Primary Hyperoxaluria Type 1 with a Novel Mutation

Sidharth Kumar Sethi, Hans R. Waterham¹, Sonika Sharma², Alok Sharma³, Pankaj Hari and Arvind Bagga

¹Divisions of Pediatric Nephrology and Genetics, ²Department of Pathology, All India Institute of Medical Sciences, New Delhi, ³Lab. Genetic Metabolic Diseases, Departments of Clinical Chemistry & Pediatrics, Academic Medical Centre, Amsterdam, The Netherlands

ABSTRACT

Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disorder caused by a deficiency of alanine-glyoxylate aminotransferase AGT, which is encoded by the AGXT gene. We report an Indian family with two affected siblings having a novel mutation in the AGXT gene inherited from the parents. The index case progressed to end stage renal disease at 5 months of age. His 4 month old sibling is presently under follow up with preserved renal function. [Indian J Pediatr 2009; 76(2): 215-217] Email : arvindbagga@hotmail.com.

Key words : Primary hyperoxaluria; Nephrocalcinosis

Type 1 primary hyperoxaluria (PH1; OMIM 259900) is a life-threatening condition due to inherited deficiency of a liver-specific enzyme (alanine:glyoxylate aminotransferase, AGT) that causes impaired glyoxylate metabolism in peroxisomes of human hepatocytes. This disorder has an autosomal recessive inheritance and is characterized by marked hyperoxaluria, calcium oxalate urolithiasis or nephrocalcinosis and progressive loss of renal function.¹ Currently, there are a total of 55 AGXT gene sequence variants reported in the Human Gene Mutation Database [http://www.hgmd.cf.ac.uk/L] of which were missense or nonsense changes.² We report an Indian family with two affected siblings secondary to a novel inherited mutation in AGXT gene. This is the first report of a genetic study in an Indian family with PH1.

CASE REPORTS

Case 1

A 5-month-old was referred for evaluation of failure to thrive and episodes of cough and fast breathing. The child was a product of fourth degree consanguineous marriage. The child was the first in birth order. On examination, he weighed 7.0 Kg, head circumference was 39.6 cm and length was 55.0 cm. The respiratory rate was 62/ minute, pulse rate 110/ minute and blood pressure 100/70 mm Hg. The patient was pale; chest examination showed wheeze and fine crackles. Investigations showed hemoglobin level of 5 g/dl; blood level of urea was 119 mg/dl, creatinine 4.6 mg/dl, sodium 142 mEq/l, potassium 5.4 mEq/l, calcium 10 mg/dl, phosphate 6.9 mg/dl, alkaline phosphatase 460 IU/l, pH 7.27, pCO₂ 19.8 mmHg and bicarbonate 11 mEq/l. Urinalysis was normal. Ultrasound of the abdomen revealed bilateral medullary nephrocalcinosis. Urinary oxalate excretion was 400 mg/1.73 m² per 24-hr (normal <40 mg/1.73 m²/24-hr). The child underwent peritoneal dialysis for metabolic acidosis, altered sensorium and uremia. Despite treatment, his condition deteriorated and he expired on day six of hospitalization because of worsening pneumonia. A post-mortem renal biopsy showed abundant oxalate crystals within the tubules (Fig. 1a), which had strong birefringence in polarized light (Fig. 1b). A diagnosis of PH1 was made in view of bilateral medullary nephrocalcinosis, raised urinary oxalate excretion and characteristic renal histology.

Case 2

Five years later, this 4-month-old girl, sibling of the first patient presented with progressive facial puffiness of 1 month duration. She was third in birth order; an elder 3-year-old male sibling was reportedly healthy. On examination, this patient weighed 5 Kg, head circumference was 38.6 cm and length was 60 cm; systemic examination was normal. Investigations showed hemoglobin level of 11 g/dl; blood level of urea was 16 mg/dl, creatinine 0.5 mg/dl, sodium 148 mEq/l,
potassium 4.8 mEq/l, calcium 9.3 mg/dl, phosphate 4.3 mg/dl, alkaline phosphatase 676 IU/l, pH 7.47 and bicarbonate 19.8 mEq/l. Abdominal ultrasonography showed bilateral medullary nephrocalcinosis. The 24-hr urinary oxalate was 242 mg/1.73 m² per 24-hr. The patient is presently on follow up with preserved renal function, and is receiving treatment with oral pyridoxine.

### Mutational analysis

Genomic DNA was extracted from peripheral blood of patient 2, her parents and the 3-yr-old live sibling. Gene sequencing for AGXT was done at the Academic Medical Centre, Amsterdam (Netherlands). Six sets of AGXT specific primers with -21M13 or M13rev extensions were used for the amplification of the entire AGXT coding region encoded by exon 1 to 11.3

Studies of the AGXT gene showed a novel missense mutation at exon 2, leading to substitution of cytosine in place of thymidine at nucleotide 302 (c.302T>C). At the amino acid level, this substitution led to formation of proline instead of leucine (L101P). The patient was homozygous for the mutation, and the parents and the unaffected sibling were carriers. Electrophoregrams of mutations in the family members and a healthy control are shown in fig. 2. The novel mutation detected was screened in 150 normal individuals and was absent in all of them.

### DISCUSSION

The primary hyperoxalurias are inborn errors of metabolism that result from specific hepatic enzyme deficiencies. In PH1, deficiency and/or mistargeting of hepatic alanine:glyoxylate aminotransferase results in metabolic overproduction of oxalate and glycolate. The excess oxalate is excreted in the urine but is of low solubility and precipitates as a calcium salt, resulting in urolithiasis, nephrocalcinosis and progressive renal insufficiency. The diagnosis of PH1 is based on the presence of nephrocalcinosis, characteristic histology on renal biopsy and elevated 24-hr urinary oxalate excretion.4 Urinary oxalate measurement may be misleading in renal insufficiency due to its decreased excretion in such cases.5 Confirmation of the diagnosis requires estimation of the enzyme, AGT on the liver biopsy, which should be transported urgently to an appropriate laboratory.4, 5

The clinical features of PH1 are variable. Some patients show symptoms in the neonatal period, which rapidly progress to renal failure during infancy. Most patients present in childhood and reach end stage renal failure between the second and fourth decades of life.6

PH1 is equally heterogeneous on the molecular level. There is no mutational hot spot, but various mutations have been found throughout the 11 exons of the AGXT gene. Fifty five mutations in the alanine:glyoxylate aminotransferase (AGXT) gene located on chromosome 2q37.3, resulting in subsequent absolute or functional enzyme deficiency, have been published. Hence, a molecular diagnosis using direct sequencing of the whole gene is recommended.3, 3 Of 55 AGXT sequence variants reported in the Human Gene Mutation Database to cause