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EDUCATIONAL REVIEW

Cystinosis: the evolution of a treatable disease

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Abstract Cystinosis is a rare autosomal recessive disorder involving lysosomal storage of the amino acid cystine due to a defect in the membrane transport protein, cystinosin. Since the introduction of kidney transplants and the availability of cystine-depleting medical therapy, this previously fatal disease was transformed into a treatable disorder. Renal allografts and medical therapy targeting the basic metabolic defect have altered the natural hisotry of cystinosis so drastically that patients have a life expectancy extending past 50 years. Consequently, early diagnosis and appropriate therapy are critically important. In this article, we offer a review of the manifestations of cystinosis, including the proximal tubular dysfunction of renal Fanconi syndrome, and discuss the prevention and treatment of the disorder's systemic complications. We focus on the nephropathic forms of cystinosis, aiming to assist nephrologists and other physicians to develop early recognition and appropriate management of cystinosis patients.

Keywords Cystinosis · Nephropathic · Fanconi · Complications · Cysteamine

Introduction

Cystinosis was the first lysosomal storage disease recognized to be due to defective lysosomal membrane transport, and it serves as a prototype for a small group of lysosomal transport disorders. The disease results in intracellular accumulation of cystine in all organs and tissues. Cystinosis has been known for over a century, but it was first described as a nosological entity in the 1930's by the distinguished European pediatrician Guido Fanconi, who categorized it as a cystine storage disease [1, 2]. The prevalence of cystinosis is approximately 1:100,000 to 1:200,000, and the disease has been found worldwide in all ethnic groups. The frequency of cystinosis in Brittany is 1 in 26,000 due to a founder effect [3]. There are three types of cystinosis. Nephropathic or classic infantile cystinosis (NC), the most severe form, inevitably leading to terminal renal failure in the first decade of life, and it is the major identifiable cause of renal Fanconi syndrome (FS) in children [4]. The intermediate form of cystinosis has all the manifestations of the nephropathic form, but its onset is generally around the time of adolescence. Non-nephropathic or ocular cystinosis is characterized only by corneal crystals and photophobia. About 95 % of cystinosis patients have the nephropathic form [4].

All three forms are allelic recessive disorders caused by mutations in the *CTNS* gene, which is 26 kb in length and has 12 exons with coding region of 1104 base pairs. At least 80 mutations in *CTNS* were reported. The most common mutation in Caucasians is 57-kb deletion and represents a founder defect [5]. The *CTNS* gene product, cystinosin, is a 367-amino acid peptide with seven transmembrane and two lysosomal targeting motifs; it is expressed in the cells of all tissues [6, 7]. Some mutant alleles are predicted to produce no *CTNS* mRNA, while others produce a truncated cystinosin, often with residual function [8]. Tissues have different susceptibilities to the accumulation of cystine: the renal tissue is one of most sensitive [3, 6, 9]. Heterozygotes for cystinosis are clinically normal, regardless of the type [3, 6].

Pathophysiology: Fibroblasts and lymphocytes isolated from patients with cystinosis manifest increased lysosomal cystine storage to approximately 100 fold those of normal individuals [10]. Cystine is poorly soluble and forms crystals in different tissues (Fig. 1c, d, e, f), but not in leucocytes [11]. An initial hypothesis of cystinosis pathophysiology suggested that the amount of intracellular cystine content would predict the severity of the phenotype [9], but this is

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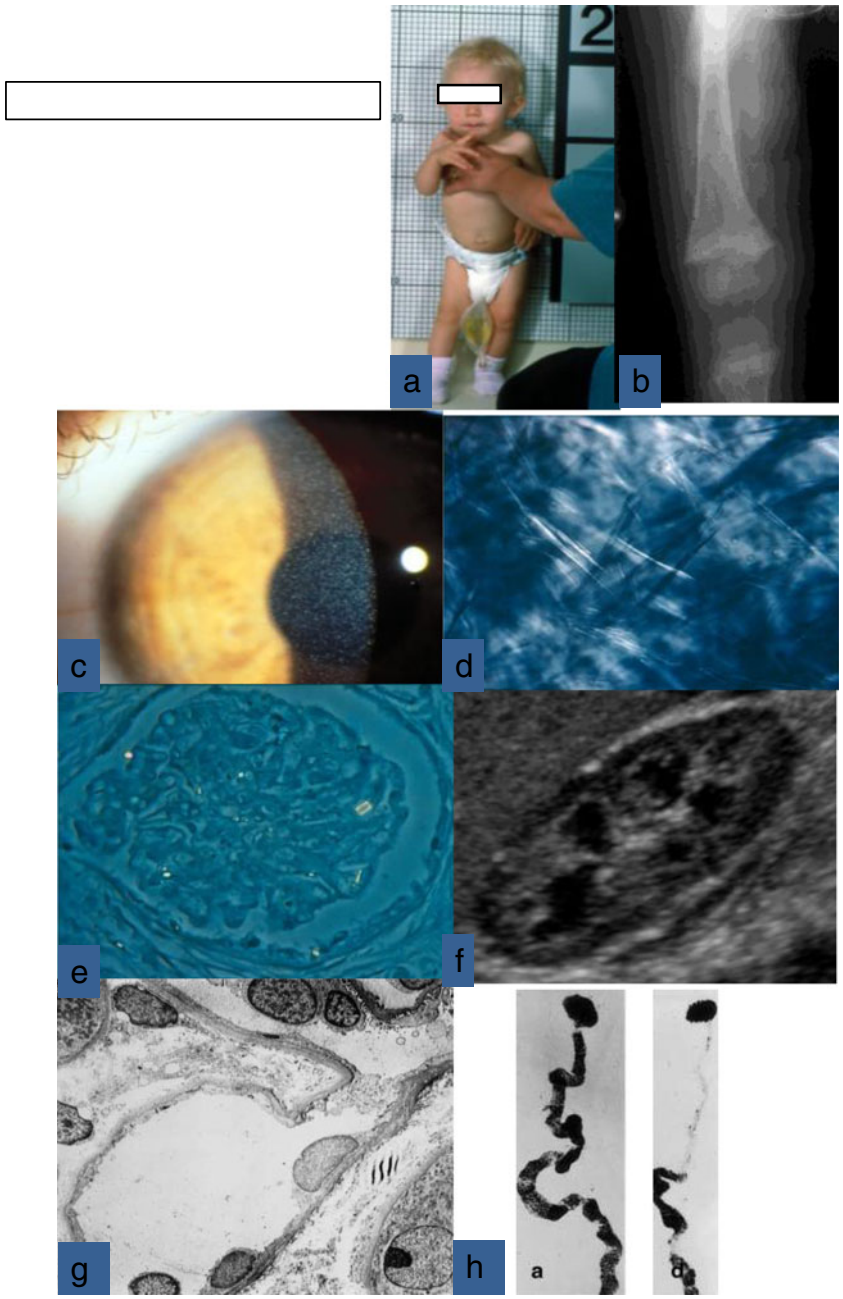

Fig. 1 Early findings in Cystinosis **a**) an infant with Fanconi syndrome (FS), **b**) Rickets, **c**) corneal crystals on Slit Lamp exam, **d**) Corneal crystals, **e**) glomerular cystine crystals, **f**) nephrocalcinosis, **g**) Electron Microscopy of the tubular epithelial cell, only the nucleus and a thin rim of cytoplasm remain. The mitochondria, lysosomes, crystalline spaces, and brush border have disappeared **h**) 'swan neck" deformities (figures used with permission from [38])

not always the case. An in vitro, cystine loading model of cystinosis failed to show a relationship between cystine storage and renal tubular dysfunction [12]. Other hypotheses link pathophysiology to aberrant energy production with diminished intracellular ATP [13], or to apoptosis, known to play a role in renal tubular dysfunction [14, 15], including that associated with allograft rejection. The most common renal symptom in mitochondrial cytopathies is proximal tubular dysfunction, suggesting that tubular cells in NC are particularly sensitive to mitochondrial injury [14]. The continued loss of proximal tubular epithelial cells could explain the morphologic hallmark of cystinosis, i.e. the "swan neck" deformity (Fig. 1h) [16].

In non-nephropathic cystinosis, the kidneys are spared, probably because the mutant allele produces residual cystinosin. [3]. Presumably the greater the expression of cystinosin, the milder the disease; this could explain the small degree of genotype-phenotype correlation both within and among cystinosis subtypes [3, 17].

Clinical characteristics of early NC

Untreated NC is associated with poor growth and proximal tubular Fanconi syndrome at 6–12 months of age, glomerular failure by age 10 years, and various nonrenal complications.

Renal Fanconi syndrome is characterized by the generalized failure of proximal tubules to reabsorb water, electrolytes, bicarbonate, calcium, glucose, phosphate, carnitine, amino acids, and tubular proteins. Renal tubular damage presents at the time of diagnosis and is largely irreversible [18]. Hyperaminoaciduria is a hallmark of FS[18]; in normal children only 1 to 6 mg of amino acids per kilogram of body weight per day are excreted, since over 98 % of the filtered load of amino acids is reabsorbed in the proximal tubules [19] . In patients with FS, the loss of amino acids is 6–16 fold normal [19]. Another hallmark of FS is glycosuria with normal serum glucose concentrations, indicating that the renal threshold for glucose is abnormally low [19]. Urine output could be as great as in nephrogenic diabetes insipidus [4], and cystinosis is occasionally mistaken for this disease. The threshold for bicarbonate reabsorption is greatly reduced in cystinosis and serum bicarbonate concentrations falls, creating metabolic acidosis, which is partially responsible for the poor growth of affected children [20]. The excess bicarbonate that reaches the distal tubule enhances potassium excretion, resulting in low serum potassium levels and, with severe hypokalemia, the risk of cardiac dysfunction [20].

Many different low-molecular weight proteins are excreted by cystinosis patients, with major loss of alpha-1-microglobulin, retinol-binding protein and beta-2-microglobulin [1]. In addition, enzymes, immunoglobulins, and hormones are frequently found in the urine of FS patients. Larger molecular weight proteins, termed glomerular proteins, are also found in the urine during the later stages of NC as glomerular dysfunction becomes more evident [19]. Profound polyuria, polydipsia, dehydration and hypochloremic metabolic acidosis commonly result in life-threatening hypovolemia and require immediate medical intervention. Failure of the proximal tubules to reabsorb phosphate and calcium leads to Vitamin D-resistant hypophosphatemic rickets in children and osteomalacia in adults [19] (Fig. 1b); hypocalcemia sometimes results in tetany. Hyperphosphaturia and hypercalciuria contribute to medullary nephrocalcinosis (Fig) [21]. Other symptoms such as anorexia, vomiting, and feeding difficulties, combined with renal losses of nutrients, cause poor nutritional status and lead to failure to thrive. Failure to grow is noticed between 6 and 12 months; infants typically fall to the third percentiles for height and weight by one year of age. Later, growth develops at approximately 60 % of the normal rate [22] (Fig. 1a). Children with FS exhibit plasma and muscle carnitine deficiency due to failure to reabsorb carnitine [23].

The renal phenotype of cystinosis consists of an overlap of the Fanconi syndrome with progressive loss of glomerular function [24]. The serum creatinine concentration often does not rise above normal until about 5 years of age, but it can increase rapidly after that. Tubular and later glomerular proteinuria presents in the nephropathic range in some individuals. Glomerular function gradually deteriorates, resulting in renal failure at the age of 7–10 years, but the rate of development of end stage renal disease (ESRD) differs among the patients. Some children reach a plateau in renal function, while others rapidly deteriorate [2]. It is possible that proximal tubular dysfunction causes a high sodium chloride concentration in the macula densa, activating a glomerular-tubular feedback mechanism and decreasing the glomerular filtration rate, resulting in glomerular failure [25, 26]. Overall, NC accounts for about 5 % of childhood renal failure [27]; it is recommended that every patient whose cause of progressive renal insufficiency is unknown should be investigated for the possibility of having cystinosis.

Without therapy, cystine accumulation occurs in virtually all organs and tissues and several complications accompany the tissues damage:

- Photophobia develops, usually at about 10 years of age, due to corneal cystine accumulation (Fig. 1c, d) [28]
- Hypothyroidism is one of the first complications, generally appearing in the first decade of life and contributing to growth failure if untreated [29]
- Heat prostration can occur due to sweating impairment [30]
- Puberty is generally delayed by one or two years. Untreated males develop primary hypogonadism [31]
- Nephrocalcinosis occurs frequently in young cystinosis patients (Fig. 1f)
- Benign intracranial hypertension presents with headaches and papilledema [32, 33]
- Cognition is normal in cystinosis, although neurobehavioral abnormalities not uncommon [33, 34]

Evaluations following initial diagnosis

Definitive diagnosis is based upon a high index of suspicion because of the clinical presentation, supported by slit lamp examination of the corneas showing crystals, which are generally present by 16 months of age [28]. Cystine measurements in a mixed white blood cell preparation enriched in polymorphonuclear leucocytes, performed using the cystine binding protein (CBP) assay or mass spectrometry in a reference lab, secure the diagnosis. Patients generally have values of 3.0–23.0 nmol half-cystine/mg cell protein (normal <0.2) [35]. Immediate nephrology consultation and cysteamine treatment are indicated. Molecular diagnosis with multiplex polymerase chain reaction (PCR) approach to test for the presence or absence of mutations is available on a clinical basis [8]. Prenatal diagnosis for families with a previous history of cystinosis can be made by measurement of the cystine level in cultural amniocytes or chorionic villi [36].

Appropriate diagnostic measures have to be taken in all individuals with cystinosis regardless of age include:

- Height and weight with maintenance of pediatric growth charts [37]

- Renal tubular and glomerular function tests: serum creatinine, phosphate, bicarbonate, potassium concentrations, alkaline phosphatase, 24 hour urine for phosphate , creatinine, protein; glomerular filtration rate [36]
- Thyroid functions studies; lipid panel; serum sex hormones: testosterone, FSH, LH (for post-adolescent males); glucose tolerance test to assess for diabetes if symptoms are present [8, 36]
- Ophthalmologic evaluation, including slit lamp examination of the cornea, funduscopic exam for possible intracranial hypertension [8, 36]
- Renal ultrasound for evaluation of nephrocalcinosis and kidney size [36]
- Renal biopsies are not indicated for diagnostic purposes, but if it is done, morphological studies usually confirm tubulointerstitial disease with deposition of birefringent, polyhedral crystals mostly identifiable focally in interstitial cells (Fig. 1.c,d, e) [37].Biopsy does not provide an explanation for progression of involution of the renal parenchyma [37]. Renal concentrations of cystine vary considerably [37] and do not differentiate between intracellular crystals accumulation and the more benign interstitial disease of children [37]. Electron microscopy of the glomeruli reveals fusion of foot processes and thickening of Bowman's basement membrane. Proximal tubular epithelial cells exhibit many signs of degeneration, with increased number of lysosomes containing dark flocculent precipitate [38]. The "swan neck" lesions involve cells containing only the nucleus and a thin rim of cytoplasm (Fig. 1j); mitochondria, lysosomes, crystalline spaces, and brush border are no longer present [38]. The end stage kidney in cystinosis is so scarred and fibrotic as to be unrecognizable on pathologic examination [19].

Treatment and prevention of early manifestations

Several therapeutic modalities are employed in the care of cystinosis patients:

- Renal Fanconi syndrome is treated by replacement of renal losses, nutritional support, free access to water and supplementation with citrate to alkalinize the blood.

  Bicarbonate, acetate or citrate salts (2–10 mEq/kg/day) are used as anions to help to control acidosis [23]. Management of metabolic acidosis could be problematic using only sodium bicarbonate or citrate; potassium supplements may be required as well [20].
  Oral phosphate replacement with the addition of Vitamin D for better absorption. Supplementation of 1–3 g neutral phosphate per day is usually required [23]. When renal failure develops, phosphate and potassium wasting might be diminished, but cystinosis patients approaching renal failure often continue to require supplementation [20].
  Thiazide diuretics increase proximal tubular reabsorption of bicarbonates and were used for FS in the past, but the use of diuretics in cystinosis has no proven benefit.
  Feeding difficulties not uncommonly required placement of a gastric tube to provide essential caloric intake for growth. Early weaning from G-tube nutritional support is recommended, and is feasible with successful cystine depleting therapy [35]
  Carnitine supplementation can increase plasma carnitine level in NC patients, but clinical benefit has not been demonstrated.

- Children benefit from growth hormone, which enhances growth [36] and may also increase phosphate reabsorption [18]; although well-treated patients usually grow well and do not require growth hormone.
- ACE inhibitors can be used to alleviate severe glomerular proteinuria, but should be indicated with caution due to blocking volume compensation mechanisms [35]
- Thyroxine replacement is frequently required [2]
- Hemodialysis and peritoneal dialysis are temporary measures for ESRD while patients are awaiting renal replacement [35].

Renal transplantation is usually indicated when the creatinine clearance falls below 20 mL/1,73 m^2) and azotemia and hypertension rapidly progress [35]. A renal allograft cures the Fanconi syndrome, but not the other multisystem complications. Symptoms often determine the exact time of transplantation, which in the past has been unavoidable by the age of ten. Live donor grafts are preferred and, with advances in antirejection management the pool of kidney donors, including heterozygous carriers, have greatly increased [35]. The advisability of native kidney nephrectomy depends on the level of polyuria and FS; the procedure may be indicated to limit urine output, electrolyte losses and chronic volume contraction [39, 40]. Cystine crystals can be observed in donor kidneys, due to invasion of host cells [35]. The transplantation experience in the cystinosis population has been equal to that of children transplanted for other causes. [10]. Post-transplant patients should be monitored for immunodeficiency and infections related to anti-rejection medications.

Cystine depleting therapy, currently administered orally in the form of cysteamine bitartrate (Cystagon), has revolutionized the management and prognosis of nephropathic cystinosis. Cysteamine enters the cystinotic lysosome and reacts with cystine, forming the mixed disulfide of half cystine (cysteine) and cysteamine; this compound exits lysosomes via the transport system for cationic amino acids [10]. The cysteamine dosage is usually 60–90 mg/kg /day or 1.35–1.90 g/m^2/day, divided every six hours [41]. Cysteamine depletes

cystinotic cells of more than 90 % of their cystine content, and cysteamine therapy should be considered for all affected individuals, regardless of age and transplantation status [42]. The free thiol does not prevent or reverse the Fanconi syndrome, but slows the glomerular damage and prevents or delays ESRD Fig. 1g [43] and the need for renal allograft. Early, right after diagnosis is made, lifelong and diligent cysteamine therapy prevents hypothyroidism, enhances growth, depletes muscle parenchyma of cystine, and prevents a host of nonrenal complications [39]. Leucocyte cystine levels, obtained 5–6 hours after a dose, are used to evaluate treatment efficacy and appropriate dosage. The side effects of cysteamine are not serious, but are common and include unpleasant taste, nausea and other digestive issues; the most frequent side effect is nausea can be alleviated with anti-emetics in the early stages of therapy initiation; some patients experienced gastrointestinal upset benefit from H2 blockers, while others do not. About one third of the patients comply strictly with the dosage regimen [42]. Topical cysteamine eye drops, administered every 1 to 2 hours, dissolve corneal crystals and ameliorate the photophobia of cystinosis within a few weeks; eye drops usually indicated upon beginning school age [28].

With early, diligent treatment, many children with cystinosis experience the growth of their renal capacity up to the age of 3 years, rather than loss of glomerular function and have survived into their twenties without need of renal transplantation [2].

Late post-transplant complications

In spite of successful treatment with cysteamine, cystine accumulation continues well after renal transplantation and leads to another set of complications at 20–40 years of age, with variable frequencies. Almost every patient who has not received early, diligent, long-term cysteamine therapy will suffered some major complication by age 30 years [35].

The late, nonrenal complications of untreated cystinosis include involvement of different organ systems, i.e., ophthalmological, musculoskeletal, gastrointestinal, endocrine, cardiovascular, neurological.

- Cystine accumulation occurs in the anterior and posterior chambers of the eye, with degenerative pigmentary retinopathy leading to retinal blindness [44]
- About one-third of adult patients are affected with progressive distal vacuolar myopathy, with severe muscle wasting in some cases (Fig. 2e, f) characterized by type 2 fiber atrophy and cystine accumulation in perimysial cells (Fig. 2a,b) [45, 46]. Oromotor problems, experienced by about 50 % of patients, result from a generalized deficit related to muscular dysfunction [46]. Extraparenchymal restrictive lung disease presents in many patients with myopathy [6].
- Renal osteodystrophy and skeletal abnormalities due to metabolic bone disease are exacerbated by the use of glucocorticosteroids [47].
- Most patients with myopathy develop swallowing dysfunction due to deterioration of oropharyngeal muscles; aspiration is a potentially fatal complication in these patients [48]. Hepatomegaly occurs in cystinosis patients with normal synthetic function; noncirrhotic portal hypertension is due to nodular regenerative hyperplasia (Fig. 2c) [49]. Severe cystinosis can be associated with inflammatory bowel disease and perforation [50].
- Hypothyroidism can progress in patients with severe or poorly treated disease. Insulin-dependent diabetes mellitus is a frequent long-term complication due to islet cell involvement [51]. Females do not have symptoms of gonadal dysfunction, and several patients with cystinosis have delivered healthy babies post-transplant. Males manifest a high incidence of hypogonadism due to testicular cystine accumulation, fibrosis and subsequent infertility [31].
- Renin-dependent hypertension can continue until a few years after transplantation [52]. Some patient have hypercholesterolemia with vascular calcifications, coronary artery involvement and cardiomyopathy [53–56]. Bone marrow biopsy reveals cells packed with cystine crystals (Fig. 2d), but hematopoietic function remains unaffected [57, 58].

Neurological complications occur in 5-10 % of adults; they are heterogeneous and include pyramidal and extra pyramidal dysfunction, hydrocephalus, personality and cognitive impairments, basal ganglia calcifications (Fig. 2h) and progressive dementia with cerebral atrophy [33].

Essential diagnostic studies recommended for management of adult, post-transplant late complications of NC include:

- Laboratory studies, including renal function tests, endocrine panels with glucose tolerance test will assess for diabetes [59]
- A leucocyte cystine measurement should be performed every 1–2 years for compliance and dose adjustment [60–62]
- Modified barium swallow video fluoroscopy is important for patients with advanced cystinosis (Fig. 2).
- Pulmonary function tests are recommended to monitor progression of extraparenchymal lung dysfunction.
- Renal ultrasound is routinely performed to determine a renal transplant survival [61].
- Bones X-ray and DEXA scans should be obtained if there is renal osteodystrophy.
- Imaging studies are important for detecting coronary artery calcifications.
- Brain MRI can reveal the cerebral atrophy or cerebral calcifications.
- Neurologic exam can identify the development of neurobehavioral complications.

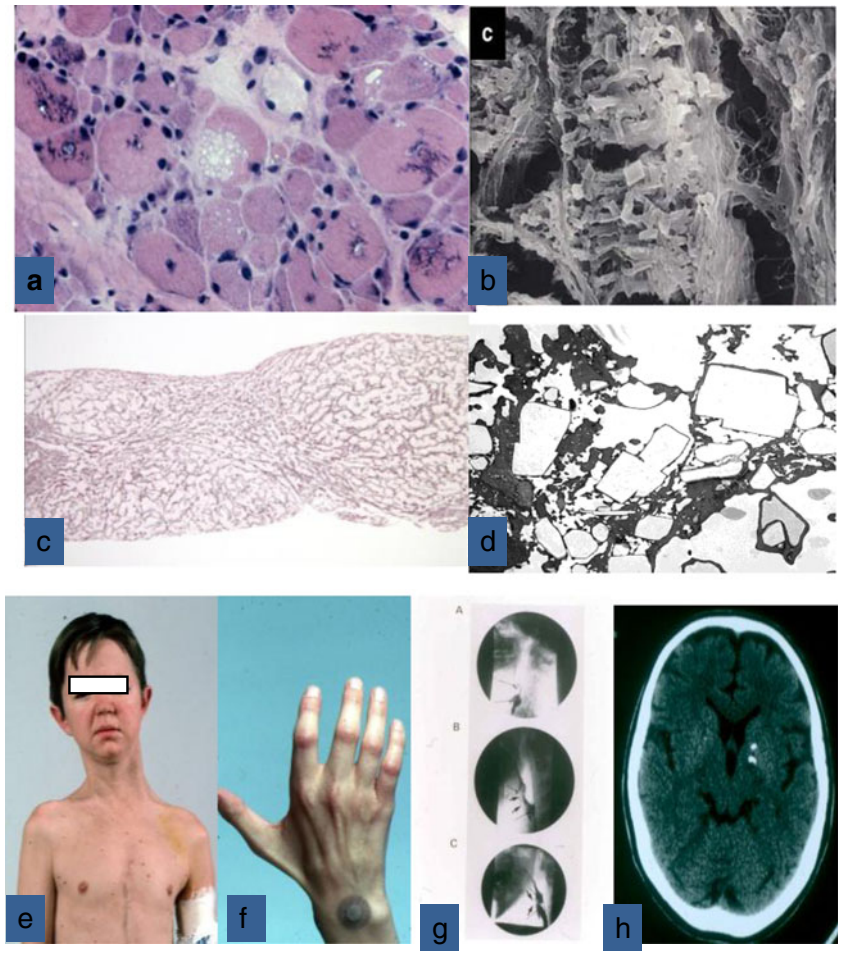

Fig. 2 Late complications of cystinosis **a**) vacuoles in the muscle cells, **b**) cystine crystals in the hand muscles, **c**) hepatic nodular hyperplasia, **d**) bone marrow cystine crystals. **e**) adult patient with trunk muscle wasting, **f**) hand muscle atrophy **g**) barium swallowing study : Pooling in valleculae and pyriform sinuses **h**) cerebral calcifications

- Regular ophthalmological exam together with slit lamp and ERG examinations are mandatory for monitoring visual impairments.
- Carrier testing can be obtained for at-risk relatives with prior identification of disease-causing mutations in the family [8]

With long-term cysteamine therapy, most late complications of cystinosis can be avoided; cysteamine now is treatment of choice for cystinosis throughout the world [60–66].

Conclusion and future prospects

Understanding molecular and pathophysiological mechanisms has opened new horizons for advances in diagnosis and treatment of cystinosis [8, 67]. Mouse model have allowed the comprehensive examination of different tissue involvement in cystinosis, including the central nervous system, and have opened possibilities for gene therapy [68]. Knowing the mechanism of apoptosis in the pathogenesis of cystinosis, involving hundreds of anti- and pro-apoptotic proteins, will potentially lead to opportunities to target control points [13]. Specific inhibition of autophagy can result in significant attenuation of cell death in NC and might attenuate renal injury in cystinosis [14]. The study of renal membrane structure and function in cystinosis patients offers great potential for solving the present enigma of the cause of FS. Newborn screening, when available, can allow earlier institution of metabolic and symptomatic therapies [35, 69, 70]. In the meantime, a multidisciplinary clinical team of geneticists, nephrologists, nutritionists, and general practitioners offer the best and the most comprehensive management and treatment of cystinosis [35, 71–73].

Multiple choice questions: answers are provided following the reference list

1) Possible mechanisms for the renal injury of cystinosis include:

   1. Renal tissue sensitivity to cystine accumulation
   2. Expression of different splice isoforms of *CTNS* in the kidney
   3. High sensitivity of the proximal tubule to apoptosis and energy depletion
   4. All of the above

2) The most frequent cause of renal Fanconi syndrome in children is:

   1. Renal transplant rejection
   2. Heavy metal poisoning, i.e. lead
   3. Oculocerebrorenal syndrome of Lowe
   4. Cystinosis
   5. Mitochondrial cytopathies

3) Which is not a common complication of NC in the first decade of life?

   1. Corneal clouding due to cystine crystal accumulation
   2. Hypothyroidism
   3. Diabetes mellitus
   4. Growth failure
   5. Hypophosphatemic rickets

4) What percent of all childhood ESRD is contributed by NC:

   1. About 10 %
   2. Based on the longitudinal multi center studies, 5-10 %
   3. Based on European Collaborative study, about 5 %
   4. Close to 0 %

5) Normal values for leucocyte cystine content are:

   1. Less than 1 nmol/half cystine/mg cell protein
   2. Less than 0.2 nmol/half cystine/mg cell protein
   3. No higher than 2 nmol/half cystine/mg cell protein
   4. Non- detectable

6) The mechanism of action of cysteamine in targeting the basic defect in cystinosis involves:

   1. Depleting cells by carrying cystine out of the cell into extracellular space
   2. Interacting with cystine and forming cystine-cysteamine disulfide, and exporting this molecule from lysosome into cytosol
   3. Restoring normal cystinosin function
   4. Protecting cells from apoptosis

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Answers:
1) 4.
2) 4.
3) 3.
4) 3
5) 2.
6) 2.