Controversies and research agenda in nephropathic cystinosis: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference

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Nephropathic cystinosis is an autosomal recessive metabolic, lifelong disease characterized by lysosomal cystine accumulation throughout the body that commonly presents in infancy with a renal Fanconi syndrome and, if untreated, leads to end-stage kidney disease (ESKD) in the later childhood years. The molecular basis is due to mutations in CTNS, the gene encoding for the lysosomal cystine-proton cotransporter, cystinosin. During adolescence and adulthood, extrarenal manifestations of cystinosis develop and require multidisciplinary care. Despite substantial improvement in prognosis due to cystine-depleting therapy with cysteamine, no cure of the disease is currently available. Kidney Disease: Improving Global Outcomes (KDIGO) convened a Controversies Conference on cystinosis to review the state-of-the-art knowledge and to address areas of controversies in pathophysiology, diagnostics, monitoring, and treatment in different age groups. More importantly, promising areas of investigation that may lead to optimal outcomes for patients afflicted with this lifelong, systemic disease were discussed with a research agenda proposed for the future.

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Nephropathic cystinosis has been recognized for well over 100 years, and by the end of the last century, was found to be due to over 100 mutations in the gene CTNS encoding for the protein cystinosin, the lysosomal cystine-proton cotransporter.[1,2] The disease is rare with yearly incidence ∼1:150,000 to 200,000 live births and prevalence ∼1.6 per million.[1] The natural history of the most common form of the disease demonstrates its earliest manifestation as a renal Fanconi syndrome in infancy, and if untreated, results in end-stage kidney disease (ESKD) by the end of the first decade of life. The disease involves most organs eventually if kidney function is maintained by dialysis or a transplant. To date, treatment has consisted of lifelong cystine-reduction therapy with cysteamine, but new treatments may be on the horizon.

To better understand the state of knowledge about this systemic disease, 49 worldwide experts including participants from patient-support groups, met in Lisbon, Portugal, in December 11–13, 2014 to iteratively discuss selected topic areas chosen by the cochairs of the conference. These groups addressed basic science aspects of cystinosin function and pathophysiology, diagnostic and monitoring biomarkers of the disease, infant and young child issues, adolescent issues including transition to adult-based practitioners, and adult

issues in those affected with the disease. The intent of this report is not to provide clinical guidelines for care but rather to highlight new areas of controversy and fruitful investigation in an effort to better understand the disease pathophysiology that might lead to improved patient outcomes.

PATHOPHYSIOLOGY OF CYSTINOSIS

The pathophysiology of cystinosis is still incompletely understood. Filling the knowledge gaps between cystinosin dysfunction on one side and multiorgan damage on the other has become an area of intense research during the last decade. Cystinosin has an established, proton-coupled, cystine transporting function, and its deficiency causes lysosomal

cystine accumulation and crystal formation.[2] More-recent studies demonstrated that cystinosin also regulates processes of intracellular vesicle trafficking, cell signaling, lysosomal dynamics, and exocytosis (Figure 1; Table 1).

Cellular events in cystinosis related to cystine accumulation

Role of cystine crystals. Because cystine is poorly soluble, cystinosin dysfunction causes lysosomal cystine crystal formation throughout the body. This prototypical abnormality was initially thought to be key to the disease process. However, the crystals are intralysosomal and solid-phase, limiting direct interaction with soluble molecules, and are not present in all cells.[1] Recently, a new pathological role of cystine crystals has been proposed. These crystals have been shown to
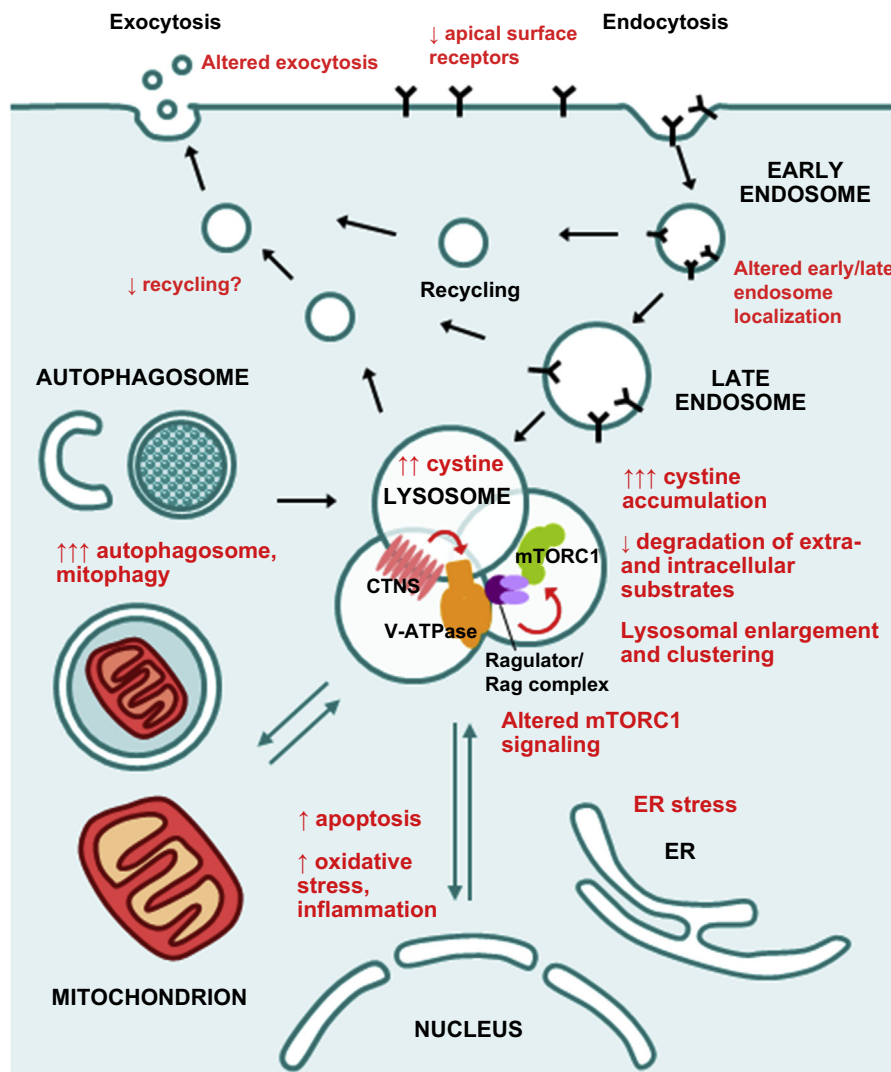

Figure 1 | Simplified scheme of the current knowledge on pathogenesis of cystinosis. Cystinosis is associated with cystine accumulation in the lysosomal lumen due to biallelic mutations in cystinosin (CTNS). Cystine crystals are mostly found in interstitial macrophages, which can lead to production of proinflammatory substances.[3,4] Lysosomal cystine accumulation also leads to altered lysosomal degradation of substrates,[21,22,25] increased oxidative stress,[10,13–17] and enhanced apoptosis.[9,10,12,24] These pathological mechanisms can be, at least partially, restored by cysteamine treatment. However, the effects of cystinosin dysfunction on the endolysosomal system appear to be broader, including the accumulation of autophagosomal markers and increased mitophagy,[11,18] impaired recycling of endocytotic receptors,[21,22,24] altered mammalian target of rapamycin complex 1 (mTORC1) signaling,[27] and impaired lysosomal morphology, intracellular dynamics, and exocytosis.[21–23,28] ER, endoplasmic reticulum; V-ATPase, vacuolar H+-adenosine triphosphatase. Adapted by permission of E. Ivanova.[91]

Table 1 | Pathophysiology of cystinosis

Pathological mechanisms	Current knowledge	Controversy and research questions
Lysosomal cystine accumulation	• Lysosomal cystine accumulation is a key feature of the disease and is a target of cystine-reducing therapy with cysteamine[1,2]	• How do solid-phase lysosomal cystine crystals cause cytosolic disturbances? • Decreased levels of ATP and reduced number of mitochondria have been demonstrated in cystinotic fibroblasts and RPTE cells.[8,11,17,18] However, *in vitro* mitochondrial ATP generative capacity and activity of Na^+-K^+ ATPase are unaltered.[92,93] In this vein, is mitochondrial dysfunction present *in vivo* and does it play a causative role and affect function of Na^+-dependent transporters in the kidney? Is this effect tissue-specific?
	• Lysosomal cystine accumulation is associated with increased oxidative stress and enhanced rate of apoptosis[8–10,12,14–17,19]	• Is *CTNS* a redox-regulated gene? Can cystinosin function influence cellular oxidative state and can cystinosin expression and function be regulated by ROS?[13] • Can adjuvant antioxidant therapy improve disease outcome?[19]
	• Cystine crystals can activate monocytes and macrophages and stimulate production of pro-inflammatory substances (IL-1β, IL-18, caspase-1, TNF-α, chitotriosidase)[3,4]	• Do cystine crystals promote chronic interstitial fibrosis in kidneys and other tissues? • Can the administration of anti-inflammatory drugs improve clinical outcome in cystinosis?
Renal proximal tubular dysfunction and atrophy are key clinical features of cystinosis[1,6]	• In the majority of cystinosis patients, generalized proximal tubular cell dysfunctions (renal Fanconi syndrome) develop during the first year of life and are morphologically associated with an atrophy of these cells[1,6]	• Why is renal Fanconi syndrome not responsive to cystine-depleting therapy and can the Fanconi syndrome be prevented by cysteamine therapy administered from birth? • Some patients with mild cystinosin mutations have mild or absent proximal tubular cell dysfunction, but severe proteinuria. Can podocyte damage be present in cystinosis without overt proximal tubular cell dysfunction?[8,94]
	• Cystinotic RPTE cells show signs of dedifferentiation and increased apoptosis[19,22,24]	• Is cell dedifferentiation in cystinosis a primary pathologic event or a compensatory mechanism of enhanced cell loss?[24] • Can inhibition of apoptosis by adjunctive therapy attenuate prognosis in cystinosis?[26]
	• Cystinotic RPTE cells show altered lysosomal morphology, dynamics, and delayed degradation of lysosomal substrates[21–23,25]	• How does cystinosin dysfunction alter lysosomal dynamics and expression of Rab GTPases, recycling of endocytotic receptors, and exocytosis?[23,25] Can stimulation of lysosomal exocytosis ameliorate RPTE cell dysfunction and improve outcomes?
Cystinosin dysfunction disturbs cell signaling mechanisms in cystinosis	• Cystinotic cells show altered mTORC1 signaling. As mTORC1 inhibits macroautophagy, altered mTOR activation can be a link between cystinosin dysfunction and increased autophagy[27] • Cystinosin is a component of vacuolar H^+-ATPase-Ragulator complex on the lysosomal membrane[27]	• Although cystinotic cells show an accumulation of autophagosomal markers, macroautophagy flux seems to be intact. Conversely, chaperone-mediated autophagy has been shown to be altered.[25] How can these findings be reconciled? • What is the molecular basis of cystinosin interaction with the components of mTORC1 and what is its role in this complex? Is cystinosin a lysosomal sensor for amino acids?[27] • What is the role of cystinosin isoform cystinosin-LKG that is not exclusively expressed on the lysosomal membrane?[95]

ATP, adenosine triphosphate; GTPase, guanosine triphosphatase; IL, interleukin; cystinosin-LKG, second cystinosin isoform; mTORC1, mammalian target of rapamycin complex 1; ROS, reactive oxygen species; RPTE, renal proximal tubular epithelial cells; TNF, tumor necrosis factor.

activate monocytes and macrophages, similarly to other toxic crystals such as urate and oxalate.[3] Accumulation of cystine crystals in monocytes increased the production of proinflammatory interleukins (ILs) and caspase-1. Similarly, *Ctns*$^{-/-}$ mice were found to have high circulating levels of IL-18 and increased renal expression of inflammasome-related genes. Other macrophage activation markers such as tumor necrosis factor-α and chitotriosidase were also augmented after phagocytosis of cystine crystals.[4] Tissue macrophages in the renal interstitium enriched with cystine crystals may trigger a chronic inflammatory process that promotes interstitial fibrosis with the progressive loss of kidney function.

Apoptosis. Several clinical observations such as the development of proximal tubular atrophy[5–7] and muscle wasting suggested an enhanced rate of cell death in cystinosis.[8] Indeed, a 300% increase in the apoptosis rate has been shown in cystinotic fibroblasts[9] and renal proximal tubular epithelial (RPTE) cells,[10,11] which could be ameliorated *in vitro* by cysteamine.[9] Lysosomal permeability and cysteinylation of proapoptotic protein kinase C-δ, which results in increased enzymatic activity, has been shown after an apoptotic stimulus.[12]

Increased oxidative stress. Several studies have shown that cystinotic cells are prone to oxidative stress and that cysteamine treatment can reduce oxidative damage.[10,13–17] Under induced oxidative stress conditions, cystinotic cells are unable to sufficiently up-regulate glutathione synthesis and such cells have elevated levels of reactive oxygen species.[10,16–18] Recently, the role of oxidative stress in the formation of proximal tubular atrophy has been strengthened by demonstrating that a small molecular antioxidant, mitoquinone, slowed down the progression of proximal tubular lesions in *Ctns*$^{-/-}$ mice.[19]

Cellular events in cystinosis likely unrelated to cystine accumulation

Emerging evidence indicates the more general role of lysosomes in regulating cell signaling cascades, nutrient-sensing, vesicle trafficking, exocytosis, and mitochondrial function.[20]

Altered lysosomal morphology and function. Both mouse and human cystinotic RPTE cells *in vitro* and human kidney tissue biopsies demonstrated abnormal lysosomal morphology.[21,22] This abnormal lysosomal phenotype could not be rescued by cysteamine treatment. On the other hand, delayed degradation of endocytosed proteins could be partially improved by cysteamine.[21]

Altered vesicle trafficking and exocytosis. Johnson *et al.*[23] observed vesicle transport defects and increased endoplasmic reticulum stress in cystinotic cells. They also found that the expression of a small Rab guanosine triphosphatase, Rab27A, was down-regulated in kidneys from *Ctns*^{−/−} mice and in human cystinotic RPTE cells. *In vitro* up-regulation of the Rab27A-dependent vesicle trafficking rescued the defective lysosomal dynamics, reduced endoplasmic reticulum stress, enhanced exocytosis, and slightly decreased lysosomal cystine accumulation.[23]

Progressive development of tubular lesions. Gaide Chevronnay *et al.*[24] delineated a sequence of events leading to proximal tubular atrophy in a *Ctns*^{−/−} mice model. At the nephron level, lesions started at the glomerulo-tubular junction and then extended distally. This was associated with progressive loss of expression of endocytotic receptors and Na^{+}-dependent transporters, suggesting that altered apical dedifferentiation accounted for Fanconi syndrome before frank atrophy. Interestingly, in the same model these changes were related to a loss of integrity of tight junctions, increased cell proliferation, and enhanced apoptosis.[22,24]

Mitochondrial and autophagosomal abnormalities. Mitochondrial dysfunction has been demonstrated *in vitro* in RPTE cells isolated from cystinotic patients, *Ctns*^{−/−} mice, and kidney biopsies of cystinosis patients showing abnormal patterns of mitochondrial autophagy and fewer number of mitochondria.[11,18] Conversely, Napolitano *et al.*[25] showed that macroautophagic flux was not impaired in cystinotic cells, but the expression and the localization of the chaperone-mediated autophagy receptor, LAMP2A, was decreased and could not be restored by cysteamine therapy.

Another recent study evaluated the expression of a protein clusterin *in vitro* in RPTE and *in vivo* in cystinotic renal biopsy tissue. Clusterin binds misfolded or heat-shock proteins in conjunction with apoptosis and autophagy proteins. In cystinotic RPTE, there are low levels of the cytoprotective secretory form of clusterin and elevated levels of the nuclear proapoptotic form; moreover, the expression of the nuclear form colocalizes with apoptotic proteins and autophagy proteins.[26]

Another possible link between altered autophagy and mutations in cystinosin has been recently provided by Andrzejewska *et al.*[27] who have shown that lysosomal cystinosin is a component of vacuolar H+–adensosine triphosphatase (ATPase)–Ragulator complex that senses nutrients and controls mammalian target of rapamycin complex 1 (mTORC1). In mouse cystinotic RPTE cells, mTORC1 activity was down-regulated and could not be restored by cysteamine treatment.[27] As mTORC1 inhibits autophagy, defective mTOR signaling in cystinosin-deficient cells might contribute to altered autophagy. Administration of 3-methyladenine, an autophagy inhibitor, rescued RPTE from apoptosis,[10] suggesting a sequence of events starting from down-regulation of mTOR, leading to increased autophagy and apoptosis.

Transcription factor EB that is inactivated by mTOR-induced phosphorylation seems to contribute to the process, as an overexpression of transcription factor EB improved abnormal lysosomal morphology and stimulated lysosomal exocytosis leading to a decrease in cystine accumulation in cystinosis cells.[28] A summary of currently known pathophysiologic mechanisms involved in cystinosis is shown in Figure 1 and Table 1.

DIAGNOSTICS AND BIOMARKER FOLLOW-UP
Rationale for biochemical testing

In cystinosis, intracellular cystine concentrations (in white blood cells [WBC] or selected WBC types) are used clinically as the only available biomarker for diagnosis and for gauging therapeutic success at the reduction in intracellular cystine accumulation. However, measurement methods vary, and therefore results may not be consistent across laboratories. Nevertheless, the consensus is that there is value to monitoring, as there is evidence that patients who are monitored in this manner have better long-term outcomes.[29–31]

Methodology for biochemical testing

Optimal techniques for WBC isolation. Granulocytes, or polymorphonuclear leukocytes, are the most informative type of leukocyte to test.[32–34] Results from mixed leukocytes are less reliable as they depend upon variable factors, including the instantaneous differential blood count, and are subject to many preanalytical processing issues. Two techniques were recommended in 2000, one for mixed leukocytes and another for polymorphonuclear cells.[35] Consensus recommendations are that:

1. Storage should follow a processing step to inhibit sulfhydryl exchange. Reagents such as *N*-ethylmaleimide are generally preferable; acidification of the cell lysate with sulfosalicylic acid may be preferential if the sample needs to be prepared at a location remote from the testing laboratory.

2. Laboratories that continue to use mixed-leukocyte isolation should investigate the reliability of also measuring differential complete blood cell count and estimate the proportion of protein in the mixed-leukocyte lysate that arises from granulocytes.

Optimal technique for WBC cystine measurement. The consensus is that tandem mass spectrometry is presently the method of choice and laboratories should participate in an

external quality control program, that is, a proficiency testing organization and/or sample exchange with other clinical laboratories.

Units for reporting WBC cystine measurement. Results should be reported in units that are directly comparable for all laboratories. The molecule of cystine is composed of 2 cysteine moieties connected by a disulfide bond. The standard nomenclature to date has been to report this as nmol $^1/_2$-cystine per milligram protein (i.e., the intracellular cysteine content, normalized to cellular protein, as cystine is reduced to cysteine prior to the measurements). There might be preference for the use of the bicinchoninic acid method[36] over the Lowry method for protein determination.[37] For mixed-leukocyte preparations in one series,[38] a correction factor of 0.65 was found to relate the response of the bicinchoninic acid assay to that of the Lowry assay. Each laboratory should establish its own reference intervals in control subjects, healthy heterozygotes, patients at diagnosis, and patients under therapy as previously recommended.[39]

Optimal timing for WBC cystine measurement. For diagnosis, there is no information to indicate any effect of the time of day for testing. For monitoring, samples should be obtained at the time of trough cysteamine, generally 6 hours after the most recent dose of the immediate-release cysteamine bitartrate and 12 to 12.5 hours after the most recent dose of the delayed-release formulation of cysteamine bitartrate.

Indications of genetic and biochemical testing for diagnosis and management

Role of genetic testing in diagnosis. Genetic analysis of the *CTNS* is recommended to confirm each new diagnosis and is particularly critical for genetic counseling.[40] Prenatal diagnosis is preferably accomplished by DNA sequencing of chorionic villus or amniocentesis samples when the mutation in an index case is known.

Role of urine testing in diagnosis. Findings of Fanconi syndrome that manifest several months after birth are very helpful in suggesting the diagnosis and may be useful in monitoring therapy. Urine sedoheptulose and erythritol[41]

may be used to suggest the diagnosis of cystinosis but only in populations with sufficient frequency to have the common 57 kb *CTNS* gene deletion as the cause of cystinosis.

Direct estimation of crystal burden. Except for the use of *in vivo* confocal microscopy and coherence tomography in monitoring ocular manifestations,[42] there is no direct evidence that monitoring of crystal burden, for example in the skin,[43] bears a relationship to systemic disease burden or its treatment.

Alternative biomarkers to monitor cystinosis. Currently there are no validated alternative biomarkers. Plasma chitotriosidase may be useful as a marker of disease activity though its universal applicability is limited by the fact that the enzyme is absent in ∼5% of the general population.[44] Trough cysteamine concentration may be of some value in determining the pharmacologic adequacy of dosage, but the correlation with intracellular cystine in WBC has not been validated.

Areas of uncertainty and research directions
Establishing therapeutic targets for intracellular WBC cystine levels requires prospective studies in which the biomarker is assessed and laboratory benchmarks are standardized to compare clinical laboratory results. The commonly used therapeutic target of <1.0 nmol $^1/_2$-cystine per milligram protein was defined by retrospective outcome analysis of a specific population of treated patients[45] in whom cystine depletion was monitored with mixed leukocytes using the Lowry assay to determine protein. However, it is not known whether this reference value adequately reflects tissue cystine depletion. A research agenda relating to diagnostic and biomarker monitoring is summarized in Table 2.

MANAGEMENT OF INFANTS AND CHILDREN WITH CYSTINOSIS
To date, there have been no prospective clinical trials in most aspects of the care and treatment for nephropathic cystinosis, but valuable data can be derived from extensive retrospective studies published by various centers.[46–48] There is little

Table 2 | Diagnostic and biomarker monitoring

Issues related to diagnosis	Research questions
• Elevated WBC cystine levels[36–39] • *CTNS* gene testing[40]	• What is the best WBC preparation and storage method for reproducibility? • Should it be evaluated along with WBC cystine levels? • Can we establish a central database of mutations in *CTNS* correlated with clinical phenotype?
• Newborn screening is currently unavailable	• What are novel methods of detection for common mutations?[41] • Is there utility for newborn screening by whole-exome sequencing or targeted genetic approach?
• Cystinosis remains an overlooked disease, especially in emerging countries[96,97]	• How can we improve awareness about cystinosis, especially for early diagnosis and treatment around the globe?
Issues related to monitoring	**Research questions**
• Titrate cystine depleting therapy to WBC cystine levels[45]	• Can standard protocols be developed for monitoring during initiation of cystine-depleting therapy? • What is the optimal number of interval samplings of WBC cystine?
• No measurement of white blood cell cystine levels achieved with specific outcomes	• Are there novel ways to gauge tissue-based cystine-lowering?[42,43]

WBC, white blood cell.

controversy that infants and children with cystinosis need great attention paid to nutrition,[49,50] fluid and electrolyte management as influenced by the Fanconi syndrome, and the use of growth hormone to promote statural growth if below genetic potential once other therapies have been optimized.[51]

Once the diagnosis of nephropathic cystinosis is confirmed, a management plan is put together with cysteamine therapy and provision of medications to provide symptomatic treatment as its main pillars. The therapeutic strategy must be regularly reviewed and updated to enhance care and address emerging issues.[46,48] This often requires frequent nephrologist visits after the diagnosis during infancy and childhood, with monitoring of serum parameters and the WBC cystine levels 3 to 4 times yearly.

Table 3 provides clinical guidance for some of the common management issues observed in infants and children. As there are still many knowledge gaps for this population, a listing of controversial issues is also summarized herein.

ADOLESCENT ISSUES IN CYSTINOSIS

Adolescence presents some specific new issues in patients with cystinosis, including treatment adherence, development of extrarenal disease,[52] progression to ESKD, the need for renal replacement therapy,[53] and the transition to adult age and adult-provider care.[54] Adolescence should be viewed as a period of fragility in patients with cystinosis for multiple reasons: the taste for risk and dangerous behaviors in adolescents; the need to control life independently of adults, parents, and doctors included;[55,56] the difficulties linked to the transfer of a complete and complex medical history to a naïve adult medical team; and the limited number of adult teams expert in pediatric chronic disease that are not

necessarily located closely to the pediatric teams.[57] Our consensus is to recommend the use of a coordinator to serve as the link between the adult team, the patient, and the pediatric team.[58] The role of this person would be to organize combined adult-pediatric outpatient dates and to coordinate subspecialist referrals as needed.[52,59,60]

Adherence to medical therapy

Adolescence poses unique challenges to those with chronic diseases and cystinosis, including a reduction in self-image, the view that being different from one's peer group is undesirable, and a rebellious nature from parental guidance and advice that altogether result in loss of medication adherence.[61–63] Missed appointments, denial of disease, and risk-taking behavior may ensue as a result.

Recognition of the systemic nature of cystinosis

Although attention early in life is directed to initiation of cysteamine therapy and restoration of fluid, electrolyte, and mineral balances, the systemic nature of cystinosis becomes more apparent to the patient and parents alike as the adolescent age approaches. It is suggested that clinicians beyond the pediatric nephrologist be involved in the care of patients with cystinosis, if not yet considered. Pubertal delay is common, although it does not affect all patients with cystinosis.[64,65] Ongoing care by an endocrinologist is therefore recommended.

Bone disease leads to the risk of early fractures in young adults.[66,67] In the infant and young child, supplementation with phosphate salts and active vitamin D are used to balance the effects of phosphate wasting and the defect in 1α hydroxylation of 25-hydroxy-vitamin D_3.[68] In adolescence, the

Table 3 | Management of infants and children with cystinosis

Management issue	Current recommendation	Controversy
Initiation of cysteamine therapy[98]	As soon as diagnosis is established: • Titrate to WBC [cystine] levels • Two dose forms are available (q6h; q12h) • Recognize maximal doses to reduce side effects	• What is the exact level of WBC [cystine] to be achieved? • When is the need for concomitant gastroprotective agents? • What is the toxicity from lifetime use?
Treatment of Fanconi syndrome: electrolytes and minerals[46,48]	• Phosphate replacement • 1,25-dihydroxyvitamin D_3 or equivalent therapy • Citrate, bicarbonate, potassium, sodium replacements	• What is the dose adjustment for phosphate as CKD ensues? • What is the role of copper deficiency? • What is the role of carnitine replacement therapy?
Treatment of Fanconi syndrome: polyuria	• Indomethacin to reduce polyuria[99]	• Are there long-term negative effects on kidney function?
Treatment of evolving proteinuria	• Questionable role for angiotensin-converting enzyme inhibition or angiotensin-receptor blockers[100]	• Can we establish databases to evaluate their long-term efficacy and safety?
Treatment of growth failure[51]	• Recombinant human growth hormone if growth failure present after optimal cysteamine therapy and treatment of Fanconi syndrome	• What is the extent of true growth hormone deficiency versus growth hormone resistance due to cystinosis or CKD?[101]
Treatment of the eye disorders[102,103]	• Topical ophthalmic solution available in some parts of the world[104]	• What is the timing of onset of therapy? • Can novel cysteamine delivery system be applied?[105]
Treatment of neurologic issues[106–108]	• Recognition of specific neurocognitive issues should lead to school counseling and learning plans optimized for each patient	• Should all children with cystinosis undergo formal neurocognitive testing?[109]
Patients with cystinosis residing in non-Western world countries	• Difficulty in diagnosis and treatment[96,110]	• How can the medical communities ensure treatment in disadvantaged populations?

CKD, chronic kidney disease; q6h, every 6 hours; q12h, every 12 hours; WBC, white blood cell.

entity of chronic kidney disease–mineral bone disorder comes into the patient's care paradigm, and it is unclear how phosphate supplementation should be adapted. Recent experimental studies suggest that the osteoblast may be particularly prone to the effects of cystinosis, and thus, low turnover renal osteodystrophy may predominate.[69] Further biopsy or advanced bone imaging studies with high-resolution peripheral quantitative computed tomography are needed. As a general principle, physical activity is highly recommended as a useful way to maintain bone health.[70]

Fertility emerges as a constant issue in male patients. It is known that adult men with cystinosis are infertile, but data are lacking about spermatogenesis and spermatozoid counts at the beginning of puberty.[71] Studies including young adolescents should be conducted and sperm freezing is obviously advised in case of evidence of living spermatozoids.

Issues specific to kidney transplantation

A preemptive renal graft remains the best choice of renal replacement therapy, compared with the morbidity of hemodialysis or peritoneal dialysis. It is widely accepted that there is no risk of recurrence as the genetic defect does not get transferred with the allograft. Cysteamine treatment is usually withdrawn for kidney transplantation but the delay in resuming treatment should be limited to a few days, along with first meals. Interactions with immunosuppressive medications have not been well studied; therefore, it might be desirable to perform pharmacokinetics and area under the curve measurements of mycophenolate, cyclosporine, and tacrolimus in each patient, when appropriate. Patients should be informed of the risk of triggering diabetes with prednisone and tacrolimus.[72] There is no consensus on immunosuppression strategies for the long term, but current approaches should favor steroid minimization. Fewer rejections after renal transplantation support less immunosuppression in the long term.[53] Studies on the occurrence of cancer and posttransplantation lymphoproliferative disease are also needed in this specific population.

Persistent polyuria after transplantation from the native cystinotic kidneys can persist in some patients. However, unilateral or bilateral nephrectomy at the time of kidney transplantation or after is rarely required.

Issues specific to dialysis

The presence of polyuria and sodium wasting[73] limits the need of ultrafiltration in cystinosis patients on hemodialysis or peritoneal dialysis in those patients with residual renal function. One case report showed that cysteamine is not extracted by hemodialysis,[74] but no data are available in peritoneal dialysis. Pharmacokinetics, as well as close monitoring of WBC cystine, would be useful for the management of dosing.[74]

Areas of uncertainty and research directions

Table 4 provides clinical guidance for some of the common management issues observed in adolescents and summarizes the controversial questions for this patient population.

ADULT ISSUES AND MANAGEMENT OF EXTRARENAL MANIFESTATIONS OF CYSTINOSIS

In light of advances in pediatric care including specific cystine-depleting therapy and successful renal transplantation, patients with nephropathic cystinosis should expect to live well into adult life. Adult services should ideally

Table 4 | Management of adolescents with cystinosis

Management issue	Current recommendation	Controversy
Adolescents with chronic diseases have reduced adherence with medications[62,63]	• Discuss adherence directly with adolescent[62,63] • Use techniques that address known adolescent developmental issues[62,63]	• Unknown whether difficulty in adherence leads to advanced kidney and extrarenal disease in cystinosis
Systemic nature of cystinosis advances[111]	• Involve multiple subspecialists (biochemical geneticists, endocrinology, pulmonary, cardiology, neurology, speech therapy for swallowing analysis)	• What is optimal WBC cystine level for slowest progression of multisystem disorders? • Are these disorders preventable in their entirety?
CKD advances[68]	• Management of CKD-MBD	• Should pharmacologic reduction in proteinuria be pursued? • Does management of bone disease differ from that in infancy and childhood?
Kidney transplantation may occur before adulthood years[53]	• Standard protocols for immunosuppression results in excellent graft survival[112] • Cysteamine therapy should be restarted in the early posttransplant period	• No published data on potential interaction of cysteamine therapy with conventional immunosuppressive agents • Should steroids as a part of immunosuppressive regimen be used in all patients?
Maintenance dialysis might be needed prior to kidney transplantation	• Polyuria may limit the need of ultrafiltration during dialysis	• There are little data on how to best use cysteamine therapy during maintenance dialysis
Fertility in male patients[64,71]	• Conventional information about infertility in all male patients is being challenged[71]	• Timing for sperm harvesting and preservation for *in vitro* fertilization is unknown
Readiness for transitioning[113]	• There is general agreement that this process should be started in mid-adolescence • Use of a care coordinator familiar with all aspects of the disease is encouraged	• Optimal transition models of care are not yet developed or often tested for effectiveness

CKD, chronic kidney disease, MBD, mineral bone disorder; WBC, white blood cell.

offer a holistic model of care including both kidney and extrarenal manifestations of the disease, which are progressive and a major feature in the adult years.

Natural history

The natural history of the disease has been described in retrospective cohorts.[52,75] The most typical chronology of organ dysfunction in patients is ESKD, progressive hypothyroidism, insulin-requiring diabetes, cardiac complications, neuromuscular complications, and central nervous system complications. However, some patients develop hypothyroidism and muscle atrophy before ESKD.

Impact of cystine depleting therapy

In the era of early and continuous treatment with cystine-reduction therapy with cysteamine compounds, this chronology is less certain. Because the life span of patients with cystinosis now extends into adulthood, there may be as yet undescribed novel manifestations in the future for early and well-treated patients. Retrospective registry data have shown that early treatment with cystine-depleting treatment delays the onset of ESKD and extrarenal complications, including hypothyroidism, diabetes, neuromuscular complications, and death.[52,75] For diabetes, hypothyroidism, and life expectancy, benefit is still observed if treatment is started beyond the early childhood years. However, there is significant interindividual phenotypic variability, the cause of which remains unexplained.

Additional complications have only surfaced in more-recent cohort analyses. Thus, late swallowing dysfunction and difficulties in phonation together with respiratory muscle weakness often lead to recurrent chest infection and poor nutrition.[76] Headaches, seizures, impaired memory, and cognitive function were observed in adults who did not receive adequate or life-long cystine-lowering therapy and could be improved in some patients by administration of cysteamine.[77,78] Visual-motor coordination may be impaired and does not seem amenable to treatment with cysteamine. Coronary calcification has been described commonly in adults with cystinosis,[79] but is likely not specific as this disease process is commonly seen in adult patients with ESKD.

Dose and monitoring of cystine-depleting therapy

The original recommended dose for cystine-reduction therapy with immediate-release cysteamine compounds was 2 g/day for patients >12 years of age or weighing >50 kg. We propose dosing based on body surface area in adults with a maximum dose of 1.95 g/m^2/day, but limited data are available. The side-effect profile of doses in excess of 2 g/day should be carefully monitored. It is strongly recommended that there be a continuation of cysteamine treatment in patients treated by dialysis or transplantation.

Treatment of extrarenal manifestations in adults

Primary hypogonadism is observed in male patients. Testosterone-replacement therapy allows pubertal development, but it does not prevent infertility. Azoospermia is observed even in patients with normal hormonal status.[71] Only 1 patient with sufficient spermatogenesis on testicular biopsy has been published. Many young men are not aware that they may be infertile, and as such, this facet of the disease requires open discussion with patients.

Female patients with cystinosis are fertile. There are no disease-specific contraindications to most forms of contraception. Clinicians should follow recommendations for renal transplant recipients.[80] A pubertal delay may be observed in female cystinotic patients, but a protective effect on gonads from early cysteamine treatment has been shown.[81] Successful pregnancies have been described and preconception counselling should be offered to all women contemplating pregnancy.[82] Because dose-dependent developmental toxicity from cysteamine has been reported in animals,[83] discussion about the optimal time for stopping cysteamine should be part of the preconception counselling.

The optimal management of neurological complications of cystinosis is uncertain and no specific recommendations for treatment are available. The management of cystinosis myopathy is also an area requiring further research. Muscle testing, electromyogram, and changes in pulmonary function should be used as endpoints for therapeutic intervention studies. Physiotherapy and exercise might be useful adjunctive therapies.

Table 5 | Management of adults with cystinosis

Management issue	Current recommendation	Controversy
Continuation of cystine depletion therapy with cysteamine compounds[1,30,45,46,48,52,98]	• Continue life-long	• Are all aspects of the medical problems seen in adults with cystinosis responsive to cystine reduction therapy? • What is dosing recommendation for adults? • What are the kinetics of cysteamine during hemodialysis or peritoneal dialysis?
Multispecialty approach to care	• Involve multiple subspecialties as medically indicated[54]	• How can multiple medical subspecialists gain better knowledge of cystinosis, a rare disorder?
Pregnancy management	• Follow recommendations for renal transplant recipients[46,80] • Stop cysteamine during pregnancy[46,83]	• Should cysteamine be stopped prior to conception or after receiving positive pregnancy test?
Neurologic complications lead to mortality[77,78]	• Continue cystine reduction therapy[68]	• Are novel therapies available from other neurologic diseases?
Adherence wanes	• Continue cystine reduction therapy[75]	• How can psychosocial aspects of this chronic disease be best addressed to improve medication adherence?

Table 6 | Emerging therapies

Novel therapy	Current knowledge	Controversy and research recommendations
HSC therapy	• Syngeneic bone marrow cell and HSC efficiently decrease cystine tissue accumulation and preserve kidney function in the Ctns^{-/-} mouse model of cystinosis[85] • Autologous HSC genetically modified to express a functional CTNS transgene using a SIN-LV are capable of decreasing cystine content in all tissues and improve kidney function in the Ctns^{-/-} mouse model of cystinosis[87] • Transplanted HSC differentiate into tissue macrophages that are able to form tunneling nanotubes reaching adjacent host cells and transferring cystinosin[114] • Paracrine microvesicles transfer is another suggested mechanism of mRNA and cystinosin transfer[115,116]	• Will autologous HSC transplantation be a valuable curative option in humans? Will the benefits outweigh the risks? • What is the optimal age of transplantation and will the effect last life-long? • How should the efficiency of transplantation be monitored? • What are the mechanisms responsible for the effect of HSC transplantation in animals? Can transplanted HSC replace damaged cells? Can nanotube transfer be the sole mechanism? Transfer of wild-type cystinosin and/or its mRNA via microvesicles shed from surface of engrafted cells has been suggested as the mode of action. As such, is microvesicle transfer of cystinosin taking place in vivo?[115,116]
Novel drugs	• Enteric-coated delayed-release cysteamine preparation is noninferior to immediate-release cysteamine efficiency in lowering WBC cystine content • Enteric-coated delayed-release cysteamine preparation improves quality of life in cystinosis patients[90,117]	• Will the use of delayed-release cysteamine bitartrate prolong kidney function survival in patients with cystinosis? • Are there alternatives to cysteamine with higher efficacy and fewer side effects? • Can halitosis and bad body odor induced by cysteamine be improved?[118]

HSC, hematopoietic stem cell; SIN-LV, self-inactivating-lentiviral vector; WBC, white blood cell.

A multispecialty approach is recommended for adults with cystinosis, including where applicable, specialists from nephrology/transplant nephrology, neurology, ophthalmology, pulmonology, endocrinology, social workers, psychology, otorhinolaryngology specialized in swallowing dysfunction, orthophonists, and physiotherapists.

Non-compliance in adults with cystinosis is frequent. Only one-half of adult patients took cysteamine with the correct number of 4 doses per day,[61] whereas another study also observed a decline in cysteamine adherence.[84] Adherence issues are often associated with depression, anxiety, and psychosocial issues, and at times memory function defects may also alter patient compliance. All of these factors may have an impact on education, employment, and quality of life. Thus, the impact of long-acting cysteamine on adherence and side-effect profile should be further investigated.

Areas of uncertainty and research directions

Table 5 provides clinical guidance for some of the common management issues observed in adults and summarizes the controversial questions for this patient population.

EMERGING THERAPIES

Despite significant improvement in overall life expectancy and long-term prognosis due to success of cysteamine therapy and kidney transplantation, there is still no cure for cystinosis. In 2009, the first step toward establishing a curative therapy was made by demonstrating the efficiency of syngeneic bone marrow and hematopoietic stem cell (HSC) transplantation in the Ctns^{-/-} mouse model of cystinosis.[85,86] Later studies demonstrated that ex vivo gene therapy in this experimental model, followed by autologous HSC transplantation, can be a valuable option in order to avoid the need for posttransplant immunosuppressive therapy and the risk of graft-versus-host disease.[85,87] Planning of the autologous HSC transplantation trial in humans with cystinosis is ongoing (Table 6).[88]

Short of a cure, improving adherence with cystine-depleting therapy itself may be beneficial for improved patient outcomes. The recent approval from the US and European Medicines Agency of a 12-hour (i.e., delayed-release drug) formulation of cysteamine bitartrate may achieve that goal. However, further studies are needed.[89,90] Lastly, alternative pharmacological interventions interfering with the disease pathogenesis are important steps in improving the clinical outcome in patients with cystinosis.

CONCLUSIONS

Although cystinosis is a rare inherited metabolic disorder, a better understanding of its pathophysiology and the development of curative therapies will have general consequences for other Mendelian disorders that share similar molecular mechanisms or require comparable models of care. By outlining areas of controversy and putting forth a research agenda for cystinosis, we hope this will not only foster fruitful collaborations in the research community but also provide an impetus for improved outcomes for afflicted patients.

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APPENDIX
Other Conference Participants

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