Common mutation underlying primary hyperoxaluria type1 in three Indian children

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ABSTRACT

Primary hyperoxaluria is an autosomal recessive disorder caused by deficiency of alanine-glyoxylate aminotransferase, which is encoded by the *AGXT* gene. We report three Indian children with primary hyperoxaluria type1 having a common mutation in this gene. All patients had evidence of chronic kidney disease at the time of diagnosis, with subsequent progression to end-stage renal disease. The detection of an identical mutation in the *AGXT* gene suggests that specific genetic screening for this mutation may be useful when considering the diagnosis of primary hyperoxaluria type1.

Key words: *AGXT* gene, chronic kidney disease, nephrocalcinosis

Introduction

Primary hyperoxaluria type1 (PH1, OMIM 259900) is an autosomal recessive disorder of glyoxylate metabolism characterized by a functional defect of the liver-specific enzyme, alanine glyoxylate aminotransferase (AGT).[1] There is an increased urinary excretion of calcium oxalate, recurrent nephrolithiasis, nephrocalcinosis and accumulation of oxalate throughout the body. The gene encoding AGT, *AGXT*, is located at 2q37.3 and consists of 11 exons.[2]

Primary hyperoxaluria type1 is suspected in patients with oxalate deposition (nephrocalcinosis, nephrolithiasis) and elevated 24-h urinary or plasma oxalate. The diagnosis is often missed in patients with renal failure since urinary oxalate may not be elevated. Levels of plasma oxalate are elevated (>60 μmol/l) in renal insufficiency and are not specific for the disease.[3] Therefore, confirmation of diagnosis requires the demonstration of reduced AGT enzyme activity in liver biopsy or whole-gene sequencing of the *AGXT* gene in the clinical setting of PH1.[2]

We report three cases of PH1, from different geographical locations within the subcontinent, secondary to an identical homozygous mutation in the *AGXT* gene. This mutation has been reported previously from our centre in two siblings with PH1.[4]

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Case Reports

Case 1

An 18-month-old child hailing from Koth, West Punjab (Pakistan), was evaluated for failure to thrive, polyuria, and polydipsia which was noted 4 months back. The child was the first in birth order in a non-consanguineous marriage without significant family history.

At 7.3kg, the boy was undernourished. Stage II hypertension (blood pressure 120/70 mmHg), pallor, and rickets were noted. Investigations revealed the following: hemoglobin, 8.8 g/dl; blood urea, 174 mg/dl; creatinine, 2.1 mg/dl (estimated GFR 18ml/minute/1.73 m^2); sodium, 144 mEq/l; potassium, 5.2 mEq/l; calcium, 8.6 mg/dl; phosphorus, 9.7 mg/dl; alkaline phosphatase, 1684 IU/l; parathormone (PTH), 788 pg/ml, pH 7.39; bicarbonate 19 mEq/l; and normal urinalysis. Abdominal ultrasonography showed bilateral medullary

nephrocalcinosis. A diagnosis of PH1 was probably considered in view of progressive renal impairment associated with nephrocalcinosis.

Case 2

A 6-month-old girl was referred for management of renal dysfunction, detected during evaluation for acute gastroenteritis, pallor and anasarca. She was born at term gestation to parents hailing from Farukhabad, Uttar Pradesh (India). Following an uneventful perinatal course, failure to thrive and developmental delay were noted. Acute peritoneal dialysis was initiated in view of deranged renal functions (blood urea, 161 mg/dl; creatinine, 5.7 mg/dl), acidosis (pH 7.28, bicarbonate 17.5 mEq/l), and dyselectrolytemia (sodium 125 mEq/l; potassium 5.4 mEq/l). The hemoglobin was 7 g/dl; calcium, 7.4 mg/dl; phosphate, 7 mg/dl; alkaline phosphatase, 129 IU/l; PTH, 563 pg/ml; and urinalysis was normal. Ultrasound suggested increased echogenicity of medullary papillae. Renal biopsy, undertaken in view of persistent azotemia, revealed that proximal and distal tubules were filled with hexagonal crystals; additional findings included tubular dilatation and atrophy, interstitial fibrosis, and periglomerular fibrosis.

Case 3

A 6-year-old girl, resident of Pauri Garhwal, Uttarakhand (India), was evaluated for recurrent abdominal pain, seizures and hypertension. Past history was significant for left percutaneous nephrolithotomy when she was 5 years old. At evaluation, undernutrition (weight, 14.5 kg; height, 112 cm) and hypertension (blood pressure 113/82 mmHg) were noted. Evaluation revealed renal dysfunction (blood urea 102 mg/dl; creatinine 5.49 mg/dl), hemoglobin 10.4 g/dl; sodium 138 mEq/l; potassium 3.4 mEq/l; calcium 8.6 mg/dl; phosphate 3.8 mg/dl; alkaline phosphatase 568 IU/l, pH 7.39; bicarbonate 21.9 mEq/l; and PTH 347 pg/ml. Ultrasonography demonstrated bilateral multiple renal pelvicalyceal stones. A diagnosis of PH1 was considered, prompting a mutational testing. As disease progressed, chronic ambulatory peritoneal dialysis was initiated.

The median urinary oxalate level in the three cases was 283.5 mg/1.73 m^2/day (normal, <40 mg/1.73 m^2/day).

Mutation analysis

Genomic DNA was extracted from peripheral blood of all three patients and from parents of case one. Sequencing for all eleven exons and flanking intronic sequences of the *AGXT* gene was performed at the Academic Medical Centre, Amsterdam (Netherlands) using six sets of *AGXT* specific primers with–21M13 or M13rev extensions.[5] All three patients were homozygous for an identical missense mutation at exon two which caused the substitution of thymidine with cytosine at nucleotide 302 (c.302T >C). Analysis on polymorphism phenotyping (PolyPhen, http://genetics.bwh.harvard.edu/pph) predicted that the locus was highly conserved and the mutation was probably damaging.[6] Genetic testing of parents of case one showed heterozygous carrier state in both parents.

Outcome

All patients had markedly low eGFR at the time of diagnosis, and subsequently were in need of dialysis. Parents of case one refused dialytic support and erythropoietin therapy when renal function deteriorated, and the boy died due to end stage renal disease (ESRD) five months after presentation. Similarly, case two succumbed to ESRD at home, six months after diagnosis. After three years on dialysis, case three is currently awaiting a combined liver-kidney transplant.

Discussion

Patients with primary hyperoxaluria type1 usually present in childhood, with progression to ESRD in 50% by the second to third decade of life. The diagnosis was considered in the present patients in view of early progressive renal impairment associated with nephrocalcinosis or nephrolithiasis. Symptoms at presentation were comparable with those described in previous cohorts.[7,8]

The observed prevalence of PH1 is clearly an underestimation given the phenotypic heterogeneity, non-specific initial symptoms, and problems with availability of definitive diagnostic tools. Though nephrolithiasis is common in Indian children, screening for hyperoxaluria is rarely performed, resulting in failure to diagnose the underlying metabolic condition. Estimation of urinary oxalate levels is unreliable in patients presenting with ESRD. Estimation of plasma oxalate is not feasible in most cases, and the levels are non-specifically elevated in patients with impaired renal excretion. As the demonstration of reduced AGT enzyme activity in liver biopsy specimen is the gold standard for confirmation of diagnosis, the investigation is not available and requires transportation of frozen biopsy specimens to laboratories outside the country.

Genetic evaluation for mutations in the *AGXT* gene is considered a specific test for diagnosing PH1. Over 146 pathogenic mutations have been reported.[9] 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.[10] These mutations, along with one third of the other documented mutations, are

located in exons 1, 4 and 7, suggesting that these exons may be hot spots for screening.[10] A recent report on 57 patients from Tunisia revealed that screening for I244T (exon 7) and 33_34insC (exon 1) accounted for 28.2% of mutations causing disease in their cohort.[11] However, since the mutational hotspots reported are few and population-specific, molecular diagnosis requires sequencing of the entire *AGXT* gene.

Sequencing of *AGXT* gene in the present cases revealed an identical missense homozygous mutation in exon two. This mutation leads to substitution of thymidine with cytosine at nucleotide 302 (c.302T > C), which is expected to form proline instead of leucine (Leu101Pro) on translation. The pathogenicity of this mutation has been previously described by Williams *et al.*[9] who demonstrated that the mutant *AGXT* gene yielded a protein having less than 1% of normal activity *in vitro*. The outcome of Polyphen, representing the differences in profile matrices between the allelic variants, calculated from position-specific independent counts scores, suggested that the mutation was probably pathogenic.[6] The same mutation was reported previously from our centre in two siblings.[4] The families of the present patients were unrelated, belonging to distinct regions in North India or Pakistan. However, suggestions of a founder effect, similar to reports from North Africa,[11] are conjectural until genetic testing of patients with PH1 in larger case series confirms that the mutation exclusively affects patients of Indian origin.

High incidence of early progression to renal insufficiency, potential for improvement with pyridoxine therapy in up to 60%, and the prognostic implications given the need for combined liver-kidney transplantation, justify the need for an early and definitive diagnosis in patients with primary hyperoxaluria type 1.[8] Genetic testing allows prenatal diagnosis if parents are confirmed heterozygous carriers of mutations in the *AGXT* gene, and is essential in patients with suspected primary hyperoxaluria type 1 planned for renal transplantation. Sequencing may also suggest prognosis, with certain mutations, such as p.Gly170Arg, being associated with a relatively favorable outcome.[9] Though limited to a small number of patients, our findings suggest the relevance of screening for a specific mutation in *AGXT* gene in Indian children when considering this condition, which is otherwise a genetically heterogeneous disease. Focused testing for this mutation in suspected individuals, potentially by a PCR-based test, is expected to be relatively inexpensive and practical when compared with genetic sequencing or enzymatic analysis in liver biopsy. However, the findings need to be examined in a large cohort of Indian patients with primary hyperoxaluria type 1.

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