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Genotype–phenotype correlation in primary hyperoxaluria type 1: the p.Gly170Arg AGXT mutation is associated with a better outcome

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We sought to ascertain the long-term outcome and genotype–phenotype correlations available for primary hyperoxaluria type 1 in a large retrospective cohort study. We examined the clinical history of 155 patients (129 families primarily from Western Europe, North Africa, or the Middle East) as well as the enzymatic or genetic diagnosis. The median age at first symptom was 4 years, and at diagnosis 7.7 years, at which time 43% had reached end-stage renal disease. Presentations included: (1) early nephrocalcinosis and infantile renal failure, (2) recurrent urolithiasis and progressive renal failure diagnosed during childhood, (3) late onset with occasional stone passage diagnosed in adulthood, (4) diagnosis occurring on post-transplantation recurrence, and (5) family screening. The cumulative patient survival was 95, 86, and 74% at ages 10, 30, and 50 years, respectively, with the cumulative renal survival of 81, 59, 41, and 10% at ages 10, 20, 30, and 50 years, respectively; 72 patients had undergone a total of 97 transplantations. Among the 136 patients with DNA analysis, the most common mutation was p.Gly170Arg (allelic frequency 21.5%), with a median age at end-stage renal disease of 47 years for homozygotes, 35 years for heterozygotes, and 21 years for other mutations. Our results underscore the severe prognosis of primary hyperoxaluria type 1 and the necessity for early diagnosis and treatment, as well as confirm a better prognosis of the p.Gly170Arg mutation.

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Primary hyperoxaluria type 1 (PH1, Online Mendelian Inheritance in Man 259900) is an autosomal recessive disorder caused by deficiency of the liver-specific, peroxisomal, enzyme alanine: glyoxylate amino transferase (AGT), involved in glyoxylate detoxification. AGT deficiency leads to oxalate and glycolate overproduction.[1] PH1 is a very rare disease with an estimated prevalence ranging from 1 to 3 per million population in Europe.[2–4] PH1 can occur either because AGT enzymatic activity is low, undetectable, or because of AGT mistargeting to the mitochondria. As calcium oxalate is poorly soluble in the urine, PH1 usually presents with symptoms referable to the kidney and urinary tract, such as stone formation and nephrocalcinosis. Both recurrent renal stones and nephrocalcinosis can lead to progressive renal impairment. Along with reduced oxalate excretion by the kidneys as glomerular filtration rate progressively declines, continued overproduction of oxalate leads to a critical saturation point for plasma oxalate, and hence oxalate deposition occurs in many organs, leading to systemic involvement defined as oxalosis.[5,6]

The genetic basis for PH1 has been identified and more than 100 mutations in the *AGXT* gene, coding

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AGT, have been published so far. In the Caucasian population, two haplotypes have been defined, referred to as minor and major allele, with a frequency of 20 and 80%, respectively.[7] The genotype–enzymatic phenotype correlation is well known, whereas the clinical phenotype–genotype correlation is more elusive. Thus, the effect of mutation analysis on defining prognosis and therapeutic options is still debated.

The diagnostic procedure has improved over the recent years and can be based on reasonable recommendations.[8,9] However, being rare, PH1 is still insufficiently known by physicians, and diagnosis is often delayed.[2,10] A significant proportion of patients are only diagnosed once end-stage renal disease (ESRD) has occurred or after disease recurrence post renal transplantation (Tx).[11,12] Considering the clinical heterogeneity of PH1 and the lack of long-term available data, more information is mandatory to understand better the clinical course of the disease and to help define therapeutic strategies and guidelines.

The objectives of this study are (1) to describe the long-term clinical course in a cohort of PH1 patients and (2) to extend the knowledge of associations between genotype and clinical outcome.

RESULTS

Population characteristics

A total of 155 PH1 patients from 129 unrelated families were identified. Diagnosis was made either by DNA analysis (79 patients), AGT enzyme activity in liver tissue (16 patients), or both (57 patients). Another three patients, siblings of confirmed PH1 patients, had clinical and biochemical phenotype of PH1 and were included in the analysis. Male-to-female ratio was 81/74 (1.09). Patients came from various countries: a Caucasian origin was reported for 52%, Arab origin (North Africa and Middle East) for 43%. A family history of PH1 or nephrolithiasis was present in 56%, and consanguinity in 46%.

Clinical features

Mean duration of follow-up since diagnosis was 7.0 ± 8.7 years (median 3 years). Median age at first clinical manifestation was 4.0 (interquartile range 0.6–8.1) years. Symptoms occurred before 1 year of age in 40 patients (26%) and after 15 years in 32 patients (21%). The median age at diagnosis was 7.7 (interquartile range 2.0–27.9) years, at which time 66 patients (43%) had reached ESRD. Mean time between initial symptoms and diagnosis was 6.2 ± 10.5 years (median 1 year). In the 40 patients who were symptomatic during infancy, mean diagnosis delay was 1.8 years (median 1 month). This mean delay was 8.0 years (median 3.5 years) for the other patients. A total of 15 patients (10%) were diagnosed after recurrence of oxalate deposition in the transplanted kidney. Among the 20 patients (13%) who were diagnosed by family screening, three were presymptomatic at the time of diagnosis. The clinical features at the time of diagnosis are summarized in Table 1.

Outcomes

Patient survival. Out of 155 patients, 20 died at a median age of 19.9 (interquartile range 3.5–47.2) years. The cumulative patient survival was 95, 86, and 74% at 10, 30, and 50 years of age, respectively (Figure 1). In a multivariate Cox regression analysis, the only factor significantly associated with a higher risk of death ($P = 0.007$) was infantile PH1 (hazard ratio (HR) 5.3, 95% confidence interval (CI) 1.6–18.2). Dialysis duration of more than 2 years was predictive of a twofold increased risk of death, although the association failed to be significant in a multivariate model ($P = 0.09$).

Renal survival. Cumulative renal survival was 90, 81, 59, 41, and 10% at 1, 10, 20, 30, and 50 years of age, respectively (Figure 2). The median survival without ESRD was 24 years (CI 20–32). We subsequently performed subgroup analyses according to geographic region, as a surrogate for access to medical care. In patients living in the European Union ($N = 133$), renal survival was 86, 62, and 49% at 10, 20, and 30 years, respectively. In the other regions (North Africa, Middle East, and Brazil), 14 out of 22 patients presented with

Table 1 | Clinical characteristics of 155 PH1 patients at diagnosis

	Median (range)	No. (%)
Total number of patients		155 (100)
Family history of PH1		57/129 (44)
Family history of urolithiasis		28/129 (22)
Age at first symptoms, years	4.0 (0.3–58.5)	142/155 (92)
History of recurrent urolithiasis		110/142 (77)
History of nephrocalcinosis		98/128 (76)
Age at clinical diagnosis, years	7.7 (0.3–67.0)	150/155 (97)
Age at definitive diagnosis, years	12.5 (0.4–73.3)	152/155 (98)
CKD 3–4 at diagnosis		109/150 (73)
ESRD at diagnosis		66/155 (43)
Extra-renal symptoms at diagnosis[a]		50/127 (39)

Abbreviations: CKD, chronic kidney disease; ESRD, end-stage renal disease; PH1, primary hyperoxaluria type 1.
[a]Including oxalate osteopathy, retinopathy, skin deposits, oxalate cardiomyopathy, and oxalate neuropathy (as reported spontaneously by clinicians).

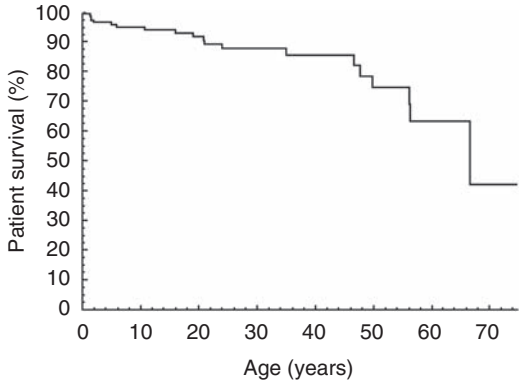


Figure 1 | Cumulative patient survival in 155 primary hyperoxaluria type 1 patients.

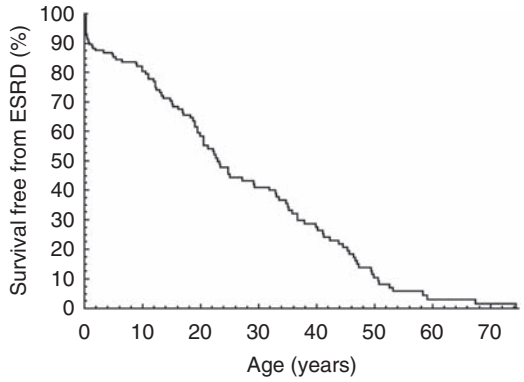


Figure 2 | Cumulative survival free from end-stage renal disease (ESRD) in 155 primary hyperoxaluria type 1 patients.

ESRD at the time of diagnosis, and renal survival was 50, 21, and 7% at age 10, 20, and 30 years, respectively. Median age of survival without ESRD was significantly different: 28 years (CI 21–35) in patients living in Europe versus 9 years (CI 4–14) in others ($P=0.002$). In a multivariate Cox regression analysis, the presence of at least one p.Gly170Arg allele was significantly associated with a lower risk of ESRD ($P=0.005$; HR 0.6, CI 0.4–0.9), whereas infantile PH1 ($P=0.001$; HR 6.0, CI 3.4–10.7) and low-income region of residence ($P=0.006$; HR 2.3, CI 1.3–4.1) were associated with an increased risk of ESRD. Chronic kidney disease (CKD) stage 3–4 at diagnosis did not remain a statistically significant predictor of subsequent ESRD ($P=0.10$; HR 1.6, CI 0.9–2.4).

At last follow-up, 45 patients (33% of survivors) were on conservative treatment, 62 (46%) had a functioning kidney graft, and 28 (21%) were on dialysis.

Transplantation strategies. A total of 97 Tx (40 kidney Tx, 52 liver–kidney Tx, and 5 liver Tx) had been performed in 72 patients. Among the 29 patients who received an isolated kidney Tx before eventual combined Tx, 26 grafts were lost to oxalate deposition in the kidney in 24 patients (83%) and two were lost to other causes (7%). The median time between the first isolated kidney Tx and graft loss was 11 months (range 1 day–12.8 years). One patient received a pre-emptive isolated liver Tx at 5 years of age,[13] but reached ESRD because of a combination of nephrotoxicity and nephrocalcinosis parenchymal injury, leading to further kidney transplant at 16 years after liver Tx. A total of 52 patients received a combined liver–kidney Tx (11 subsequently to a previous isolated kidney Tx). After 5 years of median follow-up (range 2 days–15.7 years), 34 (65%) had functioning liver and kidney grafts. Kidney graft failure occurred in nine cases (17%), liver graft failure in 10 (19%), and 11 deaths were reported (seven of whom within 3 months after Tx).

Infantile PH1. Among the 40 patients with infantile form, 29 (72%) presented with nephrocalcinosis and 19 (47%) experienced ESRD at the time of diagnosis. Seven (18%) have died during follow-up. The cumulative incidence of ESRD in this group was 50% at 3 years of age, 63% at 10 years, and 85% at 20 years. Details of infantile forms by genotype are reported in Table 2.

Table 2 | Characteristics of infantile cases of PH1 by genotype

Most common mutations	No. of patients with symptoms before 1 year (*N*=40) (homozygous/heterozygous)	No. of patients with ESRD reached before 1 year (*N*=17) (homozygous/heterozygous)
p.Gly170Arg	6 (1/5)	2 (0/2)
p.Ile244Thr	10 (6/4)	3 (2/1)
c.33dupC	3 (1/2)	2 (1/1)
p.Arg122Stop	4 (4/0)	2 (2/0)
p.Phe152Ile	2 (0/2)	2 (0/2)
p.Glu82Gln	2 (2/0)	1 (1/0)

Abbreviations: ESRD, end-stage renal disease; PH1, primary hyperoxaluria type 1.

Mutation analysis

DNA analysis was performed in 140 patients from 116 families. A minimum of one mutation was identified in 136 patients (Tables 3 and 4). A total of 222 mutations out of 232 expected alleles (116 families) were observed, corresponding to a detection rate of 95%. In all, 52 different mutations were identified. The p.Gly170Arg mutation (c.508G>A) was found in 36 patients (26%), of whom 24 were compound heterozygous and 12 homozygous (allelic frequency: 21.5%). The p.Ile244Thr mutation (c.731T>C) was detected in 27 patients (20%) with an allelic frequency of 21%. In all, 16 patients (12%) in 15 families (allelic frequency: 9.5%) carried the c.33dupC mutation and 7 patients (5%) the p.Phe152Ile mutation (c.454T>A). The p.Arg122Stop mutation (c.486C>T) was found in a homozygous state in 5 of 10 patients (7 families) from Lebanon. The majority of the remaining mutations were private mutations. There was marked intra- and inter-familial phenotypic heterogeneity in families with private mutations from ESRD in infancy to PH1 diagnosed in the second or third decade of life.

Genotype–phenotype correlations

p.Gly170Arg mutation. In patients who were homo- or heterozygous for the p.Gly170Arg mutation, 8/12 (67%) homozygous patients and 16/24 (67%) heterozygous developed ESRD, which is similar to 68/100 (68%) in patients with other mutations. However, ESRD was delayed in p.Gly170Arg patients, for which the median age at ESRD was 47 years (CI 43–50) in homozygotes and 35 years in heterozygotes, compared with 21 years in patients without p.Gly170Arg mutation (Figure 3). The cumulative survival free from ESRD was significantly different according to the p.Gly170Arg genotyping (log-rank test: $P=0.02$). Among the 12 patients who were homozygous for the p.Gly170Arg mutation, six were diagnosed when ESRD had been reached, and another already had CKD stage 3. Renal function was preserved over time in all five homozygous patients without CKD at diagnosis. One homozygous patient experienced early

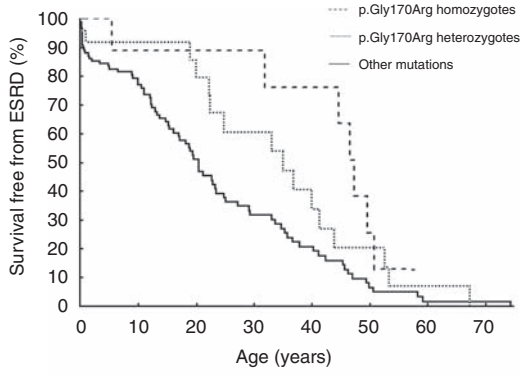
Table 3 | Percentage of the most common mutations identified from 222 alleles

Mutation	No. of patients (homozygous/ heterozygous)	Allele frequency (%)	Origin of patients (%)
p.Gly170Arg	36 (12/24)	21.5	Western Europe (97%)
p.Ile244Thr	27 (20/7)	21	North Africa (93%)
c.33dupC	16 (5/11)	9.5	Various countries
p.Arg122Stop	5 (5/0)	4.5	Middle East (100%)
p.Phe152Ile	7 (1/6)	3.5	Western Europe (100%)
Others	45	40	Various countries

Table 4 | Repartition of the most common mutations identified from 136 patients

Mutation	Total no. of patients (H/h)	No. of patients from France (H/h)	No. of patients from other countries (H/h)
p.Gly170Arg	36 (12/24)	27 (7/20)	9 (5/4)
p.Ile244Thr	27 (20/7)	22 (18/4)	5 (2/3)
c.33dupC	16 (5/11)	10 (3/7)	6 (2/4)
p.Arg122Stop	5 (5/0)	1 (1/0)	4 (4/0)
p.Phe152Ile	7 (1/6)	6 (1/5)	1 (0/1)

Abbreviation: H/h, homozygous/heterozygous.

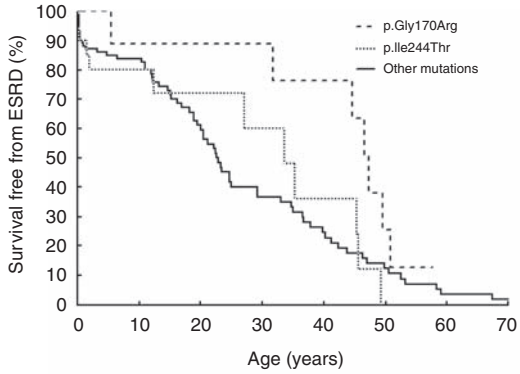


Age	10	20	30	40	50
Survival free from ESRD (no. at risk)					
Homozygous	90% (8)	90% (8)	90% (8)	78% (7)	22% (2)
Heterozygous	91% (18)	85% (14)	60% (9)	33% (5)	20% (3)
Others	78% (69)	52% (31)	31% (18)	22% (12)	7% (4)

Figure 3 | Cumulative survival free from ESRD according to genotyping for p.Gly170Arg mutation in 136 primary hyperoxaluria type 1 patients.

isolated nephrocalcinosis and presented with an infantile form of PH1 (Table 2).

p.Ile244Thr mutation. In the p.Ile244Thr group, ESRD was reached in 11/20 homozygous patients and in 3/7 heterozygotes (including one compound heterozygote, p.Gly170Arg/p.Ile244Thr). The median age at ESRD was 33 years (CI 27–46) for p.Ile244Thr homozygous patients (four of whom reached ESRD by age 2), 34 years in heterozygotes, whereas it was 23 years for others. The



Age	10	20	30	40	50
Survival free from ESRD (no. at risk)					
p.Gly170Arg Homozygous	90% (8)	90% (8)	90% (8)	78% (7)	22% (2)
p.Ile244Thr Homozygous	80% (10)	72% (6)	60% (5)	36% (3)	0% (0)
Others	82% (72)	69% (45)	39% (28)	30% (21)	13% (9)

Figure 4 | Cumulative survival free from end-stage renal disease (ESRD) according to *AGXT* mutations in 136 primary hyperoxaluria type 1 patients.

cumulative survival free from ESRD was not different according to p.Ile244Thr genotyping (log-rank test: $P = 0.88$). When comparing the survival free from ESRD among the three groups, homozygosity for p.Gly170Arg, or p.Ile244Thr mutation and other genotypes (Figure 4), the difference did not reach statistical significance (log-rank test: $P = 0.12$).

p.Phe152Ile mutation. Only one patient was homozygous for p.Phe152Ile. He had been symptomatic since 24 years of age and was clinically diagnosed as PH1 at age 49, reaching ESRD at 74.5 years. Six compound heterozygous patients with one allele with the p.Phe152Ile mutation reached ESRD at a median age of 27.5 years (range 0.3–58.1). Pyridoxine sensitivity was neither formally tested nor reported in these patients.

c.33dupC mutation. A total of five patients were homozygous for the c.33dupC. mutation, coding for a truncated protein, and three of them had had symptoms before 1 year of age. Among the five homozygous patients, two still had preserved renal function at 1 and 19 years of age, and three had reached ESRD at 3 months, 10, and 23 years of age. Two siblings of the latter with no DNA analysis had infantile form and reached ESRD at 8 months and 5 years of age.

Genotype–enzyme activity correlation. A total of 82 patients had liver AGT activity measured. Of these, 18 had AGT activity above 10% of normal activity and 14 of these 18 patients had at least one p.Gly170Arg. allele (no genotyping in two patients, one patient was homozygous for p.Ile244Thr, and one for Gly82Glu). In the 5/12 p.Gly170Arg homozygotes with AGT measured, median AGT activity was 41% (range 12–50). In the 14/25 patients heterozygous for p.Gly170Arg with AGT activity measured, median AGT activity was 14% (range 3–28). Thus, it seems

that patients with higher AGT activity belong to the group with better renal survival.

DISCUSSION

The clinical data and genotypes of a large multiethnic cohort of proven PH1 patients are presented in this study. Our data contribute to better delineate the long-term spectrum of PH1.

As previously reported,[5] PH1 could be classified into one of the five presentations: (1) an infantile form with early nephrocalcinosis and renal failure (26%), (2) a recurrent urolithiasis and progressive renal failure leading to diagnosis in adolescence or early adulthood (30%), (3) a late-onset form with occasional stone passage leading to diagnosis in adulthood (21%), (4) a diagnosis occurring on post-transplantation recurrence (10%), and (5) a diagnosis made by family screening (13%). Nephrolithiasis and nephrocalcinosis were the first manifestations of the disease in most patients, confirming that all patients with recurrent kidney stones or nephrocalcinosis should be screened for PH1. This can be carried out by determination of either urinary oxalate and glycolate excretion on 24 h urine examination or on repeated urine samples, or, in patients with ESRD, plasma oxalate and glycolate concentrations.[6,8] Crystalluria analysis and accurate analysis of the morphology and composition of stones may also yield useful information.[14,15] Approximately 40% of patients had both ESRD and extra-renal symptoms at the time of diagnosis, and the proportion increased to 60% in patients diagnosed during adulthood. Moreover, a substantial proportion of adult patients were diagnosed after oxalosis recurrence following isolated kidney Tx. The fact that most adult patients diagnosed had already developed ESRD may have several reasons: (1) urolithiasis caused by inborn errors of metabolism are still unknown or underestimated by physicians, (2) symptoms precluding renal failure may be mild in case of PH1, and (3) the only diagnosis available for PH1 was once invasive and potentially harmful after anuria had occurred and urine oxalate assessment was no longer available. We therefore believe that screening for PH1 should be performed in all ESRD of unknown origin in case of personal or familial history of urolithiasis or nephrocalcinosis. Presence of systemic oxalosis and increased level of plasma oxalate (usually $>80\,\mu mol/l$ in ESRD) provide valuable clues indicative of the necessity for diagnosis confirmation by oriented genetic testing or enzymatic diagnosis.

PH1 is a life-limiting condition. Mortality rate was 28% in the Swiss cohort[3] and 19% in the Dutch cohort.[4] The overall death rate due to PH1 or its treatment in our series was 13%. The only predictive factor for death was infantile PH1, which often presents as a life-threatening condition because of a rapid progression to ESRD as a result of a combination of early oxalate load and immature glomerular filtration rate.[16] Infantile oxalosis is a frequent cause of early ESRD in some developing countries where therapeutic withdrawal can be regarded as an acceptable option.[16] Conversely, the prognosis may be excellent in developed countries where combined liver–kidney Tx is available.[17] Although not statistically significant, patients on maintenance dialysis for more than 2 years were also at a higher risk of death. Because dialysis does not stop the formation or removes oxalate deposits, PH1 patients may die from complications of systemic oxalosis. This stresses the importance of planning either pre-emptive or at least early combined liver–kidney Tx in PH1 patients while maintaining intensive dialysis.[18,19]

In this study, the median time to ESRD since birth was 24 years. Early studies reported that 50% of patients developed ESRD by 15 years and 80% by 30 years of age.[20] The recent improvements in diagnosis procedure and PH1 management have delayed the median survival without ESRD to 33 years.[10] The shorter time to ESRD observed in this study might be because of a greater proportion of patients with ESRD at the time of diagnosis, compatible with both delayed diagnosis and management. It should be noted that, in this study, all patients who were formally diagnosed in France were included as well as a number of patients who came from countries with lower access to medical care.

In a multivariate analysis, we found that both infantile PH1 and the presence of p.Gly170Arg mutation were significantly associated with renal outcome, in opposite ways. Infantile onset of PH1 was the main predictor of progression to ESRD: 50% of affected infants experienced ESRD by the age of 3 years and two-thirds reached ESRD by 10 years of age. The presence of CKD stage 3–4 at the time of diagnosis was only a predictor of borderline significance for subsequent ESRD. Indeed, most patients with a glomerular filtration rate $>60\,ml/min$ per $1.73\,m^2$ at the time of diagnosis did not reach ESRD during follow-up. These data suggest that timely diagnosis and treatment at a stage of preserved renal function may modify the course of the disease. An aggressive conservative treatment of PH1 patients with high fluid intake ($2–3\,l/m^2$) orally, or enterally using nasogastric tube or gastrostomy placement, calcium oxalate crystallization inhibitors, and pyridoxine may improve renal survival. These treatments should therefore be started as soon as the diagnosis of PH1 has been considered.[21–23]

Our results also provide clinical evidence that the p.Gly170Arg mutation is associated with a better renal outcome even after adjustment for potential confounding variables. We found that patients homozygous for the p.Gly170Arg, and also heterozygous ones, experienced a delayed progression to ESRD, and that homozygous patients without ESRD at diagnosis have a preserved renal function during follow-up. The p.Gly170Arg mutation is the most common one in the European and North American population, and results in the singular AGT mistargeting from peroxisome-to-mitochondria. Although the mechanism is still unclear, pyridoxine decreases urinary excretion of oxalate in some patients. To date, response to pyridoxine has mostly been shown in patients with the p.Gly170Arg mutation and is suggested for the p.Phe152Ile mutation.[24–26] However, it is unknown whether pyridoxine responsiveness allows maintenance of a stable renal function over time, and

does not just delay the onset of ESRD. As pyridoxine treatment and responsiveness were not systematically recorded in our cohort, this remains to be analyzed. The fact that homozygous patients for p.Gly170Arg still reach ESRD might be attributed to late diagnosis and treatment as well as pre-existing renal lesions. No conclusions could be drawn for the p.Phe152Ile mutation as the number of patients was too small. The only other genotype that could be analyzed was the p.Ile244Thr mutations, which was highly prevalent in our study because of the great number of patient coming from Arab countries. No difference could be detected in that group one way or another. The c.33dupC mutation, although rare, was found in patients with both severe and less severe form of the disease, unlike previous reports.[25] Thus, the individual clinical benefit of mutation analysis, excluding its role in diagnosis, might still be debated.[27,28] Indeed, genotyping may be useful for prognosis in a population but should be interpreted with caution in individuals for several reasons; in addition to the large number of private mutations, most patients with PH1 are compound heterozygotes and there is a marked intrafamilial clinical heterogeneity in some pedigrees, suggesting a role for modifier genes.

Our data also confirm the poor graft survival and patient quality of life of isolated renal Tx. Previous studies have reported a renal graft survival ranging from 17 to 23% at 3 years to 48% at 8 years after Tx.[29,30] Therefore, we cannot recommend isolated kidney transplantation for PH1 patients except as a temporary solution in some countries before managing the patient in a specialized center for further liver or combined liver–kidney Tx and the possible exception of pyridoxine-sensitive patients.

Our study has some limitations. This is a retrospective study and some patients have been lost to follow-up. Data that may have an influence on renal outcome—such as urinary oxalate and pyridoxine response—were not reported in some patients. We should also not dismiss the possibility of a selection bias: patients referred for genotyping may be those with the more severe course and may not represent the true spectrum of the disease in the general population.

CONCLUSION

We have shown in this study that mutation analysis may be clinically useful for PH1 patients, as the homozygous p.Gly170Arg mutation is associated with a better prognosis, although careful consideration should be given to therapeutic strategy. Mutation analysis is an important diagnostic tool in patients with severe hyperoxaluria when dialysis or transplantation is considered. With the constitution of a European hyperoxaluria consortium (www.oxaleurope.com) and future collaboration with the Mayo Clinic registry, several hundred PH1 cases will be available for genotype–phenotype correlation studies. Collection of a large cohort of families and subsequent careful analysis may be the handle needed to tackle the problem of genetic and environmental modifiers at the root of the variability of disease expression.

MATERIALS AND METHODS

Subjects and data collection

The study design is that of a retrospective cohort of patients with PH1 diagnosed between 1993 and 2008. PH1 was defined on the following criteria: AGT enzyme activity below 50% on liver biopsy, or mutations on *AGXT* gene, or urinary biochemistry in favor of PH1 in siblings of enzymatically or genetically proven PH1 patients. Enzymatic studies and mutation analysis were performed in the same reference laboratory in Lyon, France.

Clinical and laboratory informations were collected on chart review or provided by questionnaires sent to physicians. Clinicians in charge of PH1 patients who did not respond were personally approached by the researchers. Baseline characteristics, such as sex, ethnic origin, family history of urolithiasis or PH1, and consanguinity, were recorded, as well as clinical presentation and renal outcome (urolithiasis, nephrocalcinosis, dialysis, and Tx). Data regarding conservative treatment were not systematically recorded. Extra-renal symptoms were not systematically collected but spontaneously reported by clinicians. Laboratory data included plasma creatinine levels, *AGXT* mutations, and AGT enzyme activity. Renal function was estimated by the Schwartz formula in children and the Cockcroft formula in adults.[31,32] CKD was defined as a glomerular filtration rate <60 ml/min per 1.73 m^2, that is, Kidney Disease Outcome Quality Initiative stage 3 and 4 CKD.[33] Infantile PH1 was defined as PH1 becoming symptomatic before the age of 1 year.

DNA analysis

DNA of the patients was extracted from whole blood using conventional methods. According to French law, DNA samples were obtained with an informed consent for genetic analysis. The 11 exons and exon–intron boundaries of the *AGXT* gene were amplified from genomic DNA using standard PCR procedures with in-house-designed primers. Direct bidirectional sequencing of purified PCR products was performed using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3730 genetic analyzer. Sequences were analyzed using Seqscape Analysis Software (Applied Biosystems) in comparison with GeneBank Reference genomic sequence (NT_005416).

The mutation nomenclature used in this work follows the guidelines of Human Genome Variation Society (http://www.hgvs.org) and the DNA mutation numbering is based on the cDNA reference sequence (GeneBank accession number NM_000030.2). Each novel missense sequence alteration was screened in 100 control alleles. For the patients with suspicion of large deletion, real-time quantitative PCR was performed using Light Cycler technology (Roche Applied Science, Manheim, Germany).

Statistical analysis

Results were expressed as median and interquartile range or mean $\pm$ s.d. for continuous variables, and as percentage for categorical variables. Patient and renal survival from birth were estimated using a time-to-failure Kaplan–Meier method. The end point for renal survival was defined as start of dialysis or first renal Tx. Data were censored at the time of death due to ESRD or last available follow-up. Factors associated with patient and renal survival were estimated by univariate and multivariate analyses using Cox's proportional hazard model. The age at diagnosis was considered to be that of initiation of specific management even if enzymatic or genetic confirmation was performed later. Log-rank test was used for comparison between subgroups. A P-value <0.05

was considered statistically significant. All statistical analyses were carried out using SAS software (version 8.2; SAS Institute, Cary, NC, USA).

DISCLOSURE

All the authors declared no competing interests.

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