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Short Communication

A novel 3-base deletion (IVS3+2_4delTGG) of the hydroxymethylbilane synthase gene in a Brazilian patient with acute intermittent porphyria

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Abstract

Acute intermittent porphyria (AIP, OMIM 176000) is an autosomal dominant metabolic disease caused by mutations in the gene encoding hydroxymethylbilane synthase (HMBS; EC 4.3.1.8; formely named porphobilinogen deaminase, PBGD), mapped to chromosome 11q23.3. We describe a novel mutation of the *HMBS* gene, a de novo 3-base deletion in the splicing donor site of intron 3 (IVS3+2_4delTGG) in a woman affected by AIP. RT-PCR analysis revealed an abnormal HMBS mRNA, compatible with exon 3 skipping.

Key words: acute intermittent porphyria, hydroxymethylbilane synthase, porphobilinogen deaminase, *HMBS* gene.

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Acute intermittent porphyria (AIP, OMIM 176000) is an autosomal dominant metabolic disease caused by partial deficiency of hydroxymethylbilane synthase (HMBS, EC.4.3.1.8; formerly known as porphobilinogen deaminase, PBGD). The enzyme is involved in the heme biosynthetic pathway, and the *HMBS* gene is located on chromosome 11q23.3. AIP is clinically characterized by severe abdominal pain, constipation, vomiting, neurological signs and symptoms, and the characteristic dark urine. Clinical manifestations, however, are present in only 10%-20% of mutation carriers. The disease is more prevalent in females than in males. In women, the crisis usually occurs in the premenstrual period, suggesting the influence of female hormones.

More than 200 mutations associated with AIP, including deletions, insertions, missense, nonsense and splicing mutations, have been detected throughout the *HMBS* gene, except in exon 2, and most of them have been limited to single families (Cappelini *et al.*, 2002). Here we describe a novel *HMBS* splicing-site mutation in a woman affected by AIP.

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The clinical diagnosis of AIP in the 24-year-old female was based on a psychomotor crisis induced by infection and emotional stress, which was characterized by tachycardia, labile arterial hypertension, and gastrointestinal symptoms such as abdominal colic and nausea. She also developed a neuropsychiatric syndrome, which included mental confusion, behavior changes, seizures and coma. Laboratory tests revealed that the urine turned dark when exposed to light and showed elevated urine porphobilinogen levels (50.5 mg/24 h urine, reference normal values 1.0-1.5 mg/24 h urine). A slight decrease of erythrocyte HMBS activity (122.3 pmol uroporphyrin/hour/mg Hb at 37 °C) was detected (normal range 130 to 194 pmol). The patient's parents and sister showed HMBS activity in the normal range (Figure 1A, I-1: 180, I-2: 235, II-2: 204 pmol uroporphyrin/hour/mg Hb).

Informed consent for the genetic studies was obtained from the patient and her relatives. Genomic DNA was extracted from peripheral blood leukocytes using a standard salting-out method (Salazar *et al.*, 1998). All coding exons

and exon-intron boundaries of the *HMBS* gene were amplified by polymerase chain reaction (PCR) and the fragments were submitted to Single Strand Conformation Polymorphism (SSCP) analysis (Schreiber *et al.*, 1995). DNA fragments with abnormal SSCP patterns when compared to a normal control DNA were directly sequenced, as previously described (Ribeiro *et al.*, 2002).

In the patient, the fragment containing exon 3 and its flanking regions showed atypical banding patterns (Figure 1B). This exon encodes amino acid residues, which interact with the dipyrromethane cofactor, thus pointing to its importance for HMBS function (Shoolingin-Jordan, 1995). This abnormal fragment was submitted to direct sequencing, which disclosed a 3-base deletion (IVS3+2_4delTGG) (Figure 1D). This deletion disrupts the consensus sequence of the donor-splicing site AG/GTGGGTGCA at the exon-intron junction and suggests a possible mRNA splicing abnormality. No abnormality was detected in the SSCP analysis of this fragment from the patient's parents (Figure 1B).

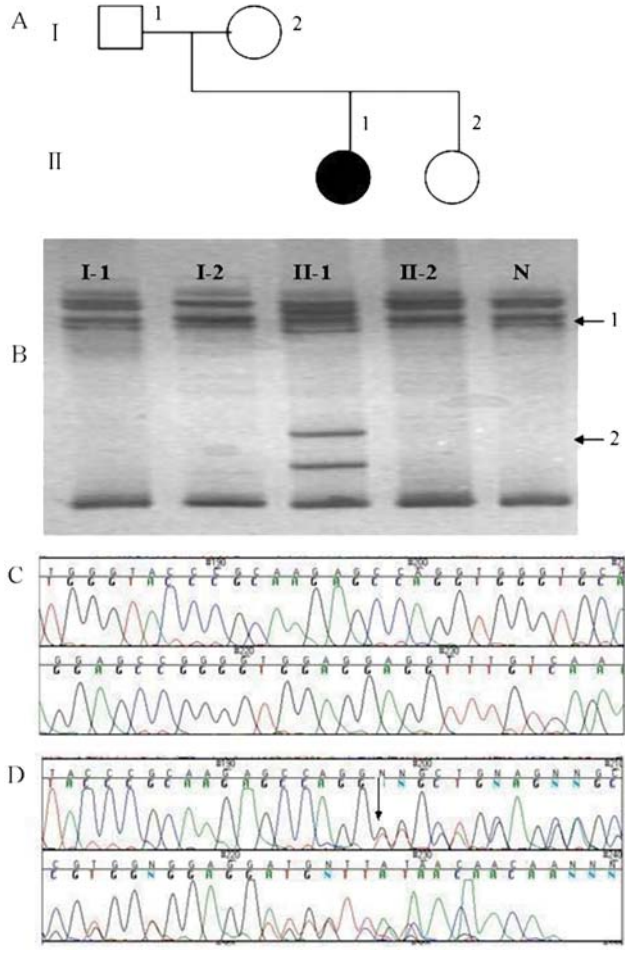

Figure 1 - **A**, Family pedigree. **B**, SSCP analysis of the exon 3/intron 3 fragment of the hydroxymethylbilane synthase gene disclosed an abnormal banding pattern in the patient (II-2). N, normal control. **C**, **D**, Electrofluorograms showing partial nucleotide sequence of the hydroxymethylbilane synthase gene in a normal control (C) and in the patient (D). The TGG deletion in the patient (arrow) disrupts a consensus sequence of the donor-splicing site G/GTGGGTGCA.

The deletion was confirmed by the amplifyication of transcription products by RT-PCR. Total RNA, obtained from peripheral blood mononuclear cells and isolated by Ficoll-Hypaque density gradient centrifugation, was reversely transcribed to cDNA using SuperScript II Rnase H-Reverse Transcriptase (Invitrogen) and random hexamers primers. Amplification of cDNA was performed by PCR with two sets of primers (Whatley *et al.*, 1999), encoding the entire *HMBS* cDNA sequence divided in two overlapping fragments of 842 and 740 base pairs, which included exons 1 to 12 and 9 to 15, respectively. The presence of a smaller transcript in addition to the expected 842 bp transcript in the patient was compatible with exon 3 skipping (Figure 2). No cDNA abnormalities were found in the patient's parents and in her sister. Polymorphic loci genotyping (D18S51, D19S253, SE-33, FGA, FESFPS, D16S539, D7S820, TPOX, F13B, D8S1179, D3S1358 and TH01; Budowle and Sprecher, 2001) of the patient and her parents resulted in a 99.9999% chance of paternity.

The mutation present in the proband was neither found in 100 normal individuals tested by SSCP screening nor in other 15 AIP patients from our laboratory. Three different mutations resulting in exon 3 skipping of the hydroxymethylbilane synthase gene have been previously described (Lundin *et al.*, 1995; Llewellyn *et al.* 1996; Mustajoki *et al.*, 1998).

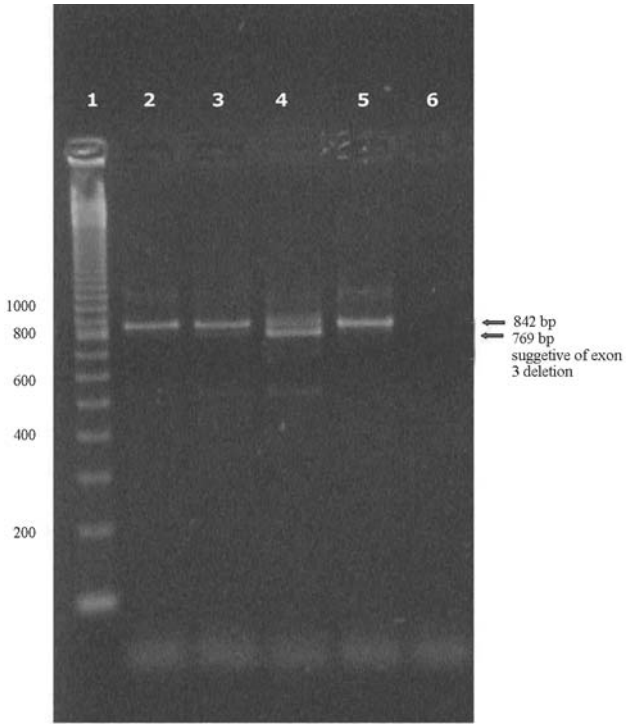

Figure 2 - cDNA analysis of exons 1 to 12 (842 bp amplification product) of the hydroxymethylbilane synthase gene. Lane 1, 100 bp DNA size marker; lane 2, normal control; lane 3, patient's mother; lane 4, patient; lane 5, patient's father; lane 6, negative control. The smaller product detected in the patient is compatible with exon 3 skipping.

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