Cystinosis

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Summary

Infantile nephropathic cystinosis is an autosomal recessive disorder caused by mutations in the *CTNS* gene that encodes for cystinosin, a lysosomal cystine/H^+ symporter. With time, cystine accumulation leads to cell dysfunction in various tissues. The kidneys are initially more involved. Most patients are asymptomatic at birth but present in the first or second

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year of life with failure to thrive, polyuria, dehydration, and/or rickets, which are secondary to the renal Fanconi syndrome. With very few exceptions, all patients have corneal cystine crystals by 18 months of age that can help in recognizing the disease. The diagnosis of cystinosis is based on the detection of increased leukocyte cystine levels and demonstration of mutations in the *CTNS* gene (detection rate > 95%). In Northern Europe, 75% of mutated alleles carry a 57-kb deletion; all other types of mutations, generally resulting in a complete or severe loss of function of the cystinosin protein, have been described.

Introduction of cysteamine treatment in the late 1980s and improvements in dialysis and renal transplantation have considerably improved the prognosis of cystinosis. Cysteamine significantly delays progression to end-stage renal kidney disease, but cannot prevent it in most cases. The majority of patients live well into adulthood but, if not appropriately treated with cysteamine, develop other symptoms related to cystine accumulation in various tissues. These include retinal degeneration, hypothyroidism, diabetes mellitus, exocrine pancreatic insufficiency, pubertal retardation and gonadal dysfunction, restrictive pulmonary disease, myopathy, neurological deterioration, and liver involvement. Two milder forms of the disease, namely, juvenile cystinosis and ocular cystinosis, caused by mutations that allow residual function of the cystinosin protein, have also been reported. These patients present with milder symptoms later in childhood or with isolated corneal cystine depositions.

Introduction

Cystinosis is an inherited autosomal recessive disease caused by mutations in the *CTNS* gene, which encodes for cystinosin, a cystine/H^+ symporter that is primarily expressed at the lysosomal membrane (Kalatzis et al. 2001). The *CTNS* gene is located on chromosome 17p13 and was cloned in 1998 (Town et al. 1998). To date, more than 140 different mutations have been identified with a detection rate that exceeds 95% (David et al. 2019).

The *CTNS* gene is composed of 12 exons and spans 23 kb. Cystinosin is composed of 367 amino acids that form a 7-transmembrane domain cotransporter with 2 lysosomal targeting motifs (Cherqui et al. 2001). The most common mutation, accounting for 75% of the affected alleles in Northern Europe, is a 57-kb deletion that removes the first nine exons and part of exon 10 of the *CTNS* gene, the upstream 5′ region of the *CARKL* gene, and the first two non-coding exons of the *TRPV1* gene (Nesterova and Gahl 2017). The function of the *CARKL* gene has been identified in the phosphorylation of sedoheptulose, an intermediate metabolite of the pentose phosphate pathway. The transient receptor potential channel vanilloid subfamily member 1 (*TRPV1*) encodes for an ion channel that is primarily expressed in sensory nerves and is activated by a wide range of chemical stimuli. Patients with a homozygous 57-kb deletion have elevated blood and urinary levels of sedoheptulose. The contribution of the *CARKL* and *TRPV1* genes to the pathogenesis of the disease in patients with homozygous 57-kb deletion has not been fully studied. No major differences in the clinical evolution, however, have been reported when comparing these patients to patients carrying other *CTNS* gene mutations (Wilmer et al. 2010). These include smaller deletions, insertions, nonsense mutations, missense mutations, mutations within the promoter region, and splice site mutations. Intronic mutations affecting normal splicing have also been reported in families whose standard genetic testing failed to identify mutations in one or both alleles, indicating that cystinosis is a monogenic disorder and that intronic regions should be analyzed when no mutations are found in the *CTNS* coding sequence (Taranta et al. 2010; David et al. 2019).

A transcript variant originating from an alternative splicing of exon 12 has been reported and was shown to be expressed also in other cell compartments, including the plasma membrane (Taranta et al. 2008). The contribution of this isoform to the physiopathology of the disease is not known yet (Wilmer et al. 2010).

The incidence of cystinosis varies among countries between 0.5 and 1.0/100,000 live births; several founder mutations have been observed in different geographic regions, such as the 57-kb deletion that originated in Germany around A.D. 500 (Nesterova and Gahl 2017). In its most frequent form, termed infantile nephropathic cystinosis (MIM 219800), children are usually asymptomatic at birth and develop normally during the first 2–3 months of life. They generally present around the age of 6 months with failure to thrive, vomiting, constipation, polyuria, excessive thirst, dehydration, and sometimes rickets (Nesterova and Gahl 2017; Elmonem et al. 2016). These symptoms are the consequence of the renal Fanconi syndrome, which is characterized by excessive urinary losses of water, amino acids, phosphate, uric acid, bicarbonate, glucose, sodium, potassium, low-molecular-weight proteins, and other solutes. Untreated children develop chronic renal failure during early childhood that progresses to end-stage kidney disease in the first decade of life. Patients with cystinosis have a

characteristic facial appearance. Typically, Caucasian children have blond hair, although children from other ethnic groups may retain normal or near-normal pigmentation. With time, cystine crystals deposit in the cornea and are nearly always observed by 18 months of age (Tsilou et al. 2002). They may cause photophobia and chronic inflammatory changes of the anterior chamber, if not treated.

The diagnosis of cystinosis is based on the measurement of leukocyte cystine levels by tandem mass spectrometry or HPLC. Abnormal results need to be confirmed by molecular analysis of the *CTNS* gene. Leukocyte cystine levels are increased up to 100-fold in affected individuals compared with control subjects; levels in heterozygous carriers are only slightly increased.

Since the late 1980s, improvements in dialysis and renal transplantation and the introduction of treatment with the cystine-depleting agent cysteamine have considerably prolonged the life expectancy of patients with cystinosis (Markello et al. 1993; Van Stralen et al. 2011). However, they have also revealed hitherto long-term complications resulting from cystine accumulation in other tissues. These include retinal degeneration, hypothyroidism, diabetes mellitus, exocrine pancreatic insufficiency, pubertal retardation and gonadal dysfunction, restrictive pulmonary disease, myopathy, neurological deterioration, and liver involvement (Gahl et al. 2007; Nesterova and Gahl 2013; Brodin-Sartorius et al. 2012). A few female patients have given birth to normal healthy children, but nearly all tested male patients with infantile cystinosis are infertile. Most late-onset complications improve or are prevented by cysteamine (Gahl et al. 2007; Nesterova and Gahl 2013; Brodin-Sartorius et al. 2012). Corneal cystine crystals do not respond to oral cysteamine and require topical administration. Cysteamine treatment should be optimized by regular measurements of leukocyte cystine levels that allow adapting the dose to target levels (Levtchenko et al. 2004).

Renal transplantation is a well-established treatment for patients reaching end-stage renal disease. Transplanted cystinotic patients have longer renal survival, probably because they have a lower risk of rejection (Van Stralen et al. 2011). They require, nonetheless, standard immunosuppression. Although cysteamine has considerably improved the outcome, progression to end-stage kidney diseases usually cannot be prevented beyond the second or third decade of life (Van Stralen et al. 2011; Nesterova and Gahl 2013).

Early recognition of complications, optimization of nutritional intake, early treatment of Fanconi syndrome and rickets, and growth hormone therapy have also considerably improved the clinical outcome.

In addition to the infantile form, two other milder variants have been identified (Nesterova and Gahl 2017). These include the "juvenile" or "intermediate" form (MIM 219900), which is usually diagnosed during childhood or adolescence and is characterized by less severe renal symptoms, and a third form that has been reported primarily in adults and is characterized by isolated ocular symptoms and is termed "ocular" or "non-nephropathic" cystinosis (MIM 219750).

The severity of the disease co-segregates within family members with very few exceptions. *In vitro* studies of residual cystine transport activity have shown that infantile cystinosis generally results from severe mutations leading to complete loss of function of cystinosin (David et al. 2019).

To date, the physiopathology of cell damage in cystinosis has not been fully elucidated. *In vitro* and *in vivo* studies have shown that cystinotic cells are more prone to apoptosis; abnormalities in glutathione and ATP metabolism, as well as defects in intracellular trafficking and mTOR signaling and impaired autophagy, have been reported (Wilmer et al. 2010; Cherqui and Courtoy 2017; Festa et al. 2018).

Nomenclature

No.	Disorder name	Alternative disorder names	Disorder abbreviation	Gene symbol	Chromosomal localization	Affected protein	OMIM #
65.1	Infantile nephropathic cystinosis		–	*CTNS*	17p13	Cystinosin	219800
65.2	Juvenile cystinosis	Intermediate form	–	*CTNS*	17p13	Cystinosin	219900
65.3	Non-nephropathic cystinosis	Ocular form	–	*CTNS*	17p13	Cystinosin	219750

Metabolic Pathways

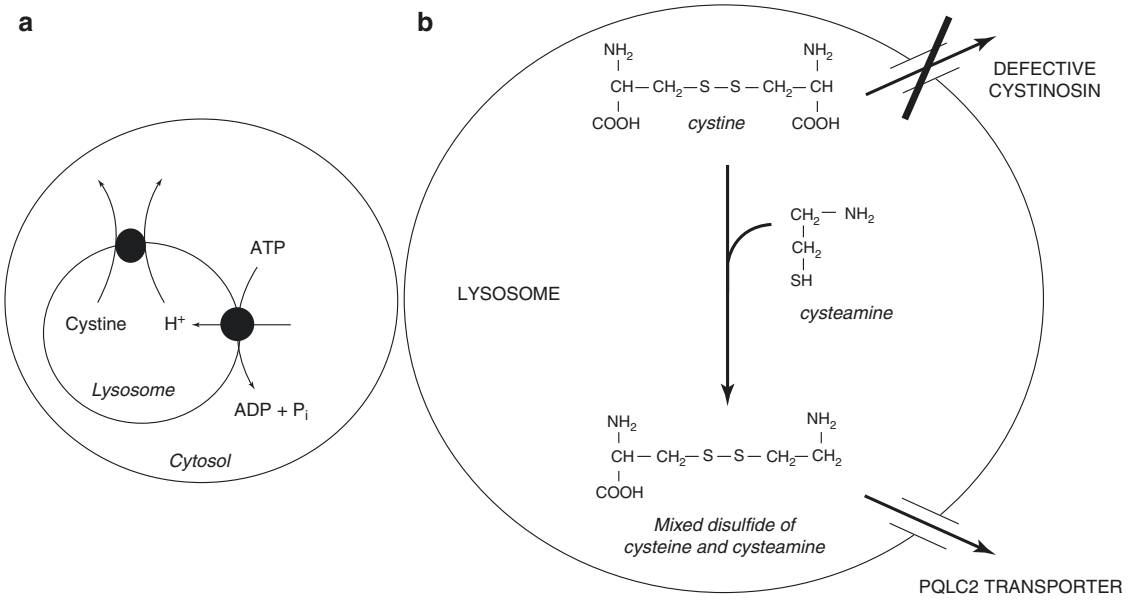


Fig. 65.1 *Panel* (**a**). Cystinosin is a lysosomal cystine carrier. Cystine efflux from lysosomes is dependent on the proton gradient generated by the vacuolar H^+ ATPase. *Panel* (**b**). Mechanism of action of cysteamine. In the presence of cystine, cysteamine forms a mixed disulfide molecule resembling lysine, which is more soluble than cystine and exits the lysosome through a PQLC2 transporter

Signs and Symptoms

Tables 65.1 and 65.2 Nephropathic cystinosis (infantile)

System	Symptom	Neonatal (birth–1 month)	Infancy (1–18 months)	Childhood (1.5–11 years)	Adolescence (11–16 years)	Adulthood (>16 years)[a]
CNS	Cognitive dysfunction			±	±	±
	Peripheral neuropathy					±
	Pyramidal signs					±
	Stroke-like episodes				±	±
	Swallowing difficulties					±
Digestive	Hepatomegaly				±	±
	Liver fibrosis					±
	Splenomegaly					±
Endocrine	Diabetes mellitus				±	±
	Hypogonadism				±	+
	Hypothyroidism			±	±	±
	Infertility (men)				+	+
	Pancreatic dysfunction, endocrine				±	±
	Puberty, delayed				+	
Eye	Photophobia			±	+	+
	Retinopathy	±	±	±	+	+
	Vision, impaired				±	±

Tables 65.1 and 65.2 (continued)

System	Symptom	Neonatal (birth–1 month)	Infancy (1–18 months)	Childhood (1.5–11 years)	Adolescence (11–16 years)	Adulthood (>16 years)[a]
Musculoskeletal	Dental defects			+	+	+
	Diaphragm dysfunction					±
	Genu valgum		±	±	±	±
	Muscle weakness					±
	Myopathy, peripheral					±
	Osteopenia			±	±	±
	Osteoporosis			±	±	±
	Pes planus			±	±	±
	Renal osteodystrophy			±	±	±
	Rickets		±	±	±	±
	Scoliosis			±	±	±
Renal	Nephrocalcinosis		±	±	±	±
	Nephrolithiasis			±	±	±
	Polyuria		+	+	+	±
	Renal failure, chronic			±	±	+
	Renal Fanconi syndrome		+	+	±	±
Other	Corneal cystine crystals		±	+	+	+
	Failure to thrive		+	+		
Routine laboratory	Albumin (U)		↑	↑	↑	
	Bicarbonate (P)		↓	↓	↓-n[b]	
	Calcium (U)		↑	↑	↑[b]	
	Creatinine (P)	n	n	n-↑	n-↑	
	Glucose (U)		↑	↑	↑[b]	
	Phosphate (P)	n	↓	↓-n	↓-n[b]	
	Phosphate (U)	↑	↑	↑	↑[b]	
	Potassium (P)	n	↓	↓	↓-n[b]	
	Potassium (U)		↑	↑	n-↑[b]	
	Sodium (U)		↑	↑	↑[b]	
	Uric acid (P)	n	↓	↓-n	n-↑[b]	
	Uric acid (U)		↑	↑	↑[b]	
Special laboratory	Amino acids (P)	n	n	n	n	
	Amino acids (U)	↑	↑	↑	n-↑[b]	
	Carnitine, total and free (P)	n	↓	↓-n	n	
	Cystine (P)	n	n	n	n	
	Cystine (WBC, FB)[c]	↑	↑	↑	↑	
	T4	n	n	↓-n	↓-n	
	TSH (S)		n-↑	n-↑	n-↑	

Patients with juvenile cystinosis present with symptoms similar to the infantile form, but usually at older age. Renal Fanconi syndrome is usually less pronounced. Non-nephropathic cystinosis 65.3 are exceptional patients that only have corneal cystine crystals.
Patients with ocular cystinosis present only with ocular complaints related to corneal cystine crystals, but not with systemic symptoms
[a]Patients have usually a kidney transplant at that age; symptoms and routine biochemical parameters depend on the graft function
[b]Biochemical features of renal Fanconi syndrome decrease during the decline of the GFR. Urinary features of renal Fanconi syndrome mostly persist even at advanced stages of renal failure and after renal transplantation if the diuresis of the native kidneys is still present
[c]Cystine measurements in cultured fi broblasts (FB) are not recommended routinely. The diagnosis of cystinosis should be confi rmed by molecular diagnosis of the *CTNS* gene

Reference Values

Reference values for leukocyte cystine

Cystine (WBC)	<0.1–0.2 nmol cystine/mg protein[a]
Cystine (PMN)	<0.1–0.2 nmol cystine/mg protein[a]

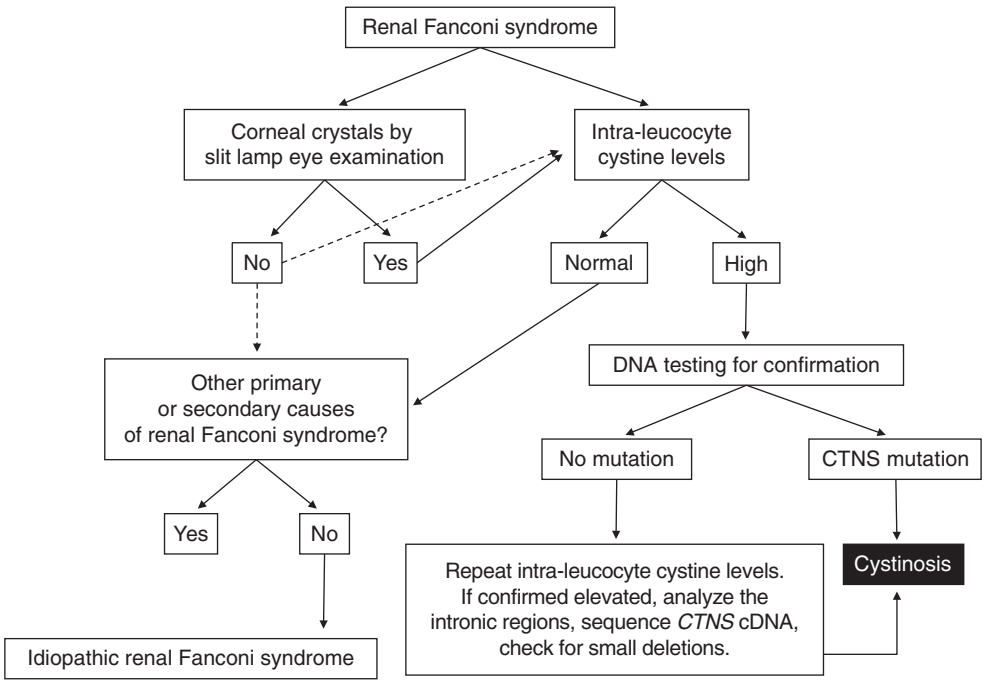
Conversion factor ½ cystine/mg protein = 2× cystine/mg protein
[a]Cystine is sometimes expressed as nmol ½ cystine/mg protein

Pathological Values

Pathological values for leukocyte cystine

Cystine (PMN)	Heterozygotes	0.09–0.65 nmol cystine/mg protein
	Patients at diagnosis	>2 nmol cystine/mg protein
	Patients under cysteamine therapy	<0.6 nmol cystine/mg protein
Cystine (WBC)	Heterozygotes	0.05–0.5 nmol cystine/mg protein
	Patients at diagnosis	>2 nmol cystine/mg protein
	Patients under cysteamine therapy	<0.5 nmol cystine/mg protein

Diagnostic Flowchart



Specimen Collection

Leukocyte cystine measurement is a particularly sensitive test that should be performed in certified laboratories. Each laboratory should produce its own reference values for healthy subjects, heterozygotes carriers, patients at diagnosis, and patients under cysteamine treatment.

Blood is usually collected in heparin or citrate tubes according to the laboratory recommendation; results are significantly influenced by the type of anticoagulant. After collecting blood samples, specimens should be processed as soon possible. The pre-analytic phase is the most delicate part of the analysis. Cystine is currently measured by tandem mass spectrometry (MS-MS) and, less frequently, by high-performance liquid chromatography (HPLC). Results are usually normalized per mg of proteins. Sample contamination with other non-leukocyte proteins (e.g., erythrocyte ghosts) may result in falsely low values. Measurements performed on the granulocyte fraction after Ficoll fractionation are more reliable than measurements on the entire leukocyte population, because granulocytes contain significantly more lysosomes than lymphocytes.

Prenatal Diagnosis

Fetal DNA sequencing allows accurate prenatal diagnosis. In the past, cystine measurements were performed on cultured amniotic cells, but this approach has been abandoned.

Treatment Summary

Oral cysteamine bitartrate efficiently depletes lysosomes from cystine (Fig. 65.1) at a recommended dose of 1.3–1.9 g/m^2/day given at 6-hour intervals (Markello et al. 1993; Elmonem et al. 2016; Gahl et al. 2007). A delayed release preparation is also available in some countries, allowing treatment every 12 hours at a dose that is usually 80% of the regular cysteamine bitartrate dose (Langman et al. 2016). Treatment should be started as soon as the diagnosis is made and continued lifelong. Trough levels should be used to monitor leukocyte cystine levels (Levtchenko et al. 2004). Side effects of cysteamine are mostly restricted to gastrointestinal discomfort, bad breath, and sweat odor. Gastric tolerance can be improved by proton pump inhibitors. Noncompliance because of foul odor and strict administration schedules (especially for standard cysteamine bitartrate) occur frequently, particularly in adolescents, and often correspond to worsening clinical symptoms and prognosis.

Symptomatic therapy includes prescription of appropriate fluid and electrolyte intake, adequate nutrition, and prevention of rickets. Patients with cystinosis should always have free access to water; prolonged heat exposure should be avoided. Young children frequently require tube feeding. Renal tubular waste is significantly reduced by indomethacin (1–3 mg/kg/day in 2–3 separate doses), but this therapy can be nephrotoxic; it can be, however, particularly useful in the first years of life. The doses of potassium, sodium, bicarbonate, and phosphate supplements should be regularly adapted; 1,25-dihydroxycholecalciferol should be prescribed very early to prevent rickets. Carnitine supplementation is recommended in some centers (Veys et al. 2017).

Treatment with recombinant growth hormone improves growth of children that are growth retarded despite cysteamine therapy and adequate metabolic control. Other complications such as hypothyroidism, diabetes, or hypogonadism are treated with L-thyroxin, insulin, or testosterone, respectively. ACE inhibitors can decrease albuminuria and may delay progression of renal failure; however, they may also cause hypotension and impair renal function in patients that are fluid and salt depleted. They should be used therefore with caution, preferably not in conjunction with indomethacin; the dose should be reduced or stopped during the hot weather season (Veys et al. 2017).

Experimental Treatment

The FDA has approved in 2019 a clinical trial in humans for the use of autologous hematopoietic stem cells after gene repair (https://clinicaltrials.gov, trial no. NCT03897361), based on encouraging results obtained in a cystinosis mouse model (Rocca and Cherqui 2018). Up to six subjects will undergo in this phase I/II trial autologous hematopoietic stem cell transplantation after ex vivo gene modification with a pCCL-CTNS lentiviral vector to express CTNS gene.

Online Resources

https://www.cystinosisresearch.org/
https://cystinosis.org/
http://cystinosis-europe.eu/network/
http://www.cystinosisfoundation.org/

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