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Primary hyperoxaluria: report of an Italian family with clear sex conditioned penetrance

G. Mandrile · A. Robbiano · D. F. Giachino · R. Sebastiano · E. Dondi · R. Fenoglio · P. Stratta · M. R. Caruso · M. Petrarulo · M. Marangella · M. De Marchi

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Abstract We report the clinical and genetic study of a primary hyperoxaluria type I (PH1) family with two sisters homozygous for p.Gly170Arg who are still asymptomatic at age 29 and 35, and two brothers, also homozygous for the same mutation, who are affected since age 27 and 30. The clear sex difference observed in this family and in others reported in the literature fits well with the prevalence of males over females in the Italian registry. In the KO model of PH1, only male mice develop renal stones, suggesting that the sex difference may affect both oxalate production and stone formation. A likely mechanism is the sex-related expression of glycolate oxidase shown in experimental animals. The stable isotope method recently developed by Huidekoper and van Woerden for in vivo assessment of the endogenous oxalate production could help to clarify the issue in humans.

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G. Mandrile · A. Robbiano · D. F. Giachino · R. Sebastiano · M. De Marchi (✉)
Departement of Clinical and Biological Sciences,
Medical Genetics Unit, S. Luigi Hospital, University of Torino,
Regione Gonzole 10, 10043 Orbassano (TO), Italy
e-mail: genmed-dscb@unito.it

E. Dondi · R. Fenoglio · P. Stratta
Nephrology Unit, Amedeo Avogadro University Medical School,
Novara, Italy

M. R. Caruso
Nephrology Unit, Ospedali Riuniti di Bergamo, Bergamo, Italy

M. Petrarulo · M. Marangella
Nephrology Unit, Umberto I-Mauriziano Hospital, Turin, Italy

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Introduction

Primary hyperoxaluria type I (PHI) is a rare autosomal recessive disease with impaired hepatic detoxification of glyoxylate, caused by deficiency of alanine:glyoxylate aminotransferase (AGT) [1]. The continuous conversion of glyoxylate to oxalate, catalysed by glycolate oxidase (in peroxisomes) and lactate dehydrogenase (in the cytosol) is not countered by the deficient glyoxylate detoxification. This leads to Ca-oxalate nephrolithiasis, end-stage renal failure and systemic oxalosis. The AGT cofactor—vitamin B6—can stabilize and rescue some of the mutated forms of AGT, notably the recurrent p.Gly170Arg mutation. Early diagnosis and genetic testing allow to predict vitamin B6 responsiveness and address responsive subjects to a conservative treatment schedule, including liquid and vitamin B6 supplementation. In contrast, liver transplant, or combined liver/kidney transplant, is performed to correct the metabolic defect in many patients, in particular those diagnosed to be non-VB6 responsive on the basis of genotypic or phenotypic assessment.

Objectives

To describe the clinical variability of PHI in a family with two sisters homozygous for p.Gly170Arg who are still asymptomatic at age 29 and 35, and two brothers, also homozygous for the same mutation, who are affected since age 27 and 30.

Patients and methods

The family pedigree and the relevant clinical data are shown in Fig. 1 and Table 1. The proband (III-3), a 33-year-old male from Albania, first experienced a renal colic at age 30, with expulsion of a Ca-oxalate stone. Urinary oxalate (UOX): 148 µmol/mmol creat (normal range: 12–55) [2]. He started vitamin B6 (600 mg/die) and K+/Mg++ supplementation, with a good response (UOX after one month: 55 µmol/mmol creat).

Family history

The proband (III-3) has two healthy children (IV-4 and IV-5), three healthy sisters (III-1, III-2, III-4) and a younger brother (III-5) who was affected by recurrent renal stones and died at age 27 for complications of pielectomy performed to remove a large cast stone of the left kidney. An episode of renal stones is reported in the proband's father (II-1). No consanguinity among parents is recorded, but both were born in the same region of Albania.

Genetic testing

Genomic DNA was extracted from whole blood by using the Maxwell®16 instrument (Promega). Exon 4 of the *AGXT* gene was amplified from 20 ng DNA in 30 µl PCR reaction containing: 0.5 pM primers (fw: ccctgctacctg-gagctgt; rev: atagcctggctctgagctgt), 0.2 mM dNTPs (Invitrogen), 1.5 mM $MgCl_2$, 1 U AmpliTaq Gold (Applied Biosystem) with the following conditions: 5 min at 94°C, followed by 38 cycles of 30 s denaturation at 94°C, 30 s annealing at 62°C, 30 s elongation at 72°C, and a final elongation of 7 min at 72°C. The PCR product was purified with MicroSpinTM Columns (GE Healthcare) and sequenced with the fw primer by the MWG Operon service (Eurofins MWG Operon).

Results

Homozygosity for the common p.Gly170Arg mutation was found in the proband's *AGXT* gene. Testing of relatives revealed—as expected—heterozygosity for the mutation in parents (II-1 and II-2) and the children (IV-4 and IV-5). Among the sisters, III-1 was heterozygous, while III-2 and III-4 surprisingly proved to be p.Gly170Arg homozygous despite the absence of any clinical symptom of hyperoxaluria. Urinanalysis of 24 h revealed: 0.42 mmol of oxalate in III-4 and 0.88 mmol of oxalate in III-2 (normal range: 0.1–0.55). Treatment with vitamin B6 and K+/Mg++ was prescribed to both sisters. Only III-4 was compliant, and indeed she showed to be responsive (0.36 mmol after 1 month of therapy).

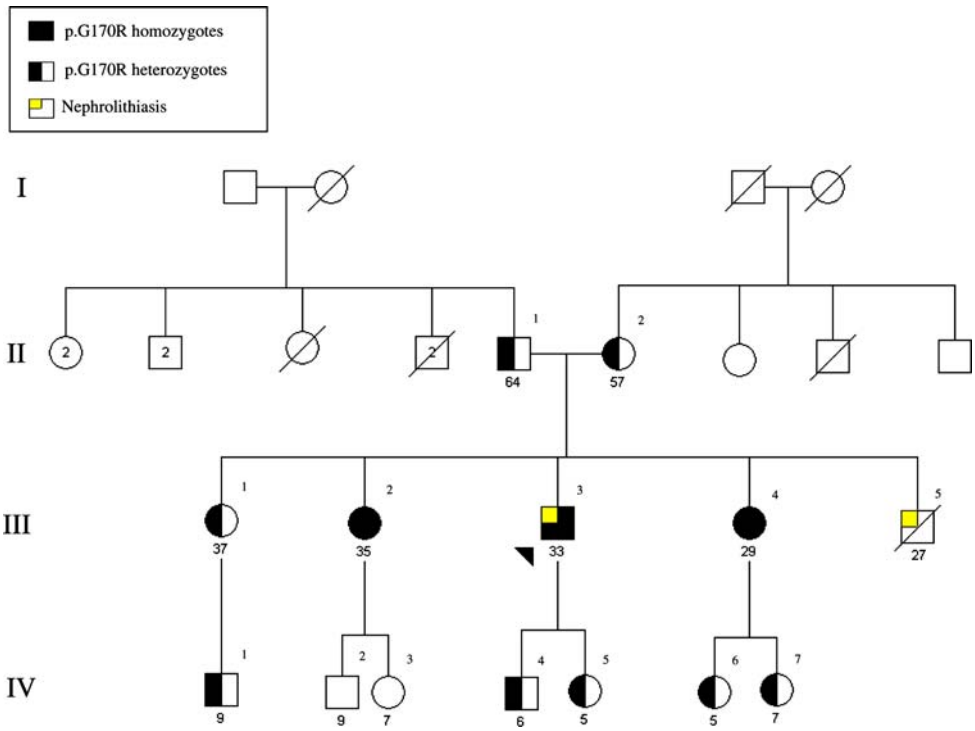

Fig. 1 Pedigree of the family. *Squares* indicate male and *circles* female members. Present age is indicated under the individual's *symbol*

Table 1 Clinical data

Individual	Present age	Symptoms at onset (age)	Symptoms at diagnosis (age)	Urinary findings at diagnosis				Urinary findings after one month VB6				
				UOx/crea (μmol/mmol)	UOx 24 h (mmol)	Ucitrate/crea 24 h (mmol/mmol)	Citrate 24 h (mmol)	UOx/crea (μmol/mmol)	UOx 24 h (mmol)	Ucitrate/crea 24 h (mmol/mmol)	Citrate 24 h (mmol)
				Normal range: 12–55	Normal range: 0.10–0.55	Normal range: 0.10–0.50	Normal range: 1–6.5	Normal range: 12–55	Normal range: 0.10–0.55	Normal range: 0.10–0.50	Normal range: 1–6.5
III-2	35	No symptoms	No symptoms (34)	NA	0.88	NA	2.15	NA	NA	NA	NA
III-3	33	Nephrolithiasis (30)	Nephrolithiasis (31)	148	2.12	0.00	0.03	55	1.06	0.04	0.09
III-4	29	No symptoms	No symptoms (28)	59	0.42	0.19	1.36	39	0.36	0.23	2.17
III-5	27	Nephrolithiasis (<27)	NA	NA	NA	NA	NA	NA	NA	NA	NA

Discussion and conclusion

A well known feature of PH1 is an extensive clinical variability, that is particularly wide for p.Gly170Arg, the most common mutation in different populations [1]. Indeed, it is associated with a vitamin B6 responsiveness and a clinically milder phenotype, later age of onset, and preserved renal function until adult age. However, a range of severity is reported in the literature among unrelated patients with the homozygous p.Gly170Arg genotype, from onset at 0.5 years to still functional at 57 years (e.g. [3, 4]). This variable expressivity might be ascribed to both different environmental factors, such as concomitant enteric or renal infections, different fluid intake and dietary vitamin B6, as well as to genetic modifiers, reviewed in [1]. The clear sex difference observed in this family and in that previously reported by Hoppe et al. [5] fits well with the prevalence of males over females in our Italian registry, 36 M/16F (sex ratio 2.25 on overall) of whom 7 M/2 F are p.Gly170Arg homozygotes (sex ratio 3.5, n.s.). Investigating the complex interplay of genetic and non genetic factors requires detailed clinical and laboratory investigations of both affected and healthy relatives, that are available only in few families. Hoppe et al. [5] described a family with two p.Gly170Arg homozygotes: a very mildly affected girl and a boy with a severe disease. Several families with related pairs, trios and a quintuplet of homozygotes were reported by Frishberg et al. [6]. Notably, in the latter family the clinical severity was not associated with the male sex (three male and one female showed a prenatal or infantile disease, while the fifth brother was asymptomatic at 20 years), possibly because they were homozygous for c.33dupC, a recurrent frameshift mutation. This class of mutations entirely precludes AGT biosyntesis, while p.Gly170Arg results in the production of still functional enzyme, that is diverted from peroxisomes and mistargeted to mitochondria [7]. Thus, the resulting phenotype will depend on a number of events, some affecting the amount of functional enzyme—such as the kinetics of AGT dimerization [8], the activity of cellular chaperones, the proteasomal degradation—and some the level of metabolic requirement. It is not clear at which level the mechanism(s) of the sex-limited or sex-related expression of PH1 are acting. The biochemical findings in the present family, indeed, showing borderline oxalate levels in the one sisters and higher levels in the other (Table 1) do not clarify whether the relative protection of females should be ascribed to a higher enzyme AGT level, lower substrate challenge, or to higher Ca-oxalate solubility in the urine.

Sex-conditioned difference of idiopathic renal stones has been reported in epidemiological studies [9]. In the KO mouse model of PHI, Salido et al. [10] found increased levels of urine oxalate in both sexes (higher in males), but

renal stones only in males, suggesting that the sex difference may affect both oxalate production and stone formation in the urinary tract. The different sex-specific expression of glycolate oxidase (GO) represent a likely mechanism. Interestingly, a higher expression of GO was found in male rats, that was ascribed sex hormones [11]. Recently, Yang et al. [12] found by microarray analysis of rat liver tissues an higher basal expression in males of the GO gene (ratio M/F = 1.42) and a lower *AGXT* gene expression (ratio M/F = 0.72). It deserves to be investigated whether also in humans these metabolic differences hold true. To better substantiate the question, the stable isotope method recently developed by Huidekoper, van Woerden et al. [13] for in vivo assessment of the endogenous oxalate production could be deserved.

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