A 43-year-old woman was admitted to our unit due to rash after sunshine since childhood while splenomegaly (4×12cm) for 13 years, in July, 2016. From 2013, the rash lasted for a longer time and the quantity increased after sunshine, companying with anaemia (Hgb, 100g/L) and liver dysfunction (ALT 77 IU/L, GGT 500+ IU/L). Normal liver function tests lasted for 2 years by taking UDCA. Liver dysfunction with ascites reappeared after stopping UDCA for 6 months. On examination, pigmentation distributed in her face and the dorsal aspect of her arms and hands. Superficial lymph nodes were palpable. The liver and spleen were both obviously palpable below the costal margin. All the non-invasive markers for the cause of liver cirrhosis were negative. Liver biopsy was performed after transfusion platelet and fresh plasma. A slew of dense, dark brown deposits in portal tracts, hepatocytes, and sinusoids were showed under optical microscope and Maltese cross birefringence crystals under the polarizing light microscope. The portal tract was enlarged and fibrotic with a ductular reaction. Plasma fluorescence emission peak was 634nm. Sequencing analysis identified c.1706-1709 del.AGTG mutation of ALAS2 gene, which had been proved a causal link between XLDP. Negative results were obtained from her daughter, mother, brother and sister. It’s the first case report of XLDP, with pathology confirmed sedimentation of protoporphyria, stage 4 biliary fibrosis of the liver and DNA sequencing identified gain-of-function mutation of ALSA2 in Chinese patient. Cholestasis liver cirrhosis with skin lesion could be the first clue of XLDP or EPP.
Acute hepatic porphyrias are potentially life threatening metabolic disorders requiring rapid diagnostic evaluation. The collection of urine samples over a period of 24 hours can lead to a harmful delay of the necessary therapeutic measures. Spot samples are considered to be sufficient to estimate the activity of acute porphyrias and allow a decision on therapeutic intervention. Nevertheless many laboratories do not accept spot samples for evaluation of the renal porphyrin precursor excretion. This is motivated by the lack of reliable Cut-off values for these specimens. Referring the measured concentrations to the amount of creatinine excreted simultaneously is an established method for different proteins or metabolites in urine spot samples. So far only a few data for the creatinine ratios of porphyrin precursors have been published. This study evaluates data of 252 well-characterized patients with acute hepatic porphyrias (AIP, VP, HCP) versus more than 1000 non-porphyric probands based on commercially available tests for 5-ALA and PBG (Recipe, Munich). The specific parameters of the ROC curve are calculated and data for positive predictive values are given. Surprisingly, 5-ALA/Crea exhibits a significantly higher AUC value and Youden Index than PBG/Crea making it a better parameter to identify acute hepatic porphyrias in our diagnostic collective with a pre-test probability of about 2%.

Based on the evaluation reference values for non-porphyric probands and reliable Cut-off values for the diagnosis of acute hepatic porphyrias are established. These data should make it easy for labs to report on urine spot samples.
A 66y male presented with photosensitivity and oedema in 2005; two years previously he had complained of a rash on his hands with pruritus and eyelid swelling after contact with the sun. Biochemically he had an elevated red cell free protoporphyrin: 10,790 nmol/L (normal <200) and was diagnosed with EPP. DNA analysis showed a single mutation on the FECH gene; a bone marrow biopsy did not show any evidence of myelodysplasia.

In 2011 he was prescribed ibuprofen and a course of UV-B therapy and developed progressive jaundice: serum bilirubin 267 µmol/L, AST 230 U/L, ferritin 135 µg/L and a normocytic anaemia (Hb = 112 g/L). A liver biopsy showed cholestasis and focal deposits of protoporphyrin. His was started on a treatment regimen of two weekly blood transfusions, monthly red cell exchange and prednisolone. After 9 months his liver function improved: bilirubin 16 µmol/L (Normal <20) but serum ferritin was 2656 µg/L (normal <300) and he commenced on desferroxamine. The exchanges and transfusions continued but when the transfusions were stopped or reduced this resulted in decreased liver function and increases in red cell and faecal protoporphyrin and total plasma porphyrins.

The monitoring period covered 5 years with well controlled protoporphyrin and mild deposition of iron in the liver and none in the heart.
The porphyrias are a group of rare, mainly inherited, diseases caused by a deficiency of one of the enzymes of the haem biosynthesis pathway. Relatively little is known about the natural history of the condition, and we have studied a cohort of patients with acute porphyria in our clinic to determine the age of the first acute attack and its significance.

The significance of the age of first acute attack in 180 patients with either AIP (137) or VP (43) was analysed using Kaplan-Meier survival analysis. 44 (32%) AIP and 33 (77%) VP patients had never had an attack. The youngest age at which a first attack occurred was 10 years in AIP and 23 years in VP. There was a significance difference (p<0.001) in the median age of first attack in AIP at 28 years (95% CI 25.4 – 30.6) compared to 49 years (95% CI 31.2 – 66.8) in VP. In AIP, a more severe clinical course was associated with a younger age at first attack: the median age of first attack in those having a single attack was 28 years (95%CI 19.0 – 36.9), 25 years (23.3 – 26.8) in those having 2 or more attacks and 22 years (19.9 – 24.0) in those having 3 or more attacks.

DNA analysis was available for 118 AIP and 35 VP patients and there was no significance difference between nonsense and missense mutations and age of first attack in AIP patients.
Since the 1950s it has been known that there is an association between PCT and diabetes mellitus (DM). From cohort studies there seems to be an increased prevalence of DM, and from small case control studies PCT patients have shown impaired glucose tolerance and hyperinsulinemia. In a retrospective study, the average time from PCT diagnosis until occurrence of glucose metabolic alterations (either DM or impaired fasting glucose) was 12.7 years. Over all, it seems that PCT patients have increased risk of getting type 2 DM (DM2).

DM2 is a serious disease resulting in increased risk of getting cardiovascular diseases, retinopathy, infections and foot ulcers. Patients with DM2 are often asymptomatic for a long time and since PCT patients have an increased risk of developing DM screening this patient group seems relevant. However, which test to choose can be difficult.

Different tests are available:

- Fasting glucose, f-glu
- Oral glucose tolerance test, OGTT
- Hemoglobin A1c, HbA1c. Recommended by WHO, when technically/biologically possible.

Which test to use depends on the patient, setting and the facilities. f-glu and OGTT requires fasting, and also OGTT is time consuming. HbA1c requires no prior preparation; however there are several contraindications to consider. Most importantly, alcoholism falsely increases while phlebotomy decreases HbA1c, factors which often apply for PCT patients.

As part of an ongoing PhD-study, we have performed the 3 diagnostic tests in, so far, 11 patients. Besides a small literature review, we will present data from 7 patients, where we have found a low diagnostic concordance in the test results.
We report 2 brothers with recurrent attacks of acute intermittent porphyria (AIP), which began in childhood in the younger brother. Both are heterozygous for the same variant in the HMBS gene, which is also present in their mother who has never had an attack. No HMBS mutations have been identified in their father or another brother. The parents are first cousins.

Case 1 was diagnosed with AIP at the age of 10 when he had a biochemically confirmed attack with seizures and hyponatraemia after an episode of gastroenteritis, and went on to develop recurrent attacks. He has been managed with prophylactic haem arginate since the age of 13. He is currently aged 23 and has had no hospital admissions for more than a year. He has treated hypertension, treated Vitamin D deficiency and iron overload, but is otherwise well and in full time employment.

Case 2 was diagnosed with AIP at the age of 16 through family screening. He had his first attack aged 19 during a period of religious fasting, and subsequently developed recurrent attacks. He has had many hospital admissions with pain and attacks despite prophylactic haem arginate. He is currently aged 26 and has recently been diagnosed with a psychotic disorder for which he is receiving treatment.

Whole exome sequencing of the 2 affected brothers, the unaffected brother and both parents is being undertaken to investigate possible genetic factors predisposing to this unusual clinical presentation.
Abstract Title | Audit of Urgent Porphobilinogen Turnaround Time in a UK Regional Porphyria Laboratory
---|---
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The 2016 British and Irish Porphyria Network (BIPNET) best practice guidelines on laboratory testing for porphyria\(^1\) state that urgent porphobilinogen (PBG) testing should be available within 24h of sample receipt at the local laboratory. An audit conducted in our lab in 2015 showed that it was difficult to accurately assess compliance with this standard. Previously, urgent PBG results were telephoned to the requestor but the time was not recorded on our LIMS. Urgent PBGs were then incorporated in the routine profile for fractionated porphyrins, meaning PBG results were only authorised from our LIMS once the entire profile was complete. To improve this system, a dedicated set code for reporting urgent PBGs was introduced in June 2016, allowing immediate authorisation. This test was also added to the LIMS telephone list to create an audit trail of the date and time the result was telephoned. Re-audit in 2016 showed that the percentage of samples that met the standard was 62.5% (compared to 4.2% in 2015). In 28% of cases, failure to meet the standard was due to delay in sample transfer from the referral laboratory, with the majority of these samples being non-diagnostic. The audit highlighted that the receipt time in the local laboratory was infrequently provided on requests to us and had to be estimated from the sample collection time. Nevertheless, simple electronic changes to the reporting system for urgent PBGs have enabled a quality improvement in the capture and monitoring of turnaround time as a key performance indicator for our service.

### Abstract Title
Genetic background influences iron imbalance due to chronic hemolysis in a mouse model of Congenital Erythropoietic Porphyria (CEP)

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Clinical severity is heterogeneous among patients suffering from CEP (Uroporphyrinogen III Synthase deficiency) suggesting a modulation of the disease by modifier genes. In a CEP patient with severe hemolysis, the occurrence of a gain-of-function mutation in ALAS2 gene, (1st step of heme biosynthesis) incite to propose ALAS2 as a modifier gene. A KI model of CEP due to a missense mutation of UROS gene present in human (P248Q), has been developed on 3 congenic mouse strains (BALB/C, C57/BL6, and 129/SV) in order to study the impact of genetic background on disease severity. To detect putative modifiers of disease expression in mice congenic strains harboring uros gene defect, hematologic data (RBC, reticulocyte and fluorocyte counts), iron parameters, porphyrin and iron content (liver, kidney, spleen, bone marrow and blood) were collected.

Although anemia had the same level in the 3 strains, various degrees of hemolysis, due to porphyrin excess in RBCs, were noted: 129/SV were the most hemolytic, BALB/C had more regenerative response to anemia, C57/BL6 were less affected. A main difference concerned iron balance: Hepcidin expression was significantly reduced in 129/SV mice compared to C57/BL6 (less pronounced reduction in BALB/C). Altered iron stores in spleen and liver macrophages resulted in iron leakage and accumulation in hepatocytes, and severe hyperferritinemia solely in 129/SV mice. In BALB/C or C57/BL6, iron accumulation in macrophages remained controlled.

These findings will be discussed in light of potential therapeutic applications in the human disease (hepcidin agonists, iron depletion)
### Abstract Title
**Missense UROS mutations causing congenital erythropoietic porphyria reduce UROS homeostasis that can be rescued by proteasome inhibition**

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In Congenital Erythropoietic Porphyria (CEP), the enzymatic defect in uroporphyrinogen III synthase (UROS) is responsible for the accumulation of photocatalytic compounds, mainly Uroporphyrin I; which accumulates in tissues inducing haemolytic anemia and skin photosensibility. Missense mutations of UROS gene are the most frequent defect, associated with a large heterogeneity in disease severity. As previously shown, 2 prevalent missense mutants retained significant intrinsic enzymatic activity but trigger severe protein instability and premature degradation.

In the present work, the molecular basis of UROS impairment associated with 29 missense mutations described in CEP was analysed. Using a computational and biophysical joint approach we predicted that most disease-causing mutations would affect UROS folding and stability. The analysis of EGFP-tagged versions of UROS enzyme confirmed the data showing that thermodynamic instability and premature protein degradation is a major mechanism accounting for the enzymatic deficiency in human cells. Using molecular dynamic simulation to rely structural 3D modification with UROS disability, we found that destabilizing mutations could be clustered within three mechanisms according to side chain rearrangements or contact alterations within UROS enzyme so that the severity degree correlated with cellular protein instability.

Moreover, proteasome inhibition using bortezomib significantly enhanced proteostasis of each unstable UROS mutant, enlightening a prevalent mechanism responsible for UROS deficiency, which may represent an attractive therapeutic option in CEP patients.
Abstract Title: Recent development of a special clinic of porphyria in Japan

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Background and aims: In Japan, porphyria is not well understood by the clinicians and hence most patients with porphyria are not properly diagnosed. Therefore, in June, 2015, a special clinic for the management of porphyria was established in Tokyo. By using the resources available in this special clinic, this study aimed to assess the present condition and problems of medical care in porphyria.

Methods: Subjects were recruited from patients of this special clinic with porphyria or suspect of porphyria. The chances of receiving consultation, ways of obtaining details of medical institutions, diagnosis of porphyria, and duration of symptoms prior to diagnosis were investigated in the subjects.

Results: In total, 51 patients (16 male, 35 female) were recruited. There were 24 patients with porphyria (5 male, 19 female; average 36 years old). For the diagnosis of porphyria, there were AIP (10), VP (3), HCP (2), EPP (8) and CEP (1) and the duration of symptoms prior to diagnosis took about 1 month to 28 years. There was 2 patient with diagnosis less than 1 years, 3 patients with diagnosis over 20 years, respectively. Most of the patients visited the special clinic after researching on internet.

Discussion: In Japan, porphyria has not been properly identified by most of the clinicians. There are limited numbers of medical institution for consultation and treatment of porphyria. In the future, it is necessary for clinicians to recognize and understand porphyria. Besides, it is important to have a medical center for the treatment and diagnosis of porphyria. It is also necessary to set up a disease network, similar to the EPNet.

Conclusion: It is imperative to promptly set up a medical system for the diagnosis and treatment of porphyria in Japan.
Microcytic hypochromic anaemia is a recognised feature of Erythropoietic protoporphyria (EPP), which in clinical practice should be monitored and distinguished from true iron deficiency or other causes requiring intervention. 22 patients (20 adults, 8 male(M), 12 female(F) and two aged <18y, 1M, 1F) attending the Salford porphyria clinic between Oct 2015-Feb 2017 were audited to assess monitoring of anaemia, indices of iron stores, prevalence of abnormalities and action taken on the results. Haemoglobin was normal in 9/9 (100%) males and 9/13 (69%) females. Overt anaemia was confined to 4 females (Hb 101-113g/L; laboratory non-pregnant female ref range 115-165g/L); 2 had true iron deficiency (one was 22/40 pregnant) and 2 had associated chronic disorders. Mean cell volume (MCV) was low (<84fl) in 7/22 (32%; 4M, 3F), red cell distribution width (RDW) raised (>14.5) in 12/22 (55%; 4M, 8F) and ferritin low in 3/22 (14%; 0M, 3F) patients. All other ferritin levels were in the lowest quartile of the sex specific reference range, apart from one outlier with raised ferritin (987ug/L) due to alcoholic liver disease. A clinical letter explaining results and actions for the GP was present in the electronic records for all patients. Two patients were currently prescribed iron; 5 were taking over the counter (OTC) multi-vitamins; 8 had taken prescribed iron or OTC supplements intermittently in the past. In this cohort, raised RDW was the commonest abnormality and a shift towards low ferritin levels was almost universal. The role of iron supplements in EPP patients with low normal ferritin levels merits further study.
Seminal studies suggested that acute intermittent porphyria (AIP) could be associated with changes in tryptophan homeostasis. To evaluate this hypothesis, we used state-of-the-art technology to study urinary metabolome of AIP patients focusing on tryptophan metabolism.

A case-control study was performed in a group of 25 AIP patients with active biochemical disease and increased excretion of heme-precursors (not in acute crisis) and 25 healthy controls. Tryptophan and related compounds and metabolites including: long neutral amino acids, serotonin, kynurenine and kynurenic acid were quantified in urine by liquid chromatography tandem-mass spectrometry (LC-MS/MS). Twenty-nine biological markers were compared between patients and controls.

Significant differences were found in the tryptophan-kynurenine metabolic pathway. In contrast, no differences were found in the serotonin metabolic pathway independently of the markers and ratios used.

The results showed an increase of kynurenine excretion and evidences of misbalance of several metabolic rations. (a) increased urinary excretion of kynurenine and anthranilic acid; (b): elevation of the kynurenine/tryptophan ratio and (c): decrease of the kynurenic acid/kynurenine ratio.

Decreased enzymatic activity in the liver of kynurenine aminotransferase and/or induction of indoleamine 2,3-deoxygenase could explain the modified ratios. We hypothesize that hepatic energy misbalance, depletion of cofactors or low-grade inflammation due to overproduction of PBG could explain the observed changes among this subgroup of AIP patients. Tryptophan metabolism during acute crisis, however, should be further evaluated.

The results confirm the utility of LC-MS/MS to characterize the urinary metabolome of AIP.
Little is known about the experiences of persons with acute intermittent porphyria (AIP) and more knowledge is warranted to ensure appropriate follow up of this patient group.

The aim of this cross sectional study was:

1) To describe quality of life (QOL) and subjective health complaints in persons with AIP
2) To investigate the association between subjective health complaints, psychological stress and QOL in persons with AIP.

140 adults registered with AIP in Norway participated in the study (response rate 53%). They answered validated and standardized written surveys on Subjective Health Complaints (SHC), generic QOL (WHOQOL-bref) and psychological stress in relation to AIP (IES). Participants were categorized as “active AIP” “manifest AIP” or “latent AIP” based on self-reported symptoms (at present, previously or never).

In general, participants reported satisfactory QOL, except on the physical domain, where participants reporting active AIP had significantly lower scores compared to the normal population.

Persons reporting active AIP had more muscular and neurological than gastrological complaints. Severity of health complaints was associated with more psychological stress and lower QOL and especially neurological health complaints showed a strong correlation with stress and reduced QOL.

Although persons with AIP reported an overall satisfactory QOL, severity of health complaints was associated with more stress and lower QOL, and additional efforts should be made to ensure optimal follow up, counseling and care during phases of active disease.
When phototesting people with erythropoietic protoporphyria (EPP), abnormally low minimal erythema doses (MEDs) are produced particularly with wavebands in the blue (wavelengths around 430nm) part of the visible spectrum. Our unit has performed monochromator phototesting on 58 people with EPP since 1971. However, we were only able to recover our records of total erythrocyte porphyrin (TEP) measured at the same time for some of these tests.

We looked at the records of 35 patients (between the ages of 5 and 49 years-old when first phototested) who had phototesting (not after phototherapy) at the same time as total erythrocyte porphyrin (TEP) was measured between 1976 and 2015 to assess any association between the MED to the waveband centred on 430nm (half-maximum bandwidth) at the most sensitive time after irradiation (usually 7 hours) and total erythrocyte porphyrin (TEP) determined at the same time. Phototesting was done as standard (MacKenzie & Frain-Bell. The construction and development of a grating monochromator and its application to the study of the reaction of the skin to light. Br J Dermatol. 1973; 89: 251-64), using geometric dose series.

Mean TEP was 19 μmol/l (standard deviation 14.8, range 2.9 to 51.9; reference range <1.3) and median MED to the waveband centred on 430nm was 10,000 mJ/cm² (interquartile range 3,900 to 27,000, range 470 to 82,000; reference range >82,000).

Using a linear regression model (log MED [in mJ/cm²] = [0.0048 x TEP in μmol/l] + 8.89) we detected no linear association beyond chance (P=0.76) and TEP concentration could only ‘explain’ 0.28% (R-squared = 0.0028) of variation in MED. These findings are likely because many factors other than TEP concentration affect skin concentration of and absorption of light by protoporphyrin and then the next steps beyond this that influence the release of mediators that cause vasodilatation (which can be perceived as threshold erythema, the MED). Our impression is that phototest MEDs may be more closely associated with clinical severity of EPP phototoxicity and that there might be a connection between TEP and MEDs within, although not between, subjects: we will prospectively assess these.
## Abstract Title
An Analysis of Healthcare Utilization and Costs Associated with Patients with Acute Hepatic Porphyria (AHP) with Recurrent Attacks in EXPLORE: A Prospective, Multinational Natural History Study of Patients with AHP

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The AHPs are characterized by disabling attacks, often requiring urgent medical care and/or hospitalization, with a significant number of patients experiencing chronic symptoms in between attacks. EXPLORE is the first, ongoing, multinational study that characterizes the natural history and clinical management of AHP patients with recurrent attacks. The objective of this analysis is to estimate healthcare utilization and costs associated with this patient population.

AHP patients with recurrent attacks (> 3 attacks/yr) or who receive prophylactic treatment to prevent attacks were enrolled in EXPLORE. Patients’ medical history, along with questionnaires on porphyria signs and symptoms, quality of life and healthcare utilization were collected at pre-specified intervals and during attacks. Costs were estimated for hospitalizations, emergency room visits, physician visits and drug use from the perspective of a payer.

A total of 112 patients have been enrolled from 13 countries with mean patient age of 39 yrs old, 89% female and 93% with AIP, 4% VP and 3% HCP. Patients reported a mean of 2.8 (0-20) ER visits and a mean of 4.6 (0-70) overnight hospitalizations, lasting on average 6 days (1-60), for porphyria-related care. 24% of patients visited their general practitioner and 36% visited a specialist monthly for porphyria-related care in the prior year.

Most porphyria attacks (76%) required urgent treatment in a healthcare setting (ER, clinic or infusion center), hospitalization and/or intravenous hemin treatment. Costs associated with AHP patient healthcare utilization during this period will be estimated and presented.
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<th>Psychosocial Issues in Erythropoietic Protoporphyria - The Perspective of Parents, Children and Young Adults</th>
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Erythropoietic protoporphyria (EPP) and X-linked protoporphyria (XLP) are rare photodermatoses, generally presenting in childhood with severe, painful phototoxicity. EPP has been reported to negatively affect quality of life (QoL), but there is limited information on the psychosocial issues faced by patients and families. To address this, an online focus group study was conducted to explore the perspective of parents of children with EPP, and young adults and children with EPP.

Five focus groups were conducted in a semi-structured format, with moderator-led discussions exploring the impact on QoL. Three focus groups included parents of children with EPP, one with children aged 10-11 years, and another with young adults aged 24-25 years, for a total of 24 participants. Thematic data analysis showed that parents experience guilt for being unable to protect their children and frustration with the current state of knowledge of EPP. Parents also admitted that the disease can lead to stress within family members which is difficult to manage. Young adults expressed embarrassment over having to explain the disease to others. They reported that the teenage years were the most difficult to navigate, however, they learned to adapt to their disease as they grew older. Children expressed that they had limited understanding of their disease and wished they were told what symptoms to expect by physicians earlier in life. Our findings emphasize the significant impact on QoL for these families and a lack of age appropriate information for children with EPP. These findings can help improve counseling and support resources for patients.
Quantification of total porphyrins from erythrocytes (TEP) can be used in the diagnosis of conditions with elevated blood porphyrins including Erythropoietic Protoporphyria (EPP) and Congenital Erythropoietic Porphyria (CEP). To quantify extracted porphyrins, sample fluorescence is compared to a porphyrin standard. As the major porphyrin extracted from erythrocytes is protoporphyrin IX (PPIX), the ideal would be to use a PPIX standard. However, historically it has been reported that PPIX solutions are less stable than those of other porphyrins, which require the use of a conversion factor to accurately determine PPIX levels, and should be prepared fresh daily.

A survey of the TEP methods used by laboratories in the British and Irish Porphyria Network (BIPNE) revealed a lack of conformity with different standards being used. A move towards standardising TEP methods and generating a PPIX standard that could be exchanged between laboratories in the absence of a commercially available traceable standard prompted a reinvestigation into the stability of PPIX.

A PPIX intermediate working standard (0.5 mg/l; 0.89 µmol/l) was prepared as described in NCCLS C42-A and aliquotted into amber vials to protect from light. Vials were kept at room temperature for up to 3 days, or in the fridge, freezer or ultralow freezer for up to 16 weeks. Fluorescence and absorbance were measured periodically and PPIX was extracted to determine its concentration. We detected no significant change in PPIX absorbance or fluorescence intensity or the amount of PPIX extracted over 3 days at room temperature or 16 weeks when stored in the fridge or freezer.
Impaired vitamin B6-status in patients with acute intermittent porphyria

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Background: Vitamin B6 is an essential co-factor for many enzymatic pathways, including transulphuration, degradation of tryptophan and heme biosynthesis. The aim of this study was to investigate vitamin B6 status, and metabolites influenced by vitamin B6 status, in patients with AIP.

Methods: Blood and urine samples were collected from 50 HMBS mutation carriers and 50 healthy controls (median age 52 years in both groups, M:29; F:21). The AIP group was divided into two subgroups based on the level of urinary porphobilinogen.

Analysis of pyridoxal 5-phosphate (PLP, the active metabolite of B6), 3-hydroxykynurenine/xanthurenic acid ratio (HK/XA, a functional B6 marker) homocysteine (tHcy), and methionine were performed by gas or liquid chromatography coupled with mass spectrometry.

Results: No significant differences in PLP levels were seen between the groups, however, HK/XA ratio, tHcy and methionine were significantly higher in AIP patients with high PBG excretion compared to the two other groups.

Conclusion: In HMBS mutation carriers with increased urinary PBG, we observed markedly increased HK/XA-ratio, tHcy and methionine levels, which might indicate a functional vitamin B6-deficiency in these patients.
A subset of patients with AIP have recurrent neurovisceral attacks (>3/yr) that result in hospitalization and significant debility. Recent data from the EXPLORE natural history study (NCT02240784) and literature suggest a significant number of patients with AIP experience chronic symptoms between attacks. The objective of this qualitative interview study was to characterize the overall symptom experience and disease impact from the perspective of AIP patients with recurrent attacks.

Patients were recruited in partnership with the American Porphyria Foundation. Qualitative phone interviews were conducted in patients with AIP who had >3 porphyria attacks in the last 12 months or who were on hemin prophylaxis to prevent attacks. Open-ended questions were used and data were analyzed qualitatively. Nineteen patients, with a mean age of 40 yrs, of which 89% were females and 57% were using hemin prophylaxis, were interviewed. Patients reported 0-20 attacks in the prior 12 months. Pain emerged as the cardinal symptom reported by all patients during acute attacks. In addition, most patients (18/19) reported chronic symptoms between attacks, with pain being the most frequent symptom reported by patients (17/19). Patients frequently reported that their symptoms had a negative impact on their lives, especially in the domains of sleep, work/education, finances, mobility and socialization.

This qualitative patient study supports the EXPLORE natural history study findings that AIP is an acute on chronic disease, and highlights pain as a cardinal disease manifestation impacting patients’ functioning during attacks and on a daily basis.
South Africa is an epicentre of the global HIV pandemic. Adult HIV prevalence is 12% and more than 3 million currently receive antiretroviral therapy (ART). Mirroring this is the 3rd highest TB burden in the world, having increased 400% over the past 15 years and driven by HIV. Approximately 73% of new TB cases are HIV co-infected. We report a 39 year old man, initially presenting in 2010 with a first acute porphyria episode including polyneuropathy, dysautonomia, abdominal pain and visual/auditory hallucinations. His father was known with AIP. Screening confirmed elevated urinary ALA and PBG concentrations and subsequently the R116W mutation in his hydroxymethylbilane synthase (HMBS) gene was identified. Haem arginate therapy was used repeatedly during his complicated hospital stay and he was diagnosed HIV positive with a preserved CD4 of 773 cells/mm³. Discharge to a rehabilitation service occurred. In 2014 he again presented with an acute attack, requiring 1 week of ventilation. He was ART naïve and sputum for TB was positive. Using an approach whereby rifampicin and isoniazid are introduced with incremental doses using urinary ALA/PBG concentration monitoring and clinical assessment with concomitant haem arginate, TB therapy was successfully introduced. Post discharge monitoring continued, and improved. Unfortunately in 2016, Efavirenz/Tenofovir/Emtricitabine based ART was inadvertently prescribed by a primary care HIV service. He presented with flaccid paralysis requiring 6 weeks of ventilation and multiple septic complications requiring several courses of haem arginate. Given a CD4 of 31, Raltegrevir/Tenofovir/Emtricitabine based ART was introduced with close monitoring. The patient was eventually discharged to a rehabilitation facility for ongoing physiotherapy. In conclusion, TB & HIV treatment in the AIP setting, requires considered management with close monitoring and carefully balancing risk with benefit.
How to manage acute liver failure in Erythropoietic Protoporphyria (EPP)?

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EPP is mainly characterised by skin photosensitivity, however 10% of patients are at risk of acute liver failure. Cholestasis is feature consistent with liver damage due to accumulation of PPIX in hepatocytes and bile canaliculi. Here we report the case of 54-years-old EPP patient who was admitted to our hospital because of jaundice, asthenia and abdominal pain. Blood tests showed elevated cholestatic liver enzymes, hyperbilirubinemia (total 11.01; conjugated 9.53mg/dl), and very high level of PPIX (286.7μg/gHb). Abdomen US showed sludge in the gallbladder and slightly splenomegaly (13cm). Endoscopic Retrograde Cholangiopancreatography showed absence of choledochal sludge; the liver biopsy revealed ductular metaplasia with cholangiolitis, lobular with canalicular bilirubin stasis and plurifocal cholestasis. The patient underwent three plasmapheresis and blood transfusion for anemia; protoporphyrin and bilirubin values rapidly decreased (166μg/gHb; 1 and 0.6mg/dl respectively). After one year the patient was again hospitalized for acute cholestatic hepatopathy and was treated with plasmapheresis, but hepatic stasis indices worsened. One week later, red blood cell (RBC) exchange therapy was performed in order to remove directly PPIX in RBC; PPIX, total and conjugated bilirubin values slowly decreased (from 213 μg/gHb, 9.86 and 7.31 mg/dl to 133, 1.13, 0.81 respectively). Plasmapheresis and RBC exchange has been reported in a small number of severe hepatic involvement. However, the effectiveness of these therapies alone or in combination has not been established by randomized clinical trials. Further investigation are needed.
### Abstract Title

**Porphyria Cutanea Tarda and Cancer Risk: A Nationwide Cohort Study**

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Our aim was to investigate the risk of cancer and all-cause mortality in people with Porphyria Cutanea Tarda (PCT) using a nationwide cohort design. The study sample consisted of all Norwegian residents aged 18 years or older. Persons with PCT were identified through the Norwegian Porphyria Centre, while patients with a cancer diagnosis were identified by linkage to the Cancer Registry of Norway and all-cause mortality by linkage to the Norwegian Cause of Death Registry. All analyses were adjusted for age, sex and education. People with PCT were at a higher relative risk of primary liver cancer, but no other cancer diagnoses. Although the relative risk was significant, the absolute risk was small. After adjusting for age, there was no increased relative risk for all-cause mortality. Our findings support previous findings that people with PCT are at an increased relative risk of PLC.
Abstract Title | Genetic counselling and lifestyle changes in acute intermittent porphyria (AIP)
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The Norwegian Porphyria Centre (NAPOS) has for fifteen years offered genetic counselling and predictive testing for acute intermittent porphyria (AIP) to healthy at risk relatives. The aim of this cross-sectional study was to investigate what motives healthy at risk persons have for undergoing genetic testing and whether they were satisfied with the genetic counselling they received. In addition, we wanted to investigate in both predictively tested and manifest patients whether receiving a diagnosis led to lifestyle changes.

The study was conducted among persons with genetically predisposed (n = 28) and manifest (n = 106) AIP. Self-administered questionnaires of motives for genetic testing, satisfaction with genetic counselling (SCS) and lifestyle changes were used.

Predictively tested patients reported that their motives for genetic testing were the possibility to prevent symptomatic disease (82 %) and the consideration of risk for children (76 %), and were highly satisfied with content of the genetic counselling. Receiving a diagnosis made both predictively tested and manifest patients become more conscious of checking their medications (92 %). Participants also changed their lifestyle in regards to eating habits (56 %), alcohol (54 %) and tobacco consumption (54 %).

Our study indicates that the genetic counselling contains elements that individuals at risk of AIP find informative. Furthermore, the possibility to prevent symptomatic disease is an important motive to get tested. Receiving the diagnosis motivates both manifest and predictively tested patients to make healthier lifestyle choices.
Porphyria is a group of metabolic disorders due to altered enzyme activities within the heme biosynthetic pathway. It is a systemic disease with multiple potential contributions to mitochondrial dysfunction and oxidative stress. Recently, it has become possible to measure mitochondrial function from cells isolated from peripheral blood (cellular bioenergetics) using the XF96 analyzer (Seahorse Bioscience). Using various inhibitors and activators of mitochondrial respiration, this technique measures various components of O₂ consumption rate (OCR) in peripheral cells such as basal, ATP linked, proton leak, maximal, reserve capacity, non-mitochondrial, and oxidative burst, all measured as pmol/min./100,000 monocytes. We performed cellular bioenergetics on 18 porphyria (9 PCT, 6 acute, and 3 protoporphyria) patients and 39 age/gender matched healthy controls. Of porphyria cases, 5 were active (1 PCT and 4 acute) and 13 in biochemical remission. Monocyte bioenergetics was significantly decreased in active porphyria vs. porphyria in remission and vs. healthy controls. Among 6 acute porphyria, a negative correlation (-0.8 to -0.93) was observed between urinary porphobilinogen and various components of monocyte OCR. In two pseudoporphyria patients, monocyte OCR was similar to healthy controls and higher than active porphyria. These novel and interesting preliminary findings suggest existence of mitochondrial dysfunction in porphyria and potential non-invasive biomarker for disease activity. Studies are suggested to examine mechanisms of these findings as basis for deriving mitochondrial based therapies in management of porphyria.
**Abstract Title**  
Secondary iatrogenic acute porphyric attack: a case report

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Porphyrias are inherited disorders due to enzyme defect along the heme biosynthesis pathway. Here, we report a 52 years-old woman, with no personal or family history of porphyria. She has been treated with a combination of carbamazepine and phenytoin since her childhood for a partial epilepsy.

She was admitted to the emergency ward with abdominal pain and constipation. No etiology was found. Biological analysis showed an increased phenytoin plasma level (2xN).

A worsening of her condition (bowel obstruction and mental disturbance) was observed after additional administration of nitrofurantoin.

Urinary PBG was at that time markedly elevated (50xN) while ALA was slightly elevated (4xN). Urinary porphyrin chromatography analysis showed 80% of uroporphyrin.

Based on these data, acute porphyric attack was diagnosed and treated with a course of heme arginate. Clinical symptoms disappeared. Urinary ALA and PBG returned to baseline values.

Unexpectedly, biochemical analysis failed to support the diagnosis of hereditary acute porphyria in this patient.

AIP was excluded on normal PBGD activity and no mutation found in HMBS gene.

CH was excluded on normal fecal porphyrins profile and no mutation found in CPOX gene.

VP was excluded on the absence of typical plasma fluorescence pic at 628 nm and no mutation found in PPOX gene.

Urinary ALA and PBG remained normal on the follow-up 3 months later.

We suggest that nitrofurantoin in combination with phenytoin overdose and carbamazepine might have produced a significant inhibition of PBGD activity in the liver to produce PBG and ALA accumulation and acute porphyric attack.
Porphyria cutanea tarda (PCT) is the most frequent porphyria with skin symptoms only. Familial PCT (fPCT; #MIM 176100) is an autosomal dominant disorder with low penetrance and a 50% uroporphyrinogen decarboxylase (UROD) deficiency while the most frequent subtype of PCT (75% of the cases) is sporadic with UROD deficiency in the liver only. Biochemical tests in blood, urine and stools allow the diagnosis of PCT. Determination of the erythrocyte UROD activity differentiates hereditary (decreased activity) from sporadic origin (normal activity). In fPCT, identification of mutations is helpful for confirmation of the disease and genetic counselling in order to prevent exposure to precipitating factors. There is no hotspot mutation in fPCT and many private mutations have been described.

Materials and Methods: From 2012 to 2016, we analyzed 233 probands with erythrocyte UROD deficiency. UROD gene molecular analysis was carried out by Sanger sequencing (coding parts and exon / intron junctions). Computational prediction tools were used to establish the pathogenicity of unknown mutations. The Genome Aggregation Database in search of their possible frequency was analyzed.

Results: Among the 233 patients, 60 new heterozygous mutations were found (25 missense, 4 nonsense, 16 small deletions, 6 small insertions, 1 indel, 8 splicing mutations).

Discussion: Although no functional analysis was performed and the segregation study not done, the concordant computer analysis obtained using several pathogenicity prediction software confirms their deleterious nature.

Conclusion: These new mutations underline the molecular heterogeneity of this disease.
Erythropoietic protoporphyria (EPP; #MIM 177000) is an inherited disorder resulting from a partial deficiency in ferrochelatase (FECH) with a complex mode of inheritance, either coinheritance of a FECH mutation in trans with a hypomorphic FECH c.315-48C allele (94 % of the cases) or less commonly (4%) homozygous or compound heterozygous FECH mutations. Other rare molecular abnormalities may be observed. Although blood tests are necessary for the diagnosis, molecular analysis is mandatory to confirm biochemical analysis, conduct a family investigation and propose genetic counseling. No hotspot mutation is found in EPP and many private mutations have been described.

Materials and Methods. From 2012 to 2016, we analyzed 62 probands with FECH enzymatic deficiency. FECH gene molecular analysis was carried out by Sanger sequencing (coding parts and exon / intron junctions). Computational prediction tools were used to establish the pathogenicity of unknown mutations. The Genome Aggregation Database in search of their possible frequency was analyzed.

Results: Incidence in EPP could be estimated to 2/10^7 per year. Among the 62 new protoporphyria patients, 15 new mutations have been found (7 missense, 2 nonsense, 1 small deletion, 1 small insertion, 1 indel and 2 splicing mutations). All the patients were heterozygous for the hypomorphic c.315-48C allele.

Discussion: Although no functional analysis was performed and the segregation study not done, the concordant computer analysis obtained using several pathogenicity prediction software confirms their deleterious nature.

Conclusion: These new mutations demonstrate once again the molecular heterogeneity of this disease.
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<th>Abstract Title</th>
<th>Physiology of Porphyrins in the amniotic fluid during the normal human pregnancy</th>
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<tr>
<td>Authors</td>
<td>Hana Manceau, Vincent Puy, Thibaud Lefebvre, Caroline Schmitt, Hervé Puy, Francoise Muller, Katell Peoc’h</td>
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The metabolism of heme by the fetal-placenta unit is not well characterized in human, as well as the porphyrins release into amniotic fluid during the pregnancy. The amniotic fluid is mainly composed of water, urines, pulmonary secretions, but also of fetal cells, and its composition, which reflects the fetal compartment, results from a tight homeostasis and a frequent turnover.

The aim of the present work was to perform a quantitative and qualitative study of porphyrins in the amniotic fluid of 37 women from week 13 to week 33 of pregnancy. Total porphyrins were measured by direct fluorescent spectroscopy, and separation of porphyrins was performed using high-pressure liquid chromatography.

We found that levels of the total porphyrins in the amniotic fluid ranged from 6 to 72 nmol/L during normal pregnancies. Mean concentrations of total porphyrins declined over the pregnancy, with a mean of 36 nmol/L at week 13 and of 11 nmol/L at week 33. The proportion of uroporphyrin compared to those of coproporphyrin increased during the time, with almost undetectable uroporphyrin at weeks 13-14 and a mean of 0.9 nmol/L at week 33.

To date, this is the first description of the physiology of porphyrins in the human amniotic fluid in a retrospective cohort of women, which evidenced that porphyrins vary quantitatively and qualitatively along the pregnancy in humans.
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<th>Abstract Title</th>
<th>Targeted re-sequencing of FECH gene shows that a single haplotype is linked to hypomorphic allele</th>
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<tr>
<td>Authors</td>
<td>Matteo Chiara(^1), Valentina Brancaleoni(^2), Luca Agnelli(^3), Valeria Fiorentino(^3), Francesca Granata(^3), Giovanna Graziadei(^2), Elena Di Pierro(^2)</td>
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It is widely established that the wild-type hypomorphic FECH allele is associated with the presence of a single c.315-48T>C polymorphism. However, the existing data do not fully explain for what reason some homozygous C>C individuals are completely asymptomatic, while others develop an overt disease.

In this study we performed high coverage targeted re-sequencing of the FECH gene using Next Generation Sequencing in order to identify possible candidate polymorphisms responsible for this variable phenotype. 15 EPP families including four subjects homozygous for the hypomorphic allele were sequenced.

A total of 683 polymorphic positions were recovered along the FECH gene of which 446 were assigned to a single haplotype by the means of the PhaseByTransmission tool from the GATK package. 176 were found to be common among patients. The comparison of the selected hypomorphic alleles showed that all patients shared an identical portion of FECH gene of about 20kb out of total 40kb. This region contains a string of 54 annotated SNPs. Among them only three known substitutions (c.-252A>G, c.68-23C>T and c.315-48T>C) were never present in trans to the mutated FECH allele in asymptomatic carriers. These results suggest that the three polymorphisms could be functionally related in reducing FECH gene expression. Extensive publicly available gene expression dataset (GTEX) also provide this evidence. At the same time no significant genetic differences were recovered between hypomorphic alleles of symptomatic and asymptomatic homozygous patients suggesting that other exogenous or endogenous factors are responsible for the variable penetrance.
**Abstract Title**  
Evaluation of inflammation markers in patients with Erythropoietic Protoporphyria after sun exposure

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To date it is not well established if photosensitivity triggers inflammation and lipid peroxidation (LPO) in erythropoietic protoporphyria (EPP) patients. Proteolytic activation of complement C3-C5 fragments was demonstrated following patients’ skin irradiation with 0.7 J/cm² at 400-410nm wavelength, corresponding to 11 minutes of exposure to sunlight. A group of 21 EPP patients and 13 healthy subjects were studied; the onset of symptoms, markers of inflammation and LPO in relation to sun exposure were assessed in all patients. We performed a survey evaluating the time of the onset of early (burning, itching, skin pain) and delayed (edema, skin lesion, petechiae, erythema, vesicles, hyperkeratosis) symptoms. Moreover, we measured C3 and malondialdehyde (MDA) in serum by colorimetric assays. Patients were stratified according to the time of onset of early symptoms into low (L), medium (M) and high (H) photosensitivity classes. The delayed symptoms resulted more common in L and M groups; C3 and MDA levels in these 2 groups were higher than in group H and in controls (significant p-values were obtained for all experiments). Concentration of free erythrocyte protoporphyrin IX (PPIX) appeared unrelated to the onset timing of symptoms. L and M patients probably expose themselves more than H patients causing the inflammation markers to increase. Differences among groups may be also affected by external factors (i.e. psychological and social). Further studies are needed to better understand the pathophysiology of skin symptoms in EPP.
Erythropoietic protoporphyria (EPP; OMIM 177000), is characterized by excess accumulation of protoporphyrin, particularly in erythroid cells. Its inheritance is complex, almost always associated with two molecular defects. In most EPP patients, clinical expression requires coinheritance of a private ferrochelatase (*FECH*) mutation trans- to a hypomorphic *FECH*|IVS3-48C allele. This leads to a decrease of *FECH* activity below the critical threshold. To our knowledge only seven families with diagnosis of EPP were diagnosed in Czech Republic. To address the question whether the relatively low incidence of EPP in the Czech Republic might be due to lower frequency of the IVS3-48C allele, we screened for the frequency of the low expression allele in a control Czech (West Slaves) Caucasian population. Such study has not been performed in any Slavic population. Among 312 control individuals, there were no IVS3-48C/C (c.68-23C-T) homozygotes; 35 IVS3-48C/T heterozygous individuals were detected. The frequency of IVS3-48C allele was thus found to be 5.5 % in the Czech population, comparable to most West Caucasian populations. Our results from the first Slavic Caucasian screening of 624 alleles in the Czech population thus indicate the overall IVS3-48C allele frequency of 5.5 %, comparable to the above-mentioned reports from other West Caucasian populations. While the frequency of the IVS3-48C allele is most likely not the reason for the low incidence of EPP in the Czech Republic, it remains to be determined whether a distinct protective variant or complex rearrangements of *FECH* or other genes involved in EPP pathogenesis underlie this phenomenon.

This work was supported by grants from Grant Agency of Czech Republic (14-36804G), Charles University in Prague (PROGRES Q26/1LF, UNCE 204011/2012), and Ministry of Health of the Czech Republic (RVO-VFN 64165/2012).
Abstract

Cytochrome P450 genes in Spaniards with acute intermittent porphyria

Authors

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Acute porphyrias present with attacks which are often provoked by drugs. Porphyrogenic drugs are known to induce or inhibit cytochrome P450 complex (CYP). 3A5, 2C9, 2C19 and 2D6 are important liver CYP, which are highly polymorphic. Different alleles lead to phenotypes from poor to ultra-metabolizers. Lower frequency of CYP2D6*4 has been reported in Argentinians AIP patients compared with control population. We aimed to study common alleles which produce decreased activity or inactive enzyme, and assess their relation with attack occurrence.

50 patients with acute intermittent porphyria (AIP) were studied, 52% symptomatic. CYP2C9*2 and *3, CYP2C19*2, CYP3A5*3, CYP2D6*4 and *5 were genotyping by TaqMan PCR. CYP2D6 duplications were analyzed by Taqman copy number assay.

Allele frequencies among symptomatic/asymptomatic were: CYP2C9*2 0.23/0.33; CYP2C9*3 0/0.02; CYP2C19*2 0.17/0.13; CYP3A5 0.94/0.96; CYP2D6*4 0.06/0.19; CYP2D6*5 0/0.02; CYP2D6 duplications 0.08/0.06.

Frequency of CYP2C9*2 was double in AIP patients compared with Spanish population while frequency of allele *3 was 8 times lower. Rest of allele frequencies was similar to those in our population. However, CYP2D6*4 and *5 was 3.5 times more frequent in asymptomatic patients than in overt AIP. Therefore, acute symptoms are more likely to occur among non-carriers of inactive alleles CYP2d6*4 and *5 than in carriers (OR: 4.60, IC 95%: 1.1-19.8).

This may suggest than inactive allele of CYP2D6 could play a protective role in precipitating attacks, although larger cohorts should be analyzed to check this association.
The authors present the medical history of a large Hungarian family with acute intermittent porphyria. The affected family members have a novel mutation of the HMBS gene (p.Ser75Lysfs*8; c.224insA; g.9354insA) which resulted in an unusually low activity of the HMBS enzyme and severe clinical manifestations, one patient with tetraplegia and the other with paresis during pregnancy.

The Hungarian AIP patients have a wide spectrum of mutations of the HMBS gene.

31 unrelated AIP families were investigated using Sanger sequencing method. Six previously unpublished mutations were observed.

Missense mutations were detected in 15/31 (48%) of investigated families. Beside them, 1/31 (3%) nonsense, 3/31 (10%) splice-site, 9/31 (30%) frameshift mutations were identified, while in three cases (10%) no alterations in the HMBS gene were found.
Abstract Title | Recurrent Attacks of Acute Intermittent Porphyria
---|---

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The main issue in the medical care of acute intermittent porphyria (AIP) patients is the occurrence of debilitating recurrent attacks. Chronically ill patients require repeated hemin infusions and develop secondary hemochromatosis and have a poorer quality of life. A follow-up study was conducted between 1974 and 2015 and included 602 French AIP patients, of whom 46 had recurrent AIP. To decipher the mechanisms underlying recurrence in AIP patients, we studied the metabolic pathways altered in 5 human explanted livers from 3 countries (Sweden, Norway and France). The introduction of hemin into the pharmacopeia has coincided with a 4.4-fold increase in the prevalence of chronic patients. We show that repeated hemin infusions trigger a high level heme oxygenase 1 (HO1) response, induce a pro-oxidative iron accumulation and a complex pattern of liver inflammation with macrophage infiltration. Conclusion: chronically heme-treated AIP patients may present with symptoms of an inflammatory disease responsible for an adaptive HO1 induction that could deplete the free heme pool inducing ALAS1. Hemin remains the most effective treatment but should be restricted to patients with severe forms of AIP to prevent chronic damage.
The PPOX gene encodes for the protoporphyrinogen oxidase, the penultimate enzyme in the heme biosynthetic pathway. An impairment in PPOX is linked with the variegate porphyria, a hepatic porphyria characterized by different symptoms such as skin photosensitivity and acute neurovisceral crises. In this study, we discovered new variants in variegate porphyria patients and characterized functions of three new variants identified in the regulatory regions of the PPOX gene. We demonstrated a lower expression of the PPOX gene through luciferase assays and RNA analysis for the c.1-883G>C promoter mutant. The c.1-413G>T and c.1-176G>A mutations in the 5' UTR of PPOX mRNA variant 2 have a post-transcriptional role. Transfection experiments of mutant -413T reporter plasmid suggest that this variant inhibits translation of the downstream firefly luciferase mRNA. In fact, the reduced firefly luciferase activity did not concord with the proportional reduction in firefly luciferase mRNA expression. Normal values of PPOX mRNA level detected in the patient carrying this substitution support this evidence. Data for the c.1-176G>A variant show that it acts as splicing variant. The qualitative RNA analysis confirms that this mutation is involved in the suppression of splicing between exon 1 and exon 2 of PPOX leading to 4 bp deletions in exon 1. The relation between these post-transcriptional alterations and the variegate porphyria remains to be investigated. This study suggests that the regulatory regions have to be considered in the diagnostic process but more studies are required to clarify their role in the disease.
First Colombian patient diagnosed with Hereditary Coproporphyria

Porphyrias are metabolic innate errors caused by mutations in genes expressing the enzymes of heme group synthesis. In Hereditary Coproporphyria (HCP) the enzyme involved is the coproporphyrinogen oxidase, encoded by the CPOX gene located on chromosome 3q11.2, it is of autosomal dominant inheritance. The clinical manifestations are characterized by acute attacks of neurological dysfunctions, which compromise the central nervous system, peripheral and autonomic. Clinical signs often present with: abdominal pain, hypertension, tachycardia, peripheral neuropathy, photosensitivity and excessive dermal fragility.

Biochemically, patients exhibit elevation of both delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) in the blood and urine, excretion of large amounts of porphyrin isomers or fractions, mainly coproporphyrin III in feces and urine. The activity of coproporphyrinogen oxidase III is generally reduced is reduced by less than 50%.

We report the case of a 23-year-old female patient who was diagnosed as a child with epilepsy from childhood, with multiple neurological manifestations and complications following administration of porphyrinogenic drugs.

This is the first patient with coproporphyria in Colombia characterized by the biochemical findings in the excretion of ALA and porphyrin isomers present in the Hereditary Coproporphyria. In the genetic characterization for the CPOX gene, three mutations were found, one of them a new mutation for HCP.
Porphyria Cutanea Tarda (PCT), represents about 70% of patients with Porphyria. Up to 2016, including Type I and Type II PCT, we diagnosed 2048 patients with overt PCT. This disease, mainly triggered by hepatotoxic drugs and hepatothropic virus, usually appears in adult life, over 40 years old, excluding Type II patients and subjects with human B and C virus infections (HBV, HCV) or human immunodeficiency virus (HIV). In the present work we have analyzed the precipitating agents in 80 women, negative for HBV, HCV or HIV infection, who developed PCT before they were 40 years old. This group represents almost 20% of total women with PCT (422). Mean age of manifestation was significantly lower (29.0±6.4, p<0.05) than in the total female PCT population (49.5±9.3). In 60% of patients hormonal treatment was the triggering factor (G1), notably different (p<0.05) respect to G2 (alcohol 10%, corticoids 5%, anxiolytic drugs 5% among others); hormones plus other risk factors were identified in 10% of these PCT women. Neither heme parameters at diagnosis nor mean age PCT manifestation showed significant differences between both groups. A comparable group of young PCT males was not found.

Hormones are a potent porphyrinogenic agent, mainly via CYP450, acting as a suicide metabolite. So, the actual high consumption of synthetic hormonal drugs would be the reason of the increment in the number of cases of early PCT manifestation. In young women undergoing hormone treatment, the presence of the typical cutaneous manifestations may be considered as a warning and early sign of a possible PCT case.
Although the characteristic biochemical abnormalities in the porphyrias are the diagnostic “gold standards”, the identification of pathogenic mutations by gene sequencing permits the diagnosis of both symptomatic and “latent” patients, especially for the Acute Hepatic Porphyrias (AHPs). During the 10-years, 2007–2016, 2,845 patients, including 2,040 unrelated probands, were referred for porphyria DNA-based diagnostic testing with informed consent; >30% were from Porphyria specialists. A total of 4,766 gene tests were performed, and 1,130 (39.7%) patients had a pathogenic mutation. Most referrals (2,004, 70.4%) were for an AHP diagnosis, either for AIP, HCP, and/or VP (1,241, 43.6%) or the “Triple Test” panel (763, 26.8%). Overall, 28.9% of AHP referrals had a pathogenic mutation. Reported/novel pathogenic mutations were identified in \textit{HMBS} (63/47), \textit{CPOX} (4/12), and \textit{PPOX} (49/21). The most common \textit{HMBS} mutations (R173W and R167Q in 13.7 and 7.7% of unrelated probands) were at CpG dinucleotides. Of 434 and 170 patients referred for EPP and XLP testing, 74.0% and 31.8% were mutation positive, respectively. All EPP patients had an IVS3-48T>C low expression allele; 19 novel \textit{FECH} mutations were identified. Targeted mutation testing for 555 AHP and 259 EPP/XLP family members resulted in 48.1% and 44.4% mutation positive, respectively. Of 372 biochemically-confirmed PCT patients, 24.7% had a \textit{UROD} mutation. For 103 CEP referrals, 70.9% were mutation positive; \textit{UROS} C73R was the most common mutation (13% of alleles). These findings indicate the value of DNA testing and the 10-year experience of a major U.S. laboratory.
Abstract Title | Harderoporphyria: clinical, biochemical and molecular features in an adult male
---|---
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**Background.** In harderoporphyria, a rare variant of homozygous hereditary coproporphyria, harderoporphyrinogen, the tricarboxyl intermediate of the 2-step decarboxylation catalyzed by coproporphyrinogen oxidase (CPOX), accumulates and is oxidized to harderoporphyrin. CPOX mutations in harderoporphyria usually affect residues D400-K404.

**Case report.** A 78 yo man had jaundice and anemia at birth, requiring transfusions, life-long cutaneous blistering and splenomegaly. Anemia was mild as an adult. Elevated porphyrins were found in his 40s but the type of porphyria could not be defined. He drank moderately until age 43 and smoked until age 53. He underwent cholecystectomy at age 75, and resection of an enlarging pancreatic cyst and a markedly enlarged spleen at 76. Studies of a son with blistering are pending.

**Methods & Results:** Biochemical testing showed: marked increases in porphyrins in plasma, urine (mostly highly carboxylated), feces and erythrocytes; normal ALA and PBG, plasma scan: peak at 620nm; RBC protoporphyrin: predominantly metal-free; RBC uroporphyrinogen decarboxylase: normal. Sequencing of UROD, FECH and ALAS2: no mutations. At this point, HPLC of RBC porphyrins showed a likely tricarboxyl porphyrin peak accounting for 24% of the total. CPOX sequencing then revealed 2 previously unreported mutations in trans: p.D233G (c.698A>G) / c.1207_1218del 12).

**Conclusions:** These clinical, biochemical and molecular findings support a diagnosis of harderoporphyria, even though residues D400-K404 were not affected. Fractionating RBC porphyrins aids in diagnosis of such cases.
**Abstract Title**  
Sex Differences in Vascular Reactivity in Mesenteric Arteries from a Mouse Model of Acute Intermittent Porphyria [AIP].

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Acute intermittent porphyria (AIP) results from a partial deficiency of porphobilinogen deaminase (PBGD). Symptomatic AIP patients, most of whom are women, experience acute attacks characterized by severe abdominal pain and abrupt increases in blood pressure. Here, we characterized the reactivity of mesenteric arteries from male (n=5) and female (n=6) AIP mice with ~30% of normal PBGD activity and wild type C57BL/6 mice male (n=5) and female (n=6). Vascular responses to acetylcholine (ACh), phenylephrine (Phe) and hemin were determined (Wire MultiMyograph). Relaxation was expressed as % of pre-constricted tone, contraction as % of maximal response (KCl 75mM, %K\text{MAX}) and sensitivity as pD\textsubscript{2} (-Log\textsubscript{10}[EC\textsubscript{50}]). Maximal relaxation to ACh was similar in males (88±5 vs 83±4%) and females (85±5 vs 80±5%), with lower sensitivity in female AIP arteries (6.9±0.1 vs 7.3±0.1, p<0.05). Female AIP arteries had increased ACh relaxation after L-NAME (60±7 vs 31±8%, p<0.05). Maximal contraction to Phe was similar in males (120±6 vs 138±10 %K\text{MAX}) and females (133±8 vs 135±11 %K\text{MAX}). Female AIP arteries had increased sensitivity to Phe (6.5±0.1 vs 6.1±0.1, p<0.05) even after L-NAME (7.1±0.2 vs 6.5±0.2, p<0.05). Hemin induced greater relaxation in AIP arteries in both males (60±7 vs 31±8%, p<0.05) and females (76±6 vs 48±6% p<0.05). Sex differences in this AIP mouse model include a pro-contractile response in females. These alterations may contribute to the increased blood pressure during an acute attack and provide a novel mechanism of action whereby heme ameliorates the attacks. Supported by NIH HL117199 to HLB and U54 DK083909 to RJD and HLB.
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<th>Abstract Title</th>
<th>Evaluation of alternative splicing and absolute FECH expression through a Digital PCR approach.</th>
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<tbody>
<tr>
<td>Authors</td>
<td>Brancaleoni Valentina, Granata Francesca, Fustinoni Silvia**, Mazza Paolo, Graziadei Giovanna, Cappellini Maria Domenica**, Di Pierro Elena.</td>
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It is known that intron 3 of FECH pre-mRNA is subjected to an alternative splicing leading to the insertion of intronic 63bp and to non-sense mediated decay of the aberrant mRNA. The c.315-48T/C polymorphism is responsible for this alternative splicing event. In this study we developed a Digital PCR assay to evaluate the absolute quantification of FECH aberrant splicing and global expression in a cohort of 91 subjects: 29 wild type, 27 EPP patients (mutation+c.315-48T/C); 13 carrying only the mutation; 17 carriers of c.315-48T/C and 5 homozygous for c.315-48T/C. We noticed that the percentage of aberrant mRNA is lower compared to data reported in literature both in wild type and c.315-48T/C carriers, even thought the last is doubled compared to the wild type. A significant difference was detected between wild type vs c.315-48T/C, vs homozygote for c.315-48T/C and vs patients. This trend was confirmed by analyzing the number of single events of insertion. There was no significant difference between wild type subjects and carriers of FECH mutations. Evaluating the total FECH expression, we noticed a significant difference between normal subjects and mutation carriers vs homozygotes for c.315-48T/C and patients. A negative correlation between PPIX vs total FECH and a direct correlation vs aberrant mRNA percentage were observed.

It is well known that c.315-48T/C is enough to reduce the global expression to a pathologic level when associated to a mutation in trans, but alone is not effective, whereas in homozygosis, the c.315-48T/C is able to reduce the FECH expression to a pathologic level, causing EPP phenotype.
In Argentina, a high association of Porphyria Cutanea Tarda (PCT) with HIV infection is found (17%), however to date, slight evidence exists about if triggering factors of PCT in HIV patients are related to the infection and/or therapy. The multidrug resistance protein (MDR1) is involved in the transport of xenobiotics and antiretroviral drugs. A number of polymorphisms in MDR1 gene were found to be of clinical importance, among them: exon 12 (c.1236C>T), 21 (c.2677G>T/A) and 26 (c.3435C>T) with high incidence in Caucasians. The frequency of these SNPs was previously studied in control, PCT and PCT-HIV individuals. The aim was to complete this research analysing also HIV patients without PCT by PCR-RFLP assay. The analysis of 1236T-2677T/A-3435T haplotypes was performed using SNPStats program. The polymorphic allelic frequencies were 0.46 (exon 26), 0.33 (exon 12) and 0.43 (exon 21). Genotypic frequencies were as follows: exon 26: CC 23.1%, CT: 61.5%, TT 15.4%; exon 12: CC 33.3%, CT 66.7%, TT 0%; exon 21: GG 32.3%, GT 47.6%, TT 19.1%. In HIV population, the frequency of T allele for exon 26 was significant higher than control, but comparable to PCT and PCT-VIH groups. For exon 21, significant differences of T allele frequency was observed for PCT-HIV respect to the other groups; while for exon 12, the mutant allele was only different in PCT cohort. In conclusion, the polymorphism of exon 21 could be involved in the manifestation of PCT in HIV individuals related to the antiretroviral therapy used in these patients, while in exon 12, T allele could be associated with another risk factors.
Abstract Title | Characterization of the Hepatic Transcriptome Following Phenobarbital Induction in the AIP Mice
---|---
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Acute Intermittent Porphyria (AIP), an autosomal dominant hepatic disorder, results from hydroxymethylbilane synthase (HMBS) mutations that decrease HMBS enzymatic activity, thereby predisposing patients to life-threatening acute attacks. Here, we characterized the hepatic transcriptome in response to porphyrinogenic phenobarbital (PB) injections in the AIP mice. Using 50% increased/decreased gene level changes as a cutoff, the expression profiles of 12,235 hepatic genes prior to treatment were very similar between AIP and wild-type (WT) mice. After PB treatments for 3 consecutive days (~120mg/kg), 644 and 369 genes in AIP mice and 284 and 84 genes in WT mice were uniquely up- and down-regulated, respectively, at False Discovery Rate (FDR) < 0.05. Expectedly, the ALAS1 expression increased 4.5X and 15X in the WT and AIP mice, respectively. Unexpectedly, however, ALA-dehydrogenase was induced 1.6X after PB treatment in the AIP mice, but was unchanged in WT mice. Of the 162 hepatic cytochrome P450 enzymes analyzed, the expression of 13 enzymes was distinctly different between AIP and WT mice, with 10 of these enzymes being elevated more than 1.5X in the PB-induced AIP mice. Notably, gene set enrichment analysis indicated that the cell cycle and mitochondria biogenesis gene-sets were significantly up-regulated in PB-treated WT and PB-treated AIP mice, respectively. Overall, we identified 1,013 and 368 genes whose expression was exclusively induced/repressed in AIP or WT mice, respectively. Further studies of the exclusively-expressed genes would increase our understanding of the hepatic changes of PB-induced AIP mice.
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<th>Abstract Title</th>
<th>The RCPA-QAP Porphyrin Quality Assurance program</th>
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<tr>
<td>Authors</td>
<td>T.Andersen, K.Barancek, S.Briscoe, V.Cronin, CM Florkowski, JA Grant, V.Poulos, CW Sies, J.Zoanetti</td>
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The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPA QAP) Porphyrin program provides a comprehensive external quality assurance program which has 55 participants in 14 countries and is now in its 27th year. The program has two components: Analytical and Patient results comments.

The analytical component offers six matched pairs of lyophilised plasma, urine and whole blood and frozen faeces for analysis. Analytes reported include urine porphobilinogen and amino laevulinic acid, and urine, faecal, plasma and whole blood total porphyrins and fractions. Samples donated by porphyric patients provide authentic profiles representative of the different types of this condition. Comprehensive statistical reports are provided that permit assessment of performance relative to all program participants.

Enrolled laboratories may also participate in a patient comment component which uses results from real patients to provide an educational self-assessment tool for individuals who normally attach comments to results from their laboratories.

The program is supported by the Australasian Association of Clinical Biochemists (AACB) Porphyrin Working Party (PWP) which is comprised of scientific and medical experts from Australia and New Zealand. The activities of the PWP include sourcing and preparing the quality control material, creating and reviewing the patient cases and providing scientific support to the RCPA QAP. This review will include recent additions to the program, other activities of the PWP and the relevance of the program in the diagnosis of porphyria within Australasia.
Abstract Title  The burden of acute intermittent porphyria in the Dutch porphyria cohort.


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Introduction  It’s known from clinical experience that patients with recurring attacks of acute intermittent porphyria (AIP) have a high burden of disease. In this retrospective longitudinal cohort study we’ve quantified and compared the burden of acute intermittent porphyria between three patient groups: asymptomatic patients / symptomatic patients / patients with recurrent attacks. Primary endpoints: healthcare costs of admissions and heme, no. of attacks, total days of hospital stay for acute attacks and no. of heme arginate infusions. Secondary endpoints: life-long prevalence of porphyric symptoms, life-long prevalence of long-term complications, and employment/marital status.

Methods:  Data was collected from 88 AIP patients using questionnaires (inclusion period 2011-2016), and historical paper/electronic patient data (1960-2016). Asymptomatic group: 53 carriers. Symptomatic group: 24 cases. Recurrent group: 11 cases.

Results:  The estimated cost of healthcare in our cohort for the recurrent group was ~€5.8 million vs ~€0.3 million for symptomatic group. Total admission days for attacks and heme therapy was 5,587 days vs 395 days. Recurrent patients suffer more symptoms (acute/pain/neurological/psychiatric) and long-term complications (hypertension/chronic kidney disease) of AIP then patients in other groups.

Conclusions:  Recurrent AIP patients porphyria have a more severe burden of disease compared to their asymptomatic or symptomatic counterparts. This is reflected by highly increased costs, extended hospital stay, high incidence of porphyria related symptoms, and high unemployment.
Abstract Title | Heme Group Biosynthesis Modulation by Porphobilinogen Deaminase Inhibition
---|---
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Congenital erythropoietic porphyria (CEP) is an autosomal recessive type of porphyria caused by a defect of the enzyme uroporphyrinogen III synthase (UROIIIS), in which the derivatives uroporphyrinogen I and coproporphyrinogen I accumulate in all the tissues, leading to haemolytic anemia, ocular complications, photosensitivity, bone fragility and mutilating skin lesions. In this work we explore the possibility of modulating the accumulation of toxic porphyrin derivates in the body by partially and reversibly inhibiting porphobilinogen deaminase (PBGD). We have developed a reliable method to screen for selective PBGD activity modulators and to identify candidates for further preclinical studies and potential clinical development. Specifically, a library of 2500 compounds was screened in vitro using the changes in the Michaelis-Menten curve of the enzyme as a reporter. With this strategy, we identified a set of compounds that efficiently inhibit the enzyme, often showing homologous chemical structure. With the aid of computational methods (docking) and high resolution NMR spectroscopy, we conclude that this inhibitors compete with the substrate for binding at the active site of the enzyme.
Two brothers of consanguineous parents (K.E., 32 years and Z.E. 36 years) were diagnosed with unexplained spastic paresis, cognitive decline and, in Z.E., prominent cystic lesions in brain white matter and deafness. Whole exome sequencing was performed. In K.E. a homozygous mutation was found in the HBMS gene (c.271G>A (p.(Asp91Asn)) and Z.E. was heterozygous for the same mutation. Heterozygous mutations in the HBMS gene are the cause of acute intermittent porphyria (AIP). However, the mutation found in the 2 brothers has not yet been published and is of unclear pathogenicity. Bi-allelic HMBS variants have been reported previously as the cause of severe encephalopathy with early childhood fatality in AIP. Furthermore, in 2016, we described a family with childhood-onset slowly progressive spastic paraparesis, cerebellar ataxia and peripheral neuropathy who had compound heterozygous missense variants in the HMBS gene. It is therefore possible that the newly identified mutation in the HBMS gene is the cause of the neurologic findings in the current 2 patients. Both patients had no clinical symptoms associated with AIP. Plasma ALA levels were normal in Z.E. (18 nmol/l) and mildly elevated in K.E. (124 nmol/l, reference value <74) but plasma PBG levels were > 100 times elevated in both brothers (1280-1540 nmol/l, reference value <12). Interestingly, PBG deaminase activity in erythrocytes was elevated (210-250 pmol/mg protein, normal value >64). Both patients were counseled according to our protocol of AIP, despite the fact that we are uncertain whether they are at risk of developing an attack.
### Abstract Title
Identification of a pharmacological chaperone that decreases UROI levels in Congenital Erythropoietic Porphyria (CEP)

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Congenital erythropoietic porphyria (CEP) is a rare genetic disease produced by deleterious mutations in UROS gene reducing the activity and stability of uroporphyrinogen III synthase enzyme (UROS), increasing the accumulation of toxic porphyrins; uroporphyrinogen I (UROI) and coproporphyrinogen I (COPROI). In previous studies, we demonstrate that the catalytic activity of UROS can be restored by incorporating residues prone to interact with the hotspot R73. Based on this proof-of-concept we propose a new therapeutic approach based on the use of pharmacological chaperones to intracellularly modulate and increase the kinetic stability of the enzyme. We have screened a library of compounds and some hit compounds stabilize the hostspot C73R in vitro, as monitored by circular dichroism (CD) and nuclear magnetic resonance (NMR). Additionally, the intracellular activity for a set of hit compounds was monitored through the analysis of EGFP-tagged version of UROS(C73R) mutant in M1 Fibroblast human cells. The results obtained at HC automated fluorescent microscope suggest that the compounds are able to interact with the protein UROS(C73R). Using the novel CRISPR-Cas9 system, we obtained HEK UROIIIS(C73R) mutant stable cell line and checked compounds selected previously like pharmacological chaperones. We found a molecule that decreases the accumulation of porphyrins by Cytometry and drastically decrease the accumulation of UROI by HPLC. Currently, we are performing the experiments in vivo with mice CEP model. All results together indicate that the molecule can be effectively used as a novel therapeutic intervention line against CEP.
**Abstract Title**  Characterization of porphobilinogen deaminase inhibitors as a therapeutic aid for congenital erythropoietic porphyria

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Congenital erythropoietic porphyria (CEP) is an autosomal recessive disorder caused by a defect of the enzyme uroporphyrinogen III synthase (UROIIIS), which results in heme precursors, uroporphyrinogen I (UROI) and coproporphyrinogen I, tissue accumulation, leading to photosensitivity, and mutilating skin lesions among others. Therapeutic interventions are limited to decrease clinical manifestations, none of them being definitive. The only curative therapy for CEP is steam cell transplantation, a high risk therapy effective as the transplanted steam cells produce normal levels of UROIIIS and reverse the clinical manifestations. Thus, further therapeutic strategies are warranted. In this work, we explore a novel putative therapeutic approach: the inhibition of the UROIIIS upstream enzyme, porphobilinogen deaminase (PBGD) to alleviate the accumulation of by-products in the body. Inhibition of PBGD would yield in a decrease amount of downstream porphyrin derivates accumulation, and therefore an important improvement in the quality life of CEP patients. We previously identified some modulators of PBGD enzymatic activity that predominantly inhibited the enzyme. We have further characterized these set of inhibitors using a relevant cellular model of CEP (HEK C73R) to observe a decrease in UROI accumulation. The same compounds have also been administered to healthy mice where a modulation of the porphyrin content can be observed in urine, serum and liver tissue as a function of the nature of the compound and its specific dose.
**Abstract Title**
Using Whole Exome Sequencing to investigate EPP patients with unknown genetic cause.

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Erythropoietic Protoporphyria (EPP) is a genetic disease characterized by photosensitivity and elevated protoporphyrin IX (PPIX) in blood, resulting from ferrochelatase (FECH) or delta-aminolevulinic acid synthase-2 (ALAS2) gene mutations. Over the last 20 years we have performed DNA testing in > 150 EPP patients. There were 7 EPP patients with no disease causing mutation in either FECH or ALAS2. The aim of our study is to identify new EPP related mutations.

We performed Whole Exome Sequencing (WES) using DNA of these 7 EPP patients and one newly diagnosed EPP patient. WES was based on the Nimblegen SeqCap EZ MedExome (47Mb) capture and Illumina HiSeq sequencing. First variant analyses show >6,000 nonsynonymous exonic and splicing variants including stop gain and loss variants in all 8 EPP patients. Excluding common variants (MAF>1%) resulted in 250-350 variants per EPP patient. In 2 of these 7 EPP patients, we identified a published splice variant (c.67+2T>G) in the FECH gene and the previously already identified common splice variant (c.315-48T>C). In the new EPP patient, we found a new nucleotide change leading to a published premature stop (c.902G>A, p.W301X), and the common splice variant. The splice variant c.67+2T>G had been missed in the past by using Sanger sequencing, which could be explained by known PCR amplification difficulties of the first exon of FECH. In the remaining 5 EPP patients further filtering and data-analyses of the WES results are ongoing.

WES is a rapid and affordable technique (€350 per exome) to find known and new disease causing mutation(s) in EPP patients.
Abstract Title:
Cytochrome P450 Isoenzymes Single Nucleotide Polymorphisms (SNPs) are Really Involved in Porphyria Cutanea Tarda Development?

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It was suggested a role for some CYP1A1 and CYP1A2 isoformes in PCT development but the results from different populations are controversial. We analysed three polymorphisms, one in CYP1A2 and two in CYP1A1 in a group of Argentinean PCT patients and in a control group. One hundred PCT patients, 37 H-PCT and 63 A-PCT were studied employing PCR-RFLP and each variant was compared with 73 controls. The Fisher exact test was used to detect differences in alleles and genotype frequencies, odds ratio and 95% confidential interval. For CYP1A2*1F (-163 A>C) the frequencies of A allele and A/A genotype were higher for both types of PCT vs control group, being the A allele associated to a more transcriptional activity of CYP1A2 gene. There were significant differences for A/A (p<0.022) and A/C (p<0.022) genotypes vs C/C genotype and also A allele vs C allele (p<0.018) for total PCT vs control group. For CYP1A1*4A or m4 (c.4487 C>A; p.T461N), C allele and C/C genotype were the most frequent for total PCT vs control group. A significant difference for C/C and C/A genotypes vs A/A genotype were found (p<0.028 and C/A p<0.014 respectively). The same was observed for the heterozygous genotype vs A/A. For CYP1A1 *2C or m2 (c.4889 A>G; p.I462V), A/A genotype and A allele frequencies were higher for all groups. In this case G allele codified for a higher enzyme activity than wild type allele. Significant differences were found only for A-PCT vs control for A/A vs A/G genotypes (p<0.0016). So, we conclude that these polymorphisms could be, among others, a risk factor for triggering PCT clinical signs in Argentinean population.
**Abstract Title**  
Cimetidine does not effectively reduce the activity of δ-Aminolevulinic Acid Synthase

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Based on case reports and animal models of heme biosynthesis, the histamine H2 antagonist cimetidine has been suggested to be effective for treatment of acute intermittent porphyria (AIP)¹, porphyria cutanea tarda² and erythropoietic protoporphyria³. The mechanism of action of cimetidine has been attributed to direct inhibition of heme oxygenase (HO), in turn increasing the free heme pool and inhibits the import of the δ-aminolevulinic acid synthase (ALAS) pre-protein into the mitochondria and/or decreases transcription rate of ALAS. Alternatively, cimetidine may directly inhibit the enzymatic activity of ALAS. Here, we report results of experiments aimed at determining whether cimetidine modulates the enzymatic activity of ALAS in vitro and in a mouse model of AIP. Incubation of recombinant human ALAS with increasing concentrations of cimetidine [0-20 uM] did not decrease enzymatic activity. When AIP mice were treated with cimetidine in dose escalating experiments [15-120 mg/kg, twice daily, i.p.] following induction of an acute biochemical attack, hepatic Alas activity and plasma aminolevulinic acid and porphobilinogen were not significantly different compared to saline-treated controls. Additionally, hepatic Alas and Ho1 mRNA levels were comparable to those of controls. Our studies show that, the activity of ALAS, the rate limiting enzyme in heme biosynthesis, is not directly affected by cimetidine.

### Abstract Title
Local Anaesthesia and Porphyria. Is Totalcaine Forte a Safe Drug for Porphyric Patients?

### Authors
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Acute attacks of Porphyria may be precipitated by exogenous drugs including anaesthetics. There is limited information in the literature about the use of some dental medicines in the acute Porphyrias. The safety of local anaesthetic agents remains a controversial issue due to experimental evidence reveals that some of them are porphyrogenic in either animal models (lidocaine) or cell culture (lidocaine, prilocaine, bupivacaine), however clinical experience has shown no notable adverse effect in patients with Acute Porphyria. Articaine (carticaine, methyl-4-methyl-3-(2-propylaminopropionamide) thiophene-2-carboxylate hydrochloride) is a relatively new local anaesthetic and still untried in Acute Porphyria, although some reports established its safety and others classified this drug as unsafe. The aim was to investigate the effect of a commercial preparation, Totalcaina Forte (carticaine chlorhidrate:L-adrenaline, Bernabo Laboratories) on heme metabolism. Animals (CF1 mice) received different doses (1, 5 and 7 mg/kg, s.c.) and were sacrificed at different times (30 and 60 minutes) after injection. 5-Aminolevulinic acid synthetase (ALA-S), Porphobilinogen deaminase (PBG-D) and Heme oxygenase (HO) were measured in liver, brain and blood. Results indicated that ALA-S and PBG-D activity remain unchanged at low doses. Although, in animals receiving 7 mg/kg a slight reduction of liver PBG-D was found. The induction of HO activity (67%, p<0.05) would indicate an alteration in redox status. These preliminary results reveal that Totalcaina Forte would be safe but at very low doses, otherwise further studies are needed.
**Abstract Title**

Effect of Vanadate on 5-Aminolevulinic Synthetase in a Murine Model of Acute Intermittent Porphyria

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Acute Intermittent Porphyria (AIP) is characterized by a reduced activity of Porphobilinogen deaminase (PBG-D), increased levels of the first and rate limiting enzyme of heme pathway, 5-Aminolevulinic synthetase (ALA-S) and the accumulation of neurotoxic precursors, ALA and porphobilinogen. ALA-S can be induced by fasting or chemical agents. We have demonstrated that vanadate prevents the transcriptional increase of ALA-S in a murine model of diabetes. The aim of this study was to investigate the effect of vanadate on ALA-S in a murine model of AIP. Male and female knockout PBG-D deficient mice (Pbgd⁻/⁻) were divided in three experimental groups: non-fasted controls (C), fasted 24 hours (F) and vanadate (0.3 mg/ml in drinking water)+fasted (V+F). V was administered during 16 or 21 days. A significant loss of body weight was observed in males of F and V+F groups respect to C group, this effect was not produced in females. Starvation increased the activity (100-500%, p<0.05) and mitochondrial protein levels (100-200%, p<0.05) of ALA-S in both male and female mice. The treatment with vanadate for 21 days attenuated the effect of starvation, producing a reduction of induced ALA-S activity in both male (A: 0.46±0.07 U/mg, V+A: 0.31±0.10 U/mg) and female (A: 0.57±0.03 U/mg, V+A: 0.41±0.10 U/mg) mice. However, when vanadate was given for 16 days this effect was only observed in females (V+A: 0.36±0.10 U/mg). Data here presented are in agreement with our previous reports about the action of vanadate preventing ALA-S increase, and showed sex-based response in a murine model of AIP.
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<tbody>
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Heme deficiency by a reduced synthesis or an accelerated catabolism would trigger severe cell damage. Previously we demonstrated that porphyrinogenic agents affected several brain metabolisms including respiratory mitochondrial chain in encephalon C57Bl/6J mice. The aim was to study the effects of volatile anaesthetics and other xenobiotics on the activity of I to IV complexes of respiratory chain in encephalon of a mouse model of Acute Intermittent Porphyria. Male knockout mice (PBGD⁻/⁻) were treated with Isoflurane (2 ml/kg), Sevoflurane (1.5 ml/kg), ethanol (30%), allylisopropylacetamide (AIA, 350 mg/kg) and Veronal (167 mg/kg), or starved 24 hours. Veronal increased the activity of II-III complexes (370%, p<0.01) and II complex (218%, p<0.01). Ethanol decreased I-III and II complexes activities (48%, p<0.01), moreover complex IV activity was induced (86%, p<0.01). Sevoflurane increased only the activity of complex II (218%, p<0.05) and AIA augmented complexes II-III activities (218%, p<0.01). The alterations observed could be the result of a deficiency of the donor reduction equivalents as a consequence of Krebs cycle alteration. So, some Krebs cycle enzymes were also measured. Fumarase activity was 60% (p<0.05) increased due to Sevoflurane while Isoflurane and ethanol caused no effect; a striking diminution was produced after veronal (87%, p<0.01), AIA (40%, p<0.01), and under starvation (55%, p<0.05). Aconitase activity was 269% (p<0.01) increased due to veronal. Results reinforce our previous reports and support the hypothesis that there would be more than one factor to explain the pathogenesis of acute attacks.
Hereditary Coproporphyria (HCP) is an acute porphyria characterised by deficient activity of the mitochondrial enzyme coproporphyrinogen oxidase (CPOX). It is inherited in autosomal dominant fashion and has low penetrance; approximately 90% of affected individuals never experience an acute attack. HCP is the rarest of the 3 principle acute porphyrias but there is no population data on pathogenic CPOX variants.

Posterior Reversible Encephalopathy Syndrome (PRES) is a clinical-radiological diagnosis. It is a disorder of usually reversible subcortical vasogenic oedema characterized by a myriad of neurological features which can include seizures and impaired consciousness. MRI will typically show bilateral subcortical oedema in the parieto-occipital region.

We report the first case of PRES in HCP. A 21 year old woman presented with severe unexplained abdominal pain and hyponatremia. She subsequently developed seizures and a prolonged delirium with Magnetic Resonance Imaging (MRI) confirming PRES. Urinary porphyrin/creatinine ratio and faecal prophyrins were markedly elevated with metabolite profiles consistent with acute coproporphyria. The diagnosis was confirmed by the demonstration of a novel missense variant in the CPOX gene c.863T>G (p.Leu288Trp).

Cascade family screening has confirmed that her mother and sister also carry the mutation. Her sister has had two confirmed acute attacks characterized by neurovisceral symptoms but no seizures, hyponatremia or PRES.
A 65-year-old man with a photosensitivity syndrome attended the dermatology clinic. At examination, he showed increased urinary excretion of isomer type-I porphyrins typical of congenital erythropoietic porphyria (CEP). Faeces showed excess of coproporphyrin I. However, uroporphyrins were not increased within the erythrocytes; URO-synthase enzymatic activity in erythrocytes was normal and genetic screening of URO-synthase gene mutations (DNA from peripheral leucocytes) was negative. Examination of peripheral blood showed only thrombocytopenia and increased erythrocyte cell volume without anemia. The patient was diagnosed of late-onset acquired erythropoietic uroporphyria secondary to a possible myelodysplastic syndrome (MDS) and referred to the haematology service. Bone marrow aspirate showed dysmegakaryopoiesis and confirmed MDS. Cytogenetic analysis of bone marrow aspirate showed a chromosomal inversion (chromosome 3; q21q26). However FISH analyses with probes addressed to MECOM gene located at 3q26.2 were normal. According to hospital protocols, the patient was treated with azacitidine with irregular response. During this period, a striking inverse relationship between (oscillating) platelets in blood and porphyrins in urine was observed. Finally and considering the progression of the disease, a bone marrow transplantation (BMT) from an antigenic 9/10 mismatched at HLA-A unrelated donor was successfully performed. Hematological evaluation three months after BMT, revealed a complete chimeraism and normal haematopoiesis, without dysplasia. Skin symptoms disappeared and porphyrin levels in urine and plasma decreased to normal. This case illustrates (a): the contribution of the porphyria laboratory to the diagnosis of a haematological disease; (b): in MDS, an unresolved link between platelet formation and porphyrin overproduction (c): clonal origin of isomer-I porphyrins in the bone marrow that were completely eradicated after BMT.
### Abstract Title

Time to Treatment in Norwegian Patients with Porphyria Cutanea Tarda (PCT)

### Authors

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### Introduction:

When the diagnosis of PCT is made, it is important that treatment is initiated quickly to reduce excess porphyrins and thus symptoms. The aim of this study was to examine how long it takes before treatment is initiated in Norwegian PCT patients by the use of data from the Norwegian Porphyria Registry, a national medical quality registry.

### Methods:

The Norwegian Porphyria Registry includes approximately 71% of all known Norwegian porphyria patients. Data from PCT patients registered from 2007-16 (n=180) were used to assess time from diagnosis to treatment and the number of patients receiving treatment within two months after diagnosis.

### Results:

More than 70% of the PCT patients had received treatment within two months after diagnosis. Maximum time from diagnosis to treatment was 20 months. Comparing data from the periods 2007-2011 to 2012-16, an increasing number of patients have had their treatment initiated within two months in the latter period.

### Conclusions:

Based on data from the Norwegian Porphyria Registry, in the majority of the PCT patients treatment is initiated within two months of the diagnosis, while for a small minority of patients, long treatment delays are seen.