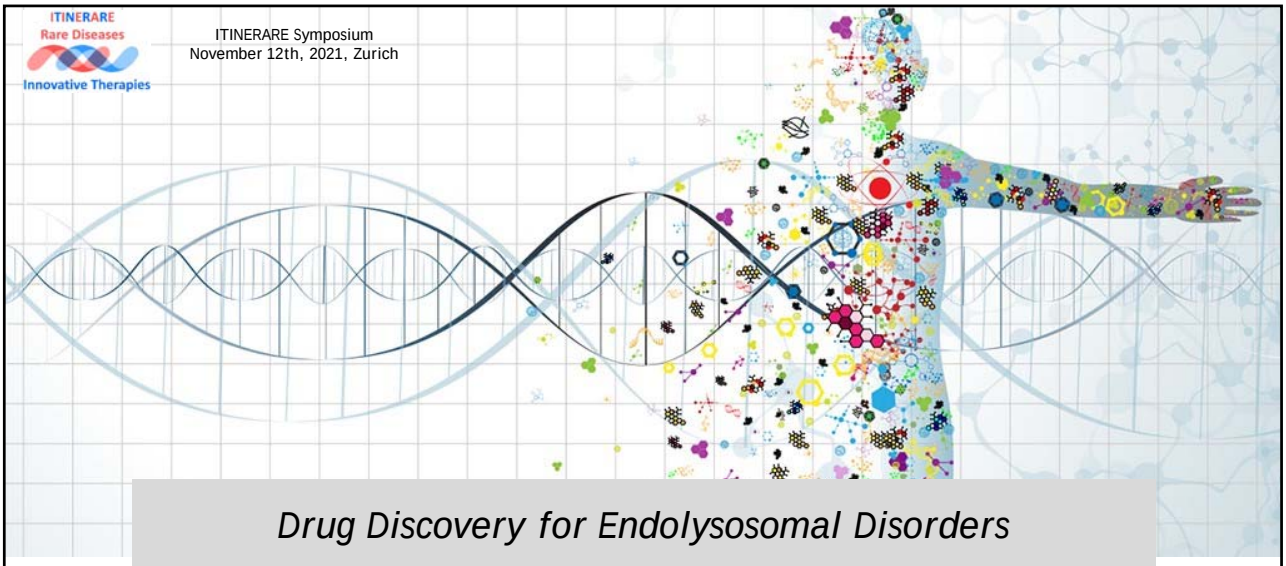
ITINERARE Rare Diseases Innovative Therapies

ITINERARE Symposium
November 12th, 2021, Zurich



Drug Discovery for Endolysosomal Disorders

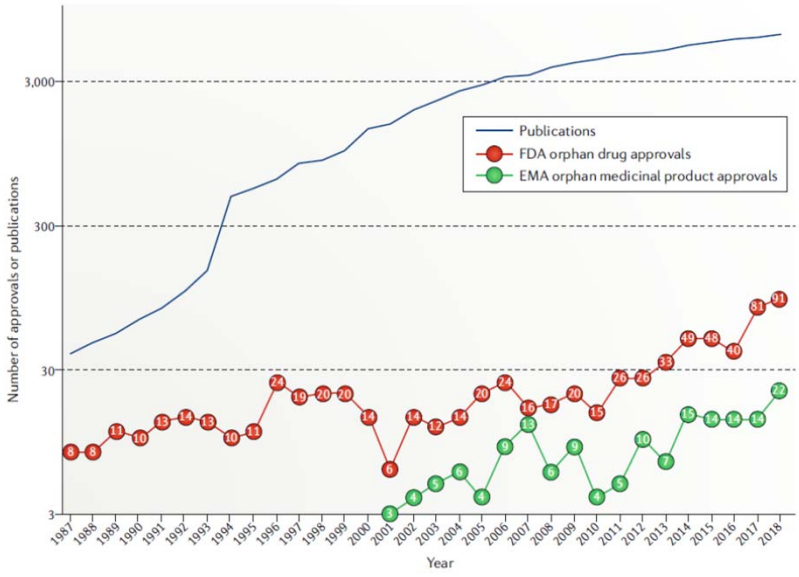
Olivier Devuyst, MD, PhD

ERKNet The European Rare Kidney Disease Reference Network

FNSNF

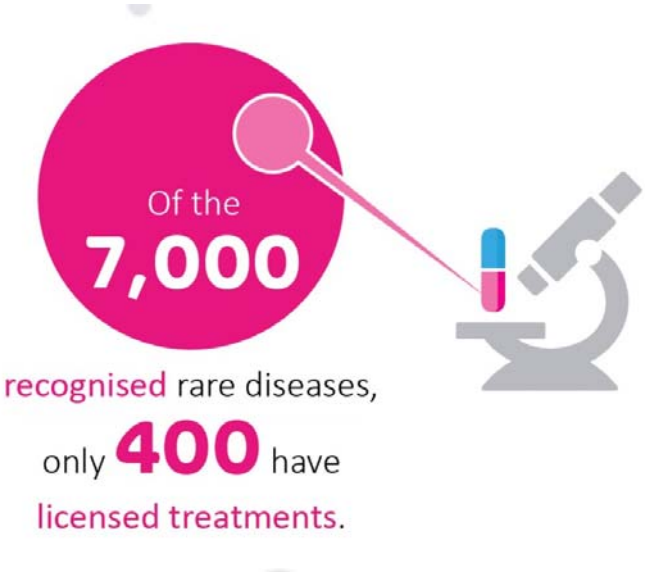
University of Zurich

Growing Gap between Science and Therapies for Rare Diseases



Tambuyzer et al. Nat Rev Drug Discov 19, 2020

Crucial Gap between Genetic Knowledge & Treatment



Nat Rev Drug Disc 2016

EXPERT OPINION ON ORPHAN DRUGS, 2017
VOL. 5, NO. 8, 611–612
https://doi.org/10.1080/21678707.2017.1341307

Taylor & Francis
Taylor & Francis Group

Check for updates

EDITORIAL

Ultra-orphan drugs: can we afford the price

Devidas Menon and Tania Stafinski

Orphan Drugs Are Driving Skyrocketing Drug Costs, AHIP Finds

September 13, 2019
Laura Joszt, MA

Average annual drug cost for orphan drug is 125,000 USD,
25 times more expensive than traditional drugs

When the Patient Is a Gold Mine: The Trouble With Rare-Disease Drugs

With a flagship treatment that helps fewer than 11,000 people, how is Alexion making so much money?

https://www.bloomberg.com/news/features/2017-05-24/

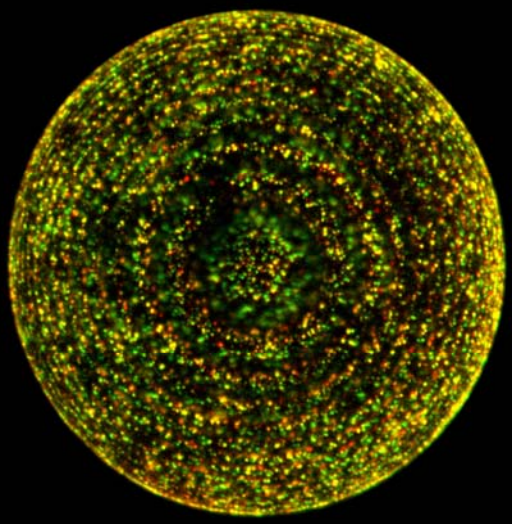
WORLD ECONOMIC FORUM

Davos, 22 Jan 2020

ITINERARE Rare Diseases Innovative Therapies

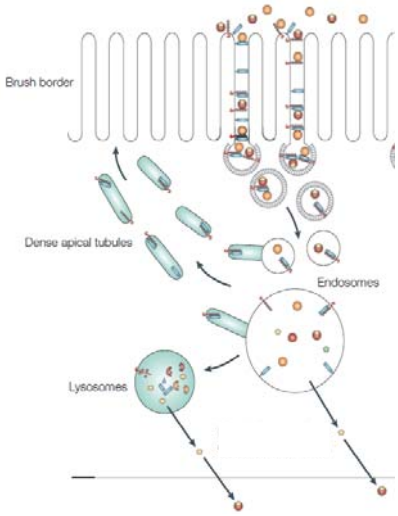
***Rare diseases:** We need new medicines to address the unmet needs*

The Secret Power of Lysosomes, the Cell's Waste Bin

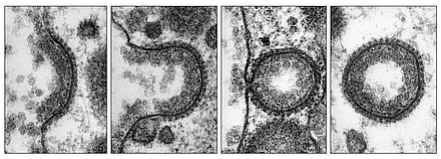


Glowing yellow dots mark where lysosomes (red) on beads in this preparation bind to the regulatory mTORC1 protein (green).
Roberto Zoncu, Sabatini Lab, Whitehead Institute

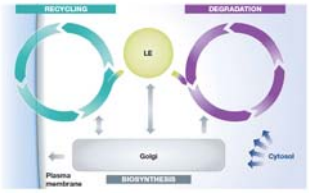
Connecting and Recycling Transport System: Recover & Process Essential Substances



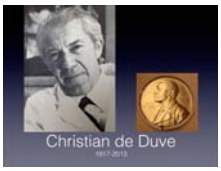
Receptors



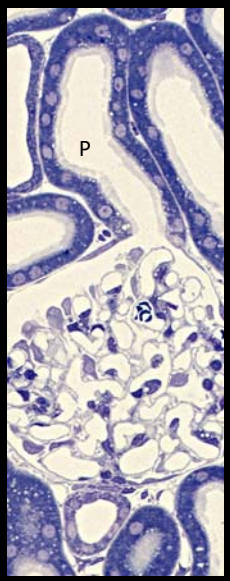
Endosomes

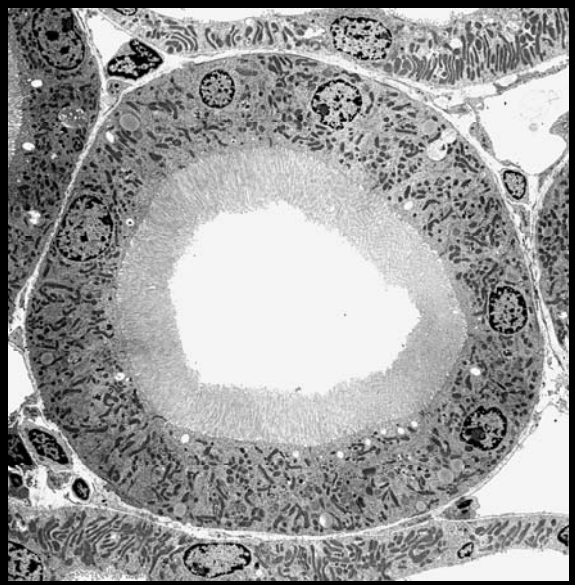


Lysosomes: degradation & recycling



Proximal Tubule of Kidney: Essential for Homeostasis

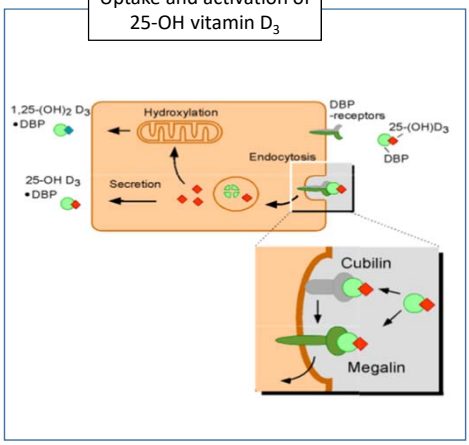




Receptor-mediated Endocytosis: Multiple Ligands

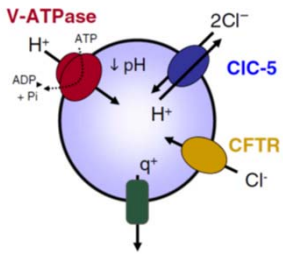
Megalin	Cubilin
Vitamin-binding proteins	
Transcobalamin–vitamin B_{12} (REE 12)	Intrinsic factor–vitamin B_{12} (REE 14)
Vitamin-D-binding protein[81]	Vitamin-D-binding protein[82]
Retinol-binding protein[79]	
Other carrier proteins	
Albumin[113]	Albumin[114]
Lactoferrin[115]	Transferrin[84]
Haemoglobin[86]	Haemoglobin[86]
Odorant-binding protein[74]	
Transthyretin[116]	
Lipoproteins	
Apolipoprotein B (REE 65)	Apolipoprotein A-I (REE 91)
Apolipoprotein E (REE 115)	High-density lipoprotein[67]
Apolipoprotein J/clusterin[117]	
Apolipoprotein H (REE 118)	
Hormones and hormone precursors	
Parathyroid hormone[89]	
Insulin[119]	
Epidermal growth factor[119]	
Prolactin[119]	
Thyroglobulin[84]	
Drugs and toxins	
Aminoglycosides[101]	
Polymyxin B (REE 101)	
Aprotinin[120]	
Trichosanthin[120]	
Enzymes and enzyme inhibitors	
PAI-1 (REE 121)	
PAI-1–urokinase[122]	
PAI-1–tPA[115,122]	
Pro-urokinase[121]	
Lipoprotein lipase[123]	
Plasminogen[124]	
β-amylase[26]	
β_1-microglobulin[74]	
Lysozyme[119]	
Immune- and stress-response-related proteins	
Immunoglobulin light chains[125]	Immunoglobulin light chains[126]
PAP-1 (REE 74)	Clara cell secretory protein[127]
β_2-microglobulin[119]	
Others	
RAP[13,115,128–130]	RAP[32]
Ca^{2+} (REE 13)	
Cytochrome *c* (REE 119)	

Uptake and activation of 25-OH vitamin D_3

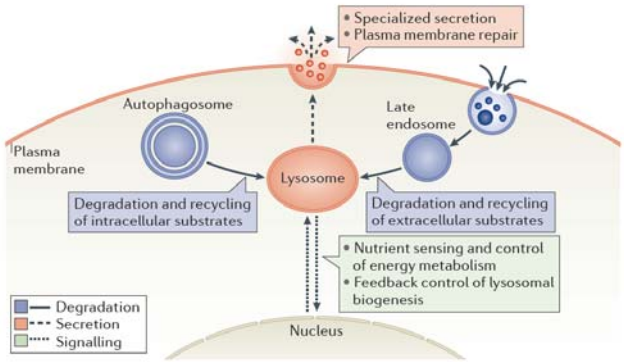


Nykjear et al. Cell 1999
Christensen Nat Rev Mol Cell Biol 2002
Vilasi et al. Am J Physiol 2007

Lysosome : Central Hub for Cell Metabolism



- End-point of RM endocytosis
- Low pH < 5.0: V-ATPase and transporters
- ~40 lysosomal enzymes
- High glycosylation - protection

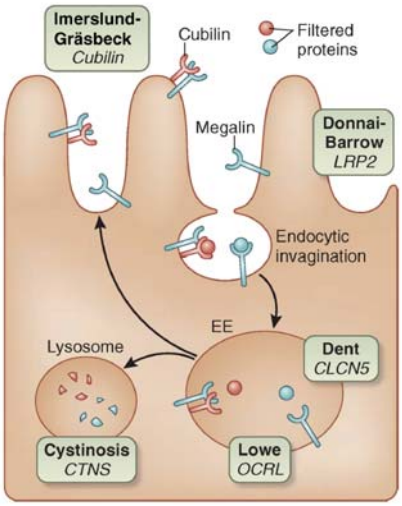


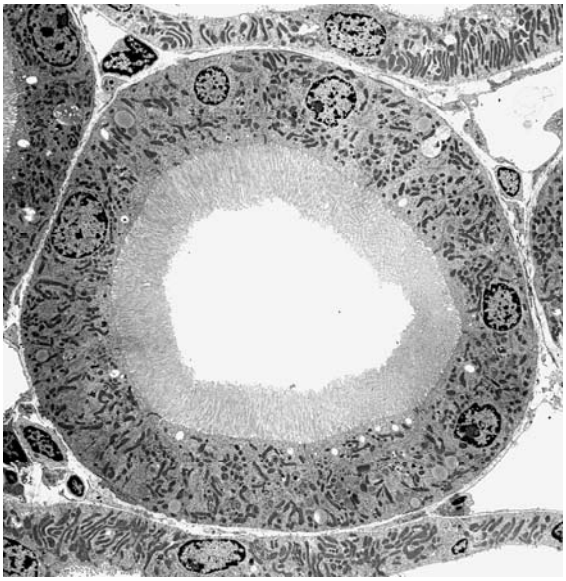
Degradation, secretion, recycling & signalling processes – nutrient sensing

Devuyst & Luciani, *J Physiol* 593, 2015

Settembre C et al. *Nat Rev Mol Cell Biol* 2013

Rare Endolysosomal Disorders Targeting Kidney Proximal Tubule





van der Wijst et al. *Physiol Rev* 99, 2019

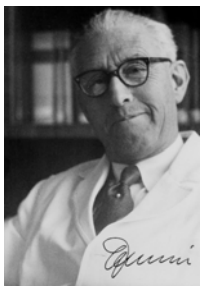
UNIVERSITÄTS-KINDERSPITAL ZÜRICH

Renal Fanconi Syndrome: Dysfunction of Kidney Proximal Tubule

Die nicht diabetischen Glykosurien *und Hyperglykämien des älteren Kindes.*
Jahrbuch für Kinderheilkunde und physische Erziehung, Wien, 1931, 133: 257-300.

- Excessive urinary wasting of solutes:

  LMW proteins, amino acids, glucose, phosphate, urate, bicarbonate, …
- Life-threatening: dehydration, hypokalemia, metabolic acidosis, hypercalciuria
- Loss of vitamin carriers, altered drug metabolization
- Failure to thrive, rickets, developmental delay



Guido Fanconi

Causes of Renal Fanconi Syndrome

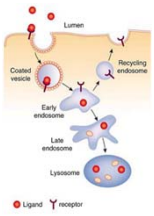
Inherited disorders
- Dent disease
- Lowe syndrome
- Cystinosis
- Galactosemia
- Hereditary fructose intolerance
- Glycogen storage disease (von Gierke disease)
- Fanconi-Bickel syndrome
- Tyrosinemia type I
- Wilson disease
- Mitochondrial diseases (cytochrome-c oxidase deficiency)
- Idiopathic Fanconi syndrome
- Sporadic Fanconi syndrome

Rare disorders:
Receptors – Endosomes – Lysosomes
Mitochondria – trafficking defects

Acquired disorders
- Glomerular proteinuria (nephrotic syndrome)
- Light chain nephropathy (multiple myeloma)
- Sjögren syndrome
- Auto-immune interstitial nephritis
- Acute tubulo-interestitial nephritis with uveitis (TINU)
- Renal transplantation
- Anorexia nervosa

Exogenous substances
- Drugs
  - Aminoglycosides, outdated tetracycline
  - Valproate, salicylate
  - Adefovir, cidofovir, tenofovir
  - Ifosfamide, cisplatin, imanitib mesylate
- Chinese herbs (aristolochic acid)
- Chemical compounds (paraquat, diachrome, 6-mercaptopurine, toluene, maleate)
- Heavy metals (lead, cadmium, chromium, platinum, uramnium, mercury)

van der Wijst et al. *Physiol Rev* 99, 2019



Endolysosomal Disorders: LMW Proteinuria and Proximal Tubule Dysfunction

Abnormality	Lowe	Dent disease 2
Extrarenal		
Cataract[a]	100%	7%
Intellectual impairment[b]	100%	27%
Growth retardation (mean height SDS)	100% (−3,7)	Frequent (−2,1)
Arthropathy[c]	Frequent	Infrequent
Elevated CPK and /or LDH[d]	98%	97%
Renal		
Nephrocalcinosis	45%	28%
LMWP	100%	100%
Albuminuria[e]	100%	NA
Lysosomal enzymuria	100%	NA
Aminoaciduria	79%	41%
Hypercalciuria	82%	78%
Metabolic acidosis	57%	4%
Phosphate wasting	51%	15%
Potassium wasting	23%	4%
Glycosuria	10%	15%

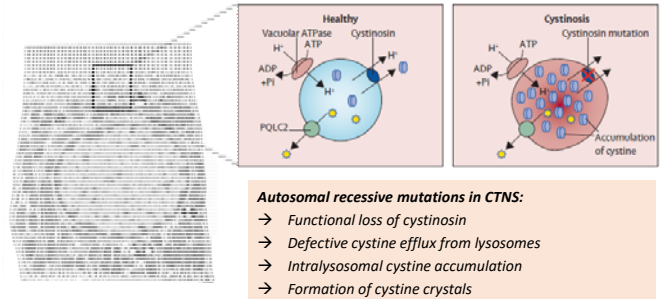
TABLE 1. AGE-RELATED CLINICAL CHARACTERISTICS OF UNTREATED NEPHROPATHIC CYSTINOSIS.

AGE	SYMPTOM OR SIGN	PREVALENCE IN AFFECTED PATIENTS
		%
6–12 mo	Renal Fanconi's syndrome (polyuria, polydipsia, electrolyte imbalance, dehydration, rickets, growth failure)	95
5–10 yr	Hypothyroidism	50
8–12 yr	Photophobia	50
8–12 yr	Chronic renal failure	95
12–40 yr	Myopathy, difficulty swallowing	20
13–40 yr	Retinal blindness	10–15
18–40 yr	Diabetes mellitus	5
18–40 yr	Male hypogonadism	70
21–40 yr	Pulmonary dysfunction	100
21–40 yr	Central nervous system calcifications	15
21–40 yr	Central nervous system symptomatic deterioration	2

Early endocytic defect - before kidney failure

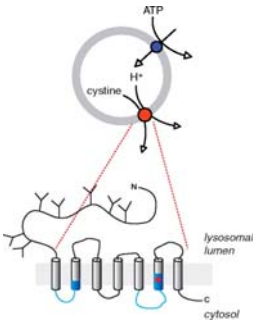
De Matteis et al. *Nat Rev Nephrol* 13, 2017
Gahl WA et al. *NEJM* 347, 2002

Cystinosis: AR mutations in cystinosin - H^+-driven export of cystine

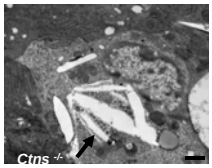


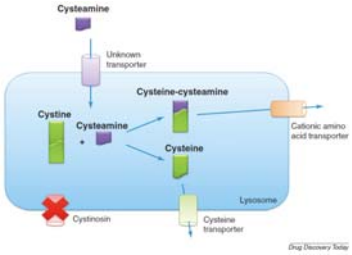
Autosomal recessive mutations in CTNS:

- → *Functional loss of cystinosin*
- → *Defective cystine efflux from lysosomes*
- → *Intralysosomal cystine accumulation*
- → *Formation of cystine crystals*



→ *Multisystemic – kidney, brain, eyes, muscles, endocrine, ...*



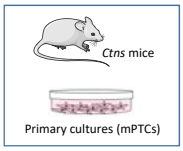


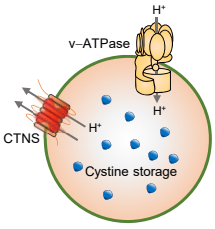
Cysteamine treatment: Help to export cystine out of lysosomes

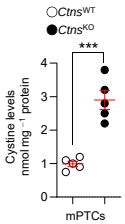
Problems:

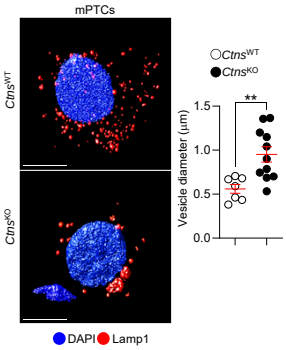
- Side-effects
- No effect on PT dysfunction

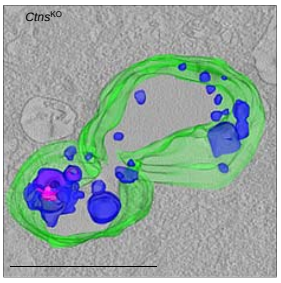
Altered Lysosomal Dynamics in Cystinosis Cells









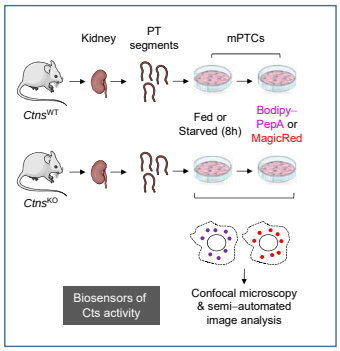


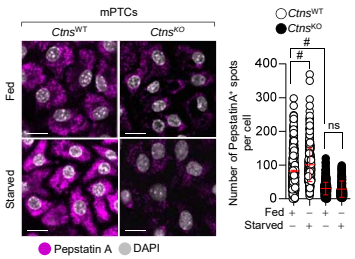
3D- Electron Tomography Microscopy

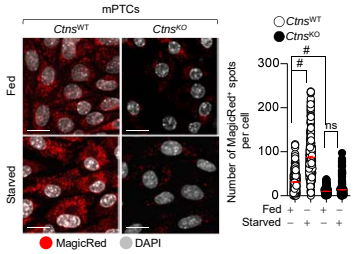
Clustering of perinuclear, enlarged lysosomes

Raggi, Luciani et al. *Hum Mol Genet* 2014; Festa et al. *Nat Commun* 2018

Defective Endolysosomal Proteolysis in Cystinosis Cells

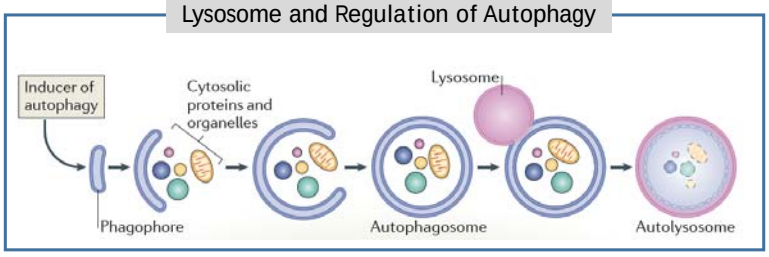


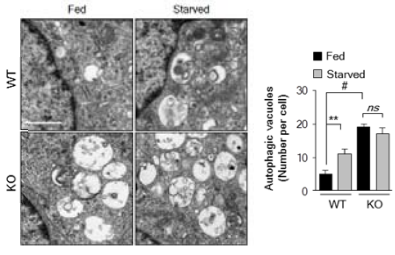


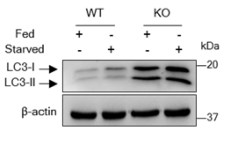


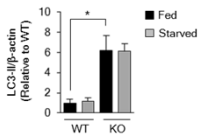
Festa B et al. *Nat Commun* 2018; 9: 161

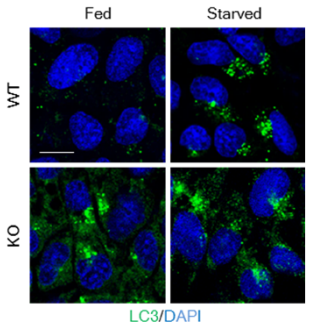
Lysosome and Regulation of Autophagy

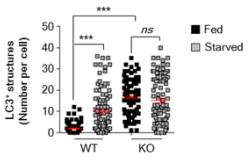








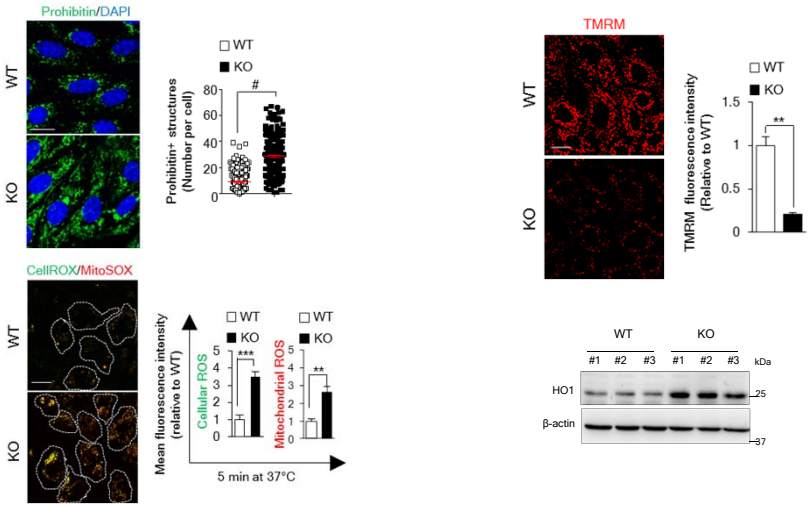




Accumulation of autophagosomes and cargo in cystinosis Cells

Festa B et al. *Nat Commun* 2018; 9: 161

Mitochondrial Accumulation & Dysfunction in Cystinosis



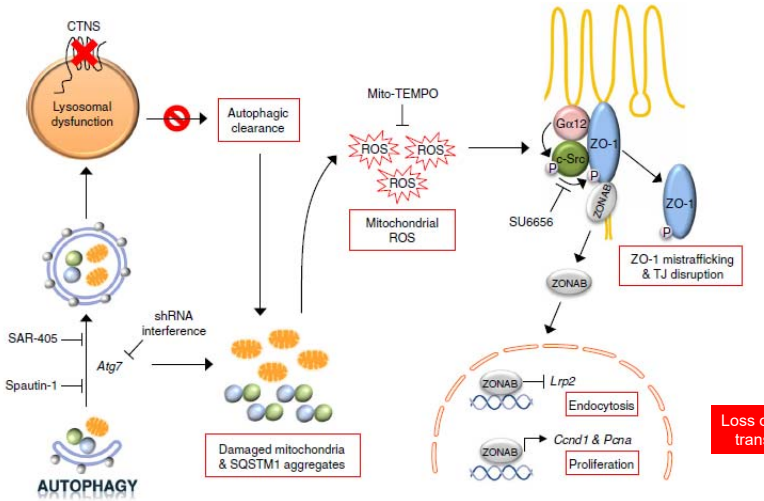
Accumulation of damaged (disorganized cristae, loss of potential), ROS producing mitochondria

Festa BP et al, *Nat Commun* 9: 161, 2018

ARTICLE

DOI: 10.1038/s41467-017-02536-7 OPEN

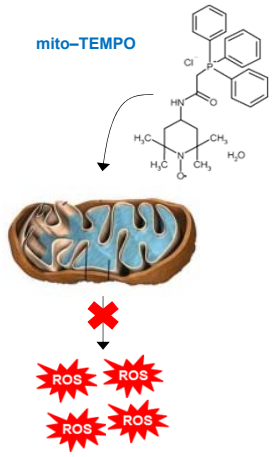
Impaired autophagy bridges lysosomal storage disease and epithelial dysfunction in the kidney



Loss of differentiation & transport properties

Festa et al. Nature Communications 9; 2018
Luciani et al. Autophagy 14; 2018

Durg Repurposing: Antioxidants Targeting Mitochondria as New Therapeutic Strategy in Cystinosis



MitoTEMPO: mitochondrially targeted antioxidant, scavenger of mitochondrial superoxide

Combination of antioxidant piperidine nitroxide + lipophilic cation triphenylphosphonium

Ability to pass through lipid bilayers and accumulates in mitochondria

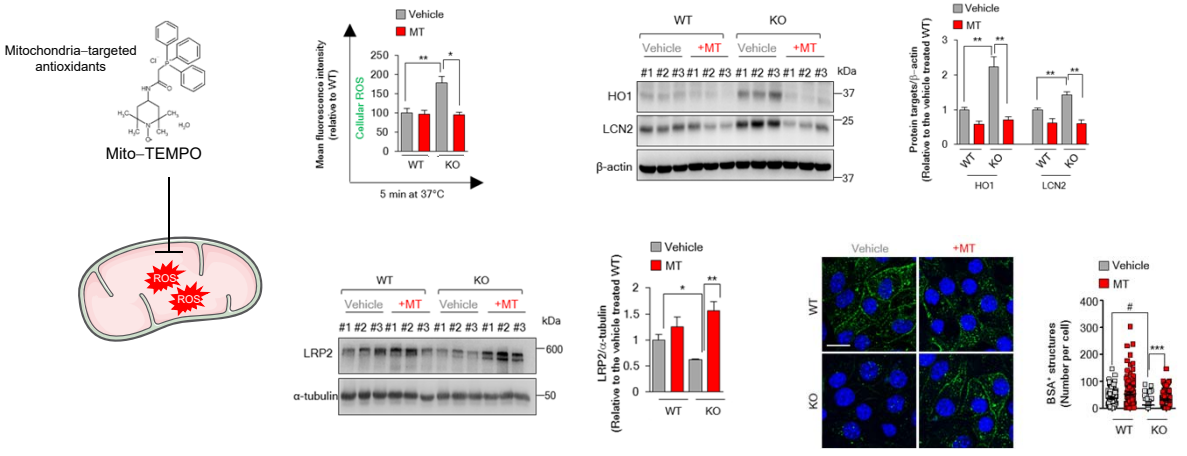
→ *Currently tested in various mitochondrial / kidney diseases*

Table 3 | **Potential approaches to target mitochondrial dysfunction**

Therapy (alternative names)	Mechanism(s) of action	Clinical trial			Refs
		Name and/or no.	Phase	Indication(s)	
CoQ10*	Antioxidant	NCT00432744	III	Mitochondrial diseases	278
		NCT00740714	III	Parkinson disease	279
MitoQ*	Mitochondrial-targeted antioxidant	NCT02364648	II	Chronic kidney disease	283
		NCT00329056	II	Parkinson disease	282
MTP-131* (SS-31, Bendavia, elamipretide)	Binds to cardiolipin, increases OXPHOS efficiency, potential antioxidant effects	NCT02367014	I/II	Mitochondrial myopathy	290
		NCT02245620	II	Age-related skeletal muscle mitochondrial dysfunction	291
KH-176	Mitochondrial-targeted antioxidant, enhances OXPHOS	NCT02544217	I	Healthy males (safety study)	293
		KHENERGY, NCT02909400	II	Mitochondrial diseases	294

Wang W et al, *Science Translational Medicine*, 2016
Forbes JM, Thorburn DR, *Nat Rev Nephrol*, 2018

Targeting Mitochondrial Stress Improves Epithelial Dysfunction in Cystinosis Cells

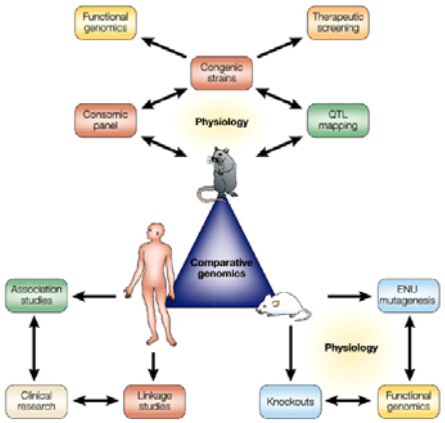


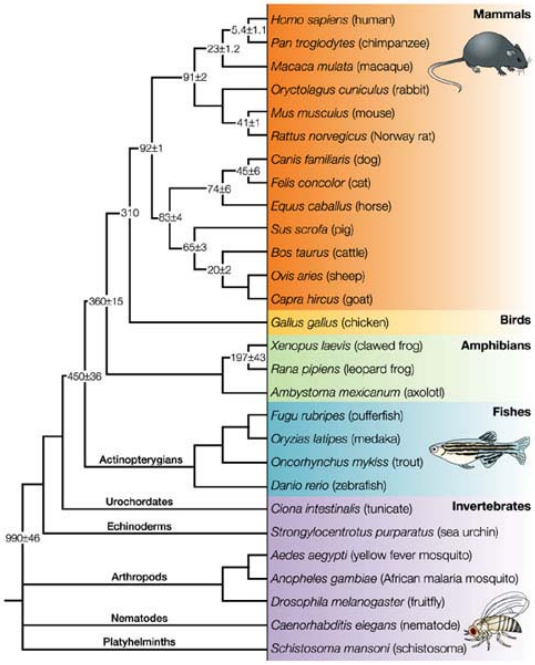
Festa et al. Nature Communications 9; 2018
Luciani et al. Autophagy 14; 2018

Which Model Organism in Rare Diseases?

Time to develop Phenotyping Space, costs

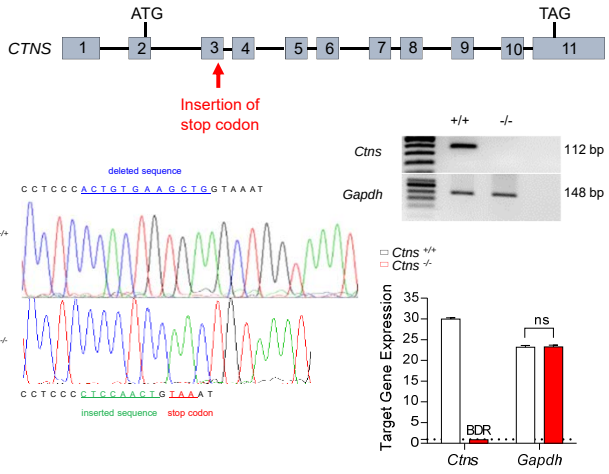
Genetic manipulation High throughput Ethics

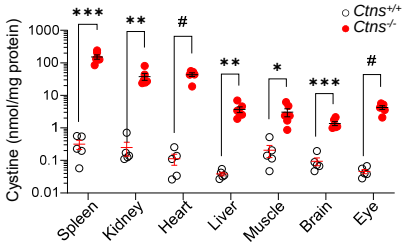




THE ORIGIN AND EVOLUTION OF MODEL ORGANISMS
Hedges, SB *Nature Reviews Genetics* **3**, 838 -849 (2002)

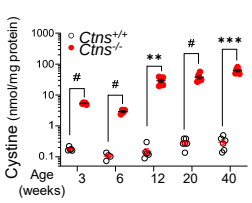
CRISPR/Cas9 Induced Generation of the *Ctns*$^{-/-}$ Rat Model



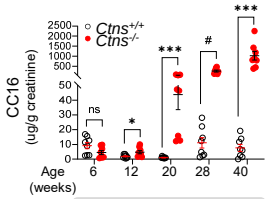


Frameshift in exon 3 resulting in premature stop codon and a complete loss of the protein
Ctns deletion leads to cystine accumulation in multiple tissues

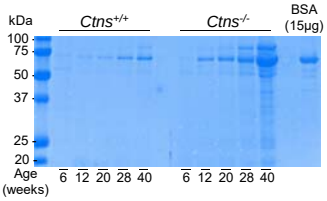
Progressive Renal Fanconi Syndrome in *Ctns*$^{-/-}$ Rats



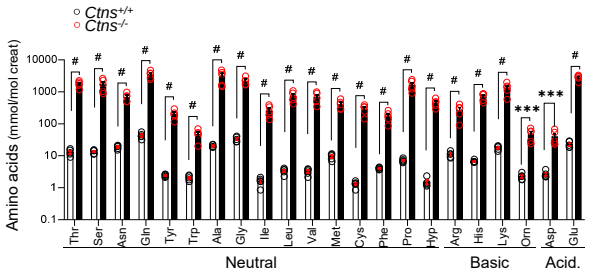
Cystine content



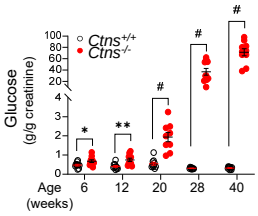
LMW proteinuria



Proteinuria

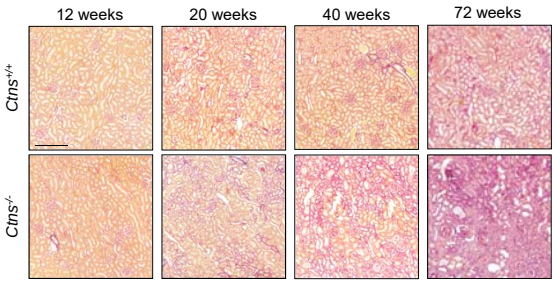


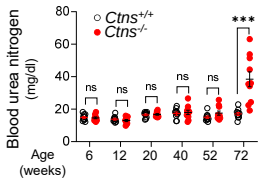
Aminoaciduria

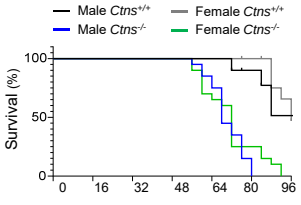


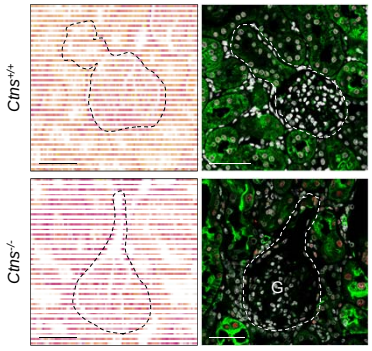
Glycosuria

Histopathological Alterations Associated with Increased Mortality









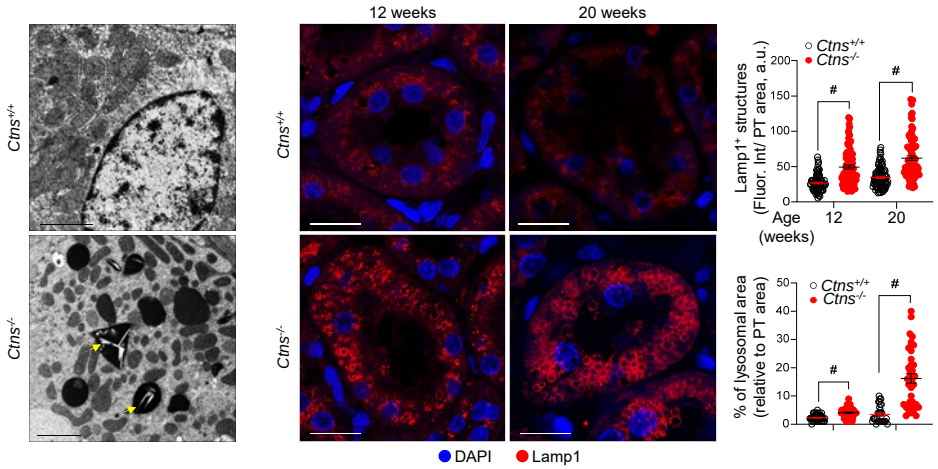
Swan-neck tubules & apoptosis

→ Development of fibrotic lesions

→ Increased BUN - kidney failure

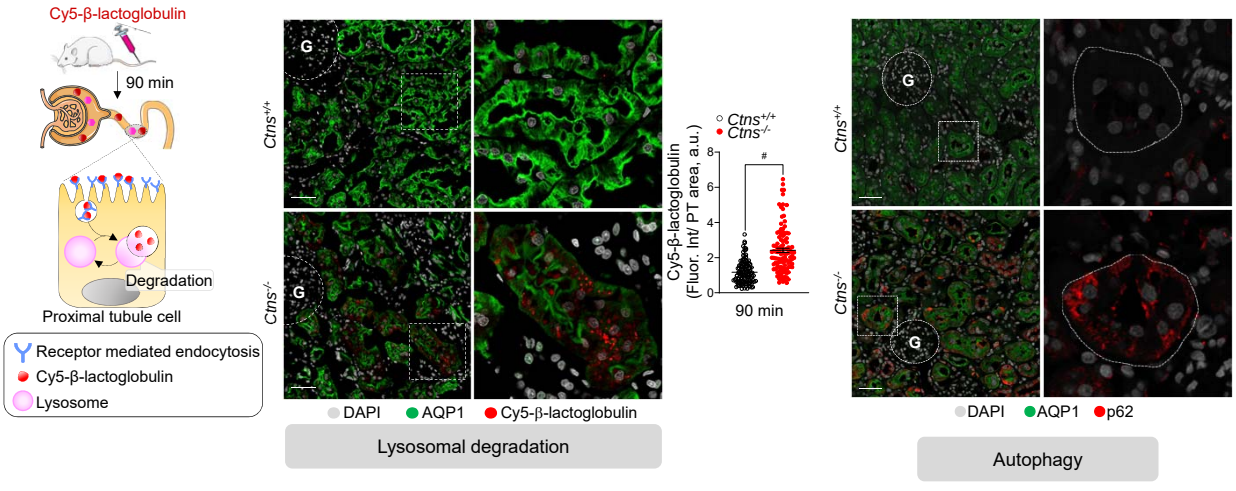
→ Decreased survival *Ctns*$^{-/-}$ rats

Impaired Lysosomal Homeostasis in *Ctns*$^{-/-}$ Rat Kidneys



Accumulation of enlarged lysosomal vesicles & crystal formation in *Ctns*$^{-/-}$ rat kidneys

Lysosomal Dysfunction in *Ctns*$^{-/-}$ Rat Kidneys



Defective lysosomal homeostasis & autophagy in *Ctns*$^{-/-}$ kidneys

NEWS & ANALYSIS

Credit: S. Harris/Springer Nature Limited

Zebrafish earn their drug discovery stripes

Nearly ten compounds from zebrafish screens are in or about to enter the clinic, and zebrafish 'avatars' are gaining traction as a tool to guide treatment plans for patients with cancers and rare diseases.

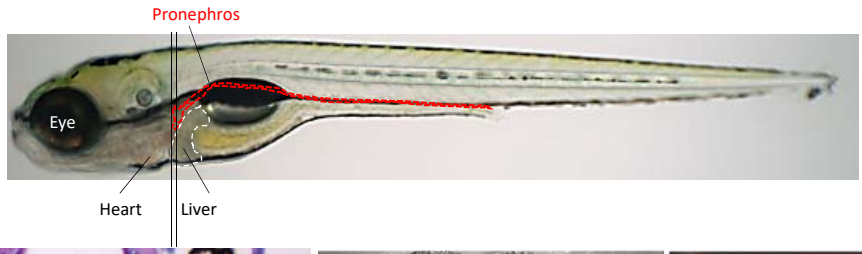
NATURE REVIEWS | DRUG DISCOVERY VOLUME 18 | NOVEMBER 2019 | 811

Advantages of Zebrafish as Model Organism

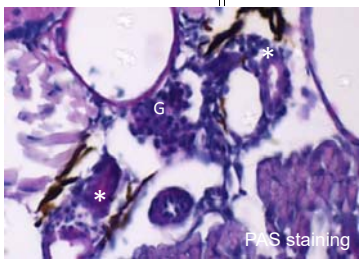
- *In vivo* model – whole organism
- Transparent
- Genome sequenced, easy to edit, multiple reporter lines
- Low cost for breeding: small size, high fertility
- Amenable to high-throughput screens
- No ethics concerns up to 7dpf
- Conservation of key transporters/receptors – patterning
- Possibility of deep phenotyping – kidney, CNS, eye, muscle

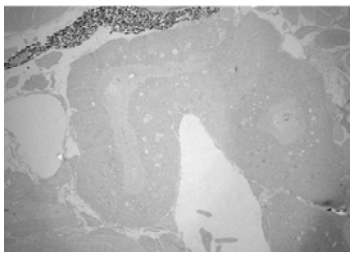
Zebrafish Larvae: Proximal Tubule Morphology

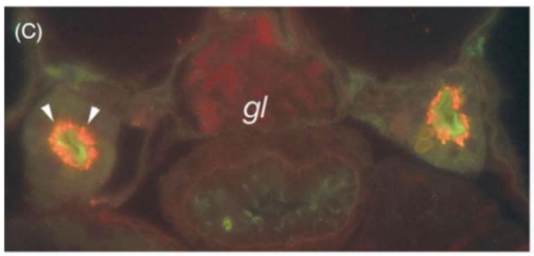
- 5dpf of larvae



Nephron segment







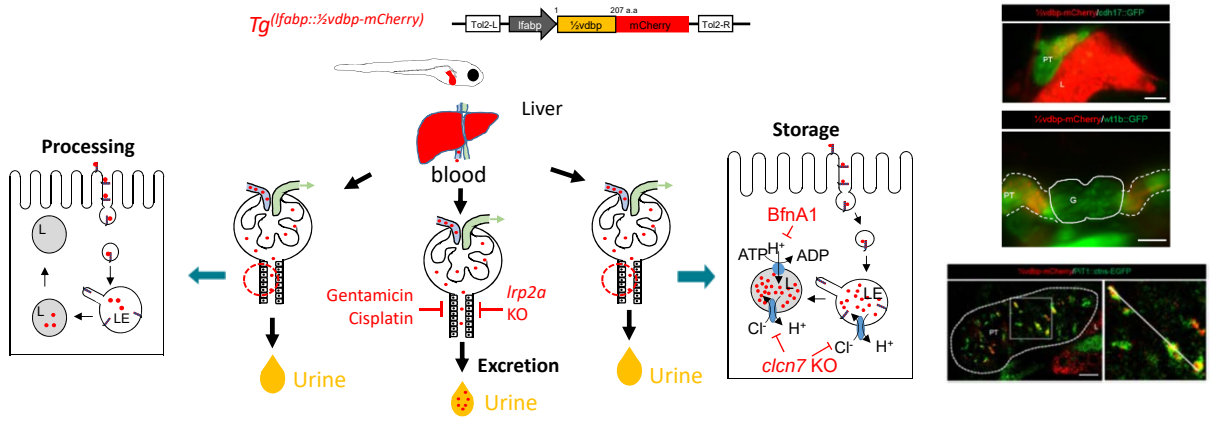
Wingert et al. Kidney Int 2016; 89: 1204-10

www.kidney-international.org basic research

Transgenic zebrafish modeling low-molecular-weight proteinuria and lysosomal storage diseases

OPEN

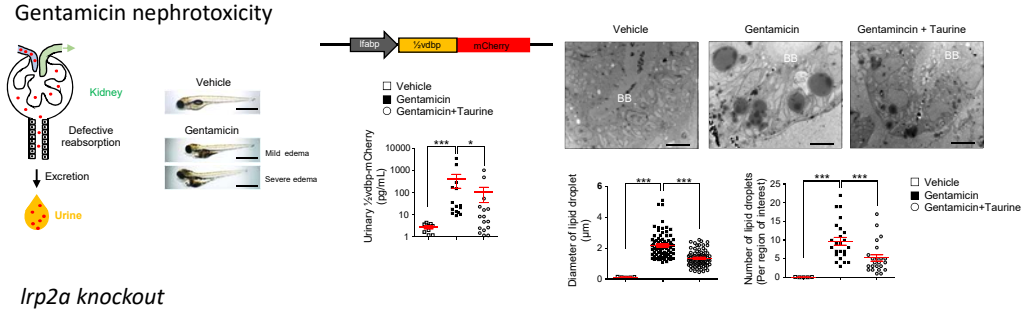
Zhiyong Chen[1], Alessandro Luciani[1], José María Mateos[2], Gery Barmettler[2], Rachel H. Giles[3,4], Stephan C.F. Neuhauss[5] and Olivier Devuyst[1]



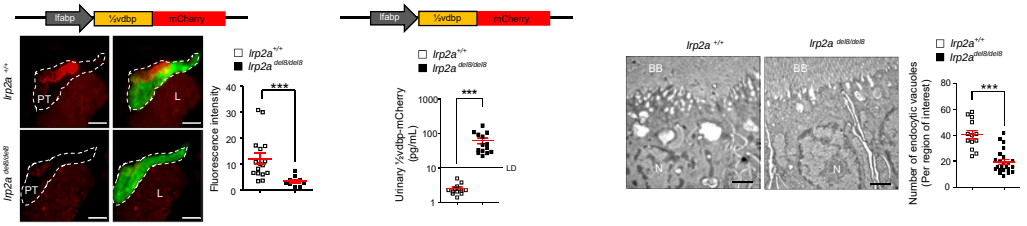
➢ Physiological condition ➢ LMW proteinuria ➢ Lysosomal storage disease

Proof-of-concept studies of LMW proteinuria

- Gentamicin nephrotoxicity

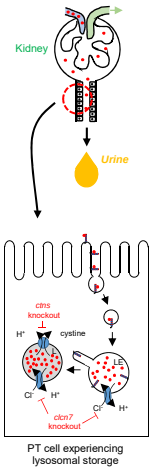


- *lrp2a knockout*

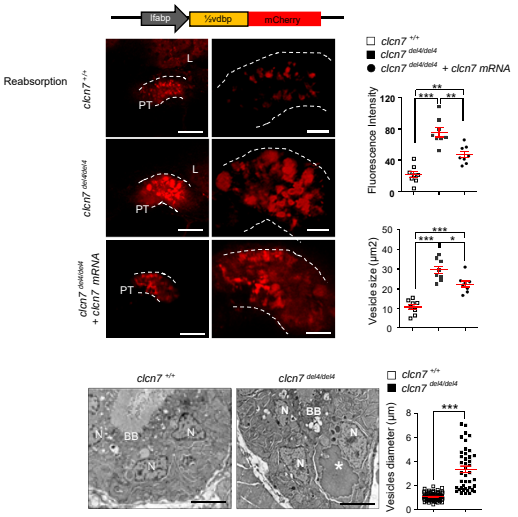


Chen Z et al. *Kidney Int* 97, 2020

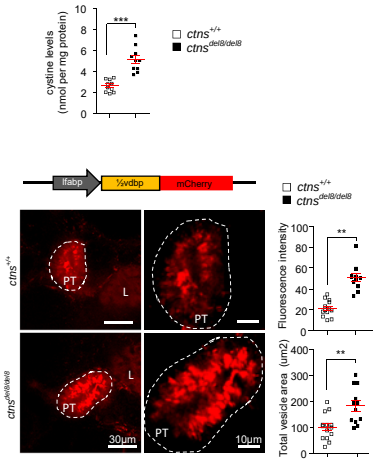
Proof-of-concept studies of lysosomal storage diseases



- *clcn7* knockout



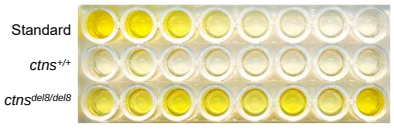
- *ctns* knockout



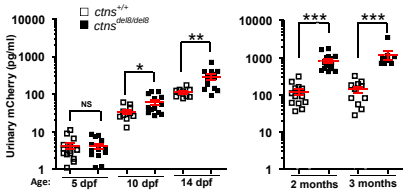
Chen Z et al. *Kidney Int* 97, 2020

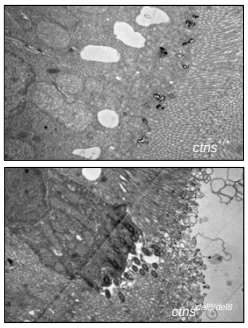
Progressive LMW proteinuria in *ctns* knockout larvae *Tg(lfabp::½vdbp-mCherry)*

- Detection of LMW proteinuria in *ctns* knockout larvae and juvenile/adult fish



- Urine collected in 40ml water
- ELISA with 50l of water



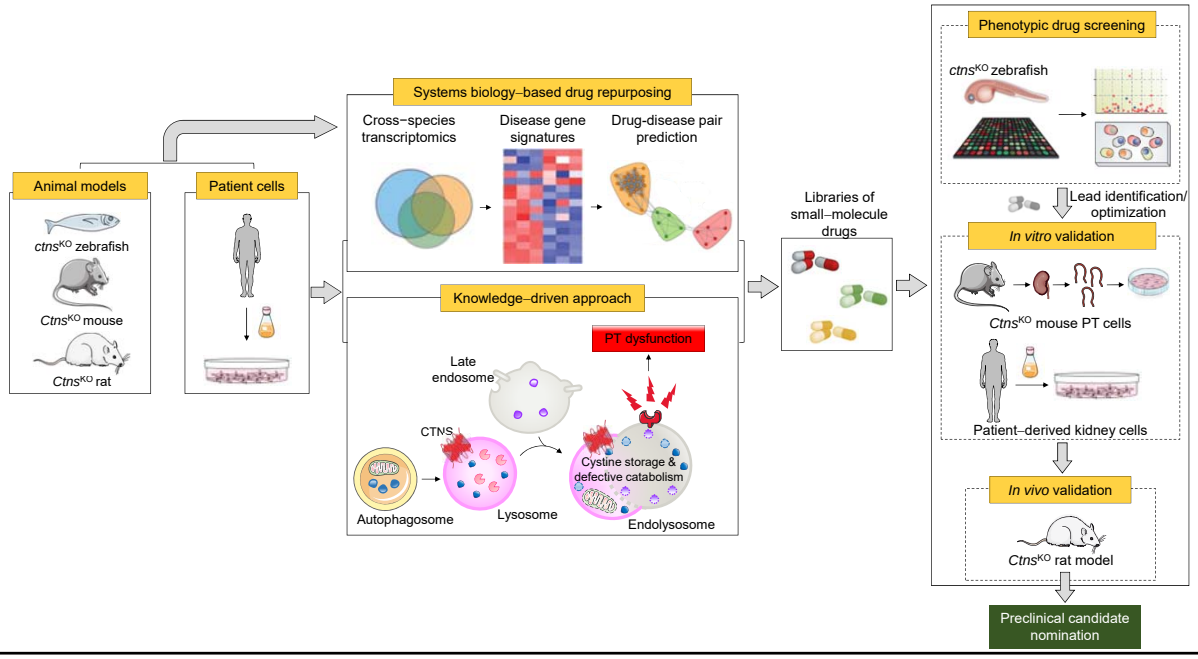


TEM, 14 dpf

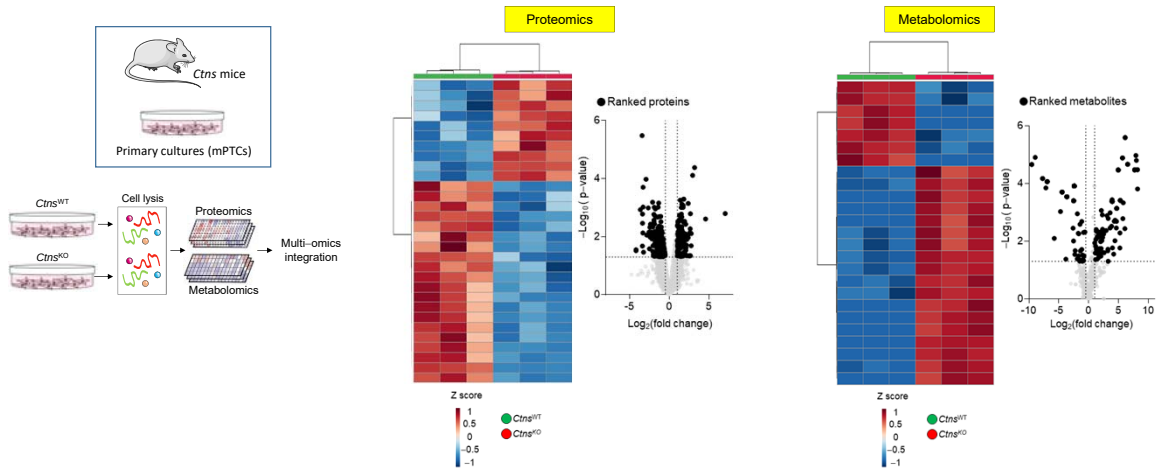
CYSTINOSIS RESEARCH FOUNDATION

Festa BP et al. Nat Commun 2018; 9: 161

An Integrated Drug Discovery and Repurposing for Endolysosomal Diseases



Integrative Omics Provide Novel Disease Mechanism and Target Discovery



Collaboration with Dr. Endre Laczko, Functional Genomics Zurich Center, UZH


Thank you !