We conclude that when interpreting plasma selenium concentrations, a marker of the inflammatory response, such as CRP, should be included to distinguish true nutritional depletion from the inherent effects of disease.

References

Mutation Screening by Denaturing Gradient Gel Electrophoresis in North American Patients with Acute Intermittent Porphyria, Niels Erik Petersen,¹ Henrik Nissen,¹ Mogens Hørder,¹ Janine Senz, Azim Jamani, William E. Schreiber²,³ (¹ Department of Clinical Biochemistry and Clinical Genetics, Odense University Hospital, DK-5000 Odense C, Denmark and The Danish Centre of Porphyria; ² Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, V6T 1W5 Canada; ³ Division of Clinical Chemistry, Vancouver Hospital and Health Sciences Centre, Vancouver, British Columbia, V5Z 1M9 Canada; * author for correspondence: fax 45-65-41-19-11, e-mail nep@ dou.dk)

Acute intermittent porphyria (AIP) is a dominantly inherited disorder of heme metabolism caused by a 50% decrease in the activity of porphobilinogen deaminase (EC 4.3.1.8). The estimated prevalence of the disease is 1:1000 to 1:5:100 000, making it the most common of the neurologic porphyrias (1). Although its penetrance is only 10–20% (2), acute attacks of AIP are disabling and can be life-threatening. Prevention of acute attacks is the most important principle in managing the disease; however, it requires prior identification of individuals at risk. Because AIP has a genetic basis, molecular analysis for this disease is ultimately the most reliable way to identify gene carriers.

More than 130 mutations in the porphobilinogen deaminase gene have been characterized to date (3). The majority have been restricted to a single family, or at most a few families, and no mutation accounts for more than a small percentage of all cases. With the exception of selected geographic areas where single mutations predominate (4,5), testing for known mutations in a patient who has not previously been investigated is likely to be negative. Molecular analysis therefore requires an efficient strategy for finding the causative mutation. Denaturing gradient gel electrophoresis (DGGE) has been used by several groups to screen the exons and flanking intronic segments of the porphobilinogen deaminase gene for variations in the wild-type sequence (6–13). This work has been carried out in European laboratories, and the mutations that have been discovered reflect the molecular defects within that population. By contrast, relatively few mutations have been described in the North American population (14–19). The goal of this study was to further characterize the spectrum of mutations in North American patients with AIP, using a well-established DGGE assay with high sensitivity to identify exons/introns that required sequencing (18). DNA samples were also analyzed by heteroduplex analysis (16) to compare its sensitivity in detecting mutations to that of DGGE.

Fifteen patients with AIP from 12 unrelated families were studied. Geographically, these patients were referred from the provinces of Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia in Canada and from the states of Kansas and Washington in the United States. The diagnosis was based on a combination of clinical and family history, increased excretion of δ-aminolevulinic acid and porphobilinogen in urine, reference value fecal porphyrins, and decreased activity of porphobilinogen deaminase in red cell lysates. Cross-reacting immunologic material status was determined in six patients from five kindreds; all were negative for cross-reacting immunological material. Procedures involving human subjects were performed in accordance with the Helsinki Declaration of 1975, as revised in 1983, and informed consent was obtained from all subjects before their inclusion in the study.

All 15 exons of the porphobilinogen deaminase gene and their surrounding intronic sequences were analyzed by DGGE as previously described (18). DGGE patterns corresponding to known polymorphisms in introns 2 and 3 (20), intron 6 (18), intron 10 (21), and exon 10 (22) were present in many of these samples. Unique patterns not associated with known polymorphisms were found in 14 of the 15 patients, and sequencing of these regions re-
revealed nine different mutations (Table 1). Two missense mutations, a C→T at position 76 (R26C) and a second C→T at position 346 (R166W) of the coding sequence, have been previously reported (23, 24). Two nonsense mutations were also detected, a previously reported C→T transition at position 673 (R225X) (23) and a new C→G transversion at position 863 (S288X). Four novel mutations located within introns were identified, at the 3’ end of intron 4, the 5’ end of intron 10, the 5’ end of intron 11, and the 3’ end of intron 11 (Table 1). All of these mutations affect either the invariant AG dinucleotide at the 3’ splice site or the invariant GT dinucleotide at the 5’ splice site of the intron. One of these mutations is a 5-bp inversion at the 5’ end of intron 10, a type of mutation that has never before been reported in AIP and would appear to be a rare occurrence in any genetic disorder. A novel 1-bp insertion in exon 15 (986insT) was also discovered in DNA from one patient. Four of the mutations eliminate restriction sites, one mutation creates a new site, and one mutation changes the location of a restriction site (Table 1). These mutations were confirmed by digestion of the appropriate PCR product with the indicated restriction enzyme. In each case, electrophoresis of the digest on an agarose gel revealed one wild-type and one mutant allele.

We also tested PCR products containing each mutation by heteroduplex analysis to look for changes in their respective patterns (16). DNA from the patient with the mutation and at least four others who did not have the mutation were analyzed side-by-side. Of nine mutations, seven produced patterns that were clearly distinct from other samples and would have led to sequencing of that exon (Table 1). Two mutations, R26C and 651+1G→C, generated patterns that were nearly indistinguishable from other samples run side-by-side. Because we knew which samples contained mutations, we had an unfair advantage in interpreting the patterns. If we had carried out the analyses without this knowledge, two of the nine mutations likely would have been missed. This limited comparison of the two screening techniques, performed as previously described, suggests that DGGE is more sensitive than heteroduplex analysis in identifying mutations among patients with AIP. Heteroduplex analysis is technically more simple to set up and run than DGGE and may therefore be preferable in a clinical laboratory. However, if no mutation is identified by heteroduplex analysis, the sample should be further analyzed by DGGE in a laboratory with experience in this technique.

Only one unique DGGE pattern, corresponding to a possible mutation, was present in any patient's DNA. Whenever such a pattern was found, sequencing revealed a change in the DNA sequence that would be expected to interfere with enzyme activity. Two mutations, 161–1G→C and R225X, were discovered in apparently unrelated families; however, all other mutations were found in a single family (Table 1). Taking all patients into account, DGGE identified mutations in 11 of the 12 families tested, leading to the identification of 9 of 10 theoretically possible mutations. For 1 of the 15 patients, neither DGGE nor heteroduplex analysis revealed a unique pattern that might correspond to a mutation. The sensitivity of our DGGE technique is therefore in the range of 90%, similar to the sensitivity reported in a recent study of a larger population in France (6). Previously, this same DGGE method correctly identified the mutation-bearing exons in all 22 samples tested with no false positives (8).

Of the nine mutations found in this study, three have been reported by investigators from Europe: R26C (23), R166W (5, 25), and R225X (23). The remaining six mutations are hitherto unpublished, although S288X, produced by a different single base substitution at position 863 of the coding sequence, was recently described (6). If we had tested only for mutations that have been reported in the past, most of the mutations in this group of North American patients with AIP would have been missed. Four of these mutations occur within introns and, in each case, affect either the invariant AG or GT dinucleotide required for normal splicing of the mRNA transcript. Splice site mutations are thought to comprise ~15% of all disease-causing point mutations (26); however, within the porphobilinogen deaminase gene >20% of the mutations published to date occur at splice sites. The higher prevalence found in this study might be representative of the overall North American population with AIP; however, the number of patients is too small to draw firm conclusions.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Detected by HA</th>
<th>Restriction site changed</th>
<th>CRIM status</th>
</tr>
</thead>
<tbody>
<tr>
<td>R26C</td>
<td>Exon 3</td>
<td>C→T at 76</td>
<td>No</td>
<td>BspMl</td>
<td>ND</td>
</tr>
<tr>
<td>161–1G→C</td>
<td>Intron 4</td>
<td>G→C at 161-1</td>
<td>Yes</td>
<td>Ddel</td>
<td>Negative</td>
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<tr>
<td>R166W</td>
<td>Exon 8</td>
<td>C→T at 346</td>
<td>Yes</td>
<td>Aci</td>
<td>Negative</td>
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<tr>
<td>612+2TAGGG→CCCTA</td>
<td>Intron 10</td>
<td>TAGGG→CCCTA at 612+2</td>
<td>Yes</td>
<td>BspMl</td>
<td>ND</td>
</tr>
<tr>
<td>651+1G→C</td>
<td>Intron 11</td>
<td>G→C at 651+1</td>
<td>No</td>
<td>Rsal</td>
<td>Negative</td>
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<tr>
<td>652-2A→G</td>
<td>Intron 11</td>
<td>A→G at 652-2</td>
<td>Yes</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>R225X</td>
<td>Exon 12</td>
<td>C→T at 673</td>
<td>Yes</td>
<td>Cac8I</td>
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<tr>
<td>S288X</td>
<td>Exon 14</td>
<td>C→G at 863</td>
<td>Yes</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>986insT</td>
<td>Exon 15</td>
<td>T insertion after 986</td>
<td>Yes</td>
<td>No</td>
<td>ND</td>
</tr>
</tbody>
</table>

*a Numbering of nucleotides is based on position in the coding sequence.
*b HA, heteroduplex analysis; ND, not determined.
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References