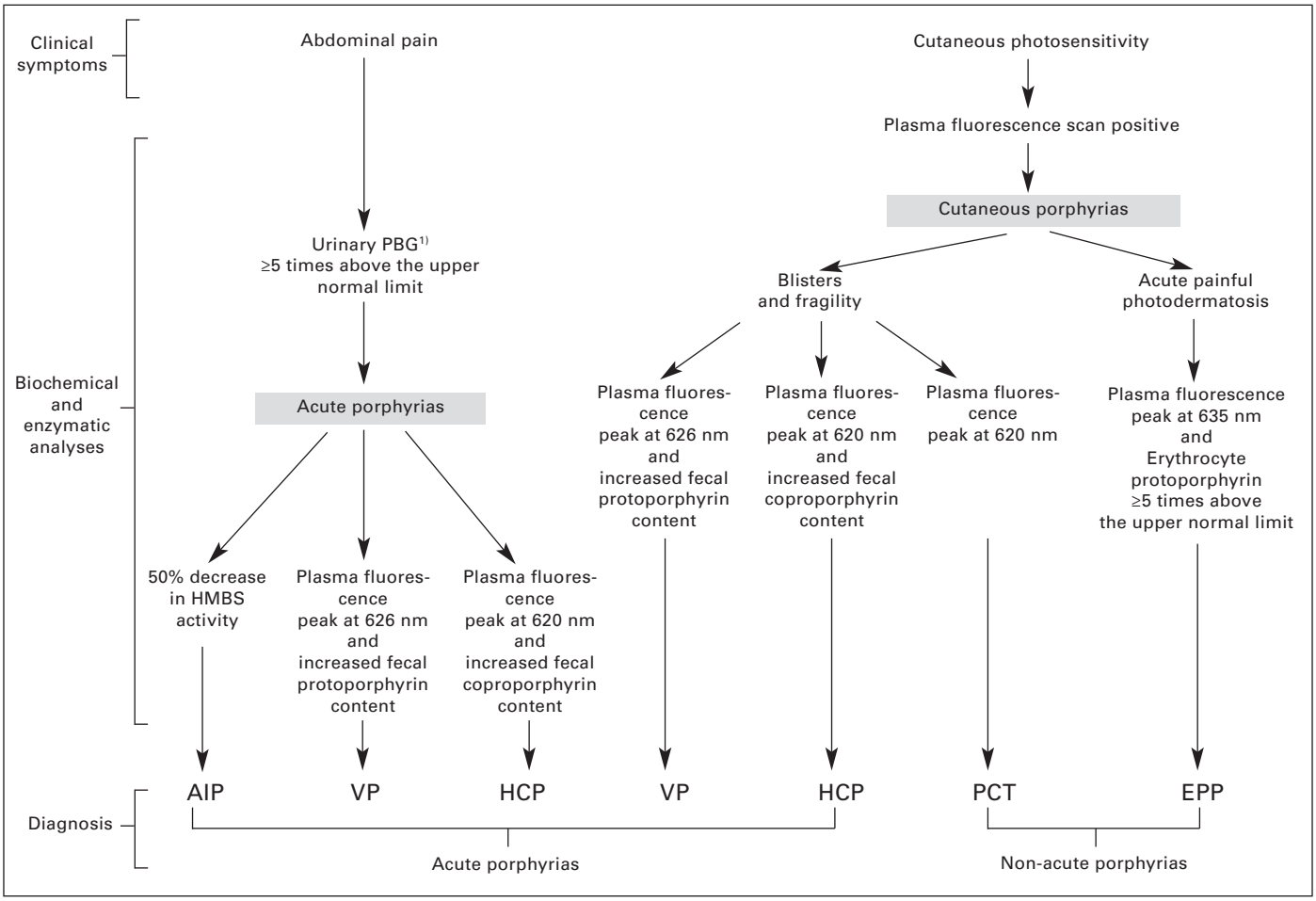


Table 2

An overview of porphyria patients in Switzerland between 1993 and 2007.

Type of porphyrias	MIM number	Number of families*	Number of symptomatic patients	Number of non-symptomatic mutation carriers
Acute porphyrias				
AIP	176000	94 (79)	111	94
ADP	125270	0	0	–
HCP	121300	2 (1)	4	0
VP	176200	30 (23)	30	7
Non-acute porphyrias				
CEP	263700	5 (5)	5	–
HEP	176100	1§ (1)	1§	–
sPCT	176090	NA	21	–
EPP	177000	38 (35)	45	–

MIM: mendelian inheritance in man; sPCT: sporadic form of PCT;
*Number of families that underwent genetic testing are given in the parentheses;
-: irrelevant clinically; §: diagnosis not confirmed; NA: not applicable.

taken to avoid the onset of the disease among asymptomatic individuals.

Plasma fluorescence scan is an effective test in the diagnosis/exclusion of cutaneous porphyrias (fig. 3; [5] and G. Biolcati, personal communication).

Types and numbers of porphyria among our patients

As the porphyria centre in Switzerland, we perform specialized analyses required for the diagnosis of all types of porphyrias, and give advice to patients, physicians and other laboratories. We therefore estimate that our data cover 80–90% of all diagnosed Swiss cases, whereby the percentage may be somewhat lower in the French- and Italian-speaking parts of Switzerland than in the German-speaking part of Switzerland.

A total of 94 AIP families have so far been documented in our centre. Among these families, the number of symptomatic patients was 111 and asymptomatic (pre-symptomatic) mutation carriers was 94. Although these numbers do not allow an accurate calculation of the prevalence of AIP, they do signify the fact that AIP is the most frequent form of porphyria in Switzerland (table 2). The prevalence of AIP in most European countries and in the US was estimated to be ~5 in 100,000. However, the prevalence is as high as 100 in 100,000 in northern Sweden [1]. Were a prevalence of 5/100,000 used in the calculation, the effective number of AIP patients in Switzerland with a population of 7 million would be 350 – more than three times that documented in our centre so far. The number of asymptomatic (pre-symptomatic) mutation carriers could be 12 times as much i.e., 4200 individuals. This number is obtained by applying a prevalence of 60 in 100,000 in the calculation according to a French study on mutation carriers among blood donors [6].

EPP, a non-acute porphyria, was the second most frequent porphyria among our patients. A total of 45 patients from 38 EPP families were documented during the past 15 years (table 2). The number of Swiss VP families was 30, slightly fewer than that of EPP. The diagnosis of *s*PCT among 21 patients was based on the clinical symptoms, family history and biochemical analyses. No genetic testing was performed among these patients. According to the literature, over 80% of PCT cases are non-hereditary. HEP, the homozygous form of hereditary PCT, is rare. Recently, an 88-year-old patient was referred to our clinic with clinical symptoms and biochemical abnormalities indicative of HEP. However, the diagnosis of HEP could not be confirmed by genetic testing since no mutations were identified in the *UROD* gene of that particular patient. Five cases of CEP, another recessive form of porphyria with clinical features similar to that of HEP, were diagnosed. HCP was found in only two Swiss families. ADP is the rarest form of all acute porphyrias. Worldwide, only seven cases have been reported. So far, no ADP cases have been identified among our patients.

Major complications associated with porphyrias

Hepatocellular carcinoma (HCC) in acute porphyrias: The acute hepatic porphyrias are risk factors for primary hepatocellular carcinoma as shown by several studies. A Swedish study found 27% of AIP carriers over the age of 50 developed HCC and HCC screening improved survival rate among studied patients [7, 8]. In a French cohort of 650 acute porphyria patients, the risk of HCC was found 36 times higher in those patients than in a non-porphyric population comparable by age and sex [9]. This carcinoma was shown to be more common in patients with manifest AIP and in women, a reversal of the usually reported gender ratio for HCC.

Over the last 15 years, we encountered a total of four cases of HCC in Swiss residents with acute porphyria, two AIP and two VP patients, and all female. In all four cases, HCC metastasized. Three patients died of HCC. Starting last year, a yearly HCC-screening regiment including ultrasonography of liver and measurement of α-fetoprotein concentration in serum, has been implemented in our centre among mutation carriers of AIP, VP and HC aged ≥47.

Renal insufficiency in acute porphyrias: Chronic renal damage including terminal renal insufficiency can occur in acute porphyrias, a complication which is less well studied. In a single published population-based study, renal insufficiency was found in 5.6% of the AIP patients i.e., 16 out of a total of 286 studied patients had a creatinine clearance of <65 mL/min/1.75 m^2 [10].

Compared to the above-mentioned Swedish study, the total number of patients we have studied so far is much smaller i.e., 35 individuals in-

cluding 25 AIP patients/carriers and 10 VP patients. All individuals tested positive for mutations in either the *PBGD* gene or the *PPOX* gene. Serum creatinine concentration was measured to allow estimation of GFR (MDRD equation). The 35 individuals, 30 female and 5 male, had a mean age of 45 years (range 18–81) at the time of analysis. The mean±SD of estimated GFR was 75 ± 19 mL min^{-1}/1.73 m^2. Eight individuals had a GFR of <60 mL/min/1.73 m^2 – the lower limit of normal (LLN) according to Levey et al [11]. These 8 individuals, aged from 27 to 81 years old, were all female. Given the limited number of individuals studied, the result i.e., a 23% affection rate, might not provide an accurate estimation of the frequency of renal insufficiency in acute porphyria. However, it did confirm that renal insufficiency is a complication of acute porphyrias and should be dealt with seriously.

In a recent study of nine AIP patients with renal disease but not hypertension, kidney biopsy found no evidence of a glomerular lesion in any of the patients, instead, it showed features of a tubulointerstitial disease that allowed the authors to conclude that the nephrotoxic effects of porphyrin precursors may contribute to the aetiology of renal damage [12].

The most severe and potentially life-threatening complication of EPP is protoporphyrin-related liver failure. Although its exact frequency is unknown, some have estimated it to be approximately 1% of all patients [13]. This terminal condition is characterised by a massive increase of erythrocytic and plasma protoporphyrin levels and a rapid deterioration of liver function. Liver transplantation is the only life-saving therapeutic option for these patients. Among the Swiss EPP patients, we are aware of two cases of terminal liver failure. The first case, a 16-year-old boy, died of liver failure 30 years ago. The second case was a female EPP patient who received liver transplantation 19 years ago at the age of 49 and has ever since retained a normal liver function ever since although her symptom of cutaneous photosensitivity remained unchanged.

Considering the potential fatal outcome of EPP liver disease, we advise all patients and their family practitioners, to monitor the liver function and to prevent any additional liver damage e.g., by avoidance of alcohol over-consumption and by vaccination against hepatotropic viruses. Annually, or twice a year in severe cases, tests including total bilirubin, ASAT, ALAT and alkaline phosphatase, and liver/abdominal ultrasound scan were recommended in these patients. During the past 15 years, no new cases of end-stage protoporphyric liver disease occurred among the EPP patients under our care. However, a third of the monitored patients (10 in 30 patients) did show slight abnormalities (within two-fold of the upper limit of normal (ULN)) in one or more of the above-mentioned four liver parameters, which indicated certain degrees of liver dysfunction among these patients. Early electronmicroscopic studies revealed that most EPP patients had minimal pathological changes in the liver cells which apparently did not lead to progressive liver damage [14].

Gallstone formation is an additional complication of EPP. Two of our patients, a 23-year-old man and a 14-year-old girl, underwent cholecystectomy because of symptomatic gallstones. None of remaining 43 EPP patients presented symptomatic cholelithiasis.

Besides liver enzymes and ultrasound scan, concentration of urinary porphyrins, especially the ratio of coproporphyrin I over total coproporphyrin, has been suggested as an early marker of liver involvement [14]. Our data showed no correlation between the coproporphyrin ratio and ASAT, ALAT, bilirubin or alkaline phosphatase (r between –0.20 and 0.06, n = 11 to18). Further, 15% of the patients had an abnormal coproporphyrin-I/total coproproporphyrin ratio without any other significant signs of EPP-induced liver disease (the ratio in percent, among 26 EPP patients was 45 ± 26 (mean ± 2SD), four patients had a ratio of >58; the reference range used in our laboratory was 28 ± 30 (mean ± 2SD)). If a ratio of 31 were used as the ULN, according to the publication of Doss and Frank, 24 of the 26 patients would have an abnormally high ratio. The significance of coproporphyrin I/total coproporphyrin ratio alone in predicting EPP liver involvement is therefore questionable. However, increased total urinary coproporphyrin excretion combined with an increased ratio seems to be a more reliable marker in this respect [15].

Based on our experience, the following abnormalities are considered as signs of liver involvement in EPP: a) signs of liver disease in ultrasound examination; b) transaminases >3 times of ULN; c) total urinary porphyrins >5 times of ULN; d) a significant increase in erythrocytic protoporphyrin concentration (>50 µmol/L).

Anaemia in EPP: According to the literature, EPP patients can present with mild anaemia featuring hypochromia and microcytosis. Abnormalities in haematologic status including haemoglobin concentration, haematocrit, MCV, MCH and thrombocyte count, were also observed among our patients. As shown in table 3, between 7–29% of the patients displayed abnormal values (i.e., below the LLN) in one or more of the five parameters. In addition, 22% of the patients (6 in 27 patients) had a serum iron concentration below the LLN which was adjusted for age and gender. 45% of the patients (13 in 29 patients) had a low ferritin level (below the LLN adjusted for age and gender).

The abnormal haematological findings in EPP patients resembled that of iron-deficiency anaemia. However, iron supplement was shown to be ineffective in improving the anaemia and in some cases, even exacerbated EPP symptoms [16]. The role of iron in the pathogenesis of EPP was

Table 3

Haematological findings in EPP patients.

Parameter	Reference range	Total number of patients	Mean±SD from patients	Number of patients with values below the LLN
Haemoglobin (g/dL)				
male	14.4–17.5	12	14.3 ± 1.0	1
female	12.0–15.8	14	12.9 ± 1.1	4
Haematocrit (%)				
male	43–53	12	41 ± 3.8	3
female	34–47	15	39 ± 2.9	2
MCV (*f*L)				
male	80–99	11	82 ± 3.6	2
female	80–99	15	85 ± 6.0	3
MCH (*p*g)				
male	27–34	11	28 ± 1.6	1
female	27–34	15	28 ± 2.6	4
Thrombocytes (10^9/L)				
male	150–400	12	181 ± 43	1
female	150–400	14	236 ± 52	1

MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; LLN: lower limit of normal

studied in a mouse model *FECH*^{m1Pas} [17]. While homozygous mice displayed no total body iron deficiency, they did show a clear-cut redistribution of iron stores from peripheral tissues to the spleen, with a concomitant 2- to 3-fold increase in transferrin mRNA and protein expression. The results suggested that elevated protoporphyrin plays a role in the orchestration of iron distribution between peripheral iron stores, the spleen and the bone marrow.

DNA-testing and counselling

In the nineties, DNA-testing was introduced to our laboratory as a diagnostic procedure. EPP and AIP were the initial candidates for molecular diagnosis because of the abundance of patients. In recent years, DNA-testing was also established for VP, CEP and PCT.

Up to now, over 75% of the families of AIP, EPP and VP, the three most frequent forms of porphyrias in Switzerland, have undergone DNA testing. The sensitivity of DNA testing i.e., percentage of families with an identifiable mutation among all clinically diagnosed families, was 95% for AIP (75 out of 79 families), 91% for VP (21 out of 23 families) and 86% for EPP (30 out of 35 families). In a recent publication, intragenic deletions of large segment of the *FECH* gene were identified in a number of EPP patients by gene dosage analysis [18]. The conventional method of sequencing such as the one used in our laboratory, which detects mutations in the coding exons and exon-intron boundaries, is incapable of identifying large intragenic deletions. A method to quantify gene dosage is currently been established in our laboratory in order to further study the "mutation-negative" patients and families.

The significance of mutation analysis in acute porphyrias, AIP and VP and HC, was to identify pre-symptomatic mutation carriers among family members. These individuals and their family physicians were consulted with the safety of using prescription drugs, since as it was mentioned earlier, certain drugs can induce acute porphyric attacks among mutation carriers (see www. porphyria-europe.org). Drug counselling, avoidance of fasting, reduction of stress, prevention and early treatments of infections are important elements in the management of acute porphyrias.

The heredity of EPP has its unique features. Mutations in the *FECH* gene are partially responsible for the enzyme deficiency in EPP patients. An intragenic single nucleotide polymorphism (SNP) IVS3-48c/t plays an additional role in the pathogenesis of EPP. As a study of Gouya et al. showed, IVS3-48c, also called the low-expressed allele, can reduce the enzyme activity by approximately 25% [19]. At the genetic level, EPP is characterised by a mutation on one *FECH* allele (M) and IVS3-48c (c) on the other. Individuals with the genotype M/c will develop clinical symptoms of protoporphyria, whereas those with the genotype M/t will be asymptomatic. The prevalence of IVS3-48c was determined to be 7% in the Swiss population [20]. Worldwide, the frequency of IVS3-48c varied from population to population with the highest of 45% found in the Japanese population and the lowest of <1% among the black Africans [21, 22]. Genetic testing in EPP therefore consisted of both mutation and SNP IVS3-48c/t analyses in the *FECH* gene. The results of these analyses provided basic information for family counselling [23].

Founder mutations in the Swiss population

Mutations in the various genes of the haem biosynthetic pathway are heterogeneous in their nature. The majority of the porphyria families studied so far carried unique mutations. The heterogeneity is reflected in the statistics on published porphyria mutations. According to the Human Gene Mutation Database, worldwide, a total of 258 different mutations have so far been identified in the *PBGD* gene, 129 mutations in *PPOX* gene, 88 mutations in the *FECH* gene, 70 mutations in the *UROD* gene, 41 mutations in the CPOX gene, 36 mutations in the UROS gene and 9 mutations in the ALAD gene (Human Gene Mutation Database, accessed in May 2008).

Table 4
Prevalent mutations among Swiss porphyria patients.

Porphyria	Affected gene	Mutations	Prevalence§
AIP	*PBGD*	W283X	56% (42 of 75 families)
CEP*	*UROS*	T228M	40% (4 of 10 alleles)
		L4F	30% (3 of 10 alleles)
VP	*PPOX*	1082-1083*ins*C	67% (14 of 21 families)
EPP	*FECH*	Q59X	30% (9 of 30 families)
		580-584*del*TACAG	27% (8 of 30 families)

* recessive porphyria;
§ in percentage of families that carry a specific mutation among all DNA-diagnosed families.

The Swiss population seemed to be an exception with respect to the heterogeneity in porphyria mutations. In 2000, we published a nonsense mutation W238X identified in 13 of the 18 AIP families studied until then, corresponding to a frequency of 72% [24]. Most of the patients lived near the Lake of Zürich and some in the central part of Switzerland. The same mutation was also found in the French AIP population. By haplotyping analysis, W283X was proven to be a founder mutation, most likely originating in Switzerland and later spreading to neighbouring France. The age of W283X was estimated to be at least 1000 years [25]. Over the past eight years, the number of genetically diagnosed AIP families has increased to 75 families. The prevalence of W283X now stands at 56% (table 4).

The phenomenon of prevalent mutation was subsequently observed in VP. In 2006, we published a prevalent mutation 1082-1083insC in the *PPOX* gene among Swiss patients [26]. Haplotyping analysis is currently been conducted to study whether the 14 VP families that carried the1082-1083insC mutation share a common ancestor. In EPP, the most common form of non-acute porphyrias in Switzerland, 57% of all families carried either the Q59X or the 580-584*del*TACAG mutation (table 4). CEP is a rare form of recessive porphyria. Most of the patients studied so far were heteroallelic for the *UROS* mutations. According to the literature, mutation C73R was found in ~33% of the CEP alleles [1]. However, this mutation was absent among the five Swiss CEP patients under our care, whereas two other known mutations T228M and L4F, were common among these patients (table 4).

Conclusion

Although porphyrias are regarded as rare or orphan diseases, a considerable number of affected patients live in Switzerland. The diagnosis and management of porphyrias require multi-disciplinary collaborations among family practitioners, dermatologists and porphyria specialists. The duty of a specialized porphyria centre is on the one hand, to provide information, services and support to both patients and clinicians, and on the other hand, to conduct clinical trails and research on the many unknown aspects of porphyrias.

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