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An international cohort study spanning five decades assessed outcomes of nephropathic cystinosis.

- Cystinosis is a rare disease secondary to mutations in the CTNS gene
- We collected data from a large cohort of 453 patients born between 1964 and 2016 and followed in:
  - Belgium
  - Germany
  - Austria
  - France
  - Italy
  - Spain
  - The Netherlands
  - Turkey
  - United Kingdom
- We investigated factors associated with kidney function and growth outcome

**Results (1)**
- Gain of 9.1 years in kidney survival from the 1970’s to the 1990’s
- No effect of the type of CTNS gene mutation

**Results (2)**
- Improved kidney survival was associated with the precocity of treatment with cysteamine and with average leukocyte cysteine levels
- Linear growth was equally improved in patients well treated with cysteamine

**CONCLUSION:** treatment with cysteamine has been the major factor responsible for improved kidney function survival in patients with nephropathic cystinosis over the past 50 years

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**Running head:** Long-term outcome of cystinosis

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ABSTRACT

Nephropathic cystinosis is a rare disease secondary to recessive mutations of the CTNS gene encoding the lysosomal cystine transporter cystinosin, causing accumulation of cystine in multiple organs. Over the years, the disease has evolved from being a fatal condition during early childhood into a treatable condition, with patients surviving into adulthood. Data on cystinosis are limited by the rarity of the disease. Here, we have investigated factors associated with kidney and growth outcome in a very large cohort of 453 patients born between 1964 and 2016 and followed in Belgium, Germany, Austria, France, Italy, Spain, The Netherlands, Turkey and United Kingdom. From the 1970’s to the 1990’s, the median increase in kidney survival was 9.1 years. During these years, cysteamine, a cystine-depleting agent, was introduced for the treatment of cystinosis. Significant risk factors associated with early progression to end-stage kidney disease assessed by Cox proportional multivariable analysis included delayed initiation of cysteamine therapy and higher mean leucocyte cystine levels. No significant effect on kidney function was observed for gender, pathogenic variant of the CTNS gene, and the prescription of indomethacin or renin angiotensin system blockers. Significantly improved linear growth was associated with early use of cysteamine and lower leukocyte cystine levels. Thus, our study provides strong evidence in favor of early diagnosis and optimization of cystine depletion therapy in nephropathic cystinosis.
INTRODUCTION

Cystinosis is a rare disease with an estimated incidence in western countries of 0.5-1:100,000 live births\(^1\)-\(^2\). The disease is caused by bi-allelic pathogenic variants of the *CTNS* gene (chr. 17p13) encoding the lysosomal cystine transporter cystinosin, and is hallmarked by lysosomal cystine accumulation in multiple organs\(^2\)-\(^3\). In its most frequent and severe form, termed infantile nephropathic cystinosis (NC) (MIM 219800), patients become symptomatic after the first months of life and present with signs of renal Fanconi syndrome\(^1\)-\(^2\). Many also have corneal cystine crystal depositions, which are always present by the age of 2 years\(^4\). If not treated with cysteamine, most patients progress to end-stage kidney disease (ESKD) around 10 years of age and develop symptoms related to cystine accumulation in various organs\(^5\). The majority of patients with NC suffer from growth stunting, which is multifactorial\(^2\),\(^6\),\(^7\).

Cysteamine was proposed in the 1970's for the treatment of NC\(^8\) and was introduced for human use from the 1980’s\(^9\),\(^10\). In conjunction with improvements in pediatric dialysis and kidney transplantation, cysteamine has dramatically changed the prognosis of NC, with most patients currently living into adulthood\(^6\),\(^11\),\(^12\). Herein, we report the results of a large international collaborative study, involving eight European countries and Turkey, which has collected data of patients born in the past five decades to evaluate factors that have improved kidney function outcome and linear growth.
METHODS

A cohort of 453 patient with nephropathic cystinosis, as the only eligibility criteria, was collected between 1970 and 2017 in nine countries, namely, United Kingdom, France, Italy, Spain, Germany, Austria, Turkey, Belgium and The Netherlands. All patients included in the study had a minimum follow-up of 3 years. Most data were collected retrospectively from medical records. All participating centers are referral centers in their countries for NC. Only data that could be reliably retrieved retrospectively were collected. These included patient age, serum creatinine levels, renal outcome (age at dialysis or pre-emptive transplantation), leucocyte cystine levels, height, and treatment. Treatments included cysteamine, indomethacin, angiotensin converting enzyme inhibitors or angiotensin receptor blockers (ACEi/ARBs), and recombinant human growth hormone (rhGH). All medications were analyzed as binary data, regardless of the dose that was prescribed. The age at which cysteamine was started was recorded for all patients. Mean leucocyte cystine levels were calculated using all levels available for each subject after starting cysteamine. The methodology for measuring leukocyte cystine levels varied among centers and over the years. In general, older measurements were performed with high performance liquid chromatography (HPLC), while measurements that are more recent were performed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Some centers performed measurements on isolated granulocytes, while other used the entire leucocyte fraction.

The study was approved by the Institutional Review Boards of participating centers according to rules in place at the last follow-up of each patient, including rules to obtain informed consent. The majority of prevalent French, Italian, Spanish, Dutch, Belgian and German patients are followed in centers that participate in the ERKNet European Reference Network for rare diseases, and have provided consent for data sharing within the ERKNet framework. The study was conducted in accordance with the Helsinki Declaration. Results were submitted and approved for publication by
the ethical committee of the corresponding author (Ref. 1640/2019).

Statistical analysis was performed with IBM SPSS Statistics 21.0.0.2 software (Segrate, Italy). All tests are two-sided and considered statistically significant for p values <0.05. Categorical data are reported as counts and percentages. Continuous normal data are expressed as mean ± standard deviation. Continuous data that did not follow a normal distribution are expressed as median value and interquartile range. Normality of data was tested with the Kolmogorov-Smirnov test. Survival data were analyzed using the Kaplan Meier method and compared using the Log Rank test. Multivariable survival analysis was performed through Cox proportional hazards regression, using variables that showed a p-value <0.2 by univariable analysis, after verifying the proportional hazard assumption. For the Cox modelling, hazard ratios were calculated for overlapping blocks of 60 patients by steps of 10 patients. The hazard ratios were then plotted against the median age of each block, and smoothed curves were generated with OriginPro 2018 software (Northampton, MA) by unweighted adjacent averaging method. A General Linear Model was used to evaluate determinants of leucocyte cystine levels and height SDS, after checking for data normality and equality of variance. Models were controlled by evaluation of residual plots.
RESULTS

Patient characteristics

The characteristics of the cohort, which included 453 patients (52% males) collected over a 37-year period, are illustrated in Figure 1 and detailed in Table 1. All patients were diagnosed based on leucocyte cystine levels and, if available, the demonstration of pathogenic variants of the CTNS gene. The median age at the last follow-up was 15.3 years [9.3-21.2]. For the analysis, patients from similar geographic regions, namely Belgium and The Netherlands, Italy and Spain, and Germany and Austria, were grouped together. Data collection periods varied slightly between countries, with the oldest patients collected in the United Kingdom and the youngest patients collected in Turkey (Supplementary Figure 1). Most patients were born between the years 1970’s and the years 2000. Longitudinal data on kidney function were available in 206 patients, while data were limited to the last follow-up or to the date at which subjects had reached chronic kidney disease stage 5 (CKD5) in the remaining patients. Due to the extended period of data collection, genetic information was not available for all patients, in particular for patients born during the initial period of the study, because they were lost at follow-up or had died prior to the identification in 1998 of the CTNS gene³. Overall, genetic data were available in 329 patients. Of these, 33% were homozygous for the common Northern European 57 Kb deletion, 23 % were heterozygous for the 57Kb deletion, and 45% had other pathogenic variants of the CTNS gene. Cysteamine was started before CKD5 in 89% of patients at a median age of 1.6 years. Additional therapies that were captured in the database included indomethacin, ACEi/ARBs, and rhGH. Patients were empirically considered to have received treatment with indomethacin or ACEi/ARB if they had been treated for more than 5 years, or for at least 50% of their life for children aged < 10 years. Overall, indomethacin was prescribed to 43% of patients, while ACEi/ARBs were prescribed to 17% of patients. Patients were considered to have been treated with rhGH if they
received treatment for at least 2 years. Overall, rhGH therapy was prescribed to 97/234 (41%) children for which longitudinal growth data were available.

**Improvement of kidney function**

The longitudinal evolution of kidney function was assessed using serum creatinine levels and stratified by decade of birth (Figure 2). Data were compared with the original data from Manz and Gretz, who reported the natural evolution of serum creatinine in Europe before cysteamine was introduced for the treatment of cystinosis. Estimated glomerular filtration rates could not be calculated because height data were not always available. As shown in figure 2A and in the survival curves of figure 2B, the prognosis of kidney function has improved steadily between the 1970’s and 1990’s (p <0.001). The median gain in renal survival was 9.1 year. Only 21 patients, mostly born in the 1960’s and 1970’s, reached ESKD in the first 8 years of life. During this period, 84 patients were censored in the survival analysis of figure 2B, with 348 remaining at risk. Cysteamine was progressively introduced for the treatment of NC in the 1980’s and 1990’s. As illustrated in figure 2C, patients received increasingly more often cysteamine and started treatment at increasingly younger age during these decades.

**Factors associated with progression of kidney disease**

We did not observed significant differences in kidney function outcome between different countries (Cox proportional hazard regression analysis - Table 2B). Next, we analyzed the impact of different pathogenic variants. These are summarized in Table 3 and are detailed in Supplementary table 1. The common Northern European 57 kb deletion encompassing the CTNS, the CARKL and the TRPV1 genes was observed in 45% of tested alleles. As expected, the deletion was mostly observed in patients from United Kingdom, Belgium, The
Netherlands, France, Germany and Austria, while it was rarely or not observed in patients from Turkey, Italy and Spain (Table 1). Missense variants, splicing variants and out-of-frame deletions were the other most frequent types of mutations, accounting for 39% of mutated alleles. It has been debated if patients harboring the classic 57 kB deletion had a worse prognosis. From the kidney standpoint, we did not observe differences in kidney survival between patients that inherited the 57 kB deletion in homozygous or in heterozygous state and patients with other pathogenic CTNS variants (Figure 3A). In addition, we tested if pathogenic variants that may potentially allow for residual activity of the cystinosin protein (missense variants, intronic variants, in-frame deletions) could be associated with better outcome, but failed to detect any differences when compared to variants that are theoretically more severe (Figure 3B). These results were confirmed by Cox proportional hazard regression analysis (Table 2A and 2B). Similarly, we did not observe an association between leucocyte cystine levels and the theoretical severity of mutations or with the presence of the 57 kb deletion (Supplementary Table 2).

A Cox proportional hazard regression was performed, excluding 71 patients that had begun cysteamine therapy after the age of 8 years (Table 2A and 2B). After this age, the relationship with the probability of CKD5 reached a plateau (Supplementary figure 2). At the univariable level, the analysis showed that patients born more recently, that were younger when cysteamine was started, and that had lower mean leucocyte cystine levels had longer kidney survival. Indomethacin and ACE inhibitors were prescribed in some centers (Table 1) to mitigate the renal Fanconi syndrome and to slow progression of CKD, respectively. Both drugs were not associated with improved outcome. At the multivariable level, the year of birth did not retain a significant effect. Only the age when cysteamine was started (H.R.: 1.24, 95% C.I. [1.9 - 1.42; p = 0.002) and the mean leucocyte cystine levels (H.R: 1.26, 95% C.I. [1.09-1.46]; p = 0.002) were associated with delayed development of CKD5. These results were further analyzed in the Cox models shown in
Modelling of the hazard ratio showed a nearly linear relationship between the age at which cysteamine was started and the likelihood of reaching CKD5 at an earlier age. This relationship was less pronounced for the mean leukocyte cystine levels, and tended to plateau for levels > 1.5-2.0 nmol ½ cystine/mg protein. The same results are also reflected in the survival curves of figure 4B and 4D. Of note, leucocyte cystine levels were not significantly associated with early cysteamine therapy (Supplementary Table 2).

Linear growth

Children with cystinosis are known to grow poorly, as confirmed in this very large cohort. The reference growth curves in figure 5A and 5D correspond to the average reference data for Northern and Southern Europeans children\textsuperscript{14}. Even considering secular growth changes during the study period\textsuperscript{14}, growth of children with NC was markedly stunted and the majority of patients grew below the 3\textsuperscript{rd} percentile (median height standard deviation scores (SDS): -2.72 [-3.64 -1.85]). Differences between patients in the length of follow-up limited the analysis. For each patient, SDS were calculated using the Northern or Southern European reference curves, as appropriate. This allowed creating the SDS curves shown in Figures 5B, 5C, 5E and 5F. Since most patients grew along a given growth channel (Supplementary Figure 3), patients were compared using their average height SDS during growth. Early initiation of cysteamine had a positive, although moderate effect on growth (Figure 5B). Children that had started cysteamine before the age of 1.5 year grew on average 0.57 SDS better compared to children that had started cysteamine later (95% confidence intervals (CI): 0.20-0.93; \textit{p} = 0.003). Likewise, children with mean cystine levels < 1.5 nmol ½ cystine/mg prot. grew on average 0.66 SDS better than those with higher levels (95% CI: 0.30-1.02; \textit{p}<0.001) (Figure 5C). On average, children treated with rhGH were shorter at young ages and showed a mean gain in height SDS during follow-up of 0.69 SDS, which was not observed
in untreated patients (Figure 5E). The catch-up growth induced by rhGH cancelled differences between treated and untreated patients resulting in similar mean height SDS in the two groups. Treatment with indomethacin had no significant influence on growth (Figure 5F). The relative contribution to height SDS of the above variables was analyzed using a General Linear Model. Only the mean leucocyte cystine levels ($p = 0.01$), and the precocity of cysteamine therapy ($p = 0.03$) were significantly associated with improved growth (Supplementary Table 3). As shown in the Cox proportional hazard regression (table 2), high cystine levels and delayed treatment with cysteamine were strong predictors of early onset ESKD, which can compromise growth per se. Accordingly, children reaching CKD5 before the age of 15 years grew on average 0.55 SDS worse compare to children that reached dialysis after their 15th birthday (95% CI: 0.09-1.01; $p = 0.03$) (Supplementary Figure 4). Because of their strong association, the relative contribution to poor growth of uremia vs. inadequate cystine depleting therapy could not be appraised. Other factors, including rickets, chronic acidosis, hyperparathyroidism, or chronic hypovolemia, may have also influenced linear growth, but could not be captured and analyzed in this study.
DISCUSSION

Before the introduction of cysteamine therapy, children with NC reached ESKD around the age of 10 years and died soon after if not treated with dialysis or kidney transplantation\textsuperscript{13}. Even if transplanted, most patients developed severe, multisystemic complications and died as young adults\textsuperscript{11}. Nowadays, patients live well into adulthood, with several having celebrated their 40\textsuperscript{th} or even 50\textsuperscript{th} birthday\textsuperscript{11,15}. These progresses could not have been made without the remarkable improvements in the treatment of children with advanced kidney failure that have taken place during these past decades. However, a major contribution to improved patient survival is represented by the introduction of cysteamine for the treatment of NC. Cysteamine is an amino thiol compound that interacts with cystine and generates a cysteamine-cysteine mixed disulfide that exit lysosomes though a cationic amino acid transporter (PQLC2), bypassing the defective cystinosin protein\textsuperscript{2,8,16}. The bitartrate salt of cysteamine was approved by the Food and Drug Administration in 1994 and by the European Medical Agency in 1997. Prior to these dates, some patients received cysteamine bitartrate in the context of clinical trials, and other were treated with variable doses of cysteamine hydrochloride or with phosphocysteamine\textsuperscript{2,17,18}. In Europe, most patients began treatment with cysteamine bitartrate in the 1990’s. This drug has side effects that limit compliance, especially in adolescents. All patients develop a foul acrid sulfur smell, and frequently complain of gastrointestinal upset\textsuperscript{4}. In addition, they need to adhere to a tight every 6-hour administration schedule. A delayed-release cysteamine bitartrate formulation that can be taken every 12 hours has recently been developed\textsuperscript{19}.

The present study took advantage of a well-established collaborative network in rare diseases\textsuperscript{20} to analyze clinical outcomes in a large cohort of European and Turkish cystinosis patients born over a period spanning 50 years. Of these, 90\% had started cysteamine prior to developing ESKD. We observed a very significant, 9.1-year increase in the median age at dialysis from the 1970’s to the
1990’s. The main gain in kidney survival corresponds to the years when cysteamine was introduced in Europe. During these years, a similar improvement was not observed in children suffering from other kidney diseases⁶, highlighting the crucial contribution of cysteamine for NC. This point is also indirectly underlined by results of the Cox proportional hazard regression showing that the year of birth, which was strongly associated with kidney survival at the univariable level, was no longer significant at the multivariable level. Our results confirm in a non-clinical trial setting and in a very large cohort of patients, the long-term results of the original National Institute of Health cohort, showing a gain of 8-10 years in the age at dialysis in 28 patients that started cysteamine before the age of 3 years². A recent retrospective French study found a gain of 6-7 years in 40 patients that were treated early with cysteamine¹². Because the size of their cohort was limited, the authors could only use arbitrary cut-offs to assess the impact of early cysteamine therapy (before or after 5 years)¹². Owing to the large number of subjects that we collected, we were able to show a nearly linear relationship between the age at which cysteamine therapy was started and kidney function outcome. This finding supports the need for early diagnosis and provides the rationale to develop newborn screening programs. To date, these have only been tested in few studies using a next generation sequencing approach²¹. Since the Fanconi syndrome associated with NC is characterized by early onset glycosuria and low-molecular weight proteinuria, a urine analysis-based screening would also allow suspecting the diagnosis very early, although not necessarily at birth. This type of screening in Japan for example, has been very successful in identifying children with Dent disease²².

Another strength of the present study was the availability of genetic information for a large subset of patients. To date, more than 160 pathogenic variants have been identified in the CTNS gene, most of which cause complete loss of function of the cystinosin protein²³. The most frequent mutation is the 57 kb deletion on chromosome 17 that arose in Germany approximately 1000
years ago and is present in heterozygous or homozygous state in 76% of patients of Norther European descent. The deletion disrupts the first ten exons of the CTNS gene, the entire CARKL gene, and the first two exons of the TRPV1 gene. CARKL encodes the enzyme sedoheptulose kinase that transforms sedoheptulose to sedoheptulose phosphate in the pentose phosphate pathway. TRPV1 encodes for a cation channel (transient receptor potential cation channel subfamily V member 1), which acts as a capsaicin and vanilloid receptor. Both genes are expressed in multiple organs. Patients carrying the 57 kb deletion in homozygous state accumulate sedoheptulose without apparent clinical consequences and respond less to capsaicin and heat stimuli. Increased morbidity and mortality has been reported in a cohort of 34 adults carrying the 57 kb deletion in homozygous state, compared to 45 subjects of the same age with other defects in the CTNS gene. Our study was limited to kidney function but failed to observe a worse outcome in patients carrying the homozygous 57 kb deletion. Unfortunately, due to the retrospective nature of the study, patient retention after reaching CKD5 was insufficient and mortality could not be assessed. In addition, we observed no effect on glomerular function or leucocyte cystine levels of CTNS variants that theoretically could have milder consequences. This suggests that all patients presenting with symptoms of infantile NC, which corresponds to the most frequent and severe form of the disease, have pathogenic variants causing complete loss of function of the protein. Conversely, patients with the juvenile or with the ocular form of cystinosis have been shown to have at least one milder variants that partially preserve the function of cystinosin.

The suggested dose of cysteamine bitartrate has not changed significantly since it was first recommended for the treatment of NC, except for the recommendation to avoid high doses after reports of toxicity, and to slightly decrease the dose when converting to the long-acting formulation of cysteamine. This latter formulation has been introduced in the market only in
recent years; its impact on kidney function outcome could not be assessed in the present study.

The suggested doses of cysteamine were initially targeted to reach leucocyte cystine levels similar to those of heterozygous carriers, which are not symptomatic\textsuperscript{4, 29}. In addition, measuring leucocyte cystine levels helps assessing patient adherence, which is a major issue in NC, particularly during adolescence\textsuperscript{30}. However, these tests are performed only in few laboratories, are expensive and cumbersome. Results are often variable and are influenced by delays in sample processing, ex-vivo anticoagulation, storage and shipping conditions, leucocyte purification modalities (i.e. entire leucocyte pool vs. purified granulocyte fractions), and analytic techniques\textsuperscript{31}. Despite these limitations, we observed an independent effect of leucocyte cystine levels on the progression of kidney failure, indicating that they have a role in monitoring and adapting cysteamine therapy. These results confirm the current recommendations to aim at levels close to heterozygous carriers\textsuperscript{29}.

Two drugs have been controversial for the treatment of NC, namely indomethacin and ACEi/ARBs. This study allowed assessing the impact of these drugs on kidney function. Indomethacin and ACEi/ARBs were prescribed according to local preferences, although we cannot exclude that more severe patients may have been more likely to receive them.

Indomethacin was introduced in the 1980’s after reports showing that it reduced tubular losses in children with severe Fanconi syndrome, promoting growth and metabolic stability\textsuperscript{32, 33}. Tubular function of NC is generally irreversibly impaired by the time that cysteamine therapy is initiated. In the past decades, indomethacin has been used in some European countries, but less in others, because of its known nephrotoxicity and gastrointestinal side effects, which may increase the gastric irritation caused by cysteamine. We did not observe a worse outcome of kidney function in patients that were treated for prolonged periods with indomethacin. Since indomethacin can help managing small children with severe renal Fanconi syndrome, our data provide reassurance for
using this drug in patients with NC. Nonetheless, it may be prudent to limit indomethacin prescription to the first years of life, when renal water and salt losses are more severe.

ACEi/ARBs are known to delay progression of kidney failure in adults and children, especially if they suffer from proteinuric glomerular diseases. In this respect, recent studies have shown podocyte dysfunction in NC. However, these drugs can also cause renal hypoperfusion in hypovolemic subjects, such as patients with NC that are often polyuric. Data from very small cohorts of children have suggested that ACEIs could delay progression of kidney failure or reduce proteinuria in NC. In our cohort, we did not observe a beneficial effect of ACEi/ARBs. The number of patients treated with ACEi/ARBs was however, limited. In addition, the age of treatment, dosages, and kidney function when treatment was initiated, were not homogeneous. Until more data are available, physicians should prescribe these drugs with caution in patients with NC.

As already shown in other studies, we observed an improvement in linear growth over the years, corresponding to the introduction of cysteamine therapy. The average height SDS in our cohort that comprised a mix of well and poorly treated patients was -2.9±0.2 (SEM); in comparison, the height SDS was -2.2±0.4 in 28 North American children well treated with cysteamine and -4.1±0.4 in 47 untreated patients from the same study. Growth in patients with NC is influenced by many factors, including cysteamine therapy, nutrition, treatment of rickets and electrolyte imbalances, chronic kidney failure, and rhGH therapy. Not all dimensions of growth could be captured in the present study. Nonetheless, we observed that early and adequate cysteamine therapy had a beneficial, but limited impact on growth. Our data confirm that on average, children with NC are shorter compared to children with kidney failure secondary to other causes. NC differs from other kidney diseases in that renal Fanconi syndrome accompanies the glomerular dysfunction and patients continue to have hypophosphatemia even with kidney failure. This could
in part account for the greater growth impairment in this disease. In addition, early Fanconi syndrome might have residual effects on later growth. In this respect, impaired bone mineralization and increased bone resorption persisting after successful kidney transplantation was recently demonstrated in patients with NC. These observations also suggest an intrinsic bone defect, which is supported by experimental data showing poor growth of cystinotic mice in the absence of glomerular and tubular damage.

The present study represents the largest cohort of patients with cystinosis assembled to date, and shows the value of collaborative networks to define the natural history of rare diseases and to identify unmet needs. It has limitations that are related to its retrospective nature. In particular, we could not assess reliably proximal tubular dysfunction and, since patients exited the study after reaching CKD5, we could not assess mortality and late extra-renal complications. Other limitations include the arbitrary definition of cut-off values to differentiate treated from untreated patients, the variability in the amount and quality of the data between the different centers, and differences in the methodology for measuring leukocyte cystine levels.

We observed a remarkable improvement over the years in the prognosis of kidney disease of patients with NC. This study demonstrates that early administration of cysteamine and optimal cystine depletion are key elements to protect kidney function and to improve growth outcome. It provides clear evidence in support of early diagnosis. However, patients still progress to ESKD, indicating the need to develop additional therapies. Despite a dense genetic information, no genotype-phenotype correlation could be evidenced.
DISCLOSURE

Francesco Emma, Dieter Haffner, Georges Deschênes, Detlef Bockenhauer, Katharina Hohenfellner, Elena Levchenko, Gema Ariceta, and Lars Pape have declared to have received in the past 36 months consulting fees and/or other honoraria for lectures, presentations, educational events, or participation to advisory boards, from one or more of the following for-profit third parties: Recordati Rare Diseases, Chiesi Pharmaceuticals, and Avrobio. Elena Levchenko is a board member of Cystinosis Network Europe, Cystinosis Group of The Netherlands & Belgium, and of the Irish Cystinosis Foundation. Francesco Emma and Corinne Antignac are members of the scientific review board of the Cystinosis Research Foundation. All other authors have declared the absence of potential conflicts of interest related to the present work.
AUTHOR CONTRIBUTIONS

The study was designed by Francesco Emma, William van’t Hoff, Patrick Niaudet, Elena Levtchenko, and Olivier Devuyst. Francesco Emma, William van’t Hoff, Katharina Hohenfellner, Rezan Topaloglu, Marcella Greco, Gema Ariceta, Detlef Bockenhauer, Koenraad Veys, Lars Pape, Sally Hulton, Suzanne Collin, Fatih Ozaltin, Aude Servais, Georges Deschênes, Robert Novo, Aurélie Bertholet-Thomas, Jun Oh, Marlies Cornelissen, Mirian Janssen, Dieter Haffner, Patrick Niaudet and, Elena Levtchenko have taken care of patients and have collected data. Chiara Bettini and Francesco Emma have created the final database and have verified all data entry. Francesco Emma, Koenraad Veys, and Corinne Antignac have reviewed and classified the genetic data. Data analysis and interpretation was performed by Francesco Emma, Elena Levtchenko and Olivier Devuyst. Statistical analysis was performed by Francesco Emma and Lucilla Ravà. The manuscript was drafted by Francesco Emma and Elena Levtchenko and reviewed by all authors. All authors have provided significant intellectual contributions to the manuscript and have approved the final version.
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REFERENCES


TABLE AND FIGURE LEGENDS

Table 1. Patient characteristics

The table reports the basic characteristics of the cohort divided by country. Longitudinal data refer to patients for which annual follow-up data were available. For the remaining patients, the outcome data were available only at the last follow-up. Transplant data (i.e. age at transplant) are limited to the first transplant for patients that have undergone more than one transplant. Parentheses indicate percentages; square brackets indicate the 25\textsuperscript{th} and 75\textsuperscript{th} percentiles.

Footnotes: (1) the median age at which patients had started cysteamine was calculated only for patients that have started treatment before reaching end-stage kidney disease; (2) mean individual leukocyte cystine levels were calculated by averaging all levels measured in each patient after starting treatment with cysteamine; (3) patients were considered to have received treatment with indomethacin or ACEi/ARB if they had been treated for at least 5 years or if treatment was prescribed for at least 50\% of the time if children were aged less than 10 years; (4) patients were considered to have received treatment with rhGH if they had been treated for at least 2 years.

Abbreviations: WBC: white blood cells; ACEi/ARBs = angiotensin converting enzyme inhibitors or angiotensin receptor blockers; rhGH = recombinant human growth hormone

Table 2. Cox Proportional Hazard Regression for the risk of CKD5

The table shows the results of the Cox Proportional Hazard Regression at the univariable and multivariable level. Variable that reached a p-value < 0.2 were introduced in the multivariable model.

(*) Severity of CTNS variants refers to the theoretical impact of mutations on the function of the cystinosin protein. For the analysis, patients with at least one allele with missense variants,
intronic variants or in-frame deletions where classified as having potentially moderate mutations; all other patients were classified as having severe mutations.

Table 3. Summary of pathogenic variants
The table summarizes the CTNS gene pathogenic variants detected in patients that had undergone a genetic evaluation, classified per type of mutation. Only variants observed in at least 10 alleles are listed in this table; a complete list is provided in Supplementary Table 1. Parentheses indicate percentages.

Figure 1. Flow diagram of the study

Figure 2. Improvement of kidney function and prescription of cysteamine therapy during the study period.
Panel A shows the evolution serum creatinine of patients for which longitudinal data were available. The shaded area indicates the 5th, 50th and 95th percentile of an historical cohort of patients not treated with cysteamine. Panel B shows the Kaplan Meier survival function for patients divided per decade of birth. Panel C shows the age at which cysteamine therapy was prescribed during the same decades.

Figure 3. Impact of different pathogenic variants on the probability of reaching CKD5.
The figure shows the Kaplan Meier survival function for patients divided according to the presence of the common Northern European 57 kb deletion in homozygosity or heterozygosity (panel A) or according to the theoretical severity of CTNS gene mutations. Patients were classified in group A if...
they had at least one allele with a moderately severe mutation, or in group B if both alleles had a severe mutation, as indicated in the graph.

**Figure 4. Impact of cysteamine therapy on the risk of progressing to end-stage kidney disease.**

Panel A and C show the hazard ratio modeling for reaching CKD5 according to the age at which cysteamine was started (panel A) or to patient mean leucocyte cystine levels (panel C) (see text for details). Shaded area indicate the 95% confidence intervals of the hazard ratio; vertical bars at the bottom of the graphs indicate individual patients. Only patients that had started cysteamine before the age of 8 years were included in this analysis. As shown, the risk of progressing rapidly to stage 5 chronic kidney disease increased steadily with delayed initiation of cysteamine (panel A), and tended to plateau for leucocyte cystine levels above 1.5-2.0 nmol ½ cystine/mg of proteins (panel C). Panels B and D shows the Kaplan Meier survival function after dividing the cohort using the cut-off values shown in the graphs for the age at which cysteamine therapy was started (panel B) and for leucocyte cystine levels (panel D).

**Figure 5. Linear growth**

Panels A and D show the 3rd, 50th and 97th percentiles for height of all patients. Numbers below error bars indicate the number of values analyzed for each data-point. Individual patient curves are available in Supplementary Figure 2. Reference curves (shaded areas) were obtained by averaging reference data for Northern and Southern Europeans children. As shown, the average height was below the normal range at all ages. Panels B, C, E, and F compare the average SDS of the cohort according to the age when cysteamine therapy was started (panel B), the mean leukocyte cystine levels (panel C), treatment with rhGH (panel E) or with indomethacin (panel F). Smoothed curves were obtained after averaging SDS values at 6-month intervals. Shaded area
indicate standard errors of the mean. P values show the results of t-tests performed using the average SDS of each patient during the entire growth period. Note that the beginning and duration of follow-up was not homogeneous (see supplementary Figure 2 and text for details).
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>United Kingdom</th>
<th>France</th>
<th>Germany &amp; Austria</th>
<th>Italy &amp; Spain</th>
<th>Belgium &amp; The Netherlands</th>
<th>Turkey</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>151</td>
<td>90</td>
<td>84</td>
<td>47</td>
<td>41</td>
<td>40</td>
<td>453</td>
</tr>
<tr>
<td>Type of data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td>93/151 (62)</td>
<td>75/90  (83)</td>
<td>0/84 (0)</td>
<td>29/47 (62)</td>
<td>9/41 (22)</td>
<td>0/40   (0)</td>
<td>206/453 (45)</td>
</tr>
<tr>
<td>Data limited to the last follow-up</td>
<td>58/151 (38)</td>
<td>15/90 (17)</td>
<td>84/84 (100)</td>
<td>18/47 (38)</td>
<td>32/41 (78)</td>
<td>40/40 (100)</td>
<td>247/453 (55)</td>
</tr>
<tr>
<td>Growth data</td>
<td>104/151 (69)</td>
<td>85/90  (94)</td>
<td>0/84 (0)</td>
<td>30/47 (64)</td>
<td>15/41 (37)</td>
<td>0/40   (0)</td>
<td>234/453 (52)</td>
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<tr>
<td>Growth data</td>
<td></td>
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<tr>
<td>Patient characteristics</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dialysis before last follow-up</td>
<td>60/151 (40)</td>
<td>47/90 (52)</td>
<td>36/84 (43)</td>
<td>24/47 (51)</td>
<td>26/41 (63)</td>
<td>13/40 (33)</td>
<td>206/453 (45)</td>
</tr>
<tr>
<td>Transplant before last follow-up</td>
<td>56/151 (37)</td>
<td>42/90 (47)</td>
<td>N/A</td>
<td>24/47 (51)</td>
<td>25/41 (61)</td>
<td>N/A</td>
<td>147/329 (45)</td>
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<tr>
<td>CTNS mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 kB deletion homozygous</td>
<td>35/95 (37)</td>
<td>25/63 (40)</td>
<td>30/59 (51)</td>
<td>4/47 (9)</td>
<td>13/31 (42)</td>
<td>0/34 (0)</td>
<td>107/329 (33)</td>
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<tr>
<td>57 kB deletion heterozygous</td>
<td>22/95 (23)</td>
<td>20/63 (32)</td>
<td>18/59 (31)</td>
<td>6/47 (13)</td>
<td>9/31 (29)</td>
<td>0/34 (0)</td>
<td>75/329 (23)</td>
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<tr>
<td>Other pathogenic variants</td>
<td>38/95 (40)</td>
<td>18/63 (29)</td>
<td>11/59 (19)</td>
<td>37/47 (79)</td>
<td>9/31 (29)</td>
<td>34/34 (100)</td>
<td>147/329 (45)</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteamine started before CKD5</td>
<td>132/151 (87)</td>
<td>79/90 (88)</td>
<td>80/84 (95)</td>
<td>42/47 (89)</td>
<td>35/41 (85)</td>
<td>37/40 (93)</td>
<td>405/453 (89)</td>
</tr>
<tr>
<td>Age when cysteamine was started (years)</td>
<td>1.7 [1.1-2.9]</td>
<td>1.6 [1.2-2.3]</td>
<td>1.4 [1.0-2.8]</td>
<td>1.7 [1.1-2.8]</td>
<td>1.3 [1.0-2.5]</td>
<td>1.5 [1.1-4.2]</td>
<td>1.6 [1.0-2.8]</td>
</tr>
<tr>
<td>WBC cystine levels (nmol ½ cystine/mg prot.)</td>
<td>1.4 [1.0-2.3]</td>
<td>1.7 [1.2-2.0]</td>
<td>1.4 [1.0-2.1]</td>
<td>1.2 [1.0-1.4]</td>
<td>1.4 [1.0-1.7]</td>
<td>1.8 [0.9-4.1]</td>
<td>1.4 [1.0-2.1]</td>
</tr>
<tr>
<td>Indomethacin²</td>
<td>75/144 (52)</td>
<td>68/89 (76)</td>
<td>8/72 (11)</td>
<td>23/47 (49)</td>
<td>2/41 (5)</td>
<td>10/40 (25)</td>
<td>186/433 (43)</td>
</tr>
<tr>
<td>ACEi/ARB³</td>
<td>0/144 (0)</td>
<td>16/89 (18)</td>
<td>17/72 (24)</td>
<td>19/47 (40)</td>
<td>7/41 (17)</td>
<td>13/38 (34)</td>
<td>72/431 (17)</td>
</tr>
<tr>
<td>rhGH⁴</td>
<td>52/104 (50)</td>
<td>17/85 (20)</td>
<td>N/A</td>
<td>21/30 (70)</td>
<td>7/15 (47)</td>
<td>N/A</td>
<td>97/234 (41)</td>
</tr>
</tbody>
</table>
Table 2A. *Cox Proportional Hazard Regression for the risk of CKD5*

**Univariable analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units/value</th>
<th>N</th>
<th>H.R.</th>
<th>95% C.I.</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td><strong>Country</strong></td>
<td>(see table 2B)</td>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>382</td>
<td>0.73</td>
<td>0.53 - 1.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Year of birth</td>
<td>Year</td>
<td>382</td>
<td>0.95</td>
<td>0.92 - 0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>57kb deletion</td>
<td>(see table 2B)</td>
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<td></td>
<td></td>
<td>0.52</td>
</tr>
<tr>
<td>Severity of CTNS variants (*)</td>
<td>Severe</td>
<td>272</td>
<td>1.09</td>
<td>0.71 - 1.69</td>
<td>0.69</td>
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<tr>
<td><strong>Treatment</strong></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Age at start cysteamine</td>
<td>Years of age</td>
<td>382</td>
<td>1.32</td>
<td>1.21 - 1.45</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean leucocyte cystine</td>
<td>nmol ½ cystine/mg prot.</td>
<td>240</td>
<td>1.24</td>
<td>1.07 - 1.44</td>
<td>&lt;0.001</td>
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<tr>
<td>Indomethacin treatment</td>
<td>Yes</td>
<td>368</td>
<td>0.95</td>
<td>0.68 - 1.34</td>
<td>0.78</td>
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<tr>
<td>ACEi/ARB treatment</td>
<td>Yes</td>
<td>366</td>
<td>0.96</td>
<td>0.64 - 1.43</td>
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</table>

**Multivariable analysis**

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<th>Parameter</th>
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<th>H.R.</th>
<th>95% C.I.</th>
<th>P value</th>
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<tr>
<td>Sex</td>
<td>Male</td>
<td>240</td>
<td>0.66</td>
<td>0.41 - 1.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Year of birth</td>
<td>Year</td>
<td>240</td>
<td>0.97</td>
<td>0.94 - 1.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Age at start cysteamine</td>
<td>Years of age</td>
<td>240</td>
<td>1.24</td>
<td>1.09 - 1.42</td>
<td>0.002</td>
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<tr>
<td>Mean leucocyte cystine</td>
<td>nmol ½ cystine/mg prot.</td>
<td>240</td>
<td>1.26</td>
<td>1.09 - 1.46</td>
<td>0.002</td>
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</tbody>
</table>

Harrell’s C concordance statistic: P = 0.68
Table 2B. *Cox Proportional Hazard Regression for the risk of CKD5: sub-analyses*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units/value</th>
<th>N</th>
<th>H.R.</th>
<th>95% C.I.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td><strong>Country</strong></td>
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<td></td>
<td>0.47</td>
</tr>
<tr>
<td>- Belgium / The Netherlands</td>
<td>Yes</td>
<td>30</td>
<td>0.73</td>
<td>0.39 - 1.34</td>
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<tr>
<td>- France</td>
<td>Yes</td>
<td>76</td>
<td>0.69</td>
<td>0.43 - 1.11</td>
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<td>- Germany / Austria</td>
<td>Yes</td>
<td>77</td>
<td>0.66</td>
<td>0.40 - 1.09</td>
<td>0.11</td>
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<td>- Italy / Spain</td>
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<td>39</td>
<td>0.90</td>
<td>0.50 - 1.60</td>
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<td>- Turkey</td>
<td>Yes</td>
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<td>0.54 - 2.08</td>
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<td>- UK</td>
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<td><strong>CTNS mutation</strong></td>
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<td>0.52</td>
</tr>
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<td>- homozygous 57 kB deletion</td>
<td>Yes</td>
<td>96</td>
<td>0.89</td>
<td>0.59 - 1.34</td>
<td>0.57</td>
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<tr>
<td>- heterozygous 57 kB deletion</td>
<td>Yes</td>
<td>62</td>
<td>0.75</td>
<td>0.45 - 1.23</td>
<td>0.25</td>
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<tr>
<td>- Other pathogenic variants</td>
<td>Reference</td>
<td>121</td>
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Table 3. *Summary of pathogenic variants of the CTNS gene*

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>N of alleles (%)</th>
<th>Most frequent mutations (&gt; 10 alleles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large deletions</td>
<td>294 (45.2)</td>
<td>287 del 57 kb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 Other</td>
</tr>
<tr>
<td>Out-of-frame deletions</td>
<td>89 (13.7)</td>
<td>44 c.18_21del - p.Thr7PhefsX7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 c.771_793del - p.Gly258SerfsX30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 Other</td>
</tr>
<tr>
<td>In-frame deletions</td>
<td>16 (2.5)</td>
<td>c.926dup - p.Ser310GlnfsX55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29 Other</td>
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<tr>
<td>Insertions</td>
<td>41 (6.3)</td>
<td>c.283G&gt;T - p.Gly95X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 Other</td>
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<tr>
<td>Nonsense variants</td>
<td>27 (4.2)</td>
<td>c.681G&gt;A</td>
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<td></td>
<td></td>
<td>16 c.681+1G&gt;A</td>
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<td>31 Other</td>
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<td>Splicing variants</td>
<td>78 (12.0)</td>
<td>c.1015G&gt;A - p.Gly339Arg</td>
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<td></td>
<td></td>
<td>10 c.473T&gt;C - p.Leu158Pro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 Other</td>
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<tr>
<td>Intronic variants</td>
<td>12 (1.8)</td>
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<td>Missense variants</td>
<td>83 (12.8)</td>
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</tr>
<tr>
<td>Not detected</td>
<td>10 (1.5)</td>
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</tbody>
</table>
United Kingdom | N = 151
France | N = 90
Germany & Austria | N = 84
Italy & Spain | N = 47
Belgium & The Netherlands | N = 41
Turkey | N = 40

Growth data: N = 234 (52%)
Total patients: N = 453 (100%)
Genetic data: N = 329 (73%)

Renal outcome data: N = 453 (100%)

Longitudinal data: N = 206 (45%)
Status at last follow-up: N = 247 (55%)

Cysteamine started before CKD5: N = 198 (96%)
Cysteamine started before CKD5: N = 207 (84%)