

Case Report

Primary hyperoxaluria type 1: a case report in a 7-month-old Chinese infant under biopsy and light microscope diagnosis

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Abstract: Primary hyperoxaluria type one (PH1) is a rare inborn autosomal recessive monogenic metabolic disorder due to hepatic alanine-glyoxylate-aminotransferase deficiency which results in excessive synthesis and urinary excretion of oxalate, inducing renal stone formation and deposition of calcium oxalate stones throughout the entire body. We present the clinical consequences in a 7-month-old male infant diagnosed with PH1. The patient was diagnosed by renal biopsy, which showed acute tubular injury with diffuse calcium oxalate crystals. The results of genomic DNA of AGXT gene were examined; the gene of the patient was similar to his mother, but different from his father and a healthy control. Mutation analysis of AGXT in the patient revealed the mutation, c.814T>GA, which was considered to be a silent mutation. During 22 days of hospitalization, supplementary pyridoxine was administered intravenously at 50 mg per day for 3 days, and then orally at 60 mg three times daily. Meanwhile the patient was treated with peritoneal dialysis and after 12 months of follow-up, he unfortunately died of refractory bacterial pneumonia.

Keywords: Primary hyperoxaluria type 1, alanine-glyoxylate-aminotransferase (AGT), oxalate, systemic oxalosis (SO)

Introduction

Human alanine glyoxylate aminotransferase (AGT; EC2.6.1.44), localized to liver peroxisomes, has a broad substrate specificity that uses glyoxylate as the preferred amino acceptor [1]. AGT catalyzes the formation of glycine and prevents the formation of oxalate from glyoxylate. In humans, hereditary deficiency of AGT results in rare clinical consequences in which excessive amounts of glyoxylate are converted to oxalate. This disorder, known as primary hyperoxaluria type 1 (PH1; MIM259900), is characterized by hyperoxalemia and hyperoxaluria with massive calcium oxalate deposition in the kidney, bone, myocardium, vessels, and throughout the entire body [2].

PH1 is a rare autosomal-recessive disorder caused by a deficiency of the liver-specific pyridoxal-phosphate dependent enzyme AGT. The

AGT gene (AGXT) mutation that underlies this disorder has been reported [3], including studies of PH1-specific mutations (G41R, F152I, G170R, and I244T) in European and North American patients [4], as well as a PH1-specific mutation (S205P) in Japanese patients [5].

Our study investigated the genetic profile of both a 7-month-old infant with PH1 and his parents to understand how this disease gene was inherited in this family.

Case report

A 7-month-old boy with intermittent diarrhea for four months was admitted to our hospital because of increased abdominal pain and watery diarrhea 7-8 times a day for 5 days, complicated with edema and oliguria. He was born after a normal pregnancy at full-term delivery with a birth weight of 3.45 kg and an uncom-

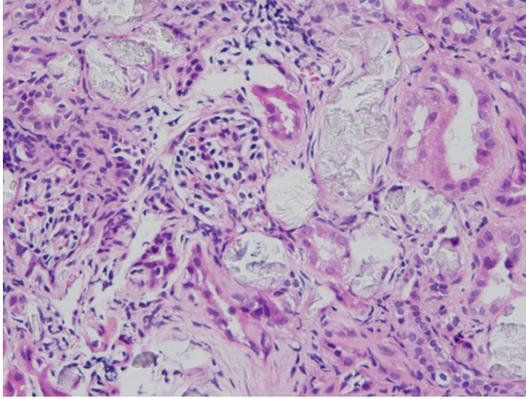


Figure 1. Renal biopsy showed acute tubular necrosis with diffuse calcium oxalate crystals (400×). The glomeruli were almost normal, but the renal tubular epithelial cell disruption was tripped. Crystals could be seen within the lumen of urinary cast. Extensively lymphocytic infiltrating foci in the renal interstitium were frequently found.

plicated neonatal course. The family history was negative with respect to renal and metabolic disease. The infant was well nourished on mixed feeding with normal growth and development.

Five months before admission, the infant had diarrhea about 5-6 times per day with unformed stools. Then he was given probiotic treatment in the hospital, but symptoms did not improve. His parents did not continue any medical treatment or investigate further examination until the infant had decreased urine output to about 100 ml per day, with facial edema 4 days before admission.

The laboratory blood examination in our hospital found Hb 55 g/L, WBC 8.67×10^9 /L, PLT 253×10^9 /L, Serum Potassium 2.93 mmol/L, Sodium 116 mmol/L, Calcium 1.85 mmol/L, Phosphate 3.60 mmol/L, Parathyroid hormone 231 pg/mL, BUN 30.93 mmol/L, Cr 662 mmol/L, Uric acid 650 mmol/L. Urine routine: pH 6.5, SG 1.010, protein (-) - (+), WBC 1-3/HP. Blood gases: pH 7.30, HCO_3^- 17.8 mmol/L, BE-10.7 mmol/L. Urine Calcium/Cr: 0.37. Ultrasound showed both kidneys were enlarged with severe parenchymal diffuse damage and enhanced echogenicity, with the left kidney sized at 7.3×3.7 cm and the right kidney sized at 7.5×3.8 cm. There were small pieces of microlith shaped stones on the left collective system of size 0.2×0.1 cm, without ureteral

dilatation. Renal biopsy was performed and the pathological results indicated normal glomeruli, but the renal tubular epithelial cells were disrupted. Large amounts of crystals could be seen within the lumen of the urinary cast. Infiltrating lymphocytes were observed in the renal interstitium (**Figure 1**). The treatment course was given without any complications.

Genome analysis

Genomic DNA of the infant and both his parents was extracted from peripheral blood samples using MagExtractor-Genome (TOYOBO, Tokyo, Japan) according to the manufacturer's instructions. The DNA encoding of AGXT gene was amplified as previously described [6]. The genomic DNA sequences were determined as follows: sense, 5'-GGGGAGAGAAAGGGGCACACAGAGT-3', and antisense, 5'-TGGGGCTVTAGT-TGGGGTTCTTGAG-3', using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA).

The molecular basis of PH1 was examined by analyzing the entire coding sequence of AGXT. Genetic mutation of GA heterozygous insertion was found in both the patient and his mother, but his father's DNA profile was normal. (Results were shown in **Figure 2**).

Treatment and follow up

The infant received anti-diarrheal treatment immediately after admission. Peritoneal dialysis (CAPD) was initiated on the second day after admission and treated for 22 days. Pyridoxine treatment was administered intravenously at 50 mg per day for 3 days, and then orally at 60 mg per time three times daily. The patient's infection was controlled with reduced symptoms of edema and diarrhea, and blood pressure was stably maintained in the 100-120/70-80 mm Hg. After 22 days, the patient was discharged, however the peritoneal dialysis was continued. Unfortunately, the infant died of refractory bacterial pneumonia 12 months later.

Discussion

Oxalate is a metabolic waste product excreted by the kidney. Hyperoxaluria is a common cause of kidney stones due to low solubility of

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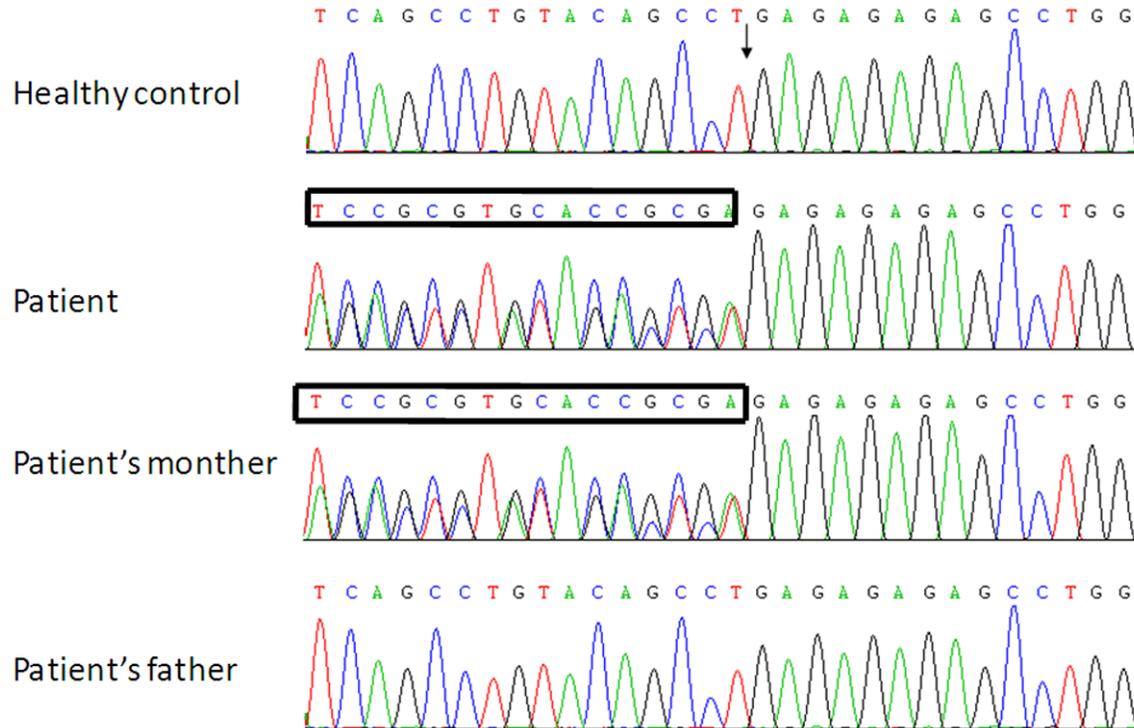


Figure 2. Genomic DNA analysis of AGXT gene. The molecular basis of PH1 was examined by analyzing the entire coding sequence of AGXT. The results of genomic DNA of AGXT gene are shown in the figure. The gene of patient was similar to his mother, but different from his father and a healthy control. Mutation analysis of the AGXT gene in the patient revealed mutation, c.814T>GA, which is considered to be a silent mutation.

calcium oxalate salts. Severe hyperoxaluria may lead to loss of renal function and systemic oxalosis. Hyperoxaluria can be caused by genetic defects that lead to endogenous overproduction or excessive oxalate absorption from the diet [7].

The primary hyperoxalurias are a group of rare hereditary calcium oxalate kidney stone diseases, the most prominent of which are primary hyperoxaluria type 1 (alanine/glyoxylate aminotransferase deficiency) and type 2 (glyoxylate/hydroxypyruvate reductase deficiency). Insoluble calcium oxalate crystallizes out in the kidney and urinary tract to cause kidney dysfunction and eventually complete organ failure. More than 100 mutations have been found in PH1, but less than 20 in PH2. Screening for the three most common mutations, c.33_34insC, c.508G>A, and c.731T>C, enables a molecular diagnosis in 34.5% cases [8].

The genomic DNA of AGXT gene was shown in **Figure 2**. Mutation analysis of the AGXT

revealed the mutation, c.814T>GA, which is considered to be a silent mutation. It is unknown whether the mother's gene will confer disease in future generations or not. Otherwise, the abnormal AGXT gene was not the contributing factor for this patient. The pathogenesis of PH1 still needs further investigation.

Since AGT is expressed predominantly in the liver, definitive diagnosis of the disease requires liver biopsy to assess AGT activity [9]. Unfortunately, we could not perform liver biopsy or exam AGT activity in our hospital.

The symptoms of the patient manifested as oliguria, mild leukocyturia, proteinuria, less microliths in the kidney, and severe renal failure. Because of the lack of certain examination facilities, we were unable to perform blood and urine oxalate tests, as well as the assessment of the AGT activity. Nonetheless, we performed renal biopsy and found diffuse crystals within the lumen of urinary cast, while the glomeruli were almost normal. These crystals were char-

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acterized as highly transparent, colorless, sub angular, and amorphous. Some of the crystals were hexagonal or had eight surfaces, and were positively birefringent on polarized light [10].

We found that the crystals were oxalate crystallization and the patient was diagnosed with oxalate nephropathy by medical history, clinical signs, laboratory tests, and the diagnostic crystals.

Since the patient's serum uric acidity was high, we should pay attention to urate nephropathy. However, there were two pieces of evidence that did not support this. First of all, the urate crystal was radiolytic and needle-like, which is different from the colorless, amorphous crystals of the infant. Secondly, the relatively high level of the patient's serum uric acidity was not significantly elevated, which may be proportionally due to the increase serum creatinine level.

Furthermore, by renal biopsy we found that another 10-month old infant had renal tubular melamine crystals, and this patient was complicated with oliguria, renal microliths, and progressive decline of renal function. But the crystals' characteristics of this case were different from the melamine crystals, which stained blue on the edge by HE stains [11]. On the basis of the renal pathology, considering the infant's early onset of only 7 months old, without any family history, and accompanied by end stage renal failure, we suggest the diagnosis is primary hyperoxaluria, and type 1 is the most likely type based on clinical signs and symptoms.

Oxalate is an end product of human metabolism, produced in the liver and excreted primarily by the kidney. Oxalate is also absorbed from the diet so that renal excretion reflects a combination of endogenous and exogenous oxalate loads. Since the child had chronic diarrhea, the hyperoxaluria could result from excess absorption from the intestinal tract, which was damaged by chronic diarrhea. After removing the allergen and relieving edema, the diarrhea improved, but the oliguria remained and aggravated renal function. This demonstrated that the hyperoxaluria might not be secondary to the diarrhea, which most likely was primary.

Oxalosis could affect many other organs as well, including bone, retina, vessel, myocardi-

um, nerve, and joint damage, which results in poor quality of life or even death [12]. However, no cardiovascular or skeletal complications were found in this case. We considered that the reason could be due to the limited duration of the disease. If the patient hadn't been treated, diverse clinical manifestations accompanied with multiple organ injury could appear after a couple of months.

To prevent exacerbating hyperoxaluria, the infant and his mother were instructed to avoid high oxalate foods, such as spinach, bran flakes, beets, potato chips, nuts, et al. The only pharmacologic intervention known to lower urine oxalate in PH is pyridoxine supplementation. Pyridoxine is a cofactor for AGT mediated conversion of glyoxylate to glycine. Approximately 25% to 30% of PH1 patients will reduce urine oxalate excretion significantly with pyridoxine [13]. The patient was given oral pyridoxine treatment but was not effective at the end, which suggested that the patient's disease was not caused by the deficiency of the pyridoxine.

The reported effective treatment is organ transplantation. Isolated kidney transplantation can reduce plasma oxalate levels, but disease recurrence often leads to poor graft survival. Liver transplantation completely corrects the enzyme defect. In Europe, with the poor results of isolated kidney transplantation reported 2 decades ago, combined or sequential liver-kidney transplantation has predominated thereafter [14]. We observed regretfully that there was no successful case with PH in children undergoing combined liver and kidney transplantation as reported in China to date.

Under conditions of limited testing for genetics, serum oxalate, urine oxalate, and AGT enzyme, we suggest that renal biopsy and light microscopic be performed together to confirm a diagnosis as soon as possible.

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Disclosure of conflict of interest

None.

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