Identification of two types of porphyria cutanea tarda
by measurement of erythrocyte uroporphyrinogen
decarboxylase

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Summary

1. Erythrocyte uroporphyrinogen decarboxylase activity has been measured in 27 patients with
porphyria cutanea tarda, of whom 11 had a family history of overt porphyria cutanea tarda.

2. Eight patients from six families had erythrocyte uroporphyrinogen decarboxylase activities that
were decreased to about half of control values. This decrease was shown by family studies to be
inherited as an autosomal dominant characteristic. Two of these patients had no family history of
overt porphyria cutanea tarda.

3. Nineteen patients had uroporphyrinogen decarboxylase activities close to or within the range
found in 18 control subjects. Of these, five patients had a family history of porphyria cutanea tarda.

4. Inheritance of an autosomal dominant gene which decreases uroporphyrinogen decarboxylase
activity in erythrocytes and liver is an uncommon cause of porphyria cutanea tarda, and may not
explain all cases of familial porphyria cutanea tarda. The hepatic enzyme defect in the common
type of porphyria cutanea tarda, in which erythrocyte uroporphyrinogen decarboxylase activity is
normal, may be caused either by inheritance of a gene whose effect is restricted to the liver or by
chemicals that selectively inhibit the hepatic enzyme.

Key words: erythrocyte, porphyria, porphyria cutanea tarda, porphyrinogens, uroporphyrinogen
decarboxylase.

Introduction

Porphyria cutanea tarda, the commonest type of porphyria in Europe and North America, is a
syndrome in which over-production of uroporphyrin and other acetate-substituted porphyrins by the liver
leads to photosensitization and the appearance of skin lesions. The characteristic pattern of porphyrin
over-production is the consequence of a decrease in the activity of uroporphyrinogen decarboxylase
(EC 4.1.1.37) in the liver (Kushner, Barbuto & Lee, 1976; Elder, 1977; Elder, Lee & Tovey, 1978).
In patients with porphyria cutanea tarda who have relatives with porphyria cutanea tarda, this enzyme
activity is also decreased in erythrocytes (Kushner et al., 1976; Benedetto, Kushner & Taylor, 1978;
Tiepermann, Topi, D’Alessandro Gandolfo & Doss, 1978; Verneuil, Aitken & Nordmann, 1978), and the low erythrocyte enzyme activity follows an
autosomal dominant pattern of inheritance in their families (Kushner et al., 1976; Benedetto et al.,
1978; Verneuil et al., 1978), which suggests that the hepatic enzyme defect is inherited similarly.
However, most patients with porphyria cutanea tarda have no family history of the disorder
(Waldenstrom & Haeger-Aronsen, 1967; Dehlin, Enerback & Lundvall, 1973; Pinol Aguade,
Herrero, Almeida, Smith & Belcher, 1975; Topi & D'Alessandro Gandolfi, 1977. Study of erythrocyte enzyme activities in these patients has produced conflicting results. Some authors have found low activities and have suggested that these cases are also the result of inheritance of an autosomal dominant gene (Kushner et al., 1976; Felscher, Norris & Shih, 1978; Tiepermann & Doss, 1978) and that the low penetrance in their families can be explained by the apparent need for interaction between the enzyme defect and other factors, in particular alcoholic liver disease and siderosis, before the condition becomes clinically manifest (Kushner et al., 1976). Other workers have found normal erythrocyte uroporphyrinogen decarboxylase activities (Blekkenhorst, Pimstone & Eales, 1976; Elder et al., 1978; Verneuil et al., 1978; Brodie, Thompson, Moore, McColl, Goldberg, Hardie & Hunter, 1979) and thus have been unable to confirm the hypothesis that the hepatic enzyme defect is inherited in this group of patients. Verneuil et al. (1978) reported that there are at least two types of porphyria cutanea tarda: a familial type, which is characterized by autosomal dominant inheritance of low erythrocyte uroporphyrinogen decarboxylase activity, and a sporadic type in which erythrocyte enzyme activity is normal. We have measured erythrocyte uroporphyrinogen decarboxylase in patients from Spain, where familial porphyria cutanea tarda is encountered more frequently than in the United Kingdom, in order to test their prediction that this measurement enables familial and sporadic forms of porphyria cutanea tarda to be distinguished.

Materials and methods

Patients and control subjects

Twenty-seven patients with porphyria cutanea tarda (26 men, one woman) and 40 asymptomatic relatives (19 men, 21 women) were studied. The patients with porphyria cutanea tarda were divided into two groups: those with (11 patients from eight families) and without (16 patients) a family history of clinically overt porphyria cutanea tarda. The clinical features of some members of family C (Fig. 2) have been described (Salamanca, Nunez-Torron, Lombera & Catalan, 1978). All patients presented with skin lesions and the diagnosis of porphyria cutanea tarda was confirmed by demonstrating characteristic porphyrin profiles in urine and faeces. At the time enzyme measurements were carried out, 17 patients had skin lesions and ten patients were clinically in remission. Ten patients had been treated by repeated venesection. Haemoglobin concentrations (range 14.0–18.5 g/dl) and packed cell volumes (range 41.6–51.4%) were normal in 26 patients. One patient had chronic renal failure and anaemia (haemoglobin 8·5 g/dl). Twenty-six patients were assessed for evidence of liver disease by clinical examination, conventional liver-function tests and, in nine patients, histological examination of needle-biopsy samples. Eighteen Spanish hospital workers without porphyria were studied as control subjects.

Methods

Venous blood for enzyme measurements was anticoagulated with dipotassium EDTA, frozen at −70°C or lower within 1 h of collection and stored at −70°C for up to 2 months. At this temperature there was no change in the activity of uroporphyrinogen decarboxylase for at least 5 months. Samples from patients and controls were collected at the same time and stored under identical conditions. They were transported to Cardiff packed in solid carbon dioxide.

Whole blood was haemolysed by freeze–thawing three times and assayed for uroporphyrinogen decarboxylase by using either pentacarboxylic porphyrinogen III (15–20 µmol/l) or uroporphyrinogen III (15–20 µmol/l) as substrate, as previously described (Elder & Tovey, 1977; Elder et al., 1978), except that the enzyme was activated by including dithiothreitol (20 mmol/l) in the incubation medium (mean increase in activity after activation was 1·5-fold). Results were expressed as pmol of coproporphyrin formed min⁻¹ mg⁻¹ of haemoglobin. All samples were measured in duplicate. Each batch of assays contained samples from controls and from patients with and without a family history of porphyria cutanea tarda and samples that were carried over from batch to batch. Within-batch and between-batch coefficients of variation were 8·5% and 9·8% respectively. Uroporphyrinogen I synthase (EC 4.3.1.8) was measured as described by Sassa, Granick & Kappas (1975), except that uroporphyrin was used as standard. Results were expressed as pmol of uroporphyrin formed h⁻¹ mg⁻¹ of haemoglobin.

Urinary and faecal porphyrin fractions were measured by solvent-extraction techniques (Rimington, 1971). Individual porphyrins in urine and faeces were separated by thin-layer chromatography
after conversion into their methyl esters, and measured by fluorescence scanning (Day, Salamanca & Eales, 1978). For comparison of urinary porphyrin patterns, the amount of each porphyrin present was expressed as a percentage of the total porphyrin and a 'porphyria cutanea tarda index' calculated by multiplying the percentage of heptacarboxylic porphyrin by 100 and dividing by the sum of the percentages of coproporphyrin and heptacarboxylic porphyrin (Salamanca, Pena, Olmos, Jimenez & Catalan, 1979).

The significance of differences between groups was assessed by the Mann-Whitney test.

Results

Enzyme measurements

Erythrocyte uroporphyrinogen decarboxylase activities, measured with pentacarboxylic porphyrinogen III as substrate in patients with and without a family history of clinically overt porphyria cutanea tarda, are shown in Fig. 1. Enzyme activities in the patients with a family history (mean 19.1, range 15.1–24.5 pmol min⁻¹ mg⁻¹) are significantly lower (P < 0.01) than in controls (mean 24.8, range 20.3–32.6 pmol min⁻¹ mg⁻¹) and patients without a family history (mean 23.0, range 14.7–26.3 pmol min⁻¹ mg⁻¹), but there is considerable overlap between the three groups.

Uroporphyrinogen I synthase activities in 23 of the patients with porphyria cutanea tarda (ten with a family history) (mean 82.5, range 61.7–108.9 pmol h⁻¹ mg⁻¹ of haemoglobin) did not differ from those in 16 control subjects (mean 78.2, range 61.6–95.0 pmol h⁻¹ mg⁻¹).

Seven patients from the group with a family history, and two from the group without, had erythrocyte uroporphyrinogen decarboxylase activities lower than the lowest value in the control group. Activities were below 19.0 pmol min⁻¹ mg⁻¹ in eight of these patients (Fig. 1). Six of these eight patients came from four families (Fig. 2: families A—D). In each family, including those of the two patients from the group without a family history (families A and B), the pattern of erythrocyte enzyme activity was consistent with inheritance of decreased activity as an autosomal dominant characteristic (Fig. 2). The families of the two remaining patients were not studied: one had a brother with porphyria cutanea tarda, and the 6 year-old niece of the other was stated to have porphyria cutanea tarda. Erythrocyte uroporphyrinogen decarboxylase measurements for these two patients and the propositi from families A—D are shown in Table 1. With pentacarboxylic porphyrinogen III as substrate, the mean activity is decreased to 66% of the control value but, when expressed as a ratio of the uroporphyrinogen I synthase activity or measured with uroporphyrinogen I₁₁ as substrate, the mean activity is half that of control subjects.

Five patients with a family history of overt porphyria cutanea tarda had uroporphyrinogen decarboxylase activities close to or within the control range (Fig. 1) and these patients could not be distinguished from those without a family history by measurements of erythrocyte enzyme activity (Table 1). They came from two families (Fig. 2: families E and F). In neither family was there any evidence for an inherited abnormality.
Family A

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Family B

Family C

Family D

Family E

Family F

FIG. 2. Pedigrees of families A–F. PCT, Porphyria cutanea tarda. Propositi are indicated by arrows and figures below symbols are erythrocyte uroporphyrinogen decarboxylase activities (pmol of coproporphyrin formed min⁻¹ mg⁻¹ of haemoglobin) measured with pentacarboxylic porphyrinogen III as substrate.

TABLE 1. Erythrocyte uroporphyrinogen decarboxylase activity in porphyria cutanea tarda

Values are means and ranges with the numbers of subjects in parentheses. Uroporphyrinogen decarboxylase activities were measured with pentacarboxylic porphyrinogen III (5-CO₂H) or uroporphyrinogen III (8-CO₂H) as substrate. The decarboxylase/synthase ratio was obtained by dividing the uroporphyrinogen decarboxylase activity, measured with pentacarboxylic porphyrinogen III as substrate, by the uroporphyrinogen I synthase activity. See the text for explanation of patient groups. *P ≤ 0.01 for the comparison with each of the other groups.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Uroporphyrinogen decarboxylase (pmol min⁻¹ mg⁻¹)</th>
<th>Decarboxylase/synthase ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-CO₂H substrate</td>
<td>8-CO₂H substrate</td>
</tr>
<tr>
<td>Proposit of families with inherited decrease</td>
<td>16.3 (6)*</td>
<td>1.7 (6)*</td>
</tr>
<tr>
<td>in erythrocyte enzyme activity</td>
<td>14.7–18.6</td>
<td>1.2–2.3</td>
</tr>
<tr>
<td>Family history of overt porphyria cutanea tarda,</td>
<td>22.2 (5)</td>
<td>2.8 (4)</td>
</tr>
<tr>
<td>erythrocyte enzyme activity normal</td>
<td>19.8–24.5</td>
<td>2.6–3.0</td>
</tr>
<tr>
<td>No family history of overt porphyria cutanea</td>
<td>24.0 (14)</td>
<td>3.7 (7)</td>
</tr>
<tr>
<td>tarda, erythrocyte enzyme activity normal</td>
<td>21.5–26.3</td>
<td>2.7–4.8</td>
</tr>
<tr>
<td>Control subjects</td>
<td>24.8 (18)</td>
<td>3.4 (7)</td>
</tr>
<tr>
<td></td>
<td>10.3–32.6</td>
<td>2.6–4.7</td>
</tr>
</tbody>
</table>

of erythrocyte uroporphyrinogen decarboxylase activity.

The parents of two patients from the group with normal erythrocyte enzyme activities and no family history were also investigated: all four had normal erythrocyte enzyme activities with pentacarboxylic porphyrinogen III as substrate (21.9–30.7 pmol min⁻¹ mg⁻¹).

Porphyrin-excretion patterns

Urinary and faecal porphyrin measurements for the patients with porphyria cutanea tarda are shown
Two types of porphyria cutanea tarda

TABLE 2. Measurements of urinary and faecal porphyrin in patients with porphyria cutanea tarda

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>Urine</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uroporphyrin fraction (nmol/l)</td>
<td>Porphyria cutanea tarda index</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Isocoproporphyrin/coproporphyrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ratio</td>
</tr>
<tr>
<td>Porphyria cutanea tarda: skin</td>
<td>17</td>
<td>1130–7570</td>
<td>43.1–94.8</td>
</tr>
<tr>
<td>lesions</td>
<td></td>
<td>22–1182</td>
<td>22.6–88.6</td>
</tr>
<tr>
<td>Porphyria cutanea tarda: clinical remission</td>
<td>10</td>
<td>&lt;19</td>
<td>&lt;18.4</td>
</tr>
<tr>
<td>Control subjects</td>
<td>109</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Clinical features of patients with porphyria cutanea tarda

Patients with porphyria cutanea tarda are divided into groups as described in the Results section. Means with ranges in parentheses are given for age at onset.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>Age at onset (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Alcohol intake &gt;50 ml/day</td>
</tr>
<tr>
<td>Inherited decrease in erythrocyte enzyme activity</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Family history of overt porphyria cutanea tarda, erythrocyte enzyme activity normal</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>No family history of overt porphyria cutanea tarda, erythrocyte enzyme activity normal</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

in Table 2. Two patients in clinical remission had isocoproporphyrin/coproporphyrin ratios within the reference range but urinary porphyrin excretion was abnormal in all patients. Porphyrin excretion was normal in 26 of 31 asymptomatic relatives from families A–F. Five relatives had increased urinary porphyrin cutanea tarda indices (mean 42.1, range 22.4–86.2), two of these had increased urinary uroporphyrin fractions (54 and 468 nmol/l) and one had an abnormal isocoproporphyrin/coproporphyrin ratio (0.9). These relatives were considered to have subclinical porphyria cutanea tarda: three had low erythrocyte enzyme activities and one, from family E, had a normal activity; enzyme measurements were not made on the fifth (Fig. 2).

Porphyrin excretion was also measured in the parents of the two patients from the group with normal enzyme activities and no family history. Although all four had normal enzyme activities, one had subclinical porphyria cutanea tarda (uroporphyrin fraction 42 nmol/l; porphyria cutanea tarda index 52.6).

Clinical features

Some clinical features of the patients with porphyria cutanea tarda, grouped according to erythrocyte uroporphyrinogen decarboxylase activities and family studies, are compared in Table 3. Three of the eight patients from families A–D presented at an early age (3, 7, 16 years) without liver disease, but the other five were adults (aged 31–55 years) clinically indistinguishable from those in the other groups (Table 3).

Discussion

These results show that measurement of uroporphyrinogen decarboxylase in erythrocytes enables a type of familial porphyria cutanea tarda, in which low uroporphyrinogen decarboxylase activity in erythrocytes is inherited as an autosomal dominant characteristic, to be distinguished from other types of porphyria cutanea tarda, in which erythrocyte enzyme activity is
normal. In this respect they confirm the work of Verneuil et al. (1978), but conflict with other studies that have shown a decrease in erythrocyte enzyme activity in all patients with porphyria cutanea tarda irrespective of whether or not they have a family history of the condition (Kushner et al., 1976; Felsher et al., 1978; Tiepermann & Doss, 1978). The reason for this difference is uncertain. Enough patients with porphyria cutanea tarda have now been studied to make it unlikely that differences in the selection of patients can be the only explanation. The standard method for the measurement of uroporphyrinogen decarboxylase used by ourselves (Elder et al., 1978) and Verneuil et al. (1978) measures the decarboxylation of pentacarboxylic porphyrinogen III to coproporphyrinogen III, which is the final reaction in the sequence of four decarboxylations catalysed by this enzyme. Felsher et al. (1978) have suggested that this method may discriminate between different types of inherited enzyme defect whereas the technique that they used, which mainly measures the decarboxylation of uroporphyrinogen to heptacarboxylic porphyrinogen (Kushner et al., 1976; Felsher et al., 1978), does not. Although the possibility that different mutations may affect different stages of the decarboxylation sequence cannot be excluded at present, this type of explanation is difficult to reconcile with the finding that our method can detect decreased activity of the hepatic enzyme in patients in whom erythrocyte enzyme activity is normal (Elder et al., 1978). Furthermore patients can be separated into the same groups whether uroporphyrinogen (Table 1), heptacarboxylic porphyrinogen or hexacarboxylic porphyrinogen is used as substrate (Verneuil et al., 1978).

Gene expression is only one of the determinants of erythrocyte enzyme activity. The activity of many erythrocyte enzymes decreases as the cells age and an inherited decrease in activity may be obscured by an increase in the proportion of young cells in the circulation. Erythrocyte uroporphyrinogen I synthase activity declines as cells become older (Anderson, Sassa, Petersen & Kappas, 1977) and increased activities have been reported in liver disease (Blum, Koehl & Abecasis, 1978) and in some patients with porphyria cutanea tarda (Brodie et al., 1979). However, all except one of our patients were haematologically normal, erythrocyte uroporphyrinogen I synthase activities were not increased, and no patients with erythrocyte decarboxylase activities within the control range were re-classified by expressing results as a ratio of synthase activity. This suggests that none of these patients had an inherited enzyme defect obscured in this way.

A number of our patients with familial porphyria cutanea tarda had erythrocyte enzyme activities within the control range (Fig. 1). In acute intermittent porphyria, where a single gene defect leads to a 50% decrease in erythrocyte uroporphyrinogen I synthase activity, some 20% of patients have enzyme activities above the lower limit of the normal range (Lamon, Frykholm & Tschudy, 1979) and a similar overlap might be expected in familial porphyria cutanea tarda (Tiepermann et al., 1978). Our patients came from two families and investigation of these failed to show any evidence for the control of erythrocyte enzyme activity by an autosomal dominant gene, which contrasts with similar studies in acute intermittent porphyria (Lamon et al., 1979). It therefore seems possible that a second type of familial porphyria cutanea tarda may exist in which erythrocyte uroporphyrinogen decarboxylase activity is normal. However, further biochemical and genetic investigations are required to exclude the presence of an erythrocyte enzyme abnormality that is not detected by our assay.

Fifteen members of families A-D were identified as carrying the gene for porphyria cutanea tarda by demonstrating decreased erythrocyte uroporphyrinogen decarboxylase activity (Fig. 2). Eleven of these had overt or subclinical porphyria cutanea tarda, which indicates a gene penetrance of 73%. Similarly high penetrance has been found in most of the small number of other families with porphyria cutanea tarda in whom enzyme measurements have been made (Kushner et al., 1976; Benedetto et al., 1978; Verneuil et al., 1978). The rare cases of porphyria cutanea tarda in children are likely to come from such families (Table 3) (Verneuil et al., 1978). In adults, a family history is the only clinical feature that points to the possible presence of this type of porphyria cutanea tarda since these patients are otherwise clinically indistinguishable from those with normal erythrocyte enzyme activity (Table 3) (Kushner et al., 1976; Topi & D'Alessandro Gandolfo, 1977; Tiepermann et al., 1978). Absence of a family history of overt porphyria cutanea tarda does not necessarily exclude the presence of an erythrocyte enzyme defect (Fig. 1). However, Fig. 1 shows that most patients who present without a family history have normal erythrocyte uroporphyrinogen decarboxylase activity, which indicates that inheritance of an autosomal dominant gene that causes decreased activity of this enzyme in both
Liver and erythrocytes is an uncommon cause of porphyria cutanea tarda in this group of patients.

The cause of the decreased hepatic uroporphyrinogen decarboxylase activity in most patients with porphyria cutanea tarda remains to be determined. This enzyme defect seems to be a prerequisite for the development of porphyria cutanea tarda in response to conditions such as alcoholic liver disease with siderosis, rather than a consequence of them (Kushner et al., 1976; Benedetto et al., 1978; Elder et al., 1978). One possibility is that the uroporphyrinogen decarboxylase defect is inherited but restricted to the liver. Our finding of normal erythrocyte enzyme activities in families E and F is compatible with this concept. The existence of a form of porphyria cutanea tarda with low enzyme activity in erythrocytes and liver shows that uroporphyrinogen decarboxylase must be at least partly under the same genetic control in both tissues. Thus restriction of an inherited abnormality to the liver requires that the enzyme contains at least two structurally different sub-units of which only one is common to both tissues, or has a liver specific isoenzyme. Human erythrocyte uroporphyrinogen decarboxylase has been partially purified (Elder & Tovey, 1977), but the presence of subunits has not been established and as yet there is no evidence for the existence of isoenzymes. If this type of inherited defect is to explain most cases of porphyria cutanea tarda, the low incidence of a family history of overt or subclinical porphyria cutanea tarda suggests that the gene penetrance must be much lower than in the less common type with low erythrocyte enzyme activity.

Another possibility is that the enzyme defect is acquired and is caused by chemicals which inhibit the enzyme in the liver but not in the erythrocytes. Thus poisoning with hexachlorobenzene or 2,3,7,8-tetrachlorodibenzo-p-dioxin is known to cause porphyria cutanea tarda in humans (Elder, 1978), and selectively to decrease hepatic uroporphyrinogen decarboxylase in animals (San Martin de Viale, Rios de Molina, Wainstok de Calmanovici & Tomio, 1977), but is unlikely to account for the disease in more than a few patients. Nevertheless, there are inherited differences in the susceptibility of laboratory animals to the porphyrogenic action of these compounds (Elder, 1978), and it is possible that certain individuals may similarly be predisposed to develop porphyria cutanea tarda when exposed to low amounts of these or related chemicals that are widely distributed in the environment.

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References


