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Infantile Nephropathic Cystinosis - Homozygous c.516dupC Mutation of the CTNS Gene

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ABSTRACT

Objective: Cystinosis is a rare, autosomal recessive, lysosomal storage disorder characterized by cystine accumulation throughout the body, due to mutations in the gene encoding cystinosin, named CTNS. Infantile nephropathic cystinosis (INC), the most severe form of the disease and the most common cause of renal Fanconi syndrome (FS), starts with proximal tubulopathy and causes renal failure and various extra-renal manifestations over the time.

Case Presentation: The authors report a 15-month-old boy of Greek origin who presented with failure to thrive over the last 7 months and was noted to have decreased weight and short stature. The metabolic control showed normoglycemic glucosuria, significant proteinuria, and generalized aminoaciduria, suggesting FS. Sequencing analysis of the CTNS gene revealed the frameshift mutation c.516dupC in the homozygous state, confirming the diagnosis of INC. Only one compound heterozygous individual for this mutation has been reported before.

Conclusion: The index case brings out a new correlation of the c.516dupC mutation in the homozygous state with a pure INC phenotype. Alongside, it reminds clinicians to consider cystinosis in the differential diagnosis of failure to thrive or short stature.

Keywords: Infantile nephropathic cystinosis, CTNS gene, frameshift mutation, cystinosin, Fanconi syndrome, short stature

INTRODUCTION

Cystinosis is a rare, autosomal recessive, lysosomal storage disease and the most common cause of inherited renal Fanconi syndrome (FS). It is caused by mutations in the CTNS gene, which is universally expressed throughout the body. CTNS encodes for a lysosomal transmembrane protein called cystinosin. This protein normally functions as a proton-cystine co-transporter, exporting the amino-acid cystine from lysosomes to the cytoplasm. Thus, in cystinosis, cystine accumulates progressively in the cells and forms crystals in every organ and tissue, leading to a multisystem disease. However, two organs are primarily affected, kidneys and eyes. The worldwide incidence of cystinosis is 1:100 000 – 1:200 000 live births (1).

There are three clinical types of the disease, based on the age of onset and severity of the symptoms:

Infantile nephropathic cystinosis (INC, OMIM 219800) is the most common (up to 95% of all cases) and severe type. It usually manifests as FS during the first year of life, characterized by proximal renal tubular dysfunction, growth retardation, hypophosphatemic rickets and episodes of dehydration and polydipsia. Without treatment, renal dysfunction leads to end-stage renal disease (ESRD) at the end of first decade of life. A variety of extra-renal manifestations can occur over time, with photophobia (due to corneal cystine deposits) being the most prominent. Other possible manifestations include primary hypothyroidism, hypergonadotropic hypogonadism (in males), central nervous system involvement, retinal damage, pancreatic (exocrine and endocrine) insufficiency, vacuolar myopathy and swallowing dysfunction (2-4). The second type, juvenile nephropathic cystinosis (JNC, OMIM 219900), represents almost 5% of all cases. Despite sharing the same spectrum of manifestations with the first type, it has a later onset (in adolescence or early adulthood) and milder clinical course. In the majority of patients, renal function is preserved by the age of thirty and their growth is only moderately affected (2-4).

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The third type, ocular or non-nephropathic cystinosis (OC, OMIM 219750), is a benign disorder manifested only by corneal deposits of cystine and the development of photophobia in adulthood, sparing kidneys and other organs (2-4). The causative gene, CTNS, was identified in 1998. It is located on chromosome 17p13 and has 12 exons (5). According to the Human Gene Mutation Database (HGMD), 172 mutations are known up to date (6).

Here we report a patient with INC homozygous for the c.516dupC mutation of the CTNS gene. Only one compound heterozygous patient has been reported before (7). Therefore, it is of particular interest the phenotype that corresponds to this genotype.

CASE PRESENTATION

A 15-month-old boy, the first and only child of a non-consanguineous couple from Greece, was admitted to our paediatric clinic for investigation because of failure to thrive over the last 7 months. An episode of metabolic alkalosis and hypokalaemia at the age of 10 months was referred. Celiac disease and cystic fibrosis had already been ruled out by previous examinations.

The boy was delivered by caesarean section (due to breech presentation), after an uncomplicated 38-week gestation. His measurements at birth were normal, all around 15th percentile: weight 2900 g, length 48 cm, head circumference 33 cm. There were no perinatal problems.

On admission, the toddler was found to have weight 8.2 kg (<3rd percentile), height 76 cm (3rd - 15th percentile), head circumference 47 cm (50th percentile), and light complexion. Growth charts showed poor weight gain since the age of 8 months, accompanied by milder decline in height percentile. The rest physical examination and the psychomotor development were normal.

Common laboratory tests demonstrated persistent hypophosphataemia (around 2 mg/dL, normal values: 4-6 mg/dL) along with a mild increase in alkaline phosphatase (ALP: 430 U/L, normal values: <300 U/L). No other electrolyte or acid-base disorders were observed in repeated measurements. Random urine specimen analysis revealed borderline proteinuria (urine protein: 30 mg/dL, normal values <30 mg/dL) and outstanding glucosuria (urine glucose: 1000 mg/dL, normal: negative for glucose) without concomitant hyperglycaemia (blood glucose: 82 mg/dL). Of note, calcium to creatinine ratio was normal (Ca/Cr: 0.27, normal values: <0.6). The rest laboratory tests (including complete blood count, common biochemical control, thyroid hormones, immunoglobulins, RAST tests, 1.25-dihydroxy-vitamin D, and parathyroid hormone) were within the normal range. Chest and femur X-rays were negative for active rickets.

The 24-hours urine collection revealed significant proteinuria (360 mg/24h, normal values < 150 mg/24h) and increased fractional excretion of phosphate (48%, normal value: 15%) and uric acid (54%, normal value: 38%). In the view of these results, along with the prominent normoglycemic glucosuria, the persistent hypophosphataemia – hyperphosphatasaemia, and the stunted growth mentioned above, renal FS was strongly suspected.

Following, the metabolic screening showed generalized aminoaciduria, particularly of aspartic acid, glutamic acid, glutamine, glycine, citrulline, valine, tyrosine, ornithine, lysine, and arginine (**Table 1**), while the respective plasma aminogram was normal. Consequently, renal FS was confirmed.

Table 1. Amino acid levels in urine showing generalized aminoaciduria

Test	Value (µmol/L)	Normal range (µmol/L)
Aspartic acid	13.00	3.00 - 10.00
Glutamic acid	17.00	0.00 - 11.00
Glutamine	172.00	62.00 - 165.00
Glycine	715.00	110.00 - 356.00
Citrulline	9.00	0.00 - 7.00
Valine	24.00	7.00 - 21.00
Tyrosine	58.00	13.00 - 48.00
Ornithine	11.00	00.0 - 8.00
Lysine	73.00	16.00 - 69.00
Arginine	15.00	0.00 - 8.00

As cystinosis represents the most common cause of the above syndrome regarding patient's age group, we proceeded with molecular genetic analysis of the CTNS gene, for the toddler and his parents. The analysis was performed by next-generation sequencing technique on exons and lateral splice regions throughout the gene [massive parallel (NextGen) sequencing]. In fact, the patient was found homozygous for the c.516dupC mutation, while the parents were heterozygous for the same mutation (**Figure 1**).

Mitochondrial DNA analysis was additionally performed and proved negative for pathological variants (mutations or polymorphisms).

Combining genotype with phenotype, the patient was diagnosed with INC. A few months later, the pathognomonic corneal crystals of cystine were found bilaterally.

Furthermore, the renal function was assessed by 99mTc-MAG3 dynamic scintigraphy. The scan showed orthotopic kidney of normal size and morphology, with adequate perfusion but mildly reduced function bilaterally (renal cortical retention index 34% left and 40% right, normal values: <30%). No drainage problems or focal parenchymal lesions were observed. As for the estimated glomerular filtration rate (eGFR), it was calculated at 63 ml/min/ 1.73m2 (normal range: 62-191 mL/min/1.73m2, mean: 127 mL/min/1.73m2), a value that imply marginally impaired renal function.

Since the diagnosis of INC was established, the patient has been receiving permanent medication with cysteamine (systemic and ocular formulations), the specific cystine-depleting therapy. Besides, supportive treatment is given to replenish deficits in fluids, electrolytes, and nutrients whenever needed. He is regularly monitored by a multidisciplinary medical team for his physical and psychomotor development, renal function, ocular course and potential complications from various systems.

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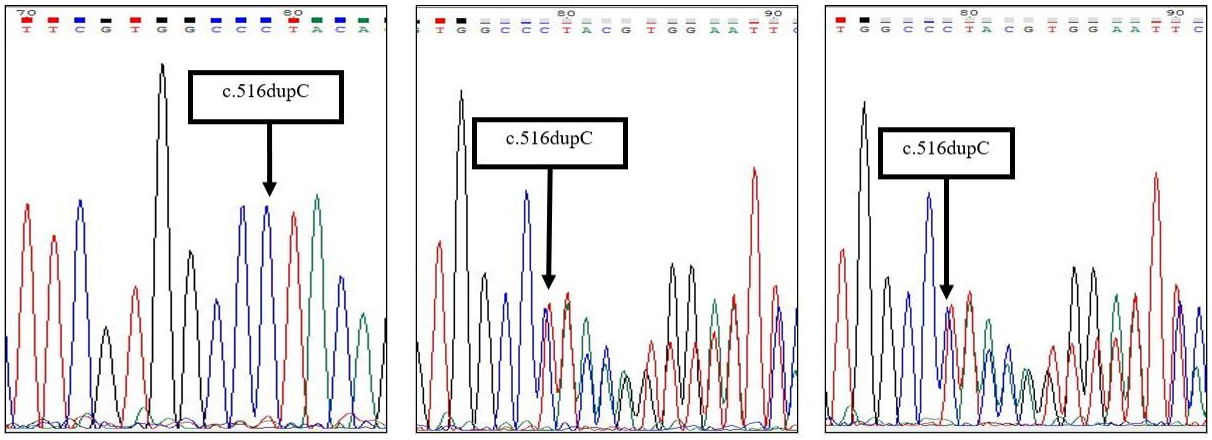

(a) (b) (c)

Figure 1. NGS analysis images of the CTNS gene showing the c.516dupC mutation in homozygous state for the patient (a) and in heterozygous state for his father (b) and mother (c).

DISCUSSION

The full name of the mutation under consideration is c.516dupC: p.Tyr173LeufsX55 (NM_004937.2). It concerns exon 8, where duplication (or small insertion) of cytosine occurs at position 516 of the gene. The normal sequence with the base that is duplicated in brackets is: TGGC[dupC]TACA. In this way, the mutation causes a frameshift, starting with codon Tyrosine 173, changes this amino acid to a Leucine residue, and creates a premature stop codon at position 55 of the new frame, denoted p.Tyr173LeufsX55. So, the c.516dupC variant is predicted to cause loss of normal protein function, either through protein truncation or non-sense mediated mRNA decay (8).

Literature review revealed that the specific mutation had been reported previously only in one individual with INC from Italy, who also harboured a second CTNS mutation (7). Moreover, it is not observed in large human genome databases (Ensembl, gnomAD).

Hence, to the best of our knowledge, our patient is the first to be reported carrying c.516dupC mutation in the homozygous state. Taking into consideration both his genotype and phenotype (growth retardation, proximal tubulopathy, fair characteristics), we interpret this mutation as a pathogenic variant causing INC. The diagnosis is further confirmed by the finding of corneal cystine crystals bilaterally.

In addition to the CTNS gene analysis, mitochondrial DNA analysis was performed, since specific mitochondrial disorders are involved in the differential diagnosis of FS (9). That is because of the impaired ATP metabolism, which leads to proximal renal tubulopathy, as it has been reported for several cases in the literature (10).

It is also worth mentioning that repeated acid-base balance testing in the patient showed no metabolic acidosis. Moreover, the patient had suffered an episode of metabolic alkalosis and hypokalaemia in the past. This means that cystinosis, in his case, came up temporarily with a Bartter-like profile. Similar sporadic clinical presentations have been referred in other patients as well (11, 12).

Consequently, normal or Bartter-like findings in acid-base ‘’snapshots’’ (instant recordings) do not rule out cystinosis. This is a tip that clinical paediatricians should keep in mind.

According to the literature, frameshift mutations, as the one under consideration, are predicted to be more severe (in comparison with missense mutations, which lead to cystinosin with impaired function), because they cause a defective cystinosin in structure. Individuals homozygous or compound heterozygous for frameshift mutations tend to perform more rapid decline in their kidneys’ function (13).

CONCLUSION

In conclusion, cystinosis is one of the few metabolic diseases with specific treatment, named cysteamine. The earlier the treatment is initiated, the better the results are for patients’ quality of life (9). The key learning points from the case are the following: The c.516dupC mutation of the CTNS gene is correlated with a pure INC phenotype. A full-blown FS is not necessary for the suspicion of cystinosis. Finally, in the differential diagnosis of failure to thrive or short stature in children, cystinosis should be taken under consideration.

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