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Correlation between biochemical findings, structural and enzymatic abnormalities in mutated HMBS identified in six Israeli families with acute intermittent porphyria

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ABSTRACT

Mutations in the hydroxymethylbilane synthase (HMBS) gene are responsible for the inherited disorder of acute intermittent porphyria (AIP). AIP is diagnosed on the basis of characteristic clinical symptoms, elevated levels of urinary porphyrin precursors aminolevulinic acid (ALA) and porphobilinogen (PBG) and a decreased erythrocytic HMBS activity, although an identifiable HMBS mutation provides the ultimate proof for AIP. Six Israeli AIP families underwent biochemical and mutation analysis in order to establish an AIP diagnosis. Variability with respect to the ALA/PBG levels and HBMS activity was found among the index patients. Indeed, each family carried a unique mutation in the *HMBS* gene. A novel missense c.95G>C (p.R32P) was shown to be a *de novo* mutation in one family, along with five known mutations p.T59I, p.D178N, p.V215M, c.730_731delCT and c.982_983delCA identified in the rest of the families. Both R32P and D178N were expressed in a prokaryotic system. Recombinant p.R32P was enzymatically inactive as demonstrated by a <1% residual activity, whereas p.D178N possessed 81% of the activity of the wild type enzyme. However, the p.D178N mutant did display a shift in optimal pH and was thermo labile compared to the wild type. Among the four missense mutations, p.R32P and p.V215M had not only harmful effects on the enzyme *in vitro* but also were associated with high levels of ALA/PBG in patients. On the other hand, the *in vitro* effect of both p.T59I and p.D178N, and the impact of these mutations on the enzyme structure and function as interpreted by the 3-D structure of the *Escherichia coli* enzyme, were weaker than that of p.R32P and p.V215M. Concomitantly, patients carrying the p.T59I or p.D178N had normal or borderline increases in ALA/PBG concentrations although they presented characteristic clinical symptoms. These findings provided further insights into the causal relationship between HMBS mutations and AIP.

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Introduction

Acute intermittent porphyria (AIP, OMIM 176000) is an autosomal dominantly inherited disorder of heme biosynthesis resulting from a ~50% deficiency of hydroxymethylbilane synthase activity (EC 2.5.1.61; HMBS). This enzyme, also known as porphobilinogen deaminase, catalyzes the head-to-tail condensation of four molecules of porphobilinogen (PBG) to form hydroxymethylbilane. Clinically, AIP is characterized by acute intermittent neurovisceral attacks that can be provoked by various factors such as drugs, hormones and alcohol [1]. Biochemical diagnosis of AIP is based on measurement of the urinary porphyrin precursors, δ-aminolevulinic acid (ALA) and PBG, in combination with the determination of erythrocytic HMBS activity.

The *HMBS* gene is located on chromosome 11q24.1–q24.2. It is approximately 10 kb long and contains 15 exons [2]. Two distinct promoters, located in the 5′ flanking region and in intron 1, respectively, generate housekeeping (contains exon 1 and 3–15) and erythroid-specific (contains exon 2–15) transcripts by alternative splicing [3,4]. Since the genetic information on the *HMBS* gene became available, DNA-analysis has been established as an important diagnostic tool for AIP [5]. Worldwide, more than 300 different mutations have been identified in the *HMBS* gene of AIP patients [6].

Recently, we published a study on two Israeli AIP patients, both of whom carried a unique missense mutation in the *HMBS* gene [7]. We performed an extensive *in vitro* characterization of the mutant proteins including residual activity and kinetic study on recombinant

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enzymes. The results of *in vitro* study helped to better understand the clinical situation and biochemical findings in these two patients. In this study, we report a systematic mutation analysis among six Israeli AIP families including family members of the two previously published cases.

Patients, material and methods

Biochemical and enzymatic analyses

A total of 26 individual from six unrelated Israeli AIP families of Caucasian origin were studied. Biochemical diagnosis of AIP was performed in the Israeli National Laboratory for the Diagnoses of Porphyrias. The diagnosis of AIP was established on the basis of reduced HMBS activity and/or increased urinary ALA and PBG. Detailed information in each family is given in Table 1. In addition, fluorescence plasma scan revealed a peak at 622 nm in a number of patients, especially during acute attacks. In all patients, the ratio of fecal coproporphyrin III/I was less than one, and total fecal coproporphyrin was normal (data not shown). The methods employed for biochemical analysis were previously described [7].

Family A

The index patient suffered from symptoms typical of acute porphyria. A massive increase in urinary porphyrin precursors i.e., 19-fold above the upper normal limit for ALA and 50-fold above the normal limit for PBG, was measured during an acute attack. HMBS activity was however within the normal range in the index patient during the latent phase. None of her family members including her parents and her younger sister, had symptoms and biochemical findings compatible with AIP. However, the father showed slightly increased urinary ALA/PBG concentrations (Table 1).

Family B and D

The clinical and biochemical characterization of AIP in the index patient from both families have been described in a previous publication [7]. In this study, we present results of biochemical analyses of family members of the two index patients. In Family B, the mother of the index did not present any symptoms of acute porphyria and had a normal HMBS activity (Table 1). Among eight family members/relatives of index D who were tested, four individuals had a decreased HMBS activity i.e., below 70% of normal. Two of these four individuals, the father and the grandfather, also showed typical symptoms of acute porphyria. The results of urinary ALA/PBG concentrations seemed to be less discriminative. In two out of the three non-affected subjects studied, urinary ALA/PBG levels were slightly increased (less than 2-fold).

Family C

Both the symptomatic patient (15 years old) and her asymptomatic mother had reduced activity of HMBS, 36% and 44% of normal, respectively. They both exhibited borderline levels of urinary ALA and PBG. No further increase in those values was noted during clinical exacerbation in the patient.

Family E

None of the tested individuals except for the index, had any symptoms compatible with AIP. The index patient and her daughter showed a clearly decreased HMBS activity and increased urinary ALA/

Table 1
Clinical, biochemical and genetic data on six Israeli AIP families

Family	Ethnical background	Age	Acute attacks	Erythrocyte HMBS activity* (>70% of normal)	Urinary ALA§ (< 38 μmol/24 h)	Urinary PBG§ (< 8.8 μmol/24 h)	Mutation status
Family A							**c.G95 > C, p.R32P**
1. Index (f)		21	Yes	85	282.2 (716.8)	238.7 (442.3)	+/-
2. Father	Ashkenazi	61	No	100	49.1	15.2	**-/-**
3. Mother	Non Ashkenazi	55	No	105	35.8	7.0	**-/-**
4. Sister		18	No	98	25.1	6.8	**-/-**
Family B	Ashkenazi						c.C176 > T, p.T59I
1. Index (f)		15	Yes	50, 63, 68	25.1 (41.2)	6.2 (8.4)	+/-
2. Mother		40	No	100	ND	ND	-/-
Family C	Jewish from						c.532G > A, p.D178N
1. Index (f)	Cochin, India	16	Yes	36	32.7 (35.8)	7.5 (11.0)	+/-
2. Mother		45	No	44	41.2	13.3	+/-
3. Father		50	No	90	ND	ND	-/-
Family D	Ashkenazi						c.643G > A, p.V215M
1. Index (f)		30	Yes	61, 50	114.4 (205.9)	97.2 (287.3)	+/-
2. Son		3	No	ND	ND	ND	+/-
3. Father		57	Yes	61	30.5	26.5	+/-
4. Grandfather		85	Yes	61	45.0	24.0	ND
5. Brother		29	No	100	64.0	17.2	-/-
6. Sister		23	No	87	62.5	14.6	-/-
7. Cousin (m)		27	No	69	54.9	23.6	+/-
8. Cousin (m)		25	No	63	62.5	30.5	+/-
9. Aunt		52	No	110	29.0	11.5	-/-
Family E	Ashkenazi						c.730_731delCT, FS244
1. Index (f)		45	Yes	42	56.4 (267.2)	42.0 (490.5)	+/-
2. Daughter		15	No	46	54.9	15.5	+/-
3. Son		16	No	92	54.0	13.0	-/-
4. Brother		50	No	ND	ND	ND	-/-
5. Niece		25	No	69	ND	ND	-/-
6. Niece		26	No	70	ND	ND	-/-
Family F	Ashkenazi						c.982_983delCA, FS 328#
1. Index (m)		30	Yes	55	76.2 (266.0)	57.0 (185.6)	+/-
2. Father		60	No	66	78	33	ND

* values measured in the latent phase; § values measured in the latent phase, those measured during acute attacks are given in the *parentheses*; #identified in Hôpital Louis Mourier, Colombes, France by the group of professor JC Deybach; Symptomatic patients are highlighted in *dark gray*, asymptomatic carriers in *light gray*. ***Bold***: new mutation; ND: not determined; +/−: a particular HMBS mutation is present in one allele; −/−: a particular HMBS mutation is absent in both alleles.

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