CEN Case Reports (2018) 7:153–157
https://doi.org/10.1007/s13730-018-0316-3

CASE REPORT

Slow progression of renal failure in a child with infantile cystinosis

Maria Bitsori[1] · **Eleni Vergadi**[1] · **Emmanouil Galanakis**[1]

Received: 25 September 2017 / Accepted: 7 February 2018 / Published online: 14 February 2018

Abstract
Cystinosis is a rare autosomal recessive lysosomal transport disorder, characterized by the accumulation of the aminoacid cystine and progressive dysfunction of several organs. Kidneys are severely affected, and the most frequent form, infantile nephropathic cystinosis, presents with growth failure in infancy, renal Fanconi syndrome and end-stage renal disease by the first decade of life. We report of a girl with infantile nephropathic cystinosis that has reached adolescence without the need of renal replacement therapy and without extrarenal manifestations despite her delayed diagnosis and treatment initiation. The girl with this intermediate phenotype was found to have compound heterozygosity of one known (1015G > A) and one novel (587_588insA) mutation in CTNS gene. Our case points to the wide clinical presentation of infantile nephropathic cystinosis and suggest that long-term outcome is not always ominous as generally thought.

Keywords Cystinosis · Novel mutation · CTNS gene · Disease progression

Introduction

Cystinosis is a rare autosomal recessive disease characterized by defective transport of the aminoacid cystine across the lysosomal membrane leading to lysosomal accumulation of cystine in many organs, mainly kidneys and eyes [1]. The disease affects one to two in 100,000 with a higher incidence in some Northern European regions [2]. Three distinct clinical forms are known: infantile nephropathic cystinosis, the juvenile or late onset nephropathic cystinosis and the benign or ocular cystinosis [2, 3]. The infantile form is the most severe, accounts for 95% of cases, and presents with Fanconi syndrome, failure to thrive, and rickets early in life and progression to end-stage kidney disease (ESKD) in the first decade of life [1]. The juvenile form is usually diagnosed later in childhood or adolescence with symptoms of mild renal disease [2, 4] and the ocular form is characterized by corneal cystine crystal depositions without renal involvement. Due to the rarity of cystinosis, the diagnosis is often delayed.

Infants with nephropathic cystinosis usually have pale blond hair and blue eyes, pointing to pigmentation defects [3]. The proximal tubule is the main target of the disease with aminoaciduria, glycosuria, tubular proteinuria and potassium, phosphate and bicarbonate losses, resulting in hypophosphatemia, hypokalemia, metabolic acidosis and rickets. Photophobia due to cystine crystal deposition in the cornea is present by 16 months of age [5]. Continuous cystine accumulation leads to endocrinologic, gastrointestinal, muscular and neurological manifestations [1, 6]. Nephropathic cystinosis has a variable severity, but despite treatment end-stage renal disease is inevitably reached by 10 years of age [6, 7]. Disease progression is slowed by cysteamine, which facilitates the elimination of cystine from lysosomes [7]. Early initiation of cysteamine has been shown to delay renal damage [8].

All forms of cystinosis are caused by mutations in the CTNS gene that maps to chromosome 17p13, has 12 exons and encodes for a lysosomal membrane protein, cystinosin, which is expressed in all tissues acting as a cystine transporter [9, 10]. More than 100 mutations have been reported, including deletions, small insertions, duplications, missense, nonsense and splice site mutations, mutations in the promotor sequence and genomic rearrangements [2, 11, 12]. Large deletions or point mutations in critical aminoacids cause infantile cystinosis, while milder mutations that do not disrupt the reading frame are observed in late-onset and ocular forms [2, 11–14]. The most frequent mutation, affecting mainly northern Europeans, is a large deletion of 57 kb involving the first 9 and part of 10 CTNS exons [2, 9, 11].

✉ Emmanouil Galanakis
emmgalan@uoc.gr

[1] Department of Paediatrics, Heraklion University Hospital, Crete, Greece

In the present report, we describe the case of a girl who, despite a delayed diagnosis of infantile cystinosis, preserved moderate renal function without extrarenal complications in her adolescence and discuss her clinical and genetic characteristics.

Case report

A 6-year-old Caucasian girl, with dark hair and brown eyes, was referred to our department for nephropathic cystinosis. Although she was symptomatic from her infancy, she had only been diagnosed at the age of 3.5 years. She was the second child, delivered after a full-term uneventful pregnancy with a birth weight of 4 kg and insignificant family history. At the age of 18 months she was unable to walk independently and was admitted in a tertiary hospital for further evaluation. Her growth was within normal limits but a fall more than 2 SDs in weight and height growth curves was noticed since the age of 10 months. On physical examination signs of rickets were identified, such as rachitic rosary along the costochondral junctions, leg bowing, double tibial epiphysis and knobby thickening of wrists. Laboratory investigation revealed hypophosphataemia, normal calcium levels, elevated alkaline phosphatase and low-normal potassium and sodium levels (Table 1). Haematologic parameters, renal and liver function tests, arterial blood gases, thyroid function tests, vitamin D and parathyroid hormone were within normal limits. In urinalysis glycosuria, proteinuria and ketonuria were noted. The 24-h urine analysis revealed polyuria (7–8 ml/kg/h), low molecular weight proteinuria, hypercalciuria, phosphaturia with reduced phosphate clearance and hyperuricosuria. Estimated GFR was 84.7 ml/min/1.73 m^2. Radiologic examination confirmed active rachitic changes on the left wrist and renal ultrasonography showed mildly echogenic enlarged kidneys.

The child was evaluated as having the typical features of renal Fanconi syndrome with consequent rickets. Slit-lamp examination of the cornea was normal and leukocyte cystine assay was not available at the time. Further metabolic investigation revealed elevated blood lactate, pyruvate and lactate/pyruvate ratio, generalized aminoaciduria and elevated urine lactate and b-hydroxybutyrate. These findings were considered as suggestive of mitochondrial disease and further investigation with muscle biopsy confirmed this suspicion with reduced activity of cytochrome oxidase and carnitine in enzyme analysis, despite the normal architecture. Under a working diagnosis of renal mitochondrial cytopathy the girl was given vitamin D, potassium, oral phosphate, carnitine and synenzyme Q10 supplements. Over the following 18 months, rickets was improved and the girl eventually walked at the age of 24 months.

Table 1 Selected biochemical results in blood serum and urine in the patient at the time of initial presentation

Laboratory test	Value	Normal range
Blood/serum		
Phosphorus	2.5 mg/dl (↓)	4.5–5.0
Calcium	9.7 mEq/l	8.8–10.4
Alkaline phosphatase	1854 U/l (↑)	150–420
Parathormone	43.6 U/l	25–65
Potassium	3.3 mEq/l (↓)	3.5–5.5
Sodium	135 mEq/l	135–145
Lactate	28.5 g/dl (↑)	4.5–19.8
Pyruvate acid	1.78 mg (↑)	0.7–1.4
Uric acid	0.7 mg/dl (↓)	1.7–5.8
%TRP	64.4% (↓)	78–90%
Urine		
Glucose	100 mg/dl (↑)	0–15
Protein/creatnine spot	1.2 (↑)	< 0.2
β2-microglobulin (24 h)	24 mg/l (↑)	< 0.2
Aminoaciduria	Positive	
Calcium (24 h)	14.8 mg/kg/day (↑)	0–4
Phosphorus (24 h)	53.5 mg/kg/day (↑)	7–20
Uric acid (24 h)	1551 mg/day (↑)	250–750
Magnesium (24 h)	39 mg/kg/day	30–100

TRP tubular reabsorption of phosphate, (↓) decreased value, (↑) elevated value

On regular reevaluation at the age of 3.5 years, cystine crystals were identified in both eyes on slit-lamp examination and the diagnosis of cystinosis was confirmed by elevated cystine levels in blood leukocytes (5.2 nmol ½cystine/mg protein, normal value < 0.15). At that time serum creatinine was 0.8 mg/dl and GFR 66 ml/min/1.73 m^2. Thyroid and parathyroid function was normal (TSH 1.8 mU/l, free T4 1.6 ng/day, free T3 5.1 pg/ml and PTH 52 pg/ml). The child was put on cysteamine, indomethacin, cysteamine eye drops, potassium chloride, carnitine, phosphate and vitamin-D supplements. After initiation of cysteamine therapy the leukocyte cystine levels were maintained to 0.68–1.3 ½cystine/mg protein, with regular monitoring targeting to < 1 nmol ½cystine/mg protein. Indomethacin was initially introduced to control polyuria and electrolyte losses [15] and it was discontinued in the age of 12 years, when the first indications of deterioration of renal function were noted. Since the start of treatment and by the time of her referral to our department, her growth, polyuria and renal function had been improved. Her weight and height were at 50th percentile, her creatinine level was 0.9 mg/gl and her GFR 75 ml/min/1.73 m^2. Currently, at 16 years of age, although her renal function has gradually deteriorated with creatinine levels of 3–3.5 mg dl and GFR of 22.5 ml/min/1.73 m^2, she maintains normal growth and vision and adequate quality

of life with no other system involvement or need for renal replacement therapy yet.

Mutational analysis and sequencing of CTNS gene revealed compound heterozygosity; one allele holds a point mutation in 1015 bp in exon 12 that resulted in aminoacid change G339R which is located in the 7th transmembrane region (TM) and the other allele holds a nucleotide insertion in at 587–588 base causing a frameshift in aminoacid sequence that results in an stop codon 32 codons downstream the insertion (Table 2).

Discussion

In this report we describe the case of a girl that presented with the typical signs of renal Fanconi syndrome and rickets at 18 months. Renal Fanconi syndrome at this age is most often associated with inherited multisystem metabolic diseases including cystinosis, Dent's disease, Fanconi–Bickel syndrome, Lowe syndrome, tyrosinemia type I, Wilson's disease, fructose intolerance, galactosemia and mitochondrial disorders [15, 16] or acquired causes from drugs and heavy metals [16, 17]. In our case the diagnosis of cystinosis was initially missed since there was a normal slit-lamp examination and leukocyte cystine assay were not available. Similarly, slit-lamp exam was also repetitively normal in a 12-year-old boy with nephropathic juvenile cystinosis, despite the presence of renal Fanconi syndrome from the age of 3 years [18]. Cystinosis is unusual in Greece and currently only four patients with a confirmed diagnosis are known, including the present case. Mitochondrial disorders and respiratory chain deficiencies are occasionally associated with renal tubular dysfunction [2, 19], but in our case there were no other systemic manifestations suggestive of mitochondrial disease. Since no other cause of Fanconi syndrome was evident at the time, the patient was considered to suffer from renal mitochondrial cytopathy.

Mitochondrial dysfunction can be a manifestation of intracellular cystine accumulation which leads to different expression of several genes that include oxidative phosphorylation, apoptosis and mitochondrial dysfunction [20]. ATP depletion and abnormal electron transport chain activity have been described in cells with cystine accumulation [21]. Moreover, in cystinosis patients, cells throughout the body including renal, have been shown to exhibit decreased levels of ATP and morphologically abnormal mitochondria [22]. Thus, the reduced COX and complex IV activity that was found in the muscle biopsy of our patient could be secondary to cystine accumulation. Still, the involvement and role of mitochondria in the pathogenesis of nephropathic cystinosis remains unclear [20, 21].

Since the introduction of cysteamine, long term prognosis of cystinosis has been improved, but even so stage IV chronic kidney disease (CKD) is usually reached by 10 years of age in > 90% of patients, whereas > 80% require renal replacement therapy before the age of 14 years [8, 23]. In a large scale study based on European registry data it was found that the mean age of initiation of renal replacement therapy is 10.8 years for the patients studied after 1995 and 12.7 years for the patients studied after 2003 [24]. Improvement in the progression of renal failure was significantly associated with introduction of cysteamine before 2 years of age with a strict administration schedule and regular dose adaptation, based on monitoring of intraleukocyte cystine levels [1, 8]. Our patient had a slow progression from stage II CKD at diagnosis (age 3.5 years) to stage IV CKD (age 16 years) and maintains adequate growth and quality of life without extrarenal manifestations.

DNA analysis of *CTNS* gene revealed compound heterozygosity to one novel and one already described mutation. In one allele of *CTNS* gene, a not previously described nucleotide insertion in nucleotide 587, located in exon 9, was found. This mutation results in frameshift and subsequent aminoacid changes from residue 196 located between the 2nd and 3rd TM region with a premature stop codon 32 residues downstream the mutation. Patients with point mutations in this *CTNS* gene region are known to have intermediate forms with late onset renal disease [13, 14, 25]. In the second allele of *CTNS*, a point mutation in 1015 bp (exon 12), results in substitution of the aminoacid glycine to arginine in the position 339 which is located in the 7th TM region. The c.1015G > A (G339R) mutation has been identified as the most prevalent in a Turkish population with cystinosis, but its origin is unclear [23]. It is considered severe mutation since it has been reported to abolish cystine transport in vitro [12–14, 26–28]. The majority of reports involve the presence of G339R mutation in one allele of *CTNS* gene with the second usually holding also a severe mutation such as the 57 kb deletion [12, 26]. Homozygosity of G339R has been also reported to cause severe phenotype [29]. However, heterogeneity in the clinical course of disease has been described in compound heterozygotes that carry G339R and

Table 2 Mutational analysis of *CTNS* gene in the patient

Location	Base change	Aminoacid change	Location	References
Exon 9	587_588insA	Asn196LysfsX32	2nd–3rd inter TM loop	Not reported yet
Exon 12	1015G > A	Gly339Arg	7th TM region	[12, 29, 30]

TM transmembrane, *fsX* frameshift, *ins* insertion

other missense mutations, ranging from severe or less severe phenotype (ESRD at 10 years or adequate renal function at 12 years old) [23]. Non-compliance to treatment was associated with worst outcome in the above compound heterozygotes to G339R mutation [23]. The slower deterioration of renal function in our patient can be attributed to her combination of mutations and her compliance to treatment. This genetic combination might also be associated with the lack of pigmentation defect, characteristic of the presented case. Cystinosin was found to be involved in melanogenesis in clinical and experimental studies, but information about the importance of this association in clinical presentation of the disease is very limited [30]. The role of modifier genes activated by the loss of cystinosin and the spectrum of molecular events associated with the variety of clinical phenotypes in cystinosis remain to be fully characterized. The patient's parents were advised to undergo genetic analysis; mother was found to be a G339R mutation carrier whereas father refused to be investigated.

In conclusion, a high index of clinical suspicion is needed for diagnosis of nephropathic cystinosis in children with renal Fanconi syndrome even with normal slip-lamp examination. Diagnosis of renal mitochondrial cytopathy should be made only if cystinosis is ruled out since secondary mitochondrial defects may be present in the cells due to intracellular cystine accumulation. Finally, in the case presented here, we describe a girl with slow progression of renal failure despite the late diagnosis and the late initiation of cystine depleting treatment.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Nesterova G, Gahl WA. Cystinosis: the evolution of a treatable disease. Pediatr Nephrol. 2013;28:51–9.
2. Emma F, Nesterova G, Langman C, Labbe A, Cherqui S, Goodyer P, et al. Nephropathic cystinosis: an international consensus document. Nephrol Dial Transpl. 2014;29(Suppl 4):iv87–94.
3. Gahl WA, Thoene JG, Schneider JA. Cystinosis. N Engl J Med. 2002;347:111–21.
4. Servais A, Moriniere V, Grunfeld JP, Noel LH, Goujon JM, Chadefaux-Vekemans B, Antignac C. Late-onset nephropathic cystinosis: clinical presentation, outcome, and genotyping. Clin J Am Soc Nephrol. 2008;3:27–35.
5. Gahl WA, Kuehl EM, Iwata F, Lindblad A, Kaiser-Kupfer MI. Corneal crystals in nephropathic cystinosis: natural history and treatment with cysteamine eyedrops. Mol Genet Metab. 2000;71:100–20.
6. Nesterova G, Gahl W. Nephropathic cystinosis: late complications of a multisystemic disease. Pediatr Nephrol. 2008;23:863–78.
7. Gahl WA. Early oral cysteamine therapy for nephropathic cystinosis. Eur J Pediatr. 2003;162(Suppl 1):S38–41.
8. Greco M, Brugnara M, Zaffanello M, Taranta A, Pastore A, Emma F. Long-term outcome of nephropathic cystinosis: a 20-year single-center experience. Pediatr Nephrol. 2010;25:2459–67.
9. Touchman JW, Anikster Y, Dietrich NL, Maduro VV, McDowell G, Shotelersuk V, et al. The genomic region encompassing the nephropathic cystinosis gene (*CTNS*): complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. Genome Res. 2000;10:165–73.
10. Town M, Jean G, Cherqui S, Attard M, Forestier L, Whitmore SA, et al. A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. Nat Genet. 1998;18:319–24.
11. Anikster Y, Lucero C, Touchman JW, Huizing M, McDowell G, Shotelersuk V, et al. Identification and detection of the common 65-kb deletion breakpoint in the nephropathic cystinosis gene (*CTNS*). Mol Genet Metab. 1999;66:111–6.
12. Attard M, Jean G, Forestier L, Cherqui S, van't Hoff W, Broyer M, et al. Severity of phenotype in cystinosis varies with mutations in the *CTNS* gene: predicted effect on the model of cystinosin. Hum Mol Genet. 1999;8:2507–14.
13. Kalatzis V, Cohen-Solal L, Cordier B, Frishberg Y, Kemper M, Nuutinen EM, et al. Identification of 14 novel *CTNS* mutations and characterization of seven splice site mutations associated with cystinosis. Hum Mutat. 2002;20:439–46.
14. Thoene J, Lemons R, Anikster Y, Mullet J, Paelicke K, Lucero C, et al. Mutations of *CTNS* causing intermediate cystinosis. Mol Genet Metab. 1999;67:283–93.
15. Emma F, Bertini E, Salviati L, Montini G. Renal involvement in mitochondrial cytopathies. Pediatr Nephrol. 2012;27:539–50.
16. Haque SK, Ariceta G, Batlle D. Proximal renal tubular acidosis: a not so rare disorder of multiple etiologies. Nephrol Dial Transpl. 2012;27:4273–87.
17. Hall AM, Bass P, Unwin RJ. Drug-induced renal fanconi syndrome. QJM. 2014;107:261–9.
18. Higashi S, Matsunoshita N, Otani M, Tokuhiro E, Nozu K, Ito S. Diagnostic challenge in a patient with nephropathic juvenile cystinosis: a case report. BMC Nephrol. 2017;18:300.
19. Mochizuki H, Joh K, Kawame H, Imadachi A, Nozaki H, Ohashi T, et al. Mitochondrial encephalomyopathies preceded by de-toni-debre-fanconi syndrome or focal segmental glomerulosclerosis. Clin Nephrol. 1996;46:347–52.
20. Wilmer MJ, van den Heuvel LP, Rodenburg RJ, Vogel RO, Nijtmans LG, Monnens LA, Levtchenko EN. Mitochondrial complex v expression and activity in cystinotic fibroblasts. Pediatr Res. 2008;64:495–7.
21. Levtchenko EN, Wilmer MJ, Janssen AJ, Koenderink JB, Visch HJ, Willems PH, et al. Decreased intracellular atp content and intact mitochondrial energy generating capacity in human cystinotic fibroblasts. Pediatr Res. 2006;59:287–92.
22. Sansanwal P, Yen B, Gahl WA, Ma Y, Ying L, Wong LJ, Sarwal MM. Mitochondrial autophagy promotes cellular injury in nephropathic cystinosis. J Am Soc Nephrol. 2010;21:272–83.
23. Topaloglu R, Vilboux T, Coskun T, Ozaltin F, Tinloy B, Gunay-Aygun M, et al. Genetic basis of cystinosis in turkish patients: a single-center experience. Pediatr Nephrol. 2012;27:115–21.
24. Van Stralen KJ, Emma F, Jager KJ, Verrina E, Schaefer F, Laube GF, et al. Improvement in the renal prognosis in nephropathic cystinosis. Clin J Am Soc Nephrol. 2011;6:2485–91.
25. Anikster Y, Lucero C, Guo J, Huizing M, Shotelersuk V, Bernardini I, et al. Ocular nonnephropathic cystinosis: clinical, biochemical, and molecular correlations. Pediatr Res. 2000;47:17–23.
26. Kiehntopf M, Schickel J, Gonne B, Koch HG, Superti-Furga A, Steinmann B, et al. Analysis of the *CTNS* gene in patients

of German and Swiss origin with nephropathic cystinosis. Hum Mutat. 2002;20:237.
27. Shotelersuk V, Larson D, Anikster Y, McDowell G, Lemons R, Bernardini I, et al. *CTNS* mutations in an american-based population of cystinosis patients. Am J Hum Genet. 1998;63:1352–62.
28. Kalatzis V, Nevo N, Cherqui S, Gasnier B, Antignac C. Molecular pathogenesis of cystinosis: effect of *CTNS* mutations on the transport activity and subcellular localization of cystinosin. Hum Mol Genet. 2004;13(13):1361–71.
29. Rupar CA, Matsell D, Surry S, Siu V. A G339R mutation in the *CTNS* gene is a common cause of nephropathic cystinosis in the south western ontario amish mennonite population. J Med Genet. 2001;38:615–6.
30. Chiaverini C, Sillard L, Flori E, Ito S, Briganti S, Wakamatsu K, et al. Cystinosin is a melanosomal protein that regulates melanin synthesis. Faseb J. 2012;26:3779–89.