Compound heterozygous hereditary coproporphyria with fluorescing teeth

M O Doss[1], U Gross[1], J Lamoril[2], Ch Kranl[3], K Jacob[4], M Doss[1], V da Silva[2], A G Freesemann[1], J-Ch Deybach[2], N Sepp[3] and Y Nordmann[2]

From the [1]Division of Clinical Biochemistry, Philipps University Hospital, Deutschhausstrasse 17½, 35037 Marburg, Germany, the [2]Department of Biochemistry, Hôpital Louis Mourier, Colombes, France, the [3]University Hospital for Dermatology and Venereology, Innsbruck, Austria, and the [4]Institute of Clinical Chemistry, University Hospital Grosshadern, Munich, Germany

In Europe, hereditary coproporphyria (HCP) is the third commonest acute hepatic porphyria after acute intermittent porphyria and variegate porphyria. It is inherited as an autosomal dominant trait and excessive excretion of coproporphyrin (copro) III in faeces is the striking biochemical abnormality.[1] Its clinical expression, with an increase in urinary δ-aminolaevulinic acid (ALA), porphobilinogen (PBG) and porphyrins, is characterized by neurovisceral and cutaneous symptoms.

PATIENTS AND METHODS

A 10-year old girl presented with skin symptoms of easy fragility, scars on hands and feet, and local reddening. Father (42 years) and mother (46 years) were clinically asymptomatic. The child's teeth were light brown and exhibited red fluorescence under long-wave ultraviolet (UV) light. Congenital erythropoietic porphyria (CEP; Günther's disease) was suspected.

ALA and PBG were determined spectrophotometrically after isolation by ion-exchange chromatography. Porphyrins were analysed spectrophotometrically as methyl esters after separation by high-performance thin-layer chromatography.[2] Porphyrin isomers and zinc protoporphyrin were determined by high-performance liquid chromatography (HPLC).[3–5]

Teeth were homogenized with a mortar and pestle in 1·5 mol/L HCl. The extract was freeze-dried and porphyrins were quantified as described above.[2] Uroporphyrinogen III synthase, ALA dehydratase, PBG deaminase and uroporphyrinogen decarboxylase were studied in erythrocytes.[6] Coproporphyrinogen oxidase was assayed using lymphocytes, according to the method described previously.[7]

The coproporphyrinogen oxidase gene was investigated by denaturing gradient gel electrophoresis (DGGE) followed by direct sequencing of the positive fragments according to the method of Puy *et al.*[8]

Correspondence: Professor Manfred O Doss MD.

E-mail: doss@post.med.uni-marburg.de

RESULTS

Metabolite studies

Table 1 shows the urinary and faecal haem precursors present in the patient's family. Urinary ALA was slightly elevated in the patient and her father, and urinary coproporphyrin concentration was enhanced in father and mother. The patient's urinary excretion of uro- and coproporphyrins were 50- and 55-fold higher than the upper limit of the reference range, respectively. In addition, urinary coproporphyrin isomer III was dominant (up to 94%), which is characteristic of an acute hepatic porphyria. Faecal coproporphyrin in the patient, the mother and the father were 76-, 7- and 5-fold higher, respectively, and more than 90% was present as isomer III. Faecal protoporphyrin was within the reference range in the family members. Zinc protoporphyrin in the patient's erythrocytes was 2·3-fold higher than the mean in healthy controls [280 (110) nmol·L^{-1}, $n = 20$].

The teeth contained uroporphyrin (2·4 nmol·g^{-1}, normal $< 0{\cdot}05$ nmol·g^{-1}), heptacarboxyporphyrin (1·3 nmol·g^{-1}, not detectable under physiological conditions) and coproporphyrin (1·5 nmol·g^{-1}, normal $< 0{\cdot}05$ nmol·g^{-1}, isomer I:III = 5:95%).

Enzyme activities

The activity or uroporphyrinogen III synthase was 2760 pkat·g^{-1} total soluble protein in the patient, 1290 pkat·g^{-1} in the mother and

TABLE 1. *Urinary and faecal haem precursors present in a family with compound heterozygous hereditary coproporphyria*

	Urine						Faeces			
Subject	ALA (μmol/ 24 h)	PBG (μmol/ 24 h)	Uro (nmol/ 24 h)	Copro (nmol/ 24 h)	Isomer I:III (%)	Tot por (nmol/ 24 h)	Copro (nmol/ g dw)	Isomer I:III (%)	Proto (nmol/ g dw)	Tot por (nmol/ g dw)
Patient	60	4	1444	6502	6:94	9727	2835	7:93	62	3045
Mother	44	4	18	189	30:70	222	266	7:93	19	312
Father	53	6	36	951	11:89	1001	189	9:91	16	243
Non-porphyric Controls	<49	<8	<29	<119	(17–31) (69–83)	<165	<37	(65–75) (25–35)	<151	<224

dw = dry weight; ALA = 5-aminolaevulinic acid; PBG = porphobilinogen; Uro = uroporphyrin; Copro = coproporphyrin; Proto = protoporphyrin, Tot por = total porphyrins.

1160 pkat·g^{-1} in the father {normal [mean (2 SD)] 1720 (280) pkat·g^{-1} total soluble protein, $n = 10$}. Thus, uroporphyrinogen III synthase was slightly enhanced in the young girl and slightly diminished in her parents.

The activities of the other cytosolic enzymes, ALA dehydratase, PBG deaminase and uroporphyrinogen decarboxylase were within the reference range.

Coproporphyrinogen oxidase activity in lymphocytes in the patient was 2% of normal (normal 138 ± 42 pkat·g^{-1} total soluble protein, $n = 50$). Copro'gen oxidase activity in the mother and father were 43% and 69%, respectively.

Molecular genetics

The coproporphyrinogen oxidase gene was studied in the patient and her parents. The child was compound heterozygous with the maternal mutation A to G at position 980 in exon 5 and the paternal single nucleotide exchange A to G at position 920 in exon 4. In addition, the mother carried a silent mutation G to A at position 612 in exon 2.

DISCUSSION

Coproporphyrin isomer III dominance in faeces, urine and teeth, as well as slightly enhanced uroporphyrinogen III synthase activity, excluded CEP. Thus, red fluorescence of the teeth could not be related to Günther's disease. The pattern of urinary and faecal excretion of haem precursors with the increase of urinary coproporphyrin isomer III and an isomer inversion in faeces shows that the child suffered from HCP. Both parents are gene carriers and expressed latent HCP. The observation of red fluorescing teeth in HCP is a surprising finding. The extremely decreased coproporphyrinogen oxidase activity (2% of controls' activity) confirms that this 10-year old girl represents a 'homozygous' case of HCP. Other porphyrias like CEP and dual porphyrias could be excluded by measurements of cytosolic enzyme activities of the porphyrin biosynthetic chain as well as urinary and faecal haem precursors. A homozygous state has been reported for HCP and its variant harderoporphyria.[9,10] In the patients mentioned above the parents were related to each other. In our patient the parents were not relatives. Molecular genetic investigations exhibited two independent mutations on each allele. This is the first description of a compound heterozygous HCP.

Acknowledgements

This work was supported by the German Research Association (grant Gr 1363) and the Hans-Fischer-Gesellschaft, Munich.

REFERENCES

1 Berger H, Goldberg A. Hereditary coproporphyria. *BMJ* 1955; **2**: 85–7
2 Doss MO. Porphyrins and porphyrin precursors. In: Curtius HC, Roth M, eds. *Clinical Biochemistry: Principles and Methods*. New York: W de Gruyter, 1974; **2**: 1323–71
3 Jacob K, Doss MO. Composition of urinary coproporphyrin isomers I–IV in human porphyrias. *Eur J Clin Chem Clin Biochem* 1993; **31**: 617–24
4 Jacob K, Doss MO. Excretion pattern of fecal coproporphyrin isomers I–IV in human porphyrias. *Eur J Clin Chem Clin Biochem* 1995; **33**: 893–901
5 Meyer HD, Jacob K, Vogt W. Ion-pair reversed-phase high-performance liquid chromatographic determination of porphyrins from red blood cells. *Chromatographia* 1982; **16**: 190–1

6 Freesemann AG, Gross U, Bensidhoum M, de Verneuil H, Doss MO. Immunological enzymatic and biochemical studies of uroporphyrinogen III-synthase deficiency in 20 patients with congenital erythropoietric porphyria. *Eur J Biochem* 1998; **257**: 149–53
7 Nordmann Y, Grandchamp B. Hereditary coproporphyria. Demonstration of a genetic defect in coproporphyrinogen metabolism. In: Doss M, ed. *Diagnosis and Therapy of Porphyrias and Lead Intoxication*. Berlin; Springer-Verlag, 1978: 77–81
8 Puy H, Deybach JC, Lamoril J, Robreau AM, da Silva V, Gouya L, *et al.* Molecular epidemiology and diagnosis of PBG deaminase gene defects in acute intermittent porphyria. *Am J Hum Genet* 1997; **60**: 1373–83
9 Doss M, von Tiepermann R, Köpp W. Harderoporphyrin coproporphyria. *Lancet* 1984; **i**: 292
10 Lamoril P, Martasek P, Deybach JC, da Silva V, Grandchamp B, Nordmann Y. A molecular defect in coproporphyrinogen oxidase gene causing harderoporphyria, a variant form of hereditary coproporphyria. *Hum Mol Gen* 1995; **4**: 275–8

Accepted for publication 13 April 1999