J Biosci (2023)48:50
DOI: 10.1007/s12038-023-00384-w
© Indian Academy of Sciences

Review

Cystinosis: Status of research and treatment in India and the world

NIKITA VASHIST[1], ANUP ARUNRAO DESHPANDE[2], ARMUGHAM KANAKARAJ[3], RAJAN RAVICHANDRAN[3] and ANAND K BACHHAWAT[1]*

[1]*Department of Biological Sciences, Indian Institute of Science Education and Research Mohali, Punjab, India*

[2]*Clinical and Translational Sciences Department, Ferring Pharmaceuticals A/S, Amager Strandvej 405, 2770 Kastrup, Denmark*

[3]*MIOT Institute of Nephrology, MIOT Hospitals, Chennai, India*

**Corresponding author (Email, anand@iisermohali.ac.in, anand.bachhawat@gmail.com)*

MS received 7 July 2023; accepted 5 September 2023

Cystinosis is an autosomally inherited rare genetic disorder in which cystine accumulates in the lysosome. The defect arises from a mutation in the lysosomal efflux pump, cystinosin (or CTNS). Despite the disease being known for more than a century, research, diagnosis, and treatment in India have been very minimal. In recent years, however, some research on cystinosis has been carried out on understanding the pathophysiology and in the development of a humanized yeast model for interrogating the CTNS protein. There has also been a greater awareness of the disease that has been facilitated by the formation of the Cystinosis Foundation of India just over a decade ago. Awareness among primary physicians is critical for early diagnosis, which in turn is critical for proper treatment. Eight different mutations have been observed in cystinosis patients in India, and the mutation spectrum seems different to what has been seen in the US and Europe. Despite these positive developments, there are immense hurdles still to be surmounted. This includes ensuring that the diagnosis is done sooner, making cysteamine more easily available, and, also for the future, to make accessible the promise of gene therapy to cystinosis patients.

Keywords. CTNS; cysteamine; cystine; cystinosis; Cystinosis Foundation of India

1. Introduction

Cystinosis is a lysosomal storage disorder in which cystine, the oxidized form of cysteine, accumulates in the lysosome. The buildup of cystine crystals in cells affects all organs and tissues, in particular the kidneys and eyes (Gahl *et al.* 2002). Although this recessive genetic disorder is rare, with an incidence of 1 in 100,000 to 200,000 live births, it is a serious disorder which manifests within the first 2 years of life, and if untreated, leads to death.

The first descriptions in literature of the clinical features of cystinosis has been from the early 1900s (Abderhalden 1903). Many decades later, the cause of cystine accumulation was determined to be in the defective cystine efflux of cystine out of the lysosome (Gahl *et al.* 1982; Jonas *et al.* 1982). The gene encoding this lysosomal cystine transporter, cystinosin (or *CTNS*), was identified in 1998 (Town *et al.* 1998).

Several excellent reviews on different aspects of cystinosis are available (Elmonem *et al.* 2016; Cherqui and Courtoy 2017; Jamalpoor *et al.* 2021). The purpose of this overview is therefore primarily to focus on

This article is part of the Topical Collection: The Rare Genetic Disease Research Landscape in India.

Published online: 25 November 2023

cystinosis-related work done in India. The attempt has been to present the perspective of the Indian situation vis-à-vis the situation across the world, both in terms of the research that has been done over the years and also in relation to the diagnosis, treatment, and management of patients with cystinosis. This includes also looking at the current scenario in the world regarding therapies that are showing immense promise. Accordingly, references have been selective or representative rather than exhaustive (figure 1).

2. *CTNS* gene encodes a proton–cystine symporter involved in efflux of lysosomal cystine

The *CTNS* gene, defects in which result in cystinosis, spans 23 kb and is composed of 12 exons. Of these, the 2 initial exons are non-coding while the remaining 10 exons encode a 367-amino-acid protein that localizes to the lysosome (Cherqui *et al.* 2001)

The *CTNS*-encoded protein has 7 transmembrane helices, with a luminal N-terminal region and a short cytosolic C-terminal tail. The N-terminal region possesses 7 glycosylation sites, while the C-terminal region contains the GYDQL motif, a lysosomal targeting motif. Deletion or mutation of this motif leads to partial misdirection of the transporter to the plasma membrane (Cherqui *et al.* 2001). In addition to the GYQDL-targeting motif, YFPQA based in the third cytoplasmic loop has been identified as a secondary motif in lysosomal targeting. Deletion of both these motifs leads to complete re-localization of *CTNS* to the plasma membrane (figure 2). Since the lysosomal lumen is topologically equivalent to the extracellular environment, *CTNS* redirected to the plasma membrane is able to take up the cystine from the extracellular milieu (Kalatzis *et al.* 2001). Using this approach, it could be established that *CTNS* is a proton–cystine symporter where the acidified lysosomal lumen actively drives the transport of cystine along with protons out of the lysosome in 1:1 stoichiometry. Recently the structures of human cystinosin in lumen-open, cytosol-open, and cystine-bound states (Guo *et al.* 2022) have been solved. The structural information should be valuable not only in understanding the mechanism by which the transporter

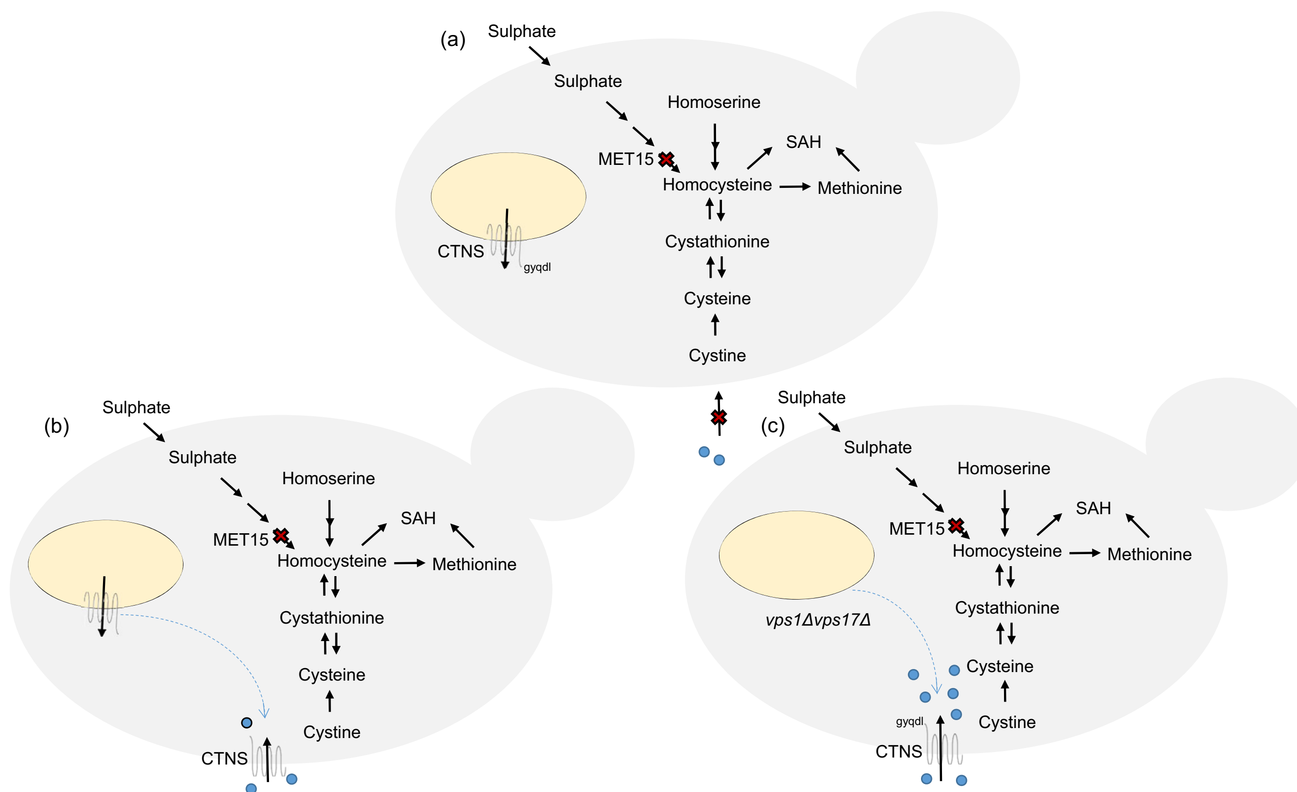

Figure 1. Schematic representation of yeast model for assaying human CTNS. (**a**) *S. cerevisiae met15Δ* cells expressing human CTNS are unable to take up cystine and grow on cystine as the sole sulfur source. (**b**) *S. cerevisiae met15Δ* cells expressing human CTNS with the targeting motif GYDQL deleted are able to allow partial growth on cystine as sole sulfur source. (**c**) *S. cerevisiae met15Δ vps1Δ vps17Δ* cells expressing human CTNS are extensively relocated to the plasma membrane and allow growth on cystine as sole sulfur source.

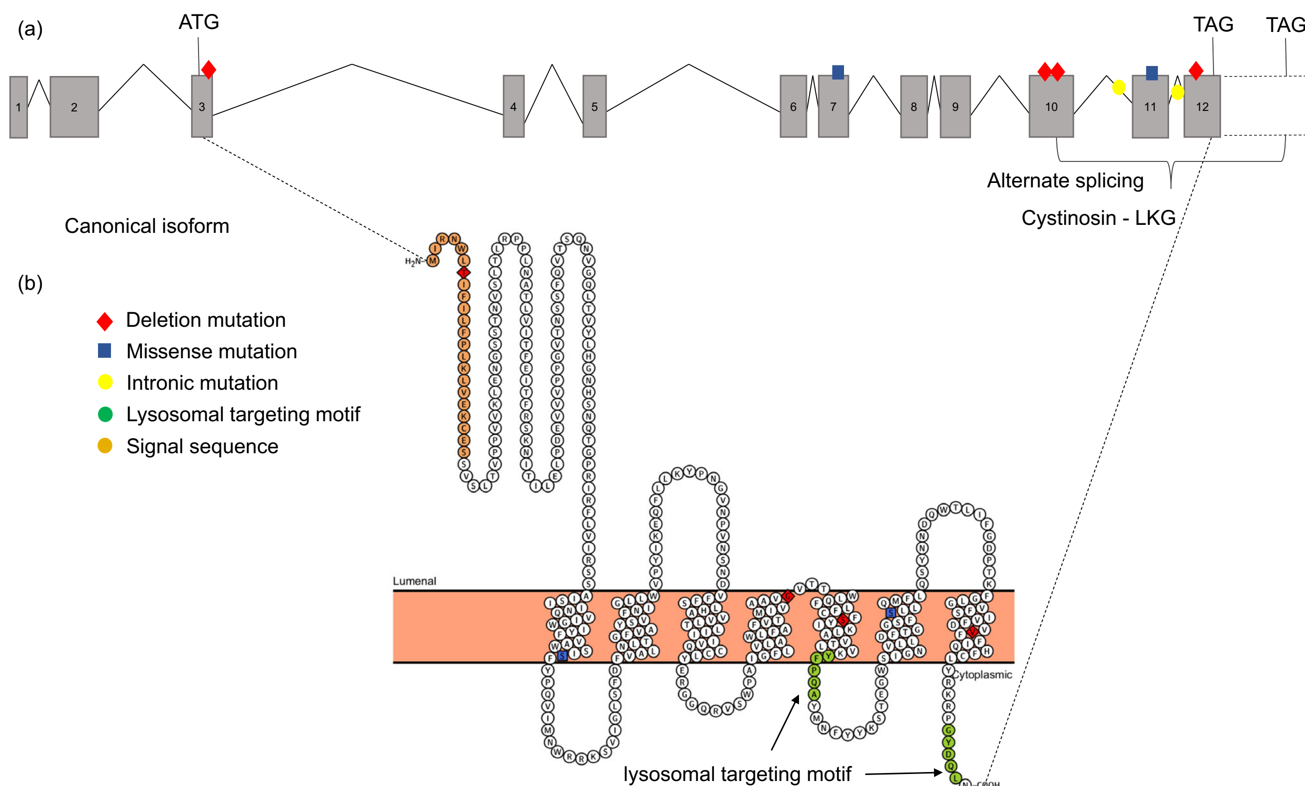

Figure 2. Schematic representation of CTNS genomic structure and the encoded protein highlighting the predominant mutations within the Indian population (**a**) CTNS genomic structure with exons, introns, and exon–intron junctions along with the splice variant CTNS-LKG (not to scale). (**b**) CTNS protein (with all 367 amino acids) depicting the 7 transmembrane helices, with a luminal N-terminal region and a cytosolic C-terminal tail. The two lysosomal targeting motifs, GYDQL at the C-terminal end and YFPQA in the third cytoplasmic loop, are highlighted in green, while the signal sequence is highlighted in orange. The deletion mutations are highlighted in red diamonds, missense mutations in blue squares, and intronic mutations in yellow circles. The image was made using the Protter software (Omasits *et al.* 2014).

functions to efflux cystine, but also to understand the effects of clinically important mutations seen in cystinosis.

3. Clinical aspects of cystinosis and its diagnosis

Based on the severity and age of onset, the disease has been classified into three clinical forms.

Infantile cystinosis, which is the most common form, leads to the most severe phenotype. These patients are asymptomatic at birth. However, between 6 and 12 months, clinical manifestation in these patients begins. Renal proximal tubule dysfunction is observed and is characterized by fluid and electrolyte loss, aminoaciduria, glycosuria, phosphaturia, hypercalciuria, renal tubular acidosis, rickets, and growth retardation. These clinical manifestations are referred to as the renal Fanconi syndrome. Cystine deposition in the cornea is the first extra-renal symptom that appears. At later ages, other organs are also affected, leading to hypothyroidism, insulin-dependent diabetes, hepatosplenomegaly, male hypogonadism, pulmonary dysfunction, myopathy, and CNS calcifications and deterioration. Renal glomerular filtration rate begins to deteriorate from the age of 5–6 years (Elmonem *et al.* 2016). Subsequently, absence of treatment leads to end-stage renal disease (ESRD) by the age of 12 years, and if untreated, proves fatal.

Juvenile (late onset) cystinosis is less frequent, and its clinical manifestations are milder and appear at a late stage and have slow progression. At 12–15 years of age, proteinuria is known to occur, and glomerular function deteriorates at a lower rate; thus, ESRD is reached at variable ages and can be functional until the age of 30–40 years. Photophobia also appears during adolescence in these individuals.

Ocular (adult) cystinosis is characterized by adult onset of mild photophobia with no systemic involvement.

The diagnosis of cystinosis should ideally begin when patients show failure to thrive, and display characteristics of the renal Fanconi syndrome. Since cystinosis is the most common form of inherited renal

Fanconi syndrome, patients showing these symptoms should be suspected for cystinosis. Identification of cystine crystals by slit lamp examination can further establish the diagnosis (Emma *et al.* 2014). Confirmation of the diagnosis can be done by cystine measurement in leukocytes using various methods (Wilmer *et al.* 2011; Elmonem *et al.* 2016). Further confirmation is by *CTNS* gene analysis. Prenatal diagnosis can also be done by genetic analysis of the *CTNS* gene using DNA samples collected from the amniotic fluid (Levtchenko *et al.* 2014).

4. Mutations associated with cystinosis

4.1 *Cystinosis mutations across the world*

More than 140 mutations have been reported worldwide. These include primarily missense and nonsense mutations (57/140) and deletion mutations (45/140). However, the mutations also include insertions, indels (insertion deletions), intronic mutations, and even a few promoter mutations (David *et al.* 2019).

About 50% of the individuals in the US and northern European countries with nephropathic cystinosis are homozygous for a 57 kb deletion covering the first 9 exons and introns of *CTNS,* a part of exon 10 and the upstream 5′ region of the *CARKL* gene, and the first two noncoding regions of the *TRPV1* (transient receptor potential channel vanilloid subfamily) gene, resulting in complete loss of the cystinosin gene (Anikster *et al.* 1999; Gahl *et al.* 2002; Wilmer *et al.* 2010). The *CARKL* gene is involved in the phosphorylation of sedoheptulose, an intermediate metabolite of the pentose phosphate pathway. Accordingly, cystinosis patients with the 57 kb deletion have increased levels of sedoheptulose in blood and urine.

4.2 *Cystinosis mutations found in India*

In India (and other developing countries), relatively fewer patients have been identified with cystinosis. The earliest report appears to be in 2004 (Phadke *et al.* 2004). After that, sporadic reports have been observed (Sharma *et al.* 2011; Kiran *et al.* 2014; Kanthila *et al.* 2015; Mittal *et al.* 2015). The first large cohort of patients was described in 2016 (Ravichandran 2016). There seems to be a very large gap between developing and developed nations in the diagnosis, awareness, and treatment of cystinosis (Bertholet-Thomas *et al.* 2014). Thus, even in the management of the disease, it was found that 7% of patients in developing nations died at a mean age of 5 years, compared with none in developed nations. Further, significantly more patients reached end-stage kidney disease earlier in developing nations (Bertholet-Thomas *et al.* 2017).

There are several practical difficulties and challenges that are being faced both by those affected by cystinosis and their treating physicians, which contributes to the large gap between India and the developed countries. Some of the challenges include: (a) the rarity of the disease and thus the need for a high degree of suspicion especially among primary care physicians, (b) the difficulty in confirming diagnosis, (c) the unavailability of the drug, cysteamine, until very recently in India, (d) the challenges in procuring it, maintaining a life-long supply, and (e) treating long-term complications. These are among the unique problems not only in the diagnosis but also in the treatment and care for these patients. In addition to these factors, there is a significant financial burden that the disease imposes and its socio-economic implications. For this reason, cystinosis needs specialized attention and a dedicated registry to better understand and treat patients effectively.

There have been limited reports on the nature of the mutation in cystinosis patients from India. A very exhaustive and useful documentation of all the cystinosis mutations around the world was tabulated a few years ago (David *et al*. 2019). However, in this report, there was no mention of any mutations from India, despite literature evidence identifying mutations of cystinosis patients from India (Tang *et al.* 2009; Swaminathan and Jeyaraman 2014; Deshpande *et al.* 2017). A larger cohort of cystinosis patients was from AIIMS, New Delhi (Raut *et al*. 2020), where genetic analysis of cystinotic patients from 12 families revealed five known variants of pathogenic nature and six patients from five unrelated families had a frameshift change of threonine to phenylalanine and premature truncation at position 7 (p.Thr7Phefs*7). As the mutation spectrum from India has not been published so far, we have tabulated here the various mutations so far documented from India (table 1). Interestingly, a Ser312Arg, and a deletion Val348del, seem not to have been reported before. The most frequently observed mutations were the frameshifts Thr7Phe*7 and Gly258Ser*30 and the missense mutation Ser141Phe and the deletion Ser270del (table 1; figure 2).

There have been a few reports of unusual presentations of the disease. Thus, in one case a 3-year-old girl with cystinosis had Bartter syndrome and

Table 1. Cystinosis mutations detected in Indian patients

Type of mutation	Position	DNA change	Patients in India	Amino acid change	Countries where seen earlier	Type	Reference
Deletion	Exon 3	C16_19delGACT	6	T7Ffs*7	UK, France, Italy, Mexico, Spain, Netherlands, Iran, Turkey, Thailand	Infantile	Raut *et al.* 2020
Deletion	Exon 10	C770_792del23	4	G258Sfs*30	UK, Tunisia	Infantile	Raut *et al.* 2020; Chadha *et al.* 2022
Deletion	Exon 10	C809_811delCTC	3	Ser270del	UK, Egypt	Infantile	Tang *et al.* 2009; Deepthi *et al.* 2022; Raut *et al.* 2020
Missense	Exon 7	C422C>T	4	Ser141Phe	France, Saudi Arabia	Infantile	Swaminathan and Jeyaraman 2014; Deshpande *et al.* 2018; Raut *et al.* 2020
Missense	Exon 11	C934A>C	2	Ser312Arg		VUS	Raut *et al.* 2020
Intronic	Intron 10	C682_685_698del	1	-		VUS	Raut *et al.* 2020
Intronic	Intron 11	C971–12G>A	1	-	UK, Thailand, S. Africa		Raut *et al.* 2020
Deletion	Exon 12	c.1042_1044del	1	Val348del		VUS Ocular	Naik *et al.* 2019

hypothyroidism (Das *et al.* 2021). In a second case, a 3-year-old boy with Hirschsprung's disease who underwent surgery was ultimately found to have cystinosis on the basis of corneal crystals (Mittal *et al.* 2015).

A very large number of patients were also observed to be born of consanguineous parents.

5. Pathophysiology of cystinosis: A still unsolved puzzle

The loss of cystinosin activity leads to accumulation of cystine in the lysosome, and this leads to a variety of cellular and physiological consequences as seen from clinical manifestations. However, even though cystine accumulation in the lysosome is the primary cause of cystinosis, recent reports suggest the involvement of other physiological processes. This fact is supported by the finding that mere depletion of accumulated lysosomal cystine does not reverse renal Fanconi syndrome-related problems. Thus, the causes behind pathophysiology of cystinosis continue to remain unanswered. Added to the complexity is the observation that there is a spliced variant of *CTNS*, *CTNS-LKG*, that is also expressed in many tissues (Taranta *et al.* 2008; figure 2). Many observations have been made regarding the physiological consequences of cystine accumulation that include ATP depletion (Levtchenko and Monnens 2006), mitochondrial dysfunction (Wilmer *et al.* 2008), abnormal cysteinylation, autophagy, apoptosis (Park *et al.* 2002; Kalatzis and Antignac 2003; Thoene 2007), and oxidative stress, with proximal tubule damage in cystinotic cells (Galarreta *et al.* 2015), unfolded protein response, and impaired lysosomal processing (Gaide Chevronnay *et al.* 2015).

Although ATP depletion is considered a key initiating event in cystinosis, this depletion does not seem to occur through a defective electron transport chain. Reduced phosphate transport in renal tubules has been one explanation (Jamalpoor *et al.* 2021). A hypothesis that we invoked to explain both ATP depletion and the various observations seen in cystinotic cells and patients proposed a 'futile ATP utilization cycle' (Kumar and Bachhawat 2010). This hypothesis, in addition to explaining ATP depletion, integrates many other observations seen in cystinosis. The key feature that leads to the origin of this futile cycle is not the accumulation of cystine in the lysosome *per se*, but the

absence of adequate cysteine in the cytosol that results from the malfunctioning of *CTNS*. Cysteine in the cytosol has essentially two destinations: protein synthesis and formation of glutathione (which accumulates at $\sim$10 mM). Owing to the insufficient supply of cysteine from the lysosome, the enzymatic reaction for the first step in glutathione synthesis (which is an ATP-dependent reaction coupling glutamate with cysteine), instead of forming γ-glu-cys (the first intermediate in glutathione), ends up forming 5-oxoproline (or pyroglutamic acid) through a secondary reaction of the same enzyme (Orlowski and Meister 1971). This is then hydrolyzed back to glutamate by another ATP-dependant reaction. The glutamate so formed, in the absence of cysteine, again forms through the incomplete action of γ-glu-cys synthase, 5-oxoproline. Thus, a futile cycle commences, both steps of which utilize ATP, leading to ATP depletion (Kumar and Bachhawat 2010). In addition to explaining ATP depletion, the hypothesis was able to explain glutathione depletion, the 60-fold excess of 5-oxoproline observed in cystinosis patients (Rizzo *et al.* 1999), as well as the tendency towards apoptosis (Rizzo *et al.* 1999). The hypothesis could further accord an explanation to the observation that Ctns−/− mice had cystine accumulation in their tissues, but did not show the severe renal defects seen in humans (Cherqui *et al*. 2002). However, whether this cycle is indeed fully, partially, or not at all responsible for the ATP depletion in cystinosis has not yet been established.

6. Models for cystinosis: Development of a humanized yeast model

6.1 *Cell line and animal models*

Much of the initial understanding of cystinosis has come from the investigation of patients. However, the pathophysiology of cystinosis and how it has such wide ranging effects on the human body has not been easy to decipher. For these reasons, different models have been developed to investigate further into the pathophysiology.

Several cell line models have been explored in cystinosis. Polymorphonuclear (PMN) leukocytes and fibroblasts from skin biopsies of cystinotic patients showed 80–100 times greater cystine than control subjects and have been used to investigate different consequences of cystine accumulation, and the effects of cysteamine treatment. Primary cell lines have also been immortalized. Thus, urine-derived cystinotic proximal tubule cells (PTC), or human kidney-2 (HK-2) transfected with HPV 16 E6/E7 for immortalization, have been developed to investigate the biochemical consequences of cellular defects. Despite all the advantages, interpretation of results with these cell lines needs to be done carefully considering the characteristics such as high growth rate, influence of external growth factors, and interference of viral proteins with the autophagy pathway. Induced pluripotent stem cells (iPSCs) and organoids also seem promising for developing combination therapies for rescuing cystinotic phenotypes (reviewed in Cheung *et al.* 2022).

Animal models have also been developed. A mouse model has been developed to investigate the consequences of a homozygous *CTNS* knockout. Although it did not completely recapitulate the human phenotype, it has been useful in developing stem cell transplants before moving towards human systems (Cherqui *et al.* 2022). Zebrafish models have been developed by knocking out the *CTNS* gene and examining renal and extrarenal consequences in adult fish (Berlingerio *et al.* 2021).

6.2 *Development of a humanized* CTNS *yeast model*

We had directed our efforts towards developing a yeast-based model to study human *CTNS* (Deshpande *et al.* 2018). The main goal of developing this humanized *CTNS* yeast model was to have a system for robust genetic analysis where one could quickly and easily isolate or analyze new mutants, and also to simplify biochemical assays on the protein. One of the limitations of the different models described above is that none of the models are amenable to either rapid genetic analysis of hCTNS or for biochemical assays.

To develop the yeast model we made use of the organic sulfur auxotrophy of a *Saccharomyces cerevisiae met15Δ* strain. This strain cannot utilize inorganic sulfate, but it can grow on organic sulfur sources such as methionine, cysteine, homocysteine, and glutathione, as it contains the plasma membrane transporters for these sulfur compounds. However, this strain cannot grow on cystine as a sulfur source since *Saccharomyces cerevisiae* lacks a plasma membrane cystine transporter (figure 1a) (Yadav and Bachhawat 2011). In the yeast model, the growth of the yeast on cystine would be dependent on a functional cystinosin that is targeted (or mis-targeted) to the yeast plasma membrane. Thus, we observed that the WT human

CTNS did not allow growth on cystine, but deleting the lysosomal targeting motif, GYQDL, led to the protein being mis-targeted to the plasma membrane, which allowed growth on cystine as the only sulfur source (figure 1b). This observation also indicated a commonality of trafficking of *CTNS* in the two organisms. Mutants of *CTNS* that were known to be non-functional were unable to grow on cystine under these conditions, indicating that this could be a simple plate-based assay for characterizing mutants. As the growth occurred after 3–4 days on plates, we also used this as a means to isolate faster growing mutants. Multiple mutants could be isolated that fell into two categories, those that were more mis-targeted and those that were more catalytically active (Deshpande *et al.* 2018).

To further improve this screen, we also screened a vacuolar protein secretion (vps) knockout library to determine if any of these vps mutants would be more effective in permitting growth of *CTNS* WT or the *CTNS-gyqdlΔ* mutant. Several vps mutants were able to enhance growth on cystine. The vps mutants were then combined, and various combinations evaluated. *vps1Δvps17Δ* appeared to be the best double mutant that allowed growth of even the WT *CTNS* (which had a GYQDL motif), and was used to further characterize the mutants (Deshpande *et al.* 2018) (figure 1c). Using this yeast model we could isolate many gain-of-function mutants. One of these included a patient mutation G197R. This is a mild mutation in humans (ocular cystinosis), and in the yeast model also we observed no significant change in Km, the Michaelis–Menten constant. However, we observed that the mutant was partially mislocalized to the plasma membrane, which may also explain its phenotype, although mild, in humans. This is an example how the yeast model can yield new insights into patient mutations. Using this yeast model we could also, for the first time, determine the kinetics of the WT *CTNS* and show that its Km was comparable to the *CTNS* bearing the GYQDL deletion that has otherwise been used in all evaluations. Prior to this, all kinetic measurements were done with the *CTNS* mutant lacking the C-terminal lysosomal targeting motif. This functional growth-based screen thus enables analysis of loss-of-function patient mutations and the isolation of new gain-of-function mutants and should be a valuable aid to determining the properties of the various mutants (Deshpande *et al.* 2018).

Incidentally, human *CTNS* has a yeast homolog, ERS1 (28% identical, 46% similarity), that is known to localize to the vacuole in *S. cerevisiae*. Although some researchers have proposed that ERS1 as a possible functional homolog of *CTNS* (Simpkins *et al.* 2016), direct biochemical evidence as a cystine transporter is lacking. Studies in our lab could not confirm the functionality of ERS1 in cystine transport despite multiple efforts (Deshpande AA, Shukla A and Bachhawat AK, unpublished observations).

7. Treatment options

7.1 *Cysteamine*

The existing treatment for cystinosis patients is the aminothiol, cysteamine, which depletes lysosomal cystine levels. Although first discovered in 1976 as a treatment option, it was approved by the FDA in 1994. However, it requires a strict regimen of high doses of cysteamine at six-hourly intervals, since cysteamine increases one hour after drug intake and declines to the original values after six hours. Cysteamine enters the lysosome by a specific transporter for aminothiols or aminosulfides (Pisoni *et al.* 1995) and reacts with cystine to produce a cysteine-cysteamine-mixed disulfide which resembles lysine and exits lysosomes through the lysosomal transporter for cationic amino acids, PQLC2, recently identified by genetic and biochemical studies (Jézégou *et al.* 2012; Liu *et al.* 2012).

Cysteamine therapy, if begun early, prolongs renal and extra-renal manifestation. However, while retarding the progression of the disease, it fails to prevent the Fanconi syndrome, ESRD, growth retardation, and male infertility (Gahl *et al.* 2002). Further, as oral/systemic cysteamine does not achieve adequate corneal concentration and hence fails to treat corneal cystine crystals, oral doses of cysteamine also have to be supplemented with eye drops of cysteamine. Development of delayed release formulation of cysteamine bitartrate (Procysbi®) has further helped decrease the frequency of drug administration from six to twelve hours.

7.1.1 *Cysteamine availability in India:* Cysteamine became available in India through the efforts of the Cystinosis Foundation of India. Currently cysteamine is still being imported. Every import requires clearances from the Drug Controller General of India as per the Drugs and Cosmetic Rules of 1945. Manufacturing the drug in India requires further clinical trials, which is not possible with such a small number of patients. Accordingly, a petition was filed by one of us (RR) (Writ petition no. 8449 of 2012) and the Madras High Court directed the Drug Controller to view the request favorably once the manufacturing is done as per the

requirements (Madras High Court judgement dated 19.06.2012). However, despite these developments, and a process for cysteamine synthesis for indigenous manufacture made available by Prof. S. Sankararaman of IIT Madras, no company has been prepared to take up the manufacture of this compound on a large scale. Thus, importing of cysteamine continues, which leads to higher costs, delays, and paperwork. The consequences for patients thus becomes difficult since drug compliance plays a significant role in long-term prognosis.

7.2 *New drug molecules for specific mutations or phenotypes*

Translational readthrough drugs allow stop codons to be read through, and thus could be applicable to patients with stop codons in the *CTNS* gene. One such drug, an aminoglycoside, ELX-02, is currently under phase 2 clinical trials for cystinosis patients (Brasell *et al.* 2019). Other drugs that target specific disrupted processes are also being researched, such as those that restore ATP, reduce oxidative stress, or reduce apoptosis (Taub and Cutuli 2012; Zhang *et al.* 2017; De Leo *et al.* 2020). Some of these drugs (if approved) could possibly be used in combination with cysteamine.

7.3 *Symptomatic therapy and kidney transplantation during end-stage renal disease*

In cases when cystinosis is identified at the later stage of the disease, or upon progression of the disease even with cysteamine treatment, symptomatic therapy is also provided for relief from general symptoms and in certain cases to rescue the side effect of cysteamine therapy. In patients who end up with Fanconi syndrome, glomerular failure, or ESRD, kidney transplant is the only choice left for ESRD. Renal disease does not recur in the transplanted kidney provided cysteamine is continued long term, to maintain a very low cystine level.

7.4 *Allogenic stem cell transplantation therapy*

Owing to the inability of the cysteamine therapy to prevent ESRD, more long-term and permanent solutions were needed. To this end, allogenic stem cell transplantation was attempted. Syngenic wild-type murine hematopoietic stem and progenitor (Ctns+/+) cells (HSPCs) were transplanted into Ctns−/− mice and were able to revert cystinosis symptoms (Syres *et al.* 2009; Rocca and Cherqui 2019). Following this success, efforts were made to translate this study to humans. Allogeneic hematopoietic stem cell transplantation (HSCT) was performed on a 16-year-old Caucasian male affected with cystinosis (Elmonem *et al.* 2018) after full HLA-matching of an unrelated healthy donor. Although there was relief in cystinosis symptoms, the patient suffered acute graft-versus-host disease and finally succumbed 35 months after transplantation.

7.5 *Current efforts towards gene therapy*

A phase 1 and 2 human clinical trial for gene therapy has been initiated after experiments in mice were found to be successful (Cherqui 2021). In this treatment, hematopoietic stem cells are collected from patients and are *ex vivo* gene-modified with the lentiviral vector, pCCL-CTNS (product name: CTNS-RD-04). After the transplantation, the patients are expected to discontinue cysteamine treatment during the assessment period of 2 years. The first patient received the transplant in October 2019, and the sixth patient received it in October 2022. Thus, all 6 patients have received the treatment, and the final assessments are awaited.

Human gene therapy trials for cystinosis took place at the University of California, San Diego (UCSD), and were led by Stephanie Cherqui at the UCSD, a pioneer in the cystinosis field. The trial was funded by the California Institute of Regenerative Medicine (CIRM), Cystinosis Research Foundation, and National Institute of Health (NIH). Eventually a partnership was established with the *ex vivo* gene therapy biotech company, Avrobio Inc. For the phase 3 clinical trials that are being initiated, Avrobio has officially announced the sale of their cystinosis program to Novartis.[1]

One point that remained was whether gene therapy would work with patients bearing the large 57 kb deletion of the cystinosis gene. This is the predominant cystinosis mutation found in the US and northern Europe. As described earlier, this deletion not only deletes the cystinosin gene but the downstream

[1] Among the six patients who received the gene therapy in phase 1/2 clinical trials, was Natalie Stack, daughter of Nancy Stack and Geoffery Stack, who have been driving the Cystinosis Research Foundation for the last 20 years, some years after their daughter was diagnosed with cystinosis. It is an example to show that a private, parent-driven foundation can have resounding achievements.

sedoheptulose kinase gene (*CARKL/SHPK*). However, experiments with mice have indicated that loss of the *CARKL* gene has no impact on the transplanted HPSCs (with restored CTNS) and thus allows Ctns−/− cells to recover from the deficiency of cystine accumulation (Goodman *et al.* 2021). Thus, the large number of patients homozygous for the 57 kb deletion could also benefit from *ex vivo* gene therapy.

8. Cystinosis registries around the world

Cystinosis registries around the world help patients with cystinosis. The Cure Cystinosis International Registry (CCIR) is the only international cystinosis registry, initiated by the Cystinosis Research Foundation, in partnership with Coordination of Rare Diseases at Sanford (CoRDS), USA. An online comprehensive questionnaire collects data on patients with cystinosis in the international community. The Cystinosis European Network was founded in 2016 by cystinosis groups from eight European countries, which helps families contact other families and clinics, and supports research projects on cystinosis. The Cystinosis Rare Disease Group of the UK Kidney Association maintains a register of patients in the UK with cystinosis and helps develop comprehensive patient information, develop best practice guidelines, improve care for patients with cystinosis, and undertake research.

9. Cystinosis Foundation of India

The rarity of the disease, and the difficulties in confirming the diagnosis as well as procuring the drug cysteamine from overseas, led to the launch of the Cystinosis Foundation of India in 2012 by the Sapiens Health Foundation, a non-governmental organization founded by one of us (RR) with donations from the public to help run the organization. Awareness of the disease was spread with the help of booklets distributed at professional meetings.

One of the aims of the foundation was to help patients with cystinosis to obtain cysteamine, which is not available in India. The foundation was successful in bringing cysteamine to India in the year 2013, almost 20 years after it was approved in the US. The Drug Controller General of India provides special permission to individual patients to import the drug. After obtaining special permission to obtain the drug from the Drug Controller General of India, the Cystinosis Foundation of India helps raising money from donations through philanthropists. The drug is then sent by courier from Orphan Europe (now Recordati Rare Diseases Foundation), which then needs to be cleared by the Customs of India (leading to delays) before finally reaching the patient. The efforts of the foundation have thus helped in the import of the drug, identification and enumeration of cystinosis patients in India, identification of important clinical mutations in the Indian population, and evaluation of patient prognosis after treatment.

The foundation has also played a key role in establishing the cystinosis registry in India. A report on the patients registered with the Cystinosis Foundation of India was published some years ago (Ravichandran 2016). A total of 41 patients were registered with the foundation until the end of 2022 (Kanakaraj and Ravichandran, manuscript communicated). Cystinosis was detected earlier in life (less than 2 years) in only 8 of them. The remaining were detected to have cystinosis much later in life, with advanced disease with Fanconi syndrome having been observed in 35 of them.

10. Concluding remarks

Although the progress with gene therapy for the treatment of cystinosis seems very promising, it is still going to be a long time before it becomes affordable and accessible to Indians, and to most other patients in developing countries. Until that time, it is important that there is a better understanding of the pathophysiology of cystinosis, and that further research is conducted on this disease. Further, what also is needed is an increasing awareness of the disease among primary physicians so that diagnosis and treatment is begun early. Easy and inexpensive access to the drug also needs urgent attention.

Acknowledgements

NV is a recipient of a CSIR Senior Research Fellowship. AKB acknowledges funding from the Cystinosis Research Foundation, USA, for part of the work carried out in his lab during 2014–2017 and currently from IISER Mohali and CEFIPRA (Project No. 6503–5).

References

Abderhalden E 1903 Familiäre Cystindiathese. *J. Biol. Chem.* **38** 557–561

Anikster Y, Lucero C, Touchman JW, *et al*. 1999 Identification and detection of the common 65-kb deletion breakpoint in the nephropathic cystinosis gene (CTNS). *Mol. Genet. Metab.* **66** 111–116

Berlingerio SP, He J, De Groef L, *et al*. 2021 Renal and extra renal manifestations in adult zebrafish model of cystinosis. *Int. J. Mol. Sci.* **22** 9398

Bertholet-Thomas A, Bacchetta J, Tasic V, *et al.* 2014 Nephropathic cystinosis-a gap between developing and developed nations. *N. Engl. J. Med.* **370** 1366–1367

Bertholet-Thomas A, Berthiller J, Tasic V, *et al.* 2017 Worldwide view of nephropathic cystinosis: results from a survey from 30 countries. *BMC Nephrol.* **18** 210

Brasell EJ, Chu L, El Kares R, *et al*. 2019 The aminoglycoside geneticin permits translational readthrough of the CTNS W138X nonsense mutation in fibroblasts from patients with nephropathic cystinosis. *Pediatr. Nephrol.* **34** 873–881

Chadha N, Anand K, Agarwal P, *et al.* 2022 POS-090 Cystinosis: A rare but treatable cause of proximal renal tubular acidosis. *Kidney Int. Rep.* **7** S37–S38

Cherqui S, Kalatzis V, Trugnan G, *et al*. 2001 The targeting of cystinosin to the lysosomal membrane requires a tyrosine-based signal and a novel sorting motif. *J. Biol. Chem.* **276** 13314–13321

Cherqui S and Courtoy PJ 2017 The renal Fanconi syndrome in cystinosis: pathogenic insights and therapeutic perspectives. *Nat. Rev. Nephrol.* **13** 115–131

Cherqui S 2021 Hematopoietic stem cell gene therapy for cystinosis: From bench-to-bedside. *Cells* **10** 3273

Cherqui S, Sevin C, Hamard G *et al.* 2002 Intralysosomal cystine accumulation in mice lacking cystinosin, the protein defective in cystinosis. *Mol. Cell Biol.* **22** 7622–7632

Cheung PY, Harrison PT, Davidson AJ, *et al*. 2022 In vitro and in vivo models to study nephropathic cystinosis. *Cells* **11** 6

Das G, Nanda PM, Kaur A, *et al*. 2021 Bartter syndrome and hypothyroidism masquerading cystinosis in a 3-year-old girl: rare manifestation of a rare disease. *BMJ Case Rep.* **14** e242954

David D, PrincieroBerlingerio S, Elmonem MA, *et al*. 2019 Molecular basis of cystinosis: geographic distribution, functional consequences of mutations in the CTNS gene, and potential for repair. *Nephron* **141** 133–146

Deepthi B, Krishnamurthy S, Karunakar P, *et al.* 2022 Atypical manifestations of infantile-onset nephropathic cystinosis: a diagnostic challenge. *CEN Case Rep*. **11** 347–350

De Leo E, Elmonem MA, Berlingerio SP, *et al*. 2020 Cell-based phenotypic drug screening identifies luteolin as candidate therapeutic for nephropathic cystinosis. *Am. J. Nephrol.* **31** 1522–1537

Deshpande AA, Ravichandran R and Bachhawat AK 2017 Molecular analysis of the CTNS gene in Indians with nephropathic cystinosis. *Indian J. Pediatr.* **84** 240–241

Deshpande AA, Shukla A and Bachhawat AK 2018 A genetic screen for investigating the human lysosomal cystine transporter Cystinosin. *Sci. Rep.* **8** 3442

Elmonem MA, Veys KR, Soliman NA, *et al.* 2016 Cystinosis: a review. *Orphanet J. Rare Dis.* **11** 47

Elmonem MA, Veys K, Arcolino FO, *et al*. 2018 Allogeneic HSCT transfers wild-type cystinosin to nonhematological epithelial cells in cystinosis: First human report. *Am. J. Transplant.* **18** 2823–2828

Emma F, Nesterova G, Langman C, *et al.* 2014 Nephropathic cystinosis: an international consensus document. *Nephrol. Dial. Transplant.* **29** iv87–iv94

Gahl W, Bashan N and Tietze F 1982 Cystine transport is defective in isolated leukocyte lysosomes from patients with cystinosis. *Science* **217** 1263–1265

Gahl WA, Thoene JG and Schneider JA 2002 Cystinosis. *N. Engl. J. Med.* **347** 111–121

Gaide Chevronnay HP, Janssens V, Van Der Smissen P, *et al*. 2015 A mouse model suggests two mechanisms for thyroid alterations in infantile cystinosis: decreased thyroglobulin synthesis due to endoplasmic reticulum stress/unfolded protein response and impaired lysosomal processing. *Endocrinology* **156** 2349–2364

Galarreta CI, Forbes MS, Thornhill BA, *et al*. 2015 The swan-neck lesion: proximal tubular adaptation to oxidative stress in nephropathic cystinosis. *Am. J. Physiol. Renal Physiol.* **308** F1155–F1166

Goodman S, Khan M, Sharma J, *et al*. 2021 Deficiency of the sedoheptulose kinase (Shpk) does not alter the ability of hematopoietic stem cells to rescue cystinosis in the mouse model. *Mol. Genet. Metab.* **134** 309–316

Guo X, Schmiege P, Assafa TE, *et al*. 2022 Structure and mechanism of human cystine exporter cystinosin. *Cell* **185** e3718

Jamalpoor A, Othman A, Levtchenko EN, *et al.* 2021 Molecular mechanisms and treatment options of nephropathic cystinosis. *Trends Mol. Med.* **27** 673–686

Jézégou A, Llinares E, Anne C, *et al*. 2012 Heptahelical protein PQLC2 is a lysosomal cationic amino acid exporter underlying the action of cysteamine in cystinosis therapy. *Proc. Natl. Acad. Sci. USA* **109** E3434–E3443

Jonas AJ, Smith ML and Schneider JA 1982 ATP-dependent lysosomal cystine efflux is defective in cystinosis. *J. Biol. Chem.* **257** 13185–13188

Kalatzis V, Cherqui S, Antignac C, *et al*. 2001 Cystinosin, the protein defective in cystinosis, is a H+-driven lysosomal cystine transporter. *EMBO J.* **20** 5940–5949

Kalatzis V and Antignac C 2003 New aspects of the pathogenesis of cystinosis. *Pediatr. Nephrol.* **18** 207–215

Kanthila J, Dsa S and Bhat KG 2015 Nephropathic cystinosis presenting as renal fanconi syndrome without glycosuria. *J. Clin. Diagn. Res.* **9** SD05–06

Kiran BV, Barman H and Iyengar A 2014 Clinical profile and outcome of renal tubular disorders in children: A single center experience. *Indian J. Nephrol.* **24** 362–366

Kumar A and Bachhawat AK 2010 A futile cycle, formed between two ATP-dependant γ-glutamyl cycle enzymes, γ-glutamyl cysteine synthetase and 5-oxoprolinase: the cause of cellular ATP depletion in nephrotic cystinosis? *J. Biosci.* **35** 21–25

Levtchenko E and Monnens L 2006 Development of Fanconi syndrome during infancy in a patient with cystinosis. *Acta Paediatr.* **95** 379–380

Levtchenko E, Van Den Heuvel L, Emma F, *et al*. 2014 Clinical utility gene card for: cystinosis. *Eur. J. Hum. Genet.* **22** 713–713

Liu B, Du H, Rutkowski R, *et al*. 2012 LAAT-1 is the lysosomal lysine/arginine transporter that maintains amino acid homeostasis. *Science* **337** 351–354

Mittal D, Bagga A, Tandon R, *et al*. 2015 Hirschsprung's disease with infantile nephropathic cystinosis. *J. Indian Assoc. Pediatr. Surg.* **20** 153

Naik MP, Sethi HS and Dabas S 2019 Ocular cystinosis: Rarity redefined. *Indian J. Ophthalmol.* **67** 1158–1159

Omasits U, Ahrens CH, Müller S, *et al*. 2014 Protter: interactive protein feature visualization and integration with experimental proteomic data. *Bioinformatics* **30** 884–886

Orlowski M and Meister A 1971 Isolation of highly purified γ-glutamylcysteine synthetase from rat kidney. *Biochemistry* **10** 372–380

Park M, Helip-Wooley A and Thoene J 2002 Lysosomal cystine storage augments apoptosis in cultured human fibroblasts and renal tubular epithelial cells. *J. Am. Soc. Nephrol.* **13** 2878–2887

Phadke K, Kumar P, Karthik S, *et al*. 2004 Infantile nephropathic cystinosis. *Indian J. Nephrol.* **14** 18–21

Pisoni RL, Park GY, Velilla VQ, *et al*. 1995 Detection and characterization of a transport system mediating cysteamine entry into human fibroblast lysosomes: specificity for aminoethylthiol and aminoethyl sulfide derivatives. *J. Biol. Chem.* **270** 1179–1184

Raut S, Khandelwal P, Sinha A, *et al.* 2020 Infantile nephropathic cystinosis: clinical features and outcome. *Asian J. Pediatr. Nephrol.* **3** 15

Ravichandran R 2016 Cystinosis a truly orphan disease - Report of the Cystinosis Foundation India. *Orphanet J. Rare Dis.* **3** 795 1–4

Rizzo C, Ribes A, Pastore A, *et al*. 1999 Pyroglutamic aciduria and nephropathic cystinosis. *J. Inherit. Metab. Dis.* **22** 224

Rocca CJ and Cherqui S 2019 Potential use of stem cells as a therapy for cystinosis. *Pediatr. Nephrol.* **34** 965–973

Sharma A, Gupta R, Sethi SK, *et al*. 2011 Giant cell transformation of podocytes: A unique histological feature associated with cystinosis. *Indian J. Nephrol.* **21** 123–125

Simpkins JA, Rickel KE, Madeo M, *et al*. 2016 Disruption of a cystine transporter downregulates expression of genes involved in sulfur regulation and cellular respiration. *Biol. Open* **5** 689–697

Swaminathan K and Jeyaraman M 2014 Cystinosis: An "eye opener." *Apollo Med.* **11** 43–45

Syres K, Harrison F, Tadlock M, *et al*. 2009 Successful treatment of the murine model of cystinosis using bone marrow cell transplantation. *Blood Am. J. Hematol.* **114** 2542–2552

Tang S, Danda S, Zoleikhaeian M, *et al*. 2009 An Indian boy with nephropathic cystinosis: a case report and molecular analysis of CTNS mutation. *Genet. Test. Mol.* **13** 435–438

Taranta A, Palma A and Emma F 2008 Pathogenesis of cell dysfunction in nephropathic cystinosis. *J. Paediatr. Child Health* **18** S51–S53

Taub M and Cutuli F 2012 Activation of AMP kinase plays a role in the increased apoptosis in the renal proximal tubule in cystinosis. *Biochem. Biophys. Res. Commun.* **426** 516–521

Thoene JG 2007 A review of the role of enhanced apoptosis in the pathophysiology of cystinosis. *Mol. Genet. Metab.* **92** 292–298

Town M, Jean G, Cherqui S, *et al*. 1998 A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat. Genet.* **18** 319–324

Wilmer MJ, van den Heuvel LP, Rodenburg RJ, *et al*. 2008 Mitochondrial complex V expression and activity in cystinotic fibroblasts. *Pediatr. Res.* **64** 495–497

Wilmer MJ, Emma F and Levtchenko EN 2010 The pathogenesis of cystinosis: mechanisms beyond cystine accumulation. *Am. J. Physiol. Renal Physiol.* **299** F905–F916

Wilmer MJ, Schoeber JP, van den Heuvel LP, *et al*. 2011 Cystinosis: practical tools for diagnosis and treatment. *Pediatr. Nephrol.* **26** 205–215

Yadav AK and Bachhawat AK 2011 CgCYN1, a plasma membrane cystine-specific transporter of *Candida glabrata* with orthologues prevalent among pathogenic yeast and fungi. *J. Biol. Chem.* **286** 19714–19723

Zhang J, Johnson JL, He J, *et al*. 2017 Cystinosin, the small GTPase Rab11, and the Rab7 effector RILP regulate intracellular trafficking of the chaperone-mediated autophagy receptor LAMP2A. *J. Biol. Chem.* **292** 10328–10346

Corresponding editor: ALOK BHATTACHARYA