Original Research

Outcome of infantile nephropathic cystinosis depends on early intervention, not genotype: a multicenter sibling cohort study

The Cystinosis sibling study

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ABSTRACT

Background Infantile Nephropathic Cystinosis (INC) is an inheritable lysosomal storage disorder characterized by lysosomal cystine accumulation, progressive kidney disease and multiple extra-renal complications (ERC). Cysteamine postpones the onset of end-stage kidney disease (ESKD) and reduces the incidence of ERCs, however, cysteamine is generally initiated upon establishment of the renal Fanconi Syndrome (FS) and partial loss of kidney function, whereas data on long-term effects of cysteamine administered from neonatal age are lacking.

Materials & Methods An international multicenter retrospective cohort study of siblings with INC was set up to investigate the outcome in relation to age at initiation of cysteamine versus *CTNS* genotype, with attention to patients treated with cysteamine from neonatal age.

Results None of the siblings treated from neonatal age (n=9; age 10 $\pm$ 6 years) had reached ESKD, while 22% of their index counterparts (n=9; age 14 $\pm$ 5 years) had commenced renal replacement therapy. Siblings treated with cysteamine from the onset of symptoms at a younger age compared to their index counterparts, reached ESKD at a significant older age (13 $\pm$ 3 years vs. 10 $\pm$ 3 years, p = 0.002). In contrast, no significant difference in ERCs was observed between sibling and index patients, independently from the age at initiation of cysteamine. The *CTNS* genotype had no impact on the overall outcome in this cohort.

Conclusions In INC, presymptomatic treatment with cysteamine results in a better renal outcome in comparison to treatment initiated from the onset of symptoms. This justifies including cystinosis into newborn screening programs.

SYNOPSIS

In infantile nephropathic cystinosis, presymptomatic treatment with cysteamine improves the renal outcome which justifies the inclusion of cystinosis into newborn screening programs.

AUTHOR'S CONTRIBUTIONS

Conceptualization: KV, EL; Methodology: KV, WZ; Software: KV, WZ; Validation: KV, EL; Formal analysis: KV, WZ; Investigation: KV, WZ; Resources: KH, DB, MJ, PN, AS, RT, MB, RN, DH, NK, LP, EW, EH, AA, PS, GA, EL; Data curation: KV, WZ; Writing-Original Draft: KV, WZ, EL; Writing -Review & Editing: KH, DB, MJ, PN, AS, RT, MB, RN, DH, NK, LP, EW, EH, AA, PS, GA, BvdH, EL; Visualization: KV, WZ; Supervision: BvdH, EL; Project administration: KV, WZ; Funding acquisition: BvdH, EL.

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DISCLOSURES – COMPETING INTEREST STATEMENT

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KV, WZ, DB, MJ, PN, DH, LP, EW, GA, BvdHn FE, AS and EL are working in reference centers for rare kidney diseases (ERKNet). Patients recruited in this cohort study were also included in the international cystinosis cohort study described by Emma *et al*.[15]

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DETAILS OF ETHICAL APPROVAL

This study was approved by the ethical board of the coordinating center UZ/KU Leuven (Ethische Commissie Onderzoek UZ/KU Leuven; study s60970) and of other collaborating centers depending on the requirements of the local authorities. Research was conducted in accordance with the last version of the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) and all applicable national and international legislation related to research involving human subjects.

PATIENT CONSENT STATEMENT

Informed consents were signed by recruited patients or their legal guardians.

DATA SHARING STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

KEYWORDS

cystinosis; siblings; outcome; genotype; newborn screening

INTRODUCTION

Infantile nephropathic cystinosis (INC) (OMIM: #219800) is a rare autosomal recessive lysosomal storage disorder, caused by bi-allelic mutations in the *CTNS* gene leading to the absence or malfunction of the cystine-proton cotransporter *cystinosin* and consecutive lysosomal accumulation of cystine, the disease's hallmark.[1,2] Infants with INC present with a generalized proximal tubular dysfunction (renal Fanconi syndrome), followed by progressive chronic kidney disease (CKD) resulting in end-stage kidney disease (ESKD).[3] The renal Fanconi syndrome (FS) is absent at birth and gradually develops during the first 6 months of life, reflecting the progressive atrophy of kidney proximal tubules.[4,5] When renal FS becomes fully established, patients become symptomatic and present with the first clinical symptoms of failure to thrive, polyuria and polydipsia, episodes of dehydration, or rickets, usually between 6 to 12 months of age. In addition, several other extra-renal manifestations develop from childhood onwards, mainly affecting the eye, the endocrine, neuromuscular, and the central nervous system.[6]

Cysteamine, a cystine-depleting drug, is currently the only available disease modifying treatment. The effectiveness and adherence to this treatment is monitored via white blood cell (WBC) cystine measurements assuming that WBCs reflect cystine accumulation in other tissues. In several large cohort studies, it has been established that cysteamine postpones the onset of ESKD, reduces the incidence of extra-renal complications (ERC), improves growth, and increases life-expectancy.[7–13] In addition, the age at introduction of cysteamine and appropriate adherence, have been associated with improved renal and extra-renal outcome.[11,12,14,15] Unfortunately, cysteamine cannot reverse the renal FS, which requires excessive supplementation of electrolytes, water, and other substances lost by the affected kidney proximal

tubules. Intriguingly, a few case reports have suggested that cysteamine might attenuate the development of renal FS when administered very early in life,[16,17] however, no long-term systematic study evaluating patients who started cysteamine from neonatal age has been performed so far. To what extent the cystinosis genotype affects the outcome on top of the age at start of cysteamine, also remains to be clarified. Previous cohort-based studies have presented contrasting results: while in the INC cohort of NIH described by Gahl *et al*. patients harboring the 57kb deletion show a higher risk for developing ERCs, no significant differences in outcome have been shown in the French cohort described by Brodin-Sartorius *et al*., despite similar age and adherence to cysteamine treatment in the groups of comparison.[9,12] Also, in a Turkish cohort, no significant differences in renal outcome have been shown in patients with a mild versus severe cystinosis genotype.[13,15]

Furthermore, while the technology for newborn screening (NBS), based on next generation sequencing (NGS), for diseases that can benefit from treatment at the presymptomatic stage is emerging in different laboratories all over the world,[18] it remains to be established whether INC should be included in NBS programs. Therefore, in this sibling study, we additionally aimed to focus on the outcome of INC patients who were initiated on cysteamine at neonatal age, following diagnosis by genetic testing or WBC cystine assay, due to the presence of an older affected sibling in the family.

MATERIALS and METHODS

Study design and population

An international multicenter retrospective cohort study was set up in collaboration with European cystinosis reference centers, and data was collected from July 2017 until April 2019.

Each pair of an index patient and sibling originating from the same family was referred to as a 'sibling versus index pair'. The first patient known to be affected by cystinosis in the family and initiated on cysteamine treatment was referred to as the 'index', while the second patient that was diagnosed with cystinosis within the same family was identified as the 'sibling' (Figure 1).

The siblings diagnosed with INC *in utero*, or during the first month of life, before any clinical signs or symptoms of the disease were present, were assigned as 'presymptomatic siblings', and together with their corresponding index patients, were referred to as the 'presymptomatic sibling vs. index pairs' (Figure 1). All the other siblings, who were diagnosed following the development of signs and symptoms of cystinosis, were referred to as 'symptomatic siblings', and together with their corresponding index patients, referred to as 'symptomatic sibling vs. index pairs' (Figure 1).

The following data were extracted from the medical records: date of birth, sex, date of last observation, cystinosis genotype, date at onset of symptoms, date at diagnosis, date at initiation of cysteamine, estimated glomerular filtration rate (eGFR) at diagnosis, final adult height, date at ESKD, date at kidney transplantation (KTx), WBC cystine levels (nmol ½ cystine/mg protein, yearly if available), presence of ERCs at last follow-up visit, date of diagnosis of extra-renal complication. $eGFR_{cr}$ was calculated using the revised Schwartz formula or CKD-EPI, depending on the age

(Table 1, 2).[19] Lifetime WBC cystine was determined as the average of all the WBC cystine values available over a patient's lifetime. The cystinosis genotype was assessed as either being the homozygous 57kb deletion versus other pathogenic variants as described by Emma *et al*.[15] According to this definition, patients with at least one allele with a missense pathogenic variant, intronic variant or in-frame deletion, were defined as having a theoretically moderate pathogenic variant; while all other patients were defined as having severe pathogenic variants (Table 2). [15] Hence, according to the cystinosis genotype, two comparative subgroups were designed: patients harboring the homozygous 57kb deletion versus other pathogenic variants, and patients harboring moderate versus severe pathogenic variants.

In order to quantify the severity of multi-systemic involvement, a 12-item composite score of ERCs was modified from Gahl *et al*..[9] The diagnostic criteria defining these complications are described in Supplementary Table S1.

Ethical approval

This study was approved by the ethical board of the coordinating center UZ/KU Leuven (Ethische Commissie Onderzoek UZ/KU Leuven; study s60970) and of other collaborating centers depending on the requirements of the local authorities. Informed consents were signed by recruited patients or their legal guardians. Research was conducted in accordance with the last version of the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) and all applicable national and international legislation related to research involving human subjects.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 9.1.2) and SAS for Windows (version 9.4). Distribution of the data was assessed, and parametric, or non-parametric tests (paired: Wilcoxon test; non-paired: Mann-Whitney) were applied accordingly. Gaussian distributed data were presented using the standard deviation (SD) and 95% confidence interval (95% CI), non-Gaussian distributed data by the median and interquartile range (IQR). Comparison of categorical data was reported via the Odds Ratio (OR) with the 95% CI, while for paired non-Gaussian distributed categorical data, a Wilcoxon signed rank test was applied.

The evolution of eGFR and the development of primary hypothyroidism in index vs. sibling patients, and the effect of the cystinosis genotype and age at initiation of cysteamine treatment on the age at ESKD, were assessed via rightward censoring of the data in a retrospective time to event analysis. In the subgroup analysis studying the cystinosis genotype, only siblings with a known genotype were included.

A univariate and multivariate linear regression analysis using linear mixed models was applied to define the significant predictors for the number of ERCs. A random effect for family was modelled to account for clustering of patients within families. Results are reported as slope (for continuous predictors) or mean difference (for categorical predictors) with 95% CI. The Mann-Whitney U-test was used to study the association between continuous predictors with genetic background.

RESULTS

Patient demographics

Patients were recruited from thirteen European cystinosis reference centers (Supplementary Table S2), yielding a total of 52 patients originating from 26 pairs of index and corresponding sibling patients. Pairs in which the index patient and sibling patient were twins, were excluded. Also, in case of a triplet of cystinosis patients within same family (one index patient with two affected siblings with cystinosis), the youngest sibling was excluded from analysis.

In three siblings diagnosed with INC *in utero* and in six siblings diagnosed during the first month of life, cysteamine treatment was initiated at neonatal age (presymptomatic siblings). All other siblings were diagnosed due to the development of signs and symptoms of cystinosis, (symptomatic siblings), even when the disease was already known in an older child of the same family. In 42 patients (21 pairs), the cystinosis genotype was known. Importantly, longitudinal data (at least 2 values on different timepoints) of the WBC cystine values were only available in 32 of the 52 patients (only 1 WBC cystine value in 12 patients and no available values in 8 patients).

1. Siblings diagnosed with cystinosis begin cysteamine therapy at a younger age

Presymptomatic siblings started on cysteamine treatment at the median age of 0.95 months (IQR: 0.2; 1.4), while their index counterparts initiated cysteamine at the median age of 22 months (IQR: 16; 28; $p = 0.004$) (Table 1).

Symptomatic siblings were diagnosed at a significant earlier age compared to their index counterparts with a median age of 10 months (IQR: 6; 17 months) versus 22 months (IQR: 18; 38 months) ($p < 0.0001$) (Table 1). Consequently, cysteamine

treatment was initiated earlier in symptomatic siblings compared to their index counterparts (median 12 months (IQR: 8; 31) versus 41 months (IQR: 21; 75); $p < 0.0001$).

2. Siblings show a slower progression to ESKD, while initiation of cysteamine treatment at neonatal age prevents ESKD until adulthood

None of the presymptomatic siblings had reached ESKD yet, with the oldest presymptomatic sibling being almost 20 years of age (Table 1; Figure 2B), while symptomatic siblings reached ESKD at the median age of 15 (Table 1; Figure 2A).

Noteworthy, the average WBC cystine levels during the patient lifetime were not different between siblings and index patients, suggesting that compliance was similar (1.43 in index patients versus 1.04 nmol ½ cystine/mg protein in siblings, $p = 0.33$), however longitudinal WBC cystine data was not available in 20 of 52 patients (Table 1). Symptomatic siblings demonstrated a significant ($p = 0.004$) slower progression towards ESKD compared to their index counterparts (average age at ESKD: 13 ± 3 years versus 10 ± 3 years; $p = 0.002$) (Table 1, Figure 2A).

3. Siblings display a similar incidence of extra-renal complications independently of age at start of cysteamine treatment

In this INC sibling cohort, siblings did not show a significant different number of ERCs compared with their index counterparts (Table 1; Figure 3). For primary hypothyroidism, the most common extra-renal manifestation, no differences were observed in the age at diagnosis between sibling and index patient in both the symptomatic (Figure 3A) and presymptomatic (Figure 3B) groups. In addition, none of

the other ERCs occurred at a significant different age in sibling vs. index pairs (Table S3).

4. In INC, the cystinosis genotype has no significant impact on the severity of the renal or extra-renal phenotype

In a time to event analysis, the age at ESKD did not show a significant difference between patients from sibling vs. index pairs harboring a homozygous 57kb deletion (n=16 patients), compared with patients from sibling vs. index pairs harboring any other pathogenic variant (n=26 patients) (Log-rank Mantel-Cox, p = 0.72) (Figure 4A, Table 3).

Using a univariate regression analysis, we confirmed the known effect of the patient's age, age at initiation of cysteamine and average lifetime WBC cystine on the extra-renal phenotype (Table 4). In this univariate analysis, the cystinosis genotype, in terms of hom 57kb del vs. other pathogenic variants, showed to be significantly associated with the extra-renal phenotype, which explains the significant higher number of ERCs in the hom 57kb del group versus other pathogenic variants (Table 4, Figure 4B). However, importantly, when correcting for the age of the patient at last observation using a multivariate analysis, the genotype was no longer significantly associated with the extra-renal outcome (Table 4).

5. Age at initiation of cysteamine treatment is only a major determinant for the renal but not for the extra-renal outcome in cystinosis siblings

In a time to event analysis, we demonstrated that initiating cysteamine treatment before the age of 10 months, is associated with an older age at attainment of ESKD (Log-rank Mantel-Cox, p = 0.002) (Figure 4C). Remarkably, at present, ESKD has

occurred only in the minority of the patients in whom cysteamine treatment was initiated before the age of 10 months (Figure 4C).

While the number of extra-renal complications was significantly lower (Mann Whitney, $p = 0.03$) (Figure 4D) in patients in whom cysteamine was initiated below the age of 10 months, these patients were however significantly younger in comparison to patients in whom cysteamine was initiated from the age of 10 months (13 $\pm$ 8 years vs. 25 $\pm$ 10 years of age, $p = 0.0001$). Indeed, in the multivariate regression analysis (Table 4), we confirmed that in this cystinosis cohort, the genotype was not a significant predictor for the number of extra-renal complications. Of note, more than half of the patients in whom cysteamine was started before the age of 10 months, were presymptomatic siblings (9/16, 56%).

DISCUSSION

In this study, we aimed to investigate the effect of initiation of cysteamine at neonatal age on the renal and extra-renal outcome in INC, by studying a unique cohort of pairs of cystinosis siblings and their corresponding index counterparts.

While previous large cohort studies have demonstrated that cystine-depleting therapy delays the progression of CKD and reduces the number of extra-renal complications, it remains unclear to what extent the cystinosis genotype is a factor herein, in contrast to timely initiation of cysteamine therapy.[9,12] As affected siblings harbor an identical genotype and are exposed to similar environmental factors, sibling studies serve as the ideal method to investigate effects related to the genotype.

The most striking and important finding of our study is that none of the *in utero* or neonatally diagnosed siblings have reached ESKD yet, with the oldest sibling reaching almost 20 years of age. These encouraging results add up to the early favorable outcome reported by Hohenfellner *et al*. in one 16-month old toddler treated with cysteamine from neonatal age.[17] In contrast, about half of the symptomatic siblings developed ESKD by the age of 13. These data indicate that the time between birth and the age at onset of symptoms is a window of opportunity during which cysteamine administration could be most efficient, albeit not solely directly based on its cystine-depleting mode of action. Indeed, the pathogenesis of the kidney disease in cystinosis is no longer regarded to be initiated by the lysosomal accumulation of cystine only. The absence of cystine crystals in human proximal tubular epithelial cells (PTEC) in young cystinosis patients,[20] and the development of the renal FS in *Ctns*$^{-/-}$ mice before the appearance of cystine crystals underscore this thesis.[21,22]

While in contrast to lysosomal cystine accumulation, some pathogenic features of cystinosis related to cystinosin function beyond cystine transport including impaired

autophagic flux and altered lysosomal distribution are not reverted by cysteamine treatment,[23–25] several others are beneficially affected by cysteamine treatment. Indeed, cysteamine has shown to reduce oxidative stress in cystinotic PTECs,[26] and significantly reduce reactive oxygen species (ROS) in mouse renal tubular cells in co-culture with cysteamine-treated macrophages.[27] Of note, in *Ctns*$^{-/-}$ mice the increase in oxidative stress precedes the swan neck deformities, which highlights the importance of oxidative stress in the initiation of the renal FS.[28] In addition, *in vitro* cysteamine treatment in PTECs reduces apoptosis,[29] while *in vivo* it attenuates macrophage infiltration, inhibits myofibroblast differentiation & reduces renal fibrosis in *Ctns*$^{-/-}$ mice.[27] Therefore, it is conceivable that presymptomatic treatment with cysteamine may beneficially attenuate the onset of the kidney disease in cystinosis and progression of interstitial fibrosis and chronic kidney disease by modulating oxidative stress, apoptosis and inflammatory responses.

In addition, another important finding of our study is that in this cystinosis cohort, we could not demonstrate that presymptomatic treatment with cysteamine, in contrast to initiation of cysteamine at the onset of symptoms, reduces the number of extra-renal complications. However, this observation might be partially explained by an important limitation of our study, which is the young age of the presymptomatic sibling vs. index pairs (10$\pm$6 and 14$\pm$3 years of age respectively). In addition, another drawback of our study is the limited availability of longitudinal WBC cystine values in only 32 of the 52 patients. Due to this limitation, a potential confounding caused by insufficient adherence to cysteamine treatment, could be underestimated. Finally, while the number of patients was low, especially in the presymptomatic treatment group (n=9 index and presymptomatic sibling pairs), the differences observed as described the results were convincingly clear.

Nevertheless, as a result, our data suggest considering the inclusion of cystinosis in newborn screening (NBS) programs in order to improve the renal outcome of cystinosis patients. In general, diseases eligible for NBS are those in which early intervention can lead to disease prevention or a considerable reduction in disease morbidity. One of the remaining requirements for establishing cystinosis as an ideal candidate for NBS, in compliance with the criteria based on the classic screening principles as defined by Wilson and Jungner,[30,31] is clear evidence demonstrating that presymptomatic initiation of disease-specific treatment results in better outcomes.[31] In this respect, this requirement underlines the importance of the data reported in this study. The practical set-up of the inclusion of cystinosis in NBS, whether via applying a biochemistry-first approach or next-generation sequencing (NGS), should be further investigated. However, a first-tier biochemical screening strategy seems reasonable. Plasma chitotriosidase enzyme activity, which is highly elevated in newly diagnosed cystinosis patients, is a promising biomarker that can be assessed on dried blood spots (DBS).[32,33] This could be a valuable tool to include in the regular inherited metabolic diseases (IMD) NBS, followed by second-tier directed genetic testing.

In conclusion, in this cystinosis sibling cohort, we demonstrated that while early initiation of cysteamine is the main determinant for the renal outcome in INC, the cystinosis genotype is not a decisive factor in the renal or extra-renal outcome. The novelty of this study is that it highlights the beneficial potential of cysteamine treatment in the presymptomatic stage on the renal outcome, which supports the consideration to include cystinosis into NBS. Furthermore, our data suggest that not all ERCs are as sensitive to cystine-depleting therapy, and more organ-specific approaches might be necessary. Finally, it is imperative that this sibling cohort are followed up in a long-term study.

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TABLES

Table 1: Demographic and clinical data of the patients recruited in the cystinosis sibling cohort

		All sibling vs. index pairs (n=52; 26 pairs)				**Symptomatic sibling vs. index pairs** (n=34; 17 pairs)				**Presymptomatic sibling vs. index pairs** (n=18; 9 pairs)			
		Index	**Sibling**	*p*	Difference (95% CI)	**Index**	**Symptomatic sibling**	*p*	Difference (95% CI)	**Index**	**Presymptomatic sibling**	*p*	Difference (95% CI)
n		**26**	**26**			**17**	**17**			**9**	**9**		
Sex	M:F	**14:12**	**8:18**	**0.15**		**8:9**	**7:10**	**> 0.99**		**6:3**	**2:7**	**0.15**	
Age at last observation	years	**23 ± 11**	**19 ± 11**	**< 0.0001**	**- 4 (-6; -2)**	**28 ± 11**	**23 ± 10**	**0.003**	**- 5 (-8; -2)**	**14 ± 5**	**10 ± 6**	**< 0.0001**	**- 4 (-6; -3)**
Genotype available	Y/N (%Y)	**42/10 (81%)**				**30/4 (88%)**				**12/6 (66%)**			
Hom 57kb del vs. other	Y/N (%Y)	**16/26 (38%)**				**12/18 (40%)**				**4/8 (33%)**			
Moderate (M) vs. Severe (S) pathogenic variant	M/S (% M)	**8/34 (19%)**				**6/24 (20%)**				**2/10 (16%)**			
Age at diagnosis	months	**22 (18; 29)**	**6 (0.2; 14)**	**< 0.0001**	**- 15 (-20; -12)**	**22 (18; 38)**	**10 (6; 17)**	**< 0.0001**	**- 13 (-15; -8)**	**22 (14; 26)**	**0 (0; 0)**	**0.004**	**-20 (-27; -12)**
Age at initiation cysteamine	months	**25 (20; 47)**	**8 (1; 19)**	**< 0.0001**	**- 16 (-27; 13)**	**41 (21; 75)**	**12 (8; 31)**	**< 0.0001**	**- 15 (-35; -9)**	**22 (16; 28)**	**0.95 (0.2; 1.4)**	**0.004**	**- 20 (-27; -14)**
ESKD	Y/N (%Y)	**16/10 (62%)**	**10/16 (38%)**	**0.17**		**14/3 (82%)**	**10/7 (59%)**	**0.26**		**2/7 (22%)**	**0/9 (0%)**	**0.47**	
Age at ESKD	years	**10 ± 3**	**13 ± 3**	**0.002**	**4 (2; 6)**	**10 ± 3**	**13 ± 3**	**0.002**	**4 (2; 6)**	**13 ± 3**	***na***	***na***	***na***
Last observed eGFR*	ml/min/1.73m2	**46 (25; 81)**	**73 (59; 93)**	**0.047**	**28 (1; 55)**	**25 (16; 91)**	**72 (48; 122)**	**0.18**	**47 (-43; 152)**	**50 (28; 78)**	**75 (64; 87)**	**0.07**	**25 (-6; 58)**
# extra-renal complications		**2 (1; 3)**	**2 (1; 2.25)**	**0.71**	**0 (0; 0)**	**2 (1; 3)**	**2 (1; 4)**	**0.63**	**0 (0; 1)**	**2 (0.5; 2.5)**	**1 (0; 2)**	**0.25**	**0.0 (-2; 0)**
Average lifetime WBC cystine	nmol ½ cystine/mg protein	**1.43 (0.87; 3.18)**	**1.04 (0.83; 2.54)**	**0.33**	**-0.02 (- 0.51; 0.2)**	**2.3 (1.04; 4.37)**	**2.08 (0.99; 3.71)**	**0.37**	**-0.1 (-1.43; 0.3)**	**1.41 (0.56; 1.83)**	**1.02 (0.71; 1.89)**	**0.64**	**0.0 (-0.51; 0.44)**

eGFR: estimated glomerular filtration rate; ESKD: end-stage kidney disease; KTx: kidney transplantation; WBC: white blood cell; information on data given: mean ± SD or median (95% confidence interval); *: only non-kidney transplanted patients are included in this analysis; *na:* not applicable

Table 2: Genotype of the cystinosis sibling cohort

Pathogenic variant	Protein	Type	Severity*	# patients (n=52) (% of total)
Homozygous del 57kb			Severe	**16 (31%)**
Heterozygous del 57kb				**10 (19%)**
del 57kb + del 13kb		Large deletion	Severe	2 (4%)
del 57kb + c.141-24t>c		Intronic mutation	Moderate	2 (4%)
del 57kb + c.314_317del	p.His105ProfsX12	Out-of-frame deletion	Severe	2 (4%)
del 57kb + c.414G>A	p.Trp138X	Nonsense mutation	Severe	2 (4%)
del 57kb + c.751_752del	p.Thr251HisfsX44	Out-of-frame deletion	Severe	2 (4%)
Other				**16 (31%)**
Homozygous				
c.1015G>A	p.Gly339Arg	Missense mutation	Moderate	2 (4%)
c.18_21del	p.Thr7PhefsX7	Out-of-frame deletion	Severe	4 (8%)
c.681G>A		Splicing mutation	Severe	4 (8%)
Ex4_Ex5del		Large deletion	Severe	2 (4%)
Heterzygous				
c.2T>C + c.518_519del	p.Met1Thr p.Y173X	Missense mutation Out-of-frame deletion	Moderate	2 (4%)
c.295_298del + c.1015G>A	p.Val99IlefsX18 p.Gly339Arg	Out-of-frame deletion Missense mutation	Moderate	2 (4%)
Unknown				**10 (19%)**

* Severity of the cystinosis genotype was defined as described by Emma *et al*.[15] Patients with at least one allele with a missense pathogenic variant, intronic pathogenic variant or in-frame deletion, were defined as having a moderate pathogenic variant; all other patients (comprising truncating and nonsense mutations) were defined as having severe pathogenic variants.

Table 3: Clinical characteristics of the homozygous 57kb deletion vs. other pathogenic variants, and moderate vs. severe pathogenic variant subgroups of all infantile nephropathic cystinosis sibling vs. index pairs with a known genotype

		All patients (n=52 of which 42 patients have a known genotype)							
		Hom 57kb del	**Other pathogenic variants**	*p*	Difference (Mean ± SD; 95% CI)	**Moderate pathogenic variant**	**Severe pathogenic variant**	*p*	Difference (Mean ± SD; 95% CI)
n		16	26			8	34		
Sex	M:F	9:7	12:14			3:5	18:16		
Age at last observation	years	27 ± 11	20 ± 11	0.07	7 ± 3 (-0.5; 14)	23 ± 13	23 ± 11	0.95	- 0.3 ± 4 (-9; 9)
Age at initiation cysteamine	months	20 (10; 38)	21 (9; 42)	0.99	- 1.1 (-12; 12)	49 (7; 150)	19 (10; 29)	0.15	- 30 (-124; 6)
Age at ESKD	years	13 ± 3	10 ± 3	0.04	3 ± 1 (0.2; 5.3)	12 ± 1	11 ± 4	0.69	-0.7 ± 2 (-4; 2.8)
# extra-renal complications		2 (2; 4.75)	2 (0; 3)	0.02	0.0 (0; 2)	0.5 (0; 2.75)	2.0 (1.0; 4.0)	0.07	1.5 (0; 2)
Average lifetime WBC cystine	nmol ½ cystine/mg protein	2.08 (0.98; 4.88)	1.43 (1.04; 2.5)	0.88	0.65 (-0.5; 1.84)	1.43 (1.05; 1.44)	1.67 (1; 3.3)	0.62	0.24 (-0.44; 3.39)

Table 4: Univariate and multivariate regression analysis of predictors for the number of extra-renal complications in all infantile nephropathic cystinosis sibling patients with a known genotype (n=42)

All patients with a known genotype (n=42)

Variable	unit	Estimate (95% CI)	*p*	# observations
Univariable analysis				
Age at last observation	years	0.09 (0.03; 0.14)	0.003 **	42
Age at initiation cysteamine	months	0.01 (0.002; 0.02)	0.02 *	42
Average lifetime WBC cystine	nmol ½ cystine/mg protein	0.47 (0.13; 0.81)	0.01 **	34
Hom 57 kb del vs. other pathogenic variants		1.76 (0.01; 3.51)	0.0486 *	42
Moderate vs. Severe pathogenic variant		-1.43 (-3.73; 0.86)	0.2	42
Multivariable analysis (model 1: Hom 57kb del vs. other pathogenic variants)				
Age at last observation	years	0.07 (-0.003; 0.14)	0.06	42
Age at initiation cysteamine	months	0.002 (-0.01; 0.01)	0.77	42
Hom 57 kb del vs. other pathogenic variants		1.31 (-0.35; 2.97)	0.11	42
Multivariable analysis (model 2: Moderate vs. Severe pathogenic variant)				
Age at last observation	years	0.08 (0.005; 0.15)	0.04 *	42
Age at initiation cysteamine	months	0.003 (-0.01; 0.02)	0.67	42
Moderate vs. Severe pathogenic variant		-1.6 (-3.65; 0.46)	0.12	42

FIGURE LEGENDS

Figure 1: Study design and definition of presymptomatic and symptomatic sibling vs. index pairs in the cystinosis sibling cohort study

This cystinosis sibling cohort is composed of siblings and corresponding index patients from within the same family, both diagnosed with cystinosis. Depending on the age of diagnosis and initiation of cysteamine treatment, presymptomatic siblings (diagnosis *in utero* or at neonatal age; initiation of cysteamine at neonatal age) and symptomatic siblings (diagnosis due to early signs and symptoms of cystinosis) are distinguished.

Figure 2: Renal outcome in cystinosis siblings

Panel A: Symptomatic sibling vs. index pairs, Panel B: Presymptomatic sibling vs. index pairs. Overall, cystinosis siblings show a slower progression of chronic kidney disease compared to the index patients, as demonstrated by a later age at achieving end-stage kidney disease in a time to event analysis. Remarkably, none of the presymptomatic siblings have reached end-stage kidney disease yet.

Figure 3: Extra-renal outcome in cystinosis siblings

Panel A: Symptomatic sibling vs. index pairs, Panel B: Presymptomatic sibling vs. index pairs. Cystinosis sibling vs. index pairs did not show significant differences in the incidence of extra-renal complications. The graphs in the left column of panel A and B display the sibling pairs via full lines connecting the index and sibling patients. Here, overlapping patients cannot be discriminated. The graphs in the middle column allow the individual patients to be discriminated in each group (index vs. sibling), however without index and sibling of each pair being connected. The horizontal line represents the median. The graphs in the right column represent a time to event analysis for the diagnosis of primary hypothyroidism, in index (black line) compared to sibling (blue line) patients. No significant differences can be observed in the age at onset of primary hypothyroidism in specific, for both the symptomatic sibling vs. index pairs and presymptomatic sibling vs. index pairs.

Figure 4: Subgroup analysis on the effect of cystinosis genotype (homozygous 57kb del vs. other pathogenic variants) and age at initiation of cysteamine (< or ≥ 10 months of age) on the renal (age at end-stage kidney disease) and extra-renal (total number of extra-renal complications) outcome in the cystinosis sibling cohort. Panel A & C: renal outcome, Panel B & D: extra-renal outcome. Panel A & B: homozygous 57kb deletion vs. other pathogenic variants; Panel C & D: age at initiation of cysteamine (< or ≥ 10 months of age). While age at initiation of cysteamine has a significant effect on the renal outcome (panel C), the increased number of extra-renal complications associated with patients in whom cysteamine was initiated from the age of 10 months was due to the older age of these patients (Table 4).

In addition, patients harboring the homozygous 57kb deletion did not show a worse renal outcome (panel A), while this genotype was associated with a higher number of extra-renal complications (panel B) also due to the older age of this patient group (Table 4). Indeed, in a multivariate regression analysis (Table 4), the genotype did not result as a significant predictor for extra-renal outcome.

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