



HHS Public Access

Author manuscript

Nat Rev Nephrol. Author manuscript; available in PMC 2017 October 26.

Published in final edited form as:

Nat Rev Nephrol. 2017 February ; 13(2): 115–131. doi:10.1038/nrneph.2016.182.

The renal Fanconi syndrome in cystinosis: pathogenic insights and therapeutic perspectives

Stephanie Cherqui¹ and Pierre J. Courtoy²

¹Department of Pediatrics, Division of Genetics, University of California San Diego, 9500 Gilman Drive, MC 0734, La Jolla, California 92093–0734, USA

²Cell biology, de Duve Institute and Université catholique de Louvain, UCL-Brussels, 75 Avenue Hippocrate, B-1200 Brussels, Belgium

Abstract

Cystinosis is an autosomal recessive metabolic disease that belongs to the family of lysosomal storage disorders. It is caused by a defect in the lysosomal cystine transporter, cystinosin, which results in an accumulation of cystine in all organs. Despite the ubiquitous expression of cystinosin, a renal Fanconi syndrome is often the first manifestation of cystinosis, usually presenting within the first year of life and characterized by the early and severe dysfunction of proximal tubule cells, highlighting the unique vulnerability of this cell type. The current therapy for cystinosis, cysteamine, facilitates lysosomal cystine clearance and greatly delays progression to kidney failure but is unable to correct the Fanconi syndrome. This Review summarizes decades of studies that have fostered a better understanding of the pathogenesis of the renal Fanconi syndrome associated with cystinosis. These studies have unraveled some of the early molecular changes that occur before the onset of tubular atrophy and identified a role for cystinosin beyond cystine transport, in endolysosomal trafficking and proteolysis, lysosomal clearance, autophagy and the regulation of energy balance. These studies have also led to the identification of new potential therapeutic targets and here, we outline the potential role of stem cell therapy for cystinosis and provide insights into the mechanism of haematopoietic stem cell-mediated kidney protection.

Introduction

Renal Fanconi syndrome presents as a generalized dysfunction of the proximal tubule, characterized by the presence of polyuria, phosphaturia, glycosuria, proteinuria, acidosis,

Correspondence to S.C. scherqui@ucsd.edu.

Author contributions

S.C. and P.J.C. contributed equally to researching data for the article, discussion of the content, and revising or editing the manuscript before submission.

Competing interests

The authors declare no competing interests.

DATABASES

MIM 219800: <http://www.omim.org/entry/219800>

MIM 219900: <https://omim.org/entry/219900>

MIM 219750: <http://www.omim.org/entry/219750>

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

growth retardation and rickets^{1–3}. The leading cause of inherited renal Fanconi syndrome in children is cystinosis, which accounts for up to 20% of cases of hereditary tubular disorders⁴.

Cystinosis is an autosomal recessive metabolic disease that belongs to the family of lysosomal storage disorders. It is characterized by an accumulation of cystine within all organs as a result of a deletion or mutations in *CTNS*, which encodes the lysosomal cystine transporter, cystinosin⁵. The most frequent and most severe form of cystinosis is infantile cystinosis (MIM 219800), which is the focus of this Review and is hereafter simply referred to as ‘cystinosis’. Children with this form of cystinosis appear normal at birth, but demonstrate failure to thrive by around 6–9 months of age. By 6–18 months of age, symptoms of kidney dysfunction, such as polyuria and polydipsia, develop, as well as rickets. Continuing loss of glomerular function eventually leads to renal insufficiency by ~10 years of age if untreated⁵ and at a median of ~20 years of age if cysteamine treatment is initiated before 5 years of age⁶. Deposition of cystine crystals in the cornea occurs early in the course of disease, causing photophobia and painful corneal erosions⁷. In their second to third decade of life, patients can develop hypothyroidism, hypogonadism, diabetes, myopathy, and deterioration of the central nervous system^{8–10}.

Juvenile (MIM 219900)¹¹ and ocular (MIM 219750)¹² cystinosis are milder and rarer than infantile cystinosis. Juvenile cystinosis usually manifests around 12 years of age and causes photophobia and kidney dysfunction at variable ages, with a combination of glomerular and tubular alterations causing marked proteinuria, eventually leading to end-stage renal disease (ESRD). Ocular cystinosis is characterized by adult-onset mild photophobia without renal manifestations.

CTNS — which encodes the lysosomal cystine–proton co-transporter, cystinosin — was first identified as the causative gene in cystinosis in 1998 (REFS 13–15). *CTNS* is expressed in all tissues, and mutations in the gene therefore eventually cause multi-systemic disease. Deletion of *Ctns* in mice leads to the development of multiple features of cystinosis, including ocular defects with deposition of corneal cystine crystals^{16,17}, bone demineralization and deformities¹⁸, muscle wasting¹⁹ and thyroid dysfunction²⁰. On certain genetic backgrounds, deletion of *Ctns* also¹⁸ leads to the development of renal pathology, in particular a renal Fanconi syndrome between 4 and 6 months of age²¹. The phenotype of the Fanconi syndrome in mice is less severe than that observed in humans; nevertheless, the mice eventually develop ESRD²¹. This animal model has been instrumental for improving our understanding of kidney pathophysiology in cystinosis. A hallmark of renal disease in *Ctns*-knockout mice is the accumulation of cystine crystals within proximal tubular cells (PTCs). Interestingly, however, evidence suggests that these PTCs become dedifferentiated before crystals accumulate, losing their brush border, becoming flattened, and eventually resting on a thicker basement membrane than do healthy PTCs^{18,21}. These tubular changes typically begin at the glomerulotubular junction and extend distally, evolving to tubular atrophy with development of characteristic swan neck deformities²². In addition, heavy inflammatory cell infiltrates can be observed in the renal interstitium^{21,23}.

Beyond supportive therapy, the only specific therapy for cystinosis is cysteamine, which acts by depleting cystine in lysosomes. Cysteamine was first used in cystinosis patients in 1976 but wasn't approved by the FDA until 1994 (REFS 24,25). Although early administration of cysteamine therapy delays the progression of cystinosis complications including ESRD^{8,10,26,27}, it has no effect on established renal Fanconi syndrome. Moreover, the renal Fanconi syndrome in cystinosis is unique in that it is typically the earliest manifestation of the disease process. In addition, in cystinotic mice, the renal Fanconi syndrome manifests before structural anomalies, such as cystine crystal deposition or PTC dedifferentiation and atrophy, are detected in PTCs. These findings raise questions as to the unique features of PTCs that explain their early vulnerability in cystinosis; the molecular mechanisms that account for the development of PTC dysfunction before the development of detectable lesions; the cellular and molecular mechanisms that link cystinosis deficiency and the resulting lysosomal defects to histopathological changes and eventual tubular atrophy; and how best to translate this knowledge into better management approaches, and potentially a cure for cystinosis.

This Review summarizes decades of research into the pathogenesis of renal Fanconi syndrome in cystinosis, focusing on findings that have provided insights into the pathogenic roles of global metabolic and oxidation defects; impaired endolysosomal trafficking, proteolysis and signalling; and apical dedifferentiation in disease processes. We emphasize the importance of cystinosis in the maintenance of cellular homeostasis, beyond its function in cystine transport and discuss current treatment approaches as well as emerging therapeutic avenues that have arisen from improved understanding of the pathophysiology of cystinosis and from studies of haematopoietic stem cell transplantation in mice.

Cystinosis: an overview

Clinical course of cystinosis

Renal Fanconi syndrome is the most common initial clinical manifestation of infantile cystinosis, typically becoming evident at 6–18 months of age^{28,29}. Although glomerular proteinuria can be detected early in the disease course (as early as 1 year of age)^{30,31}, glomerular filtration rate begins to deteriorate from 5–6 years of age, leading to the development of ESRD. According to the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) 2010–2011 annual report, cystinosis accounts for 1.4% of children on dialysis and 2.1% of paediatric renal transplant recipients in the USA³². Cystinosis is, however, a multi-systemic disease with accumulation of cystine in lysosomes throughout the body. Deposition of cystine crystals in the cornea, which begins in infancy, gradually leads to photophobia, blepharospasm, and recurrent corneal erosions⁷. Retinopathy can also develop as early as 3 years of age, and can cause blindness^{33,34}. Affected individuals often develop endocrine complications such as hypothyroidism, diabetes, pubertal delay, and male hypogonadism³⁵. Vascular damage^{36,37} and hypertension^{38,39} have also been described in relation to hyperplasia of renin-producing cells in the juxtaglomerular apparatus³⁹; chronic kidney disease and disruption of calcium, phosphate and parathyroid hormone homeostasis; and cystine accumulation in blood vessels. Cystinosis also causes rickets with bone deformities and fragility⁴⁰, which is attributed to

massive urinary phosphate loss, defective conversion of 25-hydroxyvitamin D into active calcitriol in the kidney and deposition of cystine in bone, although the exact pathophysiology is not fully understood⁴¹. Cystinosis is also associated with the development of neuromuscular and neurocognitive dysfunction including the deterioration of fine vision, motor coordination and peripheral muscle strength, and the development of swallowing difficulties^{42–47}. Impaired swallowing and chronic respiratory dysfunction favour the development of aspiration pneumonia, a major cause of death in affected patients^{48,49}. Most of these complications, with the exception of established renal Fanconi syndrome, can be delayed or even prevented with cysteamine therapy^{8,9}.

Epidemiology of cystinosis

Cystinosis is a rare, autosomal-recessive, monogenic disorder with a general incidence of 1:100,000–1:200,000 live births⁵⁰. A higher local incidence has been reported in French Brittany (1:26,000 live births)^{51,52} and Saguenay–Québec (1:62,500 live births)⁵³, due to distinct founder mutations in *CTNS* (c.898–900 + 24del27 (REFS 54,55) and p.Trp138X (REF. 56), respectively). Another founder mutation, involving a 57 kb deletion in *CTNS*, is the most common cause of cystinosis, affecting 65% of patients of northern European descent^{13,27,57} but almost never occurs in other families^{58–61}. Cystinosis is also reported in countries with high levels of consanguinity such as some countries in the Middle East, but accurate prevalence data are lacking^{58,61–64}. The low reported frequency of cystinosis in Russia⁶⁵, India⁶⁶ and China⁶⁷ likely reflects underdiagnosis.

Molecular basis of cystinosis

CTNS is located on the short arm of chromosome 17 (REF. 68) and comprises 12 exons, the two first of which are non-coding¹³. The remaining 10 exons encode a 367 amino-acid protein, called cystinosin, which is predicted to contain seven transmembrane domains, a luminal N-terminal region bearing seven *N*-glycosylation sites and a cystolic C-terminal GYDQL lysosomal targeting signal. A longer *CTNS* isoform can be generated by alternate splicing of exon 12, which removes the GYDQL motif and adds 39 amino acids to produce a 400 amino-acid protein, called cystinosin-LKG⁶⁹ (FIG. 1). Like the canonical form of cystinosin, cystinosin-LKG localizes at lysosomes, but unlike canonical cystinosin, it also resides in the secretory apparatus and the plasma membrane, where it mediates proton-coupled cystine transport⁷⁰. The cystinosin-LKG isoform represents 5–20% of all *CTNS* transcripts in most tissues (representing up to 50% of all *CTNS* transcripts in testes)⁷¹.

Subcellular localization studies have confirmed the localization of the canonical cystinosin isoform to the lysosomal membrane¹⁴. The GYDQL motif interacts with the adaptor protein complex 3 (AP-3), which is responsible for direct lysosomal targeting⁷². An additional, non-classical lysosomal targeting motif located in the fifth cytoplasmic loop, YFPQA (FIG. 1), reinforces association with lysosomes¹⁴. Paired lysosomal sorting motives are thought to ensure robust targeting of the protein to the lysosome, and are present in other lysosomal membrane proteins, such as battenin, which is involved in the neurodegenerative disorder Batten disease⁷³. The YFPQA sequence comprises part of a ‘PQ-loop’ motif, a defining feature of the PQ-loop family proteins, which are seven transmembrane helices with a duplicated region containing a well-conserved PQ-dipeptide motif⁷⁴. The cystine and

proton-binding site in cystinosin belongs to this second PQ-loop⁷⁵, as seen in other PQ-loop proteins such as the SWEET sugar transporters⁷⁶.

Cystinosin is a cystine–proton symporter that uses the proton gradient to transport cystine from the lysosomal lumen to the cytosol. The affinity of cystinosin for cystine at acidic pH¹⁵, is much higher than that of other lysosomal aminoacid transporters for their substrates. Indeed, the K_m of cystinosin for cystine is ~0.25 mM in Cos-1 cells and down to 0.075 mM when protonated, as occurs in the acidic lysosomal lumen⁷⁵, in contrast to micromolar ranges for the lysosomal amino acid transporter PQ loop repeat containing protein-2 (PQLC2; 3 mM for arginine and even higher for lysine⁷⁷), and peptide/histidine transporter-2 (PHT2; 5 mM for histidine⁷⁸). These values suggest that saturation of cystinosin occurs much below the level at which cystine saturation of lysosomes occurs (5 mM under acidic lysosomal conditions). Quantitative mass spectrometry studies further indicate low copy numbers of cystinosin at the lysosomal membrane (~10 transporters per lysosome)⁷⁹, which is compatible with cystinosin abundance being the rate-limiting step for cystine transport, consistent with reduced transport efficiency in heterozygotes carrying one defective *CNTS* allele⁷⁹. This rate-limiting effect of transporter number contrasts with lysosomal storage diseases caused by defective luminal hydrolases, whereby heterozygotes show no accumulation of substrate at 50% residual activity⁸⁰. Moreover, although a low abundance of transporters in individual lysosomes would not be expected to cause a problem as lysosomes constantly exchange their digestive load by fission and homotypic fusion, such job sharing is compromised upon rigid content overload. In addition, genotype–phenotype correlation revealed complete disruption of cystine transport in patients with infantile cystinosis associated with point mutations in *CTNS*, in contrast to the partial preservation of cystine transport observed in individuals with juvenile and ocular cystinosis⁸¹. A total loss of cystine transport might not, however, be sufficient to cause nephropathic cystinosis. For example, two missense mutations in *CTNS* — Lys280Arg and Asn323Lys — found in patients with juvenile cystinosis, result in undetectable levels of cystine transport, yet these mutated forms of cystinosin still localize at lysosomes⁸¹. This finding indicates that the presence of cystinosin without transport capability is associated with a milder phenotype than that associated with the 57 kb deletion present in most forms of infantile cystinosis, and is compatible with an important function of cystinosin beyond cystine transport (BOX 1).

Biochemistry of cystinosis

Cystine accumulation in lysosomes

Mechanism of cystine accumulation—Impaired transporter-dependent exodus of cystine across the lysosomal membrane leading to intralysosomal accumulation of cystine was until a few years ago considered to be the primary cause of all cystinosis manifestations. Studies in cultured human cystinotic fibroblasts further demonstrated that intracellular cystine does not originate from oxidation of local cysteine in lysosomes⁸² but from lysosomal degradation of disulfide-bearing proteins^{82–84}. Moreover, cystine loading in fibroblasts is proportional to the extracellular concentration of albumin⁸³ and to the intrinsic abundance of disulfide bridges in various endocytic cargos⁸².

In particular, since albumin contains 17 disulfide bonds, each degraded albumin releases 17 cystine molecules⁸³. Furthermore, a surprisingly low fraction of cystine released by albumin degradation (<1%) was retained, which was attributed to cystine exocytosis⁸³. Vesicular exodus of free cystine by exocytosis should thus be regarded as a natural clearance mechanism that considerably slows the accumulation of cystine. Quantitative estimations detailed below indicate that in PTCs from *Ctns*-knockout mice, receptor-mediated endocytosis of ultrafiltrated plasma proteins is more than a sufficient source of cystine. Albumin, the major ultrafiltrated protein, is barely detectable in urine, indicating virtually complete reabsorption. Upon selective genetic abrogation of receptor-mediated endocytosis, mice excrete on average roughly 25 μg of albumin per day⁸⁵, indicating that this amount is normally reabsorbed daily via the megalin–cubilin pathway and transferred to lysosomes, corresponding to ~ 12 nmol of half-cystine released from albumin alone in PTC lysosomes every day. Similar calculations can be extended to humans, on the basis of high levels of proteinuria observed in patients with homozygous deletion of the gene that encodes cubilin⁸⁶. Moreover, during the first months of life, when endocytic receptors are preserved, kidneys of *Ctns*-knockout mice accumulate ~ 0.1 nmol half-cystine per mg of total protein per day (~ 1.5 nmol half-cystine per day)²¹. Thus, similarly to cystinotic cells *in vitro*, where only 1% of cystine released by lysosomal proteolysis is actually retained in lysosomes⁸², the rate of lysosomal cystine generation in PTCs from *Ctns*-knockout mice exceeds the rate at which it is retained by at least sevenfold. Although these calculations are rough estimates, two conclusions can be drawn: first, albumin endocytosis and lysosomal degradation in PTCs is sufficient to account for the supply of cystine precursors; and second, the amount so supplied largely exceeds the amount being stored implying that cystine is poorly retained by endolysosomes *in vivo* and that, in addition to cystinosin-mediated transmembrane exodus, cystine can be efficiently discharged by vesicular efflux (exocytosis). These two conclusions indicate that inhibition of megalin-dependent endocytosis and triggering vesicular exocytosis could both slow the build-up of cystine in PTCs and progression of cystinosis.

Cystine accumulates as two forms in growth-arrested cells such as PTCs and interstitial macrophages. First, as soluble cystine, which in principle cannot exceed concentrations of ~ 5 mM, the limit of solubility under acidic lysosomal conditions⁸⁷. Soluble cystine can then organize into polyhedral crystals within the lysosomal matrix (FIG. 2). The theoretical limit of cystine solubility within the acidic lysosomal environment can be roughly converted into a maximal soluble cystine load per cell. Given that the typical protein concentration in kidney cells is 160 mg/ml, the 5 mM free cystine in acidic lysosomes, which typically occupy $\sim 4\%$ of cell volume, would correspond to ~ 0.2 μmol half-cystine per ml of tissue, or ~ 1 nmol half-cystine per mg of tissue protein. However, higher values of free cystine do not necessarily imply the formation of cystine crystals, since up to 20 nmol of half-cystine per mg of protein⁸⁸ has been recorded in short-lived cystinotic neutrophils (which are assessed to monitor the efficiency of cysteamine therapy), without evidence of crystals. The discrepancy between the theoretical solubility threshold of cystine and the actual load might reflect enlarged lysosomal volumes, electrostatic interaction between cystine and the charged fixed residues of lysosomal proteins enabling cystine to exit the soluble free pool, or the possible contribution of variable amounts cytosolic cystine included in the tissue cystine

assay. Of note, a 5 mM maximal concentration of cystine is too low to cause osmotic swelling, and therefore cannot be the primary cause of lysosomal enlargement.

Crystal formation—The absence of crystals in exponentially growing cultured human and murine cystinotic fibroblasts, immortalized shed urinary cells, and short-lived cystinotic neutrophils suggests that crystallization within the heterogeneous lysosomal matrix is a rather slow process. Crystals are first detected by electron microscopy in cystinotic mouse PTCs as tiny needles that are invisible by light microscopy, which then build up as polyhedra that deform lysosomes and can reach huge dimensions (>10 µm in diameter). A build-up of crystals after the onset of renal Fanconi syndrome explains the exponential increase of cystine in kidney extracts from cystinotic mice. Large crystals have a significant impact on lysosome motility (see below) and lysosome vesicular dynamics. Enlargement and content rigidification are known to impair lysosomal fusion^{89,90}. In addition, non-breakable micrometer-large crystals must prevent lysosomal fission. The combination of these effects should block interlysosomal exchange, and thereby arrest randomization of endocytic load between lysosomes, thus favour their conversion into residual bodies^{89,90}. Unlike kidneys from *Ctns*-knockout mice, in which cystine crystals are predominantly found in PTCs^{21,91}, crystals are absent or rarely found in PTCs from children with cystinosis⁹². Rather, biopsy samples from children aged 1.5–3.0 years with cystinosis show crystal deposition predominantly in interstitial macrophages³⁹. Importantly, crystals of mouse PTCs, but not of macrophages, can be disposed into the tubular lumen, either by exocytosis (in a process known as ‘lysosomal defecation’), or by apoptotic cell fragmentation⁹¹. Of note, flattened cells in swan neck deformities in cystinotic mice are devoid of crystals⁹¹. Moreover, allografts from renal transplant recipients do not show evidence of crystals in PTCs whereas crystal formation recurs in macrophages, consistent with infiltration of the allograft by cystinosin-defective host macrophages³⁹. Thus, cystine crystals are clearly pathognomonic of cystinosis, but should no longer be considered pathogenic in the initiation of renal Fanconi syndrome, which develops in *Ctns*-knockout mice before crystals appear in PTCs and is inconsistent with the absence of crystals at early stages of cystinosis in PTCs in humans. Moreover, the failure of cystine-clearing cysteamine to reverse established renal Fanconi syndrome demonstrates the involvement of additional essential mechanisms. Crystals might, however, be crucial in driving the progression of renal disease at later stages, for example, by activating the inflammasome to trigger inflammation and fibrosis⁹³.

Energy imbalance

Loading of renal tubules with cystine dimethylester was historically used to study the effects of cystine build-up *in vitro* and *in vivo*; this approach led to a drastic decrease in cellular ATP levels in renal tubules, but this effect was later attributed to mitochondrial intoxication^{94–97}. Low levels of ATP were, however, later found in fibroblasts from patients with cystinosis^{98,99}, as well as upon silencing of cystinosin in rabbit PTCs with small interfering RNA¹⁰⁰, independent of a detectable mitochondrial defect, suggesting that cystinosis is associated with altered cellular energy homeostasis. The cause of the reduced ATP levels in cystinosin-deficient cells is unknown. The presence of an energy imbalance in cystinosis is further supported by evidence that the cytosolic fuel sensor, AMP-activated protein kinase (AMPK), is activated in primary rabbit PTCs with cystinosin knockdown¹⁰¹.

However, the interpretation of this finding must be regarded with caution since AMPK is involved in kinase-based energy sensing and adaptive crosstalk, in particular with mTORC-1, a sensor of energy supply at lysosomes (FIG. 3).

Apoptosis

Cystinotic fibroblasts and PTCs are particularly vulnerable to apoptosis^{102,103}. *In vivo*, the characteristic 'swan neck' deformity of the proximal tubules is associated with local apoptosis¹⁰⁴ (FIG. 4). The propensity of PTCs to undergo apoptosis is supported by the uniform overexpression of the pro-apoptotic cysteine protease, caspase-4, in kidney biopsy samples from patients with cystinosis¹⁰⁵. Transcripts for genes encoding caspase-1, caspase-4, and caspase-12, are significantly increased in kidneys from 15-month-old *Ctns*-knockout mice, suggesting global overexpression of the apoptosis cascade (S. Cherqui, unpublished work). Increased activation of the immediate apoptosis executioner, caspase-3, was also demonstrated in scattered PTCs from *Ctns*-knockout mice, associated with luminal shedding of apoptotic bodies and a partial compensatory response through PTC proliferation and repair⁹¹. However, apoptosis is more likely to be a common end-point of disease processes than a primary cause of proximal tubule dysfunction in cystinosis. Although iterative PTC apoptosis from the swan neck could eventually cause tubular atrophy, apoptotic luminal PTC shedding can also be viewed as an initial adaptive mechanism for the selective removal of irreversibly altered and/or crystal-overloaded cells⁹¹. Indeed, each replacement of an apoptotic PTC by a proliferating neighbour implies generation of a completely new set of lysosomes. An additional possible mechanism contributing to the swan neck deformity is sliding metaplasia, whereby the flat epithelium of the Bowman capsule would extend into the convoluted segment of the proximal tubules that has been depopulated by apoptosis. This mechanism is conceptually analogous, albeit in opposite direction, to colonization of the distal Bowman capsule by PTCs.

Oxidative stress

Cystine efflux from lysosomes is widely considered to serve as a source for cysteine, which itself is used in the synthesis of the tripeptide, glutathione, via the γ -glutamyl cycle. Reduced glutathione (GSH) is the major antioxidant in the cytosol, facilitating the neutralization of free radicals such as reactive oxygen species. Accordingly, altered glutathione metabolism and increased oxidative stress have been proposed to cause the Fanconi syndrome in cystinosis (FIG. 5). A significant decrease in total glutathione content and increase in superoxide dismutase activity, which enhances oxidative stress, were observed in cultured human cystinotic fibroblasts and were corrected by administration of exogenous cysteine¹⁰⁶, raising the possibility that lowered cytosolic glutathione levels resulted from a shortage of cysteine that is normally derived from cystine (FIGS 3b,5). Further studies demonstrated altered glutathione metabolism in skin fibroblasts, polymorphonuclear cells, and cultured PTCs from patients with cystinosis^{107,108}. Fibroblasts derived from patients were unable to increase glutathione synthesis in response to oxidative stress¹⁰⁹. Under conditions of ATP inhibition, levels of 5-oxoproline, an intermediate of the γ -glutamyl cycle, were almost doubled in cystinotic cells compared to controls whereas that of γ -glutamyl cysteine remained unchanged, indicating that the cycle was impaired at the step of cysteine entry, further supporting the hypothesis that the supply of cysteine in

cystinotic cells is limited. Of note, elevated levels of 5-oxoproline have also been identified in urine of patients with cystinosis, confirming the *in vitro* results¹¹⁰. Interestingly, one study reported that PTCs extracted from the urine of patients with cystinosis had decreased basal glutathione levels, combined with lower ATP levels and a higher apoptosis rate¹¹¹, suggesting that these defects could be linked.

GSH has an important role in preventing mitochondrial oxidative damage and abnormal mitochondria have been reported in PTCs from humans and mice with cystinosis^{18,112,113}. In *Ctns*-knockout mice, the increase in oxidative stress preceded the development of swan neck deformities, which could be delayed by administration of mitoquinone, an antioxidant compound that targets mitochondria¹⁰⁴. Of interest, a pilot clinical trial in which *N*-acetylcysteine was administered for 3 months to 23 patients with nephropathic cystinosis demonstrated a significant reduction in oxidative stress and improved renal function¹¹⁴. However, additional studies with larger cohorts and longer follow-up are clearly needed to confirm this conclusion.

Together the above-described studies document alterations in glutathione metabolism and support the hypothesis that synthesis of cytoplasmic cysteine is limited when recycling of lysosomal cystine is abrogated. However, cells have alternative sources of cysteine. Indeed, cysteine can be synthesized from methionine, albeit to a limited extent¹¹⁵. More importantly, cysteine can be imported from the extracellular space¹¹⁶ especially in PTCs where cysteinylglycine is reabsorbed by the PEPT2 transporter¹¹⁷, and cystine is reabsorbed by the heterodimeric rBAT-b(0,+)-AT transporter, which are active at the apical membrane¹¹⁸. In addition, cysteamine releases equimolar amounts of cysteine from the lysosomes into the cytosol and cysteamine is also itself a potent antioxidant, shown to increase intracellular levels of GSH^{119–121}. However, the vexing inefficacy of cysteamine on established Fanconi syndrome in patients with cystinosis argues against a primary role for defective glutathione metabolism and increased PTC susceptibility to oxidative stress in the pathogenesis of Fanconi syndrome, suggesting the involvement of more complex cellular and molecular disease mechanisms.

Cell biological alterations

Trafficking and proteolysis defects

A host of structural and endocytic trafficking defects have been reported in cystinotic cells (FIG. 3b). First, in addition to severely decreased expression of the endocytic receptors megalin and cubilin in PTCs^{91,122}, impaired megalin recycling has been reported in cystinotic cells¹²³, as in two other hereditary diseases associated with renal Fanconi syndrome: Dent disease¹²⁴ and Lowe oculocerebrorenal (OCRL) syndrome¹²⁵. However, the finding that administration of cysteamine fails to control established Fanconi syndrome despite correction of megalin recycling *in vitro*¹²³ argues against a major role for recycling defects in cystinosis.

Second, cystinotic lysosomes become enlarged and cluster to the perinuclear region of cells, reflecting impaired lysosome motility and indicating reduced accessibility to endocytic and autophagic loads^{122,123}. Three possible explanations exist for the pericentriolar trapping of

cystinotic lysosomes: first, through physical retention, since enlarged lysosomes have impaired free diffusion¹²⁶ and are predicted to engage with increased numbers of microtubular contacts via their cytoskeletal motor protein, dynein, and since the microtubular network is most dense around centrioles¹²⁷; second, through altered regulation of lysosomal calcium release that controls lysosomal dynamics¹²⁷, although no change in the total level of lysosomal calcium store has been detected in cultured cystinotic cells¹²⁸; and third, through downregulation of Rab-GTPase(s) that normally catalyse lysosomal trafficking, such as Rab27a, which is specifically expressed in PTCs and is downregulated in PTCs from mice and humans with cystinosis¹²⁹.

Third, delayed transfer of endocytosed proteins into the lumen of endolysosomes that act as the degradative compartment has been documented in PTCs from *Ctns*-knockout mice, with evidence that cargo-loaded endosomes can dock without actually fusing with LAMP-1-labelled lysosomes⁹¹.

Fourth, proteolysis in endolysosomes is impaired, as was first suggested for cystinotic fibroblasts three decades ago based on ultrastructural evidence of ‘amorphous inclusions’ and high equilibrium density in subcellular fractionation studies as a result of protein accumulation¹³⁰. Direct *in situ* evidence of an impaired rate of proteolysis, independent of less frequent encounters between internalized protein cargo carried by late endosomes and the set of proteolytic enzymes brought by lysosomes, has been demonstrated in primary PTC cultures from *Ctns*-knockout mice¹²², and in human cystinotic cells¹²³. Assuming that lysosomal influx of cysteine does not increase in cystinotic cells to compensate for higher intralysosomal levels of free cystine due to impaired exodus, thus leading to luminal oxidation of lysosomal thiols, the defect in intrinsic lysosomal proteolysis could result from three possible mechanisms (FIG. 3b): first, by impairing the unfolding of disulfide-bonded substrates that are necessary to expose masked peptide bonds for endoproteolytic attack¹³¹; second, through inactivation of mature lysosomal cathepsins that belong to cysteine proteinases and critically depend on preservation of the reduced thiol in their catalytic site; and third, through impaired autoactivation and hetero-activation of pro-cathepsins by proteolytic excision of the masking pro-peptide. In turn, impaired proteolysis could affect lysosomal amino acid sensors, such as mTORC1, thereby affecting lysosomal signalling¹³² (FIG. 3b).

Signalling and autophagy defects

Lysosomes are no longer considered simply as bags for protein degradation, but rather, they are viewed as multipurpose, adaptive signalling organelles¹³³ (FIG. 3a). One major function is in the control of autophagy, and increased numbers of autophagosomes, especially enclosing mitochondria, have been reported in various cystinotic cell lines as well as in liver and kidney from *Ctns*-knockout mice^{134,135}. The lysosomal membrane is now recognized to be a regulated docking surface for the mTORC1 machinery, which integrates information on the nutritional status of the cell (that is, amino acid supply), to regulate autophagy and cell differentiation through various pathways including through transcription factor EB (TFEB) phosphorylation^{132,133}. Specifically, mTOR complexes have fundamental roles in renal physiology, highlighting the importance of this pathway in kidney cell homeostasis and

function¹³⁶. Upon stimulation by amino acids, the small GTPases Ras-related GTP-binding proteins (Rag) and the pentameric Ragulator complex recruit cytosolic mTORC1 to the lysosomal membrane, close to its activator, Rheb GTPase^{137,138}. Activation of mTORC1 by Rheb GTPase increases protein synthesis, while inhibiting autophagy. Conversely, starvation (amino acid deprivation) inactivates mTORC1, thereby triggering mobilization of nutrient reserves by activating autophagy^{139,140}. The vacuolar ATPase (v-ATPase) proton pump is necessary for mTORC1 activation by amino acids, by engaging in amino acid-sensitive interactions with the Rag–Ragulator complex^{137,141}. Co-immunoprecipitation and mass spectrometry studies have demonstrated that cystinosin interacts physically with almost all components of the v-ATPase, Ragulator complex and RagA/ RagC¹⁴²; these interactions require the fifth loop in cystinosin, which contains the PQ signal. Furthermore, PTC lines derived from *Ctns*-knockout mice showed defective lysosomal recruitment of mTOR upon nutrient shortage (FIG. 3b). Impairment of mTOR activation was confirmed in human cystinotic cells¹⁴³. Of note, as lysosomal v-ATPase–mTOR activation controls the expression of megalin and thereby regulates apical protein uptake in *Drosophila* epithelial cells and mouse PTCs¹⁴⁴, these data provide a possible explanation for the decreased megalin expression in cystinotic kidney, as discussed in further detail below.

TFEB is the master transcription factor responsible for coordinating the expression of all lysosomal constituents, including cystinosin, and therefore has a key role in regulating lysosomal biogenesis and clearance, in coordination with autophagy and anabolism¹³³ (FIG. 3b). This factor is silenced by mTOR-dependent phosphorylation, which causes it to become trapped in the cytosol, and is in turn activated by Ca²⁺-dependent dephosphorylation, which enables its nuclear translocation. Expression of TFEB mRNA and protein is impaired in PTCs deficient in cystinosin¹⁴⁵. Conditionally immortalized PTCs derived from the urine of a patient with cystinosis showed persistent nuclear translocation of TFEB, indicating stimulation of lysosomal clearance. Prolonged overexpression of exogenous TFEB or its pharmacological activation by genistein lowered cystine levels and corrected cellular alterations in endocytic trafficking and rescued deficits that could not be corrected by cysteamine, demonstrating again that the absence of cystinosin affects lysosomal signalling and pointing to the potential benefits of pharmacological intervention targeting TFEB¹⁴⁵.

Contrary to observations in many lysosomal storage disorders¹⁴⁶ and to predictions based on evidence of defective mTOR signalling in cystinosis cells¹⁴², surprising findings from one study showed that autophagosomes in fibroblasts from *Ctns*-knockout mice matured normally. These autophagosomes supported fully functional macroautophagic fluxes¹³⁴, but cells demonstrated impairment of chaperone-mediated autophagy (CMA)¹³⁴ (FIG. 3b). CMA is a process of autophagy that involves the chaperone-dependent selection of altered cytosolic proteins and their translocation into lysosomes via lysosome-associated membrane protein type 2A (LAMP2A)¹⁴⁷. In cells from *Ctns*-knockout mice, LAMP2A is mislocalized, presumably as a result of altered vesicle trafficking. In addition, CMA-dependent proteolysis by isolated lysosomes from *Ctns*-knockout mice was defective, whereas lysosomal proteases seemed to function normally. Importantly, CMA is activated during oxidative stress to facilitate the clearance of irreversibly modified molecules¹⁴⁸; therefore, impaired CMA in cystinosis could increase cell sensitivity to oxidative stress. The various autophagy defects observed in cystinosis are probably interconnected, as impairment

of CMA impairment might induce compensatory activation of other autophagic pathways^{147,149}. However, the mechanisms leading to defective CMA in cystinosis are unclear and whether cystinosis stabilizes LAMP2A, a variant of LAMP2, at the lysosomal membrane is not known. Moreover, isolated autophagy deficits observed in Danon disease, a lysosomal storage disorder caused by mutations in LAMP2 (REFS 150,151), do not lead to a renal phenotype^{150,152}, implying that this defect cannot be the primary reason for the Fanconi syndrome associated with cystinosis.

Although many questions remain unanswered with regard to the regulation and roles of autophagy in cystinosis, it is clear that cysteamine therapy does not correct many defects of cellular homeostasis, such as alterations in mTOR signalling, TFEB expression and CMA, despite improvements in cystine clearance^{134,142,145}. These findings underscore the effects of cystinosis deletion, beyond the direct effects on cystine overload (BOX 1).

Histopathological alterations

Transporters and endocytic receptors

For all their use in investigating the biochemical and cell biological alterations in cystinosis, studies with immortalized cell lines are unable to recapitulate the exquisite apical differentiation of PTCs, nor their sequential organization as tubes with segmental specialization. As the renal Fanconi syndrome is characterized by a global defect in PTC reabsorption, a global loss of solute transporters and endocytic receptors at the apical membrane of cystinotic PTCs has been hypothesized⁹¹. Indeed, careful molecular examination of early changes in kidneys from *Ctns*-knockout mice revealed a loss of the major phosphate and glucose transporters (NaPi-IIa and SGLT-2, respectively) concomitant with the detection of urinary markers of Fanconi syndrome, accounting for the functional urinary loss of solutes before any detectable (ultra)structural alterations, including at the brush border. Moreover, studies revealed a parallel spatiotemporal loss of the apical multi-ligand endocytic receptors, megalin and cubilin^{91,122}, which normally ensure near complete reuptake and lysosomal degradation of ultrafiltrated plasma proteins. This observation thus explains the characteristic tubular proteinuria of the renal Fanconi syndrome (commonly referred to as low molecular weight proteinuria). Although expression of megalin and cubilin was initially reported as being preserved in cystinosis³¹, subsequent reports found levels of these endocytic receptors to progressively decline^{91,121}. Thus, the early disappearance of several apical solute transporters and endocytic receptors, in a process that could be referred to as 'apical dedifferentiation', might provide a common functional molecular mechanism for the renal Fanconi syndrome, well before tubule atrophy occurs.

Marked cellular heterogeneity within the same histopathological section of proximal tubules is obvious in patients³⁹ and mice with cystinosis⁹¹. This heterogeneity distinguishes cystinosis from other causes of renal Fanconi syndrome, such as Dent disease¹²⁴, and calls attention to cell-autonomous responses, such as genetic regulation, in the response of individual PTCs to cystinosis deficiency. Interestingly, studies of primary PTC cultures from *Ctns*-knockout mice have demonstrated loss of megalin to be associated with the nuclear translocation of ZO-1-associated nucleic acid binding protein (ZONAB)¹²², a transcription factor that promotes cell proliferation and downregulates megalin and cubilin expression as

direct targets¹⁵³. However, ZONAB is only part of a transcriptional network that integrates with mTOR-regulated pathways, including those regulated by TFEB.

Longitudinal extension and atrophy

Over time, apical dedifferentiation of PTCs extends longitudinally from the glomerulotubular junction to the more distal PTC segments⁹¹ (FIG. 4). According to the estimates provided above, endocytosis of ultrafiltrated disulfide-rich plasma proteins, in particular albumin, is the primary source of lysosomal cystine for PTCs^{83,84}. Thus, early defective reabsorption by PTCs closest to the glomeruli followed by distal extension of the defect further suggests a causal role for defective endocytosis in disease progression. Indeed, documented transfer of endocytic load, and thus cystine insult, to more distal PTCs was predicted to cause longitudinal extension of the lesion⁹¹. Supporting this endocytosis-based model of nephropathic cystinosis, we have found that kidney-specific ablation of megalin in *Ctns*-knockout mice prevents accumulation of cystine only in the kidneys and protects nephrons from swan neck deformities¹⁵⁴. On the other hand, decreased expression of endocytic receptors in proximal PTCs could be viewed as another cell-autonomous mechanism of adaptation, by lowering protein uptake to decrease the supply of cystine¹⁵⁵. This notion is also consistent with the fact that an absence of cystinosin does not prevent primary neonatal nephron differentiation, but later induces apical dedifferentiation.

Late-stage cystinotic kidneys show extreme anatomic atrophy due to a combination of PTC atrophy with interstitial and glomerular fibrosis. Atrophied PTCs have the appearance of being replaced by flat cells resembling glomerular parietal cells, supporting the sliding metaplasia hypothesis. Interstitial fibrosis follows marked thickening of tubular basement membrane. End-stage cystinotic kidneys also show frequent glomerulotubular disconnection, due to encroachment of the junction, resulting in the development of atubular glomeruli. However, atubular glomeruli occur in a wide variety of chronic kidney diseases including glomerular, tubular, toxic obstructive and infectious syndromes¹⁵⁶; they do not, of course, contribute to urine production and therefore cannot contribute to the phenotype of the renal Fanconi syndrome.

Treatment and therapeutic perspectives

Current treatment

Beyond supportive therapy²⁸, patients with cystinosis benefit from the oral drug, cysteamine (β -mercapto-ethylamine). Cysteamine depletes lysosomal cystine by cleaving cystine into free cysteine and cysteamine–cysteine mixed disulfide, which is exported from lysosomes by the cationic amino acid transporter, PQLC2, which also belongs to the PQ-loop family⁷⁷ (FIG. 6). Although effective in delaying the complications of cystinosis including ESRD²⁶, cysteamine therapy cannot correct established renal Fanconi syndrome, nor the autophagy, mTOR, TFEB and CMA defects in cystine-deficient cells^{134,145,157}. These sobering observations highlight the fact that the pathogenic mechanisms of cystinosis involve both the lysosomal accumulation of cystine and the lack of cystinosin itself, which prevents essential, yet only partially understood, interactions with key cellular machineries. These considerations probably explain why cysteamine delays but does not prevent kidney failure

even if taken compliantly from early stages of disease. Moreover, adherence to conventional immediate-release cysteamine therapy is cumbersome. Pills must be taken every 6 h, day and night, and cause body odour, halitosis and severe gastrointestinal adverse effects^{9,28}, which hinder treatment compliance. Using enteric-release technology, a delayed and prolonged release formulation of cysteamine^{158–161} was approved by the FDA in 2013 (REF. 162). Although this new formulation needs to be taken only every 12 h, thus improving the quality-of-life of affected patients^{163,164}, the impact on the disease is expected to be similar, in delaying but not arresting the complications. Topical cysteamine hydrochloride eye drops are highly effective in reducing the density of corneal crystals and alleviating the associated symptoms^{7,165}. Treatment of the Fanconi syndrome requires careful replacement of electrolytes and vitamins. Indomethacin can reduce polyuria in patients with cystinosis, but was not adopted by consensus guidelines owing to toxicity issues^{9,28}. Taken together, the burden of cystinosis and current approaches to its management is heavy. The combination of cysteamine therapy and multi-systemic supportive treatment requires patients to take up to 60 pills per day; young children often require a gastric tube to tolerate the medications and achieve the necessary caloric intake, and patients who develop renal failure require dialysis or transplantation. Thus any novel treatment or improvement to the current treatment regimen is eagerly awaited.

New potential targets

The above-described mechanisms — involving energy depletion, oxidative stress, lysosomal enlargement, altered autophagy, lysosomal signalling and trafficking, and apoptosis — are most probably linked in the pathogenesis of the renal Fanconi syndrome in cystinosis¹⁶⁶. Interventions that improve any step in these cascades might therefore alleviate the renal Fanconi syndrome. For example, preclinical and a limited clinical study of cysteine supplements or antioxidants indicated favourable outcomes^{104,114}; however, larger studies with longer follow up are needed. Improved understanding of cellular dysfunctions induced by the absence of cystinosis has already identified other potential therapeutic targets. For example, small molecules that rescue CMA¹⁶⁷ or reduce lysosomal overload by enhancing cystine excretion¹⁶⁸ might lead to improved outcomes if they are used concomitantly with cysteamine^{129,134}. Another approach is to enhance TFEB levels, as TFEB triggers lysosome biogenesis and promotes the clearance of accumulated protein aggregates¹⁶⁹, in models of Huntington¹⁷⁰ and Parkinson disease¹⁷¹. Induction of TFEB expression, nuclear translocation and activation of TFEB-dependent lysosome biogenesis by genistein¹⁷², has been reported to decrease lysosomal storage of glycosaminoglycans due to mucopolysaccharidosis *in vitro*¹⁷³ and has been validated in a pilot clinical trial resulting in cognition improvement¹⁷⁴. These findings suggest that genistein might be beneficial in other lysosomal storage diseases such as cystinosis. Indeed, activation of TFEB by genistein in cystinotic PTCs can decrease intracellular cystine levels and lysosome enlargement and rescue endocytosis defects¹⁴⁵. Importantly, however, genistein has multiple effects, including activation of AMPK¹⁷⁵, which as mentioned earlier is activated in primary rabbit PTCs with cystinosis knockdown¹⁰¹, and inhibition of mTOR signalling¹⁷⁵, which is already reduced in cystinotic PTCs¹⁵⁷. These varied actions call for great caution before the effects of genistein are assessed in patients with cystinosis.

PTC rescue by haematopoietic stem cells

A more radical and specific approach to those described above is the direct systemic replacement of cystinosin through administration of cystinosin-producing progenitor cells. This approach has been tested in cystinotic mice, using wild-type bone marrow-derived stem cells as a vehicle to target all tissues. Total bone marrow stem cells, or purified mesenchymal stem cells (MSCs) versus haematopoietic stem cells (HSCs), were transplanted into irradiated *Ctns*-knockout mice to repopulate their bone marrow with *Ctns*-carrying donor cells. MSCs offered only short-term benefits; however, transplantation of HSCs yielded long-term rescue of the cystinosis phenotype^{23,176,177}. HSC transplantation led to stable engraftment of abundant bone marrow-derived cells in all organs, correlating with a dramatic reduction in cystine tissue levels (up to 94% clearance). Most importantly, this treatment led to a long-term improvement in kidney structure and function including the Fanconi syndrome, even though HSCs did not reprogramme into PTCs²³. As expected, effective therapy depended on strong engraftment of *Ctns*-expressing HSCs (with >50% of blood cells being donor-derived). Interestingly, kidney protection was not dependent on transplantation at a young age and was still effective in 10-month old mice, suggesting the potential for established kidney dysfunction to be rescued by stem cell therapy. Whether the observed ‘rescue’ of the cystinosis phenotype actually reflects protection of previously ‘healthy tissue’ and/or reversal of organ injury is addressed in ongoing studies.

The ability of HSCs to rescue cystinosis in mice is remarkable since cystinosin is a transmembrane lysosomal protein^{13–15} as opposed to secreted, soluble lysosomal enzymes that can be readily recaptured by receptor-mediated endocytosis in diseased cells. Indeed, cross-correction, that is, the transfer of a functional protein from wild-type cells to deficient cells, has been well demonstrated in several lysosomal storage disorders caused by defective soluble hydrolases, after bone marrow transplantation or by recombinant enzyme infusion¹⁷⁸. However, no cross-correction has been previously reported for lysosomal transmembrane proteins. A striking finding was the differentiation of tissue-engrafted HSCs into macrophages¹⁷⁹. In addition to their phagocytic function, engrafted HSCs exerted paracrine effects that were responsible for effective cystine clearance¹⁷⁹. Cystinosin-containing microvesicles and/or exosomes shed by cystinosin-expressing cells have been suggested as potential vehicles to decrease cystine in cystinotic cells^{79,180}. However, co-culture assays of *Ctns*-deficient fibroblasts with wild-type MSCs or macrophages showed low correction efficiency by shed exosomes and/or microvesicles in fibroblasts (~20% clearance) contrasting with efficient cross-correction (~75% clearance) upon cell–cell contact¹⁷⁹. Vital co-culture imaging further revealed that bone marrow-derived macrophages physically transfer cystinosin-bearing lysosomes into cystinosin-deficient cells via very long tubular extensions known as ‘tunnelling nanotubes’ (TNTs). These TNTs support long-distance intercellular communication, vesicular trafficking and pathogen spreading^{181–183}. Amazingly, cystinosin-deficient cells exploit the same route in the opposite direction to transfer cystine-loaded lysosomes into macrophages, where they can fuse with competent lysosomes, thus providing bidirectional correction.

The mechanism of HSC-mediated renal repair *in vivo* has been debated^{184,185}, in particular with regard to the route by which HSCs access PTCs across the normally continuous, thick,

dense and stiff tubular basement membrane^{186,187}. However, bone marrow-derived macrophages engrafted in cystinotic kidneys generate nanotubular extensions that readily cross the tubular basement membrane and deliver cystinosin into diseased PTCs¹⁷⁹ (FIG. 7). This novel mechanism of genetic correction, which offers long-term kidney preservation after HSC transplantation in cystinosis, opens broad perspectives for the treatment of inherited renal disorders. Although the specific signals that trigger this effect remain to be identified, we hypothesize that inflammation is a key factor since recruitment of macrophages at the injury site is necessary for therapeutic rescue (FIG. 7). Similar protection has been demonstrated in the eyes and thyroid of *Ctns*-knockout mice, leading to near complete clearance of corneal cystine crystals and preservation of corneal structure¹⁸⁸, as well as normalization of thyroid-stimulating hormone level and preservation of thyroid follicles, respectively¹⁸⁹.

Future directions

The importance of genetic context for the renal phenotype of cystinosis has long been acknowledged. In 2002, the first *Ctns*-knockout mouse was generated on a mixed C57BL6/129sv background¹⁸. Despite accumulation of cystine and crystal build-up in all tissues (including kidneys), no signs of renal Fanconi syndrome or renal dysfunction were observed. Subsequently generation of knockout mice on pure genetic backgrounds revealed that *Ctns* knockout on a C57/BL6J background led to proximal tubule dysfunction and progressive chronic renal failure, whereas *Ctns* knockout on a FVB background presented no renal anomaly despite some accumulation of cystine in the kidney²¹. These data point to the existence of modifier genes influencing the renal phenotype that could also account for the variability of disease severity in patients with identical mutations, such as patients who are homozygotes for the 57 kb deletion. Comparative genetic analyses of the two congenic *Ctns*-knockout mouse strains should enable the identification of such modifier genes and help unravel either the primary cause or aggravating as well as attenuating factors of the Fanconi syndrome in cystinosis. Another surprising finding was the existence of gender discrepancies in the cystine content of *Ctns*-knockout mice, with higher levels in females than males, specifically in the kidneys¹⁷⁶, although no evidence exists of a gender predisposition in humans. Of note, kidney function was similar in males and female *Ctns*-knockout mice, which supports the current view that cystine accumulation cannot be the only factor that leads to renal defects in cystinosis. Proteins encoded by modifier genes might be involved in apical transcriptional regulation, vesicular trafficking, lysosomal signalling and autophagy, which are all impaired in cystinotic cells. Further studies of the cause of these defects might reveal the still elusive, additional cellular functions of cystinosin. In particular, the role(s) of the second PQ-loop in cystinosin, which in addition to lysosomal targeting carries a cystine-proton binding site and includes the interaction site with mTORC1^{14,75,142}, deserves further study. The challenge remains to define the mechanisms that ultimately explain the susceptibility of PTCs to cystinosin depletion and the early onset of the renal Fanconi syndrome, and to develop rational strategies for early prevention.

Rescue of PTCs by HSCs raises a new set of questions. First, whether crossing of the tubule basement membrane (TBM) by TNTs is restricted to pathologic states such as cystinosis or

whether it is a so far unrecognized physiologic phenomenon that might support bidirectional exchange between PTCs and interstitial cell components during normal development or adult life. In this context, resident dendrite cells are known to closely surround tubules and to generate membrane extensions in the healthy kidney, suggesting a role as sensors of the microenvironment¹⁹⁰. Second, whether TNTs are able to actively perforate the TBM, or simply exploit pre-existing local TBM defects due to alterations in cystinotic PTCs requires clarification. Third is whether our improved understanding of the mechanisms by which HSCs rescue cystinotic PTCs can be used to design novel treatment for other kidney disorders, in particular proximal tubulopathies.

Given the considerable risks of morbidity and mortality associated with allogeneic HSC transplantation, the current goal is to develop autologous transplantation of gene-corrected HSCs using a lentiviral vector containing *CTNS* for the treatment of cystinosis. Preclinical studies using such an approach have been completed in *Ctns*-knockout mice and showed evidence of efficacy¹⁷⁶. Clinical trials using lentiviral vectors to transduce human HSCs have been widely undertaken in the USA and Europe, for several conditions including HIV infection, β -thalassemia, immunodeficiency diseases such as severe combined or adenosine deaminase immunodeficiency and metabolic diseases such as adrenoleukodystrophy and metachromatic leukodystrophy; no major adverse events have been reported so far, but extreme care remains necessary^{191–195}.

Conclusions

Important new findings have improved our understanding of the renal Fanconi syndrome in cystinosis, and this entity is now understood to derive from several pathogenic mechanisms, including PTC dedifferentiation, impaired vesicular trafficking, defective mTOR signalling and autophagy. Further studies will be necessary in order to completely understand the mechanisms that lead to initiation of this syndrome. Recent insights have highlighted the fact that cystinosis is not only a lysosomal cystine transporter, but also carries other functions important for PTC homeostasis and vesicular transport. Only a combination of genomic, proteomic, and metabolomic approaches along with integrated analyses using histology, cell biology and biochemistry can help elucidate the underlying mechanisms of the renal Fanconi syndrome in cystinosis, potentially leading to new therapeutic approaches. Preclinical studies of HSC transplantation in a mouse model of cystinosis have yielded promising results, and translation of this approach into a clinical trial, which will examine to what extent this approach is also beneficial to patients, is underway.

Acknowledgments

S.C. and P.J.C. are funded by the Cystinosis Research Foundation, as well as NIH grants RO1-DK090058, R21-NS090066 and grant from the Sanford Stem Cell Clinical Center (to S.C.), and Belgian F.R.S./FNRS (to P.J.C.). We acknowledge Corinne Antignac, Imagine Institute, France, for her helpful comments while assembling this Review, Heloise Gaide Chevronnay, CELL, de Duve Institute, Belgium, for her pivotal collaboration, and Patrick Van Der Smissen, CELL & PICT, de Duve Institute, Belgium, for kindly providing electron micrographs used in FIG. 2. We are also grateful to numerous colleagues who have provided so many insightful comments and contributive suggestions over the years.

Glossary

Swan neck deformities

Tubular lesions that appear in microdissected nephrons as long atrophic tubules (the ‘swan necks’), appearing below the glomeruli (the ‘swan heads’).

Founder mutations

Mutations that appear in the DNA of one or more individuals who are founders of a distinct population.

K_m

Concentration of half-maximal rate. For a transporter, the K_m value is inversely related to its affinity for its cargo.

Half-cystine

In biochemical assays, cystine values are reported as half-molecules because cystine was originally measured in reducing conditions (as cysteine), and cysteine is half a molecule of cystine. Cystine is now directly measured as cystine using a mass spectrometer but the values are still reported as half-cystine per mg of protein.

Lysosomal fusion

Fusion of acidified late endosomes carrying endocytic cargo with resting lysosomes bearing concentrated enzymes, or fusion between resting overloaded lysosomes (then also called interlysosomal fusion).

Lysosomal fission

Vesicular budding from endolysosomes (to regenerate late endosomes and resting lysosomes), or division of overloaded resting lysosomes into smaller ones to randomize residual content by interlysosomal fusion.

Residual body

A resting lysosome, filled with undigestible content, that no longer engages in vesicular trafficking.

Lysosomal defecation

Active exocytosis of overloaded lysosomes. This lysosomal clearing activity is stimulated by transcription factor EB.

Lysosomal amino acid sensors

Component or interacting partner of the mTOR-complex machinery that informs mTOR kinase on starvation or amino acid abundance.

Low molecular weight proteinuria

A commonly used term to describe tubular proteinuria due to defective proximal tubular endocytosis of plasma proteins selectively ultrafiltrated according to their size and/or sieving coefficient (thus excluding IgGs and other high molecular weight (MW) proteins), as opposed to non-selective glomerular leak. This terminology is convenient, but might be

misleading since pure tubular proteinuria typically includes relatively high MW proteins such as transferrin (80 kDa) and even larger globular proteins.

Cross-correction

Transfer of functional protein from normal cells to deficient cells.

Pathogen spreading

Transmission of bacteria, virus or parasites from one cell to another. Bacteria and virus such as HIV have been shown to colonize cells using the tunnelling nanotube route.

References

1. Fanconi G. Die nicht diabetischen glykosurien und hyperglykemiendes altem Kindes. *Jahrbuch Kinderheilkunde*. 1931; 133:257–300.
2. De Toni G. Remarks on the relations between renal rickets (renal dwarfism) and renal diabetes. *Acta Paediatr*. 1933; 16:479–484.
3. Debre R, Marie J, Cleret J, Messimy R. Rachitisme tardif coexistent avec une nephrite chronique et une glycosurie. *Arch Med Enfants*. 1934; 37:597–606.
4. Haffner D, et al. Long-term outcome of paediatric patients with hereditary tubular disorders. *Nephron*. 1999; 83:250–260. [PubMed: 10529632]
5. Gahl WA, Thoene JG, Schneider JA. Cystinosis. *N Engl J Med*. 2002; 347:111–121. [PubMed: 12110740]
6. Cohen C, et al. Excellent long-term outcome of renal transplantation in cystinosis patients. *Orphanet J Rare Diseases*. 2015; 10:90. [PubMed: 26208493]
7. Gahl WA, Kuehl EM, Iwata F, Lindblad A, Kaiser-Kupfer MI. Corneal crystals in nephropathic cystinosis: natural history and treatment with cysteamine eyedrops. *Mol Genet Metab*. 2000; 71:100–120. [PubMed: 11001803]
8. Brodin-Sartorius A, et al. Cysteamine therapy delays the progression of nephropathic cystinosis in late adolescents and adults. *Kidney Int*. 2012; 81:179–189. [PubMed: 21900880]
9. Langman CB, et al. Controversies and research agenda in nephropathic cystinosis: conclusions from a “Kidney Disease: Improving Global Outcomes” (KDIGO) Controversies Conference. *Kidney Int*. 2016; 89:1192–1203. [PubMed: 27181776]
10. Nesterova G, Gahl W. Nephropathic cystinosis: late complications of a multisystemic disease. *Pediatr Nephrol*. 2008; 23:863–878. [PubMed: 18008091]
11. Goldman H, Scriver CR, Aaron K, Delvin E, Canlas Z. Adolescent cystinosis: comparisons with infantile and adult forms. *Pediatrics*. 1971; 47:979–988. [PubMed: 5141767]
12. Cogan DG, Kuwabara T, Kinoshita J, Sheehan L, Merola L. Cystinosis in an adult. *J Am Med Assoc*. 1957; 164:394–396. [PubMed: 13415995]
13. Town M, et al. A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat Genet*. 1998; 18:319–324. [PubMed: 9537412]
14. Cherqui S, Kalatzis V, Trugnan G, Antignac C. The targeting of cystinosin to the lysosomal membrane requires a tyrosine-based signal and a novel sorting motif. *J Biol Chem*. 2001; 276:13314–13321. [PubMed: 11150305]
15. Kalatzis V, Cherqui S, Antignac C, Gasnier B. Cystinosin, the protein defective in cystinosis, is a H(+)-driven lysosomal cystine transporter. *EMBO J*. 2001; 20:5940–5949. [PubMed: 11689434]
16. Kalatzis V, et al. The ocular anomalies in a cystinosis animal model mimic disease pathogenesis. *Pediatr Res*. 2007; 62:156–162. [PubMed: 17597652]
17. Simpson J, et al. Quantitative *in vivo* and *ex vivo* confocal microscopy analysis of corneal cystine crystals in the *Ctns* knockout mouse. *Mol Vision*. 2011; 17:2212–2220.
18. Cherqui S, et al. Intralysosomal cystine accumulation in mice lacking cystinosin, the protein defective in cystinosis. *Mol Cell Biol*. 2002; 22:7622–7632. [PubMed: 12370309]

19. Cheung WW, et al. Muscle wasting and adipose tissue browning in infantile nephropathic cystinosis. *J Cachexia Sarcopenia Muscle*. 2016; 7:152–164. [PubMed: 27493869]
20. Gaide Chevronnay HP, et al. 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*. 2015; 6:2349–2364.
21. Nevo N, et al. Renal phenotype of the cystinosis mouse model is dependent upon genetic background. *Nephrol Dial Transplant*. 2010; 25:1059–1066. [PubMed: 19846395]
22. Mahoney CP, Striker GE. Early development of the renal lesions in infantile cystinosis. *Pediatr Nephrol*. 2000; 15:50–56. [PubMed: 11095011]
23. Yeagy BA, et al. Kidney preservation by bone marrow cell transplantation in hereditary nephropathy. *Kidney Int*. 2011; 79:1198–1206. [PubMed: 21248718]
24. Schneider JA. Approval of cysteamine for patients with cystinosis. *Pediatr Nephrol*. 1995; 9:254. [PubMed: 7794731]
25. Thoene JG, Oshima RG, Crawhall JC, Olson DL, Schneider JA. Cystinosis. Intracellular cystine depletion by aminothiols *in vitro* and *in vivo*. *J Clin Invest*. 1976; 58:180–189. [PubMed: 932205]
26. Cherqui S. Cysteamine therapy: a treatment for cystinosis, not a cure. *Kidney Int*. 2012; 81:127–129. [PubMed: 22205430]
27. Gahl WA, Balog JZ, Kleta R. Nephropathic cystinosis in adults: natural history and effects of oral cysteamine therapy. *Ann Internal Med*. 2007; 147:242–250. [PubMed: 17709758]
28. Emma F, et al. Nephropathic cystinosis: an international consensus document. *Nephrol Dial Transplant*. 2014; 29(Suppl 4):87–94.
29. Schulman JD, Schneider JA. Cystinosis and the Fanconi syndrome. *Pediatr Clin North Amer*. 1976; 23:779–793. [PubMed: 792782]
30. Ivanova EA, et al. Cystinosin deficiency causes podocyte damage and loss associated with increased cell motility. *Kidney Int*. 2016; 89:1037–1048. [PubMed: 27083281]
31. Wilmer MJ, Christensen EI, van den Heuvel LP, Monnens LA, Levtchenko EN. Urinary protein excretion pattern and renal expression of megalin and cubilin in nephropathic cystinosis. *Am J Kidney Dis*. 2008; 51:893–903. [PubMed: 18455850]
32. North American Pediatric Renal Trials and Collaborative Studies. NAPRTCS Annual Reports. NAPRTCS Online [online]. 2011. <https://web.emmes.com/study/ped/annlrept/annlrept.html>
33. Dufier JL, Dhermy P, Gubler MC, Gagnadoux MF, Broyer M. Ocular changes in long-term evolution of infantile cystinosis. *Ophthalm Paediatr Genet*. 1987; 8:131–137.
34. Tsilou E, Zhou M, Gahl W, Sieving PC, Chan CC. Ophthalmic manifestations and histopathology of infantile nephropathic cystinosis: report of a case and review of the literature. *Survey Ophthalmol*. 2007; 52:97–105.
35. Gultekingil Keser A, Topaloglu R, Bilginer Y, Besbas N. Long-term endocrinologic complications of cystinosis. *Minerva Pediatr*. 2014; 66:123–130. [PubMed: 24835445]
36. Besouw MT, Holeyijn S, Levtchenko EN, Janssen MC. Non-invasive measurements of atherosclerosis in adult cystinosis patients. *J Inherited Metabol Dis*. 2011; 34:811–818.
37. Ueda M, et al. Coronary artery and other vascular calcifications in patients with cystinosis after kidney transplantation. *Clin J Am Soc Nephrol*. 2006; 1:555–562. [PubMed: 17699259]
38. Dixit MP, Greifer I. Nephropathic cystinosis associated with cardiomyopathy: a 27-year clinical follow-up. *BMC Nephrol*. 2002; 3:8. [PubMed: 12425721]
39. Broyer M, Guillot M, Gubler MC, Habib R. Infantile cystinosis: a reappraisal of early and late symptoms. *Adv Nephrol Necker Hosp*. 1981; 10:137–166. [PubMed: 6791467]
40. Klusmann, M., Van't Hoff, W., Monsell, F., Offiah, AC. Progressive destructive bone changes in patients with cystinosis. *Skeletal Radiol*. <http://dx.doi.org/10.1007/s00256-013-1735-z>
41. Bacchetta J, et al. Skeletal implications and management of cystinosis: three case reports and literature review. *Bonekey Rep*. 2016; 5:828. [PubMed: 27579165]
42. Ballantyne AO, Trauner DA. Neurobehavioral consequences of a genetic metabolic disorder: visual processing deficits in infantile nephropathic cystinosis. *Cogn Behav Neurol*. 2000; 13:254–263.
43. Scarvie KM, Ballantyne AO, Trauner DA. Visuomotor performance in children with infantile nephropathic cystinosis. *Percep Mot Skills*. 1996; 82:67–75.

44. Trauner DA, Chase C, Scheller J, Katz B, Schneider JA. Neurologic and cognitive deficits in children with cystinosis. *J Pediatr*. 1988; 112:912–914. [PubMed: 3373397]
45. Trauner DA, Spilkin AM, Williams J, Babchuck L. Specific cognitive deficits in young children with cystinosis: evidence for an early effect of the cystinosis gene on neural function. *J Pediatr*. 2007; 151:192–196. [PubMed: 17643777]
46. Trauner DA, et al. Neurological impairment in nephropathic cystinosis: motor coordination deficits. *Pediatr Nephrol*. 2010; 25:2061–2066. [PubMed: 20652328]
47. Viltz L, Trauner DA. Effect of age at treatment on cognitive performance in patients with cystinosis. *J Pediatr*. 2013; 163:489–492. [PubMed: 23462307]
48. Anikster Y, et al. Pulmonary dysfunction in adults with nephropathic cystinosis. *Chest*. 2001; 119:394–401. [PubMed: 11171714]
49. Sonies BC, Almajid P, Kleta R, Bernardini I, Gahl WA. Swallowing dysfunction in 101 patients with nephropathic cystinosis: benefit of long-term cysteamine therapy. *Medicine*. 2005; 84:137–146. [PubMed: 15879904]
50. Levy M, Feingold J. Estimating prevalence in single-gene kidney diseases progressing to renal failure. *Kidney Int*. 2000; 58:925–943. [PubMed: 10972657]
51. Bois E, Feingold J, Frenay P, Briard ML. Infantile cystinosis in France: genetics, incidence, geographic distribution. *J Med Genet*. 1976; 13:434–438. [PubMed: 1018302]
52. Cochat, P., Cordier, B., Lacôte, C., Saïd, M-H. Cystinosis. Broyer, M., editor. Elsevier; 1999. p. 28-35.
53. DeBraekeleer M. Hereditary disorders in Saguenay-Lac-St-Jean (Quebec, Canada). *Hum Hered*. 1991; 41:141–146. [PubMed: 1937486]
54. Attard M, et al. Severity of phenotype in cystinosis varies with mutations in the *CTNS* gene: predicted effect on the model of cystinosis. *Hum Mol Genet*. 1999; 8:2507–2514. [PubMed: 10556299]
55. Kalatzis V, et al. Characterization of a putative founder mutation that accounts for the high incidence of cystinosis in Brittany. *J Am Soc Nephrol*. 2001; 12:2170–2174. [PubMed: 11562417]
56. McGowan-Jordan J, et al. Molecular Analysis of Cystinosis: Probable Irish Origin of the Most Common French Canadian Mutation. *Eur J Hum Genet*. 1999; 7:671–678. [PubMed: 10482956]
57. Anikster Y, et al. Identification and detection of the common 65-kb deletion breakpoint in the nephropathic cystinosis gene (*CTNS*). *Mol Genet Metab*. 1999; 66:111–116. [PubMed: 10068513]
58. Aldahmesh MA, et al. Characterization of *CTNS* mutations in Arab patients with cystinosis. *Ophthalm Genet*. 2009; 30:185–189.
59. Owen EP, et al. Common mutation causes cystinosis in the majority of black South African patients. *Pediatr Nephrol*. 2015; 30:595–601. [PubMed: 25326109]
60. Shahkarami S, Galehdari H, Ahmadzadeh A, Babaahmadi M, Pedram M. The first molecular genetics analysis of individuals suffering from nephropathic cystinosis in the Southwestern Iran. *Nefrologia*. 2013; 33:308–315. [PubMed: 23640116]
61. Soliman NA, et al. Mutational Spectrum of the *CTNS* Gene in Egyptian Patients with Nephropathic Cystinosis. *JIMD reports*. 2014; 14:87–97. [PubMed: 24464559]
62. Aly R, Makar S, El Bakri A, Soliman NA. Neurocognitive functions and behavioral profiles in children with nephropathic cystinosis. *Saudi J Kidney Dis Transpl*. 2014; 25:1224–1231. [PubMed: 25394439]
63. Elmonem MA, et al. Lysosomal Storage Disorders in Egyptian Children. *Indian J Pediatr*. 2016; 8:805–813.
64. Mirdehghan M, Ahmadzadeh A, Bana-Behbahani M, Motlagh I, Chomali B. Infantile cystinosis. *Indian Pediatr*. 2003; 40:21–24. [PubMed: 12554913]
65. Kir'ianov NA, Bazhenov EL, Stetsenko EV. Cystinosis in an adult. *Arkh Patol*. 1992; 54:34–36.
66. Tang S, Danda S, Zoleikhaian M, Simon M, Huang T. An Indian boy with nephropathic cystinosis: a case report and molecular analysis of *CTNS* mutation. *Genet Test Mol Biomarkers*. 2009; 13:435–438. [PubMed: 19580442]
67. Yang YJ, et al. First report of *CTNS* mutations in a Chinese family with infantile cystinosis. *ScientificWorldJournal*. 2015; 2015:309410. [PubMed: 25866837]

68. The Cystinosis Collaborative Research Group. Linkage of the gene for cystinosis to markers on the short arm of chromosome 17. *Nat Genet.* 1995; 10:246–248. [PubMed: 7663525]
69. Taranta A, et al. Identification and subcellular localization of a new cystinosin isoform. *Am J Physiol Renal Physiol.* 2008; 294:F1101–1108. [PubMed: 18337546]
70. Bellomo F, et al. Carboxyl-Terminal SSLKG Motif of the Human Cystinosin-LKG Plays an Important Role in Plasma Membrane Sorting. *PLoS ONE.* 2016; 11:e0154805. [PubMed: 27148969]
71. Taranta A, et al. Distribution of cystinosin-LKG in human tissues. *Histochem Cell Biol.* 2012; 138:351–363. [PubMed: 22544350]
72. Andrzejewska Z, et al. Lysosomal Targeting of Cystinosin Requires AP-3. *Traffic.* 2015; 16:712–726. [PubMed: 25753619]
73. Kyttala A, Ihrke G, Vesa J, Schell MJ, Luzio JP. Two motifs target Batten disease protein CLN3 to lysosomes in transfected nonneuronal and neuronal cells. *Mol Biol Cell.* 2004; 15:1313–1323. [PubMed: 14699076]
74. Saudek V. Cystinosin, MPDU1, SWEETs and KDELr belong to a well-defined protein family with putative function of cargo receptors involved in vesicle trafficking. *PLoS ONE.* 2012; 7:e30876. [PubMed: 22363504]
75. Ruivo R, et al. Mechanism of proton/substrate coupling in the heptahelical lysosomal transporter cystinosin. *Proc Natl Acad Sci USA.* 2012; 109:E210–217. [PubMed: 22232659]
76. Lee Y, Nishizawa T, Yamashita K, Ishitani R, Nureki O. Structural basis for the facilitative diffusion mechanism by SemiSWEET transporter. *Nat Commun.* 2015; 6:6112. [PubMed: 25598322]
77. Jezegou A, et al. Heptahelical protein PQLC2 is a lysosomal cationic amino acid exporter underlying the action of cysteamine in cystinosis therapy. *Proc Natl Acad Sci USA.* 2012; 109:E3434–3443. [PubMed: 23169667]
78. Thamotharan M, Lombardo YB, Bawani SZ, Adibi SA. An active mechanism for completion of the final stage of protein degradation in the liver, lysosomal transport of dipeptides. *J Biol Chem.* 1997; 272:11786–11790. [PubMed: 9115234]
79. Thoene J, et al. In vitro correction of disorders of lysosomal transport by microvesicles derived from baculovirus-infected *Spodoptera* cells. *Mol Genet Metab.* 2013; 109:77–85. [PubMed: 23465695]
80. Leinekugel P, Michel S, Conzelmann E, Sandhoff K. Quantitative Correlation between the Residual Activity of Beta-Hexosaminidase-a and Arylsulfatase-a and the Severity of the Resulting Lysosomal Storage Disease. *Hum Genet.* 1992; 88:513–523. [PubMed: 1348043]
81. Kalatzis V, Nevo N, Cherqui S, Gasnier B, Antignac C. Molecular pathogenesis of cystinosis: effect of *CTNS* mutations on the transport activity and subcellular localization of cystinosin. *Hum Mol Genet.* 2004; 13:1361–1371. [PubMed: 15128704]
82. Thoene JG, Lemons RM. Cystine accumulation in cystinotic fibroblasts from free and protein-linked cystine but not cysteine. *Biochem J.* 1982; 208:823–830. [PubMed: 6762213]
83. Thoene JG, Lemons R. Modulation of the intracellular cystine content of cystinotic fibroblasts by extracellular albumin. *Pediatr Res.* 1980; 14:785–787. [PubMed: 7402752]
84. Thoene JG, Oshima RG, Ritchie DG, Schneider JA. Cystinotic fibroblasts accumulate cystine from intracellular protein degradation. *Proc Natl Acad Sci USA.* 1977; 74:4505–4507. [PubMed: 270698]
85. Amsellem S, et al. Cubilin is essential for albumin reabsorption in the renal proximal tubule. *J Am Soc Nephrol.* 2010; 21:1859–1867. [PubMed: 20798259]
86. Ovunc B, et al. Exome sequencing reveals cubilin mutation as a single-gene cause of proteinuria. *J Am Soc Nephrol.* 2011; 22:1815–1820. [PubMed: 21903995]
87. Oude Elferink RP, Harms E, Strijland A, Tager JM. The intralysosomal pH in cultured human skin fibroblasts in relation to cystine accumulation in patients with cystinosis. *Biochem Biophys Res Commun.* 1983; 116:154–161. [PubMed: 6639653]
88. Elmonem MA, et al. Cystinosis: a review. *Orphanet J Rare Diseases.* 2016; 11:47. [PubMed: 27102039]

89. Futter CE, Pearse A, Hewlett LJ, Hopkins CR. Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. *J Cell Biol.* 1996; 132:1011–1023. [PubMed: 8601581]
90. Montgomery RR, Webster P, Mellman I. Accumulation of indigestible substances reduces fusion competence of macrophage lysosomes. *J Immunol.* 1991; 147:3087–3095. [PubMed: 1919007]
91. Gaide Chevronnay HP, et al. Time course of pathogenic and adaptation mechanisms in cystinotic mouse kidneys. *J Am Soc Nephrol.* 2014; 25:1256–1269. [PubMed: 24525030]
92. Gubler, MC., Lacoste, M., Sich, M., Broyer, M. Cystinosis. Broyer, M., editor. Vol. 1. Elsevier; 1999. p. 42-48.
93. Prencipe G, et al. Inflammasome activation by cystine crystals: implications for the pathogenesis of cystinosis. *J Am Soc Nephrol.* 2014; 25:1163–1169. [PubMed: 24525029]
94. Baum M. The Fanconi syndrome of cystinosis: insights into the pathophysiology. *Pediatr Nephrol.* 1998; 12:492–497. [PubMed: 9745876]
95. Coor C, Salmon RF, Quigley R, Marver D, Baum M. Role of adenosine triphosphate (ATP) and NaK ATPase in the inhibition of proximal tubule transport with intracellular cystine loading. *J Clin Invest.* 1991; 87:955–961. [PubMed: 1847941]
96. Foreman JW, et al. Metabolic studies of rat renal tubule cells loaded with cystine: the cystine dimethylester model of cystinosis. *J Am Soc Nephrol.* 1995; 6:269–272. [PubMed: 7579095]
97. Wilmer MJ, van den Heuvel LP, Levtchenko EN. The use of CDME in cystinosis research. *Neurochem Res.* 2008; 33:2373–2374. [PubMed: 18427982]
98. Levtchenko EN, et al. Decreased intracellular ATP content and intact mitochondrial energy generating capacity in human cystinotic fibroblasts. *Pediatr Res.* 2006; 59:287–292. [PubMed: 16439594]
99. Wilmer MJ, et al. Mitochondrial complex V expression and activity in cystinotic fibroblasts. *Pediatr Res.* 2008; 64:495–497. [PubMed: 18596576]
100. Taub ML, Springate JE, Cutuli F. Reduced phosphate transport in the renal proximal tubule cells in cystinosis is due to decreased expression of transporters rather than an energy defect. *Biochem Biophys Res Commun.* 2011; 407:355–359. [PubMed: 21392501]
101. Taub M, Cutuli F. Activation of AMP kinase plays a role in the increased apoptosis in the renal proximal tubule in cystinosis. *Biochem Biophys Res Commun.* 2012; 426:516–521. [PubMed: 22982317]
102. Park M, Helip-Wooley A, Thoene J. Lysosomal cystine storage augments apoptosis in cultured human fibroblasts and renal tubular epithelial cells. *J Am Soc Nephrol.* 2002; 13:2878–2887. [PubMed: 12444206]
103. Park MA, Pejovic V, Kerisit KG, Junius S, Thoene JG. Increased apoptosis in cystinotic fibroblasts and renal proximal tubule epithelial cells results from cysteinylolation of protein kinase Cdelta. *J Am Soc Nephrol.* 2006; 17:3167–3175. [PubMed: 17021265]
104. Galarreta CI, et al. The swan-neck lesion: proximal tubular adaptation to oxidative stress in nephropathic cystinosis. *Am J Physiol Renal Physiol.* 2015; 308:F1155–1166. [PubMed: 25694483]
105. Sansanwal P, Kambham N, Sarwal MM. Caspase-4 may play a role in loss of proximal tubules and renal injury in nephropathic cystinosis. *Pediatr Nephrol.* 2010; 25:105–109. [PubMed: 19705160]
106. Chol M, Nevo N, Cherqui S, Antignac C, Rustin P. Glutathione precursors replenish decreased glutathione pool in cystinotic cell lines. *Biochem Biophys Res Commun.* 2004; 324:231–235. [PubMed: 15465007]
107. Levtchenko E, et al. Altered status of glutathione and its metabolites in cystinotic cells. *Nephrol Dial Transplant.* 2005; 20:1828–1832. [PubMed: 15956064]
108. Wilmer MJ, et al. Elevated oxidized glutathione in cystinotic proximal tubular epithelial cells. *Biochem Biophys Res Commun.* 2005; 337:610–614. [PubMed: 16202976]
109. Mannucci L, et al. Impaired activity of the gamma-glutamyl cycle in nephropathic cystinosis fibroblasts. *Pediatr Res.* 2006; 59:332–335. [PubMed: 16439602]
110. Rizzo C, et al. Pyroglutamic aciduria and nephropathic cystinosis. *J Inherited Metabol Dis.* 1999; 22:224–226.

111. Laube GF, et al. Glutathione depletion and increased apoptosis rate in human cystinotic proximal tubular cells. *Pediatr Nephrol.* 2006; 21:503–509. [PubMed: 16508773]
112. Sakarcan A, Timmons C, Baum M. Intracellular distribution of cystine in cystine-loaded proximal tubules. *Pediatr Res.* 1994; 35:447–450. [PubMed: 8047382]
113. Spear GS, Slusser RJ, Tousimis AJ, Taylor CG, Schulman JD. Cystinosis. An ultrastructural and electron-probe study of the kidney with unusual findings. *Arch Pathol.* 1971; 91:206–221. [PubMed: 4322430]
114. Pache de Faria Guimaraes L, et al. N-Acetyl-cysteine is associated to renal function improvement in patients with nephropathic cystinosis. *Pediatr Nephrol.* 2014; 29:1097–1102. [PubMed: 24326786]
115. Moldeus P, Ormstad K, Reed DJ. Turnover of cellular glutathione in isolated rat-kidney cells. Role of cystine and methionine. *Eur J Biochem.* 1981; 116:13–16. [PubMed: 7250117]
116. Meister A, Anderson ME, Hwang O. Intracellular cysteine and glutathione delivery systems. *J Am College Nutr.* 1986; 5:137–151.
117. Frey IM, et al. Profiling at mRNA, protein, and metabolite levels reveals alterations in renal amino acid handling and glutathione metabolism in kidney tissue of *Pept2^{-/-}* mice. *Physiol Genom.* 2007; 28:301–310.
118. Ahmed K, Dasgupta P, Khan MS. Cystine calculi: challenging group of stones. *Postgrad Med J.* 2006; 82:799–801. [PubMed: 17148700]
119. Kessler A, et al. Antioxidant effect of cysteamine in brain cortex of young rats. *Neurochem Res.* 2008; 33:737–744. [PubMed: 17940891]
120. Okamura DM, et al. Cysteamine modulates oxidative stress and blocks myofibroblast activity in CKD. *J Am Soc Nephrol.* 2014; 25:43–54. [PubMed: 24009239]
121. Wilmer MJ, et al. Cysteamine restores glutathione redox status in cultured cystinotic proximal tubular epithelial cells. *Biochim Biophys Acta.* 2011; 1812:643–651. [PubMed: 21371554]
122. Raggi C, et al. Dedifferentiation and aberrations of the endolysosomal compartment characterize the early stage of nephropathic cystinosis. *Hum Mol Genet.* 2014; 23:2266–2278. [PubMed: 24319100]
123. Ivanova EA, et al. Endo-lysosomal dysfunction in human proximal tubular epithelial cells deficient for lysosomal cystine transporter cystinosin. *PLoS ONE.* 2015; 10:e0120998. [PubMed: 25811383]
124. Christensen EI, et al. Loss of chloride channel *ClC-5* impairs endocytosis by defective trafficking of megalin and cubilin in kidney proximal tubules. *Proc Natl Acad Sci USA.* 2003; 100:8472–8477. [PubMed: 12815097]
125. Vicinanza M, et al. OCRL controls trafficking through early endosomes via PtdIns4,5P(2)-dependent regulation of endosomal actin. *EMBO J.* 2011; 30:4970–4985. [PubMed: 21971085]
126. Bandyopadhyay D, Cyphersmith A, Zapata JA, Kim YJ, Payne CK. Lysosome transport as a function of lysosome diameter. *PLoS ONE.* 2014; 9:e86847. [PubMed: 24497985]
127. Li X, et al. A molecular mechanism to regulate lysosome motility for lysosome positioning and tubulation. *Nat Cell Biol.* 2016; 18:404–417. [PubMed: 26950892]
128. Ivanova EA, et al. Ca(2+) signalling in human proximal tubular epithelial cells deficient for cystinosin. *Cell Calcium.* 2016; 60:282–287. [PubMed: 27451386]
129. Johnson JL, et al. Upregulation of the Rab27a-dependent trafficking and secretory mechanisms improves lysosomal transport, alleviates endoplasmic reticulum stress, and reduces lysosome overload in cystinosis. *Mol Cell Biol.* 2013; 33:2950–2962. [PubMed: 23716592]
130. Schulman JD, Bradley KH, Seegmiller JE. Cystine: compartmentalization within lysosomes in cystinotic leukocytes. *Science.* 1969; 166:1152–1154. [PubMed: 5348284]
131. Kooistra T, Millard PC, Lloyd JB. Role of thiols in degradation of proteins by cathepsins. *Biochem J.* 1982; 204:471–477. [PubMed: 7052070]
132. Efeyan A, Zoncu R, Sabatini DM. Amino acids and mTORC1: from lysosomes to disease. *Trends Mol Med.* 2012; 18:524–533. [PubMed: 22749019]

133. Settembre C, Fraldi A, Medina DL, Ballabio A. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat Rev Mol Cell Biol.* 2013; 14:283–296. [PubMed: 23609508]
134. Napolitano G, et al. Impairment of chaperone-mediated autophagy leads to selective lysosomal degradation defects in the lysosomal storage disease cystinosis. *EMBO Mol Med.* 2015; 2:158–174.
135. Sansanwal P, et al. Mitochondrial autophagy promotes cellular injury in nephropathic cystinosis. *J Am Soc Nephrol.* 2010; 21:272–283. [PubMed: 19959713]
136. Fantus D, Rogers NM, Grahammer F, Huber TB, Thomson AW. Roles of mTOR complexes in the kidney: implications for renal disease and transplantation. *Nat Rev Nephrol.* 2016; 12:587–609. [PubMed: 27477490]
137. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell.* 2012; 150:1196–1208. [PubMed: 22980980]
138. Sancak Y, et al. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell.* 2010; 141:290–303. [PubMed: 20381137]
139. Laplante M, Sabatini DM. Regulation of mTORC1 and its impact on gene expression at a glance. *J Cell Sci.* 2013; 126:1713–1719. [PubMed: 23641065]
140. Martina JA, Puertollano R. RAG GTPases link nutrient availability to gene expression, autophagy and lysosomal biogenesis. *Autophagy.* 2013; 9:928–930. [PubMed: 23524842]
141. Zoncu R, et al. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H⁺-ATPase. *Science.* 2011; 334:678–683. [PubMed: 22053050]
142. Andrzejewska Z, et al. Cystinosis is a Component of the Vacuolar H⁺-ATPase-Ragulator-Rag Complex Controlling Mammalian Target of Rapamycin Complex 1 Signaling. *J Am Soc Nephrol.* 2016; 27:1678–1688. [PubMed: 26449607]
143. Ivanova EA, et al. Altered mTOR signalling in nephropathic cystinosis. *J Inherited Metabol Dis.* 2016; 39:457–464.
144. Gleixner EM, et al. V-ATPase/mTOR signaling regulates megalin-mediated apical endocytosis. *Cell Rep.* 2014; 8:10–19. [PubMed: 24953654]
145. Rega LR, et al. Activation of the transcription factor EB rescues lysosomal abnormalities in cystinotic kidney cells. *Kidney Int.* 2016; 89:862–873. [PubMed: 26994576]
146. Platt FM, Boland B, van der Spoel AC. The cell biology of disease: lysosomal storage disorders: the cellular impact of lysosomal dysfunction. *J Cell Biol.* 2012; 199:723–734. [PubMed: 23185029]
147. Kaushik S, Cuervo AM. Chaperone-mediated autophagy: a unique way to enter the lysosome world. *Trends Cell Biol.* 2012; 22:407–417. [PubMed: 22748206]
148. Kiffin R, Christian C, Knecht E, Cuervo AM. Activation of chaperone-mediated autophagy during oxidative stress. *Mol Biol Cell.* 2004; 15:4829–4840. [PubMed: 15331765]
149. Massey AC, Kaushik S, Sovak G, Kiffin R, Cuervo AM. Consequences of the selective blockage of chaperone-mediated autophagy. *Proc Natl Acad Sci USA.* 2006; 103:5805–5810. [PubMed: 16585521]
150. Balmer C, et al. Familial X-linked cardiomyopathy (Danon disease): diagnostic confirmation by mutation analysis of the LAMP2 gene. *Eur J Pediatr.* 2005; 164:509–514. [PubMed: 15889279]
151. Fanin M, et al. Generalized lysosome-associated membrane protein-2 defect explains multisystem clinical involvement and allows leukocyte diagnostic screening in Danon disease. *Am J Pathol.* 2006; 168:1309–1320. [PubMed: 16565504]
152. Horvath J, et al. Identification of a novel LAMP2 mutation responsible for X-chromosomal dominant Danon disease. *Neuropediatrics.* 2003; 34:270–273. [PubMed: 14598234]
153. Lima WR, et al. ZONAB promotes proliferation and represses differentiation of proximal tubule epithelial cells. *J Am Soc Nephrol.* 2010; 21:478–488. [PubMed: 20133480]
154. Janssens, V., et al. Fifth International Cystinosis Research Symposium. Univ. of California; 2016.
155. Chevalier RL. The proximal tubule in cystinosis: fight or flight? *J Am Soc Nephrol.* 2014; 25:1131–1132. [PubMed: 24525031]

156. Chevalier RL. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction. *Am J Physiol Renal Physiol.* 2016; 311:F145–161. [PubMed: 27194714]
157. Andrzejewska Z, et al. Cystinosin is a Component of the Vacuolar H⁺-ATPase-Ragulator-Rag Complex Controlling Mammalian Target of Rapamycin Complex 1 Signaling. *J Am Soc Nephrol.* 2015; 6:1678–1688.
158. Dohil R, et al. Long-term treatment of cystinosis in children with twice-daily cysteamine. *J Pediatr.* 2010; 156:823–827. [PubMed: 20138296]
159. Gangoiti JA, et al. Pharmacokinetics of enteric-coated cysteamine bitartrate in healthy adults: a pilot study. *Br J Clin Pharmacol.* 2010; 70:376–382. [PubMed: 20716238]
160. Dohil R, Cabrera BL. Treatment of cystinosis with delayed-release cysteamine: 6-year follow-up. *Pediatr Nephrol.* 2013; 28:507–510. [PubMed: 23001048]
161. Dohil R, et al. Twice-daily cysteamine bitartrate therapy for children with cystinosis. *J Pediatr.* 2010; 156:71–73. [PubMed: 19775699]
162. Goldenberg MM. Pharmaceutical approval update. *PT.* 2013; 38:323–324.
163. Langman CB, et al. Quality of life is improved and kidney function preserved in patients with nephropathic cystinosis treated for 2 years with delayed-release cysteamine bitartrate. *J Pediatr.* 2014; 165:528–533. [PubMed: 24948347]
164. Langman CB, et al. A randomized controlled crossover trial with delayed-release cysteamine bitartrate in nephropathic cystinosis: effectiveness on white blood cell cystine levels and comparison of safety. *Clin J Am Soc Nephrol.* 2012; 7:1112–1120. [PubMed: 22554716]
165. Kaiser-Kupfer MI, et al. A randomized placebo-controlled trial of cysteamine eye drops in nephropathic cystinosis. *Arch Ophthalmol.* 1990; 108:689–693. [PubMed: 2185723]
166. Kumar A, Bachhawat AK. A futile cycle, formed between two ATP-dependant gamma-glutamyl cycle enzymes, gamma-glutamyl cysteine synthetase and 5-oxoprolinase: the cause of cellular ATP depletion in nephrotic cystinosis? *J Biosci.* 2010; 35:21–25. [PubMed: 20413906]
167. Anguiano J, et al. Chemical modulation of chaperone-mediated autophagy by retinoic acid derivatives. *Nat Chem Biol.* 2013; 9:374–382. [PubMed: 23584676]
168. McNeill A, et al. Amroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. *Brain.* 2014; 137:1481–1495. [PubMed: 24574503]
169. Medina DL, et al. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev Cell.* 2011; 21:421–430. [PubMed: 21889421]
170. Sardiello M, et al. A gene network regulating lysosomal biogenesis and function. *Science.* 2009; 325:473–477. [PubMed: 19556463]
171. Dehay B, et al. Pathogenic lysosomal depletion in Parkinson's disease. *J Neurosci.* 2010; 30:12535–12544. [PubMed: 20844148]
172. Moskot M, et al. The phytoestrogen genistein modulates lysosomal metabolism and transcription factor EB (TFEB) activation. *J Biol Chem.* 2014; 289:17054–17069. [PubMed: 24770416]
173. Piotrowska E, et al. Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses. *Eur J Hum Genet.* 2006; 14:846–852. [PubMed: 16670689]
174. Piotrowska E, et al. Genistin-rich soy isoflavone extract in substrate reduction therapy for Sanfilippo syndrome: An open-label, pilot study in 10 pediatric patients. *Curr Ther Res Clin Exp.* 2008; 69:166–179. [PubMed: 24692796]
175. Lee KY, Kim JR, Choi HC. Genistein-induced LKB1-AMPK activation inhibits senescence of VSMC through autophagy induction. *Vascul Pharmacol.* 2016; 81:75–82. [PubMed: 26924458]
176. Harrison F, et al. Hematopoietic stem cell gene therapy for the multisystemic lysosomal storage disorder cystinosis. *Mol Ther.* 2013; 21:433–444. [PubMed: 23089735]
177. Syres K, et al. Successful treatment of the murine model of cystinosis using bone marrow cell transplantation. *Blood.* 2009; 114:2542–2552. [PubMed: 19506297]
178. Beck M. Therapy for lysosomal storage disorders. *IUBMB Life.* 2010; 62:33–40. [PubMed: 20014233]

179. Naphade S, et al. Brief reports: lysosomal cross-correction by hematopoietic stem cell-derived macrophages via tunneling nanotubes. *Stem Cells*. 2015; 33:301–309. [PubMed: 25186209]
180. Iglesias DM, et al. Stem cell microvesicles transfer cystinosis to human cystinotic cells and reduce cystine accumulation *in vitro*. *PLoS ONE*. 2012; 7:e42840. [PubMed: 22912749]
181. Onfelt B, et al. Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria. *J Immunol*. 2006; 177:8476–8483. [PubMed: 17142745]
182. Marzo L, Gousset K, Zurzolo C. Multifaceted roles of tunneling nanotubes in intercellular communication. *Frontiers Physiol*. 2012; 3:72.
183. Sowinski S, Alakoskela JM, Jolly C, Davis DM. Optimized methods for imaging membrane nanotubes between T cells and trafficking of HIV-1. *Methods*. 2011; 53:27–33. [PubMed: 20382227]
184. Humphreys BD. Kidney injury, stem cells and regeneration. *Curr Opin Nephrol Hypertens*. 2014; 23:25–31. [PubMed: 24231311]
185. Yeagy BA, Cherqui S. Kidney repair and stem cells: a complex and controversial process. *Pediatr Nephrol*. 2011; 26:1427–1434. [PubMed: 21336814]
186. Abrahamson DR, Leardkamolkarn V. Development of kidney tubular basement membranes. *Kidney Int*. 1991; 39:382–393. [PubMed: 2062031]
187. Halfter W, et al. Protein composition and biomechanical properties of *in vivo*-derived basement membranes. *Cell Adhesion Migr*. 2013; 7:64–71.
188. Rocca CJ, et al. Treatment of Inherited Eye Defects by Systemic Hematopoietic Stem Cell Transplantation. *Investigative Ophthalmol Visual Sci*. 2015; 56:7214–7223.
189. Gaide Chevronnay HP, et al. Hematopoietic stem cell transplantation can normalize thyroid function in a cystinosis mouse model. *Endocrinology*. 2016; 56:1363–1371.
190. Soos TJ, et al. CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. *Kidney Int*. 2006; 70:591–596. [PubMed: 16760907]
191. Biffi A, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science*. 2013; 341:1233158. [PubMed: 23845948]
192. Cartier N, et al. Lentiviral hematopoietic cell gene therapy for X-linked adrenoleukodystrophy. *Methods Enzymol*. 2012; 507:187–198. [PubMed: 22365775]
193. DiGiusto DL, et al. Development of hematopoietic stem cell based gene therapy for HIV-1 infection: considerations for proof of concept studies and translation to standard medical practice. *Viruses*. 2013; 5:2898–2919. [PubMed: 24284880]
194. Drakopoulou E, Papanikolaou E, Georgomanoli M, Anagnou NP. Towards more successful gene therapy clinical trials for beta-thalassemia. *Curr Mol Med*. 2013; 13:1314–1330. [PubMed: 23865429]
195. Zhang L, Thrasher AJ, Gaspar HB. Current progress on gene therapy for primary immunodeficiencies. *Gene Ther*. 2013; 20:963–969. [PubMed: 23719067]
196. Pisoni RL, Acker TL, Lisowski KM, Lemons RM, Thoene JG. A cysteine-specific lysosomal transport system provides a major route for the delivery of thiol to human fibroblast lysosomes: possible role in supporting lysosomal proteolysis. *J Cell Biol*. 1990; 110:327–335. [PubMed: 2404990]
197. Lloyd JB. Disulphide reduction in lysosomes. The role of cysteine. *Biochem J*. 1986; 237:271–272. [PubMed: 3800880]
198. Lloyd JB. Lysosomal handling of cystine residues: stoichiometry of cysteine involvement. *Biochem J*. 1992; 286:979–980. [PubMed: 1417759]
199. Pisoni RL, Park GY, Velilla VQ, Thoene JG. Detection and characterization of a transport system mediating cysteamine entry into human fibroblast lysosomes. Specificity for aminoethylthiol and aminoethylsulfide derivatives. *J Biol Chem*. 1995; 270:1179–1184. [PubMed: 7836377]

Key points

- Cystinosis is a multi-systemic lysosomal storage disease caused by inactivating mutations in, or the absence of, the lysosomal membrane exporter for cystine, cystinosin; cystinosis is the main cause of hereditary renal Fanconi syndrome
- Treatment with cysteamine efficiently depletes lysosomal cystine and delays progression to renal insufficiency; however, cysteamine does not reverse established renal Fanconi syndrome, indicating functions of cystinosin beyond cystine transport
- Insights from mechanistic studies suggest that the pathological mechanisms of Fanconi syndrome in cystinosis are multifactorial, involving oxidative stress and impaired vesicular trafficking, autophagy, and mTORC1 and TFEB signalling
- Haematopoietic stem cell (HSC) transplantation ameliorates renal Fanconi syndrome in cystinotic mice; HSCs differentiate into macrophages that transfer cystinosin-bearing lysosomes into proximal tubule cells via tunnelling nanotubes that cross the tubular basement membrane
- Since tunnelling nanotubes contain donor-derived cytosol and carry all types of organelles, this mechanism should be generic and could be used to correct other genetic diseases that affect proximal tubule cells

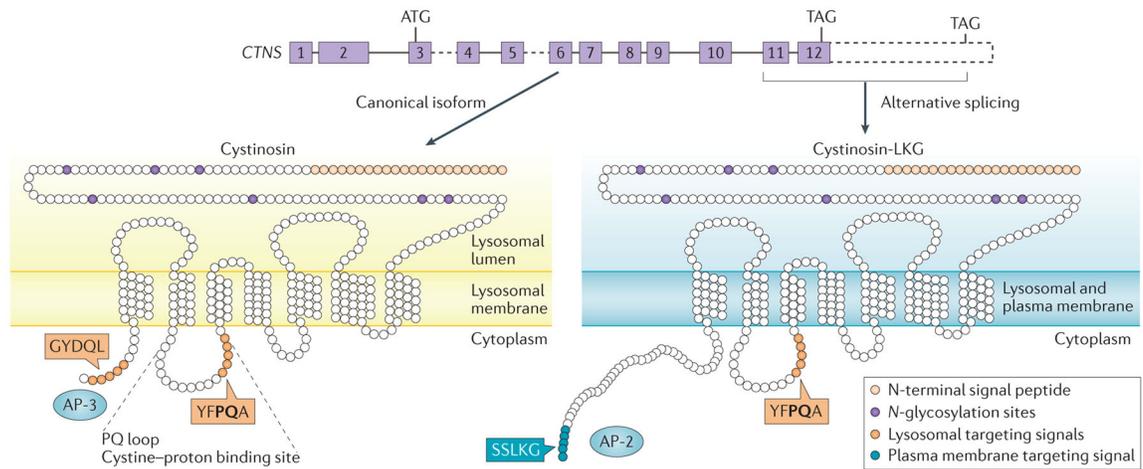


Figure 1. *CTNS* produces two isoforms with distinct subcellular localizations

CTNS, which encodes the seven transmembrane lysosomal protein cystinosin, is composed of 12 exons (solid boxes) with the start codon (ATG) in exon 3 and two alternative stop codons (TAGs) in exon 12. The common, canonical form of cystinosin is shown on the left. The 3' region of *CTNS* (dashed box) encodes the C-terminal extension of the less frequent isoform, cystinosin-LKG, which arises from alternative splicing in exon 12 (right). Canonical cystinosin exclusively localizes to lysosomes due to the concerted effect of two lysosome-targeting signals: a classic tyrosine-based C-terminal motif, GYDQL, and a non-classic motif, YFPQA, in the fifth transmembrane loop named the 'PQ loop', which also contains a cystine proton binding site. The GYDQL motif allows recognition by the adaptor protein complex 3 (AP 3), which is responsible for direct lysosomal targeting of cystinosin. The cystinosin-LKG isoform lacks the GYDQL motif but instead contains the carboxyl-terminal SSLKG sequence, which directs this isoform to the plasma membrane, with secondary endocytic trafficking to lysosomes involving AP-2 complexes.

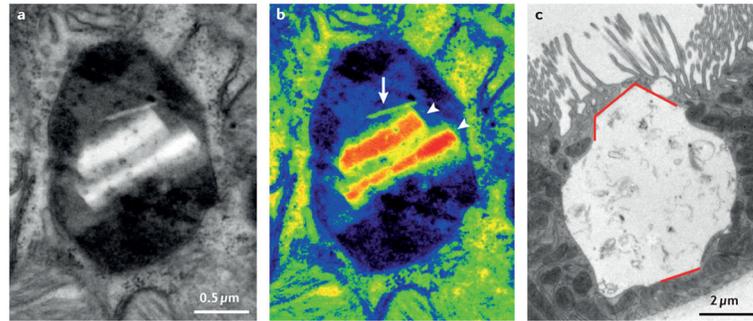


Figure 2. Cystine crystals accumulate within lysosomes of cystinotic cells

a | Conventional electron micrograph of a proximal tubule cell from an 8-month old *Ctns*-knockout mouse showing a lysosome that is about twofold larger in diameter than usual. **b** | Pseudo-colour conversion of grey levels, to better demonstrate intertwining of the normal dense matrix (dark areas) with amorphous, less electron-dense material suggestive of undigested protein (blue areas); and the two types of crystals: a sharp, needle-shaped crystal (arrow) and two polyhedral crystals (arrow heads). Such crystals are never seen before 6 months of age and needles do not usually contact the lysosomal membrane. Note the lack of luminal membrane packing that identifies autophagic structures. **c** | Conventional electron micrograph of a mouse proximal tubule cell deformed by a single huge lysosome. Red lines indicate the straight borders and angles of a polyhedral crystal, close to 10 μm in height. Note the preservation of the brush border in adjacent cells. Part c reproduced with permission from the American Society of Nephrology © Gaide Chevronnay, H. P. *et al. J. Am. Soc. Nephrol.* **25**, 1256–1269 (2014).

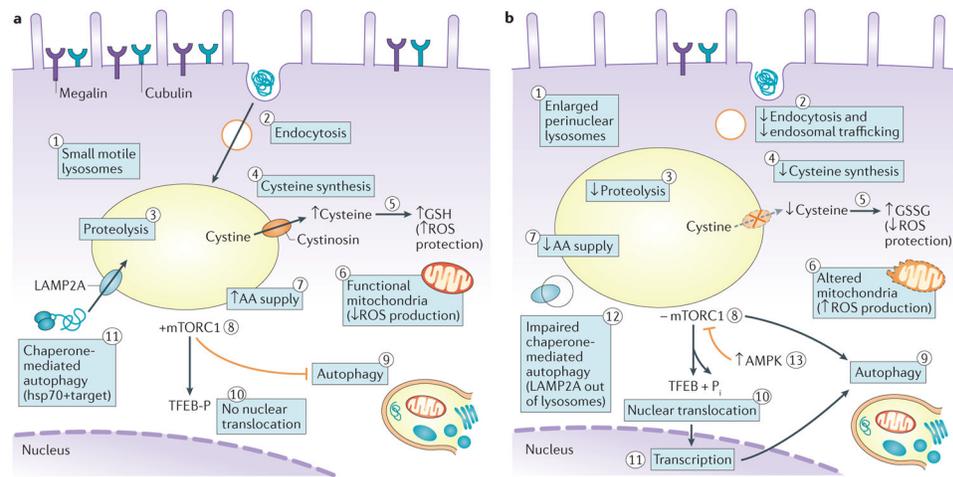


Figure 3. Cell biological alterations in cystinotic cells

a | Lysosomes of healthy proximal tubule cells (PTCs) are small, motile and dispersed throughout the cytoplasm (1), where they can readily fuse with late endosomes. High apical expression of the tandem multiligand receptors, megalin and cubilin, and their unusually fast recycling rate result in extremely active receptor mediated endocytosis (2). Endosomal trafficking, transfer into lysosomes and proteolysis (3) of ultrafiltrated disulfide rich plasma proteins ensures an ample lysosomal supply of amino acids (AAs) together with sulfur-bearing cystine. Following cystinosin-mediated export from the lysosome, cystine becomes a source of cysteine in the cytosol (4), which favours the biosynthesis of reduced glutathione (GSH), which confers protection against reactive oxygen species (ROS); (5). Moreover, functional mitochondria do not release ROS (6). An abundance of AAs (7) keeps the mTORC1 complex at the lysosomal membrane in its active kinase form (8). Crucial physical interactions between cystinosin and mTORC1 are not indicated. Lysosomal mTORC1 inhibits macroautophagy (9) and maintains TFEB phosphorylation, thereby abolishing its nuclear translocation and transcriptional effects (10). Localization of LAMP2A at the lysosomal membrane supports the clearance of altered cytosolic proteins by chaperone-mediated autophagy (11). **b** | In cystinotic PTCs, lysosomes engorged by undigested proteins (evident as amorphous inclusions) become enlarged and cluster around nuclei (1); they are less motile than lysosomes of healthy PTCs and are located further from the apical membrane, rendering them less accessible to endocytic cargo and less prone to exocytic clearance. Endocytic uptake is also decreased as expression of megalin and cubilin declines (2). Impaired proteolysis (3) lowers AA supply (7). Moreover, cystine can no longer cross the lysosomal membrane due to an absence or loss-of-function of cystinosin, which presumably limits cysteine availability (4) and therefore GSH synthesis, reversing the ratio of GSH to oxidized glutathione (GSSG), leading to increased oxidative stress by ROS (5). Concomitantly, ROS production increases as mitochondria are damaged and/or are less efficiently cleared (6). The shortage of free AAs (7) inhibits mTORC1 (8), which activates autophagic programmes, including mitophagy (9). mTORC1 inactivation also leads to dephosphorylation and nuclear translocation of TFEB (10), activating transcription programmes (11) that further promote autophagy. Chaperone-mediated autophagy of altered (including oxidized) cytosolic proteins is hampered, in part because of impaired LAMP2A

trafficking to lysosomes (12). Continuous autophagy eventually causes cell atrophy. In parallel, energy depletion activates AMPK, which inhibits mTORC1 thus activates autophagy (13). Enhanced apoptosis is not represented. Hsp70, heat shock protein-70.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

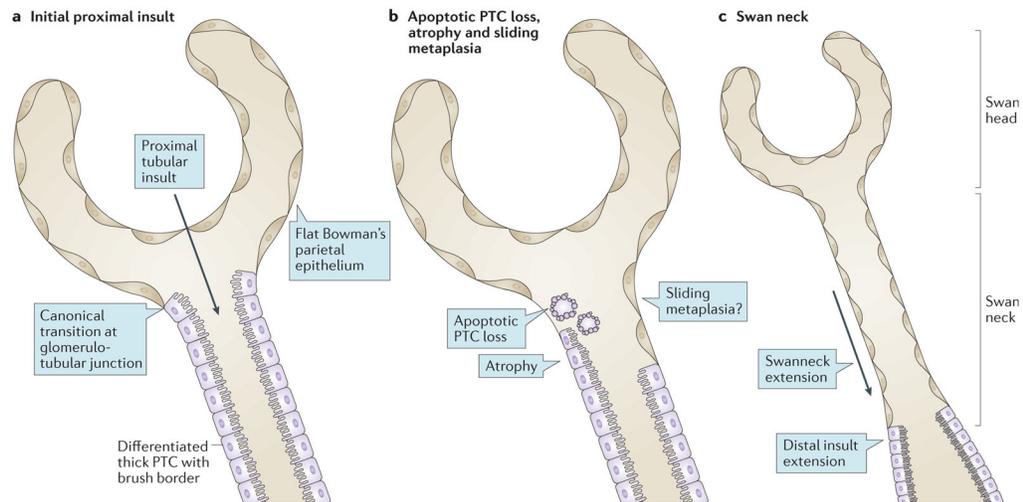


Figure 4. Origin and consequences of swan neck deformities

a | The canonical localization at the glomerulotubular junction represents the transition between the flat parietal epithelium typical of the Bowman capsule and the thick columnar epithelium lining the proximal tubules, with a highly differentiated apical pole represented by a brush border. The transition between the two types of epithelial cells can occur within the glomerulus, with the lower third of the Bowman capsule lined by thick epithelium that is undistinguishable from differentiated proximal tubule cells (PTCs). The opposite scenario (that is, a transition from thin to thick epithelium distal to the glomerulotubular junction) is never found under normal conditions. The insult here arises from the apical pole by an ultrafiltrated and reabsorbed toxic factor. We suggest that this factor could be disulfide-bearing albumin, which is the main source of cystine, and first affects the most proximal PTCs. **b** | Swan neck deformities arise from the apoptotic cell death and shedding of individual PTCs; atrophy by dedifferentiation of PTCs; and possibly by replacement of dead PTCs upon expansion of the flat Bowman epithelium from the glomerular wall into the tubules (sliding metaplasia hypothesis). **c** | The characteristic features of a microdissected nephron exhibiting a normal-sized glomerulus (swan head) and an atrophic proximal tubule (swan neck) suggest the extension of lesions to cells at the base of the neck, as they become exposed to the ultrafiltrated and reabsorbed toxic factor, are thus governed by the same mechanisms as in part b.

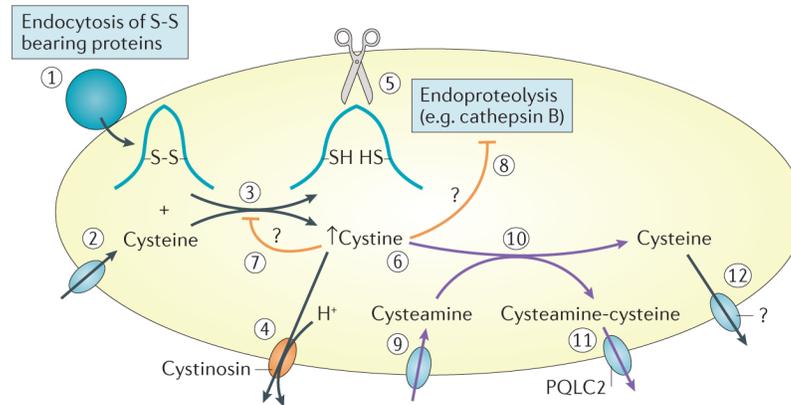


Figure 6. Lysosomal membrane transport and luminal events associated with cystinosis and cysteamine therapy

Under normal conditions (black pathways (left)), vesicular transfer into lysosomes of disulfide-bearing proteins, such as albumin (1), which is avidly taken up by receptor-mediated endocytosis into proximal tubule cells, combined with lysosomal import of cysteine by a still unknown mechanism (2)¹⁹⁶ leads to the reduction of disulfide bonds, resulting in protein unfolding and release of free cysteine (3)^{197,198}. Cystine is rapidly exported into the cytosol by the cystine proton symporter, cystinosin (4). Protein unfolding unmasks peptide bonds buried in globular proteins thus allows endoproteolytic attack by the concerted action of lysosomal cathepsins, such as the cysteine protease, cathepsin B (5)¹³¹. Free thiol in the catalytic site of cysteine proteinases is preserved by the reducing lysosomal environment. In the absence of cystinosin (orange pathways), cysteine export across the lysosomal membrane is abolished. Cystine accumulation (6) inhibits the reduction of disulfide bonds in lysosomes, which thereby is expected to slow down further cysteine generation (7) but also impairs protein unfolding and inactivates cysteine proteases (8), together leading to impairment of proteolysis (8). Cysteamine (β -mercaptoethylamine; purple pathways (right)) penetrates lysosomes by a distinct importer (9)¹⁹⁹ and reacts with cysteine to exchange its disulfide bridge into a mixed (cysteamine cysteine) disulfide (10), which exits via the cationic amino-acid exporter PQLC2 (11), and generates free cysteine, which must also exit lysosomes although this transporter is unknown (12). Question marks indicate that steps 7, 8 and 12 are still hypothetical.

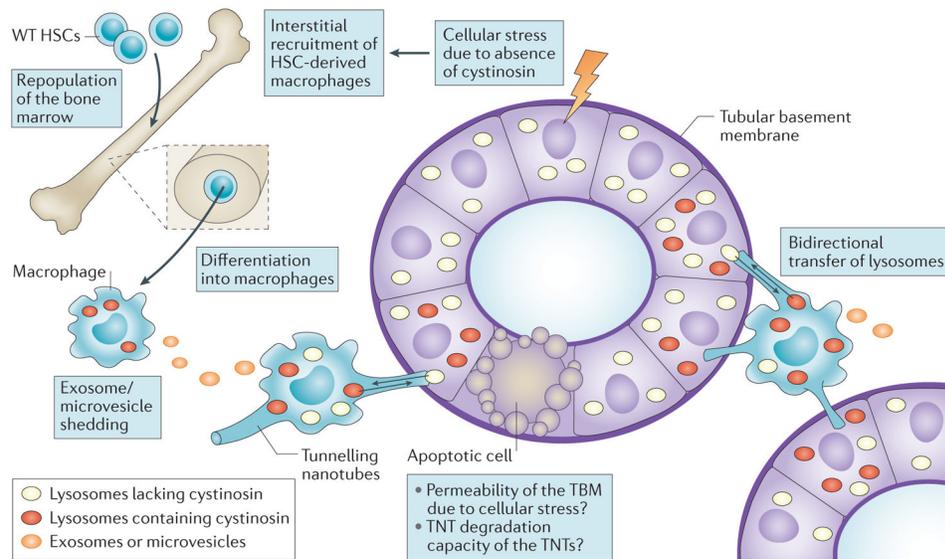


Figure 7. Proposed mechanisms by which haematopoietic stem cell (HSC) transplantation rescues cystinosis-induced Fanconi syndrome

Injury to renal cells, including proximal tubular cells (PTCs) induced by loss of cystinosin triggers an inflammatory response, which probably aggravates the injury. Transplanted wild type (WT) cystinosin sufficient HSCs repopulate the bone marrow of *Ctns* knockout mice then migrate into injured tissues, where they differentiate into macrophages. Extracellular exosome or microvesicle-mediated transfer of cystinosin might correct the cystinosin deficiency of adjacent interstitial renal cells. However, rescue of PTCs, which are embedded in the tubular basement membrane, requires tunnelling nanotubes (TNTs), which extend from the macrophages across the tubular basement membrane, and are capable of bidirectional transfer of lysosomes, cystinosin-containing lysosomes (red vesicles) from wild-type HSC-derived macrophages into PTCs and cystine-loaded lysosomes (white vesicles) from *Ctns* deficient PTCs into macrophages. A single macrophage can generate multiple TNTs that have the ability to transfer lysosomes over long distances, which makes this route of cross-correction particularly efficient, likely accounting for the observed long-term correction of the Fanconi syndrome in *Ctns*-knockout mice.