Pseudoexon Activation in the HMBS Gene as a Cause of the Nonerythroid Form of Acute Intermittent Porphyria

To the Editor:

Acute intermittent porphyria (AIP)\(^1\) (MIM 176000) is a low-penetrance, autosomal dominant disorder caused by decreased activity of the third enzyme in the pathway of heme biosynthesis, hydroxymethylbilane synthase (HMBS). Partial deficiency of HMBS can lead to episodic life-threatening neurovisceral attacks of acute porphyria, which are often provoked by drugs, alcohol, or hormonal factors\(^1\). Biochemical analyses of urinary porphobilinogen and porphyrins (fecal porphyrins and plasma porphyrins) can provide the diagnosis of AIP in symptomatic patients, but genetic testing for a defect in the HMBS gene\(^1\) is required to accurately identify asymptomatic affected family members, who can then be counseled on the lifestyle modifications required to limit the risk of attacks.

We previously reported on our experience with using direct genomic sequencing and dosage analysis of the HMBS gene for genetic analysis of AIP\(^2\). We found a 98% sensitivity for detecting disease-causing mutations in unrelated symptomatic patients with a biochemical diagnosis of AIP\(^2\), and we speculated that the cause in AIP patients in whom no mutation was found was likely to be mutations in unexplored parts of the gene, possible regulatory variants, or defects at another locus.

We investigated a 28-year-old woman who presented with a 4-day history of lower abdominal pain after a contraceptive injection of progestogen only. Our investigation showed the following: an increased urinary porphobilinogen/creatinine ratio of 62.2 (reference interval, 0–1.5 mmol/mol), confirming an acute attack; a plasma spectrofluorometric emission peak at 621 nm; a normal fecal total-porphyrin value of 56 nmol/g dry weight (reference interval, 0–200 nmol/g); and an HMBS activity of 28 nmol·h\(^{-1}\)·mL\(^{-1}\) (reference interval, 24–67 nmol·h\(^{-1}\)·mL\(^{-1}\))\(^1\). We requested mutation analysis to enable testing of family members.

The initial investigation of the patient included a denaturing HPLC evaluation to scan the HMBS gene, sequencing of all coding regions and their flanking introns, and gene-dosage analysis to look for gross deletions or insertions in the gene\(^2\). No potentially pathogenic changes or heterozygous polymorphisms were identified, and a sample of blood was requested from this “mutation-negative” patient for mRNA analysis.

Total RNA was isolated, and HMBS cDNA was amplified in 2 sections, exons 1–12 and exons 9–15\(^3\). In contrast to a normal sample, we found that the amplification product for exons 1–12 separated into 2 bands on electrophoresis (Fig. 1). Sequence analysis of the cDNA products from both alleles identified a normal sequence for all exons, although the larger band included 171 bp of extra sequence that corresponded with an isolated noncontiguous region within intron 1 that had been inserted between exon 1 and exon 3 (Fig. 1). This finding suggested a mechanism by which the splicing machinery recognized a region of intronic sequence as an exon, a so-called pseudoexon.

We amplified and sequenced intron 1 with primers 5′-GACC CAGACCAAAGACCTCG-3′ and 5′-TCAAGGGATGAGGTGACT TGC-3′. This analysis identified a single base change at position c.33+669A>G that was also present in the patient’s son. Although functional studies of this longer RNA have not been formally undertaken, the disruption caused by the inclusion of an additional 57 amino acid residues would be expected to affect protein function.

We also analyzed genomic DNA from 4 apparently unrelated patients who we had described in our previous report\(^2\). These patients had biochemical evidence of AIP, but we identified no mutation. We analyzed these patients for the intron 1 alteration (c.33+669A>G) and found this alteration in 1 patient (HMBS activity, 31 nmol·h\(^{-1}\)·mL\(^{-1}\)). This mutation is therefore a rare cause of AIP. We have identified this mutation in only 2 (0.6%) of the 322 HMBS mutations identified to date in our laboratory, but it is present in 2 (22%) of the 9 families with nonerythroid AIP\(^4\) that we have investigated.

Pseudoexon inclusion has been identified as a disease-causing mechanism in a number of disorders, including cystic fibrosis, muscular dystrophy, and neurofibromatosis\(^5\). Antisense oligonucleotides are being investigated as a possible treatment for preventing pseudoexon splicing in patients with these diseases, and such treatments may be applicable to these porphyria patients.

As our understanding of underlying molecular mechanisms improves, identifying the molecular defects in the porphyria genes is becoming more successful, thereby enabling more families to benefit from presymptomatic testing. The identification of a pseudoexon inclusion as a cause of AIP suggests that mRNA should be analyzed for all biochemically confirmed patients who are “mutation negative” by standard genomic approaches.

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\(^1\)Nonstandard abbreviations: AIP, acute intermittent porphyria; HMBS, hydroxymethylbilane synthase.
Although a rare cause of genetic
disease, pseudoexon inclusion is
being recognized in more disor-
ders, as exemplified by this first re-
port of pseudoexon inclusion caus-
ing acute intermittent porphyria.

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