HOGA1 Gene Mutations of Primary Hyperoxaluria Type 3 in Tunisian Patients

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Background: Primary hyperoxaluria type 3 (PH3) is due to mutations in the recently identified 4-hydroxy-2-oxoglutarate aldolase (*HOGA1*) gene. PH3 might be the least severe form with a milder phenotype with good preservation of kidney function in most patients. The aim of this study was to report three PH3 cases carrying mutations in *HOGA1*. **Materials and Methods:** Genetic analysis of *HOGA1* was performed in patients with a high clinical suspicion of PH after sequencing of *AGXT* and *GRHPR* genes, which was negative. Also, a complete *AGXT/GRHPR* MLPA was performed in these patients in order to detect large deletions/insertions. **Results and Discussion:** Two different *HOGA1* gene mutations were identified: the p.Pro190Leu in a homozygous state

and the p.Gly287Val in two patients in homozygous and heterozygous carriers. The median age at onset of clinical symptoms was 3.93 years. Most of the patients had a positive family history for recurrent urolithiasis. The p.Pro190Leu mutation was reported with impaired renal function at follow-up; however, the p.Gly287Val was presented with normal renal function. All patients were presented with urolithiasis, but only one had a nephrocalcinosis. **Conclusion:** This study expanded the number of PH3 patients from 63 to 66 cases. The p.Pro190Leu and the p.Gly287Val mutations found in this study can provide a first-line investigation in Tunisian PH1 patients. *J. Clin. Lab. Anal.* 31:e22053, 2017. © 2016 Wiley Periodicals, Inc.

Key words: 4-hydroxy-2-oxoglutarate aldolase; chronic kidney disease; *HOGA1*; PH; primary hyperoxaluria type 3

INTRODUCTION

Primary hyperoxalurias (PHs) are rare inborn errors of metabolism that are inherited in an autosomal recessive manner. They are the result from monogenic disorders of glyoxylate metabolism and lead to increased endogenous production of oxalate and the formation of calcium oxalate kidney stones (1, 2). Progressive renal inflammation and interstitial fibrosis from advanced nephrocalcinosis, recurrent urolithiasis, and urinary tract infections can cause reduced renal function, systemic oxalate deposition, and end-stage renal failure with time (3, 4).

To date, three genetic forms of PH have been defined: PH1–3. PH1 (MIM# 259900), the most

common and the most severe form is due to the mutations in the alanine–glyoxylate aminotransferase gene (*AGXT*) (5). PH2 (MIM# 260000) is due to mutations in the glyoxylate reductase–hydroxypyruvate reductase (*GRHPR*) (6). In general, PH2 shows a less severe phenotype with the absence of infantile oxalosis and ESRD occurring in about 20% of patients (1, 5).

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PH type 3 (PH3, MIM# 613616), accounting for 10–15% of PH cases based on analysis of local genetic background, is due to the mutations in the recently identified 4-hydroxy-2-oxoglutarate aldolase (*HOGA1*) gene (7). The *HOGA1* gene product is a mitochondrial protein, catalyzes the final step of mitochondrial hydroxyproline metabolism from 4-hydroxy-2-oxoglutarate to glyoxylate and pyruvate. Mutations in *HOGA1* cause the build-up of hydroxy-2-oxoglutarate aldolase, inhibiting GR/HPR function (8). There are 28 described mutations with two common alleles (c.700+5G>T and p.E315del), accounting for about 70% of all *HOGA1* alleles (7, 9–12).

Comparing patients with PH1 and PH2, PH3 might be the least severe form with a milder phenotype with good preservation of kidney function in most patients. The typical presentation is recurrent urolithiasis and marked hypercalciuria in the first decade, but less active stone formation later (1, 9, 11). Both PH2 and PH3 may have similar prevalence of about 10% of total genetically characterized PH cases (10). However, PH3 patients among Tunisians have never been reported. In this article, we report the first three PH3 cases carrying mutations in *HOGA1*.

PATIENTS AND METHODS

Patients

Three Tunisian subjects were enrolled in this study from three inbred families with a diagnosis of PH. Each patient was informed about the aim of the study, and the consent to genetic testing was obtained. The diagnosis of PH was based on clinical findings (urolithiasis, nephrocalcinosis, and end-stage renal failure), elevated plasma oxalate, and urinalysis (raised oxalate) in combination with spectrophotometric analysis of the calculi. None of the patients had a mutation in the *AGXT* and *GRHPR* genes. Sequencing was performed and was negative in all patients. Complete *AGXT/GRHPR* multiplex ligation-dependent probe amplification (MLPA) performed in patients in order to detect large deletions/insertions was negative.

Molecular Approach

Genomic DNA was extracted from peripheral blood leukocytes using guanidinium chloride standard procedures. Six pairs of oligonucleotide primers were generated to amplify all exons and flanking intronic regions of the *HOGA1* gene (Table 1). PCRs were performed in 25 µl of solution containing 0.2 mM dNTP, 0.02 U/µl Hot start Taq DNA polymerase (Qiagen, Center Mainz, Germany), and 2.5 µl 10. PCR buffer (with Mg 2+, Qiagen), approximately 20 ng genomic DNA and 1 mM of each primer. PCR was performed in a thermocycler, which was arranged uniformly for all reactions as followed: initial denaturation step at 95°C for 15 min, subsequently followed by 31 cycles with denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and elongation at 72°C for 30 sec. Direct sequencing of purified PCR products was performed using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI 3730 genetic analyzer. Sequences were analyzed using Seqscape Analysis Software (Applied Biosystems) in comparison with GeneBank Reference genomic sequence (*HOGA1*: NM_138413.3). The sequencing analysis was performed at service of Hereditary Diseases of Metabolism, Groupement Hospitalier Est, Bron, France.

RESULTS

Clinical Data of PH Patients

Our study was composed of three unrelated PH patients from different regions of Tunisia: three males patients, aged from 2 to 8 years. All patients are derived from a consanguineous marriage. The median age at onset of clinical symptoms was 3.93 (range: 1.5–5.5) years. Most of patients had a positive family history for recurrent urolithiasis (2/3). All patients had normal renal function at diagnosis. All patients were presented with urolithiasis, but only one had a nephrocalcinosis. Urine crystal analysis was performed in two of the PH cases, revealing monohydrated calcium

TABLE 1. PCR Primers for Directed Sequencing Analysis of *HOGA1*

Exon	Forward primer (5'–3')	Reverse primer (5'–3')	Product, bp
1	GCCAGATCCCACTGGAGATAC	CAGACCAGTCTCCCCTGTTC	687
2	GTGAACAGTCATTGCAGCTGTG	GAGACAGGACCCAAGCTAGG	361
3	CCTAGCTTGGGTCCTGTCTC	CACCACCCTATTCCCAGCTTCC	328
4 + 5	CCACTGTGATTGCTTACACTCGG	CATCCTCCTTCAGCCAACAAGG	854
6	CTCAGATCAGCCCTGAGCAG	CTCAGATCAGCCCTGAGCAG	558
7	CCAGCTTGAGGACCAATACCAC	GAGATATGCATGGGCGTGCC	619

TABLE 2. Clinical Findings and Outcome of Three Patients with Primary Hyperoxaluria Type 3

	Patient 1	Patient 2	Patient 3
Sex (M, F)	M	M	M
Age at diagnosis years	2	5	8
Age at onset of symptoms years	1,5	4,8	5,5
Consanguinity	First degree	First degree	Second degree
Symptoms	Urolithiasis and nephrocalcinosis	Urolithiasis	Urolithiasis, hematuria
Personal history	Recurrent urolithiasis, urinary infection	Recurrent urolithiasis, urinary infection	Recurrent urolithiasis, urinary infection
Renal ultrasound	- right kidney destroyed siege of nephrocalcinosis - left kidney in compensatory hypertrophy	Right kidney contains lithiasis	Urolithiasis
Urinary oxalate level	0.049 mmol	1,200 mmol/l	Not available
Stone analysis	Not available	56% whewellite	Calcium oxalate
Renal function at diagnosis	Preserved	Preserved	Preserved
Renal function at follow-up	Insufficient	Preserved	Preserved

oxalate (whewellite). Only patient 1 displayed impaired renal function at follow-up.

The characteristics and clinical features of the three patients are given in Table 2.

Identification of Mutations

Two different *HOGA1* gene mutations were identified: the c.569C>T (p.Pro190Leu) in exon 4 in a homozygous state and the c.860G>T (p.Gly287Val) in exon 7 in two patients in homozygous and heterozygous carriers. These mutations had been previously reported as disease-causing mutations (7). Electropherograms of the sequence showing the *HOGA1* mutations identified are shown in Figure 1.

Subsequent *HOGA1* sequencing of the parents of patients proved all parents to be heterozygous for the p.Pro190Leu and p.Gly287Val, respectively.

Fig. 1. Electropherograms of the sequence showing the *HOGA1* mutations identified.

The analysis showed that each mutation segregates with a specific genotypic combination. In fact, the c.834+42G>T polymorphism in intron 6 in homozygote state was associated with the c.569C>T mutation.

Moreover, we noted the intronic polymorphism c.469-31T>C in intron 3 is linked with the c.860G>T mutation.

DISCUSSION

The molecular mechanisms of PH1 and PH2 were identified in 1988 and 1999, respectively (6, 13). However, the association of PH3 and mutations in *HOGA1* gene was not established until 2010 (14). Up to now, most the described PH3 mutations came from the population of European Americans and rarely in Chinese population (12).

In this study, mutational analysis of the *HOGA1* gene showed two mutations, which have been previously reported (7), in three Tunisians patients. The first one was the p.Pro190Leu found in the homozygous state in patient 1. The second mutation was the p.Gly287Val found in the homozygous and heterozygous state in patient 2 and patient 3, respectively. This is the first report of PH3 patients among Tunisian population. PH3 was first described in Ashkenazi Jewish pedigrees (14), in Caucasians (9) and then in Chinese (12). Also, this study expanded the number of PH3 patients from 63 to 66 cases. In fact, Belostotsky et al. (14) made the first description of *HOGA1* gene mutations in PH3 patients in 2010, following by other five publications that have focused on this disease (7, 9, 11, 12, 15).

According to literature, the frequent mutation is the c.700+5G>T with an allelic frequency of 42%. However, this variation was not found in our population. So, a relationship between *HOGA1* and ethnic distribution may be suspected. The p.Pro190Leu mutation

was reported only in Arab countries such as Lebanon (9), now in a Tunisian patient. In our study, it was found in association with the c.834+42G>T polymorphism in intron 6 in homozygote state. The c.834+42G>T was listed as SNPs in databases (dbSNP, 1000 Genome Project), with a 0.01 global minor allele frequency (GMAF) and an uncertain significance. The combination with the intronic polymorphism c.834+42G>T in intron 6 and the p.Pro190Leu mutation and the historical migratory/invasive flows which took place in North Africa can be attributed for the Arab founder nature of this mutation.

The p.Pro190Leu mutation was reported in one patient who displayed impaired renal function at follow-up. This is in contrast to the previous data reporting a favorable outcome in PH3 (7, 11, 14). However, our results were similar to the study of Allard et al. (15), where reported two patient experienced impaired glomerular filtration rates (GFR), with chronic kidney disease (CKD) stage 2 occurring early during childhood in a three several affected patients in the context of a homozygous *HOGA1* mutation (p.Pro190Leu) together with a heterozygous *AGXT* mutation (p.Asp201Glu) (9). Thus, demonstrating the triallelic inheritance, defined as the presence of a disease-modulating heterozygous *AGXT* mutation. It was found in a single consanguineous family with several affected members: the father and his two daughters. Onset of recurrent urolithiasis in the two children was 0.3 and 0.6 years compared with a single stone episode requiring surgery in their father at the age of 2 years. At diagnosis, the father was clinically unremarkable aside from marked hypercalciuria, while his daughters demonstrate persistent substantial hyperoxaluria accompanied by intermittent hypercalciuria. In addition, a liver biopsy from the older girl showed reduced liver AGT activity. We report similar data for the clinical features and age at presentation in our patient and the two children. This PH3 phenotype is more severe in comparison with the adult patient (the father). As the p.Pro190Leu mutation was located in the adjacent turn and involving a proline, is likely to disrupt the active site (16), the severe phenotype can be explained by the predictor effect of this missense mutation which was pathogenic by algorithm based on sequence conservation and protein structure, and could affect the catalytic activity.

On the other hand, the p.Gly287Val mutation was identified in two patients, one in homozygous state and the other in heterozygous state. The p.Gly287Val was found in most reported studies in the heterozygous state (7, 14) and in one case reported recently in homozygous state (15). The p.Gly287Val mutation has

been identified in five Ashkenazi Jewish PH3 probands (14) and in a Caucasian patient (15). The p.Gly287Val mutation was associated with the c.469-31T>C polymorphism in intron 6, reported with a frequency equal to 0.07 in GMAF. The association between the p.Gly287Val mutation in exon 7 and the c.469-31T>C polymorphism in intron 6 is particularly noteworthy.

Our finding of *HOGA1* homozygote and heterozygote of the variant p.Gly287Val were in association with milder phenotype. All patients displayed a normal renal function and presented with common symptoms of urolithiasis. *HOGA1* heterozygosity for some other variants (c.944_946 delAGG and c.907 C_T, p.R303C) in association with milder phenotypes was reported previously (7). Thus suggesting that these hypomorphs mutations may behave a dominant negative fashion, a mechanism supported by the tetrameric structure of *HOGA1* (7). Complete sequencing and MPLA has ruled out a contribution from *AGXT* or *GRHPR* variants in our cases also, the defavorable outcome has been previously reported. Thus presents an exciting hypothesis, that the milder phenotype in heterozygous individual may be modulated by mutations in other genes affecting glyoxylate metabolism or involved by and various potential environmental factors. In fact, the heterozygous p.Gly287Val variant was detected common to both PH3 and idiopathic stone formers (7, 14), suggests that some alleles may be partially penetrant, predisposing to idiopathic calcium oxalate urolithiasis.

The milder phenotype of *HOGA1* homozygote and heterozygote of the variant p.Gly287Val underscore the favorable outcome of PH3 in the context of PH in accordance with previous studies (7, 11, 14, 15). However, we should still be cautious to call PH3 a benign condition in front of the phenotypic heterogeneity of PH3 observed. Interestingly, some *HOGA1* carriers present with mild hyperoxaluria or idiopathic urinary stone disease (unlike other forms of PH), and others patients with PH3 express a severe phenotype, linked to specific missense variants, suggesting that more penetrant mutations may have a stronger phenotypic effect. However, the renal injury observed may have, at least in part, been aggravated by nephrocalcinosis or kidney damage secondary to urological procedures or to both possibilities. Until now, no allelic correlations have been established for PH3. So metabolic studies and further functional characterization of *HOGA1* are needed to definitively confirm the role and pathophysiology of variants in PH3.

Obviously, a complete sequencing of all known genes involved in glyoxylate metabolism should be considered, notably in patients who lack a second

causative mutation or those with an atypical presentation. This would not only prevent misclassification, but furthermore allow the uncovering of multiallelic inheritance, which may constitute one of the long-sought mechanisms for inter- and intra-familial phenotype variability in PH.

In conclusion, we identified two mutations of *HOGA1* gene in Tunisians patients with PH3. This is the first report of a PH3 patient among North Africa. This study expanded the number of PH3 patients from 63 to 66 cases. The p.Pro190Leu and the p.Gly287Val mutations found in this study can provide a first-line investigation in Tunisian PH1 patients. This process may facilitate the molecular diagnosis of PH3 in our country and would allow an accurate tool for a pre-cocious diagnosis in affected families and detection of presymptomatic patients.

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