Kidney International, Vol. 54 (1998), pp. 56–61

Cystinuria subtype and the risk of nephrolithiasis[1]

Paul Goodyer, Irfan Saadi, Patricia Ong, Gay Elkas, and Rima Rozen

Departments of Pediatrics, Human Genetics, Experimental Medicine, and Biology, McGill University, Montreal Children's Hospital, Montreal, Quebec, Canada

Cystinuria subtype and the risk of nephrolithiasis.

Background. Cystinuria patients may be classified into several subgroups based on the urinary phenotype of heterozygotes. However, the relative risk for nephrolithiasis and the prevalence of SLC3A1 mutations in these subgroups are unknown.

Methods. Urinary cystine excretion, age at onset of nephrolithiasis and nature of SLC3A1 mutations were assessed prospectively in 23 cystinuria patients identified primarily through the Quebec Newborn Screening Program. Probands were classified as to cystinuria subtype on the basis of parental urinary cystine excretion.

Results. For classical Type I/I cystinuria, both parents excrete cystine in the normal range and probands carry two mutations of the SLC3A1 gene in nearly every case. Between ages 1 to 7 years, mean cystine excretion was high (4566 ± 480 μmol cystine/g creatinine) and exceeded the theoretic threshold for solubility on 70% of visits. Four of eight Type I/I patients began forming stones in the first decade. Type I/III patients ($N = 12$) excreted less cystine (1544 ± 163 μmol cystine/g creatinine), exceeded the threshold of urinary cystine solubility less frequently (22% of visits) and had no nephrolithiasis in the first decade; one formed a stone at age 16 years. Only one SLC3A1 mutation was identified in this group. Two Type II/N cystinuria children were identified. In these families, the same level of relatively high excretion (>600 μmol cystine/g creatinine) was noted in two or three generations, but no SLC3A1 mutations were identified.

Conclusions. Classical recessive Type I/I cystinuria is genetically and phenotypically distinct from the other subtypes (Type I/III and Type II/N) identified in our population.

Cystinuria is an inherited defect in the reabsorptive transport of cystine and the dibasic amino acids (ornithine, arginine and lysine) from the luminal fluid of the renal proximal tubule and small intestine. Among affected patients, nephrolithiasis occurs when urinary cystine exceeds its solubility at low pH. Certain individuals may develop massive staghorn calculi in the pediatric years, while others may form their first stone only in the second or third decade of life. Classically, at least three cystinuria subtypes have been recognized, based on the level of urinary cystine in obligate heterozygotes [1]. The heterozygous parents of our patients were classified as follows: (*a*) Type I/Normal, urine cystine = 0 to 100 μmol/g creatinine; (*b*) Type III/Normal, urine cystine = 100 to 600 μmol/g creatinine; (*c*) Type II/Normal, urine cystine = 990-1740 μmol/g creatinine.

Heterozygotes within a family tend to excrete cystine in the same range, but for Type I/Normal and Type III/Normal heterozygotes, this is not usually high enough to put them at risk for stone formation. Offspring who inherit two mutant cystinuria genes excrete much higher levels of cystine (1,000 to 8,000 μmol cystine/g creatinine)–approaching the filtered load–and this may exceed the threshold of cystine solubility as urine is concentrated and acidified in the renal collecting tubules. Since patients usually come to medical attention only after they have formed stones, the incidence of nephrolithiasis among cystinuria patients is not known. Furthermore, it is unclear whether the risk of nephrolithiasis in these patients is linked to their genotype.

Initially it was postulated that the variation among cystinuria subtypes would be explained by mild, moderate or severe mutations at a single chromosomal locus. However, we noted that affected children born of two parents excreting cystine in the normal range (Type I/Normal) had significantly higher urinary cystine than children born of matings in which one parent excreted cystine in the normal range (Type I/Normal), while the other had moderately elevated cystine (Type III/Normal) [2]. We speculated that this might be explained by genetic complementation between two distinct cystinuria loci [2].

In 1993, a human cDNA was isolated that induced uptake of cystine, dibasic and some neutral amino acids when expressed in *Xenopus laevis* oocytes [3, 4]. The gene, SLC3A1 (D2H or rBAT), was mapped by somatic cell hybridization [3, 4] and FISH analysis [5] to a region of chromosome band 2p21 containing the cystinuria locus as determined by linkage analysis [6]. Subsequently, over 20 different mutations of SLC3A1 have been identified in cystinuria families from Italy/Spain [7–9], Israel/USA [10], Japan [11] and Canada [12], and all mutations reported to date have been associated with chromosomes linked to Type I cystinuria. It has now been shown that Type III and

[1] **See *Editorial* by Chesney, p 279.**

Key words: cystinuria, nephrolithiasis, genetics, kidney stones, mutations, proximal tubule.

Received for publication November 4, 1997
and in revised form February 3, 1998
Accepted for publication February 3, 1998

probably Type II cystinuria are linked to a second cystinuria locus at chromosome 19q13.1 [13–15].

In this report, we describe long-term clinical follow-up studies of a cohort of cystinuria patients who were identified primarily via the Quebec Network of Genetic Medicine Neonatal Screening Program. The patients were classified as to cystinuria subtype on the basis of parental urinary phenotype as previously reported [2, 12] and by analysis of SLC3A1 gene mutations. Our studies suggest that Type I/I cystinuria patients have the highest risk for nephrolithiasis.

METHODS

The Quebec Network of Genetic Medicine maintains a urinary screening program for several heritable diseases, and infants with elevated excretion of cystine are identified and referred to tertiary centers for follow-up. Since some infants with heterozygous cystinuria excrete cystine and dibasic acids in the "homozygous" range during the first months of life, a final diagnosis of cystinuria is made at one year of age when maturation of normal cystine reabsorptive transport can be expected [16]. After exclusion of generalized Fanconi syndrome and other diseases, cystinuria probands excreting more than 1000 μmol cystine per gram creatinine are referred to a tertiary care center, such as the Renal-Genetics Clinic at the Montreal Children's Hospital, for investigation.

Fresh urine samples were obtained from parents and siblings referred to our clinic. Urinary cystine, ornithine, arginine and lysine were quantified by elution chromatography on ion-exchange resin columns with a Beckman 6300 amino acid analyzer (Beckman Instruments, Mississauga, Ontario, Canada). Urinary phenotype was assigned for parents according to the cystinuria subtype classification above [2], and the proband subtype was then assigned accordingly. Nine Type I/I probands, 12 Type I/III probands, and two Type II/N probands were followed for 6 to 30 years. Average urinary cystine excretion (μmol cystine per gram creatinine) was calculated from 4 to 12 random morning samples collected during routine visits to the clinic between the ages of one and seven years. These samples were also used to plot cystine concentration (μmol cystine/liter) versus urinary pH in relation to the curve describing theoretic cystine solubility. In the subset of children who formed cystine stones, successive urine samples were collected throughout the day; cystine concentration was plotted versus pH for each sample. Renal ultrasonography was performed routinely every one to three years or as needed to identify nephrolithiasis. One child, who was diagnosed elsewhere, did not have multiple urine samples analyzed during the first seven years of life. He was classified on the basis of molecular analyses and included only for the purpose of defining risk of nephrolithiasis in the first decade. For all children, parents were instructed to maintain high fluid intake, but none were treated with mercaptopropionyl glycine or penicillamine prior to onset of recurrent stones.

DNA was isolated from peripheral lymphocytes with informed consent [12]. To detect large deletions or gene rearrangements, 5 μg of DNA was digested with appropriate restriction enzymes and analyzed on Southern blots using a radiolabeled SLC3A1 cDNA probe as previously described [12]. Genomic DNA was also amplified by PCR, using primers for each SLC3A1 exon plus flanking splice junctions [12]. Heteroduplex analysis of PCR products was used to detect small insertions or deletions; SSCP analysis of radioactive PCR products was used for detection of base substitutions [12]. Following the identification of heteroduplexes or abnormally migrating bands on SSCP gels, PCR products were sequenced. Confirmation of sequence changes was performed by a new PCR with restriction analysis if the mutation created or obliterated a restriction site. When a naturally occurring restriction site was unavailable, restriction sites were artificially created during the PCR.

RESULTS

Type I/I cystinuria

Among the eight probands classified as Type I/I on the basis of parental phenotype, we identified two mutations of the SLC3A1 gene in seven of the eight patients (Table 1). In the eight patient, only one mutation was found. Since two of the probands were siblings, this represents a detection rate of 93% (13 mutations identified among 14 unrelated alleles). A ninth patient (#1440-1 referred at age 30) was homozygous for an SLC3A1 mutation, but parents were unavailable for urinary phenotyping. Detailed molecular analyses of the SLC3A1 mutations in our population are described in the accompanying manuscript [17].

Multiple random morning urine samples (N = 4 to 12) were available from seven of the nine Type I/I patients between the ages of one to seven years (data was available only after age 7 for one patient and only 3 values were available for another). The mean cystine excretion for the group was 4566 $\pm$ 480 (SEM) μmol cystine/g creatinine for the group; the mean and 95% confidence intervals for each individual are listed in Table 1. When the cystine concentration was plotted against urinary pH over the same time period, 70% of samples were found to exceed the theoretic limit of cystine solubility in aqueous solution (Fig. 1).

Four of the eight Type I/I patients followed prospectively formed kidney stones in the first decade of life. Of the four children who have not yet formed a kidney stone, three are still less than 10 years of age and were started on potassium citrate therapy between six and seven years of age because of consistent cystine excretion in the supersaturated range. All stones were associated with episodes of abdominal pain and mild microscopic hematuria, and were confirmed by renal ultrasonography or radiography.

Table 1. Type I/I cystinuria

Patient	Mutation	Age	Age at first stone	Urinary cystine excretion μmol cystine/g creatinine		
				Proband	Mother	Father
780-1	T216M/T216M	8	2	4795 (2765-4464)	58	42
782-1	974delGA/S217R	10	9	2578 (1915-3241)	80	26
782-2[a]	974delGA/S217R	8	—	5778 (2394-9163)	80	26
936-1[a]	del/del	8	—	5778 (2394-9163)	69	40
1226-1	R270L/M467T	16	—	5361 (4157-6564)	24	1
817-1	1500+1G→T/del	31	6	2482[c] (1674-3289)	26	31
818-1[a]	E483X/P337P	8	—	4255 (3116-5392)	83	46
885-1	1500+1G→T/?	9	7	5500 (3665-7330)	33	42
1440-1[b]	1810delTG/1810delTG	30	?	—	—	—

[a] Author, please define or delete
[b] Please define
[c] Please define

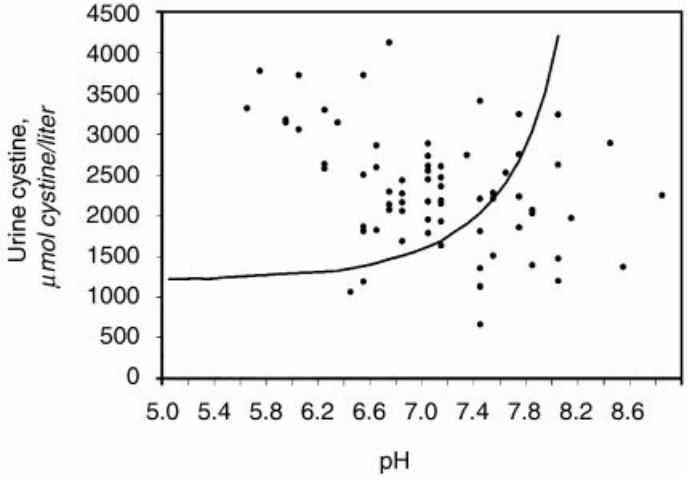

Fig. 1. Urine cystine excretion by Type I/I cystinuria probands in relation to the theoretic threshold of solubility. Urine cystine (μmol cystine/liter) of individual morning urine samples between age 1 to 7 years is plotted versus urine pH in relation to the theoretic threshold of cystine solubility.

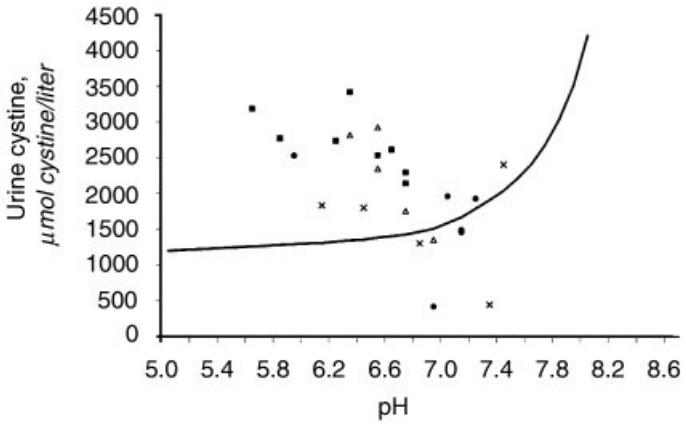

Fig. 2. Urine cystine excretion by four Type I/I stone forming cystinuria probands at various times of the day. Urine cystine (μmol cystine/liter) is plotted versus urinary pH: 5 to 7 a.m. (■), 11 a.m. to 12 p.m. (**X**), 3 to 4 p.m. (●), and 8 to 10 p.m. (△) in relation to the theoretic threshold of cystine solubility.

Four of the children who formed stones in the first decade of life and one stone-free sibling were asked to provide successive urine samples during two 24-hour periods. Urine samples were clustered into early morning (5 to 7 a.m.), noontime (11 a.m. to 12 p.m.), afternoon (3 to 4 p.m.) and evening (8 to 10 p.m.) samples, and plotted versus urine pH in relation to the theoretic cystine solubility curve (Fig. 2). In each case, cystine concentration exceeded the theoretic limit of cystine solubility to the greatest degree in early morning (8 of 8) or late evening (4 of 5) samples. During the day, urine cystine concentration tended to be below or just above the solubility threshold.

Type I/III cystinuria

DNA was available for analysis on 9 of the 12 Type I/III probands. We were able to detect only one SLC3A1 mutation among the 9 alleles contributed by the Type I/N parent (detection rate 11%; Table 2). This mutation (M467T) corresponded to the most common cystinuria allele reported in Spanish and Italian populations [7].

Average morning urinary cystine (ages 1 to 7 years) among the Type I/III children was 1544 ± 163 μmol cystine/g creatinine, which was significantly lower ($P < 0.001$, Student *t*-test) than for their Type I/I cystinuria counterparts. However, for patients in both groups, there

Table 2. Type I/III cystinuria

Patient	Mutation	Age	Age at first stone	Urinary cystine excretion μmol cystine/g creatinine		
				Proband	Mother	Father
947-1	M467T/?	8	—	2178 (1541-2814)	53	235
872-1	?/?	19	16	2714 (1900-3528)	306	29
732-1	?/?	15	—	1624 (899-2347)	417	45
1390-1	?/?	5	—	1267 (708-2087)	78	402
829-1	?/?	12	—	1688 (1110-2266)	420	47
830-1	?/?	11	—	1136 (848-1424)	120	51
979-1	?/?	13	—	1239 (970-1507)	165	70
988-1	?/?	8	—	1343 (290-2839)	577	30
1326-1	?/?	6	—	892 (455-1330)	22	212
1500-1	?/?	8	—	2210 (845-3575)	124	34
1501-1	?/?	7	—	1352 (1117-1588)	250	36
1502-1	?/?	6	—	885 (432-1337)	35	394

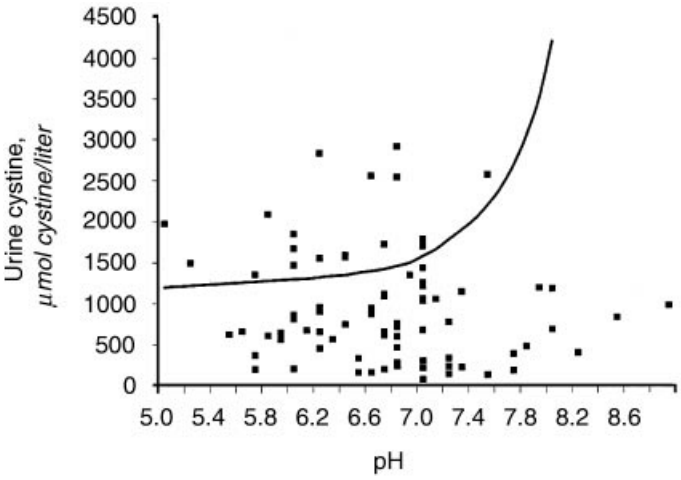

Fig. 3. Urine cystine excretion by Type I/III cystinuria probands in relation to the theoretic threshold of solubility. Urine cystine (μmol cystine/liter) of individual morning urine samples between ages 1 to 7 years is plotted versus urine pH in relation to the theoretic threshold of cystine solubility.

was considerable day-to-day variability and individual values often overlapped in the range of 2000-3000 μmol cystine/g creatinine. Thus, probands could not readily be classified as to cystinuria subtype without family studies or molecular analyses.

When cystine concentration (μmol cystine/liter) in individual morning urine samples was plotted as a function of urinary pH, 22% of samples exceeded the theoretic limit of cystine solubility (Fig. 3). Only one patient in this group has formed a kidney stone (documented by renal ultrasonography), and this was at age 16. Since the mean age of Type I/III patients was younger than that of the Type I/I group, we analyzed only the patients who had been followed for at least eight years for comparison of nephrolithiasis risk in the two groups. Five of nine Type I/I patients formed a stone in the first decade whereas all eight Type I/III patients were stone-free in this period ($P < 0.03$, two-tailed Fisher's exact test).

Type II/Normal cystinuria

Two patients who excreted >1000 μmol cystine/g creatinine at one year of age were classified as Type II/Normal cystinuria, based on parental urinary phenotype (Table 3). In each case, the level of urinary cystine excretion in the proband was comparable to that of one parent. In the 1245-1 kindred, the proband's maternal grandfather was also affected (1741 μmol cystine/g creatinine) and had a history of nephrolithiasis in adulthood. In the 918-1 kindred, the proband's affected father passed a stone at 36 years of age. No SLC3A1 mutations were identified in the two Type II/Normal patients. No nephrolithiasis was noted in the children, but the cystine concentration of morning urine samples (age 1 to 7 years) has often exceeded its theoretic solubility limit (Fig. 4).

DISCUSSION

Although three subtypes of cystinuria have been recognized since 1968 [1], prospective studies comparing the relative nephrolithiasis risk in each group have been difficult to accomplish. Most reports involve cohorts of patients who first come to medical attention only once the first stone

Table 3. Type II/Normal cystinuria

Patient	Mutation	Age	Age at first stone	Urinary cystine excretion μmol cystine/g creatinine		
				Proband	Mother	Father
1245-1	?/Normal	7	—	1777 (1314-2239)	1500	50
918-1	?/Normal	17	—	894 (707-1080)	44	843

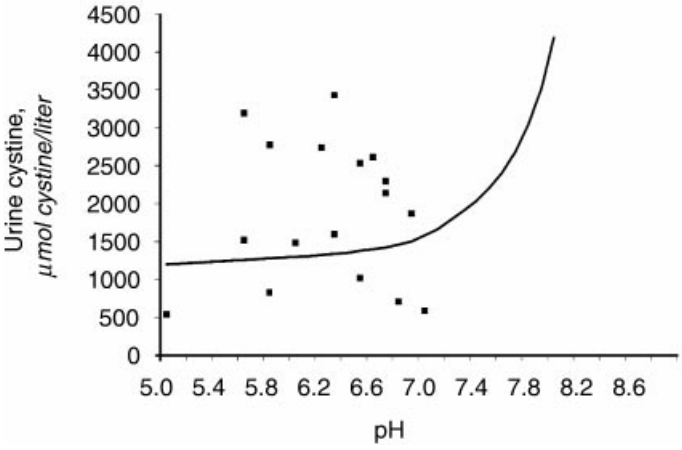

Fig. 4. Urine cystine excretion by Type II/Normal cystinuria patients in relation to the theoretic threshold of solubility. Urine cystine concentration (μmol cystine/liter) of individual morning urine samples between ages 1 to 7 years is plotted versus urine pH in relation to the theoretic threshold of cystine solubility.

is formed, which is often in adulthood when parental studies are not always possible. Consequently, it is unclear whether an appreciable number of cystinuria patients remain asymptomatic and whether the risk of nephrolithiasis is primarily determined by the nature of the cystinuria genes inherited or by other multifactorial determinants (such as, urinary stone inhibitors or environmental conditions). In this study, we have taken advantage of the Quebec Genetic Network Neonatal Screening Program to identify 22 cystinuria patients, classify them on the basis of parental urinary phenotype and SLC3A1 gene status, and collect longitudinal outcome data.

Our experience suggests that a "classic" form of cystinuria can be defined in infants excreting over 1000 μmol cystine/g creatinine at one year of age in whom both parents are completely "silent" carriers, excreting cystine in the normal range (<100 μmol cystine/g creatinine). In nearly all of these children, defective cystine excretion can be attributed to two mutations of the SLC3A1 gene. Since SLC3A1 expression has been shown to be highly restricted to the straight (S3) segment of the proximal tubule [18, 19], it is presumably the dysfunction of the shared cystine/dibasic amino acid transporter in this nephron segment which accounts for Type I/I cystinuria.

Our data show that the risk of nephrolithiasis in children with the "classic" Type I/I form of cystinuria is quite high; at least 50% will form one or more stones within the first decade of life. It should be pointed out that the incidence of early stone formation may be even greater, since three of the Type I/I probands were placed on potassium citrate by age 6 to 7 years. The nephrolithiasis risk may be attributable to the especially high level of cystine excretion associated with a pair of SLC3A1 mutations. On the other hand, this genetic burden can be modifed somewhat. The likelihood of excreting cystine in the supersaturated range appears to be greatest overnight when urine normally becomes most concentrated and acidic. Traditional strategies to dilute and alkalinize the urine might especially target this time period. Plots of urine cystine versus pH demonstrate that Type I/I patients are more likely to exceed the solubility threshold than other groups, but it is uncertain whether these plots are useful to anticipate nephrolithiasis in the individual patient.

In a second form of cystinuria, probands are born of one parent excreting cystine within the normal range and one parent with moderately elevated cystine excretion. In one of our patients, the "silent" (<100 μmol cystine/g creatinine) Type I/Normal heterozygous urinary phenotype was associated with an SLC3A1 mutation, but in most, no mutation was demonstrated. Linkage analysis has shown that a gene mapping to an entirely different locus at chromosome 19q13.1 accounts for Type III (and probably Type II) cystinuria [13–15]. Although the molecular basis for Type I/III cystinuria has not yet been unraveled, it is clear that these patients have a somewhat milder form of the disease. Thus far, none of our Type I/III patients have formed a stone in the first decade of life, and at present we advise only careful monitoring of urine cystine versus pH and occasional ultrasonography for this group. However, the risk of nephrolithiasis at later ages cannot be discounted since many of our patients have not been followed into adulthood. Stone formation has occurred in one 16-year-old Type I/III patient, and the cystine concentration exceeds the theoretic threshold of solubility in 22% of random urine samples.

Two patients identified by the Quebec Network Neonatal Screening program who excreted >1000 μmol cystine/g creatinine at one year of age were classified as Type II/Normal. In these families, cystinuria behaves as an autosomal dominant phenotype since the same relatively high range of urinary cystine was noted in two or three

generations. In both Type I/I and Type I/III families, the risk of nephrolithiasis is recessive, since only the proband excretes cystine in the supersaturated range. We were unable to identify SLC3A1 mutations in either Type II/Normal proband. Conceivably, these patients are heterozygous for a more dysfunctional or dominant-negative mutation at the 19q13.1 locus as suggested by the linkage studies of Bisceglia et al [14]. Although our two probands have not yet formed stones, urinary cystine occasionally exceeds the theoretic solubility threshold and the family history in each suggests that occasional stone formation may occur later in life. Since Type II cystinuria is quite rare in our population, the great majority of cystine stones will be formed in patients with Type I/I or Type I/III cystinuria.

Although this study does not directly address therapy, our observations suggest that patients with Type I/III or Type II/Normal cystinuria might be managed during childhood by prescription of a high daily fluid intake alone, since the risk of nephrolithiasis is low. However, for Type I/I patients, it may be warranted to introduce standard anticipatory therapies such as alkalinizing agents at age five to six years, when the risk of stone formation appears to increase. Studies are underway to determine whether nephrolithiasis can be anticipated in the individual patient by monitoring early the morning urine cystine concentration in relationship to its theoretical limit of solubility.

ACKNOWLEDGMENTS

This work was supported by the Kidney Foundation of Canada (to RR and PG) and the Réseau de Médicine Génétique Appliquée du Fonds de la Recherche en Santé du Québec.

Reprint requests to Dr. Paul Goodyer, Montreal Children's Hospital, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3.
E-mail: pgooren@mchmeds.mchis.mcgill.ca

REFERENCES

1. Rosenberg LE, Downing S, Durant JL, Segal S: Cystinuria: Biochemical evidence for three genetically distinct diseases. *J Clin Invest* 45:365–371, 1966
2. Goodyer PR, Clow C, Reade T, Girardin C: Prospective analysis and classification of patients with cystinuria identified in a newborn screening program. *J Pediatr* 122:568–572, 1993
3. Lee WS, Wells R, Sabbag RV, Mohandas TK, Hediger MA: Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic and neutral amino acid transport. *J Clin Invest* 91:1959–1963, 1993
4. Bertran J, Werner A, Chillaron J, Nunes V, Biber J, Testar X, Zorzano A, Estivill X, Murer H, Palacin M: Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in *Xenopus* oocytes. *J Biol Chem* 268:14842–14849, 1993
5. Zhang X-X, Rozen R, Hediger MA, Goodyer P, Eydoux P: Assignment of the gene for cystinuria (SLC3A1) to human chromosome 2p21 by fluorescence *in situ* hybridization. *Genomics* 24:413–414, 1994
6. Pras E, Arber N, Aksentijevitch I, Katz G, Schapiro JM, Prosen L, Gruberg L, Harel D, Liberman U, Weissenbach J, Pras M, Kastner DL: Localization of a gene causing cystinuria to chromosome 2p. *Nature Genet* 6:415–419, 1994
7. Calonge MJ, Gaspirini P, Chillaron J, Chillon M, Gallucci M, Rousaud F, Barcelo P, Estivill X, Zelante L, Testar X, Dallapiccola B, Di Silverio F, Zorzano A, Nunes V, Palacin M: Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. *Nature Genet* 6:420–425, 1994
8. Gasparini P, Calonge MJ, Bisceglia L, Purroy J, Dianzani I, Notarangelo A, Rousaud F, Gallucci M, Testar X, Panzone A, Estivill X, Zorzano A, Palacin M, Nunes V, Zelante L: Molecular genetics of cystinuria: Identification of four new mutations and seven polymorphisms, and evidence for genetic heterogeneity. *Am J Hum Genet* 57:781–788, 1995
9. Bisceglia L, Calonge MJ, Strologo LD, Rizzoni G, De Sanctis L, Gallucci M, Beccia E, Testar X, Zorzano A, Estivill X, Zelante L, Palacin M, Gasparini P, Nunes V: Molecular analysis of the cystinuria gene: Identification of four new mutations, one large deletion, and one polymorphism. *Hum Genet* 98:447–451, 1996
10. Pras E, Raben N, Golomb E, Arber N, Aksentijevitch I, Schapiro J, Harel D, Katz G, Liberman U, Pras M, Kastner DL: Mutations of the SLC3A1 transporter gene in cystinuria. *Am J Hum Genet* 56:1297–1303, 1995
11. Miyamoto K-I, Katai K, Tatsumi S, Sone K, Segawa H, Yamamoto H, Taketani Y, Takada K, Morita K, Kanayama H, Kagawa S, Takeda E: Mutations of the basic amino acid transporter gene associated with cystinuria. *Biochem J* 310:951–955, 1995
12. Horsford J, Saadi I, Raelson J, Goodyer PR, Rozen R: Molecular genetics of cystinuria in French Canadians: Identification of four novel mutations in Type I patients. *Kidney Int* 49:1401–1406, 1996
13. Calonge MJ, Volpini V, Bisceglia L, Rousaud F, De Sanctis L, Beccia E, Zelante, Testar X, Zorzano A, Estivill X, Gasparini P, Nunes V, Palacin M: Genetic heterogeneity in cystinuria: The SLC3A1 gene is linked to type I but not to type III cystinuria. *PNAS* 92:9667–9671, 1995
14. Bisceglia L, Calonge MJ, Totaro A, Feloubadalo L, Melchionda S, Garcia J, Testar X, Galucci M, Ponzone A, Zelante L, Zorzano A, Estivil X, Gasparini P, Nunes V, Palacin M: Localization, by linkage analysis of the cystinuria type III gene to chromosome 19q13.1. *Am J Hum Genet* 60:611–616, 1997
15. Wartenfeld R, Golomb E, Katz G, Bale SJ, Goldman B, Pras M, Kastner DL, Pras E: Molecular analysis of cystinuria in Libyan Jews: Exclusion of the SLC3A1 gene and mapping of a new locus on 19q. *Am J Hum Genet* 60:617–624, 1997
16. Scriver CR, Clow CL, Reade T, Goodyer P, Auray-Blais C, Giguere R, Lemieux B: Ontogeny modifies expression of cystinuria genes: implications for counselling. *J Pediatr* 106:411–416, 1985
17. Saadi I, Chen Z-X, Hediger M, Ong P, Pereira P, Goodyer P, Rozen R: Molecular genetics of cystinuria: Mutation analysis of SLC3A1 and evidence for another gene in the Type I (silent) phenotype. *Kidney Int* 54:000–000, 1998
18. Kanai Y, Stelzner MG, Lee W-S, Wells RG, Brown D, Hediger MA: Expression of mRNA (D2) encoding a protein involved in amino acid transport in S3 proximal tubule. *Am J Physiol* 263:F1087–F1093, 1992
19. Furriols M, Chillaron J, Mora C, Castello A, Bertran J, Camps M, Testar X, Vilaro S, Zorzano A, Palacin M: rBAT, related to L-cystine transport, is localized to the microvilli of proximal straight tubules, and its expression is regulated in kidney by development. *J Biol Chem* 268:27060–27068, 1993
20. Jaeger P, Portmann L, Saunders A, Rosenberg LE, Thier SO: Anticystinuric effects of glutamine and dietary sodium restriction. *N Engl J Med* 315:1120–1123, 1986