Plasma Fluorescence Scanning and Fecal Porphyrin Analysis for the Diagnosis of Variegate Porphyria: Precise Determination of Sensitivity and Specificity with Detection of Protoporphyrinogen Oxidase Mutations as a Standard

Richard J. Hift,* Brandon P. Davidson, Cornelis van der Hooft, Doreen M. Meissner, and Peter N. Meissner

Background: Variegate porphyria (VP) is the autosomal dominant disorder associated with deficiency of the enzyme protoporphyrinogen oxidase (PPOX). Plasma fluorescence scanning has been reported to be a more sensitive test for VP than traditional fecal chromatography. Previous comparisons of these techniques predated identification of the PPOX gene. We assessed these techniques in a large group of patients characterized for VP at the DNA level.

Methods: We evaluated all patients for whom the genotype and a plasma scan or fecal porphyrin result were available. Mutations were detected by restriction digest analysis. Plasma fluorescence scanning was conducted according to published methods. Fecal porphyrins were identified and quantified by thin-layer chromatography.

Results: Plasma fluorescence scanning was assessed in 679 patients (205 with VP who were carriers of a PPOX mutation, either with disease symptoms or asymptomatic) and fecal analysis in 473 (190 with VP). Sensitivity and specificity of both tests were higher in adults than in children and higher for adults with disease symptoms than for asymptomatic carriers. In a direct comparison in 168 adults (73 with VP), plasma scanning was significantly more sensitive than fecal porphyrin analysis [sensitivity, 0.96 (95% confidence interval, 0.89–0.99) vs 0.77 (0.66–0.85)]. Fecal coproporphyrin [area under the curve, 0.87 (0.83–0.90)] was a better predictor of VP than protoporphyrin [0.80 (0.76–0.84)].

Conclusions: Plasma scanning is a more sensitive and specific test for VP than fecal porphyrin analysis. Neither test is sensitive in children, and both are less sensitive in asymptomatic carriers than in symptomatic cases. DNA analysis therefore remains the preferred method for the identification of carriers, particularly in children.

© 2004 American Association for Clinical Chemistry

Variegate porphyria (VP)¹ is the autosomal dominant disorder associated with deficiency of the enzyme protoporphyrinogen oxidase (PPOX), the penultimate enzyme of the heme synthetic pathway (1). PPOX activity is decreased by ∼50% in patients with VP (2). Penetration is low, however. Clinically, patients with VP may manifest a photodermatitis characterized by skin fragility, erosions, blisters, milia, and chronic pigmented changes in sun-exposed areas, particularly the backs of the hands (3). These individuals are also at risk for acute attacks of porphyria, characterized by episodes of severe abdominal pain, autonomic disturbance, and a motor neuropathy that may progress to flaccid quadriplegia and death (3). Acute attacks frequently result from exposure to a range of drugs that share the ability to stimulate heme synthesis by induction of δ-aminolevulinic acid synthase; if these are avoided, the risk of an acute attack is lessened.

¹ Nonstandard abbreviations: VP, variegate porphyria; PPOX, protoporphyrinogen oxidase; AIP, acute intermittent porphyria; PCT, porphyria cutanea tarda; AUC, area under the curve; and CI, confidence interval.
Important biochemical features of VP include the increased biliary excretion of coproporphyrinogen and protoporphyrinogen and their corresponding porphyrins (4), which are commonly measured by fecal porphyrin analysis. When VP is fully manifested biochemically, fecal coproporphyrin and protoporphyrin are increased and pentacarboxylic porphyrin and pseudopentacarboxylic porphyrin, a hydroxylated dicarboxylic porphyrin (5), are frequently present. Chromatographically determined porphyrin isomer ratios may be disturbed, typically with increases in the fecal coproporphyrin III fraction (6, 7).

When VP is biochemically quiescent, urine porphyrin concentrations are frequently within reference values, and analysis of urine alone may cause the diagnosis of VP to be missed (8). The standard biochemical test for the diagnosis of VP involves chromatographic separation and quantification of fecal porphyrins, and is supplemented with urine and plasma porphyrin analysis and δ-aminolevulinic acid and coproporphyrinogen III measurements for confirmation of the acute attack and for exclusion of other forms of porphyria, such as acute intermittent porphoria (AIP) and porphyria cutanea tarda (PCT).

Identification of patients with overt VP is critical because treatment depends on an accurate diagnosis. Identification of asymptomatic relatives is similarly important because with proper education, particularly with regard to avoidance of porphyrinogenic drugs, the risks of developing the acute crisis can be reduced. Diagnosis may be problematic for two reasons, however. The first reason is that fecal porphyrin measurement does not detect all carriers (9). This is particularly true in children because rates of biochemical manifestation are lower before puberty (10–12). The second reason is that biochemical testing for porphyria by quantitative means may in some cases produce intermediate results, thus failing to achieve a clear separation of individuals with VP from those who do not have the disorder.

Our porphyria reference laboratory serves a large population of patients with VP, the result of a founder mutation introduced to South Africa in 1688 (13). Chromatographic fecal porphyrin analysis for the identification of VP has been offered routinely in our laboratory since 1974. Until recently, however, we lacked a gold standard for diagnosis based on the underlying genetic abnormality, which has meant that the sensitivity and specificity of the stool porphyrin analyses we use have not been known precisely.

Plasma fluorescence scanning (14) has been suggested as a more robust test than fecal porphyrin analysis for the diagnosis of VP. Patients with VP have a sharply defined fluorescence emission maximum at an excitation wavelength of 626 ± 1 nm (15); the presence of this characteristic peak efficiently separates patients with VP from those with AIP, erythropoietic protoporphoria, congenital erythropoietic porphyria, PCT, and hereditary coproporphyria (15, 16). The fluorescence maximum in the plasma samples of patients with VP does not correspond to any native porphyrin. Analysis has suggested that this fluorescence results from a mixture of porphyrins, including a mixture of uroporphyrin, harderoporphyrin, isoharderoporphyrin, protoporphyrin, and pentacarboxylic porphyrin loosely bound to proteins, coupled with a second group that may be a derivative of a dicarboxylic porphyrin tightly bound to albumin (14, 17).

The potential usefulness of plasma scanning and fecal porphyrin analysis for the diagnosis of asymptomatic carriers has been addressed in two studies (9, 12); because these predated the identification of the PPOX gene and the mutations responsible for VP, carrier status was inferred, either from statistical estimates of carrier frequency (9) or direct measurement of lymphocyte PPOX activity (12).

Identification of the cDNA for PPOX (18) and recognition that the founder R59W mutation accounts for 95% of all South African patients with VP (19, 20) have enabled the assignment of an accurate genotypic diagnosis in our patients and has in turn allowed us to determine accurately the sensitivity and specificity of biochemical testing in our population. Our findings are reported here.

**Materials and Methods**

**Patients**
We studied all individuals referred to our laboratory for porphyria diagnosis for whom both a DNA result and appropriate biochemical results (plasma scanning, fecal porphyrin chromatography, or both) were recorded (Table 1). These samples were received over the period 1997–2003, during which time our laboratory methodology remained unchanged. For the purposes of this study, all individuals 16 years or older are labeled “adult”, whereas those <16 years are labeled “children”.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the individuals studied.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic without FH*</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Mean (range) age, years</td>
</tr>
<tr>
<td>Age ≥16 years, n</td>
</tr>
<tr>
<td>Plasma scan performed, n</td>
</tr>
<tr>
<td>Fecal analysis performed, n</td>
</tr>
<tr>
<td>Both tests performed, n</td>
</tr>
</tbody>
</table>

FH, family history of VP.
CLINICAL DATA

Our laboratory serves as a national reference laboratory. Samples are referred to us from a large number of clinicians and laboratories, usually with some clinical information. To determine the relationship between test performance and clinical manifestation of VP, we reviewed all accompanying clinical information and where possible classified the individual as either symptomatic or asymptomatic. We made the assignment only if we believed the clinical data were reliable, either because individuals were known personally to us or because clinical information was sufficiently detailed and congruent—particularly in terms of the description of clinical symptoms—to allow a reasonable assessment to be made. A history considered indicative of symptomatic VP was one of typical photosensitivity, with blistering, scarring, and poor healing in sun-exposed areas, or an acute attack confirmed by the presence of increased urinary porphobilinogen concentrations. Individuals assigned the label “asymptomatic” were those in whom it was clearly stated that they were being investigated on the basis of a family history of VP alone. All other samples were excluded from the subgroup analysis designed to study the effect of presentation on test sensitivity.

MUTATION DETECTION

The uniquely high prevalence of the R59W PPOX mutation in our population justified our testing for this mutation routinely. Because this mutation abolishes an AvaI restriction cutting site, restriction analysis was performed on the exon 2 PCR product as described previously (19). Two other mutations were encountered in this series. A 537delAT mutation was detected by MspI restriction digestion of an amplified exon 6 product (21), and a c769delG; 770T>A mutation was identified by DNA sequencing of a PCR amplification product of exon 7.

PLASMA FLUORESCENCE SCANNING

Plasma was scanned for porphyrin fluorescence by the method of Poh-Fitzpatrick (15). Plasma was separated from blood collected in heparin or EDTA by centrifugation. After centrifugation, 1 mL of plasma was diluted with 9 mL of phosphate-buffered saline (9 g/L NaCl, 0.01 mol/L sodium phosphate, pH 7.4). Samples were transferred to 1-cm path-length optical glass cuvettes for spectrofluorometry. A fluorescence spectrophotometer (Hitachi 650-109 fluorescence spectrophotometer; Hitachi Koki Co. Ltd) equipped with a red-sensitive photomultiplier and chart recorder, was zeroed against phosphate-buffered saline at an excitation wavelength of 405 nm and emission wavelength of 620 nm, with the monochromator slit width set at 5 nm. The emission spectrum was then scanned from 580 to 650 nm with the excitation monochromator set at 405 nm. For the purposes of correlation with total porphyrin concentrations in plasma, urine, and stool, the peak height was scored semiquantitatively as “negative”, “low-positive”, or “high-positive”, using standard and reproducible settings.

CHROMATOGRAPHY

A thin-layer chromatographic technique was used. Urine and stool samples were prepared as follows: 3 mL of urine or ~300 mg of stool was esterified in 30 mL of a 50 g/L sulfuric acid–methanol solution overnight at room temperature in the dark. The stool esterification mixture was then centrifuged at 800g for 50 min. The dry weight:wet weight ratio of the stool sample was calculated on a separate aliquot. The esterification mixture was transferred to a separating funnel, and the pH was adjusted to neutral with a solution containing 170 g/L ammonia. The dissolved porphyrins were then extracted three times into a total volume of 20 mL of chloroform. Where large amounts of porphyrin were present, more chloroform was used. All volumes used were noted.

Immediately before chromatography, the volume of the porphyrin extract in chloroform was noted. Precise aliquots of each extract (30–50 µL of stool extract and 100–300 µL of urine extract) were applied as a spot on Merck Silica Gel-60 thin-layer chromatography plates by use of a Hamilton microsyringe. The exact volume applied was noted. A constant stream of warm air was blown over the working area to aid evaporation of the chloroform during application to the plate. On each plate, two lanes were reserved for porphyrin methyl ester calibrators (equal amounts of uroporphyrin, heptacarboxylic porphyrin, pentacarboxylic porphyrin, coproporphyrin, and mesoporphyrin methyl esters; Porphyrin Products).

Plates were placed upright in a bath containing 180 mL of separating solvent (carbon tetrachloride–dichloromethane–ethyl acetate–ethyl propionate, 2:2:1:1 by volume). After ~20 min, when the solvent front approached the top of the plate, the plate was removed and dipped briefly in a fluorescence-enhancing solution (chloroform–dodecane–hexadecane, 3:1:1 by volume) (22). The plates were then scanned on a fluoroscaning photodensitometer (TLD 100; Vitatron) connected to an integrator and recorder unit (SP 4290; Spectra-Physics). Excitation and emission filters were set to achieve wavelengths of 399–410 and 610–630 nm, respectively. Individual porphyrin esters were identified by direct comparison of the retention times with those of the calibrators. The area under the curve (AUC) for each peak of fluorescence was calculated by the integrator and compared directly with those of the calibrators on the same plate to give quantitative results.

All appropriate data, including the fluorescence detected, the fluorescence associated with each calibrator, the volume of sample applied to the plate, and the volume or mass of initial sample, were entered into a computerized porphyrin spreadsheet/database, which then calculated the concentrations of the individual porphyrins in the individual samples.
DIAGNOSTIC INTERPRETATION BY AN EXPERIENCED OBSERVER

One of us (P.N.M.) was presented with quantitative fecal chromatographic data from individuals for whom DNA status was available. All other information was withheld. He was required to flag each sample as being diagnostic of VP, unequivocally not VP, or indeterminate. The first category included all samples with increased fecal amounts of protoporphyrin, coproporphyrin, pentacarboxylic porphyrin, and pseudopentacarboxylic porphyrin. The second group included all those individuals in whom all measured porphyrin amounts were within the reference intervals. The indeterminate group included those individuals in whom one fecal porphyrin species was increased but who did not have the diagnostic pattern of increased values for all four porphyrin species. For determination of sensitivity and specificity, we explored the effects of defining strict criteria for VP, in which case all indeterminate results were classified as negative, and lenient criteria, in which all indeterminate results were classified as positive.

STATISTICAL ANALYSIS

Data were analyzed by use of Access and Excel software (Microsoft Office 2000; Microsoft Corporation). Statistical comparisons were performed with the Statistica 6.1 (StatSoft Inc.) and MedCalc (Ver. 7.2.0.0; MedCalc Software) software. The Fisher exact test was used for the comparison of categorical variables. Fecal porphyrin concentrations were compared by the Mann–Whitney U-test. The height of the plasma scan peak, measured on a semi-quantitative scale, was correlated with age and with porphyrin concentrations by Kruskal–Wallis ANOVA. Confidence intervals (CIs) for sensitivity and specificity were calculated by the Wilson score method and odds ratios were determined by the Newcombe–Wilson method with use of the Confidence Interval Calculator, Ver. 4 (Herbert R, http://ptwww.chs.usyd.edu.au/Pedro/CICalculator.xls; accessed July 18, 2003). All CIs are 95% intervals.

Results

PLASMA FLUORESCENCE SCANNING

A plasma scan and a DNA result were available for comparison in 679 individuals, of whom 205 carried a VP-associated PPOX mutation: R59W (n = 190), 537delAT (n = 14), or c769delG; 770T>A (n = 1). The sensitivity and specificity of plasma scanning were calculated for the entire group and then separately for children and adults. Reliable clinical information was available on 138 adults. Sensitivity and specificity were recorded separately for those reported to have experienced symptoms in the absence of a family history, those reported to have experienced symptoms who were aware of VP in the family, and for those asymptomatic individuals investigated on the basis of a family history only (Table 2).

We recorded a single false-positive result: a 70-year-old woman, known to belong to a well-studied family, carrying a 537delAT mutation. She carries neither this mutation nor the common R59W mutation; it is possible, although unlikely, that she carries an as yet undiscovered third mutation. One individual positive for the R59W mutation, who had strong characteristics of VP on biochemical testing, had a significant plasma porphyrin peak at 619 nm. There was no biochemical evidence to support a diagnosis other than VP; in particular there was no evidence of AIP, hereditary coproporphyria, PCT, or a dual porphyria in which certain features of PCT, notably high concentrations of urinary uroporphyrin and heptacarboxylic porphyrin and fecal isocoproporphyrin, accompany the biochemical features of VP (23). The reason for the position of the peak at 619 nm rather than 625 nm is unknown.

Of 22 others who carried a PPOX mutation but had no plasma porphyrin fluorescence peak and whose fecal porphyrin results were available, none had evidence of VP by fecal analysis.

There was a clear correlation between the height of the plasma peak assessed semiquantitatively (negative, low-positive, and high-positive) and chromatographically determined porphyrin concentrations. We observed significant correlations with total plasma porphyrin (P = 0.013), plasma protoporphyrin (P = 0.014), and plasma peak unknown (an unusual dicarboxylic porphyrin peptide migrating immediately before coproporphyrin in the plasma of patients with VP; P = 0.014); with total urine porphyrins (P = 0.04) and urine coproporphyrin (P = 0.006); and with total fecal porphyrin (P < 0.0001), protoporphyrin (P ≤ 0.0001), coproporphyrin (P < 0.0001), pentacarboxylic porphyrin (P < 0.0001), hexacarboxylic porphyrin (P = 0.04), and heptacarboxylic porphyrin (P =

<table>
<thead>
<tr>
<th>Table 2. Plasma scan results in 679 individuals with or without a VP-associated PPOX mutation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>PPOX mutation present, n</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>Specificity</td>
</tr>
</tbody>
</table>

* 95% CIs are shown in parentheses.
We observed no significant correlations with those porphyrin species not detailed above.

In the few instances where peaks were observed in children, peak height was significantly lower than in adults \((P = 0.015)\).

**CHROMATOGRAPHIC FECAL PORPHYRIN ANALYSIS**

Stool porphyrin profiles were analyzed in 190 adults carrying a VP-associated mutation (R59W mutation 180, 537delAT mutation 10) and 283 not carrying mutations. Fecal pentacarboxylic porphyrin, pseudopentacarboxylic porphyrin, coproporphyrin, and protoporphyrin concentrations were significantly higher in the VP group than in the group not carrying the mutations (Table 3). However, the diagnostic utility of this result was diminished by the large overlap in concentrations between the VP group and the control population; for coproporphyrin and protoporphyrin, this overlap amounted to \(\sim 25\%\) of cases in each group. ROC curves were constructed for coproporphyrin and protoporphyrin (Fig. 1). The AUC were 0.87 (95% CI, 0.83–0.90) for fecal coproporphyrin, 0.86 (0.82–0.89) for pseudopentacarboxylic porphyrin, and 0.80 (0.76–0.84) for fecal protoporphyrin. The AUC for both coproporphyrin \((P < 0.001)\) and pseudopentacarboxylic porphyrin \((P = 0.011)\) were significantly larger than the AUC for protoporphyrin.

These results were independently confirmed by stepwise discriminant analysis, which identified stool coproporphyrin, pseudopentacarboxylic porphyrin, heptacarboxylic porphyrin, and hexacarboxylic porphyrin as more useful predictors of a VP-associated mutation than protoporphyrin. There was a large measure of redundancy between these porphyrins. Partial Wilk \(\lambda\) values for fecal coproporphyrin, pseudopentacarboxylic porphyrin, and protoporphyrin were 0.952, 0.961, and 0.999, respectively, and any gain in sensitivity achieved by combining two or more of these values was marginal.

Reference to the ROC curve for coproporphyrin suggests a coproporphyrin concentration of 70 nmol/g dry weight as the optimum diagnostic threshold. We assessed the sensitivity and specificity of fecal porphyrin analysis for the diagnosis of VP with this cutoff. The sensitivity and specificity of fecal porphyrin analysis were determined for the entire group and then separately for children and adults. Reliable clinical information was available on 138 adults. Sensitivity and specificity were recorded separately for those reported to have experienced symptoms in the absence of a family history, those reported to have experienced symptoms who were aware of VP in the family, and those asymptomatic individuals investigated on the basis of a family history only (Table 4).

**DIRECT COMPARISON**

Direct comparison of plasma scanning and fecal porphyrin analysis was possible in 168 adults, of whom 73 carried a VP-associated \(PPOX\) mutation, for whom both tests were available. The sensitivity and specificity of plasma scanning were 0.96 (95% CI, 0.89–0.99) and 0.99 (0.94–1.00); for fecal coproporphyrin estimation, sensitivity and specificity were 0.77 (0.66–0.85) and 0.92 (0.84–0.96). Plasma scanning was significantly more sensitive than fecal analysis (odds ratio for positive test, 7.08; 95% CI, 1.98–25.4; \(P = 0.001)\). Addition of fecal analysis to plasma scanning did not increase the sensitivity obtained for plasma scanning alone.

![Fig. 1. ROC curves for fecal coproporphyrin (COPRO) and protoporphyrin concentrations (PROTO) in adults.](image)

### Table 3. Median (10th–90th percentile range) fecal porphyrin concentrations in 283 adults DNA-negative and 190 adults DNA-positive for a VP-associated \(PPOX\) mutation.

<table>
<thead>
<tr>
<th>DNA result</th>
<th>Pentacarboxylic porphyrin</th>
<th>Pseudopentacarboxylic porphyrin</th>
<th>Coproporphyrin</th>
<th>Protoporphyrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PPOX) mutation absent</td>
<td>0.0 (0.0–19.0)</td>
<td>0.0 (0.0–0.0)</td>
<td>26.7 (9.6–63.3)</td>
<td>114.8 (44.1–367.8)</td>
</tr>
<tr>
<td>(PPOX) mutation present</td>
<td>10.4 (0.0–42.3)</td>
<td>29.9 (0.0–92.3)</td>
<td>137.9 (27.3–382.6)</td>
<td>339.9 (100.6–1096.5)</td>
</tr>
<tr>
<td>(P)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(a\) An atypical dicarboxylic porphyrin appearing immediately before pentacarboxylic porphyrin on thin-layer chromatography.
Table 4. Accuracy of fecal coproporphyrin, based on a diagnostic threshold of 70 nmol/g dry weight, in predicting the presence of a VP-associated PPOX mutation.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>All adults</th>
<th>All children</th>
<th>Adults with clinical features of VP</th>
<th>Adults with symptoms and family history</th>
<th>Asymptomatic adults with a family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n</td>
<td>473</td>
<td>423</td>
<td>39</td>
<td>34</td>
<td>23</td>
<td>81</td>
</tr>
<tr>
<td>PPOX mutation present, n</td>
<td>190</td>
<td>179</td>
<td>8</td>
<td>11</td>
<td>11</td>
<td>58</td>
</tr>
<tr>
<td>Sensitivity(^a)</td>
<td>0.71 (0.64–0.77)</td>
<td>0.74 (0.67–0.80)</td>
<td>0.00 (0.00–0.32)</td>
<td>1.00 (0.74–1.00)</td>
<td>0.73 (0.48–0.89)</td>
<td>0.59 (0.46–0.70)</td>
</tr>
<tr>
<td>Specificity(^a)</td>
<td>0.92 (0.88–0.95)</td>
<td>0.91 (0.87–0.94)</td>
<td>0.98 (0.83–0.99)</td>
<td>1.00 (0.68–1.00)</td>
<td>1.00 (0.86–1.00)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 95% CIs are shown in parentheses.

**DIAGNOSTIC INTERPRETATION BY AN EXPERIENCED OBSERVER**

This exercise was undertaken to determine whether diagnosis based on fecal coproporphyrin concentrations in isolation was any less sensitive than interpretation of the whole fecal profile, including markers such as pseudopentacarboxylic porphyrin, pentacarboxylic porphyrin, and protoporphyrin, by an experienced observer. Of 221 profiles, 44 results (19.9%) were classified as indeterminate, i.e., “suggestive of VP”. This indeterminate group was nearly evenly divided between true positives and true negatives. When we used strict criteria, sensitivity was 0.69 (0.59–0.76) and specificity was 0.973 (0.92–0.99). When we used lenient criteria, the sensitivity improved to 0.87 (0.80–0.92), whereas the specificity decreased to 0.76 (0.68–0.83). This offered no advantage over the use of coproporphyrin concentration alone.

**EFFECT OF AGE ON SENSITIVITY**

The sensitivity of both plasma scanning and fecal porphyrin analysis was significantly lower in children than in adults (P < 0.0001). Fecal analysis failed to identify VP in any of 39 DNA-positive children and gave one false-positive result.

The sensitivities of plasma scanning and fecal porphyrin analysis are tabulated by decade in Table 5. Compared with adults older than 40 years, adults <40 years were more likely to have a positive fecal coproporphyrin result (odds ratio, 2.60; 95% CI, 1.27–5.30; P = 0.007) and a positive scan result (odds ratio, 2.33; 95% CI, 0.98–5.54; P = 0.04).

We noted a general trend for total porphyrin concentrations to decrease with increasing age in adults carrying a VP mutation. In DNA-positive individuals 16 years or older, there was a significant negative correlation between age and total stool porphyrin concentration [r = −0.16 (95% CI, −0.30 to −0.01); P = 0.03] and a nonsignificant negative correlation with total urine porphyrin concentration [r = −0.04 (−0.21 to 0.13); P = 0.7] and total plasma porphyrin concentration [r = −0.25 (−0.61 to −0.20); P = 0.27]. We noted an overall trend to decreasing height for the plasma peak with age, although this did not reach statistical significance (P = 0.36).

**EFFECT OF CLINICAL PRESENTATION ON SENSITIVITY**

In Tables 2 and 4, sensitivity was calculated separately for symptomatic and asymptomatic individuals in the subgroup of individuals carrying a VP-associated PPOX mutation for whom clinical information was available.

For both tests, sensitivity was highest in the group with symptoms but no family history. Although we believe this is a valid conclusion, this finding requires cautious interpretation. Because we believe that patients with VP will not show active symptoms in the absence of obvious biochemical manifestations of porphyria (9,12), it is our practice to proceed to DNA testing only if there is evidence of porphyria on biochemical screening unless there is also a family history of VP, in which case routine

Table 5. Sensitivity and specificity of plasma scanning and fecal porphyrin analysis classified by decade of life.

<table>
<thead>
<tr>
<th>Decade</th>
<th>n¹</th>
<th>Sensitivity²</th>
<th>Specificity²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma scanning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (&lt;16 years)</td>
<td>58 (13)</td>
<td>0.23 (0.08–0.50)</td>
<td>1.00 (0.92–1.00)</td>
</tr>
<tr>
<td>2 (16–24 years)</td>
<td>66 (15)</td>
<td>0.27 (0.11–0.52)</td>
<td>1.00 (0.93–1.00)</td>
</tr>
<tr>
<td>3 (&gt;24 years)</td>
<td>74 (7)</td>
<td>0.71 (0.36–0.92)</td>
<td>0.97 (0.90–0.99)</td>
</tr>
<tr>
<td>4</td>
<td>108 (46)</td>
<td>0.89 (0.77–0.95)</td>
<td>1.00 (0.94–1.00)</td>
</tr>
<tr>
<td>5</td>
<td>126 (53)</td>
<td>0.89 (0.77–0.95)</td>
<td>1.00 (0.95–1.00)</td>
</tr>
<tr>
<td>6</td>
<td>81 (25)</td>
<td>0.84 (0.65–0.94)</td>
<td>1.00 (0.94–1.00)</td>
</tr>
<tr>
<td>7</td>
<td>74 (19)</td>
<td>0.74 (0.51–0.88)</td>
<td>1.00 (0.93–1.00)</td>
</tr>
<tr>
<td>8</td>
<td>45 (13)</td>
<td>0.77 (0.50–0.92)</td>
<td>1.00 (0.89–1.00)</td>
</tr>
<tr>
<td>9</td>
<td>20 (5)</td>
<td>0.60 (0.23–0.88)</td>
<td>0.93 (0.70–0.99)</td>
</tr>
</tbody>
</table>

| Fecal coproporphyrin |
|-----------------------|-----------------|-----------------|
| ¹ Value in parentheses represents the number of individuals carrying a VP-associated PPOX mutation. |
| ² Values in parentheses represent the 95% CI. |
DNA testing is undertaken. The individuals listed in the fifth columns of Tables 2 and 4 therefore represent those who underwent DNA analysis after a positive biochemical screening test. Because the false-negative rate would be expected to be extremely low, if not zero, in individuals with active symptoms of porphyria, the sensitivity we report is likely to represent a reasonable estimation of the true sensitivity. We have not, however, reported the specificity because our selection conditions for further testing preclude us from estimating the true negative rate reliably.

Where analysis was restricted to those individuals with a family history, the sensitivity of both tests appeared higher in the symptomatic group, although the difference did not reach statistical significance. For plasma scanning, the odds ratio for a positive test if the patient had experienced symptoms was 1.76 (95% CI, 0.35–8.82; P = 0.7) compared with those who had never experienced symptoms; for a positive fecal analysis, the odds ratio was 1.94 (0.55–6.83; P = 0.4). However, when the sensitivities were compared for all individuals regardless of family history, the odds ratio for a positive test if symptoms were present vs a family history alone reached significance for fecal analysis [3.9 (1.2–12.7); P = 0.02], although not for plasma scanning [3.4 (0.7–16.0); P = 0.1].

Discussion

PLASMA SCANNING

The sensitivity we obtained for plasma scanning in asymptomatic family members of individuals with VP (0.76; 95% CI, 0.65–0.85) is slightly lower than that reported by Long et al. (9), who, using statistical estimates of expected carrier frequency, obtained a value of 0.86 (0.71–0.98) for 136 asymptomatic relatives of patients with VP. It is, however, higher than the sensitivity reported by Da Silva et al. (12), who characterized inheritance of VP on the basis of lymphocyte PPOX activities and reported a sensitivity of ~0.5 in asymptomatic carriers of the VP trait. Our somewhat more optimistic estimate may relate to our more accurate identification of cases by use of direct mutation detection rather than by PPOX activity, or it may reflect our larger sample. Although both Da Silva et al. (12) and Long et al. (9) failed to record positive scans in prepubertal children, we obtained positive scans for 6 of 25 DNA-positive children <16 years of age, the youngest of whom were 5, 8, and 8 years, respectively. The sensitivity of plasma scanning was only 0.25 (0.12–0.43), however, in children younger than 16 years.

FECAL PORPHYRIN ANALYSIS

The sensitivity we obtained for fecal coproporphyrin in asymptomatic adults [0.59 (0.46–0.70)] is significantly higher than the estimated sensitivity (0.38) reported by Long et al. (9) for 55 first-degree asymptomatic relatives. In our sample, which included both symptomatic and asymptomatic individuals, fecal porphyrin analysis had moderate sensitivity (0.74; 95% CI, 0.67–0.80) but was less sensitive than plasma scanning (0.84; 95% CI, 0.78–0.89). We confirmed the higher sensitivity of plasma scanning over fecal analysis by direct comparison of the two tests in the group in whom both tests were performed. We also learned that qualitative interpretation of the fecal porphyrin pattern is no more predictive of VP than use of the fecal coproporphyrin concentration alone. This would appear to be moot, in any event, because plasma scanning achieves the same sensitivity at a specificity that approaches 1.00.

Our results support the hypothesis that plasma fluorescence scanning is more sensitive and specific than chromatographic fecal porphyrin analysis in the diagnosis of VP. Our results suggest that an initial fecal analysis is unnecessary in the testing of family members of known VP patients in whom a plasma scan has been performed. Enriquez de Salamanca et al. (16) suggested that plasma fluorometric screening might be used as the first diagnostic approach for a patient with an active cutaneous porphyria because a negative scan excludes a cutaneous porphyria. We would agree that an initial fecal analysis may not always be necessary in the evaluation of a patient with photosensitivity, provided that urine and erythrocytes are examined to detect AIP, PCT, and the erythropoietic porphyrias CEP and erythropoietic protoporphyria and that an initial plasma fluorescence scan is performed to detect VP. Fecal analysis might then best be reserved for patients with otherwise negative tests, in whom hereditary coproporphyria would remain a possibility, or to provide supplemental information in difficult or interesting cases.

We were interested to find in this study that fecal coproporphyrin was a uniformly better diagnostic marker for VP than protoporphyrin, although the predominant porphyrinogen excreted in bile in individuals with VP is protoporphyrinogen (24). Previous reports have suggested that fecal coproporphyrin may be disproportionately increased compared with fecal protoporphyrin in individual cases, and combined increases have been suggested as useful for confirming doubtful cases (15, 25, 26). There are several mechanisms that may operate to make fecal porphyrin recovery an unreliable marker of biliary porphyrin excretion (9, 24), including variable oxidation of porphyrinogens to the corresponding porphyrins, variable reabsorption and an enterohepatic cycling of porphyrins (27, 28), the effects of microbial porphyrin metabolism within the bowel (8, 29–31), and differences in the methodologies used for recovery of fecal porphyrins. We would caution, however, that reliance on fecal coproporphyrin alone as a marker for VP might lead to confusion with hereditary coproporphyria, which is also characterized by the presence of increased coproporphyrin in stool. This we cannot address directly because our database contained no individuals with this form of porphyria, which is rare in our population.
EFFECT OF AGE ON TEST PERFORMANCE
The performance of both tests was dependent on age, with sensitivity improving during the first and second decades, peaking in the third decade, and then decreasing. This decrease with age corroborates previous reports on patients who were known to have tested positive becoming negative results in later life (9). We have now quantified the change in sensitivity with age (Table 5). We have also confirmed that for practical purposes, abnormal fecal porphyrin excretion is not seen in prepubertal children with VP.

EFFECT OF CLINICAL MANIFESTATION ON TEST PERFORMANCE
Our results suggest that the sensitivities of both tests may be lower in asymptomatic individuals with PPOX mutations screened on the basis of a family history than they are in individuals with symptomatic VP. The symptoms of porphyria are in themselves of fairly low specificity. This would explain the significant percentage of individuals reported to have symptoms who were negative for PPOX mutations. We suspect that in some PPOX mutation-positive individuals with a family history and reported to have experienced symptoms, nonspecific symptoms were ascribed in error to VP; this would account for those apparently symptomatic individuals who demonstrated negative plasma scans and fecal porphyrin values.

In conclusion, we have shown that, in this population, plasma scanning is more sensitive and specific than fecal chromatographic analysis for the diagnosis of VP. Our study thus confirms earlier reports on the potential usefulness of this technique. Although our experience suggests that plasma scanning may in some instances identify carriers of a PPOX mutation before puberty, neither scanning nor fecal analysis is sufficiently sensitive for the exclusion of the carrier state, particularly in children. We therefore concur with previous reports (9, 12) suggesting that these techniques should not be used in isolation for excluding inheritance of VP in asymptomatic family members, particularly in childhood. We recommend that DNA-based techniques be used for this purpose.

We thank Dr. Anne Corrigall and Val Hancock for the DNA analyses. This study was financed in part by the Wellcome Trust under their International Senior Research Fellowship Program, in which P.N.M. was the recipient of a Wellcome Senior Fellowship for Medical Science in South Africa, and by the South African Medical Research Council.

References


