Molecular and Biochemical Studies of Acute Intermittent Porphyria in 196 Patients and Their Families

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Background: Acute intermittent porphyria (AIP) is a metabolic disease with clinical manifestations that mimic other abdominal, neurologic, or mental crises. We studied the diagnostic accuracy of current laboratory tests during an acute attack and in remission.

Methods: Since 1966, we have studied all known Finnish AIP patients (n = 196) and their families (n = 45) and identified the porphobilinogen deaminase (PBGD) mutation in each family. Diagnoses or exclusions of AIP were based on clinical data (including family history), biochemical tests, and in 239 cases, mutation testing. We retrospectively evaluated the diagnostic accuracy of erythrocyte PBGD activity, urinary excretion of porphobilinogen (PBG) and \(-\)-aminolevulinic acid, and urinary and fecal excretion of porphyrins in these patients.

Results: Measurement of urinary PBG identified all 35 AIP patients studied during an acute attack. The mean excretion of PBG was 50-fold above the reference interval, although the intraindividual increases were modest (1.6- to 4.0-fold). In the mutation-screened population, urinary PBG analysis identified only 85% of 81 AIP patients studied during remission, but by ROC curve analysis it was nonetheless the best of the biochemical tests. It was increased <2-fold in 29% of healthy relatives. Erythrocyte PBGD activity was decreased in only 84% of AIP patients, with results within the reference interval mainly in the variant form of AIP; it was decreased in 23% of healthy relatives.

Conclusions: Measurement of urinary PBG is the best biochemical test for AIP, although it is unspecific and does not distinguish AIP from other acute porphyrias. Because the acute increase in PBG is often modest, the medical history, signs, and symptoms must be evaluated carefully during an acute attack. In addition, because biochemical analyses often remain indeterminate in remission, mutation analysis is needed to exclude or confirm the diagnosis of AIP.

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Acute intermittent porphyria (AIP)\(^1\) is a metabolic disease that results from the partial deficiency of porphobilinogen deaminase (PBGD; also known as hydroxymethylbilane synthase; EC 4.3.1.8; MIM No. 176000) (\(1\)). PBGD, which is the third enzyme in heme biosynthesis, catalyzes the polymerization of porphobilinogen (PBG), yielding a linear tetrapyrrole, uroporphyrinogen (hydroxymethylbilane). This is closed by uroporphyrinogen III synthase to form uroporphyrinogen III or, alternatively, nonenzymatically to form uroporphyrinogen I.

The PBGD gene (GenBank accession no. M95623; 10 kb) has been assigned to chromosome 11q24, and the coding sequences are spread over 15 exons (2–6). Two tissue-specific isoforms have been characterized; the two transcripts arise from two separate promoters via alternative splicing of exons 1 and 2. The mRNA of the housekeeping (nonerythropoietic) isoform contains exons 1 and 3–15, which code for an enzyme of 361 amino acids (\(M_r \sim 42\,000\)), whereas the erythroid isoform is encoded by exons 2–15 and lacks the first 17 amino acids of the NH\(_2\) terminus (\(M_r \sim 40\,000\)).

AIP is inherited as an autosomal dominant trait displaying incomplete penetrance (\(1\)). Despite \(\sim 200\) different mutations described in the PBGD gene (Fig. 1.), the clinical manifestation is relatively uniform among AIP patients. Clinical symptoms include abdominal pain, vomiting, and constipation accompanied by hypertension and tachycardia, which are signs of sympathetic activity. Peripheral neuropathy is manifested as pain in the ex-
tremities, and it may progress to a severe motor neuropathy. Cranial nerve palsies, epileptic seizures, respiratory insufficiency, abnormal sphincter function, hallucinations, and mental changes may be present. An acute attack precipitated by AIP is indistinguishable from attacks produced by variegate porphyria or hepatic coproporphyria. Acute attacks are precipitated by various exogenous factors, such as drugs, alcohol, infections, and fasting, or endogenous changes in the sex-hormone balance (7). A few homozygous patients with more severe and chronic neurologic dysfunction have been reported (8, 9).

PBGD activity is usually decreased to 50% of normal in patients’ erythrocytes (10). In the variant form of AIP, patients have a mutation in the region of the first exon or intron; thus, measurement of PBGD activity in erythrocytes is uninformative (11). In addition, AIP patients manifest overproduction and increased excretion of porphyrins and their precursors: proto- and coproporphyrins in feces; uro- and coproporphyrins in the urine and plasma (12).

In this study we used DNA technology in the diagnosis of AIP and compared the results with the data from biochemical analyses and clinical findings to evaluate the diagnostic accuracy of current laboratory tests during an acute attack and in remission.

Materials and Methods

Patients
Since 1966, we have conducted a systematic follow-up of all Finnish patients known to have AIP (13). After the diagnosis of AIP in an index case, family members were enrolled in the study during the years 1966–2001. The patients represent 45 Finnish AIP families, and the ancestry for the majority of them could be traced back to at least the 19th century by checking church registers (13). All individuals analyzed were members of the families in which AIP was confirmed by mutation analysis.
Of the 489 family members, AIP was diagnosed in 196 patients (84 males, 112 females; age range, 15–78 years; 75 symptomatic and 121 symptom free) and excluded in 293 healthy relatives (146 males, 147 females; age range, 15–61 years). We examined 239 individuals with mutation analysis and 381 individuals with biochemical testing, and 131 individuals were tested by both methods.

The diagnosis of AIP was based on mutation analysis in 120 individuals. In 66 cases (35 symptomatic and 31 symptom free), the diagnosis was based on low erythrocyte PBGD activity (<55 pmol uroporphyrin·h⁻¹·mg⁻¹) with increased (>9 μmol/L; n = 54), normal (n = 2), or undetermined (n = 10) urinary PBG excretion. In seven cases, the diagnosis was based on increased urinary PBG excretion (>47 μmol/L) with characteristic clinical symptoms (n = 5) or without symptoms (n = 2). In three cases (one symptomatic, two symptom free), the diagnosis was based on pedigree analysis. The exclusion of AIP was based on mutation analysis in 119 cases. In 139 symptom-free individuals, the erythrocyte PBGD activity was within the reference interval and the urinary PBG excretion was 0–22 μmol/L (n = 124) or undetermined (n = 15). In 35 cases, the exclusion was based on absence of clinical symptoms and normal excretion of porphyrins and their precursors. Erythrocyte PBGD activity could not be used in 16 of the individuals studied because they had the variant form of AIP. Exclusion of these family members did not affect the mean results of urinary and fecal excretions of porphyrins and their precursors in healthy relatives.

The biochemical data included data collected retrospectively and prospectively since 1966. Informed consent was obtained for all DNA testing, and the study protocol was approved by the Ethical Committee of the Department of Medicine, University Central Hospital of Helsinki.

The participants were classified into four groups based on a comparison of urinary and fecal excretion of porphyrins and their precursors with clinical manifestations: (a) AIP patients in an acute attack; (b) symptomatic AIP patients in remission; (c) symptom-free AIP patients; and (d) healthy relatives. Information about acute attacks was obtained from hospital records for all patients who had had acute attacks requiring hospitalization since 1929. The criteria for an acute attack were the acute nature of symptoms, urinary excretion of PBG that was at least five times above the upper limit of the reference interval, and severe abdominal or other pain associated with one or more additional typical porphyric symptoms (7, 11).

### BIOCHEMICAL METHODS

PBGD activity was determined from red cells separated from venous blood by centrifugation (1000g for 10 min) with heparin added as an anticoagulant (13, 14). Red cells were washed twice with 9 g/L NaCl solution and hemolyzed by freezing and thawing three times. The red cell solution was diluted with 50 mmol/L Tris-HCl, pH 8.0 (1:20 by volume). A total of 500 μL of the red cell dilution and 1 mL of 10 mmol/L PBG in H₂O were mixed before incubation for 60 min at 37 °C. The reaction was stopped with 1.5 mL of 2 mol/L perchloric acid. Coproporphyrin I formation was measured in the supernatant by spectrofluorometry (Hitachi F-4010) at an emission wavelength of 598 nm. The amount of coproporphyrin I (pmol·mg protein⁻¹·h⁻¹) formed was calculated as follows: 0.75 (conversion factor from copro- to uroporphyrin) × 1.527 (pmol/ng) × 3 (sample volume in mL) × the amount of coproporphyrin I measured (μg/L):protein (mg). The amount of protein was measured with the Bio-Rad DC Protein Assay. The reference interval was determined and monitored regularly using series of erythrocytes from 20 healthy volunteers. Two samples of erythrocytes having PBGD activities of ~70 and 40 pmol·h⁻¹·mg protein⁻¹ were used as a quality control to monitor the precision of PBGD measurements.

Excretion of urinary porphyrin precursors was measured by the δ-aminolevulinic acid (ALA)/PBG Column test (Bio-Rad), which is based on ion-exchange chromatography with measurements made by spectrophotometry. For the measurement of PBG and porphyrins, 50 mg of sodium carbonate was added to 10 mL of urine. For ALA measurements, 0.1 mL of 998 g/L acetic acid was added to 10 mL of urine (pH 5–7). The samples were preserved frozen for a maximum of 7 days before the measurement.
Excretion of urinary and fecal porphyrins was determined by HPLC (15, 16). A Varian Vista 5500 liquid chromatograph with a Shimadzu RF 530 fluorescence spectromonitor detector (Shimadzu Corporation) was used and set at an excitation wavelength of 403 nm and emission wavelength of 626 nm. Samples were loaded with an AASP Vac-Elut sample preparation system with C₁₈-bonded AASP cassettes (Varian) and run through a 3.9 × 300-mm C₁₈ Bondapak column with 10-μm particle size (Waters Corporation).

**SAMPLE PREPARATION**

We mixed 100 μL of a urine sample and 0.9 mL of 1 mol/L ammonium acetate buffer (pH 5.16) before HPLC analysis. A 10 μmol/L porphyrin standard mixture (Chromatographic Marker Porphyrin Acids; Porphyrin Products) was diluted in methanol–H₂O–acetic acid (6:4:1 by volume; 0.556 pmol/μL for each porphyrin). Coproporphyrin III standard (Porphyrin Products) was diluted with methanol–H₂O–acetic acid (6:4:1 by volume), and the final concentration was measured spectrophotometrically for each series separately. We mixed 50 μL of each standard dilution (1:1 by volume) with 0.9 mL of 1 mol/L ammonium acetate buffer (pH 5.16) before HPLC analysis. The amount of urinary porphyrins (nmol) was calculated: 10 × standard (pmol/100 μL) × sample (mm):standard (mm).

We vortex-mixed 100 mg of wet feces with 2 mL of 100 g/L trichloroacetic acid–dimethyl sulfoxide (1:1 by volume) for 3 min and separated the porphyrins by centrifugation (3000g for 10 min). We then mixed 50 μL of the supernatant with 0.95 mL of 1 mol/L ammonium acetate acid (pH 5.16) before HPLC analysis. We diluted a 10 μmol/L porphyrin standard mixture (Chromatographic Marker Porphyrin Acids; Porphyrin Products) and 2 mmol/L protoporphyrin IX and coproporphyrin III standards (Porphyrin Products) with 50 g/L bovine serum albumin in phosphate-buffered saline (1:3000) and then mixed 50 μL of this diluted standard mixture with 0.95 mL of 100 g/L trichloroacetic acid–dimethyl sulfoxide. After centrifugation, we mixed 50 μL of the supernatant with 0.95 mL of 1 mol/L ammonium acetate acid before HPLC analysis, as performed for the samples. The amount of porphyrins (nmol/g dry weight) was calculated: 10 × standard (pmol/100 μL) × [sample (mm):standard (mm)] × dry weight of feces (mg).

Lyphochek Quantitative Urine Control (Bio-Rad) was used as an assay quality control to monitor the precision of urinary measurements. For fecal measurements, two whole-blood samples having protoporphyrin concentrations of ~700 and 400 nmol/L of red cells were used as a control. The reference values for all laboratory measurements were adapted from the Mayo Medical Laboratories Test Catalog and Interpretive Handbook for 1988 (http://www.mayo.edu/mml/testcat.html) and are shown in Table 1. The biochemical tests and mutation analyses were performed independently by different laboratory technicians without knowledge of participants’ clinical status.

Plasma samples were diluted with phosphate-buffered saline (1:4 by volume) before analysis by spectrofluoro-
method quenced using dideoxynucleotide chain termination. PCR products were purified by use of the QIAquick PCR kit (Qiagen) and including 5 \( \mu \)Ci of \([\alpha-^32P]\)dATP (Amersham Pharmacia Biotech) in each sample. After electrophoresis, gels were dried (30 min at 80 °C) and autographed with Kodak Biomax MR film (Eastman Kodak). The analyses were repeated at least twice for each sample studied in the presence of positive and negative controls.

**RESTRICTION DIGESTION**

We incubated 10 \( \mu \)L of PCR product and 20 \( \mu \)L of restriction enzyme in 30 \( \mu \)L of the enzyme buffer for 1 h at the temperature specific for the enzyme (New England Biolabs). The resulting fragments were run in an ethidium bromide-stained 1–4% agarose gel (Amersham Pharmacia) and visualized with ultraviolet light. The cleavage was repeated at least twice with different samples from each patient in the presence of positive and negative controls in each series analyzed.

**Fig. 3. Correlation between urinary PBG measured from AIP patients in remission and during an acute attack.**

○, patients with low excretion (\(<150 \mu\text{mol/L}\)) in remission; ●, patients with high excretion (\(>150 \mu\text{mol/L}\)) in remission.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Leukocyte DNA was extracted from the venous blood samples with EDTA as an anticoagulant using the QIAamp Blood Kit (Qiagen). The PCR mixture contained 100 ng of DNA, 0.2 mM deoxynucleoside triphosphates, 20 pmol of primers, and 1 U of DNA polymerase (Dy-nazyme II; Finnzymes) in 50 \( \mu \)L of the enzyme buffer (18). Primer sequences have been published previously (19). The temperature profile for the PCR reactions was 2 min at 94 °C for the first denaturing step, followed by 30 s at 94 °C, 30 s at 56–60 °C, and 30 s at 72 °C for 30 cycles. The PCR products were purified by use of the QIAquick PCR Purification Kit (Qiagen). The samples were directly sequenced using dideoxynucleotide chain termination method (20) with the Amplicycle Sequencing Kit (Perkin-Elmer) and including 5 \( \mu \)Ci of \([\alpha-^32P]\)dATP (Amersham Pharmacia Biotech) in each sample. After electrophoresis, gels were dried (30 min at 80 °C) and autographed with Kodak Biomax MR film (Eastman Kodak). The analyses were repeated at least twice for each sample studied in the presence of negative and positive controls.

**STATISTICAL ANALYSES**

The Mann–Whitney test was used to assess the differences in urinary excretion of porphyrins and their precursors. ROC analyses were used to assess the validity of different laboratory tests in the diagnosis of AIP. Statistical calculations were performed using SPSS, Ver. 10.04, and NCSS 2000.

**RESULTS**

**MUTATION TESTING**

Shown in Fig. 1 are 196 mutations identified in the \( PBGD \) gene, including 26 mutations identified among Finnish AIP patients [bold; Human Gene Mutation Database (http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html)]. These mutations cover 87% of the 45 AIP families in the Finnish population of 5 million. Of the 239 individuals tested, 120 were mutation-positive, and in 119 cases, AIP could be excluded. The probability is small that a healthy relative would have a disease-causing mutation in the \( PBGD \) gene that is different from that in the proband; we thus considered that mutation analysis excluded the diagnosis of AIP in symptom-free relatives when a mutation was known in the family. Of the 131 individuals (Table 1) who underwent mutation analysis and biochemical analyses, the diagnosis or exclusion of AIP was based solely on mutation testing in 3 cases (2%).

Mutation testing had some problems. In four cases, the proband was the only patient in the family and was deceased or could not be traced back. In two additional cases, the clinical manifestations and biochemical patterns best suited AIP, but long PCR fragments and sequencing of all exons, exon-intron boundaries, and 5′- and 3′-untranslated regions did not reveal a genetic defect. In one of the latter two cases, erythrocyte PBGD activity was decreased. In the other case, six of the eight enzymes in the heme biosynthetic pathway were within the reference intervals. Her uroporphyrinogen III synthase activity was not measured, and the coproporphyrinogen oxidase activity was increased threefold during a psychotic period, suggesting secondary porphyrinuria. No other family members were affected. Although mutations could not be identified in these patients, AIP could not be excluded because of acute attacks and suitable biochemical abnormalities.

**BIOCHEMICAL METHODS**

The first PBG analysis on each patient detected 84% of 147 AIP patients screened in remission. When the analyses were repeated one to eight times during follow-up in 73 of the patients, the overall sensitivity was increased only to 88%. In the patients with increased PBG excretion (\( n = 129; 62 \) symptomatic by medical history, 67 symptom free), variation among individuals was wide (9.8–590 \( \mu\text{mol/L} \)). The mean (SD) excretion for all patients was 145 (138) \( \mu\text{mol/L} \), which was 10-fold above the upper limit of the reference interval. Excretion of PBG was also increased in 40% of 198 healthy relatives screened [\( n = 79; \)
range, 9.1–24 μmol/L; mean (SD), 13 (3.2) μmol/L], but the mean increase was markedly less (twofold). Urinary ALA was increased in 61% of 145 AIP patients screened in remission [n = 89; 50 symptomatic by medical history, 39 symptom free; range, 34–453 μmol/L; mean (SD), 130 (96) μmol/L], which is a 3.8-fold increase above the upper limit of the reference interval, and in 6.6% of 198 healthy relatives screened [n = 13; range, 34–62 μmol/L; mean (SD), 42 (8.8) μmol/L], which is a 1.2-fold increase.

Shown in Fig. 2 are the mean PBG and ALA excretions for symptomatic AIP patients during an acute attack and in remission compared with symptom-free patients and their healthy relatives. During an acute attack, excretion of PBG was increased approximately twofold (n = 29; mean values, 237–449 μmol/L) compared with the values in remission, but there was a wide interindividual variation in excretion (range, 92–1411 μmol/L). The patients with urinary PBG <150 μmol/L in remission (n = 10) had a relatively greater increase in PBG excretion during an acute attack (3.9-fold) than those who excreted high amounts of PBG (>150 μmol/L; n = 19) even in remission (1.6-fold; Fig. 3).

Never-symptomatic patients (n = 80) had significantly lower PBG excretion than symptomatic patients (n = 63) in remission [mean (SE) 72 (11) μmol/L vs 212 (18) μmol/L; P <0.000001]. Among healthy relatives, urinary

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Fig. 4. Comparison of urinary porphyrin excretion in symptomatic AIP patients during an acute attack and remission, in symptom-free patients, and in their healthy relatives.

The values for individual persons were calculated as the means of one to eight single measurements in adulthood. Dashed lines denote the upper limits of the reference intervals (36 nmol/day, 230 nmol/day, 100 nmol/g, and 130 nmol/g for urinary uro- and coproporphyrin and fecal copro- and protoporphyrin, respectively). Means (SE) are indicated.
modifying polymorphisms in other metabolic genes in these families.

Among 129 AIP patients screened in remission, 75% (n = 97; 53 symptomatic and 44 symptom free) excreted high amounts of uroporphyrins [range, 38–944 nmol/day; mean (SD), 975 (1409) nmol/day], of which ~50% were I and III isomers. Among 172 healthy relatives, 4% (n = 7; range, 36–189 nmol/day; mean (SD), 60 (57) nmol/day) excreted abnormally high amounts of uroporphyrins. Fig. 4 shows the urinary excretion of uro- and coproporphyrins and 7-, 6-, and 5C-porphyrins. In AIP patients, increased 4.2-fold [mean (SD), 1395 (1432) nmol/day vs 460 (50) nmol/day; P = 0.0005]. Urinary coproporphyrins were increased in 69% of 136 AIP patients in remission [n = 94; 52 symptomatic, 40 symptom free; range, 263-2392 nmol/day; mean (SD), 810 (476) nmol/day; 70–90% III isoform] and in 8.6% of 187 healthy relatives [n = 16; range, 235–689 nmol/day, mean, 311 (144) nmol/day], respectively. In AIP patients, excretion of urinary uroporphyrin exceeded that of coproporphyrins and 7-, 6-, and 5C-porphyrins.

Fecal coproporphyrins were increased in only 2% of 113 AIP patients screened [n = 2; range, 106–185 nmol/g; mean (SD), 145 (56) nmol/g; 30–60% III isoform], and none of 163 healthy relatives had increased fecal excretion of coproporphyrins (Fig. 4). In contrast, fecal protoporphyrins were increased in 19% of 113 AIP patients in remission [n = 21; range, 132–454 nmol/g; mean, 212 (95) nmol/g] and in 7% of 163 healthy relatives [n = 12; range, 137–286 nmol/g; mean, 180 (52) nmol/g; Fig. 4].

### Table 2. Diagnosis of AIP.

<table>
<thead>
<tr>
<th></th>
<th>Acute attack</th>
<th>Symptom free</th>
<th>Sensitivity ± 95% CI, a %</th>
<th>Specificity ± 95% CI, %</th>
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</thead>
<tbody>
<tr>
<td><strong>Mutation analysis</strong></td>
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<tr>
<td>Erythrocyte PBGD</td>
<td></td>
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<td>95</td>
<td>100</td>
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<tr>
<td>Urinary PBG</td>
<td>↑ ↑ ↑ / ↑ ↑</td>
<td>↑ / ↑ / ↑ /N</td>
<td>84 ± 8</td>
<td>77 ± 13</td>
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<tr>
<td>Urinary ALA</td>
<td>↑ ↑ ↑ / ↑ ↑</td>
<td>↑ / ↑ / ↑ /N</td>
<td>85 ± 8</td>
<td>71 ± 14</td>
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<tr>
<td>Urinary uroporphyrin</td>
<td>↑ ↑ ↑ / ↑ ↑</td>
<td>↑ / ↑ / ↑ /N</td>
<td>62 ± 11</td>
<td>90 ± 9</td>
</tr>
<tr>
<td>Urinary coproporphyrin</td>
<td>↑ ↑ ↑ / ↑ ↑</td>
<td>↑ / ↑ / ↑ /N</td>
<td>72 ± 11</td>
<td>97 ± 6</td>
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<tr>
<td>Fecal coproporphyrin</td>
<td>↑ ↑ ↑ / ↑ ↑</td>
<td>↑ / ↑ / ↑ /N</td>
<td>67 ± 11</td>
<td>87 ± 12</td>
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<tr>
<td>Fecal protoporphyrin</td>
<td>↑ ↑ ↑ / ↑ ↑</td>
<td>↑ / ↑ / ↑ /N</td>
<td>20 ± 11</td>
<td>96 ± 8</td>
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<td>Plasma fluorescence</td>
<td>+ / +</td>
<td>+ / + / +</td>
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</table>

a CI, confidence interval.

b +, mutation positive.

c ↓, decreased, N, within the reference interval; ↑ ↑ ↑, increased >10-fold; ↑ ↑ , increased 5- to 10-fold; ↑, increased 2- to 5-fold.
normal erythrocyte PBGD activity in the variant form of AIP. If those patients \( (n/H11005^8) \) were excluded, measurement of PBGD activity detected 71 (92%) of the 77 patients with the classic form of AIP. Even more troubling was the result that 23% of healthy relatives were false positive.

**Discussion**

Measurement of urinary PBG identified all AIP patients during an acute attack, and the mean excretion of PBG was markedly increased above the reference interval, although the intraindividual increases were modest, emphasizing the importance of careful evaluation of clinical manifestations. Urinary PBG analysis identified the majority of patients in remission, but \( \sim 10\% \) remained undetected. The test is sensitive for acute porphyrias in general and does not distinguish AIP from other acute porphyrias. Although many healthy relatives experienced mild increases in PBG excretion, by ROC curve analysis it was nonetheless the best of the biochemical tests for AIP. Erythrocyte PBGD activity was decreased in the majority of AIP patients, with results within the reference interval mainly in the variant form of AIP, but it was also decreased in one-fourth of healthy relatives. Because biochemical analyses remain often indeterminate, mutation analysis is needed to exclude or confirm the diagnosis of AIP in remission.

AIP is the commonest type of acute porphyria in most countries (1). The prevalence varies from 3:100,000 estimated in Finland and Western Australia (13, 21) and 5–10:100,000 in the US to 1:10,000 in Sweden (22). In northern Sweden, the prevalence exceeds 0.5–2% because of a founder effect in two small municipalities. Low erythrocyte PBGD activity is more common, \( \sim 1:500 \) when estimated among healthy Finnish and French blood donors (23, 24). Penetrance varies between 10% and 50% within the families; approximately one-half of the patients stay symptom free throughout their lives, 30–40% experience mild symptoms, and only 10–20% experience acute attacks (7). Thus, the prevalence may be much higher than previously expected. Expanded mutation screening may provide more accurate prevalence rates.

Our series includes both patients with symptoms and phenotypically normal carriers; it thus provides information about outcomes among patients with AIP in general. The proportion of the patients who had acute attacks (41%) is greater than in some extensive family studies, in which up to 80% of the diagnosed patients have been phenotypically normal carriers (25, 26). The personal contacts with every patient at the time of diagnosis and afterward explain the good collaboration, the high number of included individuals, and the relatively low number of nontraced and excluded individuals, which increased the reliability of these results. Thus, we believe that the results of our series are accurate enough to serve as a basis for conclusions concerning the diagnosis of AIP.

In our series of mutation screenings, many new symptom-free patients were identified and many healthy relatives could be excluded despite slightly increased excretion of porphyrins and porphyrin precursors. Thus, DNA analysis is the only reliable way to screen symptom-free patients to facilitate correct treatment and proper genetic counseling of family members at risk. The certain diagnosis of AIP in the early stage as well as identification of symptom-free individuals is essential for the prevention of acute attacks, which may be potentially fatal (7). The diagnosis should be confirmed in youth, so that patients

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**Fig. 5.** Comparison of sensitivity and specificity characteristics for different biochemical tests for AIP.

(A) urinary and fecal porphyrins and precursors: curve 1, urinary PBG; curve 2, urinary uroporphyrin; curve 3, urinary coproporphyrin; curve 4, urinary ALA; curve 5, fecal coproporphyrin, and curve 6, fecal protoporphyrin; (B), erythrocyte PBGD activity: curve 1, patients with variant form of AIP excluded; curve 2, all AIP patients.
been shown to have predictive value for the diagnosis of AIP in adulthood and in remission. However, clinical symptoms may be modulated by stress and may vary in intensity throughout their life span. Therefore, biochemical measurements are essential before the diagnosis of AIP can be confirmed in symptomatic patients.

In our participants, biochemical urinary and erythrocyte measurements revealed the majority of the AIP patients in remission, and urinary excretion of PBG detected all patients during an acute attack. Thus, measurement of urinary PBG is the method of choice at the symptomatic phase. PBG excretion is usually increased 20- to 50-fold during an acute attack compared with reference values. However, two-thirds of the patients excrete high amounts of PBG even in remission, and the increase may be modest during an attack, making differential diagnosis of abdominal pain more difficult. Measurement of uroporphyrin excretion during an attack provided no additional information in those patients who excreted high amounts of uroporphyrins in remission (data not shown). The plasma porphyrin fluorescence spectrum is usually only transiently characteristic for AIP during an attack. Thus, the importance of the clinical manifestations, including symptoms of porphyria, signs of neuropathy, and a history of precipitating factors, should be emphasized. Correct diagnosis is essential, and differential diagnosis of abdominal pain should be evaluated each time in AIP patients.

Although biochemical measurements of excreted porphyrins and porphyrin precursors reveal acute porphyria in the majority of cases, none of these tests is specific for AIP. Moreover, acute symptoms and signs are indistinguishable from those of other acute porphyrinas, although patients with AIP and ALA dehydratase deficiency do not manifest photosensitivity, which is common in patients with variegate porphyria and hereditary coproporphyria. Only ~40% of patients with variegate porphyria experience skin symptoms, and these appear independently from acute attacks. Excretion of fecal protoporphyrin is increased in 20% of AIP patients even in remission; thus it cannot be used to distinguish variegate porphyria from AIP. Excretion of uroporphyrin is increased in AIP, similar to porphyria cutanea tarda, which is the commonest cutaneous porphyria accompanied by mild liver failure and photosensitivity but no acute attacks. In addition, urinary excretions of coproporphyrins can be increased in many liver diseases and in heavy metal intoxication.

The use of DNA technology has its own special demands. The PCR method is prone to contamination and requires special laboratory equipment and thorough quality assurance. Mutations can be detected by various DNA methodologies, which can be chosen according to the purpose and the experience of the laboratory personnel, although sequencing is recommended. In symptom-free individuals, DNA analysis is sufficient for the diagnosis of AIP. Measurement of urinary PBG in adulthood and in remission has been shown to have predictive value. Thus, those whose urinary PBG excretion rates are within the reference interval are more likely to remain symptom free throughout their life span. However, a proper clinical investigation, detailed family history, and biochemical measurements are essential before the diagnosis of AIP can be confirmed in symptomatic patients.

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References
5. Yoo HW, Warner CA, Chen CH, Desnick RJ. Hydroxymethylbilane synthase activity may be secondary, as reported in variegate porphyria (27). The decrease in PBGD activity may be secondary, as reported in variegate porphyria patients (33). Furthermore, PBGD activity can be within the reference interval in AIP patients during an acute attack (34). In the variant form of AIP, the erythrocyte PBGD activity is within the reference interval in both the symptomatic and symptom-free phases (11).

The decrease in PBGD activity is relatively sensitive and specific for AIP, but it may be affected by various conditions, such as anemia (29), liver disease (30), malignancies (31), uremia (32), chronic polyarthritis (30), and hemodialysis, or by any other conditions in which erythropoiesis is either induced or inhibited (12). The decrease in PBGD activity may be secondary, as reported in variegate porphyria patients (33). Furthermore, PBGD activity can be within the reference interval in AIP patients during an acute attack (34). In the variant form of AIP, the erythrocyte PBGD activity is within the reference interval in both the symptomatic and symptom-free phases (11).


