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# A large deletion on chromosome 11 in acute intermittent porphyria

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**Abstract**

Acute intermittent porphyria (AIP) is an autosomal disorder caused by molecular abnormalities in the gene coding for hydroxymethylbilane synthase (HMBS), the third enzyme in the heme biosynthetic pathway. So far, more than 242 different mutations responsible for AIP have been identified in this gene. In an Italian family with typical clinical and biochemical signs of AIP, no mutation was found by direct sequencing of the entire hydroxymethylbilane synthase gene (HMBS). All the symptomatic patients showed apparent homozygosity and absence of mendelian segregation for eleven common polymorphisms along the gene. Excluding interference of polymorphisms in the primer sites, we assumed the presence of a complete HMBS gene deletion. In order to identify the size of this deletion, single nucleotide polymorphisms (SNPs) analysis was extended to flanking genes, H2A Histone Family member X (H2AFX) and Dolichyl-Phosphate *N*-Acetylglucosamine Phosphotransferase 1 (DPAGT1), downstream and Vacuolar protein sorting 11 (VPS11), upstream. Heterozygous polymorphisms in the VPS11 and DPAGT1 genes were found. Thus, we performed a Long-PCR with primers situated in regions outside the homozygous polymorphisms and we identified a double deletion with inversion on chromosome 11 (g22516974_22524062del7088, g22524062_22524278inv216, g22524278_22531093del6815). Even if the deletions include the entire HMBS and H2AFX genes and 1463 bp of the final portion of DPAGT1 gene, our patients had no other symptoms than AIP.


*Keywords:* Heme; Acute intermittent porphyria; Chromosome 11; Gene rearrangement

## Introduction

Acute intermittent porphyria (AIP, mim 176000) is an autosomal dominant disorder caused by a partial deficiency of hydroxymethylbilane synthase (HMBS, EC 4.3.1.8), the third enzyme of the heme biosynthetic pathway (also called porphobilinogen deaminase, PBGD) [1]. The clinical features of AIP are intermittent attacks of neurological dysfunctions, including abdominal pain and neuropsychiatric symptoms [2]. Most of the subjects remain asymptomatic throughout their life, but 10–20% of them have severe acute attacks [1]. AIP is normally diagnosed on the basis of clinical symptoms and urinary overproduction of porphyrin precursors, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). The erythrocyte HMBS activity supports the diagnosis but is not always informative because of the possible overlapping between carrier and normal values [3]. Only the molecular analysis of the HMBS gene confirms the diagnosis of AIP. The human HMBS gene maps to chromosome 11q23.2 with a total of 15 exons. Two distinct promoters direct housekeeping and erythroid specific mRNAs by alternative splicing [4]. So far, more than 242 different mutations have been identified among AIP patients [5]. Most of the reported mutations have been detected only in single families, showing high genetic heterogeneity [6]. In this study, we searched for molecular defects in the HMBS gene in order to identify the mutations in Italian AIP patients. A family including five patients with overt AIP and seven asymptomatic relatives was investigated. The diagnosis of AIP was based on clinical

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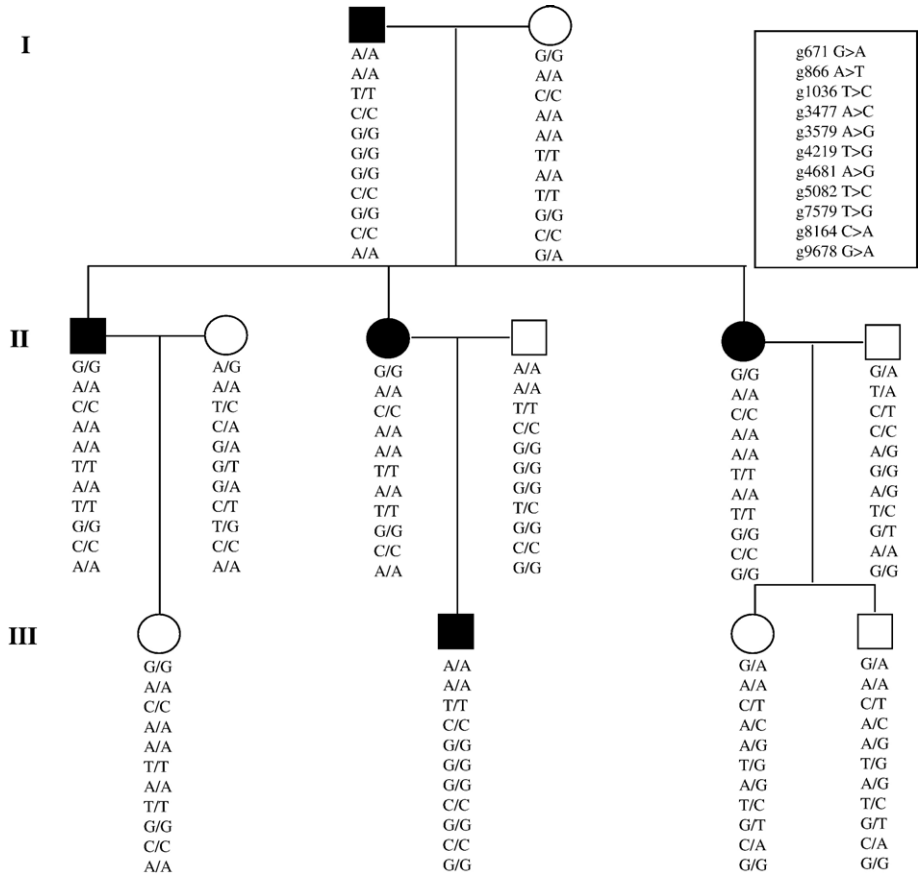


Fig. 1. Family segregation study. The figure reports the sequence analysis of eleven polymorphisms (in the box) of family members. In the symptomatic patients (in black), all polymorphisms show an apparent homozygosity and most of them show an absence of mendelian segregation. The normal relatives are represented in white.

manifestations and on increased excretion of PBG and ALA in urine during acute attacks. Moreover, in all subjects, erythrocyte HMBS activity was detected. In these patients, no mutation was found by direct sequencing of housekeeping and erythroid promoter, 5′ and 3′UTR, introns and entire coding region of the HMBS gene. All the symptomatic patients showed apparent homozygosity for eleven common polymorphisms along the gene. Further family studies established the absence of mendelian segregation (Fig. 1). Excluding interference of polymorphisms in the primer sites, we assumed the presence of a complete HMBS gene deletion.

## Materials and methods

### *DNA Analysis*

DNA was isolated from leukocytes by standard phenol-chloroform extraction. The entire HMBS gene was amplified with 14 different primer pairs (10 pmol of each primer), in the presence of 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 67 mM Tris–HCl at pH 8.8, 16 mM $(NH_4)_2SO_2$, 0.01% Tween20 and 1 U of DNA polymerase (Bioline, London, UK) in a final volume of 50 μl. The polymerase chain reaction (PCR) was carried out under the

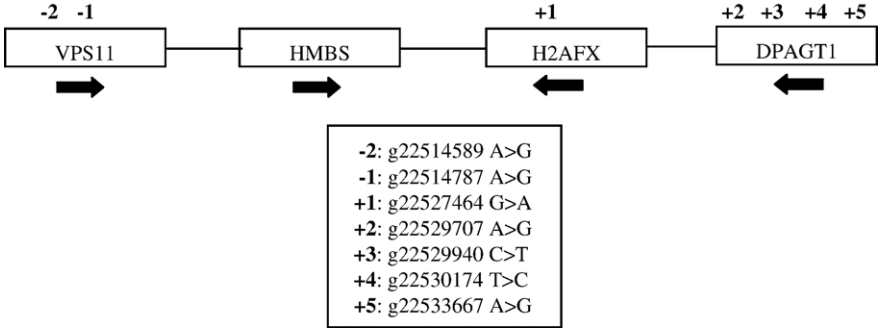


Fig. 2. Flanking genes. The figure shows the position and the orientation (bold arrow) of the genes flanking the HMBS gene. Analyzed SNPs of these genes and their positions are reported.