

Acute Intermittent Porphyria in a Child with Severe Neuropathy

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Abstract

Clinical presentation of acute intermittent porphyria before puberty is unusual. We diagnosed the non-erythroid variant form of this disease in a male child, who first presented, at the age of 6 years, with unexplained neurological symptoms and behavioural abnormalities. We also report the successful treatment, and the long-term clinical management.

Keywords: Acute porphyrias; Acute intermittent porphyria; Peripheral neuropathy; Heme; Heme arginate; Children

Abbreviations: AIP: Acute Intermittent Porphyria; ALA: Delta-Aminolevulinic Acid; ANCA: Anti-Neutrophil Cytoplasmic Antibodies; APA: Acute Porphyric Attack; CPOX: Coproporphyrinogen Oxidase; HHV: Human Herpes Virus; HMBS: Hydroxymethyl-Bilane Synthase; PBG: Porphobilinogen; PBGD: Porphobilinogen Deaminase; PPOX: Protoporphyrinogen Oxidase

Patient Presentation

A 6-year-old boy of Moroccan origin, presented with severe and progressive weakness in the upper and lower limbs. The patient had also experienced progressive behavioural changes (irritable and oppositional) and calorie restriction over a 2-year period prior to admission. The patient's vital signs and his thorax and abdominal examinations were normal; however, he appeared poorly nourished (body-mass index=14). The patient was unable to manipulate objects, with bilateral hands, he had foot drop and showed a severe steppage gait. At neurological examination, the cranial nerve function tests resulted normal, while motor strength tests reduced (Table 1). Temperature, vibration, proprioception, light touch and pinprick sensations were also normal. No abnormalities were observed at magnetic resonance imaging of the brain and spinal cord, electrocardiogram, chest X-ray and abdomen X-ray and ultrasound imaging. To test for Guillain-Barre Syndrome, a lumbar puncture together with a study of nerve conduction was performed. The cerebrospinal fluid analysis showed a glucose level of 47 mg/dL (reference values 45-80 mg/dL) and a protein level of 20 mg/dL (12-60 mg/dL) without positivity for cell or Gram's staining; protein isoelectric focusing and immunoblotting assays showed a nonspecific "mirror pattern". Electromyography was suggestive for chronic axonal motor neuropathy. The peroneal and median nerves responses were bilaterally not recordable. White and red blood counts, serum levels of folate and vitamin B12 and basal hormone levels (sexual hormones and thyroid, hypophysis, adrenal gland-related hormones) were normal. In Table 1 the results for heavy metals assessment, transaminases and lipid status are reported. Tests were negative for infectious diseases (human immunodeficiency virus, hepatitis C virus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1 and 2, human herpes virus (HHV) 6-8, *Enterovirus* and *Borrelia burgdorferi* spp., tuberculosis (quantiFERON-TB Gold In-Tube)) and for antinuclear antibodies (ANA), perinuclear anti-neutrophil cytoplasmic antibodies (ANCA), cytoplasmic ANCA, anti-gangliosides antibodies, and paraproteinemias. Screening for metabolic disorders characterized by neuropathic involvement

showed no significant alteration with regard to acyl-carnitine profile, urinary organic acids levels and amino acids serum profile. The urine analysis surprisingly revealed a high level of total porphyrins, 3479 g/g creatinine (ref.<110 g/g creatinine); delta-aminolaevulinic acid (U-ALA) 47.1 mol/mol creatinine (ref.<5 mol/mol creatinine); and porphobilinogen (U-PBG) 82.4 mol/mol creatinine (ref.<1.5 mol/mol creatinine) (measured by Chromatography methods). These data strongly suggested the diagnosis of an acute porphyria.

Genetic analysis, obtained after informed consent from the patient and his family, identified a novel splice-site mutation in *HMBS* gene, confirming the diagnosis of heterozygosity for AIP. The value of the PBGD activity in the erythrocytes was normal thus the ubiquitous promoter region including the exon 1 of the *HMBS* gene (*HMBS* RefSeq NC_000011.10 119084871-119093549), was PCR-amplified using primers and conditions as previously reported [1]. An A>G substitution in the exon 1 donor splice site of *HMBS* gene was identified (c.33+4 A>G) confirming that the patient was affected by the non-erythroid variant form of AIP. Family studies also revealed that his asymptomatic mother and brother were equally heterozygous for the splice-site mutation, never described before. In order to explain the severity of the phenotype of the patient, the entire coding regions of *HMBS* and of the genes of other acute porphyria such as delta aminolevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase (CPOX), protoporphyrinogen oxidase (PPOX) and aminolevulinic acid synthase (ALAS1) were sequenced. In order to exclude long rearrangements, the multiplex ligation-dependent probe amplification was also performed as previously described [2], but no additional abnormalities were identified. However, family segregation analysis of common polymorphisms of *HMBS* gene showed that the proband and the asymptomatic brother inherited two different paternal alleles

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	Value (reference range)
Height	113 cm (54 th percentile)
Weight	18 kg (25 th percentile)
Body-mass index	14 (10 th percentile)
Motor strength	
Upper portion of the arms	4/5
Lower portion of the arms	2-3/5
Fingers	0/5
Upper legs	4+/5
Lower legs	0-2/5
Complete laboratory testing*	
Total cholesterol	275 mg/dL (126-91 mg/dL)
High-density lipoprotein	99 mg/dL (>39 mg/dL)
Alanine transaminase	51 IU/L (8-20 IU/L)
Aspartate aminotransferase	53 IU/L (8-20 IU/L)
Heavy metal levels*	
Urinary cadmium	0.05 µg/L (0-0.2 µg/L)
Blood lead levels	1 µg/L (0-35 µg/L)

*Standard routine analysis, IU=international units.

Table 1: Patient characteristics.

(Figure 1, section A). Assuming the existence of a second mutation in the *HMBS* gene inherited from the father, a region of about 2Kb of distal promoter was also sequenced, but no additional abnormalities were identified. Moreover, no difference in common polymorphisms was found between two brothers carrying mutation, also excluding the hypothesis of an additional hypomorphic allele. In addition, *HMBS* gene expression confirmed that the proband and the asymptomatic brother had the same amount of *HMBS* mRNA.

Specific therapy by intravenous infusion of Haem arginate (Normosang[®], Orphan Europe, Paris, France) at a dose of 3 mg/kg/day by central catheter for 3 consecutive days was started. Following this infusion, the general clinical conditions of the child progressively improved, with reduction in neurological deficits and food refusal. On the basis of urinary ALA and PBG values, together with clinical incomplete remission, a maintenance scheme of one dose of Heme Arginate infusion for 2 consecutive days per week was initiated during the following months, with continuous monitoring of clinical and biochemical (U-ALA, U-PBG) parameters. **T0** at the time of diagnosis U-total porphyrins 3479 g/g creatinine U-PBG 82,4 mol/mol creatinine U-ALA 47,1 mol/mol creatinine. **T1** at 1 month of therapy. before therapy U-total porphyrins 2406 g/g creatinine U-PBG 124 mol/mol creatinine U-ALA 46,1 mol/mol creatinine; after therapy U-total porphyrins 574 g/g creatinine U-PBG 24 mol/mol creatinine U-ALA 8,2 mol/mol creatinine. **T2** at 2 month of therapy. before therapy U-total porphyrins 2687 g/g creatinine U-PBG 73,9 mol/mol creatinine U-ALA 19,2 mol/mol creatinine after therapy U-total porphyrins 1893 g/g creatinine U-PBG 22,2 mol/mol creatinine U-ALA 2,8 mol/mol creatinine. **T3** at 3 month of therapy. before therapy U-total porphyrins 2068 g/g creatinine U-PBG 88,7 mol/mol creatinine U-ALA 53,5 mol/mol creatinine after therapy U-total porphyrins 1964 g/g creatinine U-PBG 22,4 mol/mol creatinine U-ALA 6,2 mol/mol creatinine. **T6** at 6 month of therapy. before therapy U-total porphyrins 2829 g/g creatinine U-PBG 93,7 mol/mol creatinine U-ALA 34,9 mol/mol creatinine after therapy U-total porphyrins 975 g/g creatinine U-PBG 32,9 mol/mol creatinine U-ALA 7 mol/mol creatinine. **T1** food refusal disappeared and improved strength in lower limbs. **T2** almost normalized strength in lower limbs, improved strength in upper limbs almost normal behavior. **T6** upper and lower limb neurological signs

and gait anomalies were completely normalized (Figure 1, section B). After 6 months of therapy, upper and lower limb neurological signs and gait anomalies were completely normalized, together with significant improve in behaviour.

Discussion

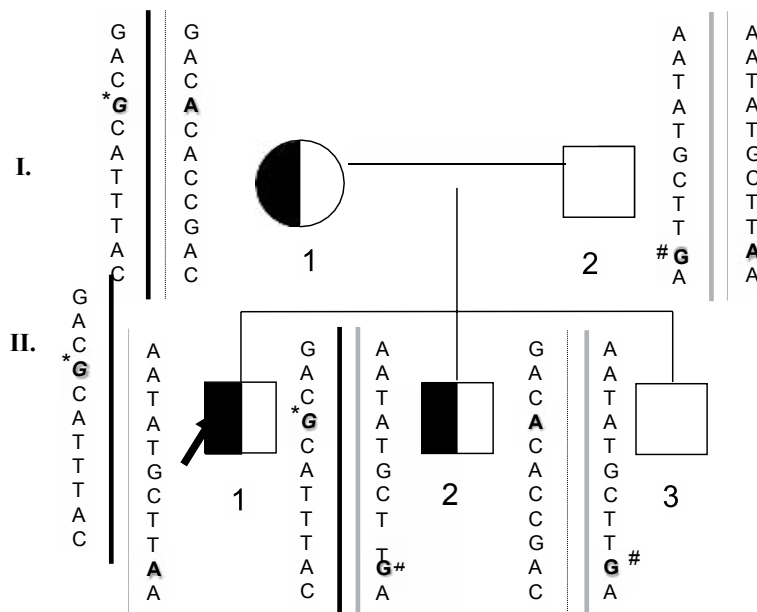
Acute Intermittent Porphyrinuria (AIP) is a low-penetrance autosomal dominant disorder of heme synthesis, characterized by a highly variable clinical presentation [3]. AIP is caused by mono-allelic mutations in the hydroxymethyl-bilane synthase gene (*HMBS*), coding for the third enzyme in the heme biosynthetic pathway. The mutation predisposes the carriers to sporadic life-threatening acute neurological attacks that require additional triggering factors [4]. Prevalent clinical features of an acute attack include severe abdominal pain, variably accompanied by neurological symptoms (mostly peripheral acute neuropathy, often-involving proximal portion of the limbs) and psychiatric abnormalities (such as behaviour alterations, agitation, hallucinations). Acute attacks may also present with cardiovascular signs (tachycardia and/or hypertension) or hyponatremia that can lead to seizures or even coma [5]. A higher incidence of seizures was reported in children who presented after treatment with porphyrinogenic anti-seizure medications [6]. Acute porphyria attacks are reported as usually occurring in second to third decades of life, very rarely in childhood and by far more common in females than males [7]. A recent revisiting of literature shows that although 70 paediatric AIP cases have been reported, the diagnosis and the management of AIP in children has been and it is still challenging [8]. We report an unusual AIP phenotype in a 6 years old child of Moroccan origin.

An acute exacerbation of AIP is diagnosed on the basis of increased urinary levels of aminolevulinic acid (ALA) and porphobilinogen (PBG) [9]. However, if specific tests are conducted delayed with respect to clinical manifestations, the measurements are inconclusive; thus a diagnosis of AIP may be based solely on reduced erythrocyte *HMBS* activity. The latter can be however normal in cases affected by the non-erythroid variant form of AIP (about 10-15% of all AIP patients). This condition is due to mutations affecting a specific region of the *HMBS* gene, thereby only affecting *HMBS* activity in non-erythroid cells [10]. Our patient presented with high urinary porphyrin (uroporphyrins), ALA and PBG levels and normal erythroid *HMBS* activity suggesting a diagnosis of non-erythroid variant of AIP. The majority of mutations causing this form of AIP are exon 1 splicing defects; seven different substitutions from +1 to +5 position in the canonical splicing donor site have been reported. All these mutations result in the activation of a cryptic splicing site 67 bp downstream in intron 1, leading to a frame shift and causing a premature stop codon in exon 4 [11,12]. It is possible that the c.33+4 A>G mutation described herein could have the same effect on *HMBS* mRNA, even if the abnormal isoform was not found during qualitative mRNA analysis.

Severe neurological involvement characterized by ataxia with progressive neurological deterioration has also been described, but mostly in rare cases of children with homozygous dominant acute porphyrias (mostly AIP) [7]. No other mutation in the coding sequence of the gene was expected in our patient given the normal *HMBS* activity in the erythrocytes. The absence of other mutations in distal promoter region of *HMBS* and in the coding regions of *ALAS1*, *ALAD*, *CPOX* and *PPOX* genes did not allow us to fully clarify the severity of the phenotype, yet. However, other paediatric cases with severe phenotype of AIP are reported carrying only one mutation in the *HMBS* gene [8].

If a second mutation should exist, it should be located in the non-

(Section A)



(Section B)

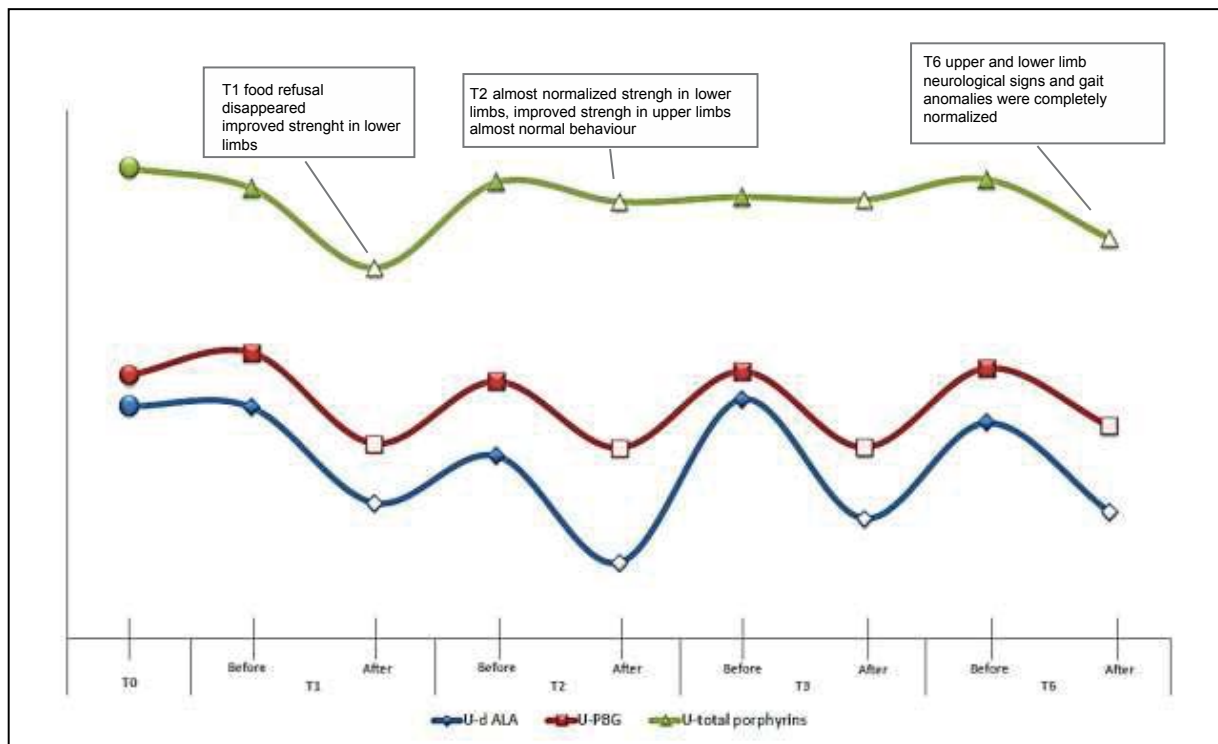


Figure 1

Section A: Genetic assessment of *HMBS* gene and family segregation studied. The arrow points to the patient described in this case report. The carriers of c.33+4 A>G mutation are depicted in black. The star indicates the mutated allele while the hashtag the difference between the two paternal alleles.

Section B: Improvement in patient symptoms [urine aminolevulinic acid (ALA), porphobilinogen (PBG), and total porphyrin levels; food refusal; motor strength; and neurological signs] throughout treatment.

analysed intron 1 of *HMBS* gene. The mutation should be carried by the paternal allele, which is the only difference between asymptomatic and symptomatic brothers carrying mutation. Anyway, the equal amount of *HMBS* mRNA found in the proband and in the asymptomatic brother excludes the hypothesis that an additional hypomorphic allele could be inherited. We can suppose that other non-porphyrin related genes perhaps located on the same chromosome of *HMBS* gene (chr11) could modulate the clinical phenotype of AIP without however altering *HMBS* gene expression.

Specific treatment for acute attack involves identifying and resolving any possible triggering factor, including stress, inadequate nutritional intake, infections and drugs or chemicals known to exacerbate porphyria. The mainstay of acute attack therapy is haem infusion [as hematin (Panhematin®) or Heme arginate (Normosang®)] at the dosage of 2-4 mg/kg intravenously once/day for 3-4 days. Heme acts through a negative feedback on heme pathway, resulting in fast reduction in ALA and PBG accumulation: it resolves most of acute crisis in 2-4 days. Whilst glucose also down-regulates the heme pathway and may be helpful in resolving acute porphyria attacks [13,14].

No literature for heme-arginate therapy in children for symptomatic AIP was available. Because of the elevated levels of ALA and PBG and the severe neurological impairment, heme-based therapy was initiated as described (by infusion through a central venous catheter) but it was prolonged to twice-a-week infusions following unsatisfactory outcomes during first-line therapy (Figure 1, section B). A gradual improvement in the patient's psycho-neurologic involvement resulted, with no acute therapy-related complications. Urinary levels of ALA and PBG dramatically decreased following each infusion but failed to reach reference intervals. AIP diagnosis in our patient was confirmed by DNA analysis, which also excluded other possible abnormalities in other genes of heme synthetic pathway [15]. The dissociation between clinical and biochemical outcome emphasizes uncertainties in pathological issues of AIP. The high urinary porphyrin, ALA and PBG levels, even outside of clinical exacerbations gives rise to the problem of a maintenance therapy.

Additional issues concerning the long-term clinical management of the patient include: how long maintenance therapy should be continued; the possibility of other treatment options; the risk of infections or other complications (thrombosis) of the central venous line; the possibility of iron overload; the effects of this experience on the psychological development of the patient.

In adult patients affected by severe forms of acute porphyrias liver transplantation has been considered an option leading to permanent cure of all acute porphyrias [16]. No literature for liver transplantation in children for symptomatic AIP is currently available. Recent experimental trials of gene-based therapy offer new promising opportunities for the treatment of acute porphyrias, even in long-term management [17].

Diagnosis of acute porphyrias is made less often than their prevalence justifies. Many patients remain undiagnosed, especially when presenting with unusual phenotypes [18]. An awareness of their possible multiform clinical presentations is imperative for successful diagnosis and treatment in order to prevent the escalation of symptoms, which may result in lethal consequences. Also paediatricians and neurologist should be made aware of insidious clinical patterns of acute porphyrias in children, presenting with, but not only, unexplained neurological symptoms.

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Financial Disclosure

The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest

Paolo Ventura has been involved as consultant in advisory boards and has received funding for consultation, research and lecturing from Orphan Europe Italy. There are no other competing interests to declare for him and for all the other co-authors.

Compliance with Ethics Guidelines

All procedures followed were in accordance with the ethical standards of the responsible committee and with the Helsinki Declaration of 1975, as revised in 2000: informed consent to make the diagnostic tests and to publish the results in this report were obtained from the people included in this study.

Contributors' Statements

Dr. Rosafio and Dr. Ventura made the diagnosis, managed the treatment and the follow up and drafted the initial manuscript; Dr. Guerra contributed to make diagnosis and to manage the treatment and the follow up; Dr. Marchini carried out the biochemical analyses; Dr. Granata and Dr. Brancaleoni carried out the genetic analyses; Dr. Di Pierro carried out the genetic analyses and reviewed and revised the manuscript; Prof. Iughetti critically reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. None of the authors received any form of payment for the production of this manuscript.

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