Infantile Nephropathic Cystinosis: A Novel *CTNS* Mutation

İnfantil Nefropatik Sistinozis: CTNS Geninde Yeni Bir Mutasyon

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ABSTRACT

Cystinosis is a rare autosomal recessive metabolic disorder characterized by the accumulation of cystine in lysosomes, which results from defects in the carrier-mediated transport protein encoded by the *CTNS* gene. Infantile nephropathic cystinosis (INC) is one of the major complications of cystinosis. It is characterized by findings of Fanconi's syndrome within the first year of life. Here we report two patients with INC presenting with signs of Fanconi's syndrome and describe a novel *CTNS* mutation.

Keywords: Nephropathic cystinosis, genetic evaluation, children

ÖZ

Sistinozis, lizozomlarda sistin birikimi ile karakterize olan ve otozomal resesif kalıtılan nadir bir metabolik hastalık olup taşıyıcı proteini *CTNS* geni tarafından kodlanan bir bozukluktan kaynaklanır. İnfantil nefropatik sistinozis (İNS) sistinozisin ana komplikasyonlarından birisidir. Bu klinik durum yaşamın ilk yılında renal Fankoni sendromunun bulguları ile karakterizedir. Biz burada renal Fankoni sendromunun bulguları ile prezente olan ve İNS tanısı konulan iki hasta sunuyor ve *CTNS* geninde yeni bir mutasyon bildiriyoruz.

Anahtar Kelimeler: Nefropatik sistinozis, genetik değerlendirme, çocuklar

Introduction

Cystinosis is a rare autosomal recessive lysosomal storage disease that results from a defect in the cystine transporter protein cystinosin, which is encoded by the *CTNS* gene. This metabolic disorder causes abnormal accumulation of cystine in lysosomes [1]. Infantile nephropathic cystinosis (INC) is one of the major complications of the disease and is characterized by failure to thrive and clinical symptoms related to Fanconi's syndrome during the first year of life [2]. The *CTNS* gene, which consists of 12 exons, is located on chromosome 17p13.3. Over 100 mutations have been reported [2]. Here, we report two patients with INC together with their molecular genetic analysis and describe a new mutation on the *CTNS* gene.

Case Reports

Case 1

A boy aged 4 years 5 months was referred to our pediatric clinic with a history of polyuria, polydipsia, intermittent vomiting, and failure to thrive from 6 months of age.

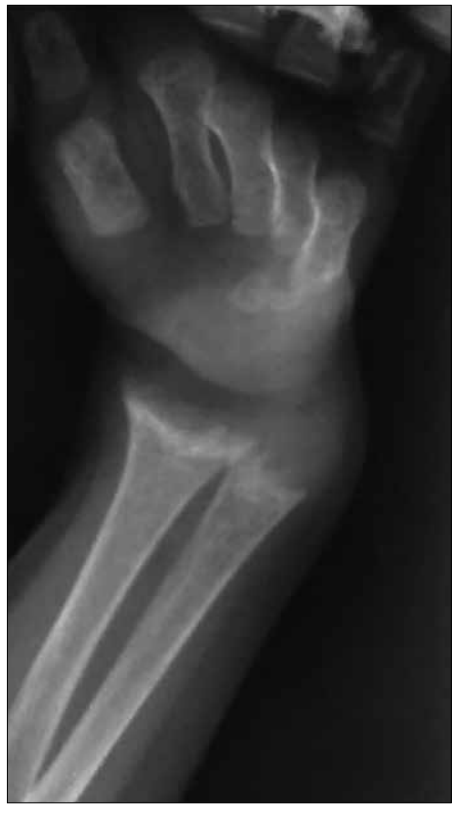

Figure 1. Rachitic changes on X-ray of the wrist.

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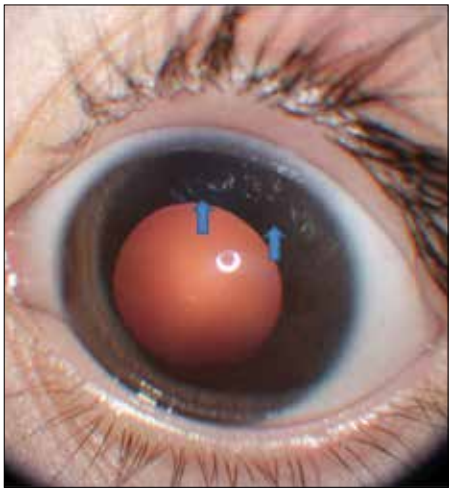

Figure 2. Cystine crystals in the cornea (arrows).

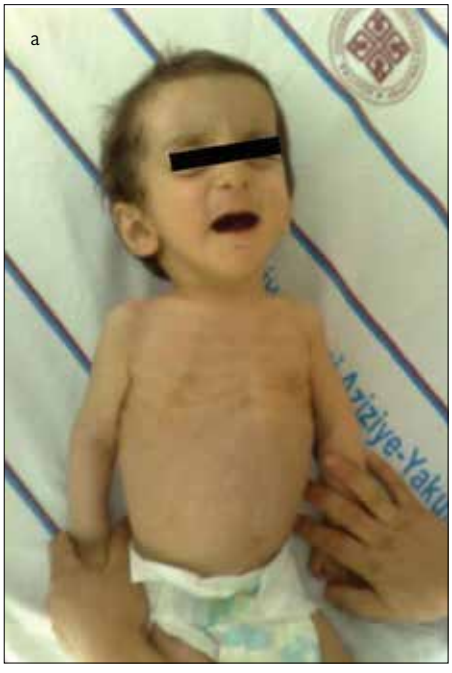

Figure 3. General appearance of case 2 (a) before and (b) after therapy.

Table 1. Hospitalization data of the two children with cystinosis

Parameters	Case 1	Case 2
Symptoms		
Restlessness	Yes	Yes
Weakness	Yes	Yes
Vomiting	Yes	Yes
Failure to thrive	Yes	Yes
Polyuria	Yes	Yes
Polydipsia	4600 mL/m^2/d	4000 mL/m^2/d
Clinical signs		
Fair skin	Yes	Yes
Blond hair	Yes	No
Growth retardation	Yes	Yes
Malnutrition	Yes	Yes
Dehydration	Yes	Yes
Rickets	Yes	Yes
Cystine crystals in the cornea	Yes	Yes
Laboratory studies*		
Urogram		
pH	6.5	6
Specific gravity	1005	1002
Glucose **	(+)	(+)
Protein **	(++)	Trace
Serum biochemistry		
Sodium (135-145 mEq/L)	118	125
Potassium (3.5-5.5 mEq/L)	2	2.4
Chloride (98-106 mEq/L)	78	91
Magnesium (1.5-2.3 mg/dL)	1.2	1.3
Calcium (8.8-10.8 mg/dL)	9.3	8.2
Phosphorus (3.7-5.6 mg/dL)	2.7	2
Alkaline phosphatase (145-420 IU/L)	400	325
Blood urea nitrogen (5-18 mg/dL)	30.5	7
Creatinine (0.3-0.7 mg/dL)	1.1	0.5
Intact parathyroid hormone (9-55 pg/mL)	107	83.8
25-hydroxyvitamin D (20-50 ng/mL)	12.4	25.6
Venous blood gases		
pH (7.35-7.45)	7.32	7.35
PCO2 (35-45 mmHg)	25	14.2
HCO3 (22-29 mEq/L)	17	9.4
24-hour urine analysis		
Generalized aminoaciduria	Yes	Yes
Ca excretion (<4 mg/kg/d)	12.5	1.8
Tubular phosphate reabsorption (>85%)	78	67
Radiology		
Rachitic changes on X-ray of the wrist	Yes	Yes
Nephrocalcinosis	No	No

*Normal ranges are provided in parentheses

**Their values are shown as quantitative measurements

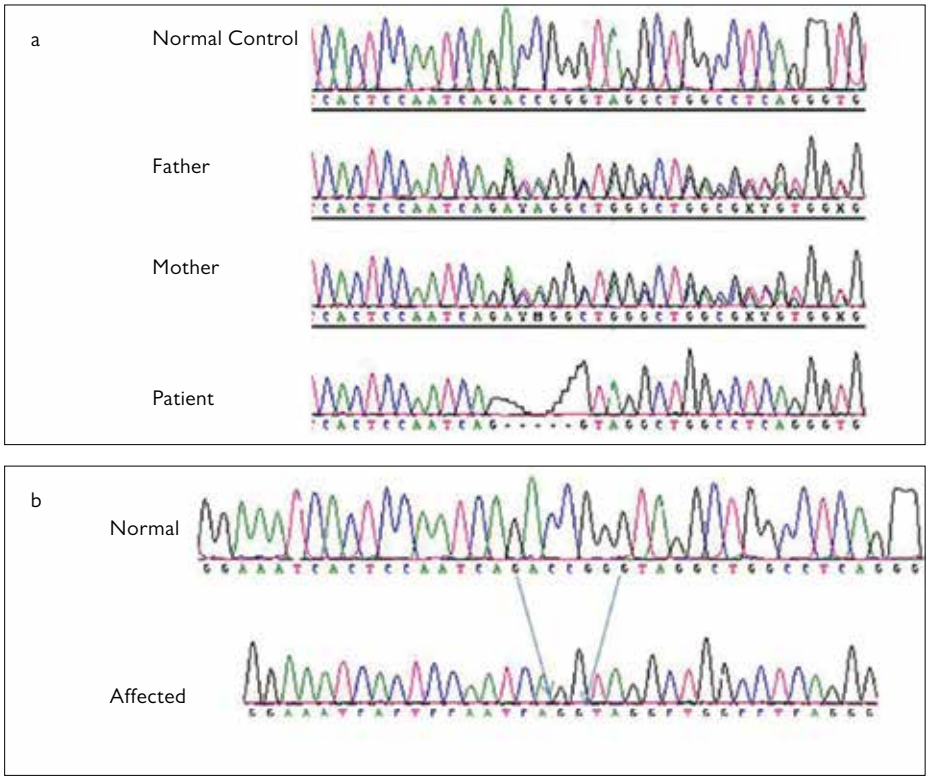

Figure 4. Sequence electropherograms of the CTNS gene for the [c.325_329del (p.Thr109ProfsX14)] mutation. For this mutation, the patient is seen to be homozygous, while his parents are heterozygous. The wild-type sequence in a healthy control is compared with the patient and his parents in the electropherogram in (a). The affected individual is homozygous for the [c.325_329del (p.Thr109ProfsX14)] mutation (b) (arrows).

Photophobia had appeared in the last year. The family history of renal or other medical disease was unremarkable. Physical examination revealed a boy with blond hair, fair skin, moderate dehydration, and severely short stature. His body weight and height were 12 kg (-1.4 standard deviation [SD]) and 83 cm (-4 SD), respectively. The typical clinical findings of rickets were noted. X-ray of the wrist showed rachitic changes (Figure 1). On laboratory evaluation, whole blood count and liver and thyroid function tests were unremarkable. Complex tubulopathy was determined (Table 1). Slit-lamp examination of the cornea revealed cystine crystals, which confirmed the diagnosis of cystinosis (Figure 2). He was treated with cysteamine (50 mg/kg/d), indomethacin (2 mg/kg/d), carnitine (120 mg/kg/d), potassium citrate (2 mEq/kg/d), oral salt (8 g/d), Shohl's solution, Joule's solution, and vitamin D. A diet high in calories and protein was given. Two months after the initiation of the above therapy, the patient has gained 1.5 cm in height and 1200 g in weight. The polydipsia has decreased (2300 mL/m^2/d), and the abnormal laboratory values, including electrolytes and renal function, have returned to the normal ranges. The family's informed consent was obtained for publication.

Case 2

A 2-year-old boy was admitted because of failure to thrive, polyuria, polydipsia, and vomiting. The family history of renal or other systemic disease was unremarkable. His height and weight were 68 cm (-4.8 SD) and 6 kg (-3.8 SD), respectively. He was irritable and weak and had clinical evidence of malnutrition, rickets, and moderate dehydration (Figure 3a). Laboratory findings, including liver and thyroid function tests and whole blood count, were normal. Biochemical findings of urine and serum tests revealed generalized proximal tubular dysfunction (Table 1). The characteristic crystals in the cornea confirmed the diagnosis of cystinosis. The patient was treated as in case 1. Forty days after therapy was started, the patient has gained 2.0 cm in height and 1500 g in weight (Figure 3b). The polydipsia has decreased (2200 mL/m^2/d), and the abnormal laboratory values have returned to the normal ranges. The family's informed consent was obtained for publication.

Genetic analysis

Genetic analyses were performed at the Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. DNA samples were obtained from the patients' peripheral whole blood using standard procedures [3]. A multiplex PCR was performed in accordance with the protocol of Forestier et al. [4]. The genomic DNA of the patients was screened for mutations in all exons of *CTNS*. Case 1 was homozygous for the c.681 G>A (p.E227E) (splice site) mutation on the *CTNS* gene. The *CTNS* gene in the parents of the proband was also analyzed in order to identify the inheritance pattern. Both parents were found to be heterozygous for the same mutation. Case 2 was homozygous for a new frameshift mutation [c.325_329del (p.Thr109ProfsX14), NM_004937.2] on the *CTNS* gene. Both of his parents were heterozygous for the same mutation (Figure 4).

Discussion

There are three different clinical forms of cystinosis according to disease severity, which depend on the age at presentation and the degree of accumulation of cystine: the infantile nephropathic form, which is the most common and most severe form of cystinosis; the late-onset adolescent form (intermediate form or juvenile nephropathic form); and the benign adult form (non-nephropathic form or ocular non-nephropathic form) [2]. The function of proximal tubular transporters is progressively lost in INC because of the accumulation of cystine, which results in Fanconi's syndrome. Thus, water, glucose, amino acids, proteins, sodium, calcium, potassium, magnesium, bicarbonate, phosphate, and many other solutes reabsorbed by the proximal tubule are lost in the urine [2]. Clinical symptoms including failure to thrive, poor feeding, vomiting, rickets, polyuria, and polydipsia and biochemical abnormalities such as hyponatremia, hypokalemia, hypophosphatemia, metabolic acidosis, and low carnitine levels can be seen. In addition, the excretion via the urine of calcium and phosphate can result in nephrocalcinosis and the formation of renal stones. Increased intracellular cystine levels in polymorphonuclear leukocytes confirm the diagnosis of cystinosis [1, 5]. However, a slit-lamp examination of the cornea revealing typical cystine crystals in patients with renal proximal tubular dysfunction is also diagnostic for cystinosis [6]. Our cases had the clinical and biochemical findings of Fanconi's syndrome. Corneal examination in both patients revealed deposits of cystine, which suggested Fanconi's syndrome developing secondarily to cystinosis. Thus, both patients were diagnosed as having INC.

The three different forms of the disease are linked to mutations of the *CTNS* gene. Patients with the late-onset adolescent and benign adult forms of cystinosis have one allele with a severe mutation and one allele with a mild mutation,

which cause impaired clearance of free cystine from lysosomes, whereas those with INC have severe mutations on both alleles, including insertion, deletion, missense, nonsense, and splicing mutations [6]. The molecular study of the *CTNS* gene in our cases confirmed the diagnosis of cystinosis. Both cases were homozygous for a mutant gene. Case 1 had the c.681 G>A (p.E227E) mutation. This mutation is located at an exon-intron boundary and disturbs normal splicing. It was first described in an Arabic population [7]. A genetic study including 12 Turkish patients with cystinosis showed that five patients had the c.681 G>A (p.E227E) mutation [6]. Of these patients, two were homozygous and three were compound heterozygous. The homozygous patients exhibited a severe disease course, as in our case. In that study, c.681 G>A (p.E227E) as a disease-causing variant was reported to be responsible for about 29% of cases. The present genetic picture suggests that the c.681 G>A (p.E227E) mutation is prevalent in the Turkish population with cystinosis. Case 2 was homozygous for a 5 bp deletion [c.325_329del (p.Thr109ProfsX14)]. To our knowledge, this mutation has not been reported in the literature. We could not perform a functional study. However, a 5 bp deletion causes a frameshift mutation and changes the gene product. We believe that it is very likely to disrupt normal gene function on the basis of formal criteria, evolutionary sequence conservation, and its absence in healthy control, as well as a gene prediction program (Figure 4). Case 2 shows us that this mutation can cause a severe phenotype of INC.

In conclusion, we found a new mutation on the *CTNS* gene. We think that further genetic analysis of other cases with similar clinical and laboratory findings may produce more useful evidence.

Informed Consent: Written informed consent was obtained from the parents of the patients who participated in this study.

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Conflict of Interest: No conflict of interest was declared by the authors.

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