Acute intermittent porphyria caused by defective splicing of porphobilinogen deaminase RNA: a synonymous codon mutation at -22 bp from the 5' splice site causes skipping of exon 3

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Abstract

Acute intermittent porphyria (AIP) results from mutations in the porphobilinogen deaminase (PBG) gene. Three of 14 randomly selected, unrelated patients with the cross reacting immunological material (CRIM) negative form of AIP were found to have previously undescribed RNA splicing defects. Defective splicing of exons 12 and 13 was caused by a C→G transversion at position -3 of the 3' splice site of intron 11 and a G→A transition at the first position of intron 13, respectively. Defective splicing of exon 3 was associated with a synonymous codon mutation (CGC→CGG, R28R) at position -22 from the 5' splice site. Our findings are consistent with previous reports indicating that about 15% of mutations in the PBG deaminase gene that cause AIP affect RNA splicing and add to the evidence that synonymous intraexonic codon mutations may cause disease.

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fragments were amplified and sequenced directly.

In three of the 14 patients with CRIM negative AIP, amplification of cDNA showed a shorter product in addition to the expected normal cDNA. Patients 1 and 2 showed defects between exons 10 and 15 with the smaller bands being 54 bp and 120 bp shorter than full length cDNA, consistent with absence of exons 13 and 12 respectively (fig 1). Sequencing of the shorter cDNAs showed splicing of exon 12 to exon 14 and exon 11 to exon 13. Amplified genomic DNA containing the relevant intron/exon boundaries from patients 1 and 2 showed heterozygosity for a G→A transition at the first position of intron 13 and a C→G transversion at position -3 of the 3' splice site of intron 11, respectively. These mutations were confirmed in the patients and their affected relatives by allele specific oligonucleotide analysis. Neither mutation was detected in 12 unrelated patients with CRIM negative AIP. Mutations at these sites are known to affect splicing of adjacent exons in other genes.^{5,6} Exon 12 contains a CAG sequence 34 bp from its 5' end but we found no evidence for use of this potential cryptic splice site. Both defects produce mRNAs with coding sequences that are in frame. Although immunochemical studies on lymphoblasts from another patient with an exon 12 splicing defect indicated that a truncated protein was produced,⁷ we failed to detect immunoreactive protein products in erythrocytes from either of our patients, suggesting that any truncated proteins are unstable in these cells.

Figure 1 cDNA for PBG deaminase from two patients with AIP. Amplification products from exons 8-15 are shown: lane 1, patient 1 (771 bp, 717 bp); lane 3, patient 2 (771 bp, 651 bp); lane 5, normal subject; lanes 2 and 4, DNA size markers.

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Acute intermittent porphyria (AIP) is a low penetrant, autosomal dominant disorder, characterised clinically by life threatening, acute, neurovisceral attacks, often precipitated by drugs, which results from deficiency of porphobilinogen (PBG) deaminase activity.¹ Over 50 disease specific mutations in the PBG deaminase gene have now been reported in AIP.^{2,3} Most decrease both enzyme activity and the concentration of cross reacting immunological material (CRIM) in all tissues; the rest are either CRIM positive or affect only the non-erythroid isoenzyme.¹ Here we report that three of 14 patients with the CRIM negative form of AIP were found to have previously undescribed RNA splicing defects.

Fourteen unrelated patients were selected at random from a group of 32 patients with the CRIM negative form of AIP. CRIM status and probabilities that asymptomatic relatives carried the AIP gene were determined as described previously.⁴ cDNAs were obtained by RT-PCR from RNA extracted from lymphoblastoid cells using primers to amplify exons 1 to 10 and 8 to 15 inclusive. Genomic DNA was extracted from whole blood; appropriate

Figure 2 Characterisation of mutation in exon 3. Digestion by *Tha*I of amplified genomic DNA containing exon 3 from patient 3 (lane 2) and normal subject (lane 4); lanes 3 and 5, undigested DNA from patient 3 and normal subject; lane 1, DNA size markers.

Patient 3 showed a defect between exons 1 and 8, the size difference (54 bp) indicating probable absence of exon 3. Sequencing of the shorter cDNA showed splicing of exon 1 to exon 4; mRNA for the ubiquitous isoenzyme of PBG deaminase does not contain exon 2. The rest of the cDNA sequence was normal. Primers in exon 2 and exon 4 were used to amplify a 630 bp genomic DNA fragment. Direct sequencing of this fragment showed no abnormalities in the exon/intron boundaries or in the branch point region in intron 2. The only base change identified between exon 2 and exon 4 was a synonymous third base mutation in exon 3 (CGC→CGG, R28R) at position -22 from the 5' splice site which abolishes a *Tha*I recognition site. Digestion of amplified genomic DNA with *Tha*I confirmed the presence of this base change in the patient (fig 2) and in six affected relatives (either symptomatic or with a probability of carrying the gene for AIP of greater than 0.80) but not in two unaffected relatives (carrier probability less than 0.10) or 19 other unrelated patients with AIP. It was also absent from 100 unrelated normal subjects. Amplification of cDNA from affected relatives confirmed defective splicing of exon 3.

Exon mutations that are distant from the splice junction consensus sequence but affect 5' splice site selection are rare, having been

reported in one human disease, mitochondrial acetoacetyl-coenzyme A thiolase deficiency,⁸ in bovine thyroglobulin deficiency,⁹ and in an in vitro mutant of the human hypoxanthine-guanine phosphoribosyl transferase gene.¹⁰ These mutations were also situated from 13 to 28 nucleotides upstream from the 5' splice site; both natural mutations altered the coding sequence.^{8,9} Intraexonic mutations of this sort are believed to affect splice site selection by altering pre-mRNA secondary structure which may interfere with assembly of small ribonucleoproteins into the spliceosome.⁸ Prediction of the secondary structure¹¹ of the mutant exon 3 showed a change in the region of the 5' splice site but it is unclear whether structure of the transcript as presented to the splicing machinery would be similarly altered. The association of this mutation only with AIP, and the otherwise normal sequence between exons 2 and 4, strongly suggest that it is the cause of exon skipping in our patient.

About 15% of the reported mutations in AIP cause exon skipping; our findings (21% in a small series) are consistent with this proportion. In addition our observation that a synonymous intraexonic codon mutation causes exon skipping adds to the growing evidence that such mutations should not be discarded as potential causes of disease until RNA processing has been investigated.¹²

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