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Mutation spectrum of primary hyperoxaluria type 1 in Tunisia: Implication for diagnosis in North Africa

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ABSTRACT

Background: Primary hyperoxaluria type 1 (PH1) is an autosomal recessive inherited metabolic disease, characterized by progressive kidney failure due to renal deposition of calcium oxalate. Mutations in the *AGXT* gene, encoding the liver-specific enzyme alanine glyoxylate aminotransferase, are responsible for the disease. We aimed to determine the mutational spectrum causing PH1 and to provide an accurate tool for diagnosis as well as for prenatal diagnosis in the affected families.

Methods: Direct sequencing was used to detect mutations in the *AGXT* gene in DNA samples from 13 patients belonging to 12 Tunisian families.

Results: Molecular analysis revealed five mutations causing PH1 in Tunisia. The mutations were identified along exons 1, 2, 4, 5 and 7. The most predominant mutations were the Maghrebian "p.I244T" and the Arabic "p.G190R". Furthermore, three other mutations characteristic of different ethnic groups were found in our study population. These results confirm the mutational heterogeneity related to PH1 in Tunisian population. All the mutations are in a homozygous state, reflecting the high impact of endogamy in our population.

Conclusion: Mutation analysis through DNA sequencing can provide a useful first line investigation for PH1. This identification could provide an accurate tool for prenatal diagnosis, genetic counseling and screen for potential presymptomatic individuals.

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1. Introduction

Type 1 primary hyperoxaluria (PH1; MIM 259900) is a rare autosomal recessive disease caused by the deficiency of the liver specific enzyme alanine glyoxylate aminotransferase (AGT; EC 2.6.1.44) (Danpure and Jennings, 1986). This enzyme in complex with phosphate, pyridoxine (vitamin B6), catalyzes the transamination of glyoxylate to glycine from alanine. This results in excessive hepatic production of oxalate and glycolate bioproducts characteristic of PH1 (Danpure and Rumsby, 2004). The precipitation of calcium oxalate (CaOx) monohydrate crystals is the origin of nephrolithiasis and/or nephrocalcinosis (NC) and in severe cases it can lead to end-stage renal disease (ESRD) (Danpure, 2001). The oxalates are then deposited in many other tissues, including bone, bone marrow, retina, cornea and heart, leading to systemic oxalosis (Rinat et al., 1999).

The incidence and the severity of the disease differ from one country to another with the highest rates found in Mediterranean countries (Cochat et al., 1999; Latta and Brodehl, 1990). In Tunisia PH1 seems to be very frequent, with almost 13% of terminal renal failure in children associated with this disease. However, the percentages in Europe and North America are not so high with 0.3% and 0.7%, respectively (Cochat et al., 1999; Rinat et al., 1999). The high prevalence of PH1 could be due to the high rate of consanguineous marriages occurring among the Tunisian population (Ben Arab et al., 2004).

AGT is encoded by *AGXT* which is composed of 11 exons. The gene spans a 10 Kb DNA segment in the chromosomal region 2q37.3 and encodes a 392 amino acid protein (Danpure, 1995). *AGXT* exists in two haplotypes: the major and the minor allele. The minor allele is a complex of three polymorphisms: P11L in exon 1, I340M in exon 10 and 74 bp duplication in intron 1. These three polymorphic variants are found in linkage disequilibrium with the minor allele haplotype (AGT-Mi), whereas the absence of these polymorphisms defines

Abbreviations: PH1, Primary hyperoxaluria type 1; AGT, alanine glyoxylate aminotransferase; vitamin B6, pyridoxine; CaOx, calcium oxalate; NC, nephrocalcinosis; ESRD, end-stage renal disease; AGT-Mi, minor allele haplotype; HGMD, Human Gene Mutation Database; SNPs, single nucleotide polymorphisms.

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the major allele. Overall, it appears that some missense mutations and polymorphisms have different impact on protein activity and stability. While p.G82Q leads to the loss of AGT catalytic activity (Danpure, 1995), the p.I244T leads to aggregation and accelerates degradation (Cochat et al., 2006).

More than 150 mutations responsible for the disease have been described so far, with all exons represented, according to the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk). The majority of them (75%) are point mutations, including missense, nonsense and nucleotide changes affecting splice-site consensus sequences. The remaining are major or minor deletions and insertions (25%) (Williams et al., 2009).

Knowing that definitive diagnosis of PH1 usually requires a needle liver biopsy (Rumsby et al., 2004); molecular genetic testing has been proved to be an efficient alternative and non-invasive approach to confirm PH1 in index case. The molecular investigation of *AGXT* in previous studies proved that the three common mutational changes c.33_34insC, c.508 G>A and c.731 T>C in exon 1, 4 and 7, respectively, are the most recurrent mutations worldwide (Monico et al., 2007; Williams and Rumsby, 2007). Other mutations were found in specific populations, such as the p.G190R (Arab), p.G82R (Vandal), p.G156R (Italian) and p.I244T (Maghrebian). The aim of our study was to characterize the mutational spectrum of PH1 in Tunisian patients. We prioritized mutation screening based on similarities of the genetic background and historical events in the Mediterranean.

2. Materials and methods

2.1. Patients

The study included 13 patients from 12 Tunisian families diagnosed with PH1. Most of the patients were consanguineous (10 of 13) with inter-marriage between first-degree cousins. Clinical data of all patients are summarized in Table 1. After obtaining informed consent, we collected blood samples from PH1 patients and their available family member.

2.2. Molecular analysis

Genomic DNA was extracted from peripheral blood leucocytes, as previously described (Miller et al., 1988). The PCR reaction was carried out in a volume of 25 μl containing 10 ng of DNA, PCR buffer (10×), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.5 μl of each primer and 0.5 U Taq DNA polymerase (Invitrogen, Foster City, CA). All PCR primers and conditions are shown in Table 2. Mutation screening was performed by direct sequencing using the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA) on an ABI prism 3130 DNA sequencer (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. Sequences were aligned against reference sequence (NM_000030.2) (http://www.ensembl.org/index.html) using BioEdit software. And the sequencing was invariably carried out on DNA strands with both forward and reverse PCR primers. To confirm the mutations detected by sequencing, we tested these variations in parents and siblings.

Table 2
Primer sequences used for PCR and sequencing.

Primer pair	Forward	Reverse	Size of the PCR fragment (bp)
Exon 1	CCGAGCACAAGCACAGATAA	TGAGACCCAGGCTCCCCGC	453
Exon 2	CCTTCCAACCTGCCTCCT	GGGCTGCCAGCTTCAAAC	494
Exon 4	CTCTGAGCTCCACCCACAG	AAGGACCAGAGGGACCAGT	223
Exon 5	AGAAGGCAACTGGCCAACTC	CTCTGGGGCATCTCAAATGT	495
Exon 7	CCGTCTCACTCCCGTGAAAC	CACCTCTCAGCCATGCCCAG	246
Exons 9–10	CAGGCAAAGTCAAACTGG	TGCACAGTCCTGCTCAAG	827

3. Results

3.1. Clinical and mutational spectrum

In this study, we report clinical and genetic investigation of PH1 in 12 Tunisian families including 13 patients. Our cohort was composed of 8 girls and 5 boys (sex ratio, 0.625) aged from 11 months to 22 years. The average age of onset was 5 years and 8 months. The examination of inbreeding in our cohort showed that out of the 13 reported PH1 patients, ten patients (77%) were born to consanguineous unions. Of these, seven were from first cousin unions. These children belong to 12 unrelated families. Two patients had the infantile form of PH1 (PH-J1 and PH-M1) and eleven had the juvenile form. Among these, five are between one and five years and six between 5 and 22 years. The molar plasma (oxalate/creatinine) was >0.07 in six cases (PH-B1, D1, D3, F1, H1, and L1). The majority of patients with ESRD presented nephrocalcinosis and/or kidney stones. For three other cases (patients PH-B1, F1 and G1), the disease was also revealed by urinary tract infections complicating nephrolithiasis.

Molecular diagnosis was performed by direct sequencing of selected exons 1, 4, 7 and 10 in order to investigate the common mutations: c.33_34insC, p.G170A and the Maghrebian mutation (p.I244T). We also tested exons 2 and 5 to identify mutations reported in the Arab population. Sequence analysis revealed five different point mutations causing PH1; all patients carried these variations at a homozygous state (Table 3). The first one detected in five affected members is a substitution c.731 T>C (p.I244T) reported as "Maghrebian mutation",

Table 1
Clinical and biochemical characteristics of PH1 patients.

Patient code	Consanguinity (F)	Age of onset (years)	Biochemical characteristics							Renal insufficiency	ESND
			Urea (mmol/l)	Blood creatinine (μmol/l)	Calcemia (mmol/l)	HCO_3- (mmol/l)	Blood oxalate (μmol/l)	Urine oxalate (mmol/l/24 h)	Urine creatinine (mmol/l/24 h)		
PH-A1	0.0156	5.58	37	540	2	10.45	222	0.59	2,5	Neph + UL	Yes
PH-L1	0.0625	1	86.7	1276	1.59	13.6	57	0.73	1.4	Neph + UL	Yes
PH-G1	0.0625	4	5.5	72	2.4	8,4	ND	0.77	2	Neph + UL	Yes
PH-I1	0.0625	11	104	18 64	1.95	11.4	86	0.15	1.8	Neph + UL	Yes
PH-D3	0.0625	5	95	1780	1.96	12.3	140	0.84	5.9	UL	Yes
PH-D1	0.0625	5	22.2	1670	2.36	5.2	ND	0.43	3.2	UL	Yes
PH-B1	0.0156	5.66	50.85	602	2.34	7.5	62	0.45	0.42	UL	No
PH-O1	0	2	101	636	2.5	11.6	120	0.79	2.3	Neph + UL	Yes
PH-J1	0.0625	0.91	38.7	463	1.85	12.8	ND	0.158	0.32	Neph	Yes
PH-M1	0.0625	4	48	900	2.12	13.2	ND	0.334	2.6	UL	Yes
PH-F1	0	5	96.7	1129	1.8	16.1	59	0.12	1.129	Neph + UL	Yes
PH-H1	0.0781	6	53.6	1010	1.38	17.6	136	0.087	0.38	Neph + UL	Yes

ND: not done; Neph: nephrocalcinosis; UL: urolithiasis; ESND: end stage renal disease.

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