Pediatr Nephrol (2002) 17:896–898
DOI 10.1007/s00467-002-0977-0

ORIGINAL ARTICLE

Danko Milosevic · Choni Rinat · Danica Batinic
Yaacov Frishberg

Genetic analysis – a diagnostic tool for primary hyperoxaluria type I

Received: 26 February 2002 / Revised: 23 July 2002 / Accepted: 24 July 2002 / Published online: 9 October 2002

Abstract Primary hyperoxaluria type I is an autosomal recessive metabolic disease in which excessive oxalates are formed by the liver and excreted by the kidneys, causing a wide spectrum of disease, ranging from renal failure in infancy to mere renal stones in late adulthood. The diagnosis may be suspected when clinical signs and increased urinary oxalate and glycolate excretion present, and is confirmed by the measurement of decreased alanine:glyoxylate aminotransferase activity in a liver sample. The enzymatic assay is not readily available to pediatric nephrologists in many parts of the world. We describe three families from Croatia in whom the diagnosis of primary hyperoxaluria was solely based on clinical findings that included nephrolithiasis and nephrocalcinosis accompanied by increased urinary oxalates and glycolate excretion, as enzymatic assays of liver samples could not be performed. Mutation analysis of the *AGXT* gene encoding the defective enzyme confirmed the diagnosis, revealing three alleles carrying the *C156ins* mutation and two the *G630A* mutation. Screening first-degree relatives for the relevant mutation disclosed an asymptomatic affected sibling. Mutation analysis of the *AGXT* gene is a non-invasive and accurate tool for the diagnosis of type I primary hyperoxaluria that may replace enzymatic assays of liver biopsies.

Keywords Primary hyperoxaluria type I · Mutation analysis · Alanine:glyoxylate aminotransferase · Liver biopsy

D. Milosevic · D. Batinic
Department of Nephrology, Children's University Hospital, Zagreb, Croatia

C. Rinat · Y. Frishberg (✉)
Division of Pediatric Nephrology, Shaare Zedek Medical Center and Hadassah-Hebrew University School of Medicine, Jerusalem, Israel
e-mail: yaacov@md.huji.ac.il
Tel.: +972-2-6666144, Fax: +972-2-6555689

Y. Frishberg
Division of Pediatric Nephrology, Shaare Zedek Medical Center, PO Box 3235, Jerusalem 91031, Israel

Introduction

Primary hyperoxaluria type 1 (PH1) is a rare autosomal recessive inborn error of peroxisomal metabolism [1]. It is caused by deficiency of the liver-specific enzyme alanine:glyoxylate aminotransferase (AGT), leading to excessive production of oxalates that are excreted by the kidneys. The elevated urinary oxalate concentration results in formation of calcium-oxalate crystals and subsequently renal stones, nephrocalcinosis, and in severe cases end-stage renal disease (ESRD) and systemic oxalosis, sometimes during early infancy [2]. The ultimate goal in treating a child with advanced renal failure is a combined liver and kidney transplantation, and therefore an early and accurate diagnosis is vital. The diagnosis of PH1 is based on clinical signs, but must be substantiated by increased urinary oxalate and glycolate excretion. This biochemical analysis is not routinely performed in many countries, including Croatia. Confirmation of the diagnosis requires the measurement of AGT enzymatic activity in liver tissue. This assay is currently performed in a few highly specialized laboratories and is therefore not readily available to many pediatric nephrologists around the world. Shipment of a frozen liver sample abroad is cumbersome and expensive, whereas a blood sample can easily be sent at no additional costs.

The AGT enzyme is encoded by a single copy gene (*AGXT*), which consists of 11 exons and spans a 10-kb DNA segment in the 2q37.3 region [3]. So far, seven polymorphisms and 35 disease-causing mutations have been identified in the *AGXT* gene [4].

The aim of this study was to confirm the diagnosis of PH1 in three index cases from Croatia, where neither biochemical analysis nor enzymatic assays are available, using mutation screening of the *AGXT* gene. Identification of a disease-causing mutation could provide an accurate tool for diagnosing PH1 and obviate the need for liver biopsy. It would also enable prenatal diagnosis in families at risk [5] and detection of presymptomatic individuals for timely medical management.

Table 1 Clinical characteristics of Croatian children with type 1 primary hyperoxaluria (*ESRD* end-stage renal disease, *Tx* transplant, *Lith* nephrolithiasis, *calc* nephrocalcinosis, *Ret* retinal involvement, *HD* hemodialysis)

Family	Ethnicity	Age at onset	Renal involvement	ESRD	Extrarenal disease	Outcome
H	Albanian	3 years	Lith+calc	No	None	Normal renal function
Z	Croatian	3 years 8 months	Lith+calc	10 years 3 months	None	Combined Tx
S	Croatian	3 years	Lith+calc	25 years	Ret	HD

Patients and methods

Patients

The study included three unrelated Caucasian families from Croatia; two native and one of Albanian ancestry. There was one known affected individual in each family, and they all presented with renal stones before the age of 4 years (Table 1). Two patients reached ESRD at the ages of 10 and 25 years, respectively. The diagnosis of PH1 was suggested based on clinical signs, and further substantiated by measuring increased urinary oxalate and glycolate excretion. Urine biochemical analysis had to be performed abroad (University Children's Hospital, Zurich, Switzerland), as it was unavailable in Croatia. Liver biopsies for the measurement of AGT enzymatic activity were not performed.

Genetic analysis

Blood samples from all affected individuals and their first-degree relatives (parents and siblings) were collected. Genomic DNA was extracted from lymphocytes using standard molecular biology techniques. Polymerase chain reaction (PCR) was used to amplify individual exons using primers based on published information regarding intron-exon boundaries [6]. Exon-specific PCR products were separated on agarose gel followed by extraction and purification (QIAgen, Hilden, Germany). Direct cycle sequencing of the entire coding region in each affected individual using ^{32}P-γATP-labeled primers was performed (Sequitherm Excell DNA, sequencing kit; Epicentre Technologies, Madison, Wis., USA).

Results

The three pedigrees are depicted in Fig. 1. Two affected individuals (H and Z) were homozygous for a mutation (one insertion and one missense) and the third was a compound heterozygote. Two patients from the same ethnic background carry the same mutation.

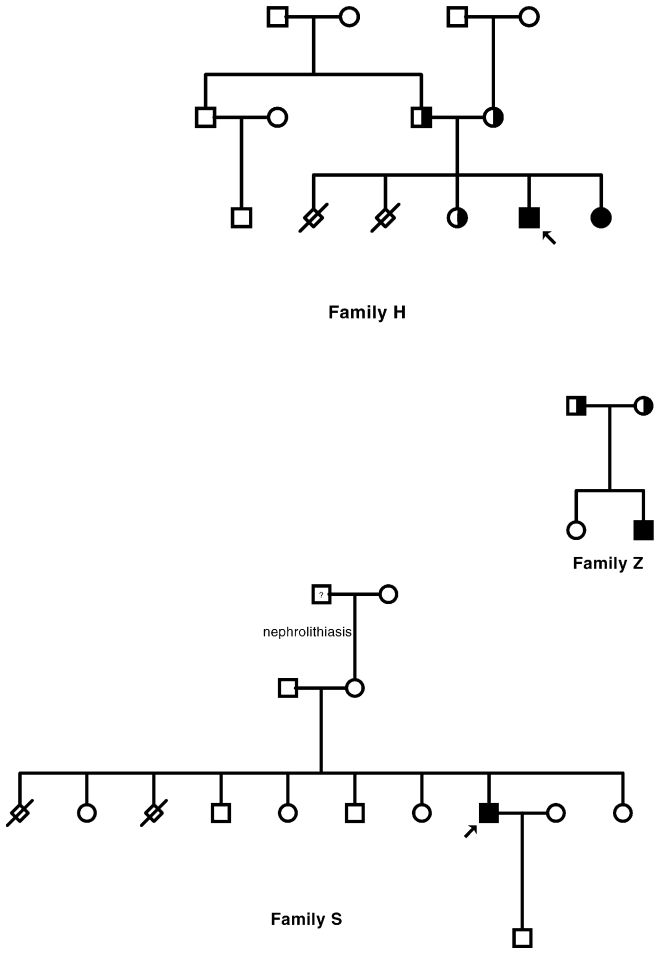

Fig. 1 Pedigrees of kindreds of Croatian origin with type 1 primary hyperoxaluria

Family Z

The proband is a native Croatian and the first patient to be diagnosed with PH1 in this country. He presented at the age of 3 years 8 months with nephrolithiasis and nephrocalcinosis. Biochemical work-up performed abroad revealed increased urinary oxalate and glycolate excretion [2.12 and 1.74 mmol/day per 1.73 m^2, respectively (normal 0.46 mmol/day per 1.73 m^2 and 0.19±0.07 mmol/day per 1.73 m^2, respectively)]. Despite treatment with pyridoxine and citrate he reached ESRD at the age of 10 years. Following a brief period on continuous ambulatory peritoneal dialysis he underwent a successful combined liver and kidney transplantation. He was found to be homozygous for the *C156ins* mutation that results in a frame shift and subsequently termination at codon 167. His mother and sister are heterozygotes for this mutation.

Family S

PH1 in the proband was diagnosed based on a medical history that was significant for renal stones and nephrocalcinosis first detected at the age of 3 years. There was increased urinary excretion of oxalate and glycolate (1.38 mmol/1.73m^2 per day and 4.9 mmol/day, respec-

tively). He is a native Croatian and his family history was remarkable for a maternal grandfather with nephrolithiasis who had died and was therefore unavailable for genetic analysis. The patient reached ESRD at the age of 25 years, and has been maintained on chronic hemodialysis awaiting a combined kidney and liver transplantation. He was found to be heterozygous for the *C156ins* mutation. The second mutation has yet to be determined.

Family H

The proband is from a non-consanguineous Albanian family who presented at the age of 3 years with nephrolithiasis and nephrocalcinosis. His maternal grandfather died in ESRD and had had recurrent nephrolithiasis of undetermined cause. He had elevated urinary oxalate and glycolate excretion (1.53 and 0.81 mmol/day per 1.73 m^2) with preserved renal function. Mutation screening revealed homozygosity for the *G630A* missense mutation, leading to the substitution of methionine for isoleucine at position 170. Screening of his first-degree relatives demonstrated that both parents and one sibling are heterozygotes, whereas his asymptomatic younger sister is homozygous for the same mutation. She was referred for a biochemical work-up but the family deferred performing these studies.

Discussion

We have identified two mutations in the *AGXT* gene in three children from unrelated families from Croatia. They all presented early in life with nephrolithiasis and nephrocalcinosis, and the diagnosis of PH1 was suspected based on their increased urinary oxalate and glycolate excretion.

The *C156ins* mutation has been previously described in Italian patients, comprising a gene frequency of 0.13 [4]. The prevalent *G630A* mutation (leading to Gly170Arg) associated with the minor allele has been shown to result in mistargeting of the AGT from the peroxisome to the mitochondria [7, 8]. This was found to be the most-frequent mutation carried by Italian PH1 patients, comprising 23.9% of all chromosomes tested. The two disease-causing mutations among the Croatian children are the two most common mutations responsible for PH1 among the Italian population. Croatia is a point of convergence of very different cultures and civilizations. The Roman Emperor Theodosius laid the border between the western and eastern Roman Empire in 395 A.D. across the Croatian territory. In later times there were additional links between the two populations. It is therefore possible that the affected individuals stem from common ancestors, a phenomenon referred to as the founder effect.

Measurement of urinary oxalate concentration presents several technical pitfalls, and much of the published information is of dubious value because of the lack of specificity of the methods used [9]. Furthermore, as urinary oxalates excretion decreases with declining renal function, the interpretation of biochemical analyses in patients suspected of having PH1 may be inaccurate. Only biochemical laboratories with the appropriate expertise and financial resources should routinely perform these studies. Many medical institutions like ours cannot undertake these assays and they are shipped abroad. The enzymatic tests of liver tissue are even more sophisticated and, in addition to being invasive, are unavailable to many pediatric nephrologists around the world. Performing genetic analysis in patients suspected of having PH1 might safely replace a liver biopsy. It also enabled us to screen first-degree relatives and to diagnose PH1 in an asymptomatic sibling. Given the fact that there seems to be a founder effect, screening individuals suspected to have PH1 is relatively simple and inexpensive, since performing sequencing of the entire *AGXT* gene is unnecessary. After diagnosis, early medical management could then be instituted, aimed at preventing the appearance of clinical signs and hopefully the gradual decline in renal function.

References

1. Watts RWE (1994) Primary hyperoxaluria type I. Q J Med 87:593–600
2. Cochat P, Koch Nogueira PC, Mahmoud A, Jamieson NV, Scheinman JI, Rolland MO (1999) Primary hyperoxaluria in infants: medical, ethical and economic issues. J Pediatr 135:746–750
3. Purdue PE, Lumb MJ, Fox M, Griffo G, Hamon-Benais C, Povey S, Danpure CJ (1991) Characterization and chromosomal mapping of a genomic clone encoding human alanine:glyoxylate aminotransferase. Genomics 10:34–42
4. Amoroso A, Pirulli D, Florian F, Puzzer D, Boniotto M, Crovella S, Zezlina S, Spano A, Mazzola G, Savoldi S, Ferrettini C, Berutti S, Petrarulo M, Marangella M (2001) *AGXT* gene mutations and their influence on clinical heterogeneity of type 1 primary hyperoxaluria. J Am Soc Nephrol 12:2072–2079
5. Danpure CJ, Rumsby G (1996) Strategies for prenatal diagnosis of primary hyperoxaluria type 1. Prenat Diagn 16:587–598
6. Rinat C, Wanders RJA, Drukker A, Halle D, Frishberg Y (1999) Primary hyperoxaluria type I: a model for multiple mutations in a monogenic disease within a distinct ethnic group. J Am Soc Nephrol 10:2352–2358
7. Purdue PE, Allsop J, Isaya G, Rosenberg LE, Danpure CJ (1991) Mistargeting of peroxisomal L-alanine:glyoxylate aminotransferase to mitochondria in primary hyperoxaluria patients depends upon activation of a cryptic mitochondrial targeting sequence by a point mutation. Proc Natl Acad Sci USA 88:10900–10904
8. Danpure CJ (1993) Primary hyperoxaluria type 1 and peroxisome-to- mitochondrion mistargeting of alanine:glyoxylate aminotransferase. Biochimie 75:309–315
9. Barratt TM, Danpure CJ (1999) Hyperoxaluria. In: Barratt TM, Avner ED, Harmon WE (eds) Pediatric nephrology. Lippincott Williams and Wilkins, Baltimore, pp 609–619