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Molecular Basis of Cystinosis: Geographic Distribution, Functional Consequences of Mutations in the *CTNS* Gene, and Potential for Repair

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Abstract
Mutations in the *CTNS* gene encoding the lysosomal membrane cystine transporter cystinosin are the cause of cystinosis, an autosomal recessive lysosomal storage disease. More than 140 *CTNS* mutations have been reported worldwide. Recent studies have discovered that cystinosin exerts other key cellular functions beyond cystine transport such as regulation of oxidative state, lysosomal dynamics and autophagy. Here, we review the different mutations described in the *CTNS* gene and the geographical distribution of incidence. In addition, the characteristics of the various mutations in relation to the functions of cystinosin needs to be further elucidated. In this review, we highlight the functional consequences of the different mutations in correlation with the clinical phenotypes. Moreover, we propose how this understanding would be fundamental for the development of new technologies through targeted gene therapy, holding promises for a possible cure of the kidney and extra-renal phenotypes of cystinosis.

Clinical Presentation of Cystinosis

Cystinosis is an autosomal recessive disease caused by mutations in the *CTNS* gene encoding the lysosomal membrane transporter cystinosin [1]. Cystinosin is a ubiquitously expressed protein, functioning as a cystine-proton co-transporter and extruding cystine out of the lysosomes; this activity is dependent on the lysosomal acidification conveyed by the H^+-ATPase [2]. Cysti-

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nosin deficiency results in lysosomal cystine accumulation and cystine crystals formation in virtually all tissues and organs [3]. While cystinosis belongs to a larger group of ~50 lysosomal storage disorders, mostly caused by lysosomal enzyme deficiencies, its clinical phenotype is distinct, with the kidney being the first affected organ.

Depending on kidney disease severity, the cystinosis phenotype is divided in 3 clinical forms [4]. The most severe variant, infantile nephropathic cystinosis (MIM219800) affects ~95% of patients and is characterized by the development of renal Fanconi syndrome during the first months of life followed by glomerular dysfunction, which if untreated, results in end-stage kidney disease (ESKD) around the age of 10 years. Late-onset juvenile nephropathic type (MIM219900) usually presents during childhood or at adolescence with mild or even absent proximal tubular dysfunction, proteinuria, which can be in the nephrotic range, and a slower rate of progression towards ESKD [5]. Non-nephropathic cystinosis (MIM219750) is a benign variant presenting with photophobia due to cystine accumulation in the cornea but causing no systemic organ damage [6].

All 3 clinical forms of cystinosis are due to bi-allelic mutations in the *CTNS* gene with a mutation detection rate >95% [7].

The first two clinical phenotypes of cystinosis are also characterized by extra-renal organ dysfunction gradually developing from childhood to adulthood. The eye is the second affected organ with cystine crystals found in the cornea and retina early in life, and progressive retinopathy eventually leading to blindness at adolescence or adult age [8]. Endocrine organs (thyroid, pancreas, and gonads), GI tract, muscles, bones, central and peripheral nervous systems are also impacted by the disease, with morbidity and mortality frequently being associated with the swallowing dysfunction and aspiration due to muscle weakness [9].

Long-term prognosis of patients with cystinosis has dramatically improved during the last 30 years by the availability of the cystine-depleting drug cysteamine and advances of kidney transplantation. Cysteamine, a small amino thiol, was first introduced for the treatment of cystinosis in 1976 [10] and later was shown to prolong kidney function survival, improve growth, and delay or even prevent extra-renal disease complications [11, 12]. The results of kidney transplantation in cystinosis are excellent with no disease recurrence and long-term graft survival being better in cystinosis compared to other renal disease patients [13, 14]. Hence, the oldest cystinosis patients passed now the age of 55, and a new generation of well-treated patients is expected to have even better life expectancy. Nevertheless, the severity of renal Fanconi syndrome with daily urine volume sometimes exceeding 10 L and resistance to cysteamine therapy, multiorgan involvement in cysteamine-treated patients, and the burden of cysteamine administration causing unpleasant body and breath odour, GI and bone complaints in some patients, point out the necessity of developing novel, less toxic and more efficient treatment strategies tackling all disease aspects.

Genetic Basis of Cystinosis

Overview of Mutations in the CTNS Gene and Geographical Distribution

Over 140 pathogenic *CTNS* mutations have been reported in cystinosis patients worldwide. These include 57 missense and nonsense mutations, 23 intronic mutations, 45 deletions, 13 small insertions, 4 indels and 3 promoter region mutations (Table 1) [15]. Overall, there is a considerable genotype-phenotype correlation of the *CTNS* mutations, with the absolute majority of pathogenic mutations causing the infantile phenotype, while only 15 and 4 mutations have been attributed to juvenile and ocular cystinosis respectively (Table 1).

Most reported *CTNS* mutations were detected in countries of Europe and North America, with only a minority of developing nations conducting genetic studies, mostly due to lacking funds to perform molecular diagnosis in potential patients [16]. Notably over the last decade, an increasing number of reports have been published on *CTNS* gene mutations in some of the developing nations' populations [17–20]. Still, large geographic regions, such as sub-Saharan Africa, South-East Asia and the Far East are underrepresented in the genetic spectrum of their cystinosis patients. Although accurate statistics are lacking in the developing world [21], recent studies from the Middle East [17–20], Mexico [22] and South Africa [23] may indicate that the incidence of cystinosis in many of these countries is expected to be higher than that of Europe and North America. Moreover, many cystinosis patients in poor countries remain undiagnosed and die at a young age due to complications of the disease [24].

The genetic landscape of cystinosis varies widely based on the ethnic and genetic makeup of each reporting population. The most common pathogenic mutations in Northern Europe and North America is a large

Table 1. Geographical distribution of all reported mutations in the *CTNS* gene

Missense/nonsense mutations						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
1	c.1A>C	p.M1L	3	Infantile	France	Kalatzis et al., 2002, Hum Mut
2	c.2T>C	p.M1T	3	Infantile	Spain	Macias-Vidal et al., 2009, Clin Genet
3	c.3G>A	p.M1I	3	Infantile	France	Kalatzis et al., 2002, Hum Mut
4	c.3 G>C	p.M1I	3	Infantile	Turkey	Topaloglu et al., 2017, CJASN
5	c.15G>A	p.W5*	3	Infantile	France	Kalatzis et al., 2002, Hum Mut
					Egypt	Soliman et al., 2014, JIMD Rep
					Turkey	Topaloglu et al., 2017, CJASN
6	c.124G>A	p.V42I	4	Juvenile	UK	Attard et al., 1999, Hum Mol Genet
					Qatar	Rodriguez-Flores et al., 2014, Hum Mut
7	c.283G>T	p.G95*	6	Infantile	UK, France	Town et al., 1998, Nat Genet
8	c.382C>T	p.Q128*	7	Infantile	UK, France	Town et al., 1998, Nat Genet
					UK	Attard et al., 1999, Hum Mol Genet
9	c.397A>T	p.I133F	7	Infantile	Canada	McGowan et al., 1999, Eur J Hum Genet
10	c.414G>A	p.W138*	7	Infantile	UK, France	Town et al., 1998, Nat Genet
					Canada	McGowan et al., 1999, Eur J Hum Genet
11	c.416C>T	p.S139F	7	Juvenile	UK	Attard et al., 1999, Hum Mol Genet
					France	Servais et al., 2008, CJASN
					Spain	Macias-Vidal et al., 2009, Clin Genet
12	c.422C>T	p.S141F	7	Infantile	France	Kalatzis et al., 2004, Hum Mol Genet
					Saudi Arabia	Aldahmesh et al., 2009, Ophthalm Genet
13	c.433C>T	p.145*	7	Infantile	Iran	Sadeghipour, 2017, Hum Genom Var
14	c.451A>G	p.R151G	7	Infantile	Turkey	Topaluglu et al., 2012, Pediatr Nephrol
15	c.470G>A	p.G157D	8	Infantile	Turkey	Topaluglu et al., 2012, Pediatr Nephrol
16	c.473T>C	p.L158P	8	Infantile	Canada	McGowan et al., 1999, Eur J Hum Genet
17	c.506G>A	p.G169D	8	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
18	c.517T>C	p.Y173H	8	Infantile	Iran	Sadeghipour, 2017, Hum Genom Var
19	c.518A>G	p.Y173C	8	Infantile	Turkey	Topaluglu et al., 2012, Pediatr Nephrol
20	c.530A>G	p.N177S	8	Infantile	Saudi Arabia	Aldahmesh et al., 2009, Ophthalm Genet
21	c.530A>C	p.N177T	8	Juvenile	France	Kalatzis et al., 2002, Hum Mut
22	c.544T>C (•)	p.W182R	8	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
23	c.560A>G	p.K187R	8	Infantile	Egypt	Soliman et al., 2014, JIMD Rep
24	c.589G>A	p.G197R	9	Ocular	USA	Anikster et al., 1999, Hum Mut
25	c.599C>T	p.P200L	9	Juvenile	France	Kalatzis et al., 2002, Hum Mut
26	c.613G>A	p.D205N	9	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
					Turkey	Topaloglu et al., 2017, CJASN
					Iran	Sadeghipour, 2017, Hum Genom Var
27	c.647C>G	p.T216R	9	Infantile	Mexico	Alcantra-Ortigoza, 2008, Hum Genet
28	c.664C>T	p.Gln222*	9	Infantile	Turkey	Topaloglu et al., 2017, CJASN
29	c.665A>G	p.Q222R	9	Infantile	France	Kalatzis et al., 2002, Hum Mut
30	c.704G>A	p.W235*	10	Infantile	Italy	Pennesi et al., 2005, Pediatr Nephrol
31	c.734G>A	p.W245*	10	Infantile	Egypt	Soliman et al., 2014, JIMD Rep
32	c.809C>T	p.S270F	10	Infantile	Spain	Macias-Vidal, 2009, Clin Genet
33	c.809C>A	p.S270Y	10	Infantile	South Africa	Owen et al., 2015, Pediatr Nephrol
34	c.839A>G (•)	p.K280R	10	Juvenile	USA	Thoene et al., 1999, Mol Genet Metab
					France	Servais et al., 2008, CJASN
35	c.850C>T	p.Q284*	10	Infantile	Thailand	Yeetong et al., 2012, Gene
36	c.861G>A	p.M287I	11	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet
37	c.864C>A (•)	p.N288K	11	Infantile	France	Kalatzis et al., 2002, Hum Mut
38	c.870C>G	p.Y290*	11	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
39	c.878G>T	p.S293I	11	Infantile	Turkey	Topaloglu et al., 2017, CJASN
40	c.890G>A	p.W297*	11	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet
41	c.893G>A (•)	p.S298N	11	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
42	c.914A>G	p.D305G	11	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
43	c.913G>T	p.D305Y	11	Infantile	UK	Attard et al., 1999, Hum Mol Genet

Table 1. (continued)

Missense/nonsense mutations						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
44	c.922G>A	p.G308R	11	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
					Italy	Mason et al., 2003, Eur J Hum Genet
					Spain	Macias-Vidal et al., 2009, Clin Genet
					Saudi Arabia	Aldahmesh et al., 2009, Ophthalm Genet
					Egypt	Soliman et al., 2014, JIMD Rep
45	c.922G>C	p.G308R	11	Infantile	Turkey	Topaloglu et al., 2017, CJASN
46	c.923G>A	p.G308E	11	Infantile	Iran	Shahkarami, 2013, Nephrologia
47	c.923G>T	p.G308V	11	Infantile	Germany, Switzerlands	Kiehntopf, 2002, Hum Mut
48	c.926G>A	p.G309D	11	Infantile	Thailand	Yeetong et al., 2012, Gene
49	c.926G>T	p.G309V	11	Infantile	Spain	Macias-Vidal, 2009, Clin Genet
50	c.969C>G (•)	p.N323K	11	Juvenile	USA	Thoene et al., 1999, Mol Genet Metab
					Thailand	Yeetong et al., 2012, Gene
51	c.1001C>A	p.T334N	12	Juvenile	France	Servais et al., 2008, CJASN
52	c.1009G>A	p.G337R	12	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet
53	c.1013T>G	p.L338R	12	Infantile	Saudi Arabia	Aldahmesh et al., 2009, Ophthalm Genet
54	c.1013T>C	p.L338P	12	Infantile	UK	Attard et al., 1999, Hum Mol Genet
55	c.1015G>A (•)	p.G339R*	12	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
					Canada	Rupar et al., 2001, J Med Genet
					Italy	Mason et al., 2003, Eur J Hum Genet
					Spain	Macias-Vidal, 2009, Clin Genet
					Egypt	Soliman et al., 2014, JIMD Rep
					Turkey	Topaloglu et al., 2017, CJASN
					Iran	Sadeghipour, 2017, Hum Genom Var
56	c.1036G>A	p.D346N	12	Juvenile	UK	Attard et al., 1999, Hum Mol Genet
57	c.1084G>A	p.G362R	12	Infantile	Egypt	Soliman et al., 2014, JIMD Rep

Intronic mutations					
No.	mutation	intron	phenotype	country of diagnosis	references
1	c.61+5G>A	4	Infantile	Mexico	Alcantra-Ortigoza, 2008, Hum Genet
				Spain	Macias-Vidal, 2009, Clin Genet
				Canada	Mucaki et al., 2013, Hum Mut
2	c.61+5G>T	4	Infantile	Egypt	Soliman et al., 2014, JIMD Rep
3	c.141-22A>G	5	Infantile	Turkey	Topaloglu et al., 2017, CJASN
4	c.141-24T>C	5	Infantile	Italy	Taranta et al., 2010, pediatr Nephrol
5	c.140+1G>T	5	Infantile	UK, France	Town et al., 1998, Nat Genet
				Turkey	Topaloglu et alv, 2017, CJASN
6	c.225+3A>T	6	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet
7	c.225+5_225+6delGTinsCC	6	Infantile	Australia	Cabrera-Serrano, 2017, Neuromuscul Disord
8	c.226-3C>G	6	Infantile	Australia	Cabrera-Serrano, 2017, Neuromuscul Disord
9	c.329G>T	7	Juvenile	France	Kalatzis et al., 2002, Hum Mut
10	c.329G>C	7	Infantile	Japan	Higashi et al., 2017, BMC Nephrol
11	c.329+2T>C	7	Infantile	Japan	Higashi et al., 2017, BMC Nephrol
12	c.329+1delG	7	Infantile	China	Yang, 2015, ScientificWorld Journal
13	c.462-10C>G	8	Juvenile	UK	Attard et al., 1999, Hum Mol Genet
14	c.562-1G>C	9	Infantile	UK, France	Town et al., 1998, Nat Genet
				Saudi Arabia	Aldahmesh et al., 2009, Ophthalm Genet
15	c.681delGT	10	Infantile	China	Yang, 2015, ScientificWorld Journal
16	c.682-1G>T	10	Infantile	Spain	Macias-Vidal, 2009, Clin Genet
17	c.681G>A (•)	10	Infantile	Saudi Arabia	Aldahmesh et al,. 2009, Ophthalm Genet
				Egypt	Soliman et al., 2014, JIMD Rep
				Iran	Ghazi et al., 2017, Nephrologia
				Turkey	Topaloglu et al., 2017, CJASN

Table 1. (continued)

Intronic mutations					
No.	mutation	intron	phenotype	country of diagnosis	references
18	c.681+1G>A	10	Infantile	UK	Attard et al., 1999, Hum Mol Genet
				Italy	Mason et al., 2003, Eur J Hum Genet
19	c.853-3C>G	11	Ocular	USA	Anikster et al., 1999, Hum Mut
				Russia	Bryzgalov, 2013, PLoS One
20	c.853-2A>G	11	Infantile	France	Kalatzis et al., 2002, Hum Mut
21	c.853-1G>A	11	Infantile	Turkey	Topaloglu et al., 2017, CJASN
22	c.971-12G>A (•)	12	Infantile	UK	Attard et al., 1999, Hum Mol Genet
				Thailand	Yeetong et al., 2012, Gene
				South Africa	Owen et al., 2015, Pediatr Nephrol
				Iran	Sadeghipour, 2017, Hum Genom Var
23	c.970+2T>C	12	Juvenile	USA	Thoene et al., 1999, Mol Genet Metab

Promoter mutations				
No.	position	phenotype	country of diagnosis	references
1	–295 G>T (–50 relative to transcription initiation site)	Infantile	USA	Phornphutkul et al., 2001, Am J Hum Genet
			Italy	Mason et al., 2003, Eur J Hum Genet
2	–295 insT (–50 relative to transcription initiation site)	Ocular	USA	Phornphutkul et al., 2001, Am J Hum Genet
3	–303 G>C (–42 relative to transcription initiation site)	Ocular	USA	Phornphutkul et al., 2001, Am J Hum Genet

Deletions						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
1	c.18_21delGACT	T7Ffs*7	3	Infantile	UK, France	Town et al., 1998, Nat Genet
					Netherlands	Heil et al., 2001, Nephron
					Italy	Mason et al., 2003, Eur J Hum Genet
					Mexico	Alcantra-Ortigoza, 2008, Hum Genet
					France	Servais et al., 2008, CJASN
					Spain	Macias-Vidal, 2009, Clin Genet
					Thailand	Yeetong et al., 2012, Gene
					Iran	Shahkarami, 2013, Nephrologia
					Turkey	Topaloglu et al., 2017, CJASN
2	c.36delT	L14*	3	Infantile	UK, France	Town et al., 1998, Nat Genet
3	c.40delC	L14*	3	Infantile	Mexico	Alcantra-Ortigoza, 2008, Hum Genet
4	c.60_61delTG	Splicing			UK, France	Town et al., 1998, Nat Genet
5	c.61_61+2delGGT	Splicing			Germany, Switzerlands	Kiehntopf, 2002, Hum Mut
6	c.120delC	N41Tfs*10	4	Infantile	Iran	Ghazi et al., 2017, Nephrologia
7	c.198_218del21 (•)	ITILELP67-73del	5	Juvenile	USA	Shotelersuk et al., 1998, Am J Hum Genet
8	c.206_210delTCCTT	I69Rfs*5	5	Infantile	USA	Shotelersuk, 1998, Am J Hum Genet
9	c.225+5_225+8delGTAA	Splicing			France	Kalatzis et al., 2002, Hum Mut
10	c.257_258delCT	S86Ffs*38			Iran	Ghazi et al., 2017, Nephrologia
11	c.260_261delTT	F87Sfs*37	6	Infantile	Egypt	Soliman et al., 2014, JIMD Rep
12	c.280delG	V94Lfs*24	6	Infantile	Germany, Switzerlands	Kiehntopf, 2002, Hum Mut
13	c.291_294delTACT	T98Ffs*19	6	Infantile	Turkey	Topaloglu et al., 2017, CJASN
14	c.295_310del16	V99Ifs*14	6	Infantile	Spain	Macias-Vidal, 2009, Clin Genet
15	c.314_317delACTC	H105Pfs*12	6	Infantile	USA	Shotelersuk, 1998, Am J Hum Genet
16	c.320_323delATCA	N107Rfs*10	6	Infantile	Spain	Macias-Vidal, 2009, Clin Genet
17	c.323delA	Q108Rfs*10	6	Infantile	Iran	Ghazi et al., 2017, Nephrologia
18	c.325_329del	Splicing	6	Infantile	Turkey	Doneray, 2017, Eurasian J Med
19	c.423delC	F142Sfs*5	7	Infantile	UK	Attard et al., 1999, Hum Mol Genet
20	c.492_515del24	FVALNLTGdel162-169	8	Infantile	Iran	Sadeghipour, 2017, Hum Genom Var
21	c.518_519delAC	Y173*	8	Infantile	Spain	Macias-Vidal, 2009, Clin Genet
22	c.519_520delCA	Y173*	8	Infantile	UK, France	Town et al., 1998, Nat Genet

Table 1. (continued)

Deletions						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
23	c.561+1delG	Splicing			UK, France	Town et al., 1998, Nat Genet
24	c.569_577delTTCTCCTCA	F190*	9	Infantile	UK, France	Town et al., 1998, Nat Genet
					Italy	Mason et al., 2003, Eur J Hum Genet
25	c.614_616delACG	D205del	9	Infantile	USA	Shotelersuk, 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
26	c.659_665delTCGTGCA	I220Sfs*31	9	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet
27	c.699_700delGT	S234Lfs*61	10	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet
28	c.741delC	F247Lfs*6	10	Juvenile	USA	Shotelersuk, 1998, Am J Hum Genet
29	c.770_792del23	G258Sfs*30	10	Infantile	UK	Attard et al., 1999, Hum Mol Genet
					Tunisia	Chkioua et al., 2015, Metagene
30	c.809_811delCTC	E270del	10		UK	Attard et al., 1999, Hum Mol Genet
					Egypt	Soliman et al., 2014, JIMD Rep
31	c.834_842del	279-281del	10	Infantile	Turkey	Topaloglu et al., 2017, CJASN
32	c.898-IVS8+24del27	Splicing			UK	Attard et al., 1999, Hum Mol Genet
33	c. 960del	Y321Tfs*8	11	Infantile	Turkey	Topaloglu et al., 2017, CJASN
33	c.992delG	G331Efs*36	12	Infantile	UK	Attard et al., 1999, Hum Mol Genet
34	c.1018_1041del24	VFSIVFDV340-347del	12	Infantile	Saudi Arabia	Aldahmesh et al., 2009, Ophthalm Genet
35	c.1027_1038del12	343-346del	12	Infantile	UK	Attard et al., 1999, Hum Mol Genet
					Italy	Mason et al., 2003, Eur J Hum Genet
36	c.1036_1047del12	344-347del	12	Infantile	France	Kalatzis et al., 2002, Hum Mut
37	>1.7kb del		1, 2	Infantile	Germany, Switzerland	Kiehntopf, 2002, Hum Mut
38	>4194bp del		1–3	Infantile	USA	Aradhya, 2012, Genet Med
39	~13 kb del		1–3	Infantile	UK, France	Town et al., 1998, Nat Genet
40					Italy	Mason et al., 2003, Eur J Hum Genet
41	~20 kb del		1–5	Infantile	Tunisia	Chkioua et al., 2015, metagene
42	~57 kb del (•)		1–10	Infantile	UK, France	Town et al., 1998, Nat Genet
					USA	Shotelersuk, 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
					USA	Anikster et al., 1999, Hum Mut
					Canada	McGowan et al., 1999, Eur J Hum Genet
					Netherlands	Heil et al., 2001, Nephron
					Germany, Switzerland	Kiehntopf, 2002, Hum Mut
					France	Kalatzis et al., 2002, Hum Mut
					Italy	Mason et al., 2003, Eur J Hum Genet
					France	Servais et al., 2008, CJASN
					Mexico	Alcantra-Ortigoza, 2008, Hum Genet
					Spain	Macias-Vidal, 2009, Clin Genet
					Netherlands	Wamelink et al., 2011, Mol Genet Metab
					Belgium	Besouw et al., 2012, Pediatr nephrol
					UK	Attard et al., 1999, Hum Mol Genet
					Netherlands	Heil et al., 2001, Nephron
					Mexico	Alcantra-Ortigoza, 2008, Hum Genet
43	~40 kb del		1–12	Infantile	Brazil	Villela et al., 2013, Genet Mol Biol
44	ex.4-5 del		4, 5	Infantile	USA	Anikster et al., 1999, Hum Mut
					Mexico	Alcantra-Ortigoza, 2008, Hum Genet
45	10 kb del, c.62-1083_551		4–8	Infantile	Turkey	Topaluglu et al., 2012, Pediatr Nephrol
Insertions						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
1	c.92insG	V31Gfs*29	4	Infantile	Iran	Ghazi et al., 2017, Nephrologia
2	c.152_153insCT	A52Lfs*5	5	Infantile	Iran	Shahkarami, 2013, Nephrologia
3	c.292dupA	T98Nfs*27	6	Infantile	Germany, Switzerland	Kiehntopf, 2002, Hum Mut
4	c.346_347insACTTC	L116Hfs*4	7	Infantile	Germany, Switzerland	Kiehntopf, 2002, Hum Mut
5	c.516dupC	Y173Lfs*55	8	Infantile	Italy	Mason et al., 2003, Eur J Hum Genet

Table 1. (continued)

Insertions						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
6	c.577_578insA	N196Kfs*32	9	Infantile	Greece	Bitsori et al., 2018, CEN Case rep
7	c.646dupA	T216Nfs*12	9	Juvenile	USA	Shotelersuk, 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
					Mexico	Alcantra-Ortigoza, 2008, Hum Genet
					France	Servais et al., 2008, CJASN
					Spain	Macias-Vidal, 2009, Clin Genet
8	c.661insT	Q222Afs*6	9	Infantile	Iran	Ghazi et al., 2017, Nephrologia
9	c.696_697dupCG	V233Rfs*21	10	Infantile	USA	Shotelersuk, 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
10	c.696dupC	V233Rfs*63	10	Infantile	USA	Shotelersuk, 1998, Am J Hum Genet
					UK	Attard et al., 1999, Hum Mol Genet
					Canada	McGowan, 1999, Eur J Hum Genet
					Italy	Mason et al., 2003, Eur J Hum Genet
11	c.829dupA	T277Nfs*19	10	Infantile	Belgium	Besouw et al., 2012, Pediatr nephrol
					Egypt	Soliman et al., 2014, JIMD Rep
					Turkey	Topaloglu et al., 2017, CJASN
12	c.926dupG	S310Qfs*55	11	Infantile	USA	Shotelersuk, 1998, Am J Hum Genet
					Netherlands	Heil et al., 2001, Nephron
13	c.1047_1048ins12	DVEF349-350ins	12	Infantile	France	Kalatzis et al., 2002, Hum Mut
Indels						
No.	mutation	protein	exon	phenotype	country of diagnosis	references
1	c.225+5_225+6delGTinsCC	Splicing		Infantile	USA	Anikster et al., 1999, Hum Mut
2	c.751_754delACCAinsCG	T251Rfs*44	10	Juvenile	Mexico	Alcantra-Ortigoza, 2008, Hum Genet
3	c.1028_1035delTCGTCTTCinsA	I343Kfs*22	12	Infantile	USA	Shotelersuk et al., 1998, Am J Hum Genet
4	c.1032delCinsTG	F345Vfs*20	12	Infantile	Egypt	Soliman et al., 2014, JIMD Rep

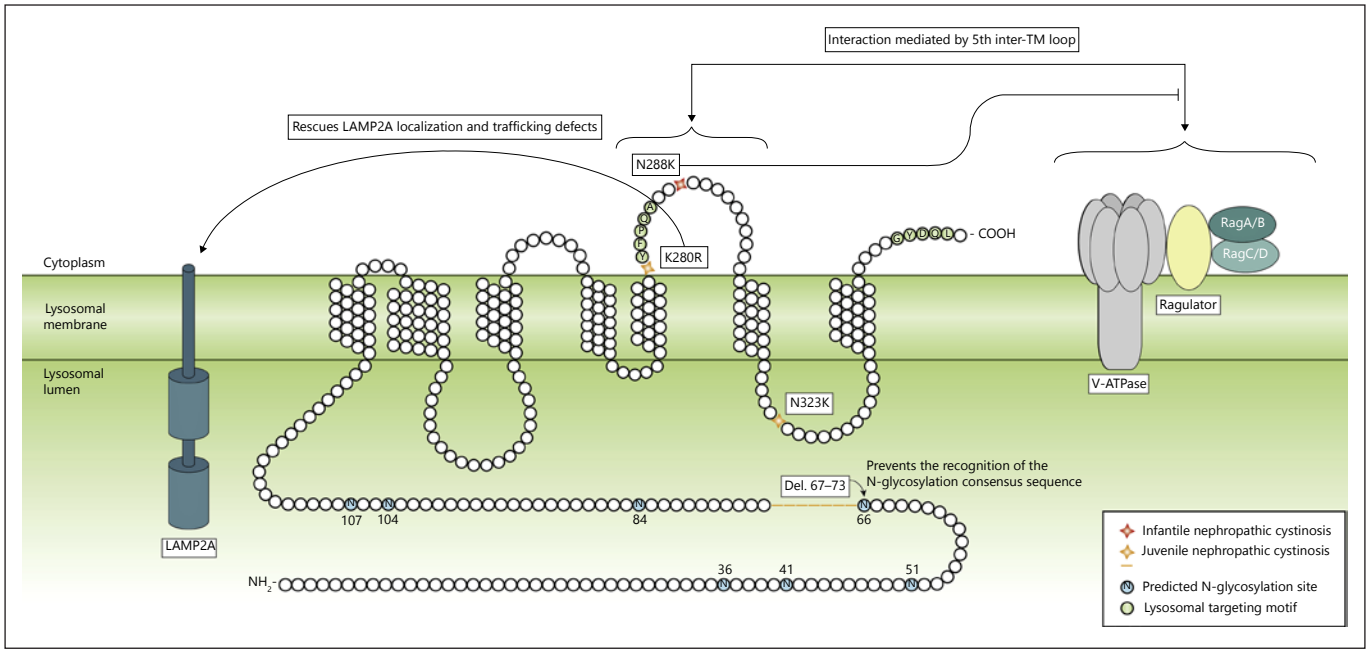
Table is updated up to July 2018 and adapted from citation [15].
(•), mutations cited in the text; * data based on public HGMD database.

deletion (57-kb del) [12] affecting the promoter region and the first 10 exons of the *CTNS* gene together with 2 upstream genes (*CARKL* and *TRPV1*) [25]. This deletion represents over 50% of mutant alleles in cystinosis patients of Northern European ancestry; however, it is completely absent in all reported patients from the Middle East, Asia and Africa [16], suggesting a founder mutation. Other important founder mutations can be encountered in certain populations or certain geographic loci, such as the c.1015G>A in the Amish population in Western Ontario, Canada [26], the c.971–12G>A in the black population of South Africa [23] and the c.681G>A in the Middle East [17–19]. A more detailed description of the genetic map of cystinosis is needed to elucidate the similarities and the differences between patients all over the world and to prepare for individualized disease treatment targeting gene repair.

Functional Characteristics of CTNS Mutations: Genotype-Phenotype Correlations

CTNS is a lysosomal 7-transmembrane protein (Fig. 1), which functions as H^+-driven cystine transporter [2]. It is predicted to have 7 N-glycosylation sites located at the N-terminal tail, residing in the lysosomal lumen. Two lysosomal targeting motifs, YFPQA (5th inter-transmembrane loop) and GYDQL (C-terminal tail), ensure localization to the lysosome [27]. An alternative splice variant termed CTNS-LKG exists, resulting in a longer protein (400 vs. 367 amino acids) with a plasma membrane targeting signal SSLKG replacing the C-terminal GYDQL [28, 29].

Kalatzis et al. [30] have studied the functional consequences of 31 *CTNS* mutations using constructs that encoded CTNS lacking the GYDQL lysosomal targeting signal, termed CTNS-ΔGYDQL. This truncation leads to a



Color version available online

Fig. 1. Structure of cystinosin with indication of *CTNS* mutations having an effect on LAMP2A localization [36, 37], interaction with V-ATPase-Ragulator-Rag complex and glycosylation status [33].

translocation of the cystinosin protein to the plasma membrane, thus enabling the monitoring of extracellular [^{35}S]-cystine influx. In particular, they measured the transport activity compared with WT CTNS in a COS-7 cell transfection model. Surprisingly, it was found that specific CTNS mutations (e.g., CTNS^{S298N} and CTNS^{W182R}) causing infantile nephropathic cystinosis, retained cystine transport activity. Conversely, mutations (e.g., CTNS^{N323K} and CTNS^{K280R}) resulting in juvenile nephropathic cystinosis, lost cystine transport activity [30]. These findings opposed the well-accepted paradigm that cystine accumulation is the primary pathogenic cause of cystinosis and suggested that CTNS exerts other key functions beyond cystine transport [2, 30]. Supporting these findings, cysteamine treatment does not alleviate the complete phenotype of cystinosis.

Indeed, other CTNS functions are being investigated, involving cell-survival pathways and the mammalian target of rapamycin complex 1 (mTORC1) activity. mTORC1 is the master regulator of cell growth, which is recruited to the lysosomal membrane in presence of nutrients, activating protein translation and inhibiting autophagy. Conversely, upon starvation, mTORC1 released from the lysosomal surface is inactivated, leading to the upregulation of autophagy and inhibition of the main anabolic pathways [31, 32]. Recently, CTNS was found to be part of the V-ATPase-Ragulator-Rag complex, which controls mTORC1 activation [33]. Both mutations CTNS^{N323K} and CTNS^{K280R} did not show any alteration in the interaction of mTORC1 with cystinosin. However, this was not true for CTNS^{N288K}, which specifically resulted in infantile nephropathic cystinosis with loss of cystine transport activity [33] (Table 2). Moreover, mouse *Ctns*$^{-/-}$ proximal tubular cells showed disturbed mTORC1 signalling and delayed docking of mTORC1 to the lysosomal surface following the re-introduction of the complete medium after starvation [5]. In addition, human cystinotic proximal tubular cells carrying the 57 kb deletion and a compound heterozygous (c.Y173X+p.G339R) showed abnormal lysosomal mTORC1 localization upon starvation, and cysteamine supplementation did not rescue the phenotype [34].

When mTORC1 is recruited to the lysosomal membrane, it also phosphorylates and inhibits transcription factor EB (TFEB). TFEB normally activates lysosomal biogenesis and autophagy-related genes, in response to starvation [32]. Human *CTNS*$^{-/-}$ proximal tubular epithelial cells (PTEC) showed lower TFEB expression, which was mainly located in the nucleus. Overexpressing TFEB in *CTNS*$^{-/-}$ PTEC rescued some of the CTNS WT characteristic such as (i) delayed lysosomal cargo processing, (ii) improved morphological irregularities of the

Table 2. *CTNS* mutations and their corresponding phenotype, cystine transport activity, location and other studied effects

CTNS mutation	Phenotype [5]	Cystine transport activity compared to WT CTNS [33]	Location	Other studied effects
N288K	Infantile	No activity	5th inter-TM loop	– Abolished interaction with V-ATPase-Ragulator-Rag complex [32] – Tendency towards increased degradation compared to WT CTNS [41] – No ER retention [41]
Y173X + G339A	Infantile	Severely inhibited (<30% of WT, homozygous G339A)	2nd TM domain + 7th TM domain	– Delayed reactivation of mTORC1 after refeeding [36] – Partial retention of mTORC1 to LE/LY after starvation [36]
K280R	Juvenile	No activity	5th inter-TM loop	– Retains interaction with V-ATPase-Ragulator-Rag complex [32] – Rescues LAMP2A localization and LAMP2A trafficking defects [38] – No ER retention [41]
N323K	Juvenile	No activity	6th inter-TM loop	– Retains interaction with V-ATPase-Ragulator-Rag complex [32] – Tendency towards increased degradation compared to WT CTNS [41]
Del. 67-73	Juvenile	Severely inhibited (<30% of WT)	N-terminal tail	– Loss of 1 consensus glycosylation site [41] – Degraded 3x faster than WT CTNS [41] – ER retention + Co-IP with calnexin [41] – Immature glycosylation [41] – Retains interaction with V-ATPase complex [41]

WT, wild type; LE/LY, late endosome/lysosome; ER, endoplasmic reticulum; TM, transmembrane.

lysosomal compartments (after >24 h) and (iii) promoted clearance of lysosomal cystine by exocytosis [35]. It would be interesting to investigate whether overexpressing (mutant and WT) CTNS in *CTNS*$^{-/-}$ PTEC could rescue this defect, as this could further confirm the altered mTORC1 signalling shown in *Ctns*$^{-/-}$ proximal tubular cells.

Another role of CTNS, in addition to its potential involvement in mTORC1 signalling, is the altered chaperone mediated autophagy, as shown by two studies from Napolitano et al. [37] and Zhang et al. [36]. They observed impaired chaperone mediated autophagy (CMA) in cystinotic mouse skin fibroblasts presenting dislocation of LAMP2A (CMA receptor) and accumulation of GAPDH (CMA substrate). Interestingly, the CTNS^{K280R} mutation rescued the localization of LAMP2A in contrast to cysteamine treatment. This was also true for CTNS-LKG, which is an alternative splice variant of CTNS, primarily targeted to the plasma membrane and other lysosomal/endosomal vesicles. Based on these results, it was suggested that CTNS is a necessary cofactor for LAMP2A trafficking [36, 37].

The lack of cystine transport activity of the CTNS^{K280R} and CTNS^{N288K} mutants may be explained by the fact that both mutations are located at the 5th inter-transmembrane loop containing the PQ motif, which is required for H^+ and cystine co-transport [38]. The difference in clinical presentation, juvenile versus infantile cystinosis for CTNS^{K280R} and CTNS^{N288K}, respectively, can thus be only explained by effects beyond cystine transport.

As mentioned earlier, CTNS is predicted to have 7 N-glycosylation sites at the N-terminal tail (Fig. 1). CTNS^{N323K} and CTNS^{N288K} do not affect glycosylation of CTNS. However, a deletion of 7 amino acids at the N-terminus, referred to as CTNS$^{Del.67–73}$ (juvenile nephropathic cystinosis with severely inhibited cystine transport), results in the loss of 1 consensus N-glycosylation site. Furthermore, this mutant protein degraded threefold faster than WT CTNS and was partially retained at the endoplasmic reticulum. Nevertheless, CTNS$^{Del.67–73}$ still interacts with the V-ATPase complex, which could explain the less severe phenotype caused by this mutation [39].

Animal Models of Cystinosis

Mouse

Sixteen years ago, Antignac's group generated the first model of cystinosis in FVB/N mice by using promoter trap approach to eliminate the *CTNS* gene [40]. Although the model showed ocular abnormalities, bone defects, behavioural anomalies and partial response to cysteamine treatment, it presented a moderate cystine accumulation compared to the affected humans without any sign of morphological or functional alterations of the proximal tubule. As the genetic background can influence the renal phenotype, the same group developed a second model in C57BL/6 mouse, and observed mild proximal tubulopathy (without the urinary loss of amino acids, bicarbonates or sodium) and focal tubular lesions. However, no podocyte damage was present [41]. The latter mouse model has been widely used by different research groups for studying the pathogenesis and treatment strategies in cystinosis [42, 43].

Yeast

Saccharomyces cerevisiae is a well-established model organism, mainly used for genetic manipulations and biochemical analyses. *ERS1*, the yeast orthologous counterpart of the human *CTNS* gene, encodes for the Ers1 protein (28% identical/46% similar to human CTNS), which localizes to the endosomes and the vacuole. *ers1Δ* mutant shows hygromycin B (hygB) sensitivity, which could be reversed only by the complementation of a functional *CTNS* human gene [44] or by overexpressing *MEH1*, which encodes a protein involved in vacuolar acidification and general amino acid permease (Gap1p) localization [44, 45]. Interestingly, Simpkins et al. [46] showed that Ers1 acts as a cystine transporter; however, the *ers1Δ* mutant does not show any detectable defect in growth and has no cystine accumulation [44], thereby supporting the evidence that other genes could compensate for its lost function [46]. Another relevant study performed in yeast confirmed that the GYQDL signature at the C-terminal end is essential for the trafficking of cystinosin; indeed, deleting the GYQDL C-terminal region of *CTNS* expressed in yeast delocalized cystinosin from the vacuole to the plasma membrane, restoring the ability to grow on cystine [47]. As the developed CTNS-ΔGYDQL transformants showed low cystine uptake, vacuolar protein-sorting deletions have been developed to increase the protein concentration at the plasma membrane, from which 2 deletions stand out: *vsp1Δ, vsp17Δ* enhancing the uptake levels. Several gain-of-function mutants were isolated, including 1 patient mutation, G197R. These data demonstrate that cystine-uptake assay in yeast cells is useful to decipher the functionality of mutant cystinosin proteins.

Zebrafish

Zebrafish is the most recent model organism to study cystinosis. Carrying the homozygous nonsense mutation in exon 8 of the *ctns*, zebrafish larvae demonstrated early cystine accumulation, enhanced deformity, apoptosis and increased mortality, which are partially responsive to cysteamine treatment [48]. Furthermore, the model is characterized by impaired glomerular permselectivity and defective tubular reabsorption. Unlike the murine model, the adult *ctns*$^{-/-}$ zebrafish kidney accumulates the highest concentration of cystine. In line with functional abnormalities, increased lysosomal size and numbers characterize larvae's PTEC, while podocytes present partial foot process effacement and narrowed slit diaphragmatic space. This last phenotype is lacking in the mouse model, but is present in humans. The abundance and localization of the megalin receptor, is altered in *ctns*$^{-/-}$ larvae compared to WT, a marker for defective tubular reabsorption, which is not restored by cysteamine treatment. Hence, the zebrafish larval model closely copies the human kidney phenotype for cystinosis and is superior to all model organisms currently available. Therefore, it will be useful to further unravel the pathophysiological aspects of cystinosis and for the in vivo screening of novel therapeutic agents [48].

A comparison of cystinosis in humans, mice, yeast and zebrafish is presented in Table 3 (adapted from [48]).

Future Perspective: Potential for Gene Repair

To date, cysteamine is the only drug available to treat cystinosis. Although cysteamine can alleviate symptoms and delay disease progression, it does not prevent the Fanconi syndrome and renal transplantation is still necessary. In addition, compliance to cysteamine is low and side effects are frequently observed [49]. Thus, there is a need for novel therapies, which not only treat, but could potentially cure cystinosis.

Since cystinosis is a multi-systemic disease, developing gene therapy is challenging as all body cells require correction. In line with the strategy applied for other lysosomal storage disorders, Syres and colleagues transplanted bone marrow-derived cells and hematopoietic stem cells (HSC) from *Ctns*$^{+/+}$ to *Ctns*$^{-/-}$ mice [50]. The

Table 3. Comparison of cystinosis in human, mouse, yeast and zebrafish

	Human	Mouse		Yeast	Zebrafish
Gene	*CTNS*	*Ctns*		*ERS1*	*ctns*
Chromosome	17	11		3	11
Exons	12 (first 2 non-coding)	12 (first 2 non-coding)		1	10 (all coding)
Ensembl gene code	ENSG00000040531	ENSMUSG00000005949		YCR075C	ENSDARG00000008890
Protein	Cystinosin	Cystinosin		Cystine transporter	Ctns
AA	367	367		260	384
UniProt code	O60931	P57757		P17261	F1QM07
		FVB/N mouse	C57BL/6 mouse		
Developmental delay					
Intrauterine	No	No	No	Not applicable	Not applicable
Infancy, childhood	Yes	No	Yes	Not applicable	Yes (early embryonic)
Embryonic mortality	Not reported	Not reported	Not reported	Not applicable	Increased
Cystine accumulations	Yes	Yes	Yes	No	Yes
Renal dysfunction	6–12 months	No	2 months	Not applicable	3–6 dpf
Renal failure	5–10 years	No	10 months	Not applicable	Not investigated
Histopathology					
PTEC changes	6–12 months	No	6 months	Not applicable	3–6 dpf
Podocyte changes	2–5 years	No	No	Not applicable	3–6 dpf

dpf, days post fertilization.

rationale underlying HSC therapy is that healthy donor cells migrate into the recipient's organs and release the missing protein locally, thereby correcting the metabolic defect [51]. However, for cystinosis it was still a question whether a transmembrane protein like CTNS could be taken up by the diseased cells [50]. In mice, bone marrow-derived cells and HSC-derived cells engrafted efficiently in the interstitial compartments of the kidney and other organs, decreased cystine accumulation and averted development of kidney dysfunction [50]. Moreover, if sufficient donor-derived blood cell engraftment took place (>50% of blood cells), the cystinotic phenotype was corrected up till 7–15 months post transplantation [52]. All together these findings provide a proof-of-principle that cystinotic mice can be treated by HSC transplantation. Elmonem et al. [53] reported the first human case which underwent allogenic HSC transplantation from a full HLA-matched unrelated donor. Although mRNA and cystinosin protein transfer from HSC to epithelial cells did occur and was able to reduce cystine crystal load, the patient still developed ESKD and died from severe graft-versus-host disease, as a complication of transplantation. Because of the inherent risks associated with allogenic transplantation, autologous *Ctns*$^{-/-}$ HSC transplantation of HSC corrected with a lentiviral vector encoding a functional copy of *CTNS* were evaluated in mice. This approach reduced cystine content in all tissues and improved kidney function in a mouse model of cystinosis [54]. In addition, cross correction of diseased cells was shown to be mediated by tunnelling nanotubes produced by the gene-corrected macrophages [54]. Both in vitro and in vivo, these tunnelling nanotubes provided diseased cells with functional CTNS bearing lysosomes. The transfer was found to be bi-directional as cystine loaded lysosomes from cystinotic cells were transferred to the macrophage as well [55].

With several gene therapeutic approaches reaching the market in the past years, gene therapy also holds promise to cure cystinosis. In line with this, the company Avrobio has planned to start Phase 1/2 human trials in 2019 (http://www.avrobio.com/pipeline/). However, the importance of *CTNS* expression levels should be noted as *CTNS* is regulated both at the transcriptional and the posttranscriptional level [56, 57]. The promoter driving *CTNS* expression in lentiviral constructs should be evaluated carefully as overexpression of CTNS has been reported to enlarge lysosomal structures of which the long-term consequence is currently unknown [27, 54]. Despite the promising results of gene therapy for other lysosomal

storage disorders [58] long-term safety and efficacy of such therapy still require to be studied in more detail before it becomes a realistic option as a first-line treatment for patients affected by lysosomal storage disorders.

Due to the risks associated with HSC transplantation, especially in young children, researchers set out to find novel gene therapeutic approaches. Arcolino et al. [59] showed that urine of preterm neonates contained kidney stem/progenitor cells that had regenerative paracrine effect and could differentiate into podocytes and proximal tubule epithelial cells. Therefore, we hypothesize that, ex vivo gene-corrected autologous cystinosis kidney progenitors could also be used as source for cell therapy that could potentially cure the kidney phenotype. As cystinosis is a multisystemic disease, this therapy should be combined with drug therapy protecting extra-renal organs.

Conclusion

Worldwide pathogenic *CTNS* mutations have been reported in cystinosis patients, but still, well-documented incidence and geographical distribution has been poorly described. Analysis of mutant cystinosin has provided the research field with a new scientific consensus in which CTNS has other critical functions beyond cystine transport. However, the extent to which defects in these other functions contribute to the overall clinical phenotype still needs further investigation. Preclinical studies using different animal models might elucidate functional consequences of specific mutations and might pave the way for a more personalized treatment depending on the correlations between genotype and phenotype.

The results of treatment with cysteamine vary widely and studies showed that cysteamine improves the outcome of cystinosis but does not provide cure. Hence, there is a need to find alternative therapeutic strategies. Over the last decades, cystinosis evolved from a paediatric lethal to a treatable disorder, with the advent of gene- and cell therapy, cystinosis might even become curable.

Ethics Statement

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Authors Contribution

D.D., S.P.B., M.A.E., F.O.A., N.S., B.V.H., R.G., and E.L. searched the literature and wrote the review text. D.D., S.P.B., and M.A.E. prepared the figure and tables.

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