Diagnostic Strategies for Autosomal Dominant Acute Porphyrias: Retrospective Analysis of 467 Unrelated Patients Referred for Mutational Analysis of the HMBS, CPOX, or PPOX Gene

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BACKGROUND: Clinically indistinguishable attacks of acute porphyria occur in acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), and variegate porphyria (VP). There are few evidence-based diagnostic strategies for these disorders.

METHODS: The diagnostic sensitivity of mutation detection was determined by sequencing and gene-dosage analysis to search for mutations in 467 sequentially referred, unrelated patients. The diagnostic accuracy of plasma fluorescence scanning, fecal porphyrin analysis, and porphobilinogen deaminase (PBGD) assay was assessed in mutation-positive patients (AIP, 260 patients; VP, 152 patients; HCP, 31 patients).

RESULTS: Sensitivities (95% CI) for mutation detection were as follows: AIP, 98.1% (95.6%–99.2%); HCP, 96.9% (84.3%–99.5%); VP, 100% (95.7%–100%). We identified 5 large deletions in the HMBS gene (hydroxymethylbilane synthase) and one in the CPOX gene (coproporphyrinogen oxidase). The plasma fluorescence scan was positive more often in VP (99% of patients) than in AIP (68%) or HCP (29%). The wavelength of the fluorescence emission peak and the fecal coproporphyrin isomer ratio had high diagnostic specificity and sensitivity for differentiating between AIP, HCP, and VP. DNA analysis followed by PBGD assay in mutation-negative patients had greater diagnostic accuracy for AIP than either test alone.

CONCLUSIONS: When PBG excretion is increased, 2 investigations (plasma fluorescence scanning, the coproporphyrin isomer ratio) are sufficient, with rare exceptions, to identify the type of acute porphyria. When the results of PBG, 5-aminolevulinate, and porphyrin analyses are within reference intervals and clinical suspicion that a past illness was caused by an acute porphyria remains high, mutation analysis of the HMBS gene followed by PBGD assay is an effective strategy for diagnosis or exclusion of AIP.

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The acute hepatic porphyrias, acute intermittent porphyria (AIP)3 (MIM 176000), variegate porphyria (VP) (MIM 176200), and hereditary coproporphyria (HCP) (MIM 121300), are low-penetrance, autosomal dominant disorders that can present with clinically indistinguishable acute neurovisceral attacks, which may be accompanied by skin lesions in HCP and VP. These acute attacks are defined biochemically by increased urinary excretion of porphobilinogen (PBG) (1). In addition, VP often presents with skin lesions alone, but this presentation is uncommon in HCP (1, 2).

Each disease results from decreased activity of the heme biosynthetic enzyme PBG deaminase (PBGD) (EC 4.3.1.8; hydroxymethylbilane synthase) in AIP, coproporphyrinogen oxidase (EC 1.3.3.3) in HCP, and protoporphyrinogen oxidase (EC 1.3.3.4) in VP. Identification of disease-specific mutations in the corresponding genes (HMBS,4 CPOX, and PPOX, respectively) is important for detection and counseling of

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3 Nonstandard abbreviations: AIP, acute intermittent porphyria; VP, variegate porphyria; HCP, hereditary coproporphyria; PBG, porphobilinogen; PBGD, PBG deaminase; TFP, total fecal porphyrin; FCR, fecal coproporphyrin isomer III/I ratio; UTR, untranslated region.

4 Human genes: HMBS, hydroxymethylbilane synthase; CPOX, coproporphyrinogen oxidase; PPOX, protoporphyrinogen oxidase; DPAGT1, dolichyl-phosphate (UDP-N-acetylgalactosamine) N-acetylglucosaminylphosphotransferase 1 (GlcNAc-1-P transferase); H2AFX, H2A histone family, member X.
asymptomatic relatives in families with acute porphyria (1). Of the at least 510 mutations that have been identified in these genes (www.hgmd.cf.ac.uk) (3–8), most are restricted to one or a few families (9, 10).

For patients who clinically manifest disease, diagnosis depends on identification of the specific patterns of heme precursor excretion and accumulation that characterize each enzyme defect, a process that is usually straightforward (1). Confirmation or exclusion of a past illness as an acute porphyria may be more difficult. In AIP, which accounts for most attacks of acute porphyria (1, 11), heme precursor excretion may become typical during clinical remission, and the PBGD assay in such cases does not adequately discriminate between affected and nonaffected individuals (12). The different investigative strategies that are available use various combinations of metabolite-, enzyme-, and DNA-based methods (1, 13–16), but there is only limited information about diagnostic accuracy to support the selection of particular strategies (12, 17–19).

We describe a retrospective analysis of 467 apparently unrelated patients with current or past symptoms consistent with an acute hepatic porphyria who were referred to our laboratory over an 18-year period for DNA-based analyses. We used data from these patients to devise a simple strategy for the diagnosis of the autosomal dominant acute hepatic porphyrias in patients with current or past symptoms consistent with an attack of acute porphyria.

Materials and Methods

Patients

We studied 467 apparently unrelated patients (459 resident in the UK) with a proven or putative diagnosis of AIP (283 patients; age range, 9–88 years), VP (152 patients; age range, 13–89 years), or HCP (32 patients; age range, 16–90 years) who were sequentially referred for mutation analysis between 1988 and 2006. EDTA-anticoagulated blood (460 patients) or preextracted anticoagulated DNA (7 patients) was received from all patients; urine and/or fecal samples were obtained from 283 patients. Mutations have previously been reported for some of these patients (2, 20–22). Clinical information was not available for all patients. The South East Wales Research Ethics Committee approved the study as a service evaluation. All patients gave informed consent for investigation.

Biochemical Methods

Urinary PBG was measured by anion-exchange chromatography with spectrophotometric measurement of the p-dimethylaminobenzaldehyde–PBG complex (23). Interassay CVs were 5.8% (7.2 μmol/L) and 5.6% (51 μmol/L). Fecal porphyrins were analyzed as described by Lockwood et al. (24). The concentration of total fecal porphyrin (TFP) was expressed in nanomoles per gram of dried feces. Interassay CVs were 7.5% (45 nmol/g) and 4.6% (718 nmol/g). Fecal porphyrins were fractionated by HPLC (25) with an Agilent 1100 system with a G1321A fluorescence detector containing a red-sensitive photomultiplier (Agilent Technologies). Fecal coproporphyrin isomer III/I ratios (FCRs) were evaluated from integrated peak areas (interassay CV, 9.1% at a ratio of 0.7). Plasma fluorescence scanning and determination of the wavelength of any fluorescence emission peak were carried out as described by Long et al. (26) with a PerkinElmer LS-50B luminescence spectrophotometer with a red-sensitive photomultiplier (Hamamatsu 928) calibrated with coproporphyrin I (180 nmol/L) in PBS (0.01 mol/L phosphate buffer, 0.0027 mol/L KCl, 0.137 mol/L NaCl, pH 7.4). PBGD activity was measured as described by Magnussen et al. (27) but with EDTA-anticoagulated whole blood of known hematocrit and fluorometric evaluation against calibration solutions of coproporphyrin I in a buffer containing 125 g/L trichloroacetic acid and 25 mmol/L Tris, pH 8.2. Interassay CVs were 9.6% (11.4 nmol·s⁻¹·L⁻¹) and 8.1% (5.3 nmol·s⁻¹·L⁻¹). Reference intervals were determined for all assays with anonymized samples from patients without porphyria or, for PBGD, from 253 members of AIP families demonstrated by mutation analysis to be unaffected.

Identification of Point Mutations and Large Deletions

Genomic DNA was extracted from whole blood and investigated for mutations in HMBS (GenBank accession no. NM_000097), PPOX (GenBank accession no. NM_000308), or CPOX (GenBank accession no. NM_000997) by direct genomic sequencing, as has previously been described (28). The following regions were sequenced: HMBS exons 1 and 3–15, 30–250 bp of their flanking regions, and 1000 bp of the 5’ untranslated region (UTR); CPOX exons 1–7, 30–200 bp of their flanking regions, and 700 bp of the 5’ UTR; PPOX exons 1–13 and 30–300 bp of their flanking regions. When bidirectional sequencing identified no mutation, quantitative PCR was used for gene-dosage analysis to identify large deletions (28).

Some AIP samples were screened for HMBS mutations by denaturing HPLC. Each exon was PCR-amplified before it was loaded onto a reversed-phase HPLC column (WAVE nucleic acid fragment analysis system; Transgenic). DNA fragments were detected with a UV detector. Traces were compared with those from both positive and negative controls obtained from samples of known sequence. Primer sequences and conditions for the PCR, denaturing HPLC, and gene-dosage analysis are available in Table 1 in the Data Supplement that
accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue7.

Nucleotides were numbered from the cDNA sequence of human *HMBS* (reference sequence NM_000190), *PPOX* (reference sequence NM_000309.2), and *CPOX* (reference sequence NM_000097.4), with the A of the ATG initiation codon designated as +1.

**STATISTICAL METHODS**

Data were analyzed with Microsoft Excel, SPSS, and Minitab (version 13) software. The significance of differences between quantitative variables and proportions was assessed with the Mann–Whitney *U*-test and the *χ²* test. The separation of PBGD activities of individuals with mutation-positive AIP and control individuals was modeled with a lognormal model, which was used to construct projected posttest risks (spreadsheet available from the authors on request). CIs for diagnostic sensitivities and specificities and for projected posttest risks were calculated by score methods (29, 30).

**Results**

Mutations were identified in 260 (91.9%) of the 283 patients referred for analysis of the *HMBS* gene; one of these patients (patient A) also had a mutation in the *PPOX* gene. Five of the 23 mutation-negative patients had biochemical data consistent with a diagnosis of AIP (Table 1), and these patients were considered to have the disease. No biochemical data supporting a diagnosis of AIP could be obtained for the remaining 18 patients. Seven individuals were asymptomatic members of families with probable AIP in which no individual with unequivocally diagnosed disease was available for mutational analysis. None of the other 11 patients had a family history of porphyria, but all had current or past symptoms that suggested a diagnosis of acute porphyria. Eight of these patients had typical values for urinary PBG (2–5 μmol/L), plasma fluorescence scans, and TFPs (19–100 nmol/g), indicating that their symptoms were unlikely to have been caused by any form of autosomal dominant acute hepatic porphyria. The 3 remaining patients had biochemical data that were less complete: All had PBGD activities within the reference interval, and urinary PBG excretion was typical in the one patient in whom it was measured. These 18 patients were considered not to have AIP.

Mutations were identified in all patients referred for mutational analysis of the *CPOX* or *PPOX* gene except for 1 patient with HCP (Table 1). All had a diagnosis of HCP or VP established by biochemical investigation either in our laboratory (HCP, 25 patients; VP, 136 patients) or in the referring laboratory. Confirmatory diagnostic criteria for HCP in our laboratory were an increase in fecal coproporphyrin III [median TFP, 1645 nmol/g (range, 189–8461 nmol/g; reference interval, <200 nmol/g); median coproporphyrin III, 95% of the TFP value (range, 31%–99%); no plasma porphyrin fluorescence emission peak at 624–628 nm]. The confirmatory diagnostic criteria for VP were a plasma fluorescence peak at 624–628 nm in either the patient or, for 2 patients, a first-degree relative. The disease in 1 patient with VP (patient B) was diagnosed by molecular analysis after a fatal acute illness suspected in retrospect as being an acute porphoria; a pre-mortem plasma sample from this patient had a fluorescence emission peak at 621 nm.

**Table 1. Biochemical and molecular evidence for HCP or AIP in mutation-negative patients.**

<table>
<thead>
<tr>
<th>Disease/patient no.</th>
<th>PBG/creatinine, μmol/mmol</th>
<th>TFP, nmol/g</th>
<th>FCR</th>
<th>Pscan,* nm</th>
<th>PBGD, nmol · s⁻¹ · L⁻¹</th>
<th>CPOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCP/1</td>
<td>0.5</td>
<td>939</td>
<td>19</td>
<td>NPD</td>
<td>—</td>
<td>NMD</td>
</tr>
<tr>
<td>AIP/1</td>
<td>2.3</td>
<td>&lt;200</td>
<td>0.9</td>
<td>NPD</td>
<td>9.7</td>
<td>—</td>
</tr>
<tr>
<td>AIP/2</td>
<td>61.4b</td>
<td>44</td>
<td>—</td>
<td>620</td>
<td>17.8</td>
<td>NMD</td>
</tr>
<tr>
<td>AIP/3</td>
<td>6.5</td>
<td>196</td>
<td>—</td>
<td>620</td>
<td>11.8</td>
<td>NMD</td>
</tr>
<tr>
<td>AIP/4</td>
<td>55.5</td>
<td>914c</td>
<td>—</td>
<td>620</td>
<td>4.5d</td>
<td>NMD</td>
</tr>
<tr>
<td>AIP/5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>NPD</td>
<td>4.7</td>
<td>—</td>
</tr>
<tr>
<td>Nonaffected individuals</td>
<td>0.08–1.45</td>
<td>14–192</td>
<td>0.3–1.4</td>
<td>NPD</td>
<td>6.1–20.0</td>
<td></td>
</tr>
</tbody>
</table>

*a* Pscan, porphyrin fluorescence wavelength; NPD, no porphyrin fluorescence peak detected; NMD, no mutation detected. Data for nonaffected individuals are the intervals in Table 3.

*b* Micromoles per liter, measured in the referring laboratory.

*c* Measured in the referring laboratory; increased uroporphyrin, coproporphyrin, and protoporphyrin.

*d* Measured in the referring laboratory; reference interval, 6.1–12.8 nmol · s⁻¹ · L⁻¹.
AIP, 98.1% (95% CI, 95.7%–99.2%; n = 265); HCP, 96.9% (95% CI, 84.3%–99.4%; n = 32); and VP, 100% (95% CI, 97.5%–100%; n = 152). Negative predictive values, calculated on the assumption that the specificity is 100% and the disease prevalence or prior risk is 0.5, were as follows: AIP, 0.98 (95% CI, 0.96 – 0.99); HCP, 0.97 (95% CI, 0.86 – 0.99); and VP, 1.00 (95% CI, 0.98 –1.00).

### Types of Mutations

We identified 123 different mutations in the HMBS gene (32% missense, 14% nonsense, 23% frameshift, 24% splice defects, 1% promoter, 4% large deletions), of which 35 have not previously been reported (see Figs. 1 and 2 in the online Data Supplement). Most mutations were present in fewer than 3 families, but one mutation (p.Arg173Trp) was present in 35 families (12%). Seven patients (3%; 95% CI, 1%–5%) had mutations in exon 3 or the promoter region that produce a variant form of AIP in which the erythroid isoform of PBGD is unaffected (20). Five patients had large deletions, 2 of which extended into the DPAGT1 gene [dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)] with complete deletion of the intervening H2AFX gene (H2A histone family, member X) (see Fig. 2 in the online Data Supplement). The breakpoints for 4 of these deletions are shown in Table 2; breakpoints were not found for the deletion encompassing exon 1 (Fig. 2 in the online Data Supplement).

We identified 28 different mutations, including 1 large deletion (Table 2; Fig. 2 in the online Data Supplement) and 15 point mutations that were reported previously (2), in the CPOX gene (60% missense, 18% frameshift, 11% nonsense, 7% splice defects, 4% large deletions). Sixty-three different mutations were detected in the PPOX gene (29% missense, 37% frameshift, 13% nonsense, 21% splice defects), of which 6 mutations (p.Leu15Phe,p.Glu189X, p.Leu295Pro, p.Gln435X, c.537_538delAT, c.807→T>C) were present in 4 –15 families, accounting for 45% of the patients with VP. Previously unreported mutations in the CPOX and PPOX genes are shown in Fig. 1 in the online Data Supplement.

### Differential Diagnosis of Acute Porphyrias

Biochemical data obtained in our laboratory for mutation-positive patients are summarized in Table 3. We used these data to determine the diagnostic accuracy of TFP, FCR, and plasma fluorescence scanning for differentiating the acute hepatic porphyrias. We selected these investigations because previous reports had indicated their usefulness for the diagnosis of one or more of the acute hepatic porphyrias (17, 31–33).

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**Table 2. Deletions identified in the HMBS and CPOX genes.**

<table>
<thead>
<tr>
<th>Deleted genes/ exons</th>
<th>Breakpoints</th>
<th>Size of deletion, bp</th>
<th>Repeat sequences</th>
<th>Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMBS/3–15</td>
<td>c.33+1356 to c.619+702</td>
<td>13 137</td>
<td>AluSx</td>
<td>cctgtaatctAGCATTGGAGAGGCC/gaggcaggtgg</td>
</tr>
<tr>
<td>H2AFX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPAGT1/9–5</td>
<td>AluJb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMBS/3–4</td>
<td>c.34–453 to c.145</td>
<td>891</td>
<td>None</td>
<td>gtcttgttgata/ataatgact</td>
</tr>
<tr>
<td>H2AFX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMBS/9–15</td>
<td>c.498+244 to c.893+15</td>
<td>7332</td>
<td>L2 LINEb</td>
<td>taatgtgtgac/aagataaaacat</td>
</tr>
<tr>
<td>H2AFX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPAGT1/9–7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMBS/3–11</td>
<td>c.34–141 to c.633</td>
<td>4032</td>
<td>None</td>
<td>agcttgtggagt/attttcat</td>
</tr>
<tr>
<td>CPOX/4–3’ UTR</td>
<td>c.812–535 to TGA+4615</td>
<td>13 247</td>
<td>AluSq</td>
<td>tgaatgagagt/ttcacctgctt</td>
</tr>
</tbody>
</table>

* Upper 2 sequences are the 5’ and 3’ breakpoints, respectively; lowest sequence is the junction sequence. Uppercase letters represent homologous sequences present at both the 5’ and 3’ breakpoints.

b LINE, long interspersed element.
We used the mutation-positive patients with other types of acute hepatic porphyria as the populations without the disease to calculate the diagnostic specificities for tests for each porphyria. Two atypical patients (patients A and B, Table 3) were excluded from this analysis.

Table 3 shows that VP was distinguished from both HCP and AIP by the wavelength of the plasma porphyrin fluorescence peak. With a cutpoint of 623 nm, diagnostic sensitivity and specificity were 98.6% (95% CI, 94.9%–99.6%; n = 11005139) and 100% (95% CI, 98.4%–100%; n = 231), respectively. Both parameters would have been 100% but for the inclusion of 2 patients with VP who had negative scan results (Table 3). Neither of these patients had ever been symptomatic, but both had a first-degree relative with overt VP. The wavelength of the plasma fluorescence emission peak did not distinguish between AIP and HCP, although the plasma fluorescence scan was positive more often in AIP cases \[P < 0.001, \text{vs} \] HCP or VP.

The FCR at a cutpoint of 1.55 differentiated AIP from HCP [sensitivity, 100% (95% CI, 87.9%–100%; n = 28); specificity, 100% (95% CI, 84.5%–100%; n = 21)] (Table 3). The FCR was 1.55 in all but one of the 33 VP patients in whom it was measured (Table 3) but tended to be lower than in HCP, so that at a cutpoint of 15.5, the FCR had a specificity of 100% (95% CI, 94.9%–99.9%; n = 139), respectively. Both parameters would have been 100% but for the inclusion of patients who had negative scan results (Table 3). Neither of these patients had ever been symptomatic, and both had a first-degree relative who had been symptomatic with the disease. Table 3 shows that VP was distinguished from both HCP and AIP by the wavelength of the plasma porphyrin fluorescence peak and a cutpoint of 623 nm. Diagnostic sensitivity and specificity were 98.6% (95% CI, 94.9%–99.9%; n = 247) and 100% (95% CI, 98.4%–100%; n = 219), respectively. Both parameters would have been 100% but for the inclusion of patients who had negative scan results (Table 3). Two typical patients (patients A and B, Table 3) were excluded from this analysis.

We next examined the diagnostic accuracy of the measurements in Table 3 for distinguishing AIP patients from those without porphyria. For the mutation-positive AIP patients investigated in our laboratory, PBG excretion was increased in 84% of the patients (95% CI, 76%–89%), the plasma fluorescence scan was positive with an emission peak at about 619 nm in 63% (95% CI, 56%–69%), and the TFP was increased in 26% (95% CI, 18%–36%). Nei-
Erythrocyte PBGD activity gave good discrimination between AIP patients and unaffected individuals, the area under the ROC curve (Fig. 1A) being significantly increased at 0.944 (95% CI, 0.918–0.962; \( P < 0.001 \)); however, our data confirm those of previous reports (1, 12, 18) that mutation detection by DNA analysis is a more effective discriminator (Fig. 1B). At a cutpoint of \( \leq 6.0 \) nmol \( \cdot \) s\(^{-1}\) \( \cdot \) L\(^{-1}\), PBGD activity is 100% specific (95% CI, 98.5%–100%) with positive predictive values as high as for mutational analysis but with a sensitivity of only 47.6% (95% CI, 40.9%–54.4%); consequently, projected negative predictive values are much lower (Fig. 1C). At a cutpoint of 7.7 nmol \( \cdot \) s\(^{-1}\) \( \cdot \) L\(^{-1}\), close to the optimum sensitivity (85.0%; 95% CI, 79.4%–89.2%) and specificity (89.7%; 95% CI, 85.4%–92.9%), diagnostic accuracy is markedly inferior to DNA analysis at all prevalences (Fig. 1D).

For mutation-negative patients, PBGD activity modifies the disease risk (Table 4). This additional step is most useful for the identification of AIP when the cutpoint is low and the prevalence is high. As the cutpoint is increased, this strategy becomes progressively more useful for excluding a diagnosis of AIP and allows quantitative determination of the disease risk.

Discussion

Reports of mutation detection in large groups of patients with AIP, VP, or HCP have relied solely on genomic sequencing to identify point mutations and have usually achieved a sensitivity of approximately 95% (2, 12, 21, 22, 35). By including gene-dosage analysis to search for deletions that are too large to be detected by sequencing alone, we increased the diagnostic sensitivity of mutation detection from 96% to 98% for AIP and from 94% to 97% for HCP. Gene-dosage analysis identified 6 deletions (Table 2; Fig. 2 in the online Data Supplement). Whereas no large deletions have been reported in the CPOX or PPOX gene, one large deletion was previously reported in the HMBS gene (36). This deletion, like that in one of our patients, involved the entire HMBS gene and the neighboring H2AFX and DPAGT1 genes, but without any modification of the AIP phenotype. Gross deletions

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**Fig. 1.** Comparison of the erythrocyte PBGD assay and mutation detection by DNA analysis for the identification of AIP.

ROC curve analysis for the PBGD assay (A) and plots of projected positive predicted value (PPV) (upper curves) and negative predictive value (NPV) (lower curves) against prevalence for PBGD with cutpoints of \( \leq 6.0 \) nmol \( \cdot \) s\(^{-1}\) \( \cdot \) L\(^{-1}\) (B) and 7.7 nmol \( \cdot \) s\(^{-1}\) \( \cdot \) L\(^{-1}\) (C), and for mutation detection by DNA analysis (D). Indicated are regions of 95% CIs. Plots that coincide with axes are indicated by lines with dots.
often arise in regions containing repetitive sequences (37), as was the case for 3 of these deletions in our patients (Table 2).

To obtain a reliable estimate of the diagnostic sensitivity of DNA analysis, we minimized selection bias by including all apparently unrelated patients referred for molecular diagnosis over an 18-year period. Determination of diagnostic sensitivity also requires accurate disease identification. This was straightforward for HCP or VP because, unlike in AIP (1, 12), abnormalities of heme precursor metabolism persist for many years after clinical remission (2, 17, 38). Establishing an unequivocal diagnosis of AIP was more difficult. For most patients, this goal was achieved by demonstrating increased urinary PBG excretion with the exclusion of VP and HCP, but a few patients were identified as having AIP only by mutational analysis. In 18 patients, we found no evidence for an acute porphyria, and these patients were excluded from our calculation of the diagnostic accuracy of mutational analysis. Because it is difficult to exclude AIP with absolute certainty, some of these patients may have had the disease. Thus, our figure for the diagnostic sensitivity of mutational analysis in AIP cases is a maximum estimate.

Our data provide evidence to support a simple diagnostic strategy for patients who present for diagnostic investigation with current or past symptoms consistent with an attack of acute porphyria (Table 5). For patients with increased urinary PBG excretion, which establishes a diagnosis of acute porphyria (1, 12), only 2 investigations, determination of the fluorescence emission wavelength and measurement of the FCR, are required to differentiate between AIP, HCP, and AIP in all but exceptional cases (Table 5). Of these tests, the FCR becomes redundant when the plasma fluorescence emission wavelength is at or close to 626 nm, because a peak at this position is diagnostic for VP (17). No additional analyses are required unless anomalous biochemical findings or unusual clinical features suggest the need for further investigation. This was the case for only 2 of our patients (patients A and B) (Table 3). Patient B had coinherited AIP and VP, whereas patient A is likely to have had large amounts of PBG-derived uroporphyrin that masked any VP plasma porphyrin complex that was present.

Typical results for PBG and 5-aminolevulinate excretion, a plasma scan, and the FCR in patients with current symptoms effectively excludes all types of acute porphyria, including the very rare autosomal recessive 5-aminolevulinate dehydratase deficiency porphyria (1), as causes of their illness. Plasma scanning and the FCR are required because PBG excretion may fall rapidly after the onset of an acute attack of HCP or VP (38).

### Table 4. Comparison of 2 strategies for the identification of AIP when concentrations of PBG and 5-aminolevulinate in urine are typical.a

<table>
<thead>
<tr>
<th>Prevalence in target population</th>
<th>Mutation detection by DNA analysis</th>
<th>DNA analysis followed by PBGD assay for mutation-negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients positive, %</td>
<td>Test positive</td>
</tr>
<tr>
<td>0.10</td>
<td>9.8</td>
<td>100</td>
</tr>
<tr>
<td>0.25</td>
<td>24.5</td>
<td>100</td>
</tr>
<tr>
<td>0.50</td>
<td>49.1</td>
<td>100</td>
</tr>
<tr>
<td>0.75</td>
<td>73.6</td>
<td>100</td>
</tr>
<tr>
<td>0.90</td>
<td>88.3</td>
<td>100</td>
</tr>
<tr>
<td>0.95</td>
<td>93.2</td>
<td>100</td>
</tr>
</tbody>
</table>

* Percentages of patients in each category are from a model (see Materials and Methods) that calculated the predicted results of applying the test strategies to 1000 individuals.
Urinary PBG decreases to typical or near typical concentrations in about 20% of AIP patients after an acute attack \((15)\), and concentrations in HCP and VP are often typical during remission \((1,38)\). Thus, a finding of typical PBG excretion does not exclude these disorders in patients who present during clinical remission. There is evidence, however, that the plasma fluorescence scan in VP and the FCR in HCP remain abnormal for many years after the symptoms have resolved \((17,26,32,33)\). Thus, these investigations are likely to identify virtually all patients with these conditions, even after prolonged remission.

Patients with typical results for PBG excretion, a plasma scan, and the FCR may have AIP in remission or an illness that is not a porphyria (Table 5). When the clinical suspicion of AIP remains high enough to justify further investigation, DNA analysis followed by PBGD assay will identify patients with AIP or, for mutation-negative patients, will give the risk of being affected (Table 4). This approach has advantages over DNA analysis alone.

Even at the high diagnostic sensitivity obtained with our mutation-detection strategy, the risk that a mutation-negative individual may have AIP remains high when the prior risk is high (Table 4). Estimation of prior risk or disease prevalence in the population from which the patient comes is thus critically important for assessing the significance of a negative DNA test result. The prevalence of AIP was 0.94 in our series of 283 referred patients; use of DNA testing alone for diagnosis would have left 23 mutation-negative patients with a 1 in 4 risk of having AIP. Among our patients with typical PBG excretion, the prevalence was 0.6. At this prevalence, DNA analysis of the HMBS gene would identify AIP in 59% of patients and leave the rest with a 1 in 36 chance of having the disease.

The sequential strategy shown in Table 5 will identify a small number of mutation-negative patients with a high risk of AIP, provided a PBGD cut-point that gives a high specificity is used. Only a single patient in our series was identified in this way (Table 1). Conversely, use of a less specific but more sensitive cutpoint is more useful for excluding AIP (Table 4); however, this strategy is unreliable unless concurrent disorders that are known to affect PBGD activity, including an ongoing severe attack of acute porphyria \((39)\), are excluded \((12)\). It is also worth noting that 3 of our patients with mutation-negative AIP (Table 1; patients 1–3) would not have been identified if only DNA analysis and the PBGD assay had been carried out. The explanation for the typical PBGD activities in these patients is unclear; only 1 patient had a urinary PBG concentration consistent with an acute attack at the time of the enzyme assay (Table 1). Other explanations include variant AIP caused by an unidentified upstream mutation disrupting production of mRNA for the ubiquitous isoform or, possibly, a mutation at another locus.

**Table 5. Key to investigation of patients with current or past symptoms consistent with attack(s) of acute porphyria.**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1.</td>
<td>Urine PBG increased → Go to 2</td>
</tr>
<tr>
<td></td>
<td>Urine PBG not increased* → Go to 4</td>
</tr>
<tr>
<td>2.</td>
<td>Plasma porphyrin peak &gt; 623 nm → VP</td>
</tr>
<tr>
<td></td>
<td>Peak &lt; 623 nm or no peak detected → Go to 3</td>
</tr>
<tr>
<td>3.</td>
<td>FCR &gt; 1.55 → HCP</td>
</tr>
<tr>
<td></td>
<td>FCR &lt; 1.55 → AIP</td>
</tr>
<tr>
<td>4.</td>
<td>Plasma porphyrin peak &gt; 623 nm → VP</td>
</tr>
<tr>
<td></td>
<td>Peak &lt; 623 nm or no peak detected → Go to 5</td>
</tr>
<tr>
<td>5.</td>
<td>FCR &gt; 1.55 → HCP</td>
</tr>
<tr>
<td></td>
<td>FCR &lt; 1.55 → AIP or illness other than porphyria (see text)</td>
</tr>
</tbody>
</table>

* Measurement of 5-aminolevulinate is required if 5-aminolevulinate dehydratase deficiency porphyria is suspected [Sassa \((1)\)].

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**References**


