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Acute Intermittent Porphyria: Diagnostic Conundrums

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Summary: Acute intermittent porphyria is a genetic disorder of haem biosynthesis caused by defects in the gene encoding hydroxymethylbilane synthase on the long arm of chromosome 11. Every effort should be made to identify gene carriers amongst the relatives of patients known to have acute intermittent porphyria as they are at risk of developing potentially fatal neurogenic attacks if exposed to precipitating factors. Erythrocyte hydroxymethylbilane synthase activity was determined in 46 members of two large well characterised families by assaying enzyme activity by both high performance liquid chromatography (HPLC) and fluorimetric assays. Additionally, hydroxymethylbilane synthase immunoreactivity was determined by a sandwich-type ELISA. Statistically significant correlations were observed between erythrocyte hydroxymethylbilane synthase activity assayed by HPLC and by the fluorimetric assay, and enzyme protein concentration ($r = 0.85$, $p < 0.001$ and $r = 0.80$, $p < 0.001$, respectively). The assay of hydroxymethylbilane synthase immunoreactive concentration in erythrocytes was useful in excluding acute intermittent porphyria in one patient in whom unequivocal assignment of porphyric status was not possible by assaying enzyme activity alone. Erythrocyte hydroxymethylbilane synthase activity assayed by HPLC and fluorimetry showed approximately equal diagnostic performances, both giving rise to a dichotomic distribution of values, with overlap zones of 6% (1/16) and 22% (2/9), respectively, at the "cut off" applied.

Introduction

Acute intermittent porphyria is an autosomal dominant disorder of haem biosynthesis in which over-production of haem precursors is associated with characteristic clinical features and a potentially fatal neuropsychiatric syndrome (1, 2). Acute intermittent porphyria is caused by defects in the gene encoding hydroxymethylbilane synthase (EC 4.3.1.8),[1]) also known as porphobilinogen deaminase (3). Hydroxymethylbilane synthase is a cytosolic enzyme and has reduced activity in abnormal gene carriers (2, 4).

It is important to investigate asymptomatic individuals for latent or subclinical porphyria and every effort should be made to identify gene carriers amongst the relatives of patients known to have acute intermittent porphyria (5). Normal haem precursors usually occur in asymptomatic gene carriers. Furthermore, normal porphyrin excretion may occur in known patients with acute intermittent porphyria during the remission periods (6). Low erythrocyte hydroxymethylbilane synthase activity is specifically diagnostic of acute intermittent porphyria (2, 7) and remains the most effective method for detecting gene carriers (8, 9). Nevertheless, unequivocal assignment confirming or refuting the diagnosis of acute intermittent porphyria cannot be achieved among some relatives of a known patient with acute intermittent porphyria due to the overlap in enzyme activities between affected patients and normal subjects (8–10).

Molecular heterogeneity in acute intermittent porphyria (11) and the large number of point mutations (12–14)

[1]) Enzyme:
Hydroxymethylbilane synthase also known as porphobilinogen deaminase (EC 4.3.1.8)

detected so far, make genetic studies in affected families an impractical option at the present time. None of the mutations so far identified has been found to occur more frequently than any of the others, and many of these mutations appear to be unique to one family. Additionally, all polymorphic sites are clustered within a small fragment (1.5×10^3 bases in the first intron) of the hydroxymethylbilane synthase gene and thus limiting the usefulness of restriction fragment length polymorphisms in identifying and differentiating the mutant allele from the normal one (15). However, restriction fragment length polymorphisms have been successfully used for haplotype analysis and heterozygote carrier detection in specific families with acute intermittent porphyria (16, 17).

In this study, the level of hydroxymethylbilane synthase gene expression in erythrocytes was determined in two large well characterised families by assaying hydroxymethylbilane synthase activity by both high performance liquid chromatography (HPLC) of the reaction products (18), and by a fluorimetric assay (19). In addition, immunoreactive enzyme was assayed by ELISA (20). The aim of this study was to assess the performance of assays measuring hydroxymethylbilane synthase activity, to document the degree of overlap in enzyme activities between gene carriers and normal subjects using different methods, and secondly, to investigate whether determination of hydroxymethylbilane synthase immunoreactivity and specific enzyme activities have a better diagnostic performance in individuals with equivocal hydroxymethylbilane synthase activities.

Patients and Methods

Patient details

We studied 46 members from two large well characterised families: 33 members aged 2 to 70 years (mean ± SEM, 34.5 ± 3.4 years) (19 females and 14 males) were investigated from family 1 (fig. 1), and 13 individuals aged 5 to 50 years (mean ± SEM, 28.2 ± 4.4 years) (7 females and 6 males) from family 2 (fig. 2) were studied.

Urinary and faecal porphyrin quantification

Urinary and faecal porphyrins were identified and quantified by high performance liquid chromatography (HPLC) according to the method of *Lim & Peters* (21). If acute porphyria was suspected, urinary 5-aminolaevulinic acid and porphobilinogen excretion were quantified after prior purification by anion-exchange chromatography (22).

Erythrocyte hydroxymethylbilane synthase activity

Hydroxymethylbilane synthase activity was assayed by the HPLC method of *Wright & Lim* (18) using fresh heparinised erythrocytes. The enzyme activity was expressed as uroporphyrin, µmol/h · l erythrocytes. The between-run coefficient of variation was 8% at a level of 25 uroporphyrin, µmol/h · l erythrocytes. Hydroxymethylbilane synthase activity was also determined by the fluorimetric assay of *Magnussen* et al. (19) with the assay of haemoglobin in the haemolysate (23). Enzyme activity was related to haemoglobin concentration and expressed as pkat/g Hb. The between-run coefficients of variation of the fluorimetric assay were 2.1% and 5.4% for healthy and porphyric subjects, respectively. The lower reference limit applied was 90 pkat/g Hb, representing a diagnostic sensitivity of 97% and a specificity of 85%. Non-gene carriers do not exhibit erythrocyte hydroxymethylbilane synthase activities below 70 pkat/g Hb, a value representing a "cut off" limit giving a sensitivity of 100%, but a low specificity of 47% (24).

Erythrocyte hydroxymethylbilane synthase concentration

Hydroxymethylbilane synthase concentration was assayed by a sandwich-type ELISA with monospecific polyclonal antiserum (IgG) raised against hydroxymethylbilane synthase, as previously described (20). Haemoglobin concentration (absorbance read at 410 nm) of the samples was determined and hydroxymethylbilane synthase concentration was expressed relative to haemoglobin concentration as µg/g Hb. Erythrocytes hydroxymethylbilane synthase specific activity was determined by dividing enzyme activity with enzyme protein concentration, and expressed as nkat/g enzyme protein (20). A lower reference limit of 110 µg/g Hb was used. This "cut off" value gives a diagnostic sensitivity of 85% and specificity of 85% (24).

Further blood analyses

All patients had assays for full blood count, white cell differential and subset assays. Biochemical profile including renal and liver function and toxicity tests was carried-out on the DAX-48 multichannel analyser (Bayer Diagnostics, Basingstoke, UK) by standard methods.

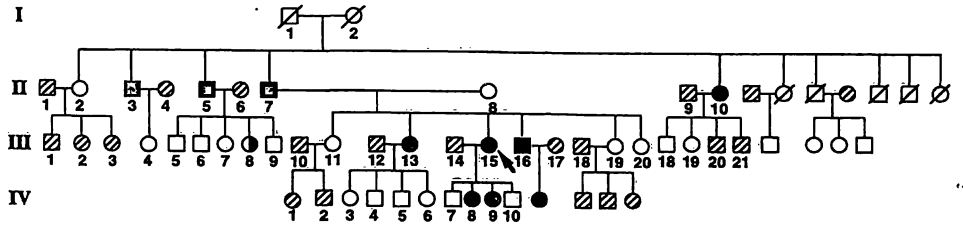

Fig. 1 Pedigree of family 1 showing autosomal dominant inheritance of acute intermittent porphyria. Affected individuals are represented by black symbols (■ ●), unaffected individuals are depicted as open symbols (□ ○), while members who were not investigated are represented by hatched symbols (▨ ⊘). Patient III:8, in whom definitive porphyric status was not possible, is represented by half-filled symbol (◑). ↖ indicates propositus.

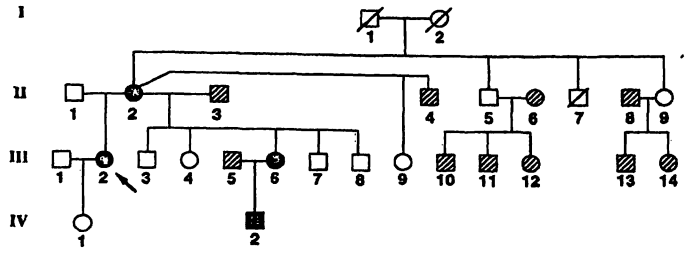

Fig. 2 Pedigree of family 2 showing autosomal dominant inheritance of acute intermittent porphyria. Affected individuals are represented by black symbols (■ ●), unaffected individuals are depicted as open symbols (□ ○), while members who were not investigated are represented by hatched symbols (▨ ⊘). ↖ indicates propositus.

Results

In the two families the propositus (patient III:15, fig. 1; patient III:2, fig. 2) presented at the age of 34 and 27 years, respectively, with a history of dieting prior to hospital admission. Both index patients were admitted to hospital with severe abdominal pain, hypertension, vomiting and constipation and had elevated urinary excretion of 5-aminolaevulinic acid, porphobilinogen, raised urinary porphyrin and normal faecal porphyrin (tab. 1). The decreased red cell hydroxymethylbilane synthase activity in both patients (assayed by HPLC, tab. 1) was consistent with the diagnosis of acute intermittent porphyria.

Assay of erythrocyte hydroxymethylbilane synthase activity (by the HPLC technique) in 33 members of family 1 (fig. 1) identified 11 (33%) individuals as gene carriers. Twenty one family members did not inherit the gene defect. In one individual (patient III:8, fig. 1) unequivocal assignment confirming or refuting the diagnosis of acute intermittent porphyria was not possible by assay of hydroxymethylbilane synthase activity alone due to the overlap in enzyme activities between affected patients and normal subjects (8–10). Of 13 members of family 2 (fig. 2) 4 (31%) patients were identified as gene carriers. There was no overlap in enzyme activity in family 2 and unequivocal assignment excluding acute intermittent porphyria was possible in the other 9 family members. All gene carriers in both families, with the exception of the two index patients, had urinary and faecal porphyrins and urinary 5-aminolaevulinic acid and porphobilinogen within the reference ranges. Of 15 gene carriers, only 2 (13%) patients (patient III:15, fig. 1; patient III:2, fig. 2) developed attacks of acute intermittent porphyria.

To characterize further the defect in acute intermittent porphyria, and establish a definitive diagnosis in the subjects with equivocal hydroxymethylbilane synthase activity, 22 members (11 from each family) were subjected to further investigations including the measurement of hydroxymethylbilane synthase immunoreactive

Tab. 1 Urinary 5-aminolaevulinic acid and porphobilinogen excretion, urinary and faecal porphyrins pattern and erythrocyte hydroxymethylbilane synthase activity in the two index patients. Hydroxymethylbilane synthase activity was assayed by the method of *Wright & Lim* (18).

	Patient III:15 (fig. 1)	Patient III:2 (fig. 2)	Reference ranges
Urine			
5-Aminolaevulinic acid	161	356	<34 µmol/l
Porphobilinogen	279	261	<8 µmol/l
Uroporphyrin I	450	2360	
Uroporphyrin III	780	2240	
Total uroporphyrin (I + III)	1230	4600	<24 nmol/l
Heptacarboxylate porphyrin	34	ND	<4 nmol/l
Hexacarboxylate porphyrin	14	ND	<3 nmol/l
Pentacarboxylate porphyrin	147	ND	<5 nmol/l
Coproporphyrin I	65	150	
Coproporphyrin III	113	131	
Total coproporphyrin (I + III)	178	281	<115 nmol/l
Faeces			
Coproporphyrin I	19	21	
Coproporphyrin III	9	9	
Total coproporphyrin (I + III)	28	30	<46 nmol/g dry stool
Protoporphyrin IX	77	32	<134 nmol/g dry stool
Blood			
Erythrocyte hydroxymethylbilane synthase activity	16	13	uroporphyrin, 20–42 µmol/h · l red cells

concentration. Additionally, enzyme activity was measured by the independent fluorimetric assay described by *Magnussen* et al. (19) and specific enzyme activity was determined. Of the 22 subjects 8 had been classified as gene carriers by the HPLC method, 13 were normal and not at risk of acute intermittent porphyria, and in one member porphyric status was questionable as erythrocyte hydroxymethylbilane synthase activity was equivocal as shown in table 2. Seven out of eight individuals diagnosed as gene carriers by the HPLC method were identified as such by the fluorimetric assay. One individual given the diagnosis of acute intermittent porphyria by the HPLC method, exhibited a value just above the reference value applied when erythrocyte hydroxymethylbilane synthase activity was assayed by the fluorimetric assay, as did the individual exhibiting equivocal result in the HPLC assay. Thus both assays show similar diagnostic efficiency and give rise to a relatively clear-cut dichotomic distribution of gene carriers and non-carriers (fig. 3).

It is clear from table 2 that all gene carriers had hydroxymethylbilane synthase concentrations below 110 µg/g Hb. The measurement of hydroxymethylbilane synthase concentration in erythrocytes was useful in excluding acute intermittent porphyria in patient III:8 (family 1, fig. 1, tab. 2) who had equivocal enzyme activity using the HPLC assay but had hydroxymethylbilane synthase concentration of 125 µg/g Hb. However, there was one overlap in hydroxymethylbilane synthase concentration in a normal subject (subject III:8, family 2, fig. 2, tab. 2) who did not inherit the gene defect but had hydroxymethylbilane synthase concentration of 90 µg/g Hb. As shown in table 2, hydroxymethylbilane synthase specific actiivty was not helpful in identifying patients at risk of acute intermittent porphyria and there was no clear separation in enzyme specific activity between gene carriers and normal subjects. Indeed, there was no correlation between hydroxymethylbilane synthase specific activity and both enzyme activity and enzyme concentration ($r = 0.42$, $p = 0.06$ and $r = 0.14$, $p = 0.52$, respec-

Tab. 2 Erythrocyte hydroxymethylbilane synthase activity, concentration and specific activity in the two families with acute intermittent porphyria.

	Age (years)	Gender	Gene carrier	Hydroxymethylbilane synthase activity (*Wright & Lim*, 1983) (uroporphyrin, µmol/h · l red cells)	Hydroxymethylbilane synthase activity (*Magnussen* et al., 1974) (pkat/g Hb)	Hydroxymethylbilane concentration (µg/g Hb)	Hydroxymethylbilane synthase specific activity (nkat/g)
Family 1 (fig. 1)							
Patient II:5	63	♂	Yes	15	72	89	806
Patient II:7	64	♂	Yes	11	87	106	823
Patient III:13	38	♀	Yes	16	79	99	801
Patient III:15*	36	♀	Yes	16	86	104	822
Patient III:16	34	♂	Yes	9	87	100	866
Patient II:8	58	♀	No	25	131	151	865
Patient III:7	37	♀	No	28	142	181	782
Patient III:11	40	♀	No	23	117	151	776
Patient III:19	31	♀	No	28	137	157	872
Patient III:20	21	♀	No	24	156	168	930
Patient III:8	39	♀	Possibly	21	96	125	786
Family 2 (fig. 2)							
Patient III:2*	28	♀	Yes	13	65	92	708
Patient III:6	24	♀	Yes	18	91	97	944
Patient IV:2	5	♂	Yes	16	54	69	782
Patient II:5	43	♂	No	36	138	148	933
Patient II:9	49	♀	No	38	183	209	874
Patient III:3	26	♂	No	25	130	145	894
Patient III:4	28	♀	No	24	113	125	905
Patient III:7	14	♂	No	29	123	127	966
Patient III:8	10	♂	No	23	95	90	1050
Patient III:9	30	♀	No	28	145	155	934
Patient IV:1	10	♀	No	24	95	118	808
Reference Range				20–42	>90	>110	

* indicates propositus

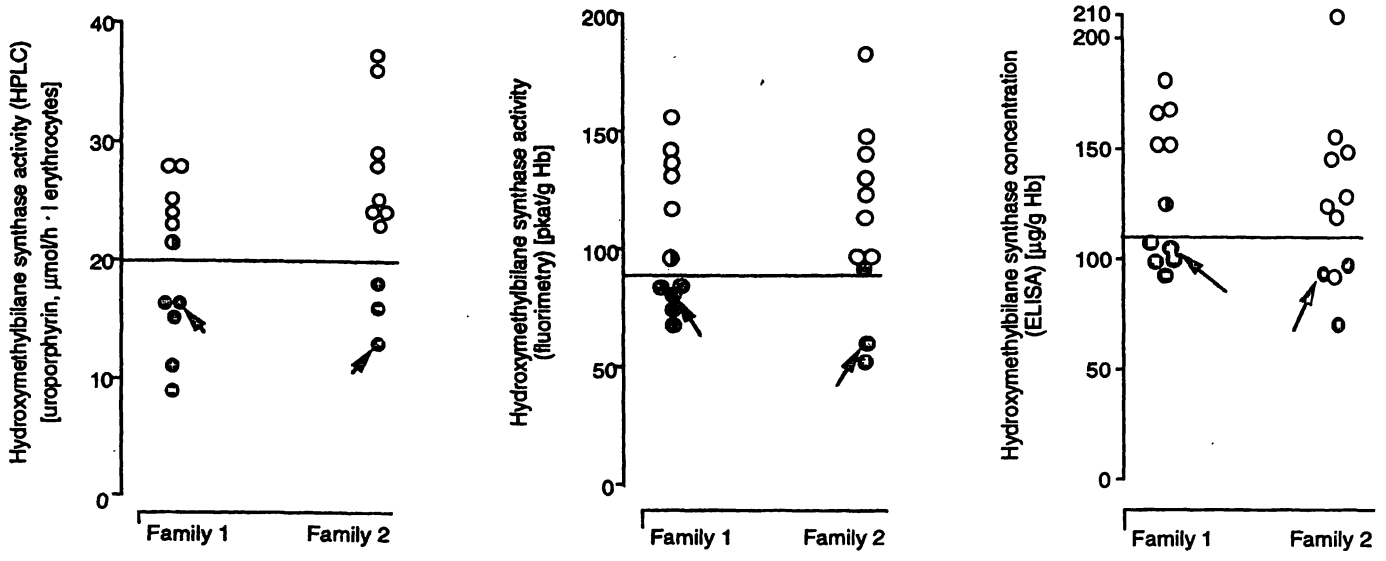

Fig. 3 Erythrocyte hydroxymethylbilane synthase activity and concentration in two families with acute intermittent porphyria. Hydroxymethylbilane synthase activities were determined by the HPLC method of *Wright & Lim*[18] and the fluorimetric method of *Magnussen* et al.[19]. Carrier status was arbitrarily assigned using the HPLC assay data. The lower limits of the reference ranges of various assays are represented with solid lines. Gene carriers (●), non carriers (○) and equivocal status (◑). ↗ indicates propositus.

tively). In contrast, statistically significant correlations were observed between erythrocyte hydroxymethylbilane synthase activity assayed by the method of *Wright & Lim* and enzyme activity determined by the method of *Magnussen* et al., and enzyme concentration ($r = 0.85$, $p < 0.001$ and $r = 0.80$, $p < 0.001$ respectively, $n = 22$). A highly significant correlation was also observed between erythrocyte hydroxymethylbilane synthase activity assayed according to the method of *Magnussen* et al. and hydroxymethylbilane synthase concentration ($r = 0.96$, $p < 0.001$, $n = 22$).

All members investigated from both families had normal biochemical profiles and haematological indices except 2 gene carriers from family 2 (patients III:6 and IV:2, fig. 2) who had iron deficiency anaemia and a repeat hydroxymethylbilane synthase activity was performed after iron replacement therapy.

Discussion

The elevated urinary excretion of 5-aminolaevulinic acid and porphobilinogen in both index patients is consistent with the diagnosis of one of the acute porphyrias (i. e., acute intermittent porphyria, variegate porphyria and hereditary coproporphyria). The raised urinary porphyrin content (tab. 1) has probably arisen by non-enzymic polymerization of porphobilinogen in the urine to form a mixture of uroporphyrin isomers. The normal faecal porphyrin excretion in both patients indicates that variegate porphyria and hereditary coproporphyria are highly unlikely, whereas the decreased red cell hydroxymethylbilane synthase activity (tab. 1) is consistent with acute intermittent porphyria.

The dominant mode of inheritance, the occurrence of asymptomatic gene carriers and the risk of developing potentially fatal attacks if exposed to a wide range of common precipitating factors, make it essential to exclude or confirm the diagnosis of acute intermittent porphyria in all relatives whenever the diagnosis has been made in one member of the family (2, 7). Patients with acute intermittent porphyria are at risk of developing potentially fatal neurogenic attacks if exposed to exogenous precipitating factors including a wide range of commonly prescribed drugs (25), alcohol, fasting, stress, hormones (2, 26) and, interestingly, if they continue to smoke (27). In our study, the index patients in both families had acute attacks of porphyria following low calorie diet for weight reduction.

In our hands the HPLC and fluorimetric assays for erythrocyte hydroxymethylbilane synthase activity showed about the same diagnostic efficiency at the "cut off" levels applied, 8 and 7 individuals, respectively, diagnosed as gene carriers. However, it is clear that, whichever assay is used, there was a small overlap between enzyme activities in normal subjects and gene carriers in our study as in previous studies (8–10). It is well established that a definitive assignment of porphyric status cannot always be made in all relatives, even when porphobilinogen excretion, 5-aminolaevulinic acid synthase activity (28) and gene dosage effect (9) are taken into account. *Lamon* et al. demonstrated that pedigree analysis with respect to the frequency distribution

of hydroxymethylbilane synthase enzyme activities in family members was successful in establishing porphyric status in 50% of those subjects with equivocal enzyme activity (9). It is noteworthy that the existence of a variant of acute intermittent porphyria has been reported where enzyme defect is not expressed in erythrocytes (29), in such families the measurement of erythrocyte hydroxymethylbilane synthase activity is unhelpful.

In this study, the measurement of erythrocyte hydroxymethylbilane synthase immunoreactive concentration was useful in excluding the diagnosis of acute intermittent porphyria in one member who had equivocal hydroxymethylbilane synthase activity. The estimation of enzyme specific activity was not useful in identifying gene carriers with no clear separation between normal subjects and gene carriers. This is hardly surprising since there is a good correlation between hydroxymethylbilane synthase activity and enzyme protein concentration. This indicates that in these two families there is either no gene product or that the protein produced is neither catalytically active nor recognised by the anti-serum used in the ELISA, i. e., we are dealing with a form of acute intermittent porphyria in which no cross reacting immunoreactive material (CRIM) is produced (CRIM negative). Since hydroxymethylbilane synthase activity decreases as red cells age any shift in their age distribution will be reflected in the enzyme activity (5), a repeat of hydroxymethylbilane synthase activity was essential in the two suspected gene carriers after the correction of the haematological abnormalities.

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