Porphyria variegata and porphyria cutanea tarda in siblings: Chemical and genetic aspects

(protocoprophyria/uro-isocoprophyria/“x” porphyrin/double heterozygosity)

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ABSTRACT

A woman aged 54 was studied because of a severe acute porphyric (neurolgic) relapse with clinical and chemical findings characteristic of porphyria variegata. During a family survey, her brother, aged 59, was found to have chemical abnormalities typical of porphyria cutanea tarda, without suggestion of neurologic manifestations. He had mild skin changes compatible with either of these forms of porphyria. The sister exhibited the protocoprophyria of porphyria variegata, together with a large amount of fecal "x" porphyrin fraction, without demonstrable isocoproporphyrins. The brother had a uro-isocopro-type of porphyria in accord with the diagnosis of porphyria cutanea tarda, and quite at variance with the sister's findings. This occurrence of porphyria variegata and porphyria cutanea tarda in siblings is thus far unique. Certain hypotheses are considered in respect to genetic aspects of the differing porphyrias in this sibling pair.

Porphyria cutanea tarda (PCT) and porphyria variegata (PV) are now generally recognized as entirely independent forms of porphyria. The genetic character of the latter is overt, that of the former relatively occult. PV was first known and is still at times referred to as South African genetic porphyria; while PCT has commonly been designated as "symptomatic" or "acquired." Nevertheless, we have observed PCT in 27 of 67 members examined, in 11 families (C. J. Watson, K. Ahmed, I. Bossenmaier, and R. Cardinal, in preparation). The same study reveals many other instances of familial occurrence in the literature. In addition to the foregoing families, 74 'sporadic' cases of PCT, apparently nonfamilial, were represented in the same roster of 464 cases of porphyria of all types observed since 1938. The relative infrequency of PCT in contrast to 'sporadic' PCT has often been ascribed to low penetrance of an autosomal dominant gene, and the phenotypic manifestations to "revealing" factors, notably alcohol, estrogens, iron overload, immunologic disturbances, and certain others (G. Gilmen, R. Cardinal, I. Bossenmaier, Z. J. Petryka, and C. J. Watson, in preparation).

The present report describes a sister and brother, the former studied first because of an almost fatal acute neurologic relapse related to chemically typical PV; the latter found to have overtaken chemical evidence of PCT with very mild cutaneous manifestations to which little attention had been given.

CASE PROTOCOLS

P430, male, 59. In the course of a detailed survey of the family of P430, it was discovered that her brother, P431, has characteristic chemical and clinical features of PCT. The latter are quite mild, consisting of distinct increase of mechanical fragility of the skin with mild epidermolysis and occasional vesicle formation, as a result of which there were a few faintly visible scars and some increase of pigmentation. The chemical data, characteristic of PCT, are also given in Table 1 under Results.

P435, a 24-year-old daughter of P430, also P447, a brother of P430 and P431, presented mild chemical evidence of latent PV, insofar as clinical and urinary porphyrin data were concerned. These four individuals are members of a large family which is being investigated as rapidly as urine and fecal samples are provided. The results of this study, when completed, will be described elsewhere (C. J. Watson et al., in preparation, see above).

MATERIALS AND METHODS

Urinary and fecal porphyrin determinations, on 24-hr collections and random samples, respectively, were carried out with the solvent fractionation method as described by Schwartz et al. (6), using ether, rather than ethyl acetate, for feces. The 5-ml modification for urine (7, 8) was used with ethyl acetate, to determine the "uroporphyrin" (7- and 8-COOH) and "coproporphyrin" fractions. The significance of these quotation marks is discussed below.

The porphyrin methyl esters of the "uroporphyrin"
(ether-insoluble) and the combined “copro” + “protoporphyrin” fractions from feces were separated by thin-layer chromatography on silica gel H (9) with Elder’s solvent system A (10). This is highly satisfactory in permitting recognition of the conventional porphyrins (2- to 8-COOH), and at the same time, the isocoproporphyrins, \( P_1 \) and \( P_2 \) (10, 11). The “uroporphyrin” fraction includes 6-, 7-, and 8-COOH porphyrins; “coproporphyrin”, in addition to coproporphyrin proper, includes the isocoproporphyrin series (see below). The “protoporphyrin” fraction includes small amounts of 3-COOH, together with the 2-COOH analogs, i.e., deuteromeso-, pempto-, and hemato-, which are readily distinguished by the Henderson-Morton method (see below). Spectrodensitometric recording of chromatograms was done with a Schoeffel Unit, model SD 3000 (Schoeffel Instrument Co., Westwood, N.J.). Alternatively, the copro- and isocoproporphyrin spots were eluted separately and quantitated fluorimetrically using the coproporphyrin standard (6). Rimington’s uresa-Triton method of extraction of the “x” porphyrin fraction from the feces of P430 (2) was unsuccessful, also after treatment with \( \text{Ag}_2\text{SO}_4 \), as according to Paul (12), intended to split a thio-ether linkage (2). The method of Hill and Keilin (13) in which \( \text{HBr} \) is used to split porphyrin \( c \) from cytochrome \( c \), was then applied to obtain the ether-soluble porphyrin component of the fecal “x” porphyrin complex, which was subjected to the Henderson-Morton method of distinction of 2-COOH porphyrins (14).

Tetracarboxylate Isocoproporphyrin Series. Elder (10, 11) observed that \( P_1 \) is a mixture of 1a and 1b, ethyl and deethylisocoproporphyrin, respectively. Both are believed formed by the action of intestinal bacteria on a primary dehydroisocoproporphyrin of hepatic origin, which Elder has observed in the bile and in trace amounts in the feces (11) of patients with PCT and in the liver bile in the experimental analog, chronic hexachlorobenzene poisoning in rats (10, 11). This monovinyl isocoproporphyrin is believed derived from the 5-COOH porphyrinogen (10, 11), having an acetate in the 5 position (15). \( P_2 \), an hydroxyethylisocoproporphyrin (16), may be derived from dehydroisocoproporphyrin by hydration, or possibly by hydrolysis of an hypothetical conjugate (10). Evidence of an hydroxy group in \( P_2 \) was provided by acetylation (17) and rechromatography (10).

The basis of the proposed deviation at the 5-COOH porphyrinogen stage has not been ascertained. Its apparent specificity for PCT, at least among the hepatic porphyrinas, is highly interesting. The development of manifest PCT is believed to depend on “revealing” factors, of which alcohol is the most frequent. Most alcoholics, however, do not form or excrete the isocoproporphyrin series (G. Gülmen et al., in preparation; see above), hence it appears that the genetic “weak spot” may be at this stage, and as Elder has suggested, the enzyme coproporphyrinogenase may be involved in the deviation or alternate pathway, as the case may be (10, 11). This enzyme is essential to the normal conversion of coproporphyrin to protoporphyrin, and quite possibly as well to that of the 5-COOH porphyrinogen to the dehydroisocoproporphyrin. For further details, Elder’s papers (10, 11, 15, 16) may be consulted.

**RESULTS**

Data for the urine and fecal porphyrins from P430 and P431 are given in Table 1, together with the normal range of values as previously documented (8, 18). The spectrodensitometric recording after thin-layer chromatography is shown in Fig. 1. It is seen that the findings in P430 are characteristic of PV (protoporphyrinogen + “x”-porphyrin peptide), those in P431 of PCT (uro-isocoproporphyrin). As mentioned in the foregoing, feces from P430 contained a large amount of ether-insoluble, tightly bound “x” porphyrin fraction (2), which, however, was only extractable in very small proportion by the urea-Triton solution (see Materials and Methods), the residual feces continuing to fluoresce intensely red in UV light. As recently emphasized (19, 20), and in accord with our own observations, the urea-Triton solution extracts ether-insoluble porphyrins indiscriminately.

The value for “x” porphyrin by Rimington’s method (2) was 3.4 \( \mu g/g \) dry wt. of feces, well within his normal range. Rimington and coworkers (2) described evidence that the “x” porphyrin was a peptide complex. We have not attempted to identify a binding peptide, but consider it probable that a peptide was involved despite the failure of the urea-Triton to extract the complex, and of \( \text{Ag}_2\text{SO}_4 \) intended to split a thio-ether linkage (12), to yield hematoporphyrin. Nevertheless, HBr liberated the bound porphyrin, which

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**Table 1. Chemical data for P430 and P431 and their respective asymptomatic relatives, P435 and P447**

<table>
<thead>
<tr>
<th></th>
<th>P430</th>
<th>P435</th>
<th>P431</th>
<th>P447</th>
<th>Normal&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine “uroporphyrin” (( \mu g/\text{day} ))</td>
<td>289</td>
<td>66</td>
<td>4331</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>“coproporphyrin” (( \mu g/\text{day} ))</td>
<td>1043</td>
<td>120</td>
<td>360</td>
<td>62</td>
<td>290</td>
</tr>
<tr>
<td>Feces “uroporphyrin” (( \mu g/\text{dry wt.} ))</td>
<td>188</td>
<td>18</td>
<td>184</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>“coproporphyrin” (( \mu g/\text{dry wt.} ))</td>
<td>1064</td>
<td>109</td>
<td>248</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>“protoporphyrin” (( \mu g/\text{dry wt.} ))</td>
<td>1451</td>
<td>145</td>
<td>40</td>
<td>184</td>
<td>107</td>
</tr>
<tr>
<td>“isocoproporphyrin” (( \mu g/\text{dry wt.} ))</td>
<td>0</td>
<td>0</td>
<td>159</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>“x”-porphyrin peptide (( \mu g/\text{dry wt.} ))</td>
<td>3.4*</td>
<td>23</td>
<td>3.0</td>
<td>6.5</td>
<td>8.0</td>
</tr>
</tbody>
</table>

For explanation of material in quotation marks, see Materials and Methods.

* “x”, fraction in feces in large amount, unextracted by urea-Triton solution (see text).
was then identified as hematoporphyrin (see Materials and Methods). In all likelihood, this was derived from a native protoporphyrin peptide, in accord with the foregoing, and with Rimington's observations (2). Why it was not extracted by the urea-Triton solution, nor affected by Ag$_2$SO$_4$, is not clear.

Careful study of the feces of P430 by Elder's methods (see above) failed to demonstrate the presence of an isocoproporphyrin. This was in marked contrast with P431 whose feces, in addition to a considerable excess of "uroporphyrin" contained P$_1$ and P$_2$ (see above), the latter in smaller amount. The P$_2$ behaved entirely as the hydroxyisocoproporphyrin, with the characteristic change in thin-layer chromatography behavior, on acetylation (10).

**DISCUSSION**

The striking differences in the various porphyrin data easily served to distinguish the two forms of porphyria, PV in P430, PCT in P431. The clinical features are in accord. Special note may be taken of the respective presence and absence in P431 and P430 of the isocoproporphyrin series in the feces of these siblings. This finding reinforces the distinction of the two forms on the basis of the conventional porphyrin data, especially the large excesses of 8- and 7-COOH porphyrin ("uroporphyrin" group in PCT, as contrasted with PV). Our results in the study of additional cases of these and other forms of porphyria confirm Elder's belief that insofar as hepatic porphyria is concerned, the isocoproporphyrin series is characteristic of PCT. Nevertheless, smaller though significant amounts of P$_1$ have now been encountered in feces from patients with hepatic acute intermittent porphyria. This is to be described elsewhere. Also, others have observed members of the series in an atypical case of congenital erythropoietic porphyria (21, 22). We have identified P$_2$ in the feces in three typical examples of the latter disease, a finding also to be reported separately. In these cases, however, P$_2$ was not accompanied by P$_1$, whereas, the latter is the dominant isocoproporphyrin in PCT. The basis of this difference has not been determined. While it has been suggested that the isocoproporphyrin series is purely hepatogenous, even in congenital erythropoietic porphyria (21, 22), a normoblastic origin in this disease may deserve consideration.

The genetic problem

The genetic aspect of the present sibling pair poses important questions. Three possibilities may be considered briefly. (i) It might be assumed that the PCT of P431 is acquired rather than genetic in origin. As mentioned at the outset, there are compelling reasons for the belief that PCT is an "occult" genetic disease, possibly because of low penetrance of an autosomal dominant gene. It might be questioned whether some cases are acquired, others of genetic origin, but this only narrows the same difficulty encountered in considering PCT at large, as acquired or "symptomatic." There is general agreement that the occurrence of PCT is relatively frequent in association with chronic alcoholism, yet most alcoholics do not develop PCT. Waldenström and Haeger-Aronsen (23) found that among 360 cases of alcoholic cirrhosis gathered by Hallén and Krook (24), there were only seven of PCT. Among 60 chronic alcoholics recently studied by Gulmen et al. (see above), only one had the characteristic chemical findings of PCT, i.e., marked excess of "uroporphyrin" in the urine, red fluorescence of the liver biopsy in UV light due to great excess of porphyrin, and the presence of isocoproporphyrin in the feces. Despite this outspoken evidence of PCT, the porphyria was still latent, no skin lesions having been observed.

In the following, we shall regard PCT as a conditioned genetic or constitutional disease and refer to a hypothetical gene for PCT as contrasted with one for PV, the genetic character of which is well established (3-5).

(ii) One might consider that P430 is heterozygous for PV, P431 for PCT. In this connection, With (25) has recently proposed that PV and PCT simply represent two alleles among many. This theory, however, may be inadequate in explaining the unique character of the present sibling pair.

(iii) A genetic heterogeneity represented by a double heterozygosity is an interesting possibility in which differing mutant alleles, at the same or differing loci, would relate respectively to heterozygosity for PV and for PCT in P431, while the sister and index case, P430, is believed heterozygous for a PV and for a normal gene. A double heterozygosity would be analogous to S/C disease or other, similar hemoglobinopathies. Recently, a somewhat analogous situation has been described in which genetic heterogeneity was evident in two forms of familial hypercholesterolemia (26). In these forms of double heterozygosity, however, there is no need to postulate competition for substrate for the respective gene products, but in PCT and PV, the early and late segments of the porphyrin and heme biosynthetic pathway, respectively, are involved. In PCT, the early segment is abnormal in respect to decarboxylation from uroporphyrin to coproporphyrin, at the 5-COOH, 8- and 4-COOH, including a specific, major deviation from the 5-COOH porphyrinogen to the isocoproporphyrin series (10, 11, 27-30). This might be expected to minimize the excessive formation of coproporphyrin and protoporphyrin (4- and 2-COOH) as other pairs for PV. In PCT the fecal protoporphyrin ratios are generally normal or low. Thus, it is reasonable to believe that a partial failure of decarboxylation of uroporphyrinogen to the 5-COOH porphyrinogen and diversion of the latter to isocoproporphyrin (10, 11) imposes sufficient interference in the formation of coproporphyrinogen and protoporphyrin to produce an excretory porphyrin pattern more in accord with PCT than PV. In this connection, we have reported elsewhere (31) that the isocoproporphyrin/coproproporphyrin ratio studied serially in the feces of a case of PCT varied in a range of 2.5-6.5/1.0, which points to a highly significant reduction in substrate for the formation of protoporphyrin. In Fig. 1 this ratio is also evident in the data for P431 as compared with P430. In the foregoing latent cases, P435 and P447, especially the latter, because he is comparable in age with P431, alcoholism might be expected to reveal a PCT gene, if present, and thus produce a phenotype indistinguishable from that of P431, even though P447's phenotype is in accord with latent PV. There is reason to believe that P447 is a mild chronic alcoholic, but it is quite likely that he, like his sister, P430, is heterozygous for PV and a normal gene, but not for PCT. As noted above, a detailed examination of additional members of this family is still in progress. Up to the present, 23 of 27 living family members have been screened for porphyria, revealing six cases, including P430 and P431. Five of these six had PV as in P430, the index case, only P431 exhibiting the chemical changes characteristic of PCT. Our experience with families of PCT and their relative infrequency, has already been mentioned (see above) in connection with the question of low penetrance of a dominant gene.
The effect of a “double heterozygosity” on the PV phenotype, when the PCT gene is revealed by chronic alcoholism or by other “revealing agents,” must for the present, remain purely speculative.

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