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Clinical indications for the investigation of porphyria: case examples and evolving laboratory approaches to its diagnosis in New Zealand

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Abstract

Patients with porphyria present in a diverse and unusual variety of ways and most clinicians will see only a few cases, if any, during their professional lives. Porphyria may present (1) with acute symptoms, which may be abdominal pain, neurological or psychiatric; (2) with skin rash or photosensitivity; or (3) with a putative family history. Screening for latent porphyria has been greatly facilitated by fluorescence emission scanning of plasma and by mutational analysis. Our reference laboratory has recently diagnosed several cases of the less common types of porphyria, which we postulate is due to the availability of these methods and to the changing population of New Zealand. Accurate screening and diagnosis of porphyria is important, as an acute porphyric attack is life-threatening and preventable. Retrospective diagnosis may be difficult.

The porphyrias are a diverse group of metabolic diseases affecting haem synthesis. A traditional classification is acute (acute intermittent porphyria [AIP], variegate porphyria [VP] and hereditary coproporphyria [HC]) versus chronic (porphyria cutanea tarda [PCT] and erythropoietic protoporphyria [EPP]). This is of limited practical value, in our opinion, and investigation is best guided by which of three presenting clinical scenarios is present.

Possible clinical indications for the investigation of porphyria:

- Patients presenting with acute symptoms, which may be abdominal pain, neurological, or psychiatric. Some patients presenting with acute abdominal pain may require a general anaesthetic, which could potentially include drugs that exacerbate acute porphyria.
- Patients presenting with vesicles, bullae, hyperpigmentation, milia, hypertrichosis, or increased skin fragility on sun-exposed skin. This photosensitivity is probably the most common indication for investigation.
- Screening of family members where there is a positive family history—testing for latent porphyria.

An acute attack begins with minor behavioural changes such as anxiety, restlessness, and insomnia—and proceeds rapidly to symptoms of autonomic and sensomotoric neuropathy. Abdominal pain (major presenting feature), usually followed by vomiting and constipation is common and severe, mimicking acute abdominal crisis.

Pain in the back or in the extremities is frequently present. Hypertension and tachycardia, which are signs of increased sympathetic activity and are associated with the activity of the disease, should be followed up together with visual assessment

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scaling of pain. Pain declines within a week, but if an additional precipitating factor is administered, the progressing sensorimotor neuropathy manifests and can proceed to respiratory paralysis. Central nervous system (CNS) impairment can manifest with convulsions and confusion. The combination of polyneuropathy and focal CNS involvement is unusual for other polyneuropathies and should alert a doctor to look for porphyrias.[1,2]

Random urine porphobilinogen (PBG) is the most important test for the diagnosis or exclusion of acute porphyria. If urine PBG is negative at the time of acute symptoms, then that excludes porphyria as cause of those symptoms. All acute hospitals should have access to urine PBG screening,[3] and a positive PBG test should be followed by further tests on urine, faeces, and blood to differentiate the type of acute porphyria.

For patients presenting with a skin rash or photosensitivity, it is preferable to send (protected from light) a complete set of samples of urine, faeces, and blood.

The commonest porphyria (90% of all cases) is PCT where the diagnosis is made on positive faecal and urine porphyrins, with characteristic patterns on high performance liquid chromatography (HPLC) analysis.

Screening of family members for latent porphyria, where there is a positive family history, is the most difficult clinical scenario to manage. The approach hinges on validating the diagnosis in the index case, which can be difficult when that case may be deceased or living overseas.

A valid diagnosis in an index case enables the diagnostic tests to be focused to those pertinent to the porphyria in question. In the case of AIP, for example, tests for latent disease include measurement of PBG deaminase activity (see below) or mutational analysis.

For VP, fluorescence emission spectrophotometry is gaining acceptance,[4] although it has limited sensitivity. Mutational analysis is the definitive way to confirm or refute a diagnosis of latent porphyria, but depends on the identification of a mutation in the index case. This approach can also identify asymptomatic carriers who may never have clinical manifestations of the disease.

We have witnessed a significant increase in the number of less common types of porphyria. This may, in part be due to the changing population of New Zealand, but may also be attributed to evolving methods of investigation, particularly the introduction of plasma fluorescence scanning.[4]

This review provides background information on the different clinical types of porphyria, some explanation of the diagnostic tests available, and finally some case examples encountered through our practice as a reference laboratory.

Biosynthesis and metabolism of haem precursors

Each type of porphyria results from decreased activity of one of the enzymes of haem biosynthesis as outlined in Figure 1. The porphyrinogen intermediates are unstable and readily autoxidise to porphyrins within tissue and after excretion. In normal circumstances most of the α-aminolaevulinate (ALA) produced is converted to haem, with only small amounts of intermediates being lost from the pathway and excreted either unchanged or after oxidation to porphyrins.

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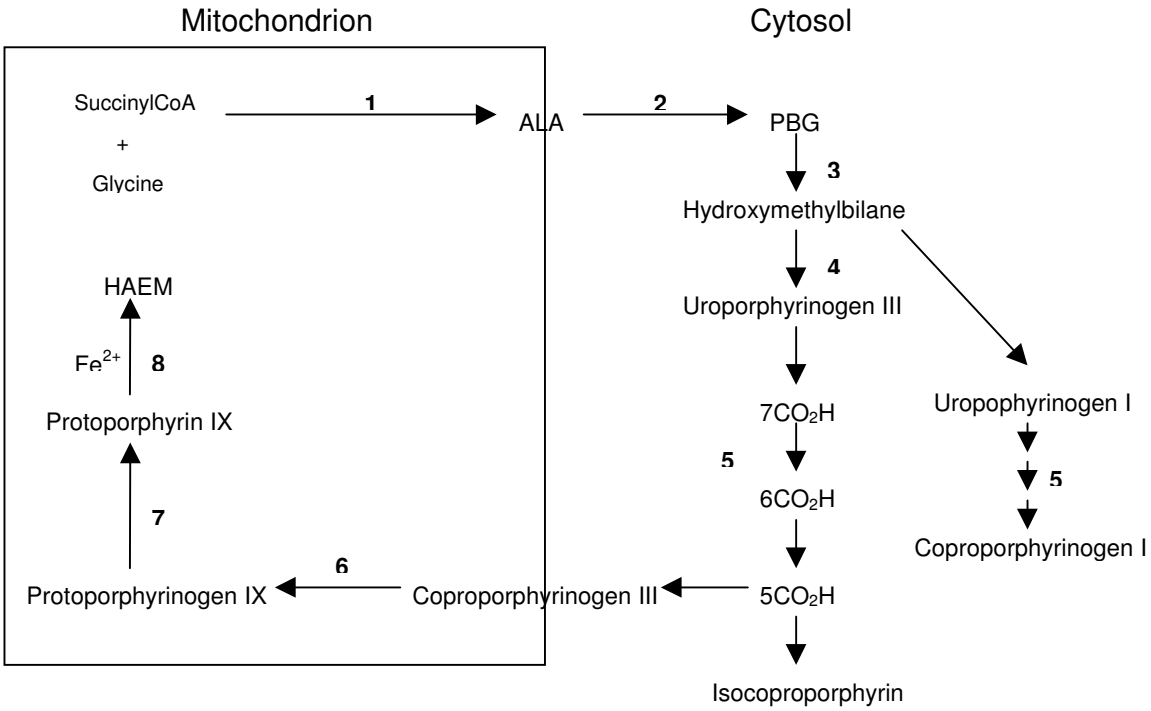
Figure 1. The pathway of haem biosynthesis. The reactions are catalysed by α-aminolaevulinate (ALA) synthase (1), porphobilinogen (PBG) synthase (2), PBG-deaminase (3), uroporphyrinogen-III synthase (4), uroporphyrinogen decarboxylase (5), coproporphyrinogen oxidase (6), protoporphyrinogen oxidase (7), and ferrochelatase (8)



The route of excretion is largely determined by the solubility of the compound. Generally, intermediates from ALA to coproporphyrin are water soluble, and are excreted in urine, while those from coproporphyrin to protophyrin are lipophilic and excreted in bile (faeces). Decreased enzyme activity (at any one point) results in a build-up of the preceding intermediates, while intracellular accumulation and subsequent excretion of the substrate produces a characteristic pattern of plasma, erythrocyte, and excretory abnormalities for each enzyme deficiency.

The different types of porphyrias (in order of enzyme defect in haem biosynthesis) are:

Acute intermittent porphyria (AIP) or Swedish porphyria

- Inherited in an autosomal dominant fashion.
- 1–2 carriers per 100,000 people, of which >80% do not develop symptoms. AIP is latent before puberty; symptoms are more frequent in females than males. Hormonal, drug (lists of safe and unsafe drugs are available)[5] and nutritional factors may aggravate the disorder.
- There are latent and acute stages.

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- Symptoms are neurological without skin photosensitivity. Acute abdominal pain occurs in 85–95% of cases. Symptoms may include: abdominal pain, nausea, vomiting, diarrhoea, constipation, ileus, dysuria, muscle hypotonia, respiratory insufficiency, sensory neuropathy, seizures.
- As for all acute porphyrias, urine ALA and PBG are increased in the acute state, slightly elevated or normal in the latent state. Urine porphyrins are often raised, while faecal and erythrocyte porphyrins are normal.

Congenital erythropoietic porphyria (CEP) or Gunther disease

- Inherited in an autosomal recessive fashion.
- Very rare with only 130 cases reported prior to 1997.
- The age of onset and clinical severity is highly variable, in most cases photosensitivity develops soon after birth. Blistering and scarring of exposed areas may lead to mutilating deformity. Symptoms may include: bullae, crusts, scar formation, sclerodermoid, hyper- and hypopigmentation, hypertrichosis, erythrodontia, haemolytic anaemia, splenomegaly.
- Urine ALA/PBG are not raised; urinary, faecal, and erythrocyte porphyrins are raised and are predominantly of the I isomeric form. Protection of the skin from sunlight is essential. Repeated blood transfusions can suppress erythropoiesis and may decrease porphyrin production, thus reducing photosensitivity.

Porphyria cutanea tarda (PCT)

- Two clinically indistinguishable types: Type I is sporadic and occurs as a result of inhibited or inactivated uroporphyrinogen decarboxylase (UROD) activity only in the liver due to: excessive hepatic iron, ethanol use, hepatitis C, HIV infection, or oestrogen administration. Type II is less common and inherited in an autosomal dominant fashion and results in systemic UROD deficiency.
- PCT type I is the most common form of porphyria and is estimated to occur at 1 in 25,000 people.
- Chronic blistering lesions (bullae) develop on sun-exposed areas of skin. Symptoms may include: skin fragility, crusts, sclerodermoid, hyper- and hypopigmentation, hypertrichosis.
- There are no neurological features.
- Urine ALA/PBG are not raised; urine and faecal porphyrins are raised, with a typical pattern on High Pressure Liquid Chromatography profiling.

Hereditary coproporphyria (HCP)

- Inherited in an autosomal dominant fashion.
- Prevalence is less than 1 in 250,000 people.
- HCP symptoms may include those of AIP and PCT. HCP is precipitated by the same factors as AIP.

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- As for other acute porphyrias, urine ALA and PBG are increased in the acute state and may be slightly elevated or normal in the latent state. The most predominant finding is the increased urinary and faecal coproporphyrin III, which may be slightly raised in the latent state.

Variegate porphyria (VP) or South African type

- Inherited in an autosomal dominant fashion.
- Especially common in South African whites (approximately 3 per 1000) and can be traced to a common founding couple who emigrated from Holland in the seventeenth century. As many as 20,000 South Africans may carry this trait due to a founder effect.
- The disease is termed variegate because it can present with a variety of symptoms, these may be neurological and or photosensitivity, and are the same as those seen in AIP and PCT. Provoking factors are similar to those of AIP.
- As for all the acute porphyrias, ALA and PBG are increased in the acute presentation and may be slightly elevated or normal in the latent state. Urine and faecal porphyrins are increased, mainly as coproprophyrin III, with a large amount of protoporhyrin IX in the faeces. Plasma has a specific fluorescence peak at 626 nm.

Erythropoietic protoporphyria (EPP) or protoporphyria

- Inherited in an autosomal dominant fashion.
- Prevalence is 1 in 250,000 people, with no racial predilection.[6]
- The major clinical features of EPP are cutaneous photosensitivity, which begins in childhood, with sun-exposed areas developing itching and swelling within minutes of sun exposure, often mimicking very severe sunburn. Symptoms may include: burning sensation, oedema, erythema, itching, scarring, vesicles.
- Protoporphyrin accumulates primarily in erythroid cells in the bone marrow, and appears in excess in plasma, circulating erythrocytes, bile, and faeces. Plasma has a specific fluorescence peak at 632 nm.
- Zinc oxide cream and avoidance of sunlight are essential.[7]

Laboratory investigations

Specimen requirements

Specimens should be protected from light and received by the laboratory within 24 hours.

Urine—Fresh random urine is preferred to 24-hour collections, and is ideally collected during an acute episode if acute porphyria is suspected.

Faeces—A random 10 g sample of faeces is required.

Blood—Whole blood (EDTA or heparinised).

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Laboratory tests

Urine porphobilinogen (PBG) screen[8]—PBG is raised in patients with acute attacks of hepatic porphyrias AIP, VP, and HCP. It is not raised in PCT or the erythropoietic porphyrias. Absence of PBG in the urine collected when a patient has abdominal pain excludes porphyria as a cause of abdominal pain. PBG, however, may disappear from the urine between acute attacks (latent phase).

All patients undergoing porphyria testing presenting with abdominal pain should also be questioned with regard to their previous (and family) history of skin and neurological symptoms.

Urine total porphyrins—Concentrations will be increased in patients with current symptoms of PCT, VP, HCP, AIP, and CEP.[9]

Faecal total porphyrins—Faecal porphyrin concentrations are increased in hepatic porphyrias (except AIP), EPP, gastrointestinal bleeding, and very high meat diets.[9]

High pressure liquid chromatography (HPLC) profiling—When there is a elevated excretion in urine or faeces, HPLC separation of the isoforms must be undertaken to differentiate the type of porphyria. The specific pattern of the different porphyrins (in both the urine and faeces) is diagnostic of the type of porphyria, and it is not usually necessary to quantitate the individual porphyrins.

Blood porphyrins

Plasma

The plasma of patients with overt and latent VP contains a porphyrin protein complex (thought to be a porphyrin molecule attached to a short amino acid chain) that has a specific fluorescence emission peak for the excitation wavelength of 405 nm.[10] An emission peak at 626 supports VP and one at 632 supports EPP.[11] An emission peak at 619 nm may occur with AIP or PCT, although it is frequently non-specific.[3]

Whole blood

Red blood cell porphyrin levels are raised in EPP, CEP, HEP, VP, lead poisoning, iron deficiency, and anaemia. In EPP and CEP, there is an excess production and accumulation of free protoporphyrin. Lead poisoning, which can mimic a porphyria, may also present with abdominal pain and increased urine porphyrins. The ferrochelatase activity is inhibited and results in the formation of zinc protoporphyrin. This is also produced in iron deficiency anaemia as a result of the lack of available iron.

Red cell enzyme levels

The measurement of the enzymes of the haem biosynthesis are rarely essential for the accurate diagnosis of overt porphyria. Enzyme assays, however, are useful for the detection of latent porphyria in family members of an index case, especially where the family members have either no symptoms, or are pre-puberty and often thus biochemically normal. In particular, it is useful to measure PBG-deaminase for the diagnosis of AIP (90% of AIP patients have mutations that decrease red cell PBG-

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deaminase activity, with some overlap of enzyme activity between the normal and affected population).

Mutation analysis

Similar to the blood enzyme studies, mutational analysis can be used to confirm clinical/biochemical diagnosis or for family studies. For VP, there is the well studied *p.R59W* founder mutation in South Africa. There are also founder mutations in Finish (*p.R152C*) and Chilean (*c.1241_1245del*) populations, as well as 60 novel mutations identified in a recent study of 104 unrelated patients from Western Europe.[12] For suspected latent AIP and VP, mutation analysis offers a means of making a definitive diagnosis in families of an index case, where the gene defect is known.[13,14] Mutation analysis for AIP and VP is now readily available through our laboratory.

Clinical and biochemical studies of specific porphyrias

Variegate porphyria

Case 1 (atypical presentation)—A South Africa-born 52-year-old female presented with severe migraine to an emergency department, she was admitted for observation overnight. The next day her migraine had improved and she was discharged into her GP's care, no diagnosis of cause was made. Her GP, also South African, considered VP as a possible diagnosis. High levels of urinary PBG, ALA, and total porphyrins along with a specific plasma fluorescence peak indicated acute VP, and this was confirmed by detection of the *p.R59W* mutation. Follow-up found that this acute episode occurred several days post-anaesthetic for dental surgery. Her mother and two sisters had been previously diagnosed in South Africa. Mutation analysis was performed for her two symptom-free daughters (aged 17 and 20). and one daughter was found to be heterozygous for the *p.R59W* mutation. Neither daughter had the characteristic plasma fluorescence peak.

Case 2 (typical presentation)—A 48-year-old male presented with abdominal pain and constipation of 7 days' duration. The pain was colicky, central, and associated with anorexia, bloating, and vomiting. One month earlier, he had been started on phenytoin following his first generalised seizure, and after this developed insomnia, anxiety and urethral discharge. Infection, vasculitis, and thromboembolism were excluded. The patient's wife noticed that the urine in the 24-hour collection bottle darkened on standing. Laboratory tests identified raised urinary PBG and total porphyrins, raised faecal porphyrins with a low CI:CIII ratio on HPLC, and a specific plasma fluorescence peak—thus indicating acute VP. Family studies were offered to his three symptom-free sons (aged 14, 19, and 21). The father and eldest son were found to be heterozygous for the *c.461_483del* mutation of the PPOX gene. The eldest son also had raised total faecal porphyrins and an abnormal plasma fluorescence peak.[15]

Case 3 (atypical presentation)—A 25-year-old female of Pacific Island descent was referred to a dermatologist with blistering lesions on sun exposed skin. Raised urinary PBG, ALA, and total porphyrins; raised faecal porphyrins; and a specific fluorescence peak indicated acute VP. Family studies were offered to the extended family, some of whom had been assigned a diagnosis of PCT (probably erroneously without biochemical porphyrin studies), although this offer was not accepted.

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Discussion—While VP is a relatively common type of porphyria in South Africa, there has been a significant recent increase in cases in our local experience. This can, in part, be attributed to the significant increase in the New Zealand population of South African immigrants; 14,727 new immigrants between 1996 and 2001, a 130% increase, (Census 2001 statistics). Other *non-p.R59W* cases have also been diagnosed; this has mainly been through the use of the plasma scanning technique on all samples with slightly raised erythrocyte porphyrins; where in some cases the more traditional screening tests have been normal.

Some groups have found this plasma scanning technique to be 100% specific, with a sensitivity of 86% (95% confidence interval: 71-98%) for asymptomatic adults with VP[11]. In contrast, the sensitivity of faecal porphyrin analysis as a test for adult gene carriers was 36%. Plasma porphyrin analysis has been suggested as an adequate substitute for faecal porphyrin measurement as a front line test, and is particularly useful for distinguishing VP from other bullous porphyrias, of which PCT is by far the most common.[16] Neither test is as sensitive in children, where if the mutation can be detected in an index case, DNA analysis remains the preferred diagnostic tool.[4]

Erythropoietic protoporphyria

Case 1 (typical presentation)—A 5-year-old male presented with sudden severe photosensitivity on sun-exposed skin. Symptoms included itching, painful erythema, and swelling. Urinary PBG and total porphyrins were normal; erythrocyte protoporphyrins were raised at 81 μg/100 mL; a specific plasma fluorescence peak at 632 nm indicated acute EPP. Two siblings were diagnosed with EPP on the grounds of their raised erythrocyte porphyrins, plasma fluorescence peak, and reduced ferrochelatase activity. The mother did not have a history of photosensitivity, which was consistent with her normal red cell and plasma porphyrins (despite a very low ferrochelatase activity).

It has been reported that clinical expression of EPP requires a defective ferrochelatase (FECH) gene on one allele and a common low expression FECH variant on the other. Thus they are compound heterozygotes for a mutation and a polymorphism.[17] Her husband was found to carry the commonly occurring low-expression FECH polymorphism, thus explaining his wife's lack of symptoms, and the clinical expression of EPP in their child.

Case 2 (atypical presentation)—An 80-year-old female with history of photosensitivity and ulceration; according to he physician she had 'always had porphyria.' Normal urinary PBG and total porphyrins, a specific plasma fluorescence peak, and raised erythrocyte porphyrins indicated EPP. Family studies were offered to the extended family, but were not accepted.

Discussion—In both these cases, traditional (urine and faecal porphyrins) screening strategies would have missed the diagnosis of EPP. While erythrocyte porphyrins are raised, this test is not specific, as they can be raised in conditions such as; high blood lead levels, chronic infection, malignancy, or iron deficiency anaemia.[18] However, by following up raised levels with plasma fluorescence, a clear diagnosis can be achieved. This characteristic fluorescent peak at 632±2 nm, has been found to be particularly useful for the diagnosis and screening for this type of porphyria. Plasma porphyrins may be increased in conditions where porphyrin excretion is impaired,

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such as renal failure and cholestasis, but the specific peak at 632±2 nm has only been reported in association with EPP.[9,16] Furthermore, the identification of the two polymorphic variant sequences associated with this low expression allele now enables improved predictive counselling for couples where one partner has EPP.[19]

Conclusion

The porphyrias are relatively uncommon disorders that require specialised investigation for precise diagnosis. Every effort must be made to establish an accurate diagnosis during presenting illness—as retrospective diagnosis may be difficult. All laboratories should be able to identify acute porphyria, with ready access to urine PBG testing. Confirmation of the type of porphyria will require more specialised assays including HPLC profiling, plasma fluorescence scanning, and mutational analysis. For definitive investigation and diagnosis, a full set of blood, urine, and faecal samples should be sent to a reference laboratory.

Although PCT is the most common type of porphyria in New Zealand, the other less common types (especially AIP, VP, and EPP) must be considered when the appropriate clinical symptoms are present. Additionally, with the recent increased immigration rate from a country with a high known prevalence of VP (South Africa), there is likely to be an increased rate of presentation here in New Zealand.

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