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Fecal Coproporphyrin Isomers in Hereditary Coproporphyria

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To see whether the fecal coproporphyrin III:coproporphyrin I (CIII:CI) ratio (determined by HPLC) would be suitable for screening patients at risk of hereditary coproporphyria (HC), we compared such ratios with the lymphocyte coproporphyrinogen oxidase (EC 1.3.3.3) activities (COOX) in 38 subjects from one large family and two smaller families with HC. The CIII:CI ratio was normal (<1.3) in adults with normal COOX (>180 nmol/g of protein per hour) and high (>2) in those with low COOX. Results were difficult to interpret in six of 10 children, who had borderline or low COOX but normal fecal CIII:CI ratios. Five subjects with low COOX and abnormal fecal CIII:CI ratios had normal fecal total porphyrin, indicating that the latter investigation alone is inadequate for family studies. The sample for determining the fecal CIII:CI ratio is easier to obtain and the assay is technically less demanding than COOX. We found the fecal CIII:CI ratio suitable for investigation of adults in a family study, but its usefulness in children needs to be established.

Additional Keyphrases: *heritable disorders · coproporphyrinogen oxidase · pediatric chemistry*

The porphyrias are inherited and acquired disorders of the heme biosynthetic pathway. Hereditary coproporphyria (HC) results from a deficiency in the activity of the mitochondrial enzyme coproporphyrinogen oxidase (COOX; EC 1.3.3.3), which converts coproporphyrinogen III to protoporphyrinogen IX.[1] This acute porphyria is inherited in an autosomally dominant mode. Clinically, the disease is similar to acute intermittent porphyria (AIP), although it is often milder, and, unlike AIP, may be associated with photosensitivity (*1*). Abdominal pain, vomiting, constipation, and psychiatric manifestations are most common, and the cutaneous features are found in about 30% of the patients (*1*). Attacks are commonly precipitated by drugs, most commonly barbiturates and oral contraceptives. Some female patients have attacks related to the menstrual cycle. It is therefore necessary that family studies be done to identify patients at risk.

Acute porphyrias are diagnosed by the presence of high concentrations of delta-aminolevulinic acid, porphobilinogen (PBG), and uroporphyrin in urine during an acute attack. In HC the urinary findings may be normal between attacks and the only clue to diagnosis is an increase in fecal porphyrin, predominantly coproporphyrin.

This finding distinguishes HC from porphyria variegata, where protoporphyrin predominates, and from AIP, where the amount of fecal porphyrin excreted is normal [i.e., <200 μmol/kg dry wt. (*2*)]. The total fecal porphyrin concentrations of prepubertal family members are usually normal; the youngest patient described with increased concentrations of this marker was 12 years old (*3*). The predominance of coproporphyrin III in the feces and urine in HC has been described (*1*). The activity of COOX is decreased in the leukocytes in patients with HC (*4, 5*).

We analyzed for fecal porphyrin and quantified COOX in the lymphocytes of three Australian families with HC to evaluate the suitability for family studies of determining the ratio of coproporphyrin III:coproporphyrin I (CIII:CI) in feces.

Materials and Methods

Patients

We analyzed the lymphocyte COOX, total fecal porphyrins, and the CIII:CI isomers pattern in 32 members of a large family (Family 1). We studied 17 females and 15 males, of whom 23 were adolescents or adults (ages 13–78 years) and nine were children (ages 5–12 years). Here we present the case studies of proband M.C. and her cousin S.H.

We also studied two other women—S.C., age 32 years, and M.P., age 30 years—who had clinically and biochemically diagnosed HC; S.C.'s parents and brother (Family 2); and M.P.'s daughter (Family 3).

Case 1 (the proband). In 1975, when she was 22 years old, M.C. presented with a 10-week history of polyarthritis, fever, and a 10-day history of numbness of the soles of both feet; she had had five grand mal convulsions four days before admission. At the time of admission to the Royal Melbourne Hospital, she was taking prednisolone, 40 mg daily, and phenytoin, 100 mg thrice daily. She was afebrile, had a blood pressure of 210/120 mmHg, and had decreased sensation in the sole of her left foot.

She had an erythrocyte sedimentation rate of 23 mm/h and a urinary protein excretion of 0.4 g/day. (reference range <0.15 g/day). She had negative test results for anti-nuclear antibodies and rheumatoid factors, no lupus erythematosus cells, and a normal muscle biopsy. A renal biopsy showed conditions "consistent with poly arteritis nodosa," which was the clinical diagnosis at the time.

Porphyria was looked for as part of the differential diagnosis of polyneuropathy with hypertension. She had a negative screening result for PBG in her urine and an

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[1] Nonstandard abbreviations: HC, hereditary coproporphyria; COOX, coproporphyrinogen oxidase; AIP, acute intermittent porphyria; PBG, porphobilinogen; and CIII:CI, ratio of coproporphyrin isomers III and I.

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increase in total urine porphyrin to 720 nmol/L (reference range <300 nmol/L), which was predominantly coproporphyrin (602 nmol/L). Her feces had been examined for porphyrins in 1978: total porphyrins were 874 μmol/kg (dry wt.), of which 848 μmol/kg was coproporphyrin; her PBG test at that time was also negative. A repeat renal biopsy in 1981 was more suggestive of systemic lupus erythematosus (largely inactive), although results of her blood tests and serology never substantiated it. In May 1988 her total porphyrin in feces was 190 μmol/kg (within the reference range), but her CIII:CI ratio was 6. None of her symptoms and signs were at any stage attributed to her accompanying HC, although her biochemical investigations have been consistent with HC.

Case 2. S.H. was born in 1960. She was admitted to the psychiatry unit of the Royal Melbourne Hospital in February 1978 and again in June 1978 with a diagnosis of schizophrenia. She had a history of a depressive illness, having been treated at age 13 at the psychiatry unit of the Royal Children's Hospital in Melbourne. On discovery of her relationship to the proband (second cousins), she was investigated for porphyria. Her parents dated the onset of her illness to November 1977, when she had received a general anesthetic for a nose operation and had started taking oral contraceptives soon after. She had no cutaneous or abdominal symptoms. In June 1978 her urine was positive for PBG; total urinary porphyrin was 1425 nmol/L, with coproporphyrin at 1176 nmol/L. Her total fecal porphyrin was 2825 μmol/kg (dry wt.), predominantly coproporphyrin. She was readmitted in January 1981, when an alternative diagnosis of hypomania was considered. Examination of her urine and feces at intervals over the following years always showed very high concentrations of fecal and urine porphyrins. Reexamination of her urine and feces in 1990 gave the following results: urine porphyrin 805 nmol/L, PBG 18 μmol/L (reference range <10 μmol/L), and total fecal porphyrin 4079 μmol/kg (dry wt.); the CIII:CI ratio was 15.8. Her lymphocyte COOX activity was 90 nmol/g of protein per hour. She has remained well since 1981, and gave birth to a baby girl in 1983. However, she does get attacks of lower abdominal pain accompanied by distension about once every six months, which she says occurs about two weeks after a menstrual period.

Apparatus

We used a Model 3000 spectrophotometer (Milton Roy, Rochester, NY) to scan fecal acid extracts for the Soret peak. To estimate percentage dry weight, we dried the feces in a carousel microwave oven.

The HPLC system used comprised Model 114M pumps (Beckman Instruments, Fullerton, CA), a Kortec 65B Autosampler (ICI, Sydney, Australia), a Model LS40 fluorescence detector with a red-sensitive photomultiplier tube (Perkin-Elmer, Palo Alto, CA), and a Perkin-Elmer Nelson Integration system (Model 2600 chromatography software version 5.1.5). The HPLC column used was a Nova-pak C_{18} (3.9 × 150 mm; Waters Co., Konigstein, F.R.G.).

Reagents and Materials

All reagents were analytical grade or HPLC grade: acetonitrile ("Hypersolv"), ammonium acetate, ammonium chloride, copper sulfate, diethyl ether, dimethyl sulfoxide, Folin & Ciocalteu's phenol reagent, sodium carbonate, and trichloroacetic acid (all from BDH, Poole, U.K.); sodium hydroxide (May and Baker, Sydney, Australia); methanol (Mallinkrodt, Paris, KY); porphyrin acid chromatographic marker kit, coproporphyrin III, mesoporphyrin, protoporphyrin IX (Porphyrin Products, Logan, UT); 5-μm (pore-size) filters (Argyle, St. Louis, MO); Ficoll–metrizoate mixture ("Lymphoprep"; Nycomed, As, Oslo, Norway); and potassium sodium tartrate (Merck, Darmstadt, F.R.G.).

Procedure

Fecal total porphyrin was assayed by the method of Lockwood et al. (*2*). The only modification to the assay was to estimate the percentage dry weight of the feces by drying a weighed sample in a microwave oven for 15 min. The interassay CV for total porphyrin at 507 μmol/kg dry weight is 12.5%.

Fecal coproporphyrin isomer separation was by the HPLC method of Lim and Peters (*6*) with the following modifications: we used a C_{18} Nova-pak column, continued the gradient to 100% solvent B (methanol/acetonitrile, 90/10 by vol) instead of 65% B, and detected coproporphyrin by fluorescence (excitation 395 nm, emission 620 nm). The interassay CV% was 8.0% for a fecal CIII:CI ratio of 3.9. The reference range for this assay was determined by analyzing 269 samples from subjects without porphyria; 95% of the results were between 0.4 and 1.3 (Figure 1). This population included 22 healthy laboratory staff, who as a subgroup had values <0.7. The reference range for children, determined for 23 normal children, ages one through seven years, was also <0.7, not significantly different from the adults.

Fecal coproporphyrin was obtained by multiplying the total fecal porphyrin by the sum of the percentages of CI and CIII determined by HPLC.

COOX activity was determined by the method of Guo et al. (*7*), with the following modifications: lymphocytes, prepared by density gradient separation ("Lymphoprep"), were used as the enzyme source and stored in glycerol–Tris buffer, 20 mmol/L, pH 7.2 (per liter, 200 mL of glycerol and 800 mL of Tris buffer) at −20 °C until analysis (*8*); enzyme incubation was reduced to 20 min; protoporphyrin standard was prepared in dimethyl sulfoxide to improve stability; and the fluorometer was optimized to detect the protoporphyrin product (excitation 400 nm, emission 631 nm). The quality-control and patients' samples were prepared by freeze–thawing, assayed in duplicate, and the results averaged. The quality-control material was a preparation of lymphocytes obtained from leukophoresis of a patient with leukemia, freeze–thawed, aliquoted, and stored at −20 °C. The interassay CV for COOX at an activity of 248 nmol of protoporphyrin per gram of protein per hour

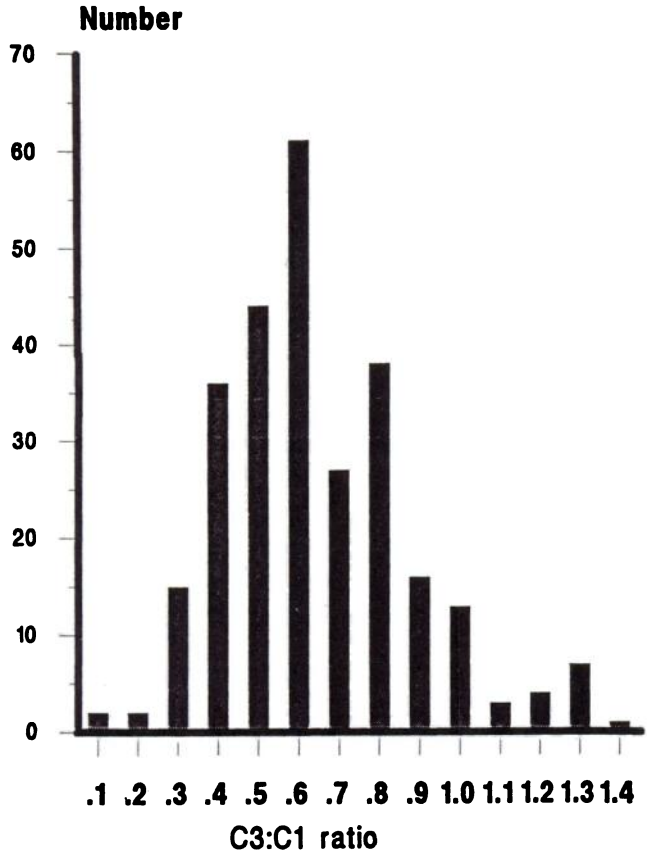

Fig. 1. Histogram of the fecal CIII:CI (C3:C1) ratio in 269 subjects without porphyria

was 14.0%. The reference range, determined by analyzing 20 samples from healthy laboratory workers (14 women, six men, ages 23 to 49 years), was 180 to 312 (mean 269) nmol of protoporphyrin per gram of protein per hour.

Results

Clinical findings. In the large kindred, S.H. (patient 23) had clinical HC. Only one other person (patient 11) may have had an acute attack of porphyria; however, this cannot be confirmed because one week after the illness her urine PBG was normal.

Biochemical findings. The biochemical findings for Family 1 are listed in Table 1. We found normal COOX activity [>180 nmol/g per hour] in 12 persons. Sixteen persons had low activity [<150 nmol/g per hour]. Four children (ages five to 10 years) had borderline activities, between 150 and 170 nmol/g per hour. Eleven of the 32 family members in the study had an increased fecal porphyrin content in at least one examination.

Fifteen of the 32 members of the family had abnormal (>2) CIII:CI ratios. Table 2 summarizes the possible effect of age on the CIII:CI ratio in seven persons in whom it was measured more than once.

Lymphocyte COOX activity and fecal CIII:CI ratio. The correlation between these two measurements is shown in Figure 2. All persons with normal COOX activity had normal CIII:CI ratios. All persons with an abnormal CIII:CI ratio had low COOX activity.

Table 1. Biochemical Investigations: Family 1

Patient no.	Age, y	Sex	Fecal porphyrin: Total porphyrin, μmol/kg	Fecal porphyrin: Coproporphyrin, μmol/kg	CIII:CI ratio	Lymphocyte COOX, nmol/g per hour
1	5	M	69	21	0.9	150
2	7	F	284	45	1.0	122
3	7	M	126	82	2.1	113
4	9	M	157	24	0.9	157
5	9	M	226	77	3.3	133
6	10	F	43	13	0.5	162
7	11	M	217	72	2.5	87
8	12	M	10	6	0.6	215
9	12	M	76	12	0.5	170
10	13	M	26	13	0.6	313
11	14	F	104	78	8.4	70
12	17	F	168	25	0.5	197
13	19	F	10	4	0.6	206
14	19	F	92	69	5.2	117
15	19	M	89	24	0.8	310
16	19	F	129	22	0.5	194
17	20	M	204	55	0.8	238
18	20	M	74	26	0.6	225
19	22	F	69	15	0.5	267
20	15	M	159	143	17.0	93
21	27	F	1868	1774	30.7	75
22	27	M	533	474	2.1	131
23	32	F	4079	3956	15.2	90
24	36	F	10	6	0.4	226
25	38	M	5	4	0.7	271
26	40	M	4020	3417	5.5	90
27	42	F	1033	785	18.0	143
28	43	F	1000	960	4.7	133
29	46	F	189	53	1.2	223
30	49	F	109	76	18.0	88
31	58	F	126	96	4.1	113
32	77	F	233	175	8.4	130

M, male; F, female.

All subjects older than 12 years who had a low COOX activity also had an abnormal CIII:CI ratio. One child with a low COOX activity (121 nmol/g per hour) and four children with borderline low COOX activity had normal CIII:CI ratios.

Total fecal porphyrin and fecal CIII:CI ratios. Of the 11 subjects with increased fecal porphyrin excretion, nine also had an abnormal CIII:CI ratio. The other two had predominantly protoporphyrin in their feces. Five persons with normal total porphyrin had abnormal CIII:CI ratios.

Results for Families 2 and 3. Results for fecal porphyrin and lymphocyte COOX in the two unrelated families are shown in Table 3. The correlation between the different investigations was as found in Family 1. M.P.'s child shows discrepant values for COOX activity and the isomer ratio.

Discussion

The large family we studied is remarkable in that there is hardly any clinical expression of the disease.

Table 2. Effect of Age on CIII:CI Ratio

Patient no.	Age, y	Fecal porphyrin: Total porphyrin, μmol/kg	Fecal porphyrin: Coproporphyrin, μmol/kg	CIII:CI ratio	Lymphocyte COOX, nmol/g per hour
12	5	60	27	0.7	
	17	168	25	0.5	196
14	7	99	54	2.4	
	19	92	69	5.2	117
15	7	113	24	1.1	
	19	89	24	0.8	310
16	7	24	12	0.5	
	19	129	22	0.5	194
18	9	77	25	0.9	
	20	74	30	0.6	225
20	8	95	83	3.4	
	15	159	143	17.0	93
Female child of 23					
	7 wks	39	33	1.2	
	0.5	27	26	2.1	
	6	137	122	6.4	—
	7	160	162	8.0	

The proband was discovered by accident; her clinical symptoms had not been attributed to the disease. Only patient S.H. had clinical HC.

The results of the two assays showed good concordance in adults (see Figure 2). All adults with normal COOX had their CIII:CI ratio within the reference range. All adults in the study with a low COOX had a CIII:CI ratio >2. There was no overlap.

The interpretation of the findings in children was difficult (Figure 2). In the large kindred, one child (patient 8) had normal COOX and normal fecal isomer ratio; three children (patients 3, 5, and 7) had low COOX (87–133 nmol/g per hour) and abnormal CIII:CI ratios (2.1–3.3). These results are as would be expected. However, the remaining five children had COOX activity less than the adult reference range, but CIII:CI ratios were normal: one seven-year-old (patient 2) had an activity of 122 nmol/g per hour and a CIII:CI ratio of 1.0; the other four had COOX activities between 150 and 170 nmol/g per hour. These were within the adult reference range but were still higher than the activity in any adult with an abnormal CIII:CI ratio or other biochemical findings of HC. One child (patient 6) with a COOX activity of 162 nmol/g per hour and a CIII:CI ratio of 0.5 is an obligate normal because her father (patient 25) had normal COOX activity and CIII:CI ratio. We were unfortunately unable to establish a COOX reference range in children. This makes interpretation of the COOX activity in childhood difficult.

It is tempting to speculate that the reference range for COOX in children may be lower than in adults. The reference range for fecal CIII:CI ratio in childhood is not significantly different from adults.

In the course of our follow-up of this family, we analyzed the feces more than once in seven persons (Table 2). These samples had been stored at −20 °C for

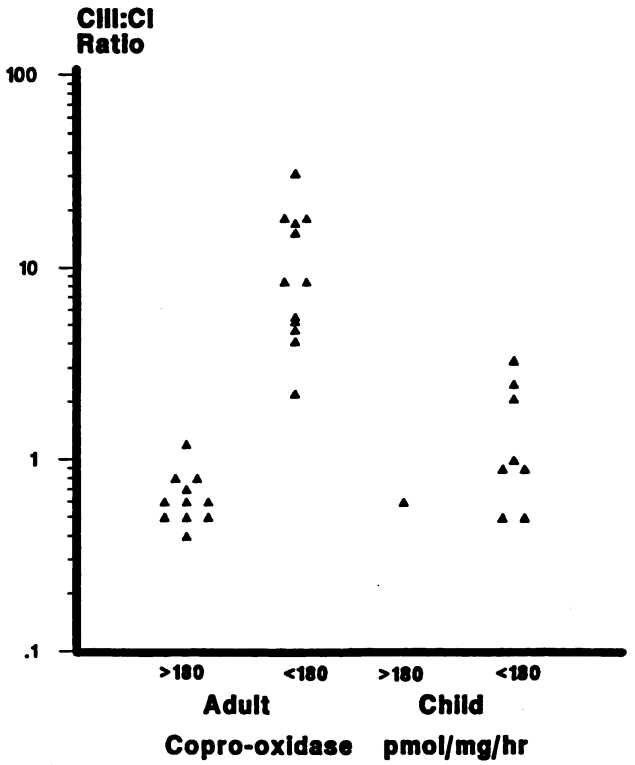

Fig. 2. Scattergram showing the relationship between normal (>180 nmol/g per hour) and abnormal (<180 nmol/g per hour) lymphocyte COOX activity and the fecal CIII:CI ratio in adults (*left*) and in children (*right*)

Table 3. Biochemical Investigations: Families 2 and 3

Age, y	Sex	Fecal porphyrin: Total porphyrin, μmol/kg	Fecal porphyrin: Coproporphyrin, μmol/kg	CIII:CI ratio	Lymphocyte COOX, nmol/g per hour
Family 2					
28	M	62	17	1.0	251
32	F	1945	1886	23.2	35
63	M	302	283	12.0	79
64	F	222	18	0.6	183
Family 3					
5	F	49	35	1.2	89
30	F	3228	3034	17.8	81

as long as 12 years. In the three with abnormal ratios, we noticed that the ratio increased with age. The ratios of the other four actually decreased. However, the number of subjects was too small for statistical analysis.

In these three families we found five persons with normal total fecal porphyrin, but a high CIII:CI ratio and low COOX activity. Two more persons had an increase in total fecal porphyrin, with the predominant porphyrin being protoporphyrin, perhaps as a result of a high meat diet or bleeding into the gut. Hence, total fecal porphyrin is inadequate for screening for HC. Normal total fecal porphyrin in patients with low COOX has been described by others (*3*, *4*).

Total coproporphyrin in the feces, either as a concentration or as a percentage of the total fecal porphyrin,

was high, especially in those with increased total porphyrin. However, there was a substantial overlap between those with low and normal COOX activity and those with low and normal fecal isomer ratios, especially if the total fecal porphyrin was low. Therefore, quantifying total coproporphyrin is also inadequate for screening for HC.

The determination of the fecal CIII:CI ratio offers many advantages in conducting a family study of HC. The samples are less invasive to obtain and more stable, and hence easier to transport than blood samples for COOX. Determination of the CIII:CI isomer ratios is also technically easier than quantifying the lymphocyte COOX activity.

The presumed reason for the abnormal isomer ratio is that, under normal circumstances, CI is excreted in the feces, being no further utilized in the heme pathway. However, in HC, CIII accumulates and is excreted, and hence alters the isomer ratio. We have found abnormal (increased) CIII:CI isomer ratios in some patients with porphyria variegata as well, perhaps from accumulation of metabolites proximal to the block. Hence, the CIII:CI isomer ratio also cannot be used alone to diagnose an isolated case of HC.

The CIII:CI isomer ratio is as reliable as a measurement of COOX for a family study of HC in adults. However, the interpretation of a normal CIII:CI isomer ratio in young children is difficult.

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