



## THE VERY FIRST DESCRIPTION OF A PATIENT WITH HEPATOERYTHROPOIETIC PORPHYRIA IN ARGENTINA. BIOCHEMICAL AND MOLECULAR STUDIES

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**Abstract** – Hepatoerythropoietic Porphyria (HEP) is the rare homozygous form of Porphyria Cutanea Tarda (PCT). It is characterized clinically by the early onset of severe skin manifestations which can be confused with Congenital Erythropoietic Porphyria (CEP) or with PCT when the symptoms are mild. We describe the case of a 14 year-old child with skin manifestations similar to those observed in PCT. The biochemical assays ruled out a CEP as well as they suggested the development of a HEP. Although his symptoms were not severe enough to be HEP, the enzymatic activity was dramatically reduced to a 5% of normal values and the molecular analysis revealed the presence of two already known different mutations on the patient's URO-D gene, c.703 C>T and IVS9-1. Each parent carry one of the mutations, but they were absent in the brother. This is the first Argentinean HEP case ever described which appeared in a compound heterozygous form and less residual URO-D activity but associated to a mild phenotype.

**Key words:** Hepatoerythropoietic Porphyria, Uroporphyrinogen Decarboxylase, mutations, compound heterozygous.

### INTRODUCTION

Porphyrias are metabolic disorders caused by a specific enzyme deficiency along the heme biosynthetic pathway. Uroporphyrinogen Decarboxylase (URO-D; EC. 4.1.1.37) is the fifth enzyme of this pathway and catalyzes the sequential decarboxylation of uroporphyrinogen III (urogen III) to yield coproporphyrinogen III (coprogen III). Deficiencies in this activity are responsible for two types of porphyria: Porphyria Cutanea Tarda (PCT), the commonest form of these diseases, which has a frequency of 1:5000

**Abbreviations:** CEP, Congenital Erythropoietic Porphyria; **Copro I**, Coproporphyrin I; **Copro**, Coproporphyrin; **Coprogen III**, Coproporphyrinogen III; **DNA**, Desoxyribonucleic acid; **Phyria**, Phyriaporphyrin; **HBV**, Hepatitis B virus; **HCV**, Hepatitis C virus; **HEP**, Hepatoerythropoietic Porphyria; **Hexa**, Hexaporphyrin; **Isocopro**, Isocoproporphyrin; **mRNA**, Messenger ribonucleic acid; **PCR**, Polymerase chain reaction; **PCT**, Porphyria Cutanea Tarda; **Penta**, Pentaporphyrin; **PPI**, Plasma porphyrin index; **RBC**, Red blood cells; **Uro**, Uroporphyrin; **URO-D**, Uroporphyrinogen Decarboxylase; **Urogen III**, Uroporphyrinogen III.

to 1:25000 (1); and Hepatoerythropoietic Porphyria (HEP), the rare homozygous form of PCT, in which only about 30 cases have been described worldwide (2, 4-6, 9, 10, 14-17, 19, 20, 26, 28, 31, 32).

There are two main forms of PCT, an inherited (type II, familial PCT) or an acquired (type I, sporadic PCT) one. In the first case the URO-D deficiency is present in all tissues while in the second case the deficiency is only present in the liver. Familial PCT is inherited as an autosomal dominant trait and the URO-D activity is reduced approximately to a 50% (24). PCT usually occurs in adult life and clinically is characterized by skin photosensitivity with blistering on sun exposed areas of the body, skin fragility, hypertrichosis and hyperpigmentation (24, 30). The clinical manifestation of this disease is frequently associated with the exposure to precipitating agents including polyhalogenated aromatic hydrocarbons, alcohol abuse, oestrogen ingestion, iron overload, and infection with hepatitis C virus (HCV) and, less frequently, hepatitis B virus (HBV); all of these factors

cause liver dysfunction, a common sign in PCT patients (24). Biochemically, it is characterized by high levels of highly and partially carboxylated porphyrins, principally uroporphyrin (uro) and phyriaporphyrin and (phyria), in urine and plasma (22), and isocoporphyrin (isocopro) in faeces (30). There is also a form of familial PCT called type III, in which a family history of PCT is observed, but subnormal URO-D activity is restricted to the liver (8, 13).

HEP is inherited as an autosomal recessive trait and patients can be either homoallelic or heteroallelic for mutations in the URO-D gene. The enzymatic activity decreases between 3 and 25% of normal value (18). Unlike most cases of PCT, HEP appeared during early childhood (24). Skin lesions resemble those of PCT but they often lead to severe scarring so that patients may become as disfigured as in Congenital Erythropoietic Porphyria (CEP) (2). However, the typical symptoms of CEP, such as erythrodontia and haematological abnormalities, are not usually present in HEP (18, 27). Moreover, URO and Coproporphyrin of type I are found in urine of CEP patients. Biochemically it can be distinguished from PCT due to the fact that in HEP elevated porphyrin levels can be found in erythrocytes, besides urine, faeces and plasma and in general the chromatographic behaviour of urinary porphyrins is slightly different in both cases. The human URO-D gene has been mapped to 1p34 (11, 22) and about 80 different mutations responsible for PCT and HEP have been identified (Human Genome Mutation Database, <http://www.hgmd.org>).

In the present study we describe the case of a 14 year-old boy who showed skin manifestations since early childhood, similar to those observed in PCT. The biochemical parameters ruled out a CEP as well as they strongly suggested the development of HEP,

which was confirmed by molecular studies. This is the description of the first Argentinean case of HEP, which appeared in a compound heterozygous form and with a mild phenotype.

## MATERIALS AND METHODS

### Case report

A 14 year-old patient presented since infancy skin manifestations such as skin fragility, photosensitivity, hypertrichosis, blistering on sun exposed areas of the body and dark urine. He was first referred to the Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP) as a CEP patient. Neither his parents nor his brother presented any symptom. There was no history of exposure to drugs, chemicals, pesticides or alcohol abuse. The parents were not consanguineous. Informed consent was obtained from this family prior to their inclusion in the study. The protocols used were approved by the Ethical Committee of the Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP, CONICET, Hospital de Clínicas, UBA)

### Biochemical measurements

The biochemical assays were performed according to Batlle (3).

### URO-D assay

Enzymatic activity of URO-D was measured according to Méndez *et al* (23).

### Genetic study

Genomic DNA was isolated from EDTA-collected blood samples using GFX Genomic Blood Purification Kit (GE Healthcare). The URO-D gene was amplified by polymerase chain reaction (PCR) in five different fragments in order to generate overlapped products of 1 Kb approximately according to Méndez *et al* (23), introducing some modifications. The amplification was performed in a final volume of 50µl containing 0.2mM of each dNTP, 0.2µM of each primer, between 10 and 50µg/ml of DNA and 0.05U/µl of *Taq Polymerase Recombinant* (Invitrogen). The concentration of Mg<sup>2+</sup> and the primers used are summarized in Table 1. After an initial denaturation at 94°C for 3 min, 30 cycles were performed as follows: 94°C for 60 sec, 60°C for 60 sec, 72°C for 90 sec; and a final extension at 72°C for 5 min. Every PCR fragment was automatically sequenced (ABI3730XL – Macrogen) using the primers listed in Table 1.

**Table 1.** Primers sequences <sup>(23)</sup> and Mg<sup>2+</sup> conditions used to amplify every URO-D fragment.

Genomic region	Primer forward	Primer reverse	[Mg <sup>2+</sup> ] (mM)
5'-exon 2	tatggacctggctggataagactgttggt	ctcgcaggaatgtgtcattcttcagcctc	2
Exon 1-exon 4	agttacagacagctgacc	aatgatggcagcatccagagggaa	2
Exon 3-exon 6	cgggctgccagactttttcagcacg	ttgtcctaccagatatgggaccagagc	2
Exon 5-exon 8	ggacgtgtgccgctgattggctttgct	ccagctcctccagggcaaatgccatcct	2
Exon 7-3'	tggccaagcaagtgaaggccaggttgcggga	ctgaggatatgggacaatctttcac	2.7

## RESULTS

The patient's and relative's biochemical pattern obtained is summarized in Table 2. All biochemical values are abnormal in the proband and they strongly suggest the development of HEP since we found high blood porphyrin levels unusual urinary porphyrin excretion and less residual URO-D activity (5%). Nevertheless, neither relative showed any biochemical abnormality, except by the decrease in the URO-D activity to about 50% of normal value found in his parents.

The molecular analysis revealed the presence of two different mutations, one on each

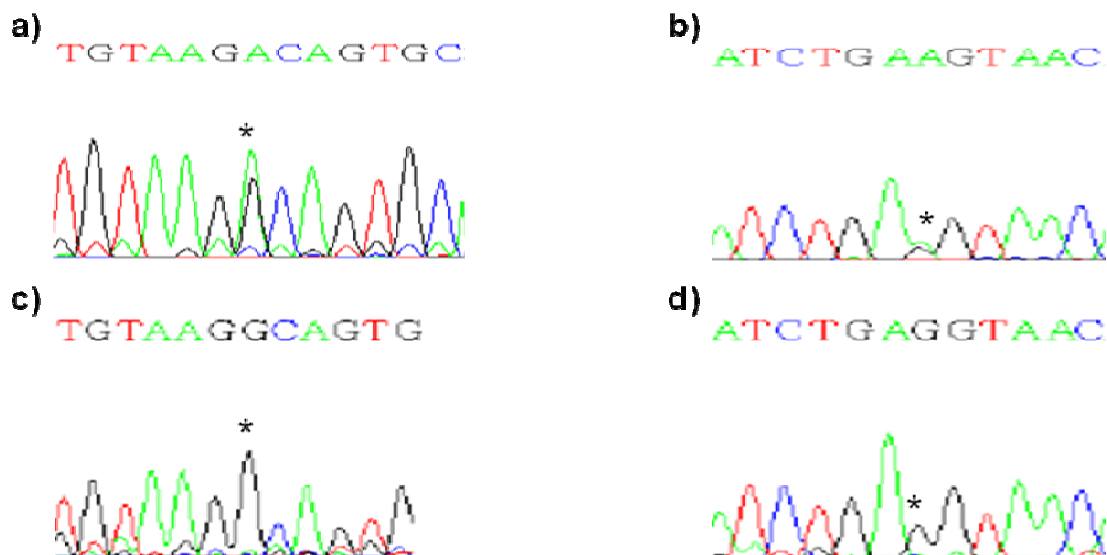
allele, in the proband's URO-D gene. One of them was a point mutation in the exon 7 (c.703 C>T) (Fig. 1a) which leads to the amino acid substitution P235S already described by Cappellini *et al* (7) in two familial PCT patients. The other one is another point mutation in the exon 9-intron 9 boundary (Fig. 1b), this substitution alters the exon 9 splicing donor site. This mutation was described by Méndez *et al* (23) in a familial PCT patient.

The c.703 C>T mutation was found in heterozygosis on the father, while the IVS9-1 G>A mutation was found in heterozygosis on the mother. Neither mutation was found on the brother.

**Table 2:** Biochemical data of the proband and his family.

Patient	Urine						PPI ( $\lambda=619$ )	BP ( $\mu\text{g}/100\text{ml}$ of RBC)	FP ( $\mu\text{g}/\text{g}$ )	URO- D act (%)
	P ( $\mu\text{g}/24\text{hs}$ )	%u	%ph	%h	%p	%c				
Proband	12072	33	41	5	14	7	10.33	390	935	5
Father	42					100	1.30			55
Mother	30					100	1.26			45
Brother	63					100	1.26			110
Normal value	20-250					100	$\leq 1.30$	$150\pm 40$	30- 130	100

P: porphyrin, u: uro, ph: phyria, h: hexa, p: penta, c: copro, PPI: plasma porphyrin index, BP: blood porphyrins, FP: faecal porphyrins, URO-D act: URO-D activity in % (normal value:  $16.35\pm 3.56$  nmol copro III/hour/ml of RBC).



**Figure 1.** Mutations found on the URO-D gene. a) c.703 C>T, b) IVS9-1 G>A and their corresponding normal sequences (c) for c.703 CT and d) for IVS9-9). The asterisks indicate the position of the substitution. The sequences a) and c) are shown in antisense orientation, b) and d) are shown in sense orientation.

## DISCUSSION

The clinical manifestations exhibited by the patient were, at first, considered by the MD to be consistent with CEP. However this was immediately ruled out because he did not present erythrodontia, the phenotype was not severe enough and it was more similar to a PCT phenotype. The elevated blood porphyrin levels found showed that the development of HEP was more likely than PCT. This was then partially confirmed by URO-D activity value which was reduced to a 5% of normal values. When the genetic studies were performed two mutations in heteroallelic state were found on the patient's URO-D gene previously described in PCT patients: c.703 C>T (7) and IVS9-1 G>A (23).

The missense mutation found in exon 7, which leads to the P235S substitution, has been described in two PCT patients who showed an URO-D activity of approximately 50% (7). The 235 residue is related to the active site of the enzyme (23), so the change from a hydrophobic amino acid to a hydrophilic residue, could affect the protein architecture resulting in loss of enzyme activity. Besides, this residue falls in conserved regions in several organisms suggesting an important role in catalysis or in the protein structure stabilization (7). Moreover, since the URO-D protein is dimeric (29), missense mutations could affect the enzymatic activity, the association between subunits and the stability of the dimer (25).

The splicing defect (IVS9-1 G>A) according to Méndez *et al* (23) leads to the deletion of exon 9 during the splicing of URO-D mRNA. This abnormality generates a premature stop codon leading to a loss of 20% of the protein amino acids; therefore the stability and activity of the enzyme could be seriously affected.

So the patient described carries two mutations each responsible for about a 50% residual URO-D activity when presented separately on PCT patients (7, 23), which was consistent with the URO-D activity value showed by his parents. A more severe decrease in the proband's URO-D activity was found, however his phenotype was mild as it has been described by Ged *et al* for a homozygous HEP case (15). Both parents were healthy carriers of one of the URO-D gene mutations due perhaps to the fact that they have not been exposed to any of the known PCT precipitating agents.

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