

Long-term outcome of nephropathic cystinosis: a 20-year single-center experience

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Abstract Nephropathic cystinosis (NC) is a severe disease that is complicated by early-onset chronic renal failure (CRF) and other complications related to cystine deposition in tissue. Since the 1980s, the prognosis of NC has dramatically improved after the introduction of cysteamine treatment. Limited data are available documenting improvement in prognosis. We reviewed our long-term data (follow-up 6.3–27.8 years) on 23 patients followed in the past 26 years. Overall, stage III CRF was reached at 10 years of age in >90% of patients, whereas >80% reached end-stage renal disease before the age of 14 years. Three patients died during the follow-up. Our analysis shows a clear improvement in renal outcome ($p=0.001$) and linear growth ($p=0.04$) in patients treated more recently. Improvement in the evolution of renal function was significantly associated with early initiation of cysteamine ($p=0.006$), with the dose of cysteamine ($p=0.04$), and with the use of angiotensin-converting enzyme inhibitors ($p=0.01$). Nonrenal long-term complications are similar to previously reported data. Of note, 3/23 patients developed rare

forms of primary tumors that were successfully treated. In conclusion, our experience shows a significant improvement in the renal and nonrenal complications of cystinosis over the past decades and highlights the importance of early diagnosis in order to initiate cysteamine as soon as possible.

Keywords Nephropathic cystinosis · Cysteamine · Growth · Chronic renal failure

Introduction

Cystinosis is an inherited autosomal recessive disease caused by mutations in the *CTNS* gene, which encodes for the cystinosin protein that is primarily expressed at the lysosomal level [1]. Disease incidence varies in different countries between 1 and 2:100,000 live births; a founder effect has been frequently observed [2]. Of the three known forms of the disease, the infantile form, also termed nephropathic cystinosis (NC), is by far the most common and is characterized by early-onset Fanconi syndrome within the first year of life [2]. With time, most patients develop tubulointerstitial and glomerular diseases, leading to chronic renal failure (CRF) and ultimately end-stage renal disease (ESRD) [2–4]. Renal transplantation is a well-established treatment for patients with NC. It is well tolerated and may be associated with a lower rate of rejections [5].

Unfortunately, cystine accumulates in other tissues, causing cell dysfunction that leads to the development of other complications and symptoms, including photophobia, anterior chamber abnormalities, retinal degeneration, hypothyroidism, diabetes mellitus, exocrine pancreatic insufficiency, pubertal retardation and gonadal dysfunction, restrictive pulmonary disease, myopathy, neurological deterioration, and liver involvement [2, 6–8]. In recent decades, NC prognosis has

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considerably improved due to the introduction of treatment with 2-mercaptoethylamine, or cysteamine, which allows cystine clearance from the lysosomal compartment [2, 9–11]. Treatment with cysteamine has been further optimized by methods to monitor intraleucocyte cystine (ILC) levels that allow dose adaptation of cysteamine to maintain target ILC levels of ILC [11–14]. Most likely, nutritional intake optimization, growth hormone therapy, and improvement in other replacement therapies have also contributed to lengthen renal survival and improve quality of life of cystinotic patients [15, 16].

Nonetheless, NC remains a very severe disease. End-stage renal failure (ESRF) cannot be prevented in most patients but is only postponed to the second or third decade of life; late-onset complications ultimately develop in a substantial proportion of patients [11–13, 17]. In this retrospective study, we reviewed the outcome of 23 patients with NC who were followed continuously in our unit from 1987 to 2006. We documented their evolution and identified factors that contributed to improved outcomes.

Materials and methods

Twenty-three patients (12 males and 11 females) with NC who were followed at our institution for at least 5 years were included in the study. NC diagnosis was based on elevated ILC levels and genetic testing in all but one patient. Genomic DNA was extracted from whole blood with the QIAamp blood DNA kit (QIAGEN, Milan, Italy), followed by direct sequencing of all exons and neighboring intronic regions and promoter region of the *CTNS* gene using primers and amplification conditions previously described [18]. All genomes were tested for the 57-kb deletion, as previously described [1]. Mutations in 13/23 patients were previously reported as part of a large multicentric Italian study [18].

Thirteen patients were initially diagnosed at our unit, and ten children were referred to us for treatment and follow-up after being diagnosed at other institutions. Most patients were followed on a monthly or bi-monthly basis. Medical records were reviewed retrospectively to collect data on renal function, growth, treatment, ILC levels, and complications related to NC. Data were averaged at 6-month intervals for analysis. ILC levels were performed in all patients at least once a year from 1993 and every 3–4 months after 1997 using a semiautomated high-performance liquid chromatography (HPLC) method with fluorescent detection (FD), as previously described [19]. Briefly, polymorphonuclear neutrophils were collected from 5 ml of blood in a heparin tube and were sonicated in a phosphate buffer containing 5 mmol/l N-ethylmaleimide (NEM). Cell proteins were precipitated with sulfosalicylic acid and were measured after centrifugation with bicinchoninic acid (BCA) protein assay (Pierce,

Rockford, IL, USA). The acid-soluble fraction was derivatized with buffers (detailed in [19]), and analyzed by HPLC/FD on a Agilent Technologies Model 1100 instrument (Agilent Technologies) equipped with a Hypersil-ODS chromatographic column (Thermo-Hypersil, Bellefonte, PA, USA). Results were expressed as nanomoles (nmol) $\frac{1}{2}$ cystine per milligram of protein. All measurements were performed when patients attended our outpatient clinic; the preparative phase was always performed within 6 h from blood-sample collection.

Height measurements were expressed as height standard deviation scores (HtSDS) based on the Tanner-Whitehouse reference growth curves [20]. With the exception of cysteamine, drug variables were expressed as dichotomous variables (yes/no) when patients had taken a given medication for at least 12 months. Glomerular filtration rate (GFR) was calculated from serum creatinine using the Schwartz formula [21]. The evolution of GFR was compared using the Manz reference curves based on a historic population of 205 NC patients who did not receive cysteamine [3]. GFR levels of each patient were plotted against age and fitted with a first-degree exponential curve to extrapolate the age at which patients reached different stages of CRF. Hypothyroidism was defined as the need for thyroxin therapy, diabetes mellitus as the need for insulin therapy, and exocrine pancreatic dysfunction as the need for oral pancreatic enzyme supplementation (prescribed after documentation of steatorrhea). All adult male patients underwent a sperm analysis to define hypogonadism. The diagnosis of myopathy was based on clinical assessment of muscular strength. Electromyography or muscle biopsy studies were not routinely performed. Lung function tests were not available in a majority of patients and were therefore not included in the analysis. All patients had ocular symptoms. Unfortunately, medical records did not allow grading these symptoms or subclassification of ocular lesions for statistical analysis. Although most patients were poorly compliant with cysteamine eye drops in the first years of life, most did not develop a band keratopathy and other severe anterior-chamber abnormalities. One patient had corneal lesions related to herpes simplex infection after renal transplantation, one developed glaucoma, and two developed retinopathy. Likewise, most patients had enamel defects and other dental abnormalities, as previously reported [22]. Despite all having been routinely followed by a dentist, a detailed description of their oral and dental abnormalities was not available.

Statistical analysis

Statistical analyses were performed with the SPSS 11.5.1 for Windows software (SPSS Inc. Chicago, IL, USA). Unadjusted analyses of independent variables were performed by logistic regression for dichotomous variables

and by Cox Mantel or multiple regression analysis for survival and discrete variables, respectively. Multivariate analysis was not performed due to the limited number of cases, which would generate type 1 errors. All values are two-sided and statistical significance was assumed for *p* values <0.05.

Results

Patient characteristics and genetics

Patient characteristics and treatment are summarized in Table 1. Twenty-three patients were enrolled in the study, including 12 males and 11 females. The majority of patients were diagnosed before the age of 2 years (median age at diagnosis 21 months, range 3–60). The primary reason for diagnosis was failure to thrive, which was observed in 19/23 patients. Eight patients had a GFR>90 ml/min/1.73 m² when they first presented. The median duration of follow-up was 17.6 years (range 6.3–27.8 years). At the last follow-up, one patient had normal renal function at 12 years of age, six had CRF, and 16 were transplanted. Of these, 11 had a functional graft and five re-entered dialysis after graft failure. Three patients died. One patient (patient 8) died at age 15 of unspecified cause while on home peritoneal dialysis, one (patient 9) died at age 21 of severe pulmonary infection, and one (patient 11) died on hemodialysis at age 25 in very poor clinical condition.

CTNS mutations were detected in 43/44 tested alleles. A distinct founder effect was observed in patients originating from central Italy (Lazio, Abruzzo, and Marche regions),

where the c.681 +1 G>A mutation in intron 9 was detected in 16/32 alleles. In Northern and Southern Italy, the c.1015 G>A mutation was predominantly detected (7/12 alleles). Genetic tests were not performed in patient 8, who died at age 15 years. Parental consanguinity (second and fourth degree) was present in two families.

Evolution of renal function

The evolution of renal function is illustrated in Fig. 1. Most patients reached stage III CRF at the age of 10 years, and >80% initiated dialysis by the age of 14 years. These evolutions were not homogeneous. A distinct improvement was observed in children treated in more recent years, as indicated by Cox regression analysis (Table 2). Specifically, the analysis shows increased risk of reaching earlier stage III CRF in older patients and in patients who initiated cysteamine therapy later in life. Conversely, higher doses of cysteamine, particularly when expressed as g/m²/day, and the use of angiotensin-converting enzyme inhibitors (ACEi), were protective against a rapid deterioration of renal function.

To highlight these results, the cohort was divided in two groups according to age at which cysteamine was initiated (before or after 2.5 years of age). Patient 1 had taken cysteamine in a single daily dose until the age of 8.2 years, and patient 11 was treated with <0.3 g/m² until the age of 7.2 years. These two patients were classified in the second group despite having started cysteamine before 2.5 years and, for the purpose of the analyses, the age at which they were treated appropriately was assumed to be the age at which they started treatment. ILC levels for patients of each

Table 1 Patient characteristics and treatments

Patient characteristics		
Gender	12 M:11 F	52%:48%
Age at diagnosis (months)	21	3 - 60
Abnormal renal function at diagnosis	15/23	65%
Years of follow-up	17.6	6.3-27.8
Treatment with cysteamine		
Age at the beginning of treatment (years)	1.7	0.3 - 21
Patients treated before 2.5 years	11/23	48%
Patients treated before ESRF	21/23	91%
Dose (mg/Kg)	53.5	±17.8
Dose (g/m ²)	1.35	±0.49
Other treatments		
ACEi (ramipril)	7/23	30%
rhGH	11/23	48%
Indomethacin	10/23	43%
Thyroxin	13/23	57%
Insulin	4/23	17%
Pancreatic enzymes	4/23	17%

Categorical data are expressed as absolute count and percentage; continuous data are expressed as mean±SD, or median and range if data did not fit a normal distribution
ACEi angiotensin-converting enzyme inhibitor, *rhGH* recombinant human growth hormone

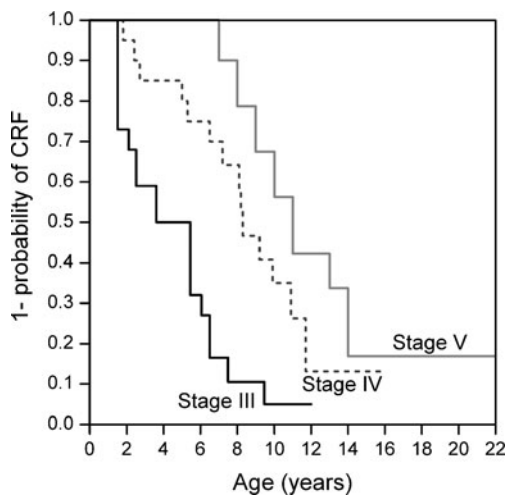


Fig. 1 Evolution of renal function. Kaplan–Meyer analysis indicating the age at which stages III–V chronic renal failure (CRF) were reached in all 23 patients. Stage III: glomerular filtration rate (GFR) 30–59 ml/min/1.73 m², stage IV: GFR 15–30 ml/min/1.73 m², stage V: GFR <15 ml/min/1.73 m²

group are detailed in Fig. 2a. All patients treated before 2.5 years of age (group 1) had a median ILC level <1.5 nmol ½ cystine/mg protein, and in most patients, >75% were below this threshold. Conversely, several patients in group 2 had suboptimal ILC levels and were less frequently monitored.

Patients 3, 7, and 11 admitted poor compliance. After excluding these patients and calculating the mean doses of cysteamine and mean ILC levels per patient-year, a negative correlation was observed between ILC levels and cysteamine

dose, which reached statistical significance when the dose was expressed in mg/kg (Fig. 2b, c). The dose needed to reach target ILC levels varied considerably. No clear trend related to age was observed (data not shown). The evolution of renal function in groups 1 and 2 is illustrated in Fig. 3a, b using the Manz curves as reference [3]. A majority of patients treated early with cysteamine maintained a GFR close to normal values in the first decade of life. Four patients reached ESRD more rapidly. Of these, three (16, 17, 21) had begun monitoring their ILC levels only 3.6–7.8 years after starting treatment (Fig. 2a). The evolution of GFR in group 2 patients fitted the evolution predicted by Manz and coworkers [3].

ACEi were prescribed to seven patients since 2002. All received ramipril at a dose of 2–5 mg/m²/day. Treatment was not started before 6 years of age, and no attempt was made to increase the dose due to the risk of acute renal failure in polyuric patients with Fanconi syndrome. Indomethacin was stopped prior to ramipril initiation. To analyze the effect of ACEi, the delta GFR per year was compared between patients treated with or without ACEi during stages I, II and III CRF (mean creatinine clearance was similar between the two groups). The average changes in GFR were +0.06±0.78 ml/min/1.73 m²/year in treated patients (19 patient-years) and -0.27±0.91 ml/min/1.73 m² in nontreated patients (79 patient-years). These differences did not reach statistical significance (*p*=0.17) and were not controlled for other covariates due to the limited number of patients. In addition, a clear but modest trend in decreased proteinuria was observed in most patients (data not shown). Unfortunately, differences in timing and modality of monitoring

Table 2 Variables associated with chronic renal failure or poor growth

Ind. variables	Reference/units	Stage III CRF			HtSDS<-2SD		
		H.R.	95% C.I.	<i>p</i>	O.R.	95% C.I.	<i>p</i>
Gender	male	1.16	0.39-3.47	0.80	0.60	0.10-3.72	0.58
Age at last F/U	years	1.26	1.10-1.45	0.001	1.23	1.01-1.50	0.04
Serum creatinine at 5 years of age	mg/dl				2.23	0.77-6.44	0.14
cysteamine: age started	years	1.32	1.09-1.61	0.006	1.18	0.84-1.64	0.34
cysteamine: dose	mg/Kg/day	0.97	0.93-1.00	0.07	0.98	0.92-1.04	0.51
cysteamine: dose	g/m ² /day	0.19	0.04-0.89	0.04	0.71	0.07-6.89	0.77
ILC levels	nmoles ½ cystine / mg prot	2.61	0.63-10.83	0.19	8.10	0.51->100	0.14
	%>1.0	1.11	0.07-18.26	0.94	>100	0.00->100	0.14
	%>1.5	2.71	0.04-172.5	0.63	8.83	0.03->100	0.46
Indomethacin	yes	1.10	0.35-3.36	0.87	0.30	0.05-1.94	0.20
ACEi	yes	0.15	0.03-0.68	0.01	0.14	0.01-1.55	0.10
rhGH	yes	0.66	0.23-1.90	0.44	1.00	0.17-5.99	1.00
Thyroxin	yes	0.71	0.22-2.31	0.58	0.47	0.07-3.34	0.44

The first block of results, labeled “Stage III CRF”, reports the hazard ratios (H.R.) for the Cox-Mantel analysis and the 95% confidence intervals (C.I.). The second block of results, labeled “HtSDS<-2SD”, indicate the odds ratios (O.R.) for the logistic regression analysis and the 95% C.I. Significant results are highlighted in bold

F/U, follow-up, ACEi, angiotensin converting enzyme inhibitors, rhGH, recombinant human growth hormone

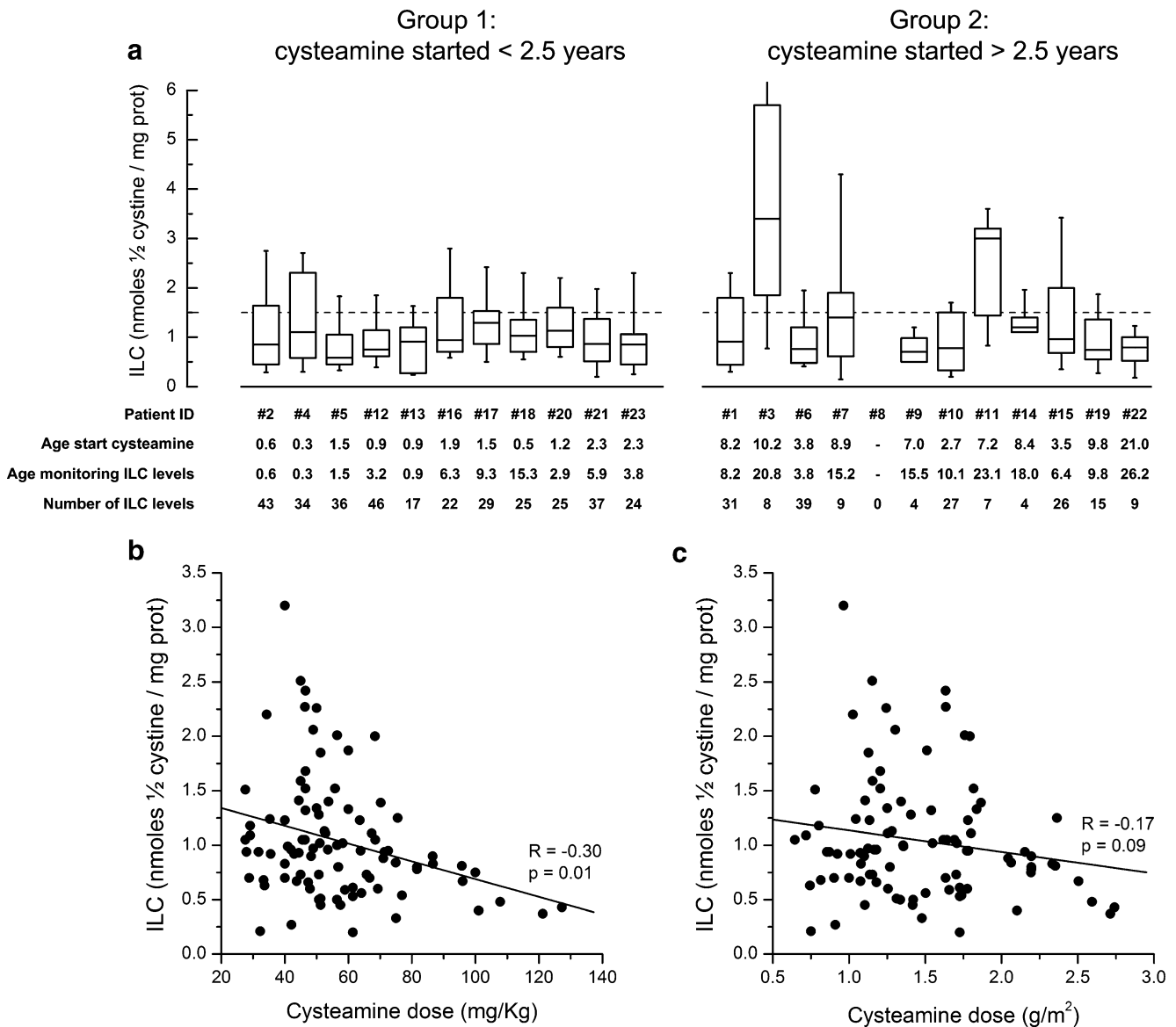


Fig. 2 Intraleucocyte cystine (ILC) levels. **a** Patients were divided in two groups depending on the age at which they started appropriate cysteamine treatment (see text for details). The 25th, 50th, and 75th percentiles (box) and the 5th and 95th percentiles (error bars) are

reported for each patient. The age at which cysteamine was started and at which ILC levels were monitored are indicated in years. **b,c** Correlation between cysteamine dose, expressed in mg/kg/d or g/m²/d, and ILC levels. Each point represents one patient-year

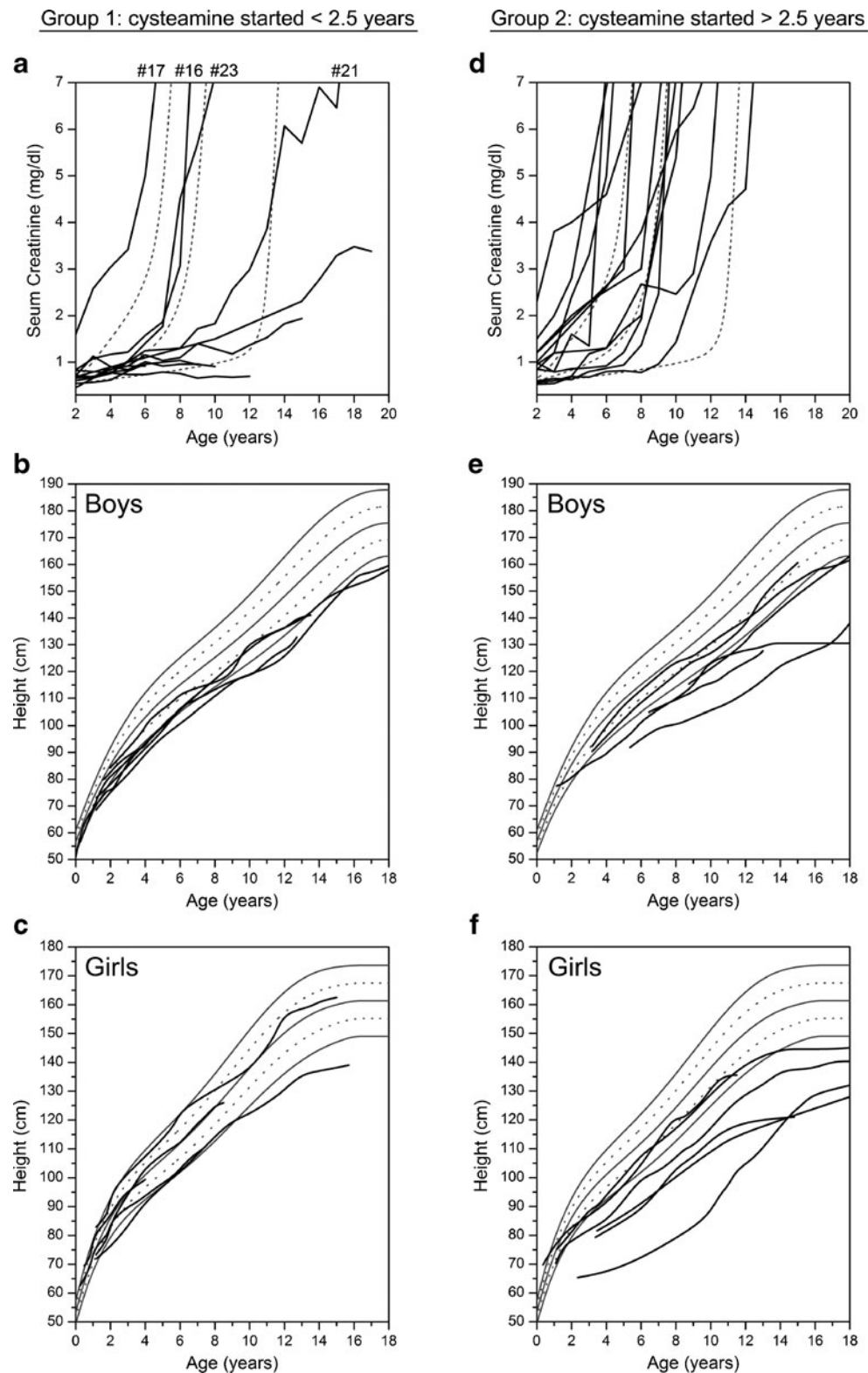
proteinuria between patients and within patients prevented reliable quantification of the effect of ramipril on protein excretion.

Growth

Growth curves are reported in panels b, c, e, and f of Fig. 3. As shown, patients in group 1 grew better in comparison with patients in group 2. By logistic regression analysis (Table 2), the likelihood of having a HtSDS below -2 SD at the last follow-up was significantly associated with patient’s age, indicating that older patients grew worse. As

discussed above, these patients were more likely to have received suboptimal cysteamine treatment. Annual changes in HtSDS were also analyzed by multiple regression analysis, including the same independent variables reported in Table 2. Positive changes in HtSDS were statistically associated with the use of recombinant human growth hormone (rhGH) ($p < 0.001$), which was prescribed to eight patients who had fallen below the -2 SD threshold. The positive association between gain in HtSDS and rhGH treatment highlights catch-up growth secondary to hormonal therapy but does not indicate that rhGH was a prognostic factor for better linear growth.

Fig. 3 Panels **a, d** Evolution of serum creatinine in children treated with cysteamine before or after 2.5 years of age. *Hashed lines* indicate the 3rd, 50th, and 97th percentiles of the reference population reported by Manz et al. [3] (see text for details). **b, c, e, f** Growth charts for the same patients



Other complications

The prevalence of diabetes mellitus (1/22 before 10 years of age, 4/18 between 10–18 years, and 5/11 older than 18 years) and hypothyroidism (7/22 before 10 years of age, 10/18

between 10–18 years, and 9/11 older than 18 years) increased progressively with age. Pancreatic enzyme supplementation was prescribed to 3/11 adult patients. Clinical evidence of myopathy and/or epilepsy was observed in 5/11 and in 1/11 patients older than 18 years, respectively. Male hypogonadism

was diagnosed in all five tested adult male patients. Three patients died during follow-up. Of note, we observed four tumors. One patient (23) was diagnosed with a cerebral medulloblastoma at age 4 that was successfully removed by neurosurgery. He is now doing well but has residual facial hemiparesis. One patient (11) underwent surgery at age 13 for a rare ankle hemangioblastoma. One patient (10) developed hyperthyroidism secondary to a thyroid follicular adenoma at age 23, which was surgically removed. Detailed history of this patient has been reported elsewhere [23]. He also developed Epstein Barr virus (EBV)-related posttransplant lymphoproliferative disease at age 14 and is in complete remission with a functioning graft.

Discussion

To date, NC remains a severe condition. The overall prognosis has considerably improved since the 1950s–1960s, when Fanconi syndrome began to be recognized and treated appropriately, allowing children to survive beyond the first year of life. However, most patients developed ESRD by the age of 10 years [3]. Cysteamine treatment was introduced in the 1980s and has considerably improved renal survival, as well as the development of symptoms related to cystine accumulation in other organs [7, 12, 24, 25]. Fanconi syndrome, on the other hand, does not respond to cysteamine [26]. With increased life expectancy, early focus on renal disease is progressively shifting to other complications that develop later in life. Data accumulated in the past decade indicate that cysteamine not only delays ESRD but improves and potentially prevents most other nonrenal symptoms of NC [7, 12, 13]. Patients treated very early in life were entering adulthood in the last decade. More long-term data are needed to verify whether positive effects of cysteamine will last into mid- and late adulthood or whether symptoms—in particular, neurological and muscular symptoms—are only postponed.

In this study, we reviewed our experience over more than 20 years. This period encompasses the introduction of cysteamine therapy at our unit in the late 1980s, followed by routine measurement of ILC levels since 1998, allowing treatment optimization. Eventually, nearly all our patients received cysteamine, but a significant proportion was born before treatment was available and/or ILC levels were not monitored until several years after therapy initiation. Overall, our experience is similar to that reported by others and confirms the dramatic improvements in renal prognosis and linear growth of patients with NC [24]. The major factor in improving renal function was early initiation of cysteamine treatment. ILC levels, on the other hand, did not correlate well with evolution. This is most likely attributable to a lack of power of our analysis because levels were not

available for most patients with poor renal evolution. Nonetheless, our results highlight the importance of rapidly recognizing the disease and immediately initiating treatment with cysteamine [24, 26, 27]. Patients 6, 10, and 15 for example, began treatment between 2.5 and 4.0 years of age and rapidly developed ESRD despite being treated with appropriate doses of cysteamine and being monitored regularly with at least three ILC levels per year (Fig. 2a). The importance of taking cysteamine every 6 h and monitoring ILC levels has been well documented [7, 28] and is confirmed in our cohort. Specifically, we observed that levels were not monitored for several years in three out of the four patients who had unfavorable renal evolution despite starting treatment before 2.5 years of age (Fig. 3a). The clinical relevance of monitoring ILC level is further emphasized by the weak correlation between cysteamine dose and ILC levels, indicating a large variability in the pharmacokinetics of the drug among individuals.

The second factor that improved renal function was treatment with ramipril. ACEi are known to decrease intraglomerular pressure and inhibit glomerular fibrotic pathways, allowing delay of CRF progression in children with proteinuric renal diseases [29]. Proteinuria in NC is of mixed tubular and glomerular origin [30]. The antiproteinuric effect of ACEi in NC has already been documented, emphasizing the importance of the glomerular component in NC proteinuria [16]. In other renal diseases, reducing urine protein excretion with ACEi treatment has been shown to correlate with delayed CFR progression [29]. Likewise, our results suggest that decreased proteinuria after ACEi therapy, as previously documented by Levchenko et al. [16], translates into delaying CFR progression. The number of patients in our cohort was too small to draw definitive conclusions and did not allow controlling for covariates. In particular, we only began treating patients with ramipril after 2002. Therefore, patients receiving this treatment were also more likely to have been treated earlier and better with cysteamine. Treatment with ACEi in polyuric patients may be dangerous and may cause acute renal failure because these drugs decrease renal perfusion [31]. For these reasons, we did not combine ramipril with indomethacin therapy, which has similar hemodynamic effects on the renal microcirculation [31], and did not increase doses to maximize the antiproteinuric effect of ACEi. Also, we only started ramipril after the age of 5 years. The efficacy of ACEi in delaying CRF in NC, as well as the optimal dose and timing for initiating treatment, require further studies.

Poor growth is a classic symptom of NC; most of our patients were diagnosed after consulting for failure to thrive. Growth retardation in NC is multifactorial. In addition to rickets, poor feeding, hypothyroidism, CRF, and steroid treatment after transplantation, patients may also suffer from cystinosis-specific bone abnormalities, as suggested by data

on CTNS-knockout mice, which displayed decreased bone density and thinner cortical bones despite normal renal function and the absence of significant urinary phosphate wasting [32].

Our experience shows a very clear improvement in linear growth over the years. Most of our recently treated patients have normal or near-normal linear growth. In our view, this is related to early initiation of cysteamine, as previously reported [24, 26, 27], despite the fact that this association did not emerge from our analysis, most likely due to lack of power. On the other hand, our analysis may also reflect the importance of other covariates, including better management of rickets, prompt initiation of enteral feedings, hormonal therapies with rhGH and/or thyroxin, and overall improvement in renal prognosis, which decreases the impact of CRF on linear growth.

Finally, the prevalence of other symptoms related to cystinosis, including male infertility, was similar to data reported by others [33]. The number of patients was too small to appreciate changes over time. Of note, however, we observed a significant incidence of tumors. Other benign or malignant tumors were reported in patients with NC, including a renal cell carcinoma, a prolactinoma, and an odontogenic cyst [34–36]. The risk of developing tumors in these patients has not been established, although higher prevalence has not been reported. Large registries are needed to address this question.

In conclusion, our experience confirms previous reports showing a significant improvement in renal and nonrenal outcomes of cystinosis over the past decades. Despite limitations related to the number of patients in our cohort, our data highlight the importance of early diagnosis in order to initiate cysteamine as soon as possible. Prospective data are needed to confirm the positive effect of ACEi on the progression of renal failure.

References

1. Town M, Jean G, Cherqui S, Attard M, Forestier L, Whitmore SA, Callen DF, Gribouval O, Broker M, Bates GP, han't Hoff W, Antignac C (1998) A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat Genet* 18:319–324
2. Gahl WA, Thoene JG, Schneider JA (2002) Cystinosis. *N Engl J Med* 347:111–120
3. Manz F, Gretz N (1994) Progression of chronic renal failure in a historical group of patients with nephropathic cystinosis. European collaborative study on cystinosis. *Pediatr Nephrol* 8:466–471
4. Norden AG, Lapsley M, Lee PJ, Pusey CD, Scheinman SJ, Tam FW, Thakker RV, Unwin RJ, Wrong O (2001) Glomerular protein sieving and implications for renal failure in fanconi syndrome. *Kidney Int* 60:1885–1892
5. Niaudet P, Tete M-J, Broyer M (1999) Renal disease in cystinosis. In: Broyer M (ed) *Cystinosis*. Elsevier, Paris
6. Kalatzis V, Antignac C (2002) Cystinosis: from gene to disease. *Nephrol Dial Transplant* 17:1883–1886
7. Theodoropoulos DS, Krasnewich D, Kaiser-Kupfer MI, Gahl WA (1993) Classic nephropathic cystinosis as an adult disease. *JAMA* 270:2200–2204
8. Robert JJ, Tête MJ, Guest G, Gagnadoux MF, Niaudet P, Broyer M (1999) Diabetes mellitus in patients with infantile cystinosis after renal transplantation. *Pediatr Nephrol* 13:524–529
9. Pisoni RL, Thoene JG, Christensen HN (1985) Detection and characterization of carrier-mediated cationic amino acid transport in lysosomes of normal and cystinotic human fibroblasts. Role in therapeutic cystine removal? *J Biol Chem* 260:4791–4798
10. Schneider JA, Clark KF, Greene AA, Reisch JS, Markello TC, Gahl WA, Thoene JG, Noonan PK, Berry KA (1995) Recent advances in the treatment of cystinosis. *J Inher Metab Dis* 18:387–397
11. Gahl WA, Reed GF, Thoene JG, Schulman JD, Rizzo WB, Jonas AJ, Denman DW, Schlesselman JJ, Corden BJ, Schneider JA (1987) Cysteamine therapy for children with nephropathic cystinosis. *N Engl J Med* 316:971–977
12. Gahl WA, Balog JZ, Kleta R (2007) Nephropathic cystinosis in adults: natural history and effects of oral cysteamine therapy. *Ann Intern Med* 147:242–250
13. Nesterova G, Gahl W (2008) Nephropathic cystinosis: late complications of a multisystemic disease. *Pediatr Nephrol* 23:863–878
14. Levchenko E, de Graaf-Hess A, Wilmer M, van den Heuvel L, Monnens L, Blom H (2004) Comparison of cystine determination in mixed leukocytes vs polymorphonuclear leukocytes for diagnosis of cystinosis and monitoring of cysteamine therapy. *Clin Chem* 50:1686–1688
15. Haycock GB, Al-Dahhan J, Mak RH, Chantler C (1982) Effect of indomethacin on clinical progress and renal function in cystinosis. *Arch Dis Child* 57:934–939
16. Levchenko E, Blom H, Wilmer M, van den Heuvel L, Monnens L (2003) ACE inhibitorenalapril diminishes albuminuria in patients with cystinosis. *Clin Nephrol* 60:386–389
17. Geelen JM, Leo AH, Monnens, Levchenko EN (2002) Follow-up and treatment of adults with cystinosis in the Netherlands. *Nephrol Dial Transplant* 17:1766–1770
18. Mason S, Pepe G, Dall'Amico R, Tartaglia S, Casciani S, Greco M, Bencivenga P, Murer L, Rizzoni G, Tenconi R, Clementi M (2003) Mutational Spectrum of the CTNS gene in Italy. *Eur J Hum Genet* 11:503–508
19. Pastore A, Lo Russo A, Greco M, Rizzoni G, Federici G (2000) Semiautomated method for determination of cystine concentration in polymorphonuclear leukocytes. *Clin Chem* 46:574–576
20. Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170–179
21. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics* 58:259–263
22. Bassim CW, Gautam P, Domingo DL, Balog JZ, Guadagnini JP, Gahl WA, Hart TC (2010) Craniofacial and dental findings in cystinosis. *Oral Dis* 16:488–495
23. Guzzo I, Di Zazzo G, Grossi A, Greco M, Dello Strologo L (2010) Radiotherapy-induced hyperthyroidism in a cystinotic kidney transplant patient with Hodgkin lymphoma. *Pediatr Transplant*. doi:10.1111/j.1399-3046.2009.01274.x
24. Markello TC, Isa B, Gahl WA (1993) Improved renal function in children with cystinosis treated with cysteamine. *N Engl J Med* 328:1157–1162
25. Kimonis VE, Troendle J, Rose SR, Yang M, Markello T, Gahl W (1995) Effects of early cysteamine therapy on thyroid function and growth in nephropathic cystinosis. *J Clin Endocrinol Metab* 80:3257–3261

26. Kleta R, Bernardini I, Ueda M, Varade WS, Phornphutkul C, Krasnewich D, Gahl WA (2004) Long-term follow up of well-treated nephropathic cystinosis patients. *J Pediatr* 145:555–560
27. Gahl WA (2003) Early oral cysteamine therapy for nephropathic cystinosis. *Eur J Pediatr* 162:S38–S41
28. Levchenko EN, van Dael CM, de Graaf-Hess AC, Wilmer MJ, van den Heuvel LP, Monnens LA, Blom HJ (2006) Strict cysteamine dose is required to prevent nocturnal cystine accumulation in cystinosis. *Pediatr Nephrol* 21:110–113
29. ESCAPE Trial Group, Wühl E, Trivelli A, Picca S, Litwin M, Peco-Antic A, Zurowska A, Testa S, Jankauskiene A, Emre S, Caldas-Afonso A, Anarat A, Niaudet P, Mir S, Bakkaloglu A, Enke B, Montini G, Wingen AM, Sallay P, Jeck N, Berg U, Caliskan S, Wygoda S, Hohbach-Hohenfellner K, Dusek J, Urasinski T, Arbeiter K, Neuhaus T, Gellermann J, Drozd D, Fischbach M, Möller K, Wigger M, Peruzzi L, Mehls O, Schaefer F (2009) Strict blood-pressure control and progression of renal failure in children. *N Engl J Med* 361:1639–1650
30. Wilmer MJ, Christensen EI, van den Heuvel LP, Monnens LA, Levchenko EN (2008) Urinary protein excretion pattern and renal expression of megalin and cubilin in nephropathic cystinosis. *Am J Kidney Dis* 51:893–903
31. Abuelo JG (2007) Normotensive ischemic acute renal failure. *N Engl J Med* 357:797–805
32. Cherqui S, Sevin C, Hamard G, Kalatzis V, Sich M, Pequignot MO, Gogat K, Abitbol M, Broyer M, Gubler MC, Antignac C (2002) Intra-lysosomal accumulation in mice lacking cystinosin, the protein defective in cystinosis. *Mol Cell Biol* 22:7622–7632
33. Besouw MT, Kremer JA, Janssen MC, Levchenko EN (2010) Fertility status in male cystinosis patients treated with cysteamine. *Fertil Steril* 93:1880–1883
34. Besouw MT, Levchenko EN, Willemsen MA, Noordam K (2008) Growth hormone producing prolactinoma in juvenile cystinosis: a simple coincidence? *Pediatr Nephrol* 23:307–310
35. DeVilliers P, Gutta R, Szymela VF (2008) Cystinosis, Fanconi syndrome, and odontogenic cysts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 106:866–871
36. Cameron C, Greenbaum L, Sato T, Trost B, Lundeen B, Kelly ME (2008) Renal cell carcinoma in a patient with cystinosis and inflammatory bowel disease: a case report. *Pediatr Nephrol* 23:1167–1170