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Genetic heterogeneity in acute intermittent porphyria: characterisation and frequency of porphobilinogen deaminase mutations in Finland

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Abstract

The occurrence of different porphobilinogen deaminase mutant types in 68 patients with acute intermittent porphyria from 33 unrelated families in Finland was studied with biochemical and immunological techniques. In this fairly homogenous population four different porphobilinogen deaminase mutant types were identified and their frequencies determined. Most (about 80%) of the mutations were cross reacting immunological material (CRIM) negative, including a large kindred with normal erythrocyte porphobilinogen deaminase activities. The remainder of the families had CRIM positive mutations, including an unusual type (type 2) that had an immunoreactive, non-catalytic porphobilinogen deaminase level considerably greater than the maximal theoretical ratio of CRIM to activity of 2.0 for a single mutant allele.

Correlations of the amount of residual porphobilinogen deaminase activity and the occurrence of acute clinical manifestations in each mutant type suggested that CRIM positive type 2 patients may have fewer acute symptoms.

Introduction

Acute intermittent porphyria is a dominantly inherited, inborn error of metabolism that results from half normal activity of

the third enzyme in the haem biosynthetic pathway, porphobilinogen deaminase, and the accumulation of the porphyrin precursors, δ -aminolevulinic acid and porphobilinogen (fig 1).¹⁻⁵ The clinical onset of the disease usually occurs during or after puberty and is characterised by intermittent attacks of neurological dysfunction, including abdominal pain and other gastrointestinal complaints, hypertension, tachycardia, and various peripheral and central nervous system manifestations. The acute attacks are precipitated by environmental factors—for example, certain drugs, various hormones and steroids, infections, and starvation—which induce hepatic δ -aminolevulinic acid synthase activity, resulting in the increased production and accumulation of δ -aminolevulinic acid and porphobilinogen and the precipitation of acute symptoms of disease. Normally the excess porphobilinogen would be converted by porphobilinogen deaminase into the porphyrins uroporphyrinogens I and III (fig 1).^{6,7} The half normal hepatic porphobilinogen deaminase activity in patients with acute intermittent porphyria, however, is insufficient to prevent the pathological accumulation of this porphyrin precursor.

Recent studies have focused attention on the nature of the enzymatic defect in this hepatic porphyria. The physicochemical properties of porphobilinogen deaminase in erythrocytes from patients with acute intermittent porphyria and normal subjects have been characterised,^{8,9} and the structural gene encoding the monomeric enzyme and the mutations of the disease has been localised to the distal long arm of chromosome 11.⁹ Anderson *et al* produced monospecific polyclonal antibodies against human porphobilinogen deaminase and used immunological techniques to characterise the defective enzyme in 22 unrelated families with acute intermittent porphyria.⁸ Two major classes of enzyme defects were identified from the amount of immunologically cross reacting enzyme protein (CRIM) in erythrocyte lysates of acute intermittent porphyria: one class (designated CRIM negative) in which the amount of CRIM was proportional to the enzymatic activity, and a second class (designated CRIM positive) in which non-catalytic, mutant enzyme protein was present. The CRIM positive patients had ratios of CRIM to activity of about 1.6, showing the presence of non-catalytic enzyme protein produced by the

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mutant porphobilinogen deaminase allele. A more recent study identified a second and unusual CRIM positive mutation in which the amount of non-catalytic, mutant enzyme protein was greater (ratio ≈ 5.6) than that expected for a single mutant allele (ratio = 2.0). Characterisation of this CRIM positive mutation showed that the mutant enzyme was stabilised by bound substrate, which protected it from intraerythrocytic proteolysis.¹⁰

In Finland over 150 patients with acute intermittent porphyria were identified in a nationwide survey of porphyrias,¹¹ and a large kindred with acute intermittent porphyria was discovered in which the erythrocyte porphobilinogen deaminase activity was normal.¹² Affected members in a family had the

mittent porphyria of Finnish and Lappish ancestry from 33 unrelated families, including affected subjects from the family with acute intermittent porphyria who had normal erythrocyte porphobilinogen deaminase activities.

Patients and methods

We studied 158 patients with acute intermittent porphyria from 33 unrelated families in Finland. This was 94% of all known families with acute intermittent porphyria in Finland. In all patients the clinical diagnosis of acute intermittent porphyria was documented by half normal porphobilinogen deaminase activities or evidence of

FIG 1—Current concept of human haem biosynthesis. At each step in the pathway the deficient activity of a haem biosynthetic enzyme (italics) results in the indicated porphyria.

typical clinical manifestations exhibited by patients with acute intermittent porphyria with half normal porphobilinogen deaminase activity and excreted large amounts of porphobilinogen and δ -aminolevulinic acid, especially during acute attacks. Studies of these patients showed normal erythrocyte δ -aminolevulinic dehydratase, uroporphyrinogen III cosynthase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase, and ferrochelatase activities. In addition, typical profiles of urinary and faecal porphyrins in acute intermittent porphyria were observed during remission and acute attacks as well as after a provocative oral loading test of δ -aminolevulinic acid.¹³

To investigate the nature of the enzymatic defect underlying acute intermittent porphyria in Finland biochemical and immunological studies were undertaken to characterise the defective porphobilinogen deaminase activity in affected subjects. Here we describe the occurrence of genetic heterogeneity among 68 well documented patients with acute inter-

increased urinary excretion of porphobilinogen and δ -aminolevulinic acid, or both. The prevalence of acute attacks that required admission to a hospital was determined from hospital records or personal interviews, or both. Pedigrees were constructed for each family; data from the nineteenth century were available for every family, and, in many, from the eighteenth century. No known relationship existed among the families. Of the 33 families, 31 were of Finnish, one of Skolt Lappish (a small inbred group of about 600 people in northern Finland), and one of mixed Finnish and Lappish ancestry. Thirty one of the families have been reported in an earlier survey on porphyrias in Finland.¹¹

For biochemical and immunological studies heparinised venous blood was obtained from 68 patients with acute intermittent porphyria from the 33 families when they were asymptomatic; all had normal packed cell volumes and reticulocyte counts. The separated erythrocytes were washed three times with saline buffered with phosphate and stored at -20°C for a maximum of three months before analysis. Erythrocyte porphobilinogen deaminase activity was determined in Helsinki by the method of Ford *et al*¹⁴ and in New York by the method of Anderson and Desnick.¹⁵ Protein concentration was determined

by the fluorescamine procedure.¹⁶ Urinary δ -aminolevulinic acid and porphobilinogen and faecal porphyrins were quantified as described previously.¹¹

Monospecific rabbit antihuman porphobilinogen deaminase IgG was prepared and used for quantitative rocket immunoelectrophoresis, immunotitration, and crossed immunoelectrophoresis as previously described.^{8,10} The antibody recognised equally each of the porphobilinogen deaminase intermediates in normal erythrocytes.

The relative amounts of porphobilinogen deaminase CRIM in erythrocyte lysates from each patient with acute intermittent porphyria and normal subjects were determined by subjecting equal amounts of enzyme activity to rocket immunoelectrophoresis.⁸ The ratio of CRIM to activity for porphobilinogen deaminase in normal erythrocytes was defined as 1.0—that is, the amount of immunologically detectable enzyme protein was directly proportional to the amount of enzyme activity applied. In CRIM positive patients with acute intermittent porphyria non-catalytic enzyme protein encoded by the mutant allele was immunologically detectable. If the non-catalytic mutant protein had normal intraerythrocytic stability then the maximal theoretical ratio of CRIM to activity would be 2.0, the normal and mutant alleles contributing equal amounts of enzyme protein but only the normal allele encoding active enzyme molecules. Analogously, CRIM negative patients had ratios of 1.0, indicating the absence of immunologically detectable mutant enzyme protein.

Results

Figure 2 shows the four major mutant types of porphobilinogen deaminase that were distinguished when equal amounts of erythrocyte lysate activity from the 68 patients with acute intermittent porphyria and from normal subjects were subjected to rocket immunoelectrophoresis. The amount of immunoreactive porphobilinogen deaminase

FIG 2—Rocket immunoelectrophoresis of erythrocyte porphobilinogen deaminase from the four mutant types of acute intermittent porphyria. Each well contained equal amounts of erythrocyte lysate activity, except as indicated. Wells were as follows: normal subjects (wells 1, 5, and 11), a CRIM negative type 1 patient (well 2), a CRIM negative type 2 patient (well 3), a CRIM positive type 1 patient (well 4), and a CRIM positive type 2 patient (well 6). Wells 7 and 8-10 contained the indicated dilutions of lysate activity from a normal subject and a CRIM positive type 2 patient respectively.

activity in 40 patients from 25 unrelated families (including the family of Skolt Lappish ancestry) was essentially identical with that observed for an equal amount of activity in lysates from normal subjects. Thus the mutant allele in these patients with acute intermittent porphyria did not produce an enzyme protein that was immunologically detectable (ratio of CRIM to activity=1.0). The enzyme defect in these families was therefore designated CRIM negative type 1. Characterisation of the erythrocyte enzyme in lysates from five affected members of a family with acute intermittent porphyria with normal erythrocyte porphobilinogen deaminase activity showed a ratio of CRIM to activity of 1.0, indicating that this porphobilinogen deaminase mutation also was CRIM negative (type 2).

Two types of CRIM positive porphobilinogen deaminase mutations were identified among the patients with acute intermittent porphyria. Ten affected subjects from four unrelated families had immunoreactive porphobilinogen deaminase levels that were almost two times greater (ratio of CRIM to activity about 1.6-1.8) than those observed in normal subjects (designated CRIM positive type 1). A second type, designated CRIM positive type 2, included 13 patients from three unrelated families with acute intermittent porphyria (one of

mixed Finnish and Lappish ancestry), who had ratios of CRIM to activity of about 4.9-6.0.

Immunotitration experiments confirmed the quantitative rocket immunoelectrophoretic studies. CRIM negative type 1 and 2 patients had immunotitration curves that were essentially identical with those obtained with the same amounts of porphobilinogen deaminase activity in erythrocyte lysates from normal subjects or with the purified normal enzyme. In CRIM positive patients the amount of antibody required to precipitate half of the erythrocyte activity was about 1.6-1.8 (type 1) or 4.9-6.0 (type 2) times that required to precipitate the same amount of normal lysate activity (data not shown).

FIG 3—Map of Finland showing origins of families of CRIM negative type 1 (●) and type 2 (▲) and CRIM positive type 1 (□) and type 2 patients (○). Dashed line shows Finnish boundaries before the second world war.

Within each family the data for rocket immunoelectrophoresis and immunotitration for affected relatives were essentially identical.

Table I shows that 25 of the 33 families with acute intermittent porphyria had porphobilinogen deaminase mutations that were classified as CRIM negative type 1, whereas the CRIM negative type 2 mutation was observed only in one large kindred, who had normal porphobilinogen deaminase activity. Of the remaining seven families, four were CRIM positive type 1 whereas three were CRIM positive type 2. Figure 3 shows the demographic origins of the 33 families with acute intermittent porphyria. The origins of the CRIM positive type 1 and 2 families were concentrated in northern and southern Finland, respectively, whereas those of the CRIM negative families were distributed throughout the country.

Efforts were made to detect biochemical or clinical features, or both, that further differentiated the four mutant types of acute intermittent porphyria identified by these studies. Table I shows

TABLE 1—Classification of the porphobilinogen deaminase mutations in 68 patients with acute intermittent porphyria from 33 families in Finland

Mutant type	No of patients	No of families	Mean (SD) enzymatic activity* (nmol/min/ml)
CRIM negative patients:			
Type 1 (half normal erythrocyte porphobilinogen deaminase activity)	40	25	1.43 (0.27)†
Type 2 (normal erythrocyte porphobilinogen deaminase activity)	5	1	2.33 (0.19) (NS)
CRIM positive patients:			
Type 1 (ratio of CRIM to activity = 1.6-1.8)	10	4	1.29 (0.28)†
Type 2 (ratio of CRIM to activity = 4.9-6.0)	13	3	1.64 (0.20)†
Normal subjects	35	35	2.50 (0.31)

CRIM = Cross reacting immunological material.

*Assayed by method of Ford *et al.*¹⁴†Compared with mean control values, these mean porphobilinogen deaminase activities in patients with acute intermittent porphyria were significant at $p < 0.001$.

that the mean porphobilinogen deaminase activities for CRIM negative type 1 and CRIM positive type 1 and 2 patients were similar, although there was a range of activities for each type (fig 4). The mean porphobilinogen deaminase activity for CRIM negative type 2 patients was similar to that for normal subjects.¹² Interestingly, the mean erythrocyte activity in the 13 CRIM positive type 2 patients was slightly higher than that observed for CRIM negative type 1 ($p < 0.01$; $t = 2.57$) and CRIM positive type 1 patients ($p < 0.0025$; $t = 3.54$).

Table II summarises the 24 hour urinary excretion of δ -aminolevulinic acid and porphobilinogen collected during an asymptomatic period from patients with each mutant type of acute intermittent porphyria. Although differences were observed in the mean urinary concentrations of these compounds, no significance was placed on these findings as they were highly variable as reflected by the large range for each mutant type of acute intermittent porphyria. Faecal coproporphyrin and protoporphyrin concentrations were within their respective normal ranges or only slightly increased with no distinction among the different mutant types of acute intermittent porphyria

FIG 4—Erythrocyte porphobilinogen deaminase activities in patients with the four porphobilinogen deaminase mutant types. (Bars denote means (SD).)

(data not shown). In addition, oral loading tests of δ -aminolevulinic acid were performed in two CRIM negative type 2 patients.^{13 17} The profiles of urinary and faecal excretion of δ -aminolevulinic acid, porphobilinogen, and porphyrins were similar to those observed previously in CRIM negative type 1 patients.^{11 17}

Table III shows the occurrence of acute attacks of abdominal pain, with or without neurological symptoms, determined for 158 affected subjects from 33 families with acute intermittent porphyria who were admitted to hospital. These data suggest that acute symptoms may occur less often in CRIM negative type 2 and CRIM positive type 2 patients.

TABLE II—Urinary excretion of porphobilinogen and δ -aminolevulinic acid in 62 patients with acute intermittent porphyria (aged ≥ 15) during an asymptomatic period of disease

Mutant type	No of cases	Mean (range) porphobilinogen (μ mol/24 h)	Mean (range) δ -aminolevulinic acid (μ mol/24 h)
CRIM negative type 1	37	210 (3.9-614)	117 (8.6-599)
CRIM negative type 2	5	87 (30-199)	56 (27-84)
CRIM positive type 1	7	143 (11-283)	97 (9.1-214)
CRIM positive type 2	13	49 (6.7-202)*	54 (15-200)*
Normal subjects	35	< 15	< 30

CRIM = Cross reacting immunological material.

*Values calculated for seven symptomatic CRIM positive type 2 patients during an asymptomatic period were 83 (8.4-202) and 78 (15-200) μ mol/24 h, respectively.

TABLE III—Occurrence of acute attacks in 158 patients in 33 families with acute intermittent porphyria

Mutant type	Total No of patients investigated	% of patients who had had acute attacks
CRIM negative type 1	114	51
CRIM negative type 2	12	33
CRIM positive type 1	16	50
CRIM positive type 2	16	25
Total	158	47

CRIM = Cross reacting immunological material.

Discussion

Four different types of porphobilinogen deaminase mutations were identified by immunological and enzymatic characterisation of the erythrocyte enzyme in patients with acute intermittent porphyria from 33 unrelated families in Finland. These studies showed two CRIM positive¹⁰ and two CRIM negative mutations. The CRIM negative mutations were subclassified into two types based on the presence of half normal or normal porphobilinogen deaminase activity in erythrocytes from affected subjects. Within families the enzymatic and immunological properties of erythrocyte porphobilinogen deaminase in each affected relative were the same, consistent with the existence of at least four different, dominantly inherited mutations and the occurrence of genetic heterogeneity at the locus for porphobilinogen deaminase.

Almost 80% of the families studied (including the Skolt Lappish family) were CRIM negative, which indicated that non-catalytic porphobilinogen deaminase protein encoded by the mutant allele was not immunologically detectable in the erythrocyte lysates. The possible molecular lesions responsible for CRIM negative mutations include complete or partial gene deletions as well as point mutations or insertions that alter the processing or stability of mRNA, create chain terminating codons, or appreciably alter the conformation or stability of the mutant protein. The CRIM negative mutations are probably heterogeneous at the molecular level, and therefore further characterisation will require the use of appropriate cDNA and genomic probes for the porphobilinogen deaminase structural gene analogous to the elucidation of the molecular defects in the β^0 -thalassaemias.¹⁸

The previously reported family with acute intermittent porphyria with normal erythrocyte porphobilinogen deaminase activity was also classified as CRIM negative (ratio of CRIM to activity=1.0). The clinical manifestations as well as the urinary and faecal excretion of haem precursors and porphyrins in affected members of this family were similar to those observed in patients with acute intermittent porphyria with half normal porphobilinogen deaminase activity. In addition, the results of a provocative oral loading test of δ -aminolevulinic acid were consistent with a defect in porphobilinogen metabolism.¹³⁻¹⁷ Moreover, the activities of five other haem biosynthetic enzymes were normal. These findings were not limited to this Finnish family, as American and other European kindreds with acute intermittent porphyria with normal porphobilinogen deaminase activities have been identified¹⁰ (M Doss, personal communication). The nature of the CRIM negative mutations in these families remains unknown. Hepatic and erythrocytic porphobilinogen deaminase isozymes are unlikely to exist under separate genetic control as the enzyme deficiency has been shown in both sources in the same patients with acute intermittent porphyria^{6,7,9} and the enzyme activities in erythrocytes and liver are immunologically identical.¹⁰ Thus further characterisation of the genetic defect in this kindred with acute intermittent porphyria will also require molecular genetic techniques to determine whether the porphobilinogen deaminase structural gene is intact or whether other, undefined mechanisms are responsible for the acute attacks in these patients.

The two CRIM positive defects presumably represent different point mutations in the structural gene for porphobilinogen deaminase. Previous studies of the non-catalytic enzyme protein from a CRIM positive type 1 family indicated that this mutation resulted in a kinetically defective enzyme with a slightly decreased stability.⁸ Immunological investigation of the non-catalytic porphobilinogen deaminase in the CRIM positive type 2 families showed an unusual mutation—that is, the levels of immunoreactive, non-catalytic porphobilinogen deaminase were considerably greater than the maximal theoretical ratio of CRIM to activity of 2.0 for a single mutant allele. Another study has shown that the non-catalytic enzyme protein was bound to substrate,¹⁰ presumably owing to a mutation that increased substrate binding or prevented product release, or both. The mutant enzyme bound to substrate was more resistant to intraerythrocyte proteolysis than the normal enzyme protein, thereby increasing the ratio of CRIM to activity with erythrocyte age. It is intriguing to speculate that if the “super stable” mutant enzyme retained even a fraction of normal activity then the residual activities in erythrocytes and liver would be slightly greater than those in CRIM negative type 1 and CRIM positive type 1 patients and could result in a clinically milder disorder. The mean porphobilinogen deaminase activities in erythrocyte lysates from CRIM negative type 1 and CRIM positive type 1 and type 2 patients support this hypothesis (see table I). The observed incidence of acute symptoms in CRIM positive type 2 patients also was lower than that in CRIM negative type 1 and CRIM positive type 1 patients (see table III). Further support for this hypothesis requires evidence of stable non-catalytic enzyme in the liver of patients with the CRIM positive type 2 mutation as well as slightly increased hepatic activity. Such biochemical findings would be consistent with a

milder disease phenotype, except when exacerbated by selected drugs.^{1,2}

Characterisation of porphobilinogen deaminase activity in erythrocytes from families with acute intermittent porphyria in Finland provided information concerning the frequency of the various mutations in this fairly homogenous population.¹⁹ Of the 33 families studied, 25 were CRIM negative type 1. Of the seven CRIM positive families, four (12% of all families studied) were CRIM positive type 1 and three (9%) were CRIM positive type 2. For comparison, the frequencies of CRIM negative type 1 and 2 and CRIM positive type 1 and 2 mutations in a larger, random sample composed of North and South American patients from various ethnic and demographic ancestries were 90, 3, 2, and 5% respectively.¹⁰ Thus this study documents the molecular genetic heterogeneity of the mutations causing acute intermittent porphyria, even in the fairly homogenous population of Finland.

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